Epiphytic Lichen Monitoring at the
***rare* Charitable Research Reserve**

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Introduction

Air Quality Monitoring

Air quality monitoring plays a critical role in identifying changes in atmospheric pollutants and other chemicals, which can have an effect on ecosystem integrity and human health. Results from air quality monitoring studies can influence policy and decision makers towards regulations that seek to ameliorate deteriorating air quality conditions (Environment Canada, 2013).

In Canada, air quality monitoring and research is intended to identify atmospheric pollutants and track them, which can ultimately be used to evaluate the effectiveness of current policies (Environment Canada, 2013). Due to the transboundary nature of atmospheric pollutants, international agreements and protocols have also been created in places like North America and Europe. An example of such protocols is the *Protocol to the 1979 Convention on Long-Range Transboundary Air Pollution to Abate Acidification, Eutrophication and Ground-Level Ozone* created by the United Nations Economic Commission of Europe (UNECE) (Vipond, 2003). This type of protocol was developed in recognition "…that nitrogen oxides, sulphur, volatile organic compounds and reduced nitrogen compounds have been associated with adverse effects on human health and the environment…" (UNECE, 1999).The directives outlined in this protocol have managed to reduce emissions from pollutants like Nitrogen Dioxide (NO_2) and Sulphur Dioxide (SO_2) by 40 per cent and 70 per cent respectively in Europe (Vipond, 2003).

Air Quality Monitoring at *rare*

Similar to other ecological monitoring activities, air quality monitoring can rely upon indicator organisms from which inferences can be made. Lichens serve as ideal organisms for monitoring changes in air quality because of their ability to absorb pollutants into their tissues through air and rainfall (Brodo, Duran Sharnoff, & Sharnoff, 2001; EMAN, 2002). Lichens have often been described as the *canaries in the coal mine* for air quality because of the sensitivity of certain species to environmental change (Jovan, 2008). Lichens are very responsive to deteriorating air quality (Will-Wolf, n.d.), which enables them to serve as an early warning system for air pollution (Cameron, n.d.). In addition, lichens can be found on every continent, making them readily accessible for air quality monitoring studies (Rossbach & Lambrecht, 2006). Indeed, lichens have long been used in air quality monitoring studies in North America and Europe (Brodo, Duran Sharnoff, & Sharnoff, 2001; MacDonald & Coxson, 2012).

Lichen monitoring at ***rare*** began in 2003 as a component of a senior undergraduate thesis supervised by Greg Michelanko and former ***rare*** Environmental Advisory Committee member Larry Lamb (Weaver, 2003). The study was designed following the protocols of the Ecological Monitoring and Assessment Network (EMAN). Following Weaver's study, the lichen monitoring program at ***rare*** continued with an adjusted protocol that better suited the needs and resources of ***rare*** and incorporated the use of new technology (see *Methods* below). For this reason, data collected from the 2003 pilot year is inconsistent with data collected in subsequent monitoring seasons and is not used in the database compilation.

The lichen monitoring program at ***rare*** is one of several long term ecological monitoring programs which yield valuable baseline data and can help to identify critical changes in the ecosystem. Long term monitoring is especially appealing for the ***rare*** **Charitable Research Reserve** because of its unique location within several urban municipalities. Lichen monitoring at ***rare*** acts as an opportunity to investigate the effects of urban development outside the periphery of the reserve. Waterloo Region is one of the largest expanding urban areas in Canada, expected to grow in population by 30% in the next 20 years. In particular, new housing developments are planned in the city of Cambridge that will border the southeastern edge of ***rare***. This development will likely bring added pressures to ***rare*** from increased traffic, water consumption, and recreational use of the property. Maintaining a monitoring program to establish baseline data prior to such developments will be essential in identifying critical changes to local ecosystem functioning and integrity. The results of air quality monitoring at ***rare*** could thus be a valuable resource for management of other natural areas situated within urban centres.

Lichen Taxonomy

Lichens are a unique group of organisms that represent a mutualistic relationship between a fungus and an alga (Walewski, 2007). The fungal partner in this relationship, also known as the mycobiont, provides the physical structure of the lichen, while the algal partner, also known as the photobiont, generates sustenance through photosynthesis (Walewski, 2007). Although somewhat inconspicuous, lichens play an integral role in ecosystems as forage for certain wildlife species, particularly in the winter when food is scarce, and nest material and important habitat for some birds and other animals (Walewski, 2007).

The composition of a lichen community can be very informative with respect to inferences about air quality. A common approach to air quality monitoring using lichens is to record the presence or absence of certain species (Wolseley, n.d.). This can infer air quality conditions because different lichen species have variable tolerances to common air pollutants (i.e. sulphur dioxide and nitrogen dioxide). Thus, the presence of highly sensitive species can be indicative of low atmospheric pollution and hence better air quality.

Lichen communities can be placed into three broad categories: nitrophytic, neutrophytic, and acidophytic (Wolseley, n.d.). Nitrophytic lichen species are those that benefit from excess nitrogen in their environment, such as vehicle exhaust (NO_2) and intensive agriculture (NH_3) (van Herk, 2001). Acidophytic lichen species are generally very sensitive to pollutants, and are scarce in areas of higher pollution (i.e. urban areas) (van Herk, 2001). Neutrophytic lichens are moderately sensitive to pollution (van Herk, 1999). By classifying lichen into these three community types, inferences can be made regarding the air quality of an area based upon the presence and absence of certain species. In more sophisticated air quality monitoring programs, chemical analysis of lichen tissues can be employed for a detailed description of pollutants, however this method can be time and resource intensive. It should be cautioned, however, that pollutants can directly affect the lichen, but also have an indirect effect by causing changes to bark pH. Bark pH is associated with lichen community type (British Lichen Society, 2003; van Herk, 2001).

