Soil Lichen Communities of the New Jersey Pinelands

and their Effects on Belowground Patterns and Processes

By

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ABSTRACT OF THE DISSERTATION

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In this dissertation, I describe some of the role that lichens play in the New Jersey Pinelands ecosystem. I begin with a review of worldwide lichen communities, and discuss some mechanisms through which lichen communities have previously been found to be influential in the ecosystems where they occur. In the first chapter, I have quantified the structure of the lichen communities where extensive lichen mats are present. I found that extensive lichen mats occur in areas with low soil organic matter content, that have experienced no recent fires, and that exhibit wide ranges of canopy cover. Most lichen mats communities were similar to each other in composition, except for the southernmost site, the Manumuskin River Preserve.

In my second chapter, my collaborators and I investigated the influence of these lichen mats on trends in soil moisture, soil chemistry, soil microbial community activity, and soil arthropod presence, through a transplant experiment. We found that the influence

of lichens on soils varies with soil conditions and with climate conditions. In low moisture conditions lichens contribute to soil retention of moisture, and when soils have higher inorganic phosphorus availability, lichens significantly reduce extractable phosphorus concentrations. Lichens also promoted higher densities of collembolans in the summer. The microbial community activity did not respond dramatically to lichens; although lichens are known to leach phenolic compounds into the soil, lichens did not promote increased production of any enzymes associated with degradation of recalcitrant carbon compounds. The lichens did not have significant effects soil ammonium or nitrate levels or on microbial community activity.

My third chapter tests whether lichens can mediate human-induced environmental changes by investigating how lichens process excesses of nitrogen; we found that at high loads of nitrogen, lichens can depress the quantities of total nitrogen delivered to the soil and to the groundwater that leaches through it. Lichens prevent ammonium from being accumulated in the soil although not significantly more than any aboveground cover. Results from these studies clarify some of the functional significance of temperate forest lichens.

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1 Introduction: Lichens in Natural Ecosystems.

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Fungi are understood to be critical players in global nutrient cycling, promoting plant growth and primary production, decomposition and nutrient cycling, serving as food for animals, and regulating populations through pathogenicity. However, lichens, whose feeding mode involves no living or dead plant or animal tissue (instead deriving their carbon from products of their photosynthetic symbionts), are often overlooked in discussions on ecosystem processes.

There are over 18,000 described species of lichenized fungi (Feuerer and Hawksworth, 2007). Although the lichen biomass mostly consists of fungi, the lichen entity is an emergent property of the interactions between the photosynthesizing organism(s), the fungi, and the associated microbes. The photobiont in a lichen thallus may be either a green algae or a cyanobacteria (a cyanolichen), or both (a tripartite lichen). The fungal biont is typically an Ascomycete, but Basidiomycete lichens also occur.. The interaction between the photobiont and the fungal component lies along the parasitism to mutualism continuum, and is different for different lichens (Honegger, 1991). Lichenization may have evolved several times (Gargas et al., 1995) so the relationship between these organisms can be characterized in many different ways: as a fungal feeding strategy, a mutualism, a parasitism, an agricultural system, an algal habitat or a micro-ecosystem (Goward, 2009; Sanders, 2001). In fact, lichenized fungi interact with the photobionts in ways that are comparable to the interactions between fungi and

higher plants (Sanders, 2001). In this chapter, we will summarize research on lichen community structure and function, and on current issues in lichen conservation, including public engagement in lichenology.

This introduction discusses lichens in natural systems at many different scales, beginning with an examination of the role and development of lichen communities worldwide. The community within the lichen is discussed in the following section on the lichen microbiome. Section (3) covers and changing paradigms on allelopathy and lichens, leading to discussion in section (4) on the role of lichens in food webs, and primary productivity in lichen communities. We then turn to (5) the role of lichens in pedogenesis and nutrient cycling. Our final sections are devoted to human uses of lichens, as (6) indicators of ecosystem change and as a nexus for forest conservation, and as (7) an outreach tool to increase peoples' understanding and appreciation of the ecosystems around them. We have minimized our discussion of lichens as tools for biomonitoring, since that material is covered extensively by Wolseley et al. (2008) and others.

(1) Lichens in Ecosystems

Boreal Forests. Lichens play a major role in boreal forest ecology (Kershaw, 1978), with broadly similar trends seen in successional development of lichen communities across the circumboreal north. As in the NJ Pinelands, stand-destroying fires are a major part of the natural disturbance ecology in boreal forests, with stand succession often described as the maturing of monospecific post fire stands (Johnson et al., 1995). It is worth noting the comment of Johnson (1981) that changes in lichen

community composition as boreal forest stands age are more likely explained by the habitat requirements of individual lichens, rather than pre-determined successional sequences.

Initial lichen communities on newly burned soil surfaces in the boreal forest are typically dominated by crustaceous lichens such as *Lecidea granulosa* and *L. uliginosa* (Maikawa and Kershaw, 1976), species that can tolerate extreme soil surface microclimate conditions and are also present in the NJ Pinelands. This is followed by a successional stage dominated by cup-lichen species such as *Cladonia gracilis* and *C. cornuta*, (Johnson, 1981). Usually within 40-50 years of stand development reindeer lichens become increasingly common, including species such as *Cladonia arbuscula*, *C. rangiferina*, and *C. uncialis* (Coxson and Marsh, 2001). Depending on forest type and site microclimate, some boreal forest stands see a further transition to forest floor surfaces dominated by feather-moss mats (Coxson and Marsh, 2001; Maikawa and Kershaw, 1976). Jonsson Čabrajić et al. (2010) found that optimal terrestrial lichen growth occurring in stands with less than 60% canopy cover and predicts that in absence of re-occurring fires or stand thinning, many northern Scandinavian lichen woodlands will see declining terrestrial lichen abundance. In other cases, especially in cool maritime lichen woodlands, terrestrial lichen mats can dominate the forest floor surface well into the second century of stand development (Morneau and Payette, 1989), and similarly terrestrial lichens can still be abundant in mature forests in the NJ Pinelands.

Temperate Rainforests. Temperate and boreal rainforests represent only a small proportion of the earth's surface, less than 2% by recent estimates (DellaSala, 2011); however, they are an important repository for lichen diversity and contain many shared

floristic elements. One of the most extensive temperate rainforest ecosystems globally is found in western North America, where the composition and function of canopy lichen communities has been extensively studied (Rhoades, 1995; Sillet and Antoine, 2004). An indication of the richness of these west coast lichen communities is provided by Spribille et al. (2010), who described 766 taxa of lichens and lichenicolous fungi from the Klondike Gold Rush National Historic Park in Alaska, in an area of only 53 km^2. Disturbance regimes in cool temperate rainforests, as in the boreal, can include stand destroying events such as fire, although these historically occurred at very long time intervals. Gavin et al. (2003) found that 20% of temperate rain forest stands on western Vancouver Island had not burned in the past 6000 years, and that the mean time since the last fire ranged from 4410 years on valley-bottom terraces to 740 years on hill slopes. This long site continuity is an important factor in the accumulation of old-forest dependant species, many of which have limited dispersal abilities (Sillett et al., 2000). It also fosters the development of complex environmental gradients within stands, both vertically within the canopy (McCune, 1993), and horizontally along the branches of individual trees (Lyons et al., 2000).

Deciduous Forests. Deciduous forest biomes occur in eastern North America, Europe, and eastern Asia, as well as in southern Chile and southeastern Australia. Like wet temperate rainforests, they can support rich lichen communities. Lichen diversity in these forests tends to be highly correlated with site continuity and tree age (Fritz et al., 2008, Li et al., 2013). The extent of deciduous forests has declined dramatically in past centuries, due to agriculture, urbanization, and logging (Gustafsson et al., 1992). A further stressor on lichen communities in many deciduous forests is the effect of air

pollution exposure, both current and historic (Hawksworth and Rose, 1970.). The impact of air pollution on lichens and their use as biological monitors is dealt with elsewhere in many reviews (e.g. Nimis et al., 2002).

Tropical Forests. Tropical forest ecosystems can support high lichen species richness; however, they are poorly described compared to most temperate forest ecosystems. Current estimates suggest that there may be 4000 or more undescribed lichen species, especially on the bark and leaves of tropical primary forests (Sipman and Aptroot, 2001). Wolf (1993) found that diversity of foliose lichens increased rapidly at elevations above 1000m in the northern Andes, while crustose lichens were more uniformly distributed across all elevation zones. A major concern for lichens in tropical ecosystems is loss of habitat and deforestation. Gradstein (2008) found that lichen communities in 50 year old secondary montane forests in South America were highly impoverished compared to undisturbed ecosystems. Cáceres et al. (2000) similarly found dramatically reduced richness of foliicolous lichen species richness in forest remnants from the Atlantic rain forest in Brazil.

