THE DISTRIBUTION OF MOSS IN STREAMS AT HUBBARD BROOK EXPERIMENTAL FOREST

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Abstract. Moss has long been an understudied part of aquatic stream ecosystems. Although it is known that mosses play a crucial role in ecosystems, providing habitat, nutrients, and influencing stream flow, there is much less known about its distribution across the landscape and the long-term dynamics in moss cover in response to changing climate and stream chemistry. In this study, we measured moss cover at Hubbard Brook Experimental Forest (HBEF) in 2019 to better understand its distribution. Where possible, we compared these current day moss distributions to data collected over the last 50 years. The questions we addressed with these data include: 1) How is stream moss distributed across Hubbard Brook?; 2) Can we detect any temporal changes in moss distribution, possibly associated with long-term trends in climate and acid deposition?; and 3) does stream moss harbor benthic algae and does the amount of algae in moss vary across space? Moss cover ranges widely across streams at Hubbard Brook, with some areas having little to no moss to other areas with upwards of 80-90% cover. A strong driver of moss cover at Hubbard Brook appears to be the physical habitat, although these data demonstrate that there has been variability in moss cover over the past 50 years at Hubbard Brook. This implies there are interactions between the physical template and other conditions, such as possibly stream water chemistry and winter scour. More research is needed to tease apart these dynamics. Understanding the dynamics of mosses in the northeast will help to build a greater understanding of how moss in temperate aquatic stream ecosystems may respond to long-term changes in climate and acid deposition.

INTRODUCTION

Deciduous forests and the stream ecosystems that drain them are responding to many anthropogenic stressors including, but not limited to, climate variability and change, acidic atmospheric deposition, and species invasions. HBEF, a site of extensive ecological research since the 1950s, provides a unique location to understand how the forest ecosystem is responding to these stressors over decadal time scales. The changes that have been documented thus far include increasing temperature—particularly in the spring months—as well as a decrease in ice cover duration in nearby Mirror Lake, with the timing of ice-out becoming about 12 days earlier (Likens 2011). Precipitation has also increased, particularly in the winter months in the form of rain (Campbell et al. 2010). Additionally, as precipitation increases, there has been an increase in streamflow (Bailey et al. 2003). One of the most prominent abiotic changes to Hubbard Brook has been the increase in pH within the streams. This occurred due to the passage and implementation of the US Clean Air Act, which included legislation specifically designed to combat acid deposition. With the amelioration of acid deposition, the pH values in the streams have begun to rise and return to a less acidic conditions.

Because the aquatic ecosystem is an important habitat to many organisms, it is important to understand how these ecosystems are responding to long-term change. In forested headwater stream ecosystems like those at Hubbard Brook, aquatic mosses are an important part of aquatic ecosystems. For example, moss is an important habitat for invertebrates (Wulf and Pearson 2017). In addition, moss has the ability to utilize organic phosphorus via phosphatase activity when inorganic phosphorus is limiting, allowing moss to live in extremely oligotrophic streams (Tessler et al. 2014). An early study found that mosses were the only primary producer in a Hubbard Brook stream (Fisher and Likens 1973). Mosses can influence current velocity and stream bed stability (Stream Bryophyte Group 1999). Algae are also known to be present in stream mosses and may grow in these stream bryophytes quite well. This interaction can greatly benefit the algae and it is thought that the presence of moss can increase algae growth (Alvarez and Peckarsky 2013). Algae were previously not present in the Hubbard Brook streams (see Fisher and Likens 1973), but in the past 20 years, algae have become more common, possibly due to an association with stream mosses. Although moss is an important habitat in Hubbard Brook streams, there is still limited research investigating how moss responds to long-term abiotic changes that have been observed at Hubbard Brook, like climate changes and increasing stream water pH.

Another important aspect of aquatic moss that is not well understood is the spatial variation of stream moss that exists within a particular landscape. Since streams are so dynamic and varying in terms of their habitat characteristic even within the same stream, the things that reside within them, including aquatic moss, also varies. There has been some work characterizing the preferences that moss has regarding differing habitats. For example, it was found that due to the CO_2 and O_2 exchange, aquatic mosses are best suited for waterfalls (Stream Bryophyte Group 1999), where they can receive the CO_2 they need as well as continual moisture. The substrate in waterfalls is also the most stable, allowing the bryophytes to grow most successfully. Due to the slow growing nature of moss, it is thought they prefer stable substrates such as bedrock and boulders over smaller more mobile substrate such as cobbles and pebbles where they are more likely to be disturbed (Stream Bryophyte Group 1999). However, there has not been much work done to further characterize these habitat preferences and explore the impact of this on moss cover over large stretches of stream.

Overall, moss has been found to be very dynamic and responsive in the few experiments that have altered abiotic factors to influence moss. Increasing temperatures, particularly summer temperatures, have been found to increase the moss cover in streams (Thiemer et al. 2018). However, as forests experience more extreme weather patterns and more intense precipitation, scour has been shown to reduce moss cover by 50% (Lind et al. 2014).

We took advantage of moss surveys that go back 50 years at Hubbard Brook, and conducted our own survey this year, to investigate how the moss habitat has changed over time. We conducted surveys over vast spatial reaches in order to understand and characterize the moss variation that occurs. This work will be conducted within the Hubbard Brook Valley, which is located in the White Mountain National Forest in north central New Hampshire. The HBEF was established in 1955 by the U.S. Forest Service to monitor and build research on temperate ecosystems in the Northeast and is now one of the most comprehensively studied ecosystems in the country (Likens 2013). Within the HBEF, there are nine distinct watersheds that have been experimentally manipulated (Table 1).

Our research builds on a number of historic data sets including an energy budget conducted by Fisher and Likens at Hubbard Brook in 1973 (with field work done the summer of 1969). As part of that energy budget they did a moss survey and found that 2% of stream cover at Bear Brook (part of one of the watersheds) was moss (Fisher and Likens 1973). Further, in 1997, there was a project known as the Lotic Intersite Nitrogen eXperiment (LINX) (WEBSTER et al. 2003). In order to better understand the habitat prior to the addition of nitrogen they also conducted a moss survey in Bear Brook using lateral and longitudinal transects (W. B. Bowden, *personal communication*). Using their methods, they found anywhere from a 5-10% moss cover in Bear Brook. Lastly, in 2000, there was a valley-wide survey that was conducted across the HBEF to investigate large-scale water chemistry and watershed attributes (Likens and Buso 2006). Unpublished data that were obtained in this survey included percent moss cover that was collected in 100-meter transects throughout the streams at Hubbard Brook. These data suggest that moss was very common in Bear Brook. Our research will further investigate the changing dynamics of moss, exploring mechanisms for the change as well as document any changes in moss cover that have occurred from 2000 to 2019.

Some of the unpublished historic moss surveys captured the extreme variation of moss cover that occurs within a small section of a stream. However, only the valley-wide survey from 2000 fully encapsulated the variation that is found across large reaches of stream and within a watershed. Our research furthered knowledge regarding moss habitat and what determined moss growth on the landscape level. The research also provided data on what type of variation occurs regarding that growth within a particular stream in temperate ecosystems.

Informed by moss studies in HBEF streams and in other ecosystems, we seek to answer the following questions 1) How is stream moss distributed on the Hubbard Brook landscape? and 2) Can we detect any temporal changes in the stream moss? Lastly 3) Has a change in stream moss cover been associated with a change in algae? To address these questions, we plan to replicate the historical stream moss studies of 1969, 1997, 1998, and 2000 through qualitative surveys and lateral and longitudinal transects, as well as to conduct destructive moss sampling in order to determine algal biomass in moss habitats. We predict that either moss cover has increased as a result of rising air temperatures, or has decreased in response to scour. Since moss can provide nutrients and a habitat for algae, we also expect that moss will contain algal communities and that changes in moss cover translate to changes in associated algal biomass.