Methods

Site Description

The lichen monitoring plots at ***rare*** are located within Indian Woods, an old-growth mixed deciduous forest. The forest is co-dominated by Sugar Maple (*Acer saccharum*) and American Beech (*Fagus grandifolia*), and houses trees with a range of diameters. For a full description of the Indian Woods species composition and size classes refer to the *Forest Canopy and Tree Biodiversity Monitoring* chapter. The four monitoring plots are located in the southeastern portion of Indian Woods, approximately 50 metres east of the Grand Allée (Figure 1.1). The plots surround an ephemeral pond that is a part of the existing Plethodontid salamander monitoring program at ***rare***.

Monitoring Protocol

Following the EMAN protocol for lichen monitoring, four plots were established in 2008 in the Indian Woods, with four trees selected in each plot for a total of sixteen trees to be re-sampled through time. Trees were selected based on diameter at breast height, with the largest trees in each plot being targeted. Large diameter trees are preferred in this monitoring program because the curvature of the tree will have less impact on capturing a consistent depth of field, which is an important consideration when monitoring lichens using photographs (Parker et al., 2003). Larger trees are also more likely to have well established lichen communities on their trunks than smaller, younger trees because they have had more time for colonisation by lichens. The EMAN protocol for lichen monitoring also recommends that a site have at least five Sugar Maple, Red Maple, and/or Silver Maple present. Should a site have fewer than this recommended number Ash, Basswood, and Elm trees are acceptable. If none of these species are available, then the largest trees with the greatest abundance of lichens should be selected. Tree species in the lichen monitoring program at ***rare*** include Sugar Maple (*Acer saccharum*), Red Maple (*Acer rubrum*) American Beech (*Fagus grandifolia*), and Red Oak (*Quercus rubra*).

The GPS coordinates of the centre tree locations for each of the four plots and a map of the lichen monitoring plots is provided below in Figure 1.

Plot	GPS Coordinates
1	N 43 22.508 W 80 21.943
2	N 43 22.523 W 80 21.909
3	N 43 22.499 W 80 21.924
4	N 43 22.480 W 80 21.924

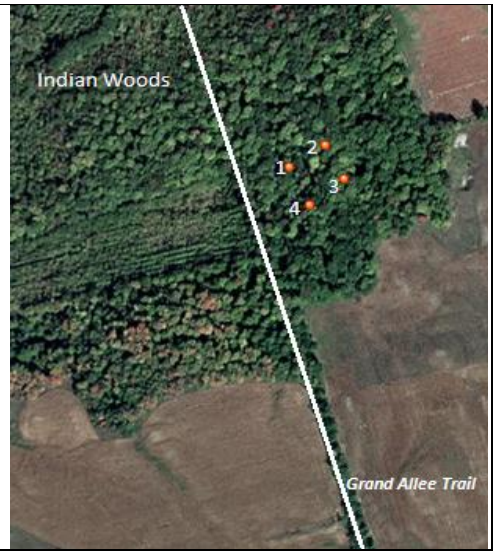


Figure 1 GPS coordinates for centre tree location and diagram depicting their location in Indian Woods in each lichen monitoring plot