Alpine and Polar Lichen Communities. High tolerance of freezing, ability to rapidly resume metabolic activity when conditions allow, and low mineral nutrient demand all contribute to the success of lichens in polar and alpine environments (Kappen, 2000; Lange and Kappen 1972). Adaptations of morphology, such as mats or cushions, are often used by lichens in alpine and polar environments (as well as in the boreal) to modify boundary layer conditions. Another significant environmental variable in alpine and polar environments is the depth and persistence of winter snowpack; the changing nature of winter snowpack with climate change may be a critical factor for lichen

communities. Benedict (1990) used transplant experiments to determine that many alpine lichen species were intolerant of burial under late snowmelt patches. A greater frequency of rain and freeze-thaw events in winter, leading to encapsulation of alpine and polar lichens in ice, may be highly detrimental to cold-adapted lichen communities (Bjerke, 2011).

Grasslands and Deserts. Soil surface lichen communities are abundant in many semi-arid and arid landscapes, often as a part of biological soil crusts (biocrusts), a mixture of soil surface algae, lichens, mosses, liverworts, and cyanobacteria (Belnap, 2003). Lichens in biocrusts play an important role in stabilizing and enhancing soil properties (Belnap, 2003).

(2) The lichen microbiome

Changes to microbial communities within lichens may change many lichen properties, including potential niches, secondary compound production and susceptibility to pathogens. The non-photosynthetic organisms associated with lichens are now known to include disparate taxa of bacteria, eukaryotes, and archaea (Schneider et al., 2011). This wide variety of lichen associates should not be surprising; Grube and Berg (2009) suggest that the associations of bacteria with the lichen are as old as the lichen symbiosis itself. In this section, we discuss organisms that constitute the lichen microbiome and their function within lichens.

The bacterial groups associated with lichens are diverse and abundant. Grube et al. (2009) found 1.6×10^4 to 4.7×10^7 colony forming units of bacteria within lichens, with the soil-inhabiting *Cladonia arbuscula* hosting the highest densities. Lineages of

Alphaproteobacteria consistently represent the highest abundance and diversity of sequences (Hodkinson and Lutzoni 2009) and the Rhizobiales, an order that includes many N-fixers, is the most diverse and abundant group within this class (Bates et al., 2011; Cardinale et al., 2012). The importance of the Alphaproteobacteria in lichens should not be surprising since they are also involved in other fungal symbioses (Barbieri et al., 2005). Lichens also may include lineages of Acidobacteria, Firmicutes, Actinobacteria, Bacteriodetes, Verrumicrobia, Betaproteobacteria, Deltaproteobactiera, Gammaproteobacteria, and Chlorflexi, though abundance varies by lichen taxon (Cardinale et al., 2012; Schneider et al., 2011). Besides these bacterial groups, many non-bacterial microorganisms have been found associated with lichens (Bates et al., 2011). Schneider et al. (2011) found that the most abundant sequences in lichens could be attributed to the mycobiont and photobiont of the lichen, but many other groups were present, including green plants, animals, viruses and archea.

The nutrient-acquisition capabilities of lichen-associated microbial communities led Hodkinson et al. (2012) to suggest the microbial flora may allow the lichen to exploit more nutrient-deficient habitats. Three pieces of evidence that lichen-associated bacteria are involved in nutrient scavenging include: Grube et al. (2009) found that of the 10% of culturable lichen-associated bacteria they found, most had lytic capabilities and 23% of the strains were able to solubilize phosphate; Liba et al. (2006) also found that bacteria affiliated with cyanobacterial lichens are able to release amino acids and to solubilize phosphate; and Sigurbjörnsdóttir et al. (2014) found that the bacteria associated with lichens had the enzymatic capability to harvest phosphorus from the seawater. The endolichenic community likely has other functions besides nutrient acquisition; they

produce allelopathic compounds themselves (Gonzalez et al., 2005) and may influence secondary compound production in the lichen (Grube and Berg, 2009).

(3) Secondary compounds and allelopathy

The secondary compounds produced by lichens represent a critical juncture in the feedback between lichens and their communities. A wide array of secondary compounds (which are compounds deposited outside the fungal cell) are produced by lichens (Molnár and Farkas, 2010). Although the best studied lichen secondary compound is usnic acid (Cocchietto et al., 2002), there are countless other secondary compounds many of whose functions are less well understood. Most of the over 1050 secondary compounds found in lichens are fungal in origin and most are unique to lichenized fungi; less than 10% of these compounds are found in non-lichenized fungi or in higher plants (Elix and Stocker-Worgotter, 2008). These secondary compounds can have profound effects on the abiotic conditions the lichen can survive and on the organisms with which the lichens interact.

The biological activities of secondary compounds, reviewed by Huneck (1999), include preventing herbivory by animals, preventing bacterial and viral invasions, preventing the growth of potential competitors, and managing the interaction with the photobiont. For some lichen compounds, the location within the lichen is related to the function; for example, Gauslaa (2009) points out that UV protective compounds are all located in the cortex of the lichen, while antiherbivory compounds may be located in the medulla.

There are many studies that document the potential antiherbivory function of lichen secondary compounds. Secondary compound removal from lichens increases

palatability of lichens for snails (Asplund and Wardle, 2013) and mammals (Nybakken et al., 2010) and increases survival of moth larvae using the lichen as a food source (Pöykkö et al., 2005). As a wide variety of organisms are known to feed on lichens (reviewed by Gerson and Seward, 1977), it comes as no surprise that lichen herbivory in some areas may be so intense as to dictate lichen community composition (Asplund et al., 2010; Gauslaa, 2009) and may drive lichen evolution to produce more of these secondary compounds (Gauslaa, 2005).

Lichen secondary compounds also have antibacterial and antiviral activity. Hodkinson and Lutzoni (2009) suggest that some of the secondary compounds allow the lichen to tailor its microbial flora, preferentially selecting beneficial groups and dictating which taxa can live in the lichen. There are many studies that demonstrate antibacterial capabilities of lichens (reviewed by Shrestha and St. Clair, 2013). Antiviral activities are also well documented in lichens, as recently reviewed by Odimegwu et al. (2015).

Some lichens also have antifungal and antilichen compounds. In their review of competition between lichens Armstrong and Welsh (2007) describe that allelopathy is only one of several factors, including growth rate, senescence rate and growth form, that drive competitive interaction between lichens. Lichen extracts are able to prevent germination of fungal spores (Votintseva and Mukhin, 2004). Lichens also may decrease mycorrhizal colonization of roots below them (Sedia and Ehrenfeld, 2003).

There have been many well executed laboratory studies in which lichens or their extracts produced allelopathic effects on plants and mosses. Huneck (1999), in his review of the activity of lichen substances, presents 15 studies that demonstrate that lichen acids

inhibit growth of a variety of plants, and lichens themselves can inhibit germination of many plant taxa. In their 2002 review, Cocchietto et al. (2002) detail some examples in which the (-) enantiomer of usnic acid serves as a natural herbicide; however, studies in the field have not shown definitive allelopathy; Kytöviita and Stark (2009) found that neither fragments of *Cladonia stellaris* nor usnic acid extracts reduced germination or nitrogen uptake of pines.

There are many potential reasons for the apparent lack of lichen inhibition of germination in the field, including the heterogeneity of substrate and field conditions that influence lichen activity (Favero-Longo and Piervittori, 2010). Field weather conditions are also important - leaching of secondary compounds is negligible within an hour after rainfall (Dudley and Lechowicz 1987), so inhibition effects can be highly intermittent. Environmental factors also affect the quantity of compounds that lichens produce (Vatne et al., 2011).

(4) Primary production of lichens and lichens as foundations of food webs

When ecologists consider net primary production of ecosystems, plants are often the major generators of fixed carbon in the system, but lichens can also contribute meaningfully to carbon balances in many ecosystems. In Svalbard, Uchida et al. (2006) found that lichen primary productivity was 5.1 g dry weight $\cdot$ m^2, representing 29% of moss and 5% of vascular plant primary productivity in the study sites. The ability of lichens to utilize both water vapor (in lichens with green-algal symbionts) and liquid water (in lichens with either green- or cyanobacterial photobionts) allows growth and

reproduction in a broad range of habitats, often under conditions where metabolic activity in higher plants would not be possible (Lange et al., 1986; Lange et al., 1994).

Lichen productivity is highly variable, but can be particularly important in ecosystems facing high abiotic stress. Bohuslavová et al. (2012) documented 65 kg · ha^{-1} standing biomass for Antarctic lichen communities. In boreal forests, McMullin et al. (2011) found 1-9677 kg · ha^{-1} of terrestrial lichens, with an average value of 3124 kg · ha^{-1} . Ellis (2012), in a comparison of standing biomass in North American epiphytic lichen communities, demonstrated that many forests had over 1000 kg·ha^{-1} standing biomass, with up to 2500 kg·ha^{-1} found in wet-temperate rainforest stands in Oregon. Biomass accumulation in epiphytic forests is highly sensitive to rates of turnover, with decomposition possible both within the canopy, and after lichens fall to the forest floor surface.