MATERIALS AND METHODS

Large- scale Moss Cover Percent Qualitative Quantification (Valley-wide)

In order to replicate the year 2000 valley-wide moss survey, we conducted the 2019 moss survey using the same methods (T. Wooster, *personal communication*). Using UTM coordinates from the valley-wide project in 2000 and the QGIS program, we made maps for the different watersheds that contain points spaced 100 meters apart (Figures 1, 2). We inputted the maps into the app Avenza in order to determine when 100 meters was reached in the field. We walked the streams of watersheds 1-6 and 8-9 at Hubbard Brook and every 100 meters we estimated the percent cover of moss in the preceding 100-m reach. In the 2000 valley-wide survey, the surveyors recorded if moss was absent, rare, common, and abundant. In order to more quantitatively measure we recategorized these into percent cover 0%, 5%, 27.5%, 50%, 65%, and 80%+, respectively based on conversations with T. Wooster. While surveying, every 100 meters we also recorded conductivity, pH, water temperature, and canopy cover.

Lateral Transects

To additionally quantify the moss growing in the Hubbard Brook watersheds we conducted lateral transects along the streams. In watersheds 8, 9, and Bear Brook we measured moss cover using lateral transects along four 25-m stretches. In the remainder of the locations we measured moss cover using lateral transects along two 25-m stretches. We selected the location of the transects by evenly splitting the stream area into two or four sections respectively (based on whether we were doing two or four 25-m stretches), and then used a random number generator to determine the starting location of the lateral transect. The random number generator assigns which 100-m section to begin at within the stream. For example, if there were 500 meters in the section, and the random number generator gave us the number two, we would start the lateral transect at 200 meters. For each 25-m reach, every 5-m, we laid a rope marked with 5-cm segments across the stream width. In transects perpendicular to the stream flow, the stream biota, habitat type, and substrate were recorded at 5 cm intervals.

Longitudinal Transects

Longitudinal transects were conducted to compare against the lateral transects and gain a more comprehensive view of the moss cover, replicating what was performed in 1997. We used a random number generator to determine which 100-m reaches of the stream were selected for longitudinal surveys. To ensure that the stream was properly characterized, we blocked longer streams (watersheds 8, 9, and Bear Brook) into 6 reaches and shorter streams (watersheds 1, 2, 3, 4, 5, 6, and Paradise Brook) into three reaches. At the randomly selected point, the presence of moss, stream habitat, and substrate was noted every meter for 30 m.

Algae Quantification

We randomly took ten destructive moss samples from watersheds 8, 9, Bear Brook and Paradise Brook in order to quantify algal biomass (as chlorophyll-a) living within the moss. We also took three destructive moss samples below the weir in watersheds 1-6. To sample, we placed a 40 mL specimen cup firmly against a moss-covered rock. We then took a flat-ended X-Acto knife and scraped the moss along the outside of the cup, then used a metal paint scraper to dislodged attached moss and other material from under the cup, quickly turning it over so the moss and water falls into the cup. Upon return to the lab, we manually agitated the moss sample using our fingers for 20 seconds, and then put it through a 500-micron sieve, removing the moss from the liquid. We dried and measured the ash-free dry mass of the moss using standard techniques. The remaining supernatant was put through a glass fiber filter (nominal pore size ~0.7 μm) and chlorophyll-a on the filter was measured on a fluorometer using the ethanol extraction method (Azar and Collins 1997).

Data analysis

First, we organized our data into a carefully organized Excel document. Then, in order to statistically compare the percent cover of the moss among the various watersheds we ran an ANOVA test in R on the data to see if the watersheds were different from one another. We then ran a Tukey (HSD) test to see what the specific differences among the watersheds were (R studio team 2018). We also did this for the data that looked at substrate and habitat regarding moss cover. To compare the percent cover between 2019 and 1997 and 2000, we graphed the percent moss points using excel and then visually compared using a 1:1 line placed on the graph. To represent the moss cover points on the map we used QGIS (QGIS Development Team 2019) to map the percent cover over every 100-m transect. This was also used with the 2000 valley-wide data in order to see differences among years looking specifically at percent cover.

RESULTS

Moss differences among watershed

Across the watersheds of the Hubbard Brook Valley, there is a significant statistical difference in the moss fraction seen (Figures 3 & 4). We initially made a map of the large-scale survey of the Valley-wide points that were collected in 2019 using QGIS and noticed the variability seen (Figure 2). After running an ANOVA test on figures 3 and 4 to compare the mean moss fractions among the various watersheds, and a Tukey(HSD) test to see which watersheds were different from one another, there was a p-value<0.05, indicating that there is a significant statistical difference between the various sites. In figure 3 (lateral transects), watershed 2 was different from the other sites and had a high moss fraction observed. Figure 4 (longitudinal transects) was also statistically significant among watersheds with a p- value of less than 0.05. In figure 4, watershed 1 was an outlier of a low moss fraction and significantly different from PB, WS 2, and WS 4. Watershed 2 was an outlier of a high moss fraction and was significantly different from BB, WS 1, WS 5, WS 6, and WS 9.

Effect of substrate and habitat on moss cover

Across all the substrates that are evident at Hubbard Brook, we looked to see if there was a significant difference in the moss fraction that grows on the various substrates for both lateral and longitudinal surveys. The different substrates included in the surveys were boulders, bedrock, cobbles, pebbles, sand and wood (represented in that order on the graphs). In the longitudinal transects (Figure 7), the boulders were significantly different from cobbles and pebbles as the boulders had a greater moss fraction, and the bedrock was different from cobbles, pebbles and wood ($p<0.05$) as the bedrock had a great moss fraction. In figure 6, the boulders and bedrock are both significantly different from the cobbles and pebbles ($p<0.03$) with the boulders and bedrock having a higher moss fraction.

Across the habitats that are evident in the Hubbard Brook Valley (cascade, pool, riffle, run), there was a significant difference between the fraction moss cover and the habitat. We used an ANOVA test to test significance and Tukey (HSD) to see what was significant between the different categories. In the lateral transects, the cascades had a significantly greater fraction moss than the pools, and riffles ($p< 0.005$). In the longitudinal transects, the riffles had a significantly higher moss fraction, and the pools had a significantly lower moss fraction ($p<0.001$).

Chlorophyll a

Between the different sites at Hubbard Brook, there is a significant difference of chlorophyll a ($p<0.05$). We ran an ANOVA test to find that significance. Specifically, watersheds 8 and 9 have a significantly higher concentration of chlorophyll a while Paradise Brook has a significantly lower concentration of chlorophyll a.

Moss cover data over time

Looking at the Valley-wide survey over time, figures 11 and 14 show what the moss cover looked like in 2000. The maps were made in QGIS, and each point represents 100 meters along the stream. The colors are correlated to the fraction of moss seen over that last 100 meters. The white dots are correlated with low moss cover fraction seen (starting with 0.00), and the red dots are correlated with a high moss cover fraction seen (0.80). Figures 12 and 13 show what the moss cover looked like in 2019. The scale is the same between the different maps. Figures 11 and 12 show a zoomed in map of Bear Brook while figures 13 and 14 show zoomed in versions of watershed 8 (on left) and watershed 9 (on right). Watersheds 9 and Bear Brook show a decreased in moss cover. Watershed 8 shows a fairly consistent moss cover.

As also shown in the previous maps, there are differences between the percent moss obtained from the Valley-wide surveys in 2000 and 2019. In figure 15, a 1:1 line was drawn in the graph. Each dot represents moss cover. Since the points are more concentrated below the 1:1 line, the data show a decrease in moss cover.

Figure 16 is set up in the same way as figure 15. The line drawn is a 1:1 line representing the way the moss cover was compared between 1997 and 2019. Each point represents the moss cover in 1997 compared with the moss cover in 2019. The data show that there was an increase in moss cover between 1997 and 2019 as the points are concentrated above the 1:1 line. The data was taken from a 160-meter section of Bear Brook.