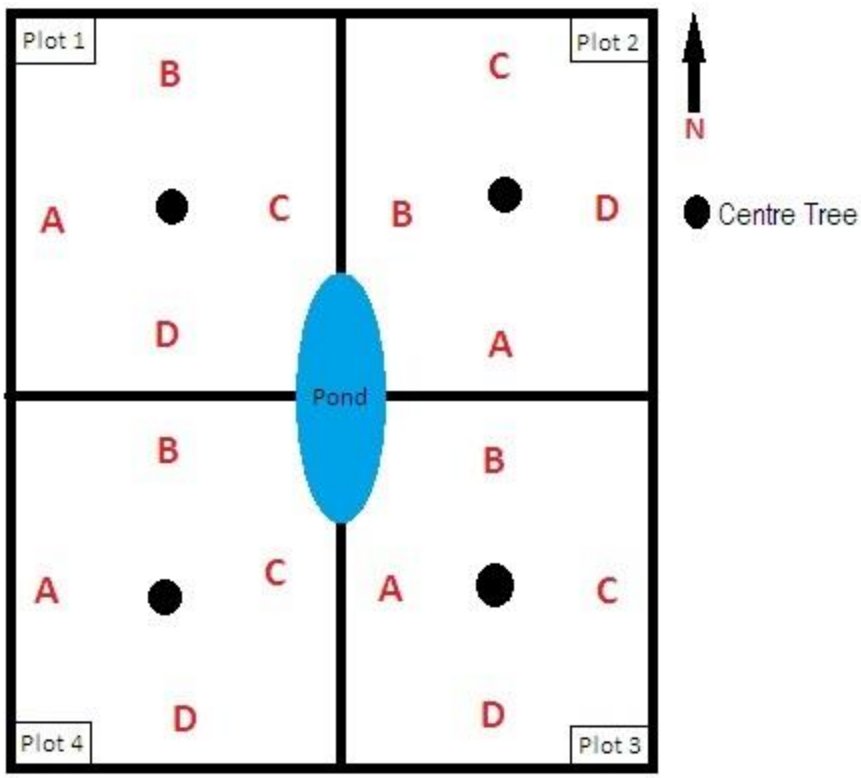


Figure 2 Tree arrangements in Indian Woods Lichen Monitoring Plots

Within each plot, the four trees are situated in an ‘x’ shape pattern about the centre trees (Figure 2) Each of the trees in the monitoring program were assigned a letter, such that each plot had four trees labeled A through D. In most of the plots, tree A was found to be the tree the furthest west and the subsequent three trees were situated clockwise from tree A (see Figure 2). Plot two however had tree B as the tree furthest west therefore a more complete diagram of the tree arrangement in the lichen monitoring plots is provided above (Figure 2).

Lichen monitoring occurs once every five years, as per the EMAN protocol. Because lichens are long-lived, growth occurs very slowly, and little change is observable from year to year. Monitoring more frequently than once every five years is not necessary to capture change in lichen communities.

Each tree included in the lichen monitoring program is marked by a nail in its trunk at each of the four cardinal directions at a height of 1.3 metres from the ground. At each nail, an EMAN visual estimation colour chart (Figure 3) is aligned such that the nail goes through the hole at the top of the chart. The window within the chart samples 200 square centimetres of the tree trunk at each of the four nails for a total of 800 square centimetres per tree included in the lichen monitoring program. Using the photos from previous monitoring years, the chart is adjusted to capture the same field of view as the previous monitoring season. To facilitate a quick comparison between monitoring seasons in the field, photos from previous years can be loaded onto the iPad.

The chart and the lichen(s) framed within it are photographed using a digital SLR camera at a 50 millimetre focal depth. Photos should also be taken in a RAW format so that post sampling modifications can be made to the image without compromising the image quality. It is essential that prior to each photograph the information on the right hand side of the EMAN visual estimation colour chart be updated to reflect the tree number and aspect. An example of how the fields should be completed is provided in Table 1.2. Plots are numbered one through four, and individual trees within each plot are assigned a letter, A through D. Additionally, each tree has photos taken in each of the four cardinal directions, meaning a total of 64 photos are required. To ensure accuracy, photos should be taken in triplicate. Given the nature of the monitoring tasks, a minimum of two people are required.

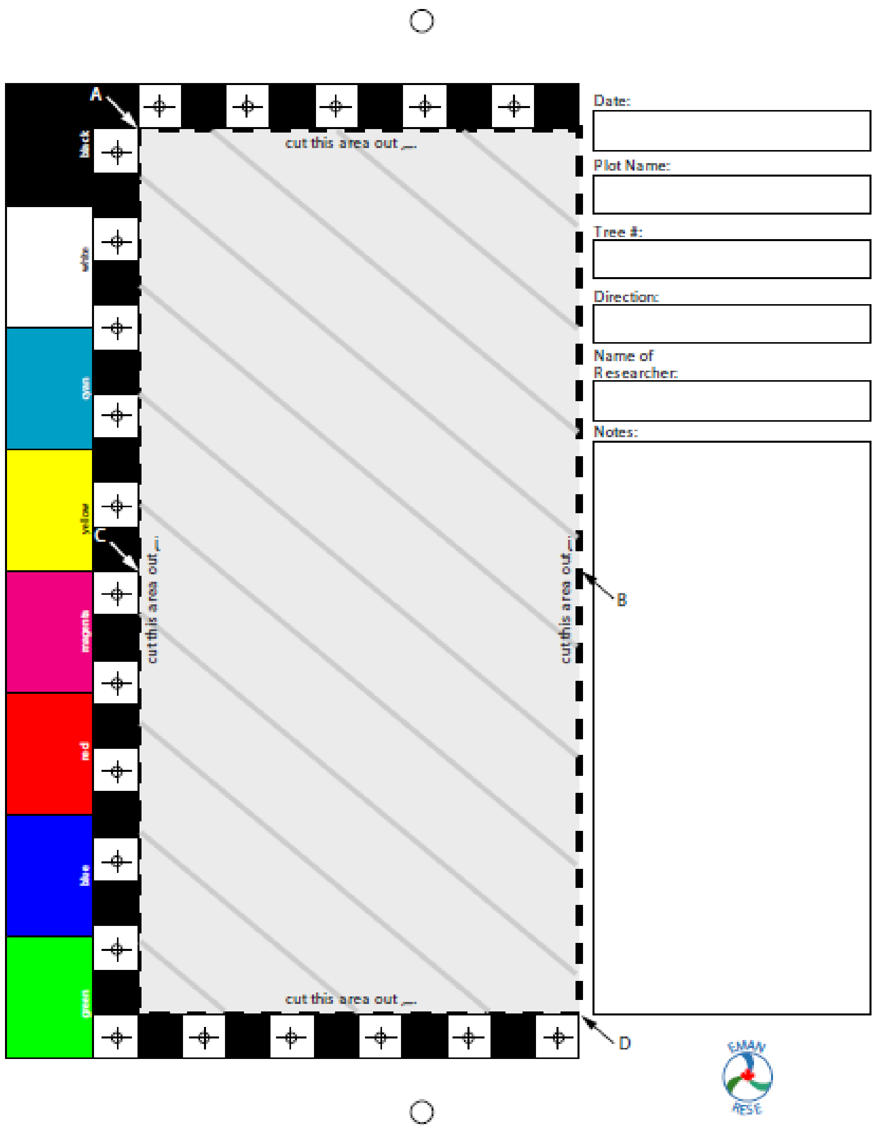


Figure 3 Example of the EMAN visual estimation colour chart (not to scale)

After photographing all four sides of a tree included in the lichen monitoring program, the diameter at breast height (dbh) is measured and recorded. Following this, the next tree in the monitoring plot can be sampled, until all the sixteen trees have been photographed at each cardinal direction and all dbh measurements have been recorded. An example data sheet can be found in Appendix A.