Lichens represent important food sources for many taxa. Isotopic analysis of litter-dwelling collembolans revealed that algae from lichens made up an important part of their diets (Chahartaghi et al., 2005) and Erdmann et al. (2007) used similar analyses to circumscribe a feeding guild of oribatid mites that specialized on lichens. Indeed, lichen traits including nutrient status have been found to influence community structure of many invertebrate groups, including mites, springtails, and nematodes (Bockhorst et al., 2015). The importance of lichens to mammal food webs has long been understood by the people who depend on, who research, and who manage caribou and reindeer herds. In the winter, lichen represent 60-80% of the fecal matter of caribou in west-central Alberta (Thomas et al., 1994), and the caribou prefer *Cladonia* and *Bryoria* species to mosses and

shrubs (Danell et al. 1994). More detailed reviews of forage lichens are provided by Esseen and Coxson (2015) and Thompson et al. (2015).

(5) Soil formation and Biogeochemical Cycling

Even where lichens do not make important contributions to ecosystems through biomass buildup they influence the system through soil formation, soil retention, and influencing nitrogen cycling. Lichens are often portrayed as initial colonizers, or pioneers of bare rock and mineral soils, but actually succession usually begins with microbial communities including filamentous cyanobacteria, small cyanobacteria and algae (Ashley and Rushforth, 1984) and only later do lichens and mosses begin their own successional sequences (Belnap, 2003).

Once lichens and mosses have become established, they can chemically and physically transform rock substrates (Syers and Iskandar, 1973). Some mosses and lichens also appear to have complementary capabilities in weathering; in a study on gneiss, lichens produced more rapid chemical weathering of silicate minerals to clays, but mosses degraded the silicates and the clays more thoroughly (Jackson, 2015). Lichens can also contribute to retention of sandy, erosion-prone soils (Abed et al., 2013), with lichen rhizomorphs serving as soil-anchoring structures in biological soil crusts (Belnap et al., 2003).

An important contribution of lichens in ecosystems, especially cyanolichens, is their role in nitrogen fixation. Sollins et al. (1980) measured 2.8 kg kg $N \cdot ha^{-1} \cdot yr^{-1}$ nitrogen fixation by epiphytic cyanolichens in old-growth Douglas Fir forest, with lichens leaching 2.1 kg $N \cdot ha^{-1} \cdot yr^{-1}$, comparable to atmospheric deposition at that site (2 kg N ·

$ha^{-1} \cdot yr^{-1}$). Similarly, Forman (1975) found high levels of N fixation in Columbian rain forests, from 1.5-8 kg $N \cdot ha^{-1} \cdot yr^{-1}$. However, other studies have found much lower N fixation rates by lichens (Cusack et al., 2009; Kurina and Vitousek 2001). In several cases, the N fixation of cyanobacterial lichens (reviewed by Ellis et al., 2012) has translated to increases in N-availability in the soils around the lichens.

Even green algal lichens can intercept airborne N and slowly release it during the decomposition of the lichen thalli, increasing the available N in the soils (Knops et al., 1996). The presence of mature soil crusts, including lichens, may prevent the spaces in between desert plants from becoming depauperate in soil nutrients, organic matter, or soil animals (Housman et al., 2007). Other studies have shown that lichens have the opposite effect on N-availability in soils: epiphytic lichens can absorb ammonium and nitrate from rainwater, depleting the throughfall of N and decreasing N availability in the throughfall that reaches the ground (Lang et al., 1976; Reiners & Olson, 1984).

(6) Conservation and Climate change

With the advent of the 6th major planetary extinction, there have recently been high levels of interest in biodiversity conservation. As habitat loss is generally considered the greatest threat to lichen conservation, lichen conservation efforts should focus on habitat quality, connectivity, and patch size (Scheidegger and Werth, 2009). However, habitat quality for lichens is different than for other groups; alarmingly, Lendemer and Allen (2014) found that patterns in lichen diversity do not mirror patterns in diversity of other groups usually used for conservation prioritization (birds, mammals, and vascular plants), and lichens and other biodiverse but understudied groups (including bacteria,

microinvertebrates, fungi, and mosses) are often not considered explicitly in conservation assessments. Further, lichens may be more threatened than other taxa; Lendemer and Allen (2014) found that the sites in the Mid-Atlantic coastal plain of the United States with the highest lichen diversity were concentrated in the lowest elevation areas, areas that climate models predict to be inundated even in the most conservative sea level rise scenarios. An increased focus on patterns in lichen threat and biodiversity is therefore urgently needed.

Lichen conservation measures are necessary because lichen cover and diversity has decreased significantly and consistently when climatic changes have been combined with other human-induced ecosystem changes. A large range of responses lichens to climate warming alone has been found: some studies find decreases in lichen species richness (Lang et al., 2012) and cover (Cornelissen et al., 2001; Walker et al., 2006) others found increases in lichen cover (Biasi et al., 2008), others find no difference in lichen cover or richness (Alatalo et al., 2014), and some studies also identify particular taxa that are vulnerable to climate change (Song et al., 2012). However, the combination of climate change with other human-induced ecosystem changes is universally detrimental. For example, the spread of invasive insects (Ellis et al., 2014) led to most notable epiphyte declines in species poor, dry forests of Great Britain; richer, moister forests had more resilient epiphyte communities. The disappearance of sensitive cyanolichen species from some forests in the Pacific Northwest of the U.S. was strongly associated with the combined effects of climatic variables and pollution (Geiser and Neitlich, 2007). Increased nutrient inputs combined with warming treatments led to dramatic decreases in lichen abundance in northern Sweden (Jägerbrand et al., 2009), and

a changing climate along with increased herbivory (Klein and Shulski, 2011) led to such losses of lichen cover in Arctic Canada that reintroduction was not viable. Ellis & Coppins (2007) found that lichen diversity in Scotland is influenced jointly by land use variables and climate variables, so future models of climate effects on lichen communities should include both types of variables.

Much conservation-related work on lichens involves preservation of old growth forests as lichen habitat since the oldest forests often harbor the highest lichen diversity (Marmor et al. 2011; Nascimbene et al. 2010), but new evidence is emerging that not only preservation of old trees, but also the preservation of tree diversity is important for maintenance of epiphytic diversity (Ellis et al. 2014). Management of forests with substantial human impacts can still serve as important resources for forest biodiversity; for example logging without herbicides (McMullin et al., 2013) and logging in winter (Larsson et al., 2014) can both be ways of maintaining higher epiphyte diversity in logged forests. Nascimbene et al. (2014) suggest that preservation of many different habitat types, including the grazed larch forest, a habitat type maintained by traditional farming techniques, is important to maintaining the lichen diversity in Italy. Comparably, (Li et al., 2013) found that 3 types of secondary forests in China contained equivalent species richness to an old growth forest, and also harbored unique communities, so secondary AND primary forests should both be important in conservation agendas there. Esseen and Coxson (2015) review how alternative forest management practices can be used to maintain lichen biodiversity and minimize negative impacts from factors such as edge effects in managed forests.

Lichen conservation can also benefit from new technology. An important advance in lichen habitat mapping has been the ability to remotely sense lichens as described by Nelson et al. (2013); hotspots of lichen cover changes can be identified and conservation measures may be more timely and more effective. Although not a long-term solution, lichen transplantation, a technique used on many occasions to monitor air pollution (Kularatne and De Freitas, 2013; Odiwe et al., 2014), has in the short term been shown to be viable method for transporting populations out of threatened areas for fruiticose lichens (Zarabska-Bożejewicz et al., 2015; Lidén et al., 2004). Allen and Lendemer (2015) point out that one of the keys to advancing a fungal conservation agenda is through building widespread knowledge of fungi in our society; one way to accomplishing this is through outreach and education programs.

(7) Outreach and lichens

Lichens have long been used as indicators of environmental change (Hawksworth & Rose, 1970; Nimis et al., 2002), and as rates of environmental change are increasing, it is important to build a wider network of people who have the expertise and interest in carrying out local monitoring projects. Brodo (2000) suggested that as professional lichenologists focus more on understanding molecular biology of lichens, publication of local floras is done more and more by agencies, museums and amateurs. So public groups dedicated to lichenology are becoming more important for building motivation for and establishing mentorship networks for these necessary lichen related projects. Some strategies for building the wider network of lichenology enthusiasts this include institution of citizen science programs, incorporating lichens into general nature-

appreciation programs, and increasing the number of lichen-related education in schools; we discuss examples of each of these strategies below.