DISCUSSION

Moss differences between watersheds

As we saw through three different methods of comparison: valley-wide large-scale surveying, lateral surveys and longitudinal surveys, there is a statistical difference in moss cover between the various watersheds at Hubbard Brook. This lends itself to the first question we had: How is stream moss distributed on the Hubbard Brook landscape? We found through the survey done this summer that the moss is distributed variably and that there are differences between the moss cover at the different sites (figures 3, 4, 5). There are a number of possible mechanisms for this difference that was evident. As seen in figures 6, 7, 8, 9, there is a correlation between the habitat and substrate and the moss cover that is seen. Therefore, the physical habitat of each watershed could play a role in that difference. It was found that more stable substrate and cascades and riffles have the most moss growth. Another possible mechanism that one could explore through future analysis of my data is the possibility that the other watershed differences such as pH, nutrient availability, hydrological flashiness and the other distinctions that are seen from the manipulations that took place between watersheds at the Hubbard Brook Experimental Forest have had an impact on these varying moss cover percentages seen.

Moss differences over time

The moss cover in the streams at Hubbard Brook has been compared from 1972, 1997, and 2000. Referring to figures 11, 12, 13, and 14 it is possible to see how moss has changed on a more crude, broad scale. Based off the map comparisons, it looks as though some of the watersheds (9 and Bear Brook) have decreased in moss cover. Watershed 8 seems to have stayed fairly consistent. Figure 15 corroborates this general finding; based on the valley-wide survey from 2000 it seems as though moss has decreased in Hubbard Brook streams between those two years. Figure 16 tells a slightly different story. Based on the LINX data from 1997, there seems to be an apparent increase in moss cover between then and now. However, this data was taken only in 160 meters of Bear Brook. The scale of this data is different and was taken much more quantitatively. Therefore, to answer the question: can we detect any temporal changes in the stream moss is not an easy straightforward answer. It does seem as though there has been some temporal changes in the moss cover but there is still much more to understand about these temporal differences. The decrease that might be evident could be caused from increased scour events due to an increasingly intense climate in the face of climate change. The apparent increase in the LINX data could be due to the fact that that survey comes from only a small part of the stream. The variability of moss throughout the streams (figure 3) means that when only a small part is surveyed the larger story may be missed. Another survey that occurred in the summer in 1969 (Fisher's dissertation), found that Bear Brook contained 2% moss cover (Fisher, S. G., and G. E. Likens). Though this number seems particularly low, it has been communicated that that winter might have been particularly intense with lots of potential for scour events (G. E. Likens, *personal communication*). This means that it's possible that much of the moss may have been physically removed. The moss in Bear Brook has undoubtedly increased since then (referring to 1997, 2000 and 2019), but more research into the mechanism of this change needs to be explored.

Chlorophyll a (algae) in the streams at Hubbard Brook

The way that algae is measured in stream ecology is often through measuring the chlorophyll a seen in the samples of the stream. In the case of the moss that I sampled, the chlorophyll a ideally correlates with the algae in the stream. Therefore, the implication is that figure 10 shows that there are significantly different amounts of algae in the various watersheds at Hubbard Brook and that there is a presence of algae. This also implies the fact that stream moss is an important habitat for filamentous algae and diatoms in these relatively low production streams. Regarding whether there is a correlation with moss to account for these differences, it doesn't seem as though there is. The highest quantities of algae occurred in watersheds 8 and 9, and the lowest in Paradise Brook. The highest amount of moss occurred in watershed 2 and the lowest in watershed 1. However, more research can be done regarding these correlations. Other mechanisms to explain this algae difference that can also be explored is the difference in organic content in the water.

Watershed 9 is an outlier for increased organic content. Additionally, light present in the streams could play a large factor.

Greater Implications

The greater implications for this project regarding the future of Hubbard Brook, aquatic moss and algae are quite numerous. This project has established the fact that aquatic stream moss is extremely variable so thus requires sampling techniques that account for this variability. Additionally, it cannot be understated the importance of this moss to the ecosystem and the role that it has in forming habitat for the life of the Hubbard Brook streams. In order to fully understand the life in the streams at Hubbard Brook we need to make an effort to better understand the distribution and presence of aquatic moss. This project seeks to build and establish this understanding a bit more thoroughly and extensively.

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APPENDIX

TABLE 1. Experimental effects on watershed 1-9, Hubbard Brook Experimental Forest. (Hubbard Brook Ecosystem Study 2019)

Watershed 1	In November 1999, wollastonite ($CaSiO_3$) was applied by helicopter in order to combat the effects of acid rain that had depleted the Ca over the last 50 years.
Watershed 2	From 1965-1967 watershed 2 was devegetated, and during the second two years herbicide treatments were applied to prevent regrowth.
Watershed 3	Hydrologic reference watershed.
Watershed 4	A 3-phase strip cut treatment was carried out in 1970, 1972, and 1974 to study how strip cutting affects nutrients and hydrological cycling and regeneration.
Watershed 5	Whole tree harvest occurred here during the dormant season of 1983-1984.
Watershed 6	Biogeochemical reference watershed.
Watershed 7	No experimental treatments.
Watershed 8	No experimental treatments.
Watershed 9	No experimental treatments.

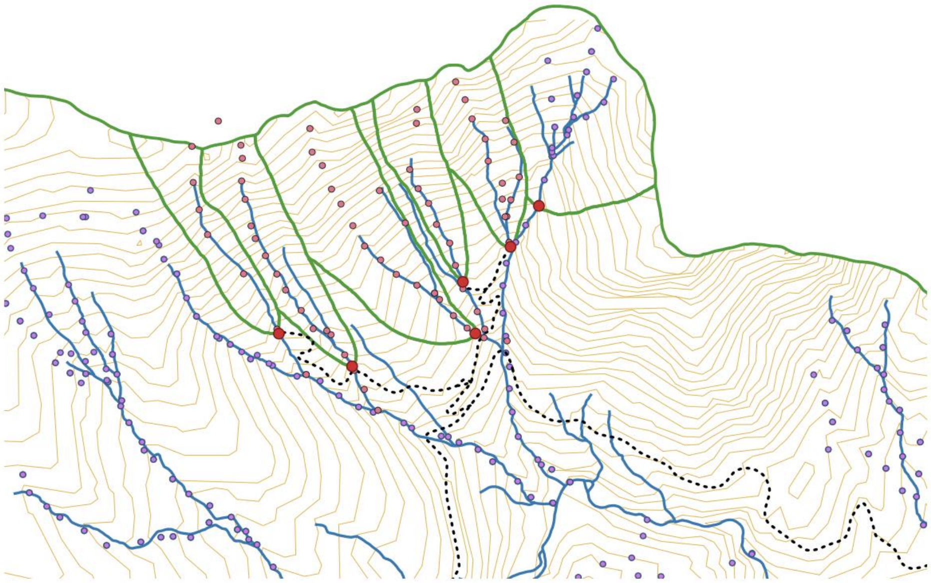

FIGURE 1. Sampling map for watersheds 1-6. (Campbell 2004, Campbell 2016, Likens and Buso 2015)