The EMAN protocol streamlined the process of inferring air quality by establishing a suite of 17 key lichen species, most of which are readily identifiable in the field. This means that a more general statement can be made about the air quality within a given area based upon the assemblage of lichen species observed. For the purposes of the lichen monitoring program at

rare, inferences about air quality are made using the (i) the EMAN approach of identifying the presence or absence of the selected 17 lichen species with known sensitivity levels, and (ii) sensitivity assessment of each lichen species based upon the compiled list of Ontario lichen species from the Natural Heritage Information Centre (NHIC) in the Ontario Ministry of Natural Resources.

Lichens that were photographed during monitoring were identified after the field work was completed using the *Identifying Urban Lichens Reference Notebook* for the EMAN lichen biomonitoring program by Irwin Brodo. Lichens that were not included in the suite of 17 species were identified if possible using *Lichens of the North Woods* by Joe Walewski. Some lichens were identified in a consultation with Dr. Dan McCarthy, an Associate Professor at Brock University. Presence of lichen species that are particularly sensitive to pollutants were seen as an indication of relatively good air quality, whereas the presence of particularly pollution tolerant lichen species and the absence of sensitive species was seen as an indication of poor air quality.

Conclusions on overall community sensitivity to pollution were based on a qualitative assessment in which most of the species within the community were of similar sensitivity.

Results

2008 Lichen Monitoring Results

In 2008, a total of nine different species of lichens were recorded of which four species were listed in the EMAN suite of 17 common urban lichens reference guide. These four species are listed by EMAN as common bark-dwelling species. All of the nine species with the exception of one (*Conotrema urceolatum*) are considered non-sensitive by the NHIC and their occurrence in southern Ontario ranges from rare to very common (Table 1). The lichen communities observed in 2008 are typical of a nitrophytic composition, and many species observed (*Candelariella efflorescens*, *Physcia millegrana*, and *Physcia adscendens*) are commonly found in urban areas.

Table 1 Species composition for 2008 lichen monitoring session. Sensitivity of each lichen species is given based upon the compiled list of Ontario lichen species from the Natural Heritage Information Centre in the Ontario Ministry of Natural Resources and EMAN

Scientific Name	Common Name	Sensitivity	Occurrence
Buellia stilligiana	Common Button lichen	Non-sensitive	Frequent
Candelariella efflorescens	Powdery Goldspeck lichen	Non-sensitive	Common
Conotrema urceolatum	Can-of-Worms lichen	Medium	Rare
Flavoparmelia caperata	Common Greenshield lichen	Non-sensitive	Very common
Lepraria incana	Dust lichen	Non-sensitive	Infrequent
Lepraria lobificans	Fluffy Dust lichen	Non-sensitive	Common
Phlyctis spp.	Whitewash lichens	Non-sensitive	Infrequent
Physcia millegrana	Mealy Rosette lichen	Non-sensitive	Very common
Physcia adscendens	Hooded Rosette lichen	Non-sensitive	Very common

Of all the lichen species recorded in 2008, *P. millegrana* was the most frequently observed, occurring on 14 of the 16 trees in the monitoring plot. The next two most frequently occurring lichen species were *Phlyctis spp*. and *C. efflorescens* which were observed on 11 and 10 of the 16 trees respectively. Of the nine species observed, three are confirmed nitrophytes: *P.millegrana*, *C.efflorescens*, and *P. adscendens* (Tulumello, 2010); these three species made up the majority of observed lichen cover. The remaining six species are suspected nitrophytes or neutrophytes (Tulumello, 2010).

Cardinal direction appeared to have no impact on lichen community distribution or composition on a tree, however some species were more frequently observed on all sides of the tree (*P. millegrana* and *Phlyctis spp*.).

2014 Lichen Monitoring Results

In the 2014 monitoring season, a total of seven species were observed, of which three are listed in the EMAN suite of 17 common urban lichen species. Two (*L. incana* and *P. adscendens*) of the species observed in 2008 were not recorded in 2014.

