**Abstract**
Green roofs are constructed ecosystems that provide ecosystem services in urban environments. Shallow substrate green roofs subject the vegetation layer to desiccation and other environmental extremes, so researchers have evaluated a variety of stress-tolerant vegetation types for green roof applications. Lichens can be found in most terrestrial habitats. They are able to survive extremely harsh conditions, including frequent cycles of desiccation and rehydration, nutrient-poor soil, fluctuating temperatures, and high UV intensities. Extensive green roofs (substrate depth <20cm) exhibit these harsh conditions, making lichens possible candidates for incorporation into the vegetation layer on extensive green roofs. In a modular green roof system, we tested the effect of *Cladonia* lichens on substrate temperature, water loss, and albedo compared to a substrate-only control. Overall, the *Cladonia* modules had significantly cooler substrate temperatures during the summer and significantly warmer temperatures during the fall. Additionally, the *Cladonia* modules lost significantly less water than the substrate-only control. This implies that they may be able to benefit neighboring vascular plant species by reducing water loss and maintaining favorable substrate temperatures.
Introduction
Green roofs are constructed ecosystems, designed to provide ecosystem services such as the reduction of heat flux through the roof, the capture of storm water, and the provision of habitat for animals. Green roofs consist of a vegetation and growing medium layer (substrate) over engineered layers that provide a root barrier, drainage, and/or water retention layers. The majority of green roofs constructed in temperate climates are “extensive” green roofs, characterized by shallow growing media (<20cm), which minimizes the weight added to the building. Shallow growing media in such systems have led to a reliance on succulent plant species in the vegetation layer, to ensure survival during drought conditions. Other plant growth forms have also been used on extensive green roofs, with grasses, forbs, and mosses among the most frequent. Lichens are often found on conventional roof surfaces and form an important component of cryptogamic crusts in terrestrial ecosystems. Cryptogamic crusts in arid environments perform a range of ecosystem services, such as stabilizing soil, fixing nitrogen, and enhancing soil water holding capacity, but have not been intentionally planted in green roof ecosystems.

Lichens are symbiotic organisms, an association between a fungus (the mycobiont) and one or more algal and/or cyanobacterial photobionts. Typically the mycobiont forms 95% of the lichen body. They are the dominant plant life in harsh environments such as the Arctic, Antarctic, mountains, and dry land crusts. Lichen species are widespread and can be found from the Arctic to deserts; they can survive frequent cycles of desiccation and rehydration, nutrient-poor soil, fluctuating temperatures, and UV light intensities. They can survive and grow on the bare surface of rocks and in poor soils such as heathlands, peat lands, sand dunes, and toxic spoil heaps. Selection of vegetation types appropriate for extensive green roofs often involves identifying local habitats that have characteristics in common with green roofs (shallow soil, harsh abiotic conditions); lichens are a common component of rock barrens, dunes, and heathland habitats that can be a source of plants for green roofing projects.

Lichens are lightweight and can be found growing naturally on bare tile or slate rooftops. This could make them a candidate for roofs on buildings with low weight-loading capabilities. Species of the genus Cladonia, large fruticose lichens that colonize bare soils, are common in cold temperate climates. These lichens produce bundles of hyphae that stabilize the soil and add organic matter. The light color of the lichen can reflect solar radiation, keeping the soil cool and moist. These characteristics indicate the possible utility of Cladonia as a component of extensive green roofs. In this study, the performance of key green roof ecosystem functions in green roof modules planted with live Cladonia lichens was compared with substrate-only controls.

Methods
The study site was located on the east side of the roof of the five-story Atrium building at Saint Mary’s University in Halifax, Nova Scotia, Canada (44°39’N, 63°35’W) (Figure 1). Data on soil temperature and water loss were collected between July and October 2012. Albedo was measured in September 2013. During the 2012 study period, the weather station on the adjacent green roof testing facility (~50m away from the study site) recorded the minimum monthly temperature as 6.7–20.7°C and the monthly maximum as 12–30°C. The monthly precipitation recorded from the green roof weather station averaged between 1.7 and 11.59mm. Albedo was collected on September 28, 2013 (average temperature: 14.12°C, total precipitation: 0mm).

Figure 1. Placement of lichen trial on the east side of the roof of the five-story Atrium building at Saint Mary’s University in Halifax, Nova Scotia, Canada (44°39’N, 63°35’W).