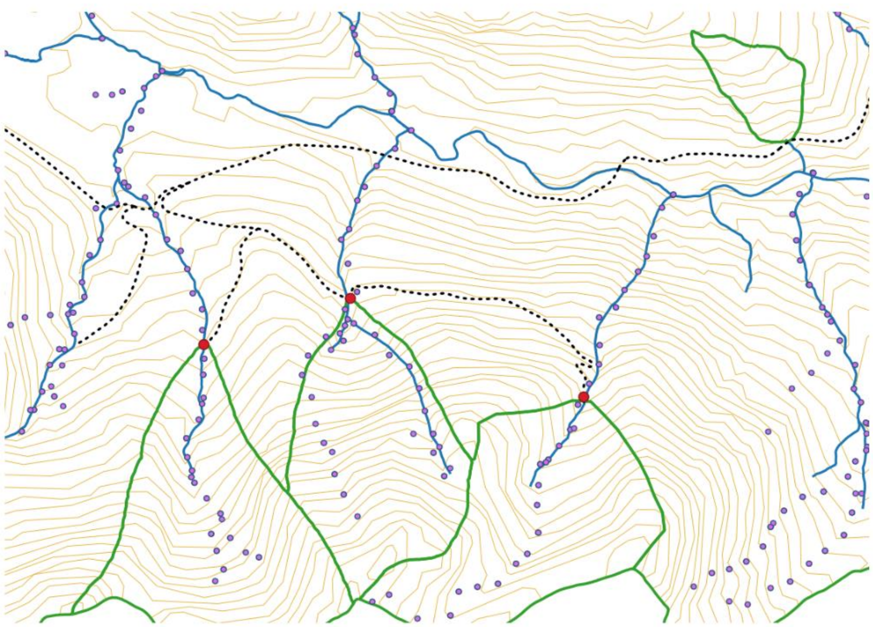

FIGURE 2. Sampling map for watersheds 7-9. (Campbell 2004, Campbell 2016, Likens and Buso 2015)

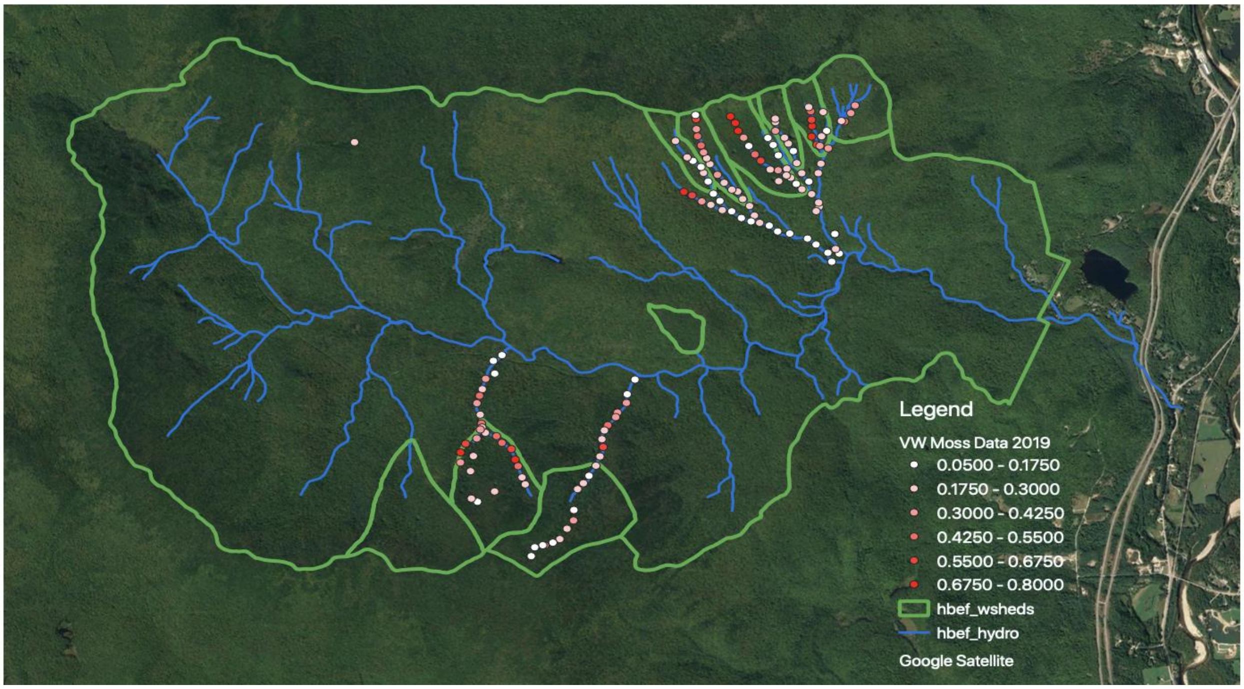

FIGURE 3. Valley-wide spatial stream moss distribution for 2019.

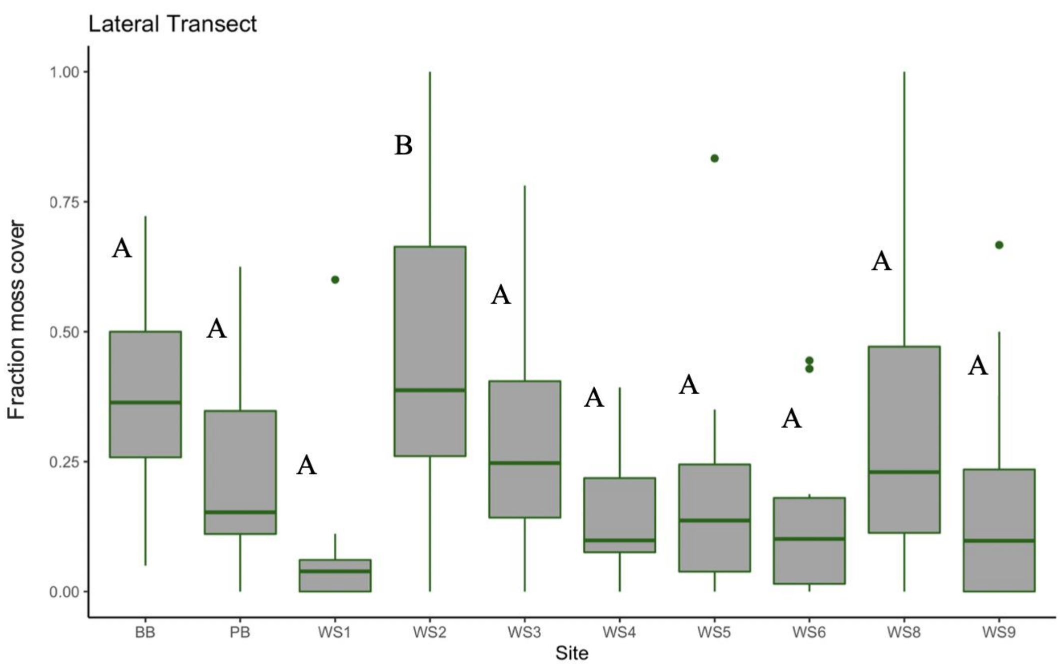

FIGURE 4. Fraction moss differences among watersheds (lateral transects). There was a significant difference between watershed 2 and the remainder of the watersheds $F(9, 115) = 2.992$, $p<0.05$.