Table 2 Species composition for 2014 lichen monitoring session

Scientific Name	Common Name	Sensitivity	
Buellia stillingiana	Common Button lichen	Non-sensitive	Frequent
Candelariella efflorescens	Powdery Goldspeck lichen	Non-sensitive	Common
Flavoparmelia caperata	Common Greenshield lichen	Non-sensitive	Very common
Lecanora spp.		Non-sensitive	Rare to common
Lepraria lobificans	Fluffy Dust lichen	Non-sensitive	Common
Phlyctis spp.	Whitewash lichens	Non-sensitive	Infrequent
Physcia millegrana	Mealy Rosette lichen	Non-sensitive	Very common
Unknown	Sterile Black Crust	Unknown	Unknown

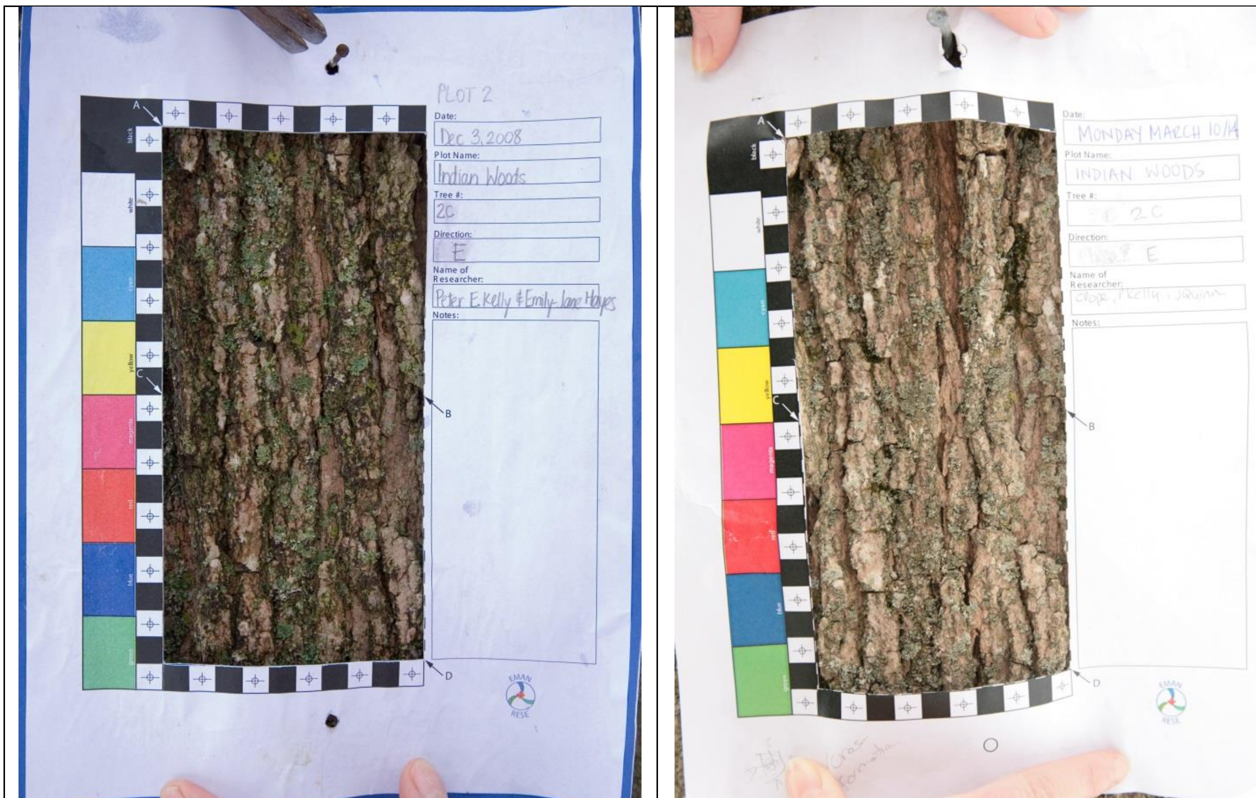
Similar to the 2008 results, the most frequently observed lichen species was *P.millegrana*, which occurred on all 16 trees in the monitoring program. On seven of the trees it was observed in all four sampling positions (north, south, east and west aspects). As in 2008, the next two most frequently observed lichen species in 2014 were *Phlyctis spp.* (recorded on 11 of 16 trees) and *C.efflorescens* (recorded on 10 of 16 trees). Two species that were observed in 2008 were not recorded in 2014 (*C.urceolatum*, *P.adscendens*, and *Lepraria incana*), however one new genus and an unknown species were observed in 2014 (*Leconora spp*. and a Sterile Black Crust). The Sterile Black Crust was identified as such through personal communications with Dr. Dan McCarthy of Brock University.

As in 2008, cardinal direction in 2014 appeared to have no effect on the lichen community composition or distribution, with *P.millegrana* and *Phlyctis spp.* being observed more frequently on all sides of the tree. The confirmed nitrophytic lichen species are identical to those in 2008, with the exception of *P. adscendens*, which was not observed in 2014. The lichen communities resemble those observed in 2008 in that they are nitrophytic and are commonly found in urban areas. All of the observed species in 2014 are listed as non-sensitive by the NHIC with the exception of the sterile black crust, which does not have a sensitivity rating because it is unidentifiable. In the future, it may be possible to identify this species through chemical analysis of a sample.

Discussion

Lichen Communities at *rare*

The species observed during the 2014 lichen monitoring season are nearly identical to those recorded in 2008. Two species that were found in 2008 were not observed in 2014 (*Conotrema urceolatum,* and *Physcia adscendens*). This absence was not attributed to damage sustained to trees between monitoring seasons, but rather could be a result of lichen mortality or difficulties with identification. These two species did not represent a large portion of the lichen communities observed in 2008 as each of them have only two recorded observations.



Tree 2C East in 2008 | Tree 2C East in 2014

Figure 4 Example of decreased lichen surface area coverage between monitoring years

Figure 1.4 Example of decreased lichen surface area coverage between monitoring years.