Citizen science, which aims to improve public understanding of environmental problems, engages members of the public in science programs. Tregidgo et al. (2013) described a citizen science program in England involving 650,000 participants and found that a simplified monitoring program was effective for detecting large changes in air pollution. Another study provided estimates of lichen biodiversity from datasets collected by 20 citizen science photographers in Washington D.C; the species estimates generated from these data were comparable to species counts from a reference dataset generated by students (Casanovas et al. 2014).

Lichens have also been included in outreach programs geared less toward generating scientific results and more toward increasing awareness and appreciation for lichens. Lichens are cataloged by general natural enthusiasts on the website iNaturalist, on botanical foray reports (Moore, 2004), and in organized bioblitzes. Bioblitzes (which are short term, intensive surveys of an area involving taxonomists from as many disciplines as possible, and citizen volunteers) have engaged general nature enthusiasts in lichenology at US National Park Service's Yellowstone National Park, Sandy Hook National Recreation Area and The Great Smoky Mountains National Park (Keller et al. 2007). These activities indicate that there is interest in lichenology among the general community of botany enthusiasts.

Teachers at all levels of education have found ways to integrate the study of lichens into their curricula. At the undergraduate level, lichen inventories can be included as part of botany classwork and as independent projects (Struwe et al., 2014). At the

middle school levels, lichen biomonitoring projects can be used to teach hypothesis testing, collecting and analyzing data, and drawing conclusions (Smith and Baker, 2003). The British Lichen Society in collaboration with the Association for Science Education, for instance, developed a successful program for primary school students on lichens in churchyards, in which they covered the concepts of habitats and scientific names (Oldershaw, 2010).

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2 Soil Lichen Community Composition and Disturbance in the NJ Pinelands Natural Reserve

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Abstract.

Among the many unusual features of the NJ Pinelands Natural Reserve is the diversity and abundance of its lichens. Throughout the reserve there are areas in which lichens form extensive mats on the sandy soils. In this study, we describe the different land uses and fire histories of 5 such sites across the NJ Pinelands and compare the lichen communities and the soil processes taking place at each site. Our hypothesis was that lichen mats with more local disturbance would be significantly different structurally and functionally than lichens from less disturbed habitats. We conducted lichen community surveys at each site and measured soil properties including soil moisture, soil organic matter, soil nutrient availability, and soil micro-arthropod abundance. We found that the lichen communities and the soils below them were similar across different land use histories, with the exception of the Manumuskin River Preserve and Wharton State Forest. Both of these sites had higher soil moisture content than the other sites and the Manumuskin River Preserve site had more soil organic matter than the other sites. The Manumuskin River Preserve also had significantly higher predatory mite and collembolan densities than the other sites. This is important work because it identifies an unusual lichen community at the Manumuskin River Preserve and these findings

demonstrate that lichen communities in the New Jersey Pinelands can be functionally robust in areas with historic disturbances.

Keywords: lichen ecology, *Cladonia*, soil arthropods, Pinelands National Reserve, Manumuskin River Preserve, Wharton State Forest, Makepeace Lake, Crossley Preserve.

Introduction:

The lichen flora of New Jersey is diverse and abundant; Lendemer (2006) found 190 species of lichens and lichenicolous fungi in Wharton State Forest in NJ. Wright et al. (2005) found that in some areas of the NJ Pinelands, the cryptogams (mosses and lichens) on the forest floor constituted a fuel reserve of 1.05 tons/acre, slightly more than the conifer litter (1.02 tons per acre), and representing 43% of the surface material on the forest floor. The diversity of the lichen flora of NJ is low compared to the diversity of exceptional areas in European and North American National Parks (reviewed by Spribille et al. 2010). However, the persistence of diverse lichen communities in NJ is noteworthy because of the high human population density there. Lichen cover and diversity has repeatedly been found to inversely correlate with population density (Brodo, 1968) and with industrial air pollution (reviewed by Conti & Cecchetti, 2001). New Jersey is the most densely populated state in the nation (2010 U.S. Census), and many areas have legacies of industrial air pollution, but in the Pinelands National Reserve, which has low human population densities, lichen proliferate.

Much of the lichenological work previously done on the Pine Barrens of New Jersey (Evans, 1935; Forman, 1979; Lendemer 2006) has been accomplished through targeted inventories aimed at capturing the species richness of the area. This work is

critically important in determining biodiversity hotspots and areas of conservation importance. However, we are most interested in how lichens contribute to ecosystem function (in particular nutrient cycling and maintenance of soil biodiversity) so our aim is to quantify how lichen communities are structured, and which species are most dominant in their communities. This study describes how the lichen community structure differs in the extensive lichen mats that ground dwelling lichens form across the soil across the Pine Barrens.

Aboveground community structure of lichens can be important to soil function. For example; Bowker et al. (2011) found that different lichen species in a biological soil crust have different effects on carbon cycling and phosphorus cycling in the soils below them, so that shifts in dominance of individual lichens in the soil crust have implications for ecosystem function. Studies that have investigated the ecological role of the New Jersey Pine Barrens soil lichen mats found the lichens to be important in water retention (Bernard, 1963) and soil nutrient cycling (Sedia and Ehrenfeld 2005). With different species making different functional contributions, the community structure may be important to function of the lichen mats, and mats with different communities may have different effects on the ground below them. With this in mind, we sought to characterize the communities in different lichen mats across the pinelands and measure various soil properties in the soils below the lichen mats to see whether distinctive lichen mats were present in areas with distinctive soil properties.

Aboveground community structure of plants has been shown to influence below-ground community structure, and thereby have indirect effects on soil function. Many studies have demonstrated ways in which resource complexity of plant material

aboveground leads to soil community diversity (reviewed in Wardle et al., 2004) In general, primary consumers (including fungi, bacteria and plant feeders) seem to be more responsive to changes in plant diversity and identity than secondary (microbe-feeders) and tertiary consumers (DeDeyn et al., 2004). These influences of plant diversity on microbial and protozoan diversity and abundance are likely mediated by changes in living root biomass, root exudates and/or litter chemistry (Scherber et al., 2010).

Because of these effects of plant chemistry on soil biological activity, we might expect lichens to also have important effects on soil communities. Though they do not produce roots, lichens are very chemically active and manufacture an array of secondary compounds that may serve in UV protection, herbivore deterrence, or antimicrobial functions (reviewed by Huneck 1999). The presence of aboveground lichens and the chemical compounds they produce could therefore strongly influence the primary consumer populations in soil animal communities; some oribatid mites (Materna, 2000; Chahartaghi et al., 2005; Behan-Pelletier et al., 2008) and some collembolans (Leinaas and Fjellberg, 1985) have been found to be strongly affiliated with lichens. So, in quantifying the structure of the different lichen communities, we also investigated the structure of the corresponding belowground soil animal communities in order to see if changes in lichen community structure represented changes in other belowground properties (soil animal diversity and soil chemistry).

MATERIALS AND METHODS

Study Area

Each of the 5 sites we chose had patches of extensive vegetation cover, but, they all represented different land use histories. Two of the sites where the lichen patches existed

we have characterized as historically disturbed with continuing local disturbance the Crossley Preserve site (powerline right of way with active unpaved road) and Makepeace Lake Wildlife Management Area site (former sand road with active unpaved road adjacent to it). Three of the sites were relatively undisturbed, the Warren Grove FAA tower site (lichens were growing near, but not in, the area of land disturbance for infrastructure construction) Batsto and Manumuskin River Preserve. They were all upland forest sites, but the Manusmuskin River preserve and the Makepeace Lake Sites were close to major bodies of water. The 5 sites we chose also represent a gradient of nitrogen deposition (increasing towards the south; (Dighton et al. 2004)), and if our data had shown trends along a north-south gradient, such trends might have been driven by nutrient availability. The five sites are displayed in Figure 1.

The northernmost site, the Crossley Preserve, is in Tom's River, Ocean County, NJ (at lat: 39.952414, long: -74.286287), 40 m south of Westbrook Dr. in the Holiday City development. The Crossley Preserve was the former site of a clay mining town, then a right of way for the Pennsylvania Railroad, and is now a powerline corridor. The area is protected because of the presence of the federally endangered Knieskiern's beaked rush (*Rhynchospora knieskiernii*), the state endangered Pickering's morning glory (*Stylisma pickeringii*), and the Pine Barrens tree frog (*Hyla andersonii*). (Natural Lands Trust, 2004). The lichen covered areas occur between the power line clearings and the treeline, (mostly *Pinus echinata*). Several lichen patches occur in the area, in long narrow strips, approximately 200m x 10m in extent. The lichens are growing intermingled with perennial grasses, sedges, and forbs. Since the area we studied is immediately adjacent to a residential area, it has not been subject to documented wildfires or prescribed burns

since record keeping began in 1924 (Forest Fire Service, 2011) The soils there are Lakewood Sand, which are sandy and excessively drained, with a pH of 3.6-4.4 (USDA Soil Survey, 2015).