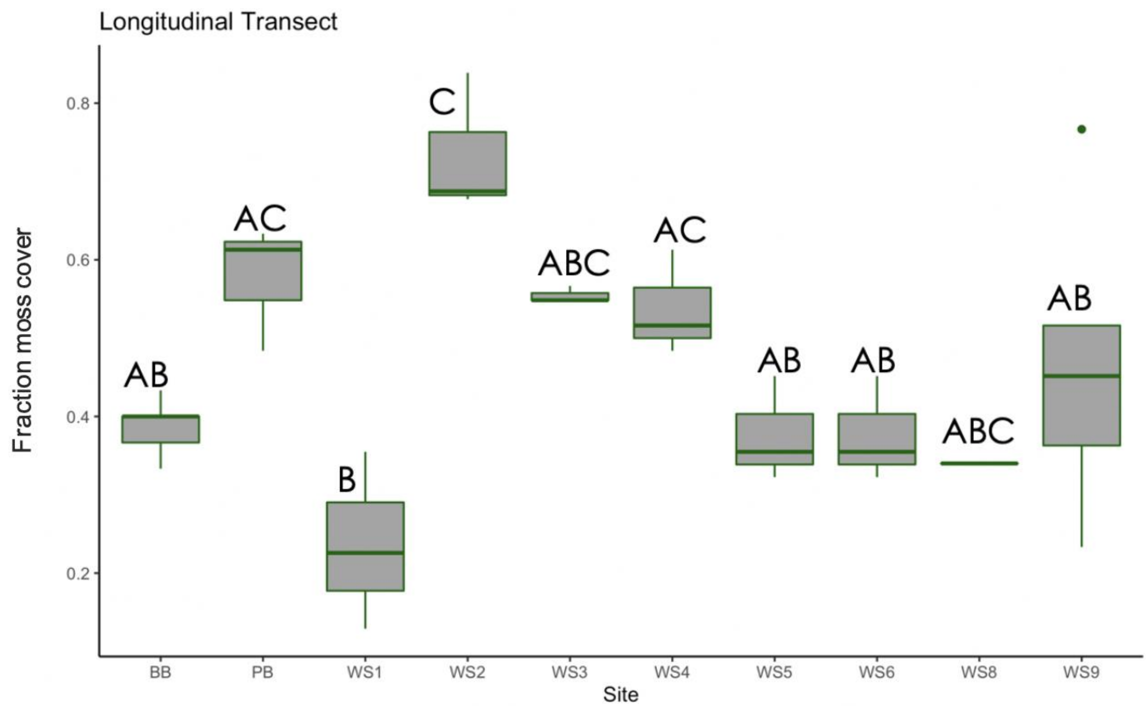

FIGURE 5. Fraction moss differences among watersheds (longitudinal transects). There was a significant difference between many of the watersheds at Hubbard Brook, specifically looking at WS 1 and WS 2 $F(9, 23) = 5.434$, $p<0.05$.

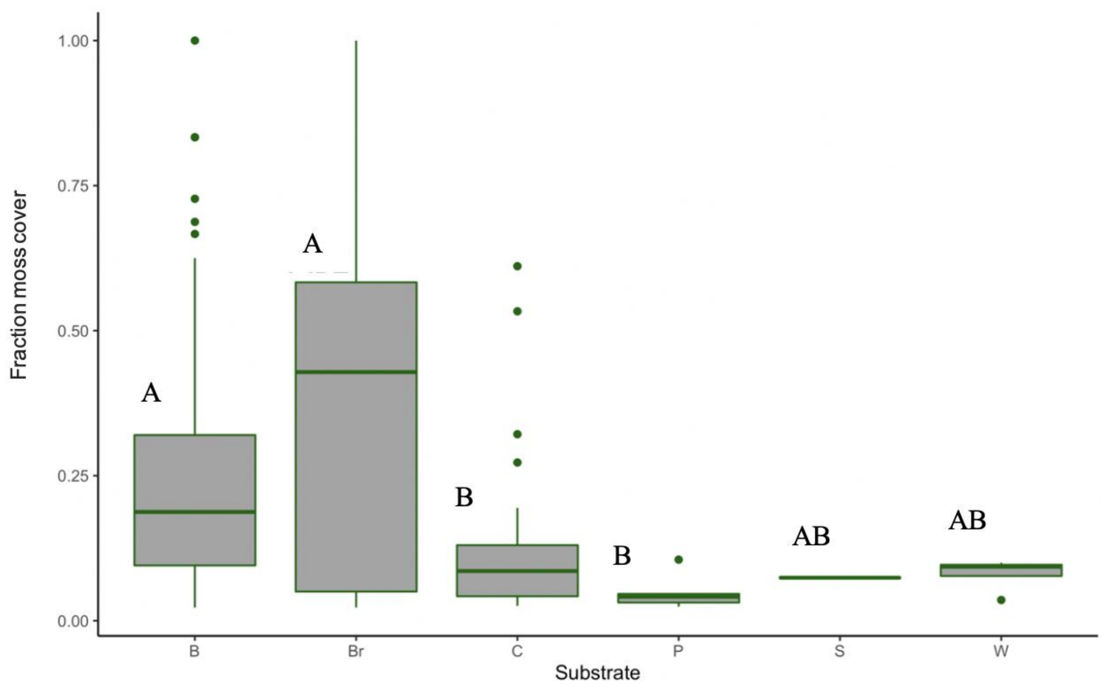

FIGURE 6. Lateral transects, substrate related to fraction moss cover. There was a significant difference between different substrates as related to moss cover $F(5, 147) = 6.565$, $P<0.03$.

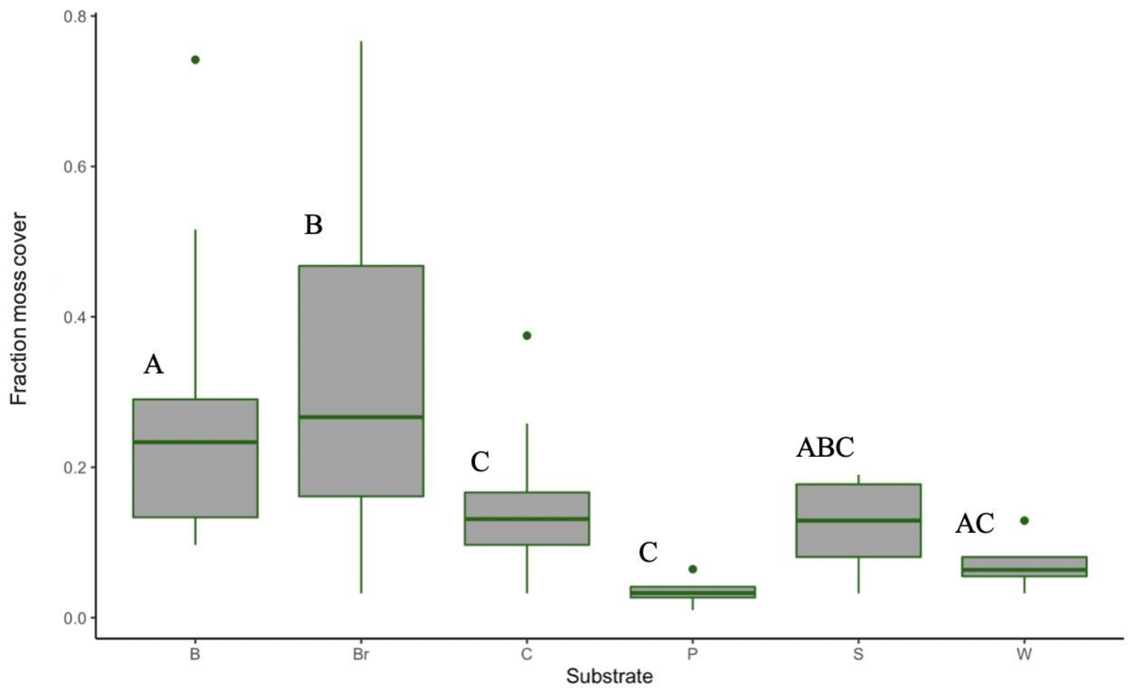

FIGURE 7. Longitudinal transects, substrate related to fraction of moss cover. There was a significant difference among the different substrates and its effect on moss cover F(5, 70) = 5.529, p<.05.