Figure 1.4 is one example of a change in lichen surface area coverage between monitoring years. It is unknown at this time what may have caused the changes in lichen surface area coverage however lichen mortality can be a result of increased air pollution (Will-Wolf, n.d.; Hutchinson, Maynard, Geiser, 1996) or possible herbivory (Walewski, 2007).

Of the suite of 17 lichen species prepared by EMAN, three were recorded in 2014, two of which were clearly dominant within the lichen communities (*C. efflorescens* and *P. millegrana*). This suite of lichen species were selected because of their sensitivity to air quality and their simplicity in identification (EMAN, 2002). The overall low number of lichen species observed at ***rare*** that are included in this suite could be indicative of a number of factors such as the ongoing influence of urban pressures on the reserve (i.e. pollution and dust from vehicle traffic, aggregate extraction etc.). Often in urban lichen monitoring studies, specimens become distorted and/or discoloured due to air pollutants (Dan McCarthy, personal communication, May 28th 2014). This makes accurate identification of lichens outside of the EMAN suite of arboreal lichen species in the monitoring program especially challenging however this could be improved by collecting samples from unknown species.

Another possible explanation is the limited sampling area used in the lichen monitoring program. The protocol for lichen monitoring developed by EMAN had a total sampling area of 500 square centimetres (five 10x10 cm windows) (Parker et al., 2003). This method was originally used in the undergraduate project in 2003, the data from which is not kept in the ***rare*** database. As lichen monitoring transitioned from this method to one based on photographs, the EMAN colour visual estimation chart was adopted. The photometric method was used in 2008, and remains the current methodology for lichen monitoring. The species data collected in 2008 are stored in the ***rare*** database. The sampling window area of 200 square centimetres effectively reduced the sampling area by 60%. This creates the risk of omitting lichen species that may typically occur on other parts of the tree (i.e. the base of the trunk or on branches). In effect, limiting the sampling area to 200 square centimetres at a height of 1.3 metres from the ground will completely miss lichen communities further up or down the trunk. The impact of vertical distribution on lichens is variable in the literature, depending on a suite of factors including latitude and tree species (Boch et al. 2013; Fritz 2009; Marmor et al. 2013). It is unknown whether or not the sampling method at ***rare*** is representative of the entire lichen community, however it is imperative that sampling remain consistent from year to year to ensure comparability of results. See *Recommendations* below for suggestions on how to improve the lichen monitoring program at ***rare.***

The majority of the lichen species observed in both 2008 and 2014 are not a part of the EMAN collection of common urban lichen species, and they presented a challenge in identifying them to a species level. For the current purposes of lichen monitoring at ***rare***, it is only imperative that any lichen species that are included in this suite be identified because of its broad implications about local air quality. Identification of other species that are not included in the EMAN suite is still advantageous however, especially when considering the addition of more sophisticated data analysis in future years (i.e. species richness and diversity).

The results from the 2014 lichen monitoring program at ***rare*** are indicative of a nitrophytic lichen community, which is a group of lichens that can do well in conditions of excessive nitrogen input (British Lichen Society, 2003; Frati et al., 2005; Marmor & Randlane, 2007;

Tulumello, 2010). Typical nitrophytic lichen species include *Physcia millegrana*, *Candelaria concolor*, and *Physcia aipolia* (Tulumello, 2010) all of which have been observed at ***rare***.

A nitrophytic lichen community, like that observed at ***rare***, is typical of urban areas, where pollution from vehicle traffic (NO_2 and SO_2), agricultural (NH_3), and industrial activities (SO_2 and NO_2) can result in excess pollutants within a regional area. Although ***rare*** itself is 900+ acres of protected greenspace, it is surrounded by urbanization, agriculture, and gravel extraction, including a 3.5 kilometre road that bisects the property and sees traffic upwards of 11,000 cars each day. In general, an increasing abundance of nitrophytic lichen species is associated with a decrease in acidophytic lichen species, which are particularly sensitive to sulphur dioxide (SO_2) (van Herk, 2001; Tulumello, 2010). Van Herk (2001) attributes this phenomenon to the higher tolerance of SO_2 pollution of nitrophytic lichen species and their affinity for a high bark pH. Essentially, an observed change to nitrophytic lichen community and lack of acidophytic lichen species could be indicative of elevated bark pH as a result from pollutants. It should be noted that nitrophytic lichens have been observed on trees that were impregnated with calcareous dust which can effectively raise the bark pH (van Herk, 2001). The underlying geology of ***rare*** and the aggregate industrial operations may have influenced the lichen community composition in this way.

Tree Species and Bark pH

Nitrophytic lichen species are associated with a higher bark pH, which may play a more influential role in determining lichen community assemblages than the direct effect of NH_3 on the lichen (van Herk, 2001; British Lichen Society, 2003). Acidophytic lichen species abundance has been observed to decline in areas with increased bark pH because they require a more acidic substrate (British Lichen Society, 2003; Tulumello, 2010). Ultimately, a decline and eventual disappearance of acidophytic lichen species will result in a lower number of lichen species and lower species richness (British Lichen Society, 2003).