The site further south was at the Warren Grove FAA Tower in Ocean County (at lat: 39.753082, long:-74.388776), 80m southwest of the junction of Beaver Dam Rd. and Radio Tower Rd. The lichens in an approximately 200m x 300m are visible in satellite imagery, as the site has an open canopy, and lichens are growing in and around several small shrubs including beach heather (*Hudsonia ericoides*), bearberry (*Arctostaphylos uva*-ursi), huckleberry *(Gaylusaccia baccata*) and the state endangered broom crowberry (*Corema conradii*). The area had a spring wildfire in 1936 (NJ Forest Fire Service, 2011), and the construction of Federal Aviation Administration tower represents a nearby construction disturbance. Additionally the area was cleared and disked in 1940, then cleared again in 1961 (Levin 1966). The soils there are characterized as Woodmansie Sand, which is a well-drained, sandy soil with pH 3.6-4.4 (USDA Soil Survey, 2015).

The 3rd site, near Batsto Village, is about 350 m N of the Batsto-Pleasant Mills United Methodist Church on Pleasant Mills Rd. (lat: 39.644100, long: -74.660965) in Mullica, Ocean County NJ. Lichen patches occur on both sides of the sand road, but the extensive lichen patch we studied was approximately 150m x 75m in extent, and the lichens were growing intermingled with greenbriar vines (*Smilax glauca*), and *Carex sp*. Two rivers run nearby, the Nescochague Creek and Mullica River, and there is extensive blueberry production downstream near Hammonton NJ, but the part of the watershed with the study site is relatively undisturbed. The Wharton State Forest, in which the site lies, is an 110,000 acre (44,515 Ha) parcel operated by the NJ State Parks Department since 1954.

The village nearby was used for bog iron extraction and glassmaking, and the land was bought by industrialist Joseph Wharton in 1876 for its groundwater resources. The area has not had a fire since before 1925 (NJ Forest Fire Service, 2011). The soils are moderately well drained Lakehurst Sand, a well-drained, sandy soil with a pH 3.6-4.3 (USDA Soil Survey, 2015).

Makepeace Lake Wildlife Management Area is an over 10,000-acre parcel now managed by the NJ Department of Environmental Protection. The 300-acre Makepeace Lake was formed by damming and flooding the old Bozarth cranberry bogs in the 1930s. Now the area includes many different habitat types, but these lichen patches were growing extensively in disused roadcuts (lat: 39.537486, long:-74.747578), 1km east of the intersection of Egg Harbor City Rd and Elwood Rd, in Hamilton Township, Atlantic County, NJ. The canopy cover in this site was *Quercus sp*. and *Pinus sp*. There were a fires here in the summer of 1977 and in the spring of 1925 (NJ Forest Fire Service, 2011). The soils, like those at Batso, are Lakehurst Sands (USDA Soil Survey, 2015).

The Manumuskin River Preserve is a 3500-acre preserve in Atlantic County New Jersey, and our samples were collected at the N end of Barth Rd., Millville (at lat: 39.332115, long: -74.973186). The preserve was established in 1983 to protect the federally threatened sensitive joint-vetch, *Aeschynomene virginica*. The preserve also hosts other rare plants, animals, and plant communities, and the lichen carpet there extends to within 20m of the Manumuskin River, a mostly undisturbed river with few residential homes alongside it. There is no recorded history of fire at this site since before 1925 (NJ Forest Fire Service, 2011). The canopy at the site is dominated by pitch pine, and the soils are excessively drained Evesboro Sands, pH 4.6-5.2 (USDA Soil survey,

2015). The Manumuskin River Preserve is part of the Atlantic Coastal Plain physiogeographic province; its parent material may be different in important ways from the other sites, even though all are well drained, sandy soils with low pH.

Field Sampling:

Soil community characterization.

Two of the most commonly used in lichen community assessments are quadrat and point-intercept methods (Rosentreter & Eldridge, 2002). Point-intercept methods can be less subjective than % cover estimates in a quadrat, but generally estimation of cover in a quadrat captures more species than the point-intercept method (McCune and Lesica, 1992). In recent literature on lichen communities, researchers have used quadrat areas from 4.2 cm^2 (Eldridge at al. 2000) to sample microlichens to 3782 m^2 (Root, McGee, and Nyland 2007) to monitor lichen community response to landscape-scale gradients, although most studies use quadrats of intermediate size. Since the terrestrial lichen flora of the NJ Pinelands are dominated by members of the Cladoniaceae, which are macrolichens, we concluded that using an area quadrat (1m) would be more appropriate to link community composition to other spatially related information on soil chemistry and fauna.

We created a 50m transect through two different lichen patches at each site. We divided each transect into 5 10-meter sections, and in each section randomly chose a meter number and a side of transect to sample from, using a $1m^2$ quadrat, giving 5 replicate samples per lichen patch. Since members of the Cladoniaceae are identified primarily through the stalks (podetia) that may be capped with fruiting bodies, or have distinctive branching patterns, we identified most of the specimens in the field. The cup

lichens that we were unable to distinguish in the field, we labeled *Cladonia chlorophaea* group in our analyses; this included: *C. chlorophaea, C. cryptochlorophaea,* and *C. grayii.* We also did not distinguish between various types of red-apotheciate sorediate lichens without cups and without usnic acid: these were designated *C. macilenta* group and included *C. macilenta, C. didyma,* and *C. floerkeana.* We followed the nomenclature provided by Esslinger (2015), followed species concepts as described by Brodo et al. (2001) and collected specimens as vouchers which are stored in the Chrysler Herbarium at Rutgers University (CHRB).

In the lichen sampling, we did not include lichens that were found in only one plot, operating on the assumption that the most functionally important members of the community would be the most abundant ones. However, Jain et al (2014) point out that rare species can be important for several reasons: (1) they may increase in abundance with environmental changes (as demonstrated by Esteban et al. 2015) (2) any functional redundancy they provide may become more important in conditions of community stress, and contribute to system resilience (3) they may provide unique functions. For example, Youngster et al. (2010) were able to characterize slow growing microbes that poses the capabilities to degrade MTBE, an environmentally important pollutant, and Musat et al (2008) found that the least abundant organism in an oligotrophic lake microbial community was responsible for 70% of the system carbon uptake and 30% of the nitrogen uptake there. However, as Gaston (2011) describes, more common species are more likely to be influential in system-wide energy balances, food webs, and ecosystem engineers. So we chose to focus on the more abundant species, which admittedly reduced the value of our study as a biodiversity inventory, but we contend that our method

suitably captures the key aspects of community structure at these sites.

Soil chemistry and environmental variables:

Soil moisture, loss on ignition (LOI), available soil nitrogen, and available phosphorus, in the 5cm below the lichens were determined from 5cm diameter soil cores to a depth of 5 cm, with 4 replicates per site. For the soil moisture and LOI measurements, we used protocols described by Roberston et al, (1999). Soil samples were homogenized and stored in airtight plastic bags. To calculate percent moisture and loss on ignition, after weighing a field moist subsample of about 5g, we oven dried the subsample at 70°C to determine the moisture content, then put a dried subsample in a crucible to heat in the muffle furnace at 550°C for 2 hours, after which we recorded the ash-free dry weight. We sampled soil chemistry as described by Dighton et al. (2004). Briefly, to determine soil ammonium and nitrate content, 10g of a homogenized field moist sample was incubated with KCl and shaken for 1 hour, the filtrate was collected from a Whatman 42 filter, and the filtrate was frozen for storage and subsequently analyzed in an Astoria pacific autoanalyzer for NH_4+ and NO_3-. For soil phosphorus determinations, soils were also extracted as described by Gray and Dighton (2009). We used 5g of a field moist, homogenized sample incubated with, Bray extracting solution (Bray and Kurtz, 1945), shook the solution for 1 hour at 25° C and filtered using a Whatman 42 filter, and the solution was stored frozen before conducting a colorimetric analysis using the ascorbic acid method. We also measured lichen mat height of six samples by choosing the closest *C. subtenuis* specimens to the 50 m point of the transect in each quadrat (N, E, S, W) and the closest *C. uncialis* to the 25m point and the 0m

point.