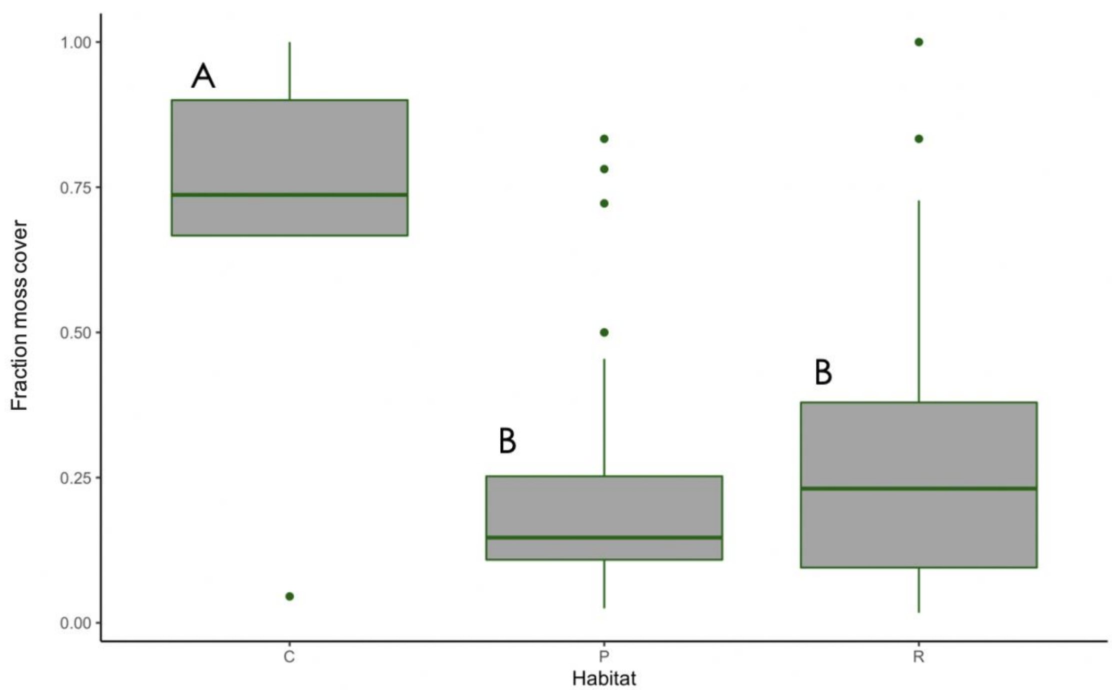

FIGURE 8. Lateral transects, habitat related to fraction moss cover. There was a significant difference among the different habitats and its effect on the fraction moss cover evaluated F(2, 114) = 10.84, p<0.005.

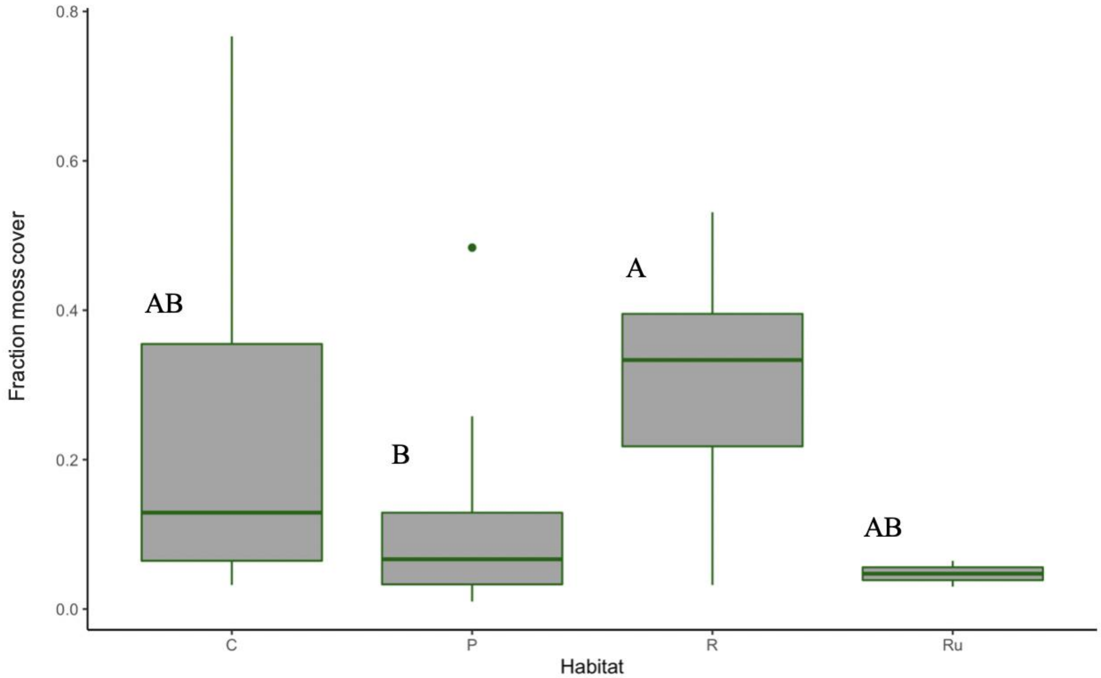

FIGURE 9. Longitudinal transects, habitat related to fraction moss cover. There was a significant difference among the different habitats and its effect on the fraction moss cover evaluated F(3, 67) = 9.749, p<0.001.

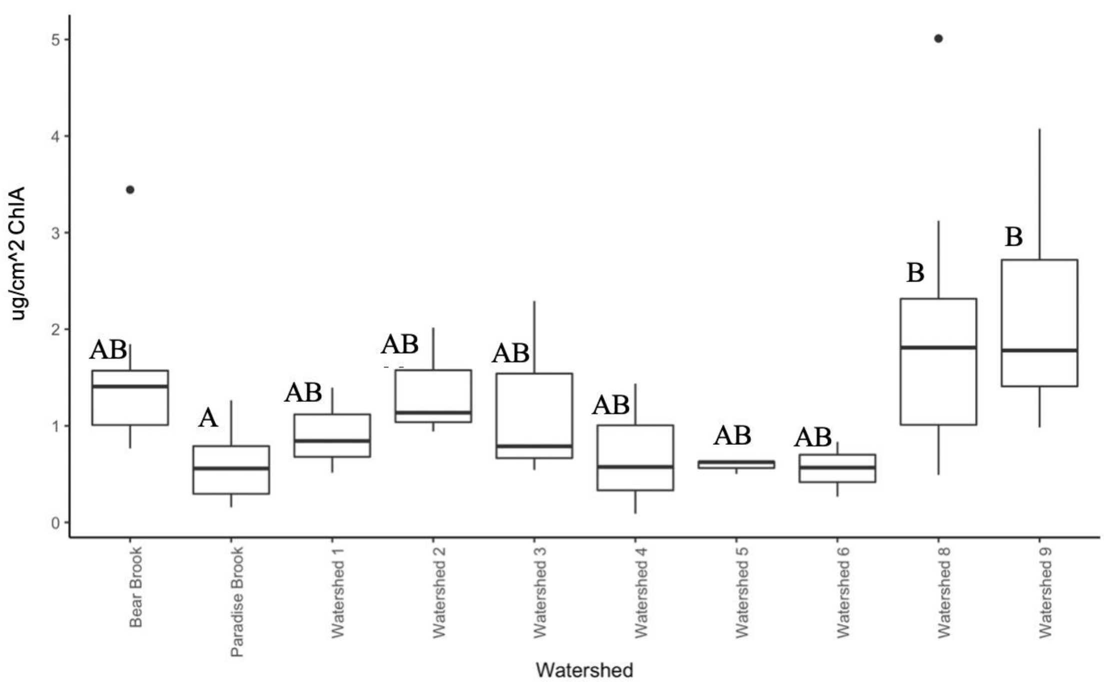

FIGURE 10. Concentration of chlorophyll a per watershed. The chlorophyll a is significantly different amongst the different watersheds F(9, 48) = 3.708, p<0.05.