The tree species included in this lichen monitoring program are Sugar Maple, Red Maple, American Beech, and Red Oak. Despite the variety of tree species, it is now thought that bark pH has a greater influence on lichen community assemblage, rather than species alone (van Herk, 2001). Unfortunately there is a lack of literature regarding the differences in bark pH between different species; however there is some evidence for changes within a particular species of tree. The physical and chemical properties of the bark changes with age which can influence of tree-dwelling lichen communities. For example, young maples have smooth bark that becomes more cracked and softens as it ages, and oozes nitrogenous compounds (Walewski, 2007), which could be a possible explanation for the nitrophytic lichen communities observed on the maples included in this program. We cannot exclude then that the lichen communities observed reflect tree age and not pollution levels.

Most of the lichen species observed in this monitoring program were not found to be selective of tree species (i.e. found on any of the four species of trees). One species, *Flavoparmelia caperata,* was only observed on maple species; however this could be a false indication of tree species preference because of its low abundance throughout the plot. Information on tree preference related to this species could not be found in the literature.

In the context of ***rare***, the lichen monitoring plots are situated within Indian Woods, which is hemmed by aggregate pits, agricultural fields, and busy roads. In addition to the possible influence of air currents carrying pollutants from elsewhere in the province, these local conditions create an environment favourable for the proliferation of nitrophytic species. In the absence of evidence for a change in community composition, however, it cannot be stated that local pollutants are the cause of the nitrophytic communities observed. Furthermore, the relatively small size of Indian Woods made the selection of a truly interior forest lichen monitoring plot challenging, and thus impacts of surrounding urban activities may have a greater impact than they would had the forest plot been located further from a forest edge.

Air quality at *rare*

No acidophytic lichen species were observed in 2014, suggesting that the local air quality may have a high enough concentration of SO_2 that it is having deleterious effects on acidophytic lichen communities, or that bark pH levels are sufficiently high (either naturally by species or promoted by the geology of southern Ontario) or that atmospheric NH_3 levels are high (direct N impact or raising of bark pH). Atmospheric pollution, local conditions, and bark pH may have a complex effect on lichen communities. Data from the Environment Canada air quality monitoring network indicate that southern Ontario has an ambient SO_2 concentration greater than the national average by greater than 60 per cent, 1.76 and 2.85 parts per billion respectively (Environment Canada, 2013b). Similarly, average nitrogen dioxide (NO_2) concentrations in southern Ontario exceed that of Canada for 2011 (11.12 and 10.63 parts per billion respectively) (Environment Canada, 2013b). These data are indicative of how industry and development influence the regional air quality of southern Ontario, which may have a direct effect on the lichen communities.

An inherent issue of lichen based air quality monitoring is the broad influence from the surrounding environment. Air borne pollutants can be carried great distances and have an impact on lichen communities far from the pollution source. As baseline data continues to be amassed from the lichen monitoring program, it is anticipated that few changes may be observed in light of upcoming housing developments in ***rare's*** periphery. As a result of these new housing developments, it is expected that there will be an increase in vehicle traffic, and thus vehicle emissions. This could further reinforce the dominance of nitrophytic lichen communities at ***rare***, but would exhibit little to no change from its current composition. Consideration of how ***rare*** might measure changes in lichen communities from local sources of pollution is needed and may contribute to an understanding of how the monitoring plots reflect local versus regional conditions. One approach for determining the influence of local air quality conditions is to undertake a broader epiphytic lichen mapping initiative which could be done in conjunction with local post-secondary institutions.

Despite this challenge, baseline data can still be valuable in tracking changes as the community surrounding ***rare*** develops. For instance, should Blair Road be closed in the future the reduction in vehicle traffic and exhaust in the immediate vicinity of ***rare*** could enable more sensitive species to become established. The baseline data therefore would provide a clear example of meaningful improvements as a result of such an endeavour. The reverse of this situation is also true, in that an increase in vehicle traffic on Blair Road can influence the local air quality and the data being currently collected will be a valuable baseline.

Recommendations

Tree mortality poses a challenge for maintaining meaningful results between monitoring sessions. Unless a dead tree has fallen over or lost a substantial amount of bark as a result of its mortality, new trees need not be added to the monitoring program. Substantial loss of bark may have impacted results between 2008 and 2014; at least three trees had lost all bark at one of sampling windows, along with the established lichens recorded in 2008. Because these trees have not lost bark at all of the sampling locations (i.e. at each cardinal direction) it is not necessary to replace imminently, however this should be kept in mind for the lichen monitoring season in 2019. Replacement trees for trees that have fallen need to meet the same requirements for initial selection (see section 6.1.3). It is strongly recommended that new trees comply with the EMAN recommended tree species (i.e. Sugar Maple, Silver Maple, and/or Red Maple) It is also recommended that general notes about tree health be included in each monitoring session to be aware of likely tree mortalities before the next monitoring sessions. Tree health notes should include any stem defects, or visible damage to the tree that could have some impact on its survivorship.

To facilitate faster and easier sampling between monitoring sessions, some upkeep of lichen monitoring plots is necessary. Each tree included in the monitoring program should have any missing nails replaced, and the north and south nails should be reflagged with flagging tape.

With respect to the monitoring process, the EMAN visual colour estimation chart (see appendix A) should be laminated so that it is resistant to moisture while in the field. Furthermore, dry-erase markers should be used to complete the fields to the right of the sampling window, which will enable easier relabeling between monitoring trees.

Some of the trees included in this monitoring program were observed to have sustained some damage and/or scarring from the stainless steel nails used to mark the cardinal directions of the tree. Unfortunately, there is a lack of literature that investigates the impact of nail scarring on tree trunks upon lichen community composition. This topic should however be further investigated along with the lichen monitoring program to better ascertain the effects of stainless steel nail markers on monitoring results.