Soil Animals:

Soil invertebrates were collected from 5cm (diameter) soil cores, in 3 cores per site, and the animals were collected by dynamic extraction from soil into a solution of 70% ethanol and 5% gycerol using a Tullgren extractor. The organisms were identified to morphogroups, as described in Dighton et al. (2012). For the analysis, we divided these into 4 major groups; oribatid mites (including adults and juveniles), predatory mites (prostigmatid and mesostigmatid mites), collembolans, and other organisms (including ants, spiders, pseudoscorpions, and insect larvae). Animal abundance in the sample was converted to animal density per m^2 of soil in the top 5cm, as density changes in soil animal communities more closely follow changes in soil function than species richness values do (Nahmani and Lavelle 2002).

Data analysis:

We analyzed the data on the lichen communities using non-metric multidimensional scaling analysis and canonical correspondence analysis in the Vegan program in R (Oksanen et al., 2013). We compared the environmental variables and soil arthropod communities at each using multivariate analysis of variance, also in R. Our stepwise multiple regression was conducted using PHREG process in the SAS program.

RESULTS:

In the sites that we studied, the most commonly occurring lichens were *Cladonia uncialis* and *Cladonia subtenuis* (Table 1). *C. uncialis* was the most common lichen at the Crossley Preserve, FAA Tower, and Makepeace Lake sites, *C. subtenuis,* was the most common lichen at Batsto (although it was an important component of the lichen

community at every site). *C. submitis* was the most common lichen at Manumuskin River Preserve, but it was not present at any other site, and C. submitis was mostly responsible for the lichen community differences between this site and the others (Figure 2). *Placynthiella sp*. were important only where the total lichen cover was lower, they represented the second and third most common lichen taxa at Crossley Preserve and Makepeace lake, respectively. *C. dimorphoclada* was present only at these two sites. *C. chlorophaea* and *C. macilenta,* were present in low levels at every site.

The three relatively undisturbed sites (Batsto, FAA, Manumuskin) had higher average lichen cover, and higher tree and shrub cover than the two more disturbed sites (Crossley, Makepeace; Table 1 and Figure 3).

The Batsto and Manumuskin River Preserve communities were distinctly different from the communities at the other sites (Figure 2). Analysis of variance of site scores on the NMDS axes revealed that there were significant differences between the lichen communities along both axis (NMDS Axis 1: F-value = 16.18 $p < 2.79 \times 10^{-08}$; NMDS Axis 2: F-value = 14.333, $p < 1.276 \times 10^{-07}$). On Axis 1, Batsto was significantly different from all the other sites, and FAA and Manumuskin were significantly different from Crossley and Makepeace. Along Axis 2, Manumuskin was significantly different from all the other sites. *C. submitis* was present only at Manumuskin, and represented a large proportion of the cover there. *C. fimbriata* was also present in this community and absent elsewhere. Corticolous lichens, *C. rangiferina* and *C. rappii* also made up much more cover here than at any of the other site (Table 1). The lichen community at Batsto was also distinct. It had less *C. uncialis* and more *C. submitis* cover than the other sites.

The Manumuskin River Preserve and Batsto had significantly higher percent moisture values than the other sites (Table 2 and Figure 4). Lichen mat height was significantly higher at Batsto and soil available phosphorus was higher at Manumuskin River preserve than at the other sites we studied. Soil nitrate was higher at the FAA and Batsto sites, but these levels were very low, and there was no significant difference between the levels of available ammonium.

There were significantly more collembolans and predatory mites under the lichens at Manumuskin Rivers Preserve (Figure 5). Oribatid mites, which was the most abundant group, and other organisms, which was the least abundant group, did not show site-specific changes in density.

DISCUSSION

Results from Figure 2 suggest that the the Manumuskin River Preserve and the Batsto sites have distinct lichen communities from the lichen communities present at the other sites. This is likely driven by the importance of *C. submitis* at Manumuskin and by the decreased presence of *C. uncialis* and increased importance of *C. subtenuis* in the community at Batsto (Table 1). Since there was no significant difference between lichen communities at other sites regardless of historic soil disturbance or fire history, we might conclude that the lichen communities are robust to historic disturbances. These disturbances at each of these 3 sites though (Makepeace Lake, Brendan Byrne and Warren Grove) were distant in time; in areas with more recent disturbance, including frequently used roads or vehicle tracks, there were no lichens growing, so a lichen community characterization would not have been possible. A valuable research question

to follow this one could be: what intensity of disturbance can the lichen mats survive on the sandy soils of the NJ pinelands?

Fire history at these sites did not appear to influence the lichen mat community as much as we originally assumed. The Makepeace Lake site was the only one with a fire in the last 50 years (1977), and it was not distinct from the other sites studied. Johnson (1981) found that in the terrestrial lichen communities he studied in the Northwest Territories, the lichen community was more strongly influenced by habitat conditions such as forest type, substrate, topographic position, and aspect, than by fire interval. In his study, most species found in older forest stands were already present in the first years after fires, and species abundance was better explained by habitat requirements rather than by position along a successional trajectory. Ground lichens were found in all parts of the fire gradient, from *Cladonia cornuta* and *Cladonia coccifera*, which disperse by soredia, at the earliest stages post fire, to *Cladonia rangiferina* and *Cladonia mitis*, which reproduce by fragmentation and colonize later. In Boudreault's (2002) study in Ontario and Quebec also found no significant lichen communities in forests between 80-200 years old. Our data makes sense in this context since we found few differences in community composition between sites with different disturbance regimes.

However, a more recent study investigating fire influence on lichen communities in Quebec over a longer time scale, from 43-355 years, found that lichen abundance and species composition responded more strongly to time since fire than to any habitat variable (Zouaoui, 2014). The disturbance gradient in our study was small; since we chose sites with obvious and robust lichen mats, any sites with low cover due to recent and intense disturbance would not have been included in our study, and since

anthropogenic influence in the NJ Pinelands has been long and thorough, even our less disturbed sites were not undisturbed. Fires still may be important for clearing vegetation to enable the lichen proliferation initially.

Lichen mats were present in some areas with high percent cover of vascular plants. Lichen mats were associated with a wide range of canopy coverage (60% at Manumuskin River Preserve to 1% at warren grove), suggesting that the forest openings that lichen mats are often associated with in the NJ pinelands (Forman, 1979), may actually have considerable canopy coverage. Lichen mats were also present in areas with robust shrub cover (1-57% cover). In the area with the highest cover of shrubs, the Warren Grove FAA tower, lichens were growing directly underneath the shrub cover, mostly *Corema conradii* (41.7%), *Hudsonia erecoides*(11.5%), and *Arctostaphylos uva-ursi* (2.9%). In contrast, there was relatively low range of herbaceous plant cover (1% at the Warren Grove FAA tower and Manumuskin River Preserve to 12% at Crossley Preserve); lichen cover and lichen species richness has been found to be negatively correlated to the cover of vascular plants in some grass-dominated areas (Barger et al. 2006). Our Canonical Correspondence Analysis (Figure 3) visually represents those trends, with lichen cover being negatively associated with herbaceous plant and moss cover, but not with tree or shrub cover.

The presence of roads used by vehicles within 20m of the lichen mats at the Crossley Preserve, the Makepeace lake Wildlife Management Area, and the Manumuskin River Preserve did not lead to their having significantly different lichen communities from the lichen communities at Batsto and at Warren Grove, with no directly adjacent used road. However, we did not have data on the frequency of road use, or the frequency

of human visitation at each of these sites. Direct trampling has a huge influence on the structure and function of desert lichen mats (Barger et al. 2006), so perhaps the presence of an adjacent road is less important than use of the site exactly where the lichen is growing.

Natural Land Trust's Manumuskin River Preserve has lichen mats that provide distinct habitats compared with lichen mats from those in the rest of NJ. They have different lichens, the soils have more organic matter and support more predatory mites and collembolans. Both this site and the Batsto site were distinct from the others in their soil moisture and their proximity to major waterways. The climatic conditions surrounding these waterways might lead to different air moisture conditions. We suggest that the lichens and their associated belowground microfauna represent further good reasons to maintain protections on the Manumuskin River Preserve site.

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Table 1: Vegetation and lichen communities present at each site. Results are presented as mean and standard deviation of % cover of vascular plants, lichens and mosses in 10 quadrats at each site.