In order to quantify the influence of Cladonia on green roof substrate, a trial was set up to determine the effects of Cladonia on soil temperature, water loss, and albedo. Ten green roof modules were placed on the Atrium roof on top of the roof surface, which was made up of grey concrete pavers. Each module had a length and width of 36cm, with a freely draining base (Polyflat®, Stuewe & Sons Inc., Oregon, USA). Modules contained a root barrier/water retention fleece (length and width 36cm) over the base (EnkaRetain and Drain 3111®, Colbond Inc., North Carolina, USA) with 6cm depth of Sopraflor X substrate, purchased in 2011 (Soprema Inc., Drummondville, QC, Canada), over the root barrier/water retention layer. A soil test describing the composition of the Soprema X substrate can be seen in Supplementary Table 1. This experiment consisted of two substrate-only controls and eight modules covered 100% in Cladonia lichen approximately 6cm thick (Figure 2). The lichen was collected from a coastal barrens site (Chebucto Head (44°30’N, 63°31’W)) in May 2012 and placed on the surface of the substrate. A mix of two lichen species, C. terranova and C. boryi, was used; both species have similar colors and growth forms, and co-occur in lichen mats. Lichens were alive when transplanted and shoot tips were marked to determine incremental growth over the experiment, but any such growth was undetectable and these results are not included here.
Substrate temperature and volumetric water content (VWC)

Substrate temperature was recorded using a Taylor 9878 Slim-Line Pocket Thermometer Probe (Commercial Solutions Inc., Edmonton, Alberta, Canada) once a month throughout the growing season. The temperature was recorded from the center of each module approximately 2 cm below the substrate surface when exposed to full sun, no more than two hours from solar noon. Only one measurement was recorded for each module on the day of measurement. These spot measurements represented maximal substrate temperatures and were collected during hot sunny conditions to provide maximum contrast between controls and planted green roof modules. This technique does not characterize the long-term provisioning of thermal benefits but has been used to contrast performance of different plant species or vegetation types during hot conditions. Volumetric water content of the substrate (VWC: water volume/soil volume x 100%) was recorded one day after a rain event and again one day later if no new showers were observed. Water loss was determined by subtracting the day one VWC from the day two VWC, which provides an index of net evapotranspiration over 24 hours.

Albedo

Albedo was not originally part of this trial, it was added later in order to get a better understanding of how Cladonia lichen could affect green roof substrates; visually, the lichens were very similar throughout both 2012 and 2013. Albedo was measured on September 28, 2013. The information was gathered from five lichen modules (100% cover) from the previous season and five substrate only controls created by removing vegetation from the old lichen modules (due to wind damage, only five lichen modules could be salvaged). Albedo was measured with a single LI-COR pyrometer sensor and LI-250A light meter (LI-COR Biosciences, Lincoln, Nebraska, United States) attached to a fixed position 35 cm above the soil surface. The incoming solar radiation (W/m²) was measured by pointing the sensor 180° from the roof surface. The reflected radiation was measured by rotating the sensor 180° to face each module. Albedo was calculated by dividing the reflected radiation by the incoming radiation, and a one-tailed t-test was used to compare the control and lichen modules. A p-value < 0.05 was considered significant.

Results

The albedo of the Cladonia modules was significantly higher than the control modules (Table 1, Data Set 1). Substrate temperatures for July and August were significantly lower in the Cladonia modules compared to the controls (Table 1). In September, there was no significant difference in soil temperature between the Cladonia modules and the controls. In October, the Cladonia modules had significantly higher substrate temperatures than the controls (Table 1, Data Set 2). Regarding water loss, there was no significant difference between the Cladonia modules and the control for August, but in September and October, Cladonia modules lost significantly less water than the controls (Table 1, Data Set 3).
**Data Set 1.** Raw albedo data for the lichen modules and substrate only controls taken on September 28, 2013. Albedo was calculated by dividing the reflected radiation (Wm\(^{-2}\)) by the incoming radiation (Wm\(^{-2}\)).

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<th>Treatment</th>
<th>Incoming</th>
<th>Reflected</th>
<th>Albedo</th>
</tr>
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<td>116.7</td>
<td>0.379019</td>
</tr>
<tr>
<td>Lichen</td>
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<td>117</td>
<td>0.39747</td>
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<td>0.368788</td>
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<tr>
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<td>309</td>
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<td>0.366019</td>
</tr>
<tr>
<td>Lichen</td>
<td>312</td>
<td>119.1</td>
<td>0.381731</td>
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<td>Control</td>
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<td>Control</td>
<td>298.3</td>
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<td>Control</td>
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<td>Control</td>
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<td>102.06</td>
<td>0.33342</td>
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</table>

**Discussion**

Compared to the substrate-only controls, the *Cladonia* modules were cooler during hot conditions and retained more moisture in the substrate. These cooler temperatures may have been a result of shading and higher albedo. Even though albedo was only measured in 2013, the *Cladonia* lichen always had a lighter coloring than the bare substrate. Lighter colors are associated with higher albedo. Likewise, the lichen cover resulted in less net evapotranspiration compared with the bare substrate treatment. Interestingly, the soil temperature during October (average air temperature on date of measurement: 17.9°C) was significantly warmer in the *Cladonia* modules compared to the controls. This implies that a *Cladonia* mat may help reduce heat loss during the winter and help cool the roof during the summer.