Future lichen monitoring studies at ***rare*** may be enhanced by incorporating an analysis of bark pH for each of the trees included in the monitoring program (e.g., Marmor and Randlane, 2007). This could provide more meaningful information with regards to air quality monitoring because bark pH is thought to have a greater influence on lichen communities than air or water contaminants (British Lichen Society, 2003). Furthermore, bark pH is less variable than contaminant concentrations in air and water because of the long term effects and delayed response of bark pH to environmental change (McCarthy, personal comm., 2014). Indeed, understanding the buffering capacity of bark could be highly informative of bark interactions with epiphytic lichen species (Enns, 2001). A recommended method for testing bark pH is provided in Appendix B. This method provides a detailed description of a method similar to that employed by Marmor and Randlane (2007).

Another potential avenue for lichen monitoring at ***rare*** is the calculation of indices through the interpretation of quantitative data. These indices include the Lichen Diversity Value (LDV), Lichen Diversity Width (LDW), and the Index of Atmospheric Purity (IAP) (MacDonald & Coxson, 2012). These indices may be a possible calculation in future lichen monitoring seasons, however there are insufficient data currently to be able to identify long term changes in air quality. With increased staffing in the land management department, and the help of interns, additional measurements might be feasible.

Finally, it is recommended that data on weather conditions be recorded at the time of sampling from the Waterloo Region Airport. Moisture can affect the colour and appearance of epiphytic lichens so days with high precipitation should be avoided for consistency in data collection.

Conclusion

Lichen monitoring can provide some inferences about local air quality based upon the presence and absence of identified key species. Streamlined monitoring, like that proposed by the EMAN program, allows for fast and rapid sampling of lichens within an established plot, which enables the program to take place with minimal cost, aside from staff time. The results from the 2008 and 2014 lichen monitoring program demonstrate that the lichen communities are nitrophytic in overall composition and have a low sensitivity to common urban pollutants. Agriculture, busy roads, and industrial operations (i.e. aggregate pits) may all be contributing excess inputs of pollutants into the local air and water, which are considerations for explaining the abundance of nitrophytic lichens observed.

It is recommended that lichen monitoring at ***rare*** continue because of its low cost and relative ease of implementation. With the recommendations listed in the previous section, the lichen monitoring program could be improved and yield more meaningful results. Baseline data are still being amassed for lichen monitoring at ***rare*** and thus monitoring should continue to enhance this resource.

Literature Cited

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APPENDIX A – Sample Lichen Monitoring Data Sheet

LICHEN DATA SHEET

Location

Site name:	Site Number:
Observer Name(s):	Date:
Air Temperature (C):	
Precipitation (mm):	

Condition

Tracks and Trails	None	Faint Trails	Well Marked	Tracks/Roads
Extent	None	Local	Widespread	Extensive
Urbanization/Development	None	Light	Moderate	Intensive
Extent	None	Local	Widespread	Extensive
Other	None	Light	Moderate	Heavy
Extent	None	Local	Widespread	Extensive

Tree Species		**Tree Number:**		**Diameter (cm):**
Lichen Species	% North Cover	% East Cover	% South Cover	% West Cover

Tree Species		**Tree Number:**		**Diameter (cm):**
Lichen Species	% North Cover	% East Cover	% South Cover	% West Cover

Tree Species		**Tree Number:**		**Diameter (cm):**
Lichen Species	% North	% East	% South	% West

	Cover	Cover	Cover	Cover

APPENDIX B: Recommended Methodology for Measuring bark pH

Materials:

- Stanley Surform Shaver (retails at under $10.00)
- 62 small newspaper packets
- 64 50mL Specimen Tubes
- Digital scale
- Forceps
- Distilled Water
- Quanta Water Quality Meter
- Data sheet

This method has been outlined in *Bark pH determination for the Bryophyte Habitats Survey* (Bates, n.d.) and is paraphrased below. The original document is available on the ***rare*** server.

1. Bark samples are to be collected from trees included in the monitoring program from each cardinal direction. It is necessary to collect the bark sample from outside the lichen monitoring viewing window. To collect the bark sample, it is recommended to use a wood file, such as the Stanley Surform Shaver (retails at under $10.00) as it will only collect the top layer of bark and limit injury to the tree. Samples should be placed in newspaper packets and laid out to air dry for 1-2 weeks.
2. Once the bark samples are dry, weigh out 0.5 g of each bark sample into clean specimen tubes (50mL) using forceps. To each of the specimen tubes, add in 10 mL of distilled water to the wood samples. Replace the specimen tube lid and gently shake to ensure the sample is thoroughly wet. Allow the samples 1 hour to steep with occasional shaking.
3. While samples are steeping, the Quanta Water Quality Meter should be calibrated against buffers of pH 7 and pH 4. Once calibrated rinse the pH electrode before measuring a sample.
4. Once the hour is complete, samples should be given a final shake and insert the pH electrode into one specimen tube at a time and stir the solution gently. Once the readings on the pH meter stabilise the pH reading can be recorded. Clean the pH electrode with distilled water between samples

Sample Data Sheet for Recording pH values

Tree ID	Species	Aspect	Sample Weight (g)	pH
1A	*Sugar Maple*	*North*	*0.5*	*6.0*