	Crossley		FAA Tower		Batsto		Makepeace		Manumuskin	
	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev	Mean	St dev
Vegetation										
Arctostaphylos uva ursi (L.) Spreng.	0.02	0.06	2.9	4.48	0	0	0	0	0	0
Artimesia spp.	0.09	0.28	0	0	0	0	0	0	0	0
Atrichum sp.	0.2	0.38	0	0	0.68	1.56	0.91	1.91	0	0
Carex sp.	1.85	3.28	0	0	4.55	5.67	1.57	3.04	0.65	0.78
Comptonia peregrina (L.) J.M. Coult.	1	3.16	0	0	0	0	0	0	0	0
Corema conradii (Torr.) Torr. ex Loudon	0	0	41.7	33.42	0	0	0	0	0	0
Gaylusaccia baccata (Wangenh.) K. Koch	0.01	0.03	1.3	2.83	1.3	3.2	0	0	0	0
Hudsonia ericoides L.	0.75	1.87	11.5	25.61	0.02	0.05	0.66	1.41	0	0
Juncus sp.	0.01	0.03	0	0	0	0	0	0	0	0
Leucobryum sp.	0	0	0	0	1.86	2.44	0	0	3.6	4
Pinus spp.	21.45	39.58	1	3.16	32	22.51	9	22.34	61.62	32.44
Poaceae	9.43	16.58	0.72	2.21	0.9	1.91	0	0	0.01	0.02
Polytrichum sp.	9.11	8.11	12.63	20.09	0.49	1.26	2.92	5.27	0.01	0.02
Pteridium aquilinum (L.) Kuhn	0	0	0	0	0.1	0.32	0	0	0	0
Quercus sp.	0.03	0.08	0.01	0.03	0	0	22.55	41.55	0	0
Smilax spp.	0.08	0.17	0	0	6.18	7.5	0.46	0.95	0.46	1.26
Lichens										
Cladonia atlantica A. Evans	0.12	0.28	2.89	6.23	1.03	2.36	1.79	4.51	1.16	1.81
Cladonia chlorophaea gr.	2.52	5.02	1.72	1.94	0.04	0.08	0.04	0.09	0.3	0.5
Cladonia cristatella Tuck.	0.01	0.01	0	0	0.55	1.57	0	0	0	0.01
Cladonia dimorphoclada Robbins	0.1	0.32	0	0	0	0	0.22	0.63	0	0
Cladonia macilenta gr.	0.37	0.7	0.11	0.31	0.13	0.41	0.13	0.21	0.03	0.04
Cladonia rangiferina (L.) F. H. Wigg.	0	0	5	15.81	0	0	0	0	0.01	0.02
Cladonia rappii A. Evans	0.05	0.14	1.07	3.14	0.01	0.03	0	0	0	0
Cladonia submitis A. Evans	0	0	0	0	0	0	0	0	35.2	27.41
Cladonia subtenuis (Abbayes) Mattick	0.43	0.67	9.98	17.76	38.7	14.75	2.23	3.34	9.85	17.83
Cladonia uncialis (L.) Weber ex F. H. Wigg.	12.49	16.36	35.6	33.95	0.9	1.13	9.32	8.3	2.48	4.82
Placynthiella sp.	0.47	0.75	0.14	0.32	0	0	2.3	6.29	0	0
Corticolous lichens	0	0	1.1	3.14	0.04	0.11	0	0	0.06	0.08
Summary statistics										
Lichen total	16.56	15.04	57.61	31.77	41.39	14.68	16.01	12.4	49.08	21
moss	9.31	8.14	12.63	20.09	3.03	2.44	3.83	5.06	3.61	4
shrub/vine	0.86	1.83	57.4	33.61	7.5	7.78	1.12	1.52	0.46	1.26
grass/forb	12.38	17.43	0.72	2.21	5.55	5.8	1.57	3.04	0.66	0.79
tree	21.48	39.64	1.01	3.16	32	22.51	31.55	47.11	61.62	32.44
Total cover	60.58	40.45	129.4	33.45	89.46	26.46	54.08	41.14	115.4	33.97

Table 2. Chemical properties of soil at each site. Results are presented as mean and standard deviation of measurement.

	Crossley		FAA		Batsto		Makepeace		Manumuskin	
	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
% moisture	7.37	0.88	10.13	2.77	17.4	5.65	10.13	3.79	20.78	6.08
% loss on ignition	4.62	1.2	5.08	0.64	6.46	1.29	5.43	1.9	10.46	5.11
NH_4-N (ug/g dry soil)	1.1	0.43	1.24	0.52	1.9	0.56	1.79	0.73	2.89	1.48
NO_3/NO_2 (ug/g dry soil)	0.34	0.02	0.51	0.09	0.51	0.02	0.38	0.03	0.41	0.1
PO_4-P (ug/g dry soil)	2.57	0.39	3.07	1.64	2.78	0.82	1.54	1.06	4.5	1.27
height of C. subtenuis (cm)	3.43	1.58	3.18	0.33	7.83	1.28	3.68	1.52	4.53	0.71

Figure 1. Five sites involved in the study, from north to south: Crossley Preserve, FAA tower at Warren Grove, Pleasant Mills church at Batsto, Makepeace Lake Wildlife Management Area, Manumuskin River Preserve.

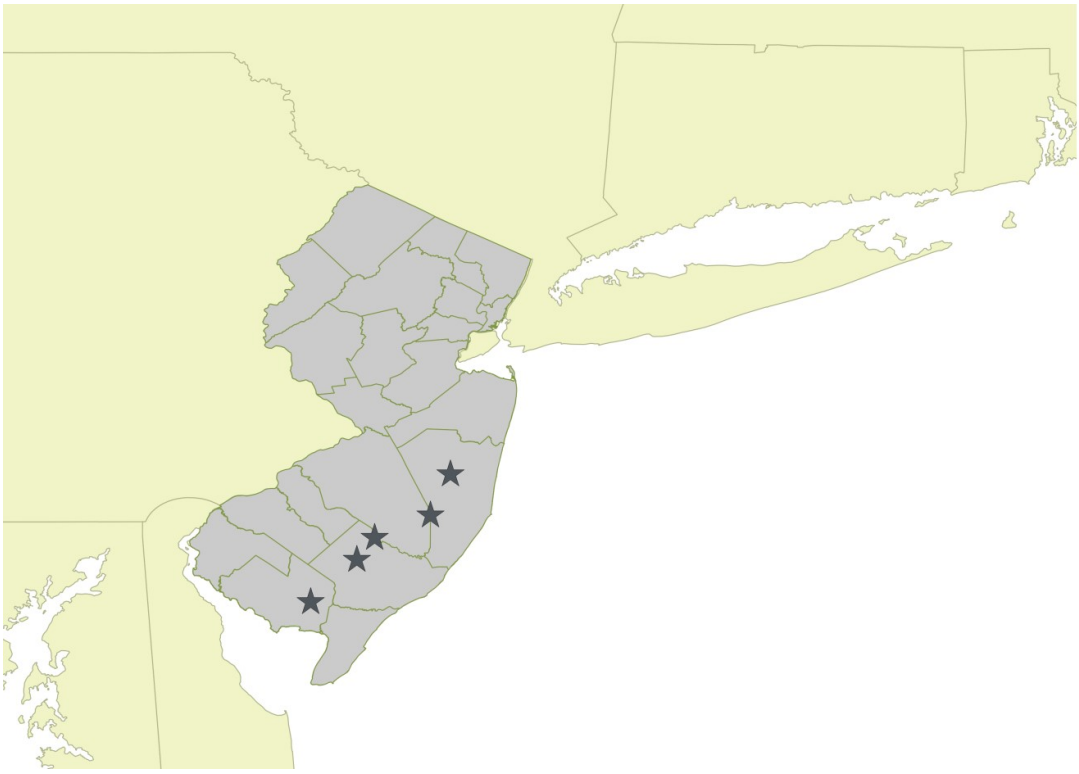


Figure 2. Non-Metric Multidimensional Scaling ordination of lichen communities. Lichen communities are presented (using a Euclidian distance measurement). Quadrats are designated from each site as follows: Crossley preserve (white diamond: ◇); FAA Tower (asterisk: ✳), Batsto (light grey square: ▪), Makepeace Lake (dark grey circle: ●), Manumskin (black triangle: ▲) . The stress for the ordination is: 0.0864296. The root-mean squared error is 0.0008385144, and the maximum residuals are: 0.005459069. The Batsto site is significantly different from the others on axis 1, (F-value = 16.18 p value $=2.8 \cdot 10^{(-08)}$) and the Makepeace lake site has significantly different axis scores than the other sites on axis 2 F-value = 14.351, p value $< 1.257 \cdot 10^{(-07)}$). Lichens are indicated as follows: Lplacy= *Placynthiella sp*.; LCunci = *Cladonia uncialis*; LCatl = *Cladonia atlantica*; LCrapii = *Cladonia rapii*; LCmac = *Claconia macilenta*; LCcris = Cladonia cristatella; LCsubt = *Cladonia subtenuis*; LCsubm= *Cladonia submitis.*