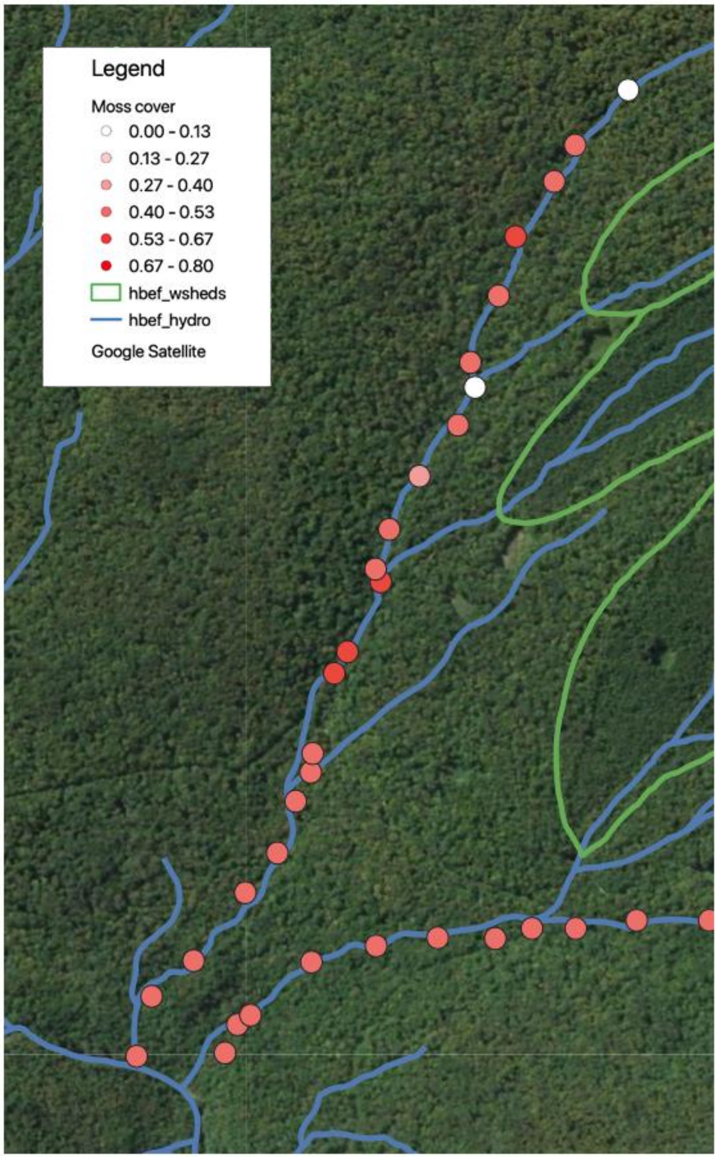

FIGURE 11. Bear Brook valley-wide moss distribution in 2000.

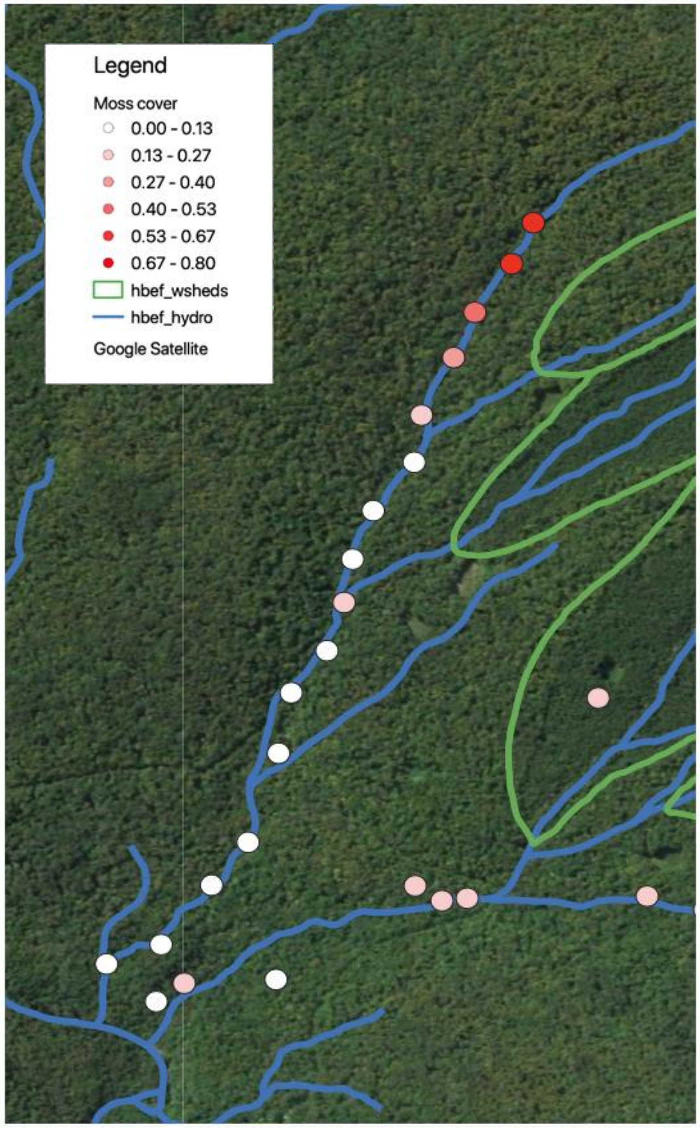

FIGURE 12. Bear Brook valley-wide moss distribution in 2019.

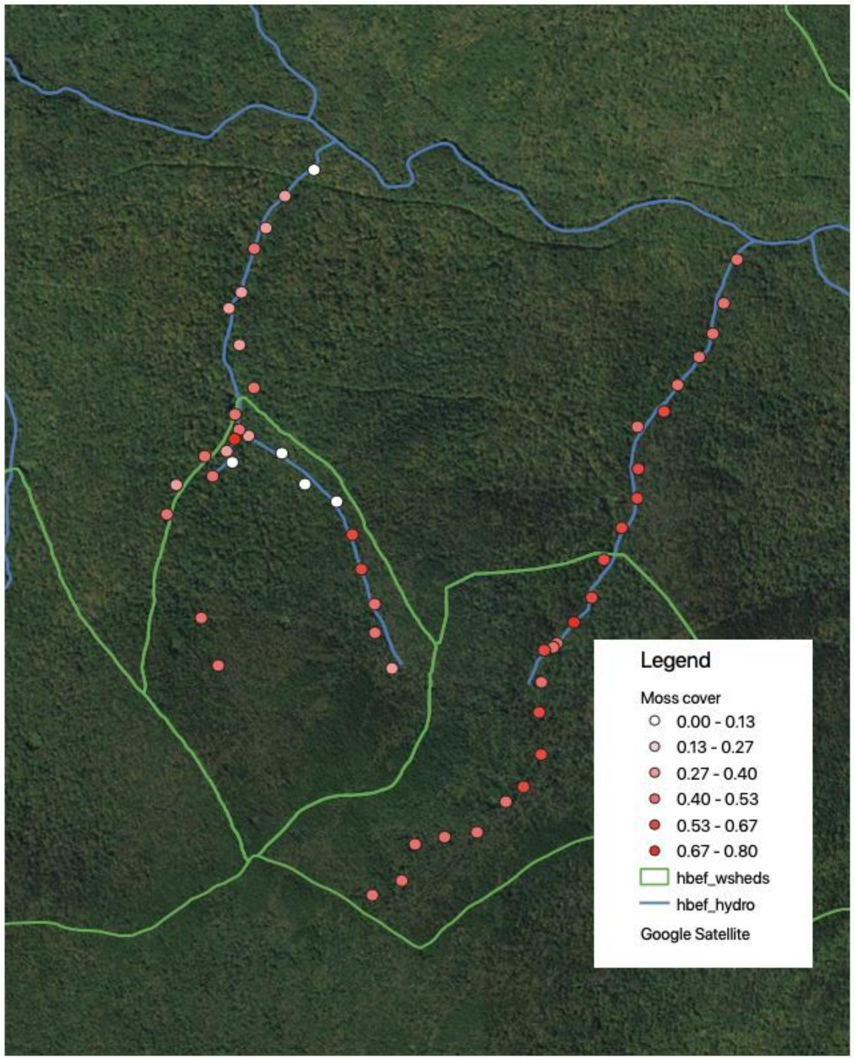

FIGURE 13. Percent moss cover in 2000 for Watershed 8 & Watershed 9.