Plants surrounded by *Cladonia* may benefit from such temperature regulation (cooler temperatures in the summer, warmer temperatures in the winter) and greater water availability. Other studies have shown that plants with mat-forming growth can benefit less drought-tolerant species\(^\text{16}\). In addition to this, *Cladonia* lichens do not appear detrimental to plant growth. Vascular plant species growing out of these lichen mats is a natural occurrence on the coastal barrens of Nova Scotia and, during the trial, seedlings of trees and grasses were observed growing out of these modules, although control modules also had such seedling growth. Some lichen species also play a key role in soil development in conditions of high abiotic stress\(^\text{8,17}\). If lichen inclusion on a green roof could improve the substrate properties for neighboring species, the entire system would benefit.

In this study, while the lichens remained in the roof modules for the duration of the experiment, it is not clear that the lichens remained alive. More research is required to determine whether this method of transplanting lichens onto green roof substrates results in viable lichen populations. Further, our transplanting method...
involved sourcing lichens from the wild and while these species are very common in the region this kind of collection would clearly not be feasible on an industrial scale. Finally, while green roofs are becoming more popular in all regions, lichens, in varying degrees, are sensitive to air pollution and many species are likely to perform poorly where air quality is low. Our study establishes that Cladonia lichens could provide roof cooling and water retention services in a green roof environment, but more work is necessary to explore the long-term viability of fruticose lichens in these systems.

Author contributions
A. Heim contributed to experimental design, collected the data, analyzed data, wrote the paper; J. Lundholm contributed to experimental design and wrote the paper. Both authors checked and agreed to the final publication of the manuscript.

Competing interests
No competing interests were disclosed.

Grant information
This research was funded by NSERC Discovery Grant 311788-2010 to J. Lundholm.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments
We thank Emily Walker for critical edits on the manuscript.

Supplementary material

Supplementary Table 1. A soil test conducted by Nova Scotia Agriculture describing the elements present in the Soprema X 2011 substrate.

<table>
<thead>
<tr>
<th>Soprema X 2011</th>
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<th>Sample 2</th>
<th>Sample 3</th>
<th>Average</th>
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<tr>
<td>Organic Matter (%)</td>
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<tr>
<td>P₂O₅ (kg/ha)</td>
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<tr>
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<td>Lime Required (t/ha)</td>
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References

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Version 1

Reviewer Report 23 December 2013

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Martin Hermy
Division of Forest, Nature and Landscape, KU Leuven, Flanders, Belgium

The title and abstract cover the contents of this original work well. The study design, methods and analysis seem appropriate. Overall I do agree with the conclusions. However, one crucial element should be mentioned (which is recognized by the authors).

The fact that it is not sure that the lichens still were alive after the experiment puts considerable constraints about the applicability of these organisms in extensive green roof systems. My experience with lichens is that they normally grow on nutrient poor, dry, acidophilous soils. I've seen Cladonia's enter extensive green roof systems after a number of years (when substrate pH dropped). Green roof substrate used here (which is the standard) is indeed nutrient poor, but definitely not acid (see info). That makes the point whether these lichens are still alive or not even more important. One could eventually use dead lichen material and or other dead materials with similar properties (e.g. albedo), e.g. white pebbles.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 12 Sep 2017
Jeremy Lundholm, Saint Mary's University, Canada

The lichens were alive after all. We have since observed the same species colonizing an adjacent green roof from fragments originating in the experiment described in this paper. (there are no other local sources of these species)

Competing Interests: No competing interests were disclosed.

Reviewer Report 17 December 2013
Richard Sutton
Department of Agronomy & Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA

The Title is appropriate, the Abstract is useful and the article clearly constructed. The experimental design also appears appropriate.

I am pleased to see complete data collection information, enabling the experiment to be duplicated, however, although it is required as a part of the F1000Research format, the raw data could be more easily understood if graphed.

I have the following comments about the Conclusion/Discussion section:

1. The last part of last sentence in the Methods section should go into the Discussion and lead on to a more detailed discussion of the lichen’s growth (or death).

2. This following sentence is unclear. It implies that the non-planted controls had seedling growth. How could this be possible if it was simply straight substrate? “[D]uring the trial, seedlings of trees and grasses were observed growing out of these modules, although control modules also had such seedling growth.” How would the Sopraflora-X get seeds?

3. While beyond the scope of the study, could a line be added about transplanting/regrowth-rate/propagation? Those features have an important impact on the future use of lichens. In my experience, Lichen growth under “normal” conditions is very slow.

4. The authors should talk more about the wind damage which might be an indication that the lichens have not attached themselves. This is important because most green roofs are very windy places.

5. It is also worth noting that displaced lichen cover would function less effectively.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
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