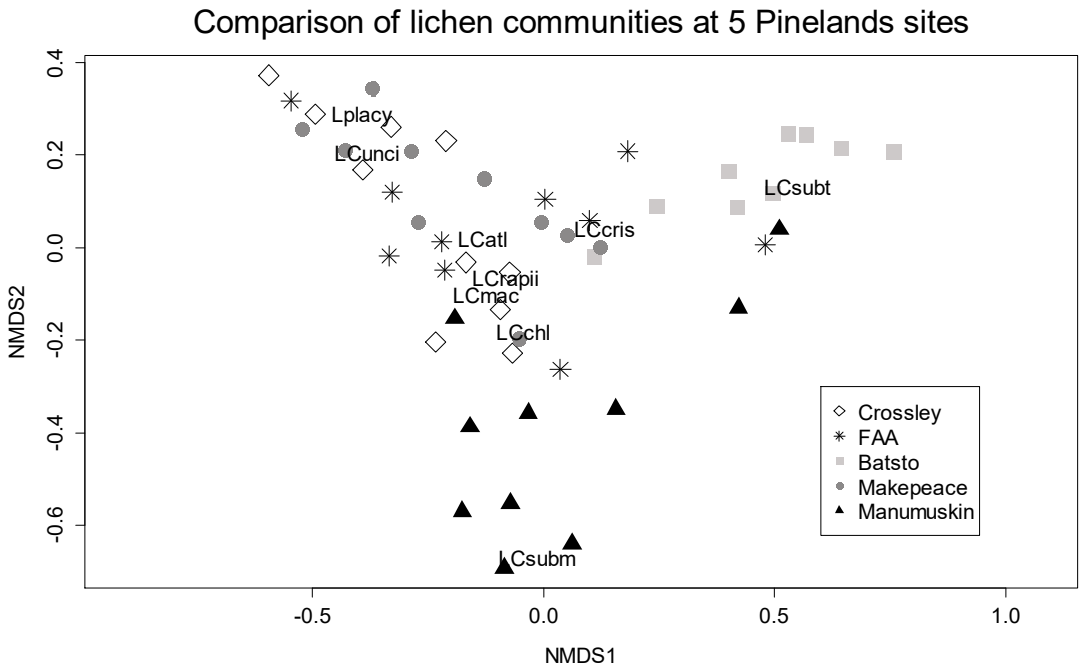


Figure 3. Canonical Correspondence Analysis of lichen communities with respect to vegetation communities at 5 sites. The diagram represents a constrained ordination in which only variation aligning with the described vegetation cover is presented. The environmental variables represent total percent cover of the following groups: lichens, mosses, herbaceous plants, shrubs + vines, and trees. Quadrats are designated from each site as follows: Crossley preserve (white diamond: ◇); FAA Tower (asterisk: ✳), Batsto (light grey square: ▫), Makepeace Lake (dark grey circle: ●), Manumuskin (black triangle: ▲) .

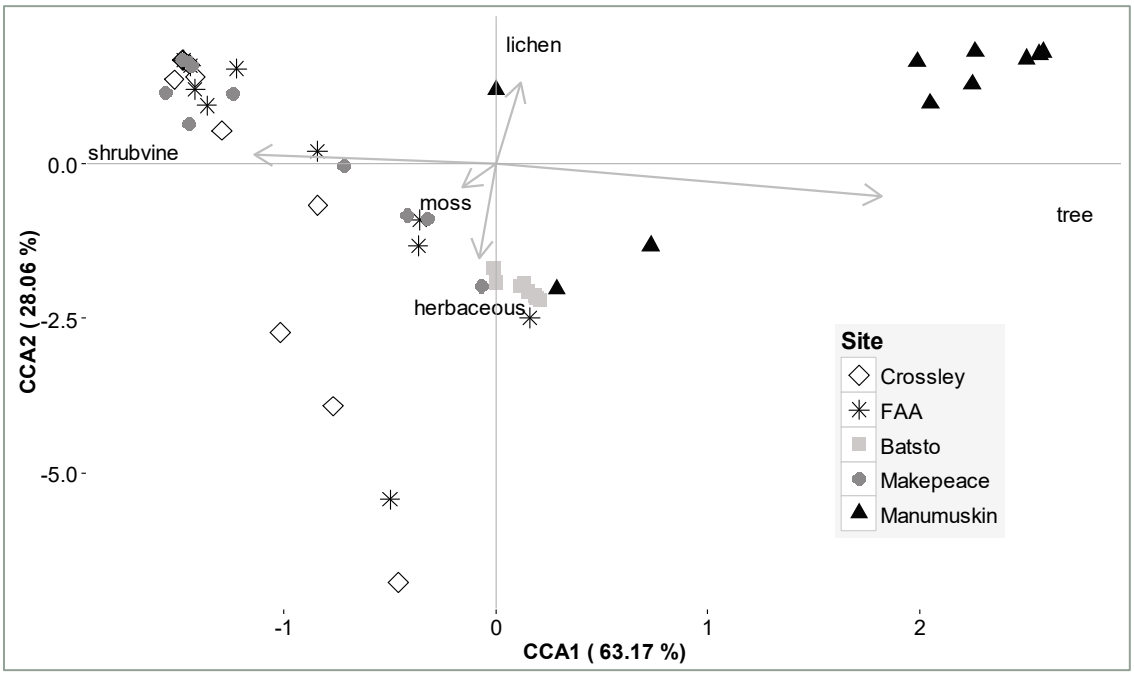


Figure 4. Comparison of soil properties at the 5 sites. 4(a) Soil extractable ammonium values presented in μg NH_4^+-N / g dry soil; 4(b) Soil extractable nitrate – nitrite values in μg NO_3^--N, NO_2^--N / g dry soil; 4(c) Soil extractable phosphate in μg PO_4^--P / g dry soil; 4(d) Percent moisture; 4(e) percent mass loss on ignition; 4(f) Lichen mat height in centimeters. (average of 6 measurements on *C. subtenuis*). Horizontal lines in the center of the boxplots represent the mean value (n=5), and the vertical lines represent the standard error. Values with different letters represent significantly different quantities as determined by the Tukey HSD test.

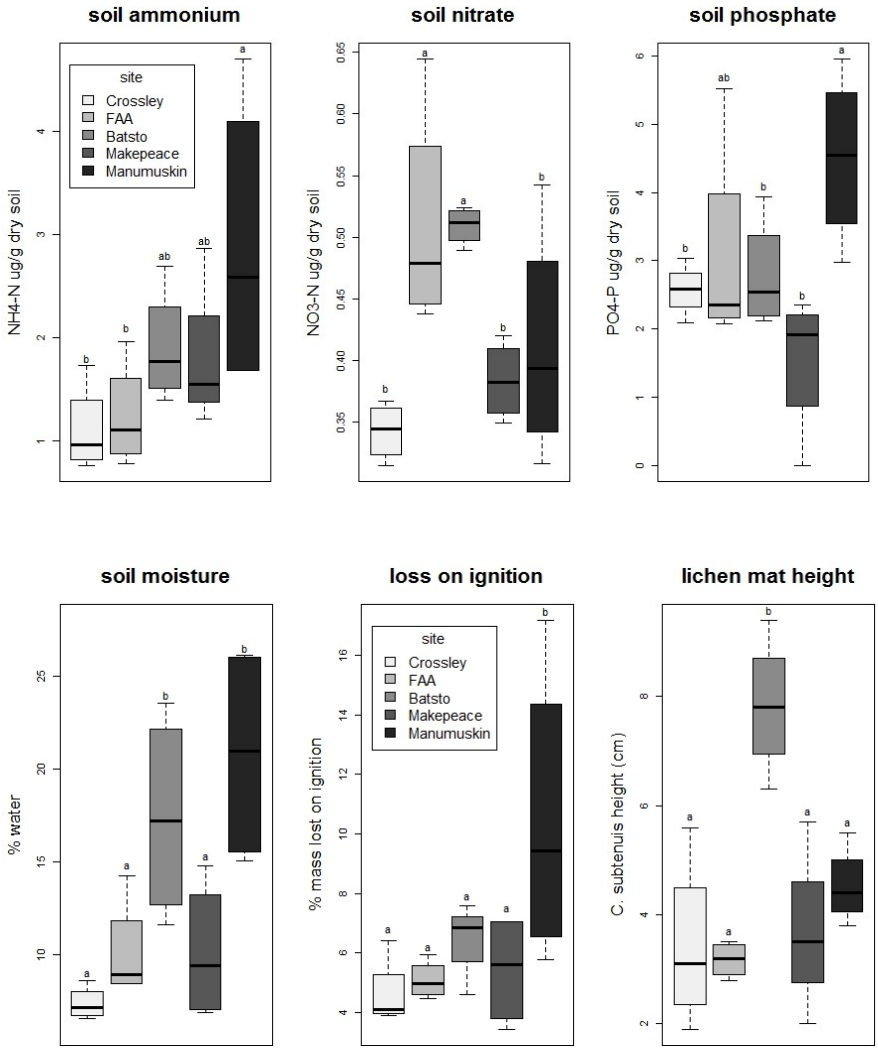