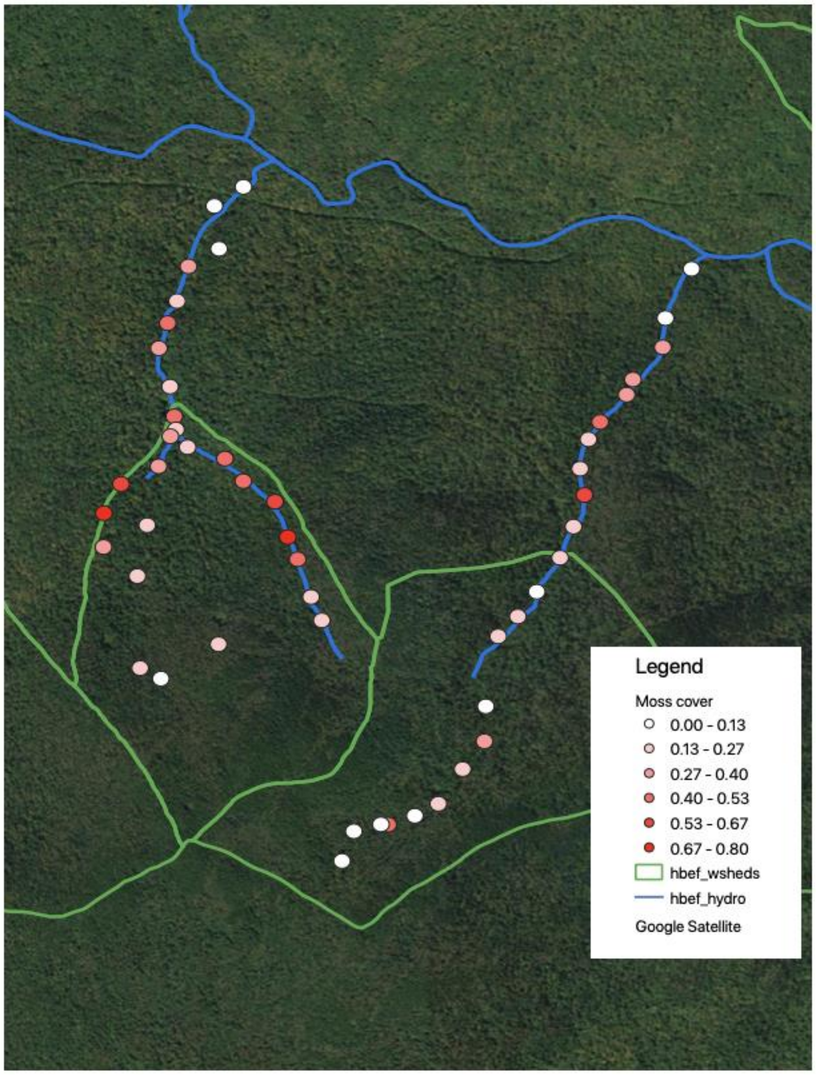

FIGURE 14. Percent moss cover in 2019 for Watershed 8 & Watershed 9.

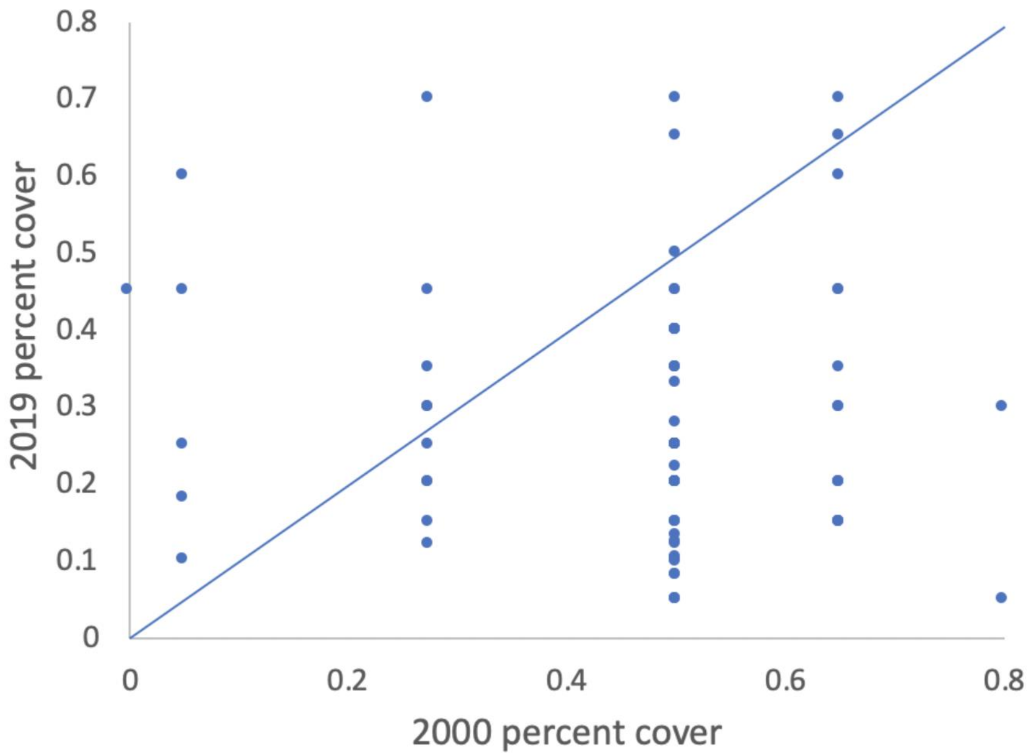

FIGURE 15. Valley-wide moss cover data compared between 2000 and 2019.

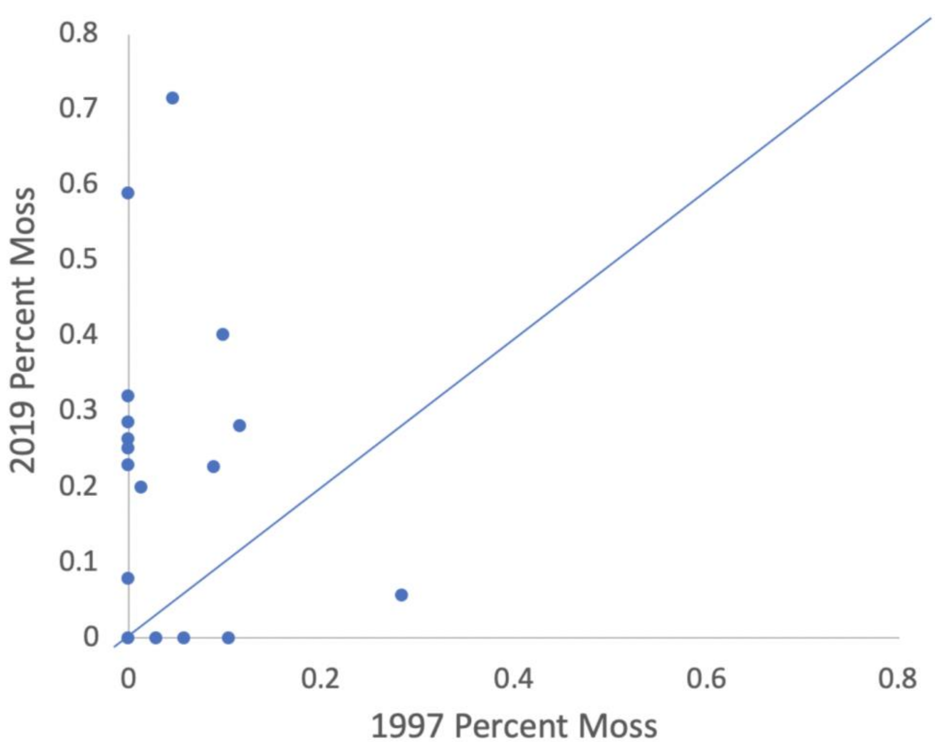

FIGURE 16. LINX data for Bear Brook in 1997 and 2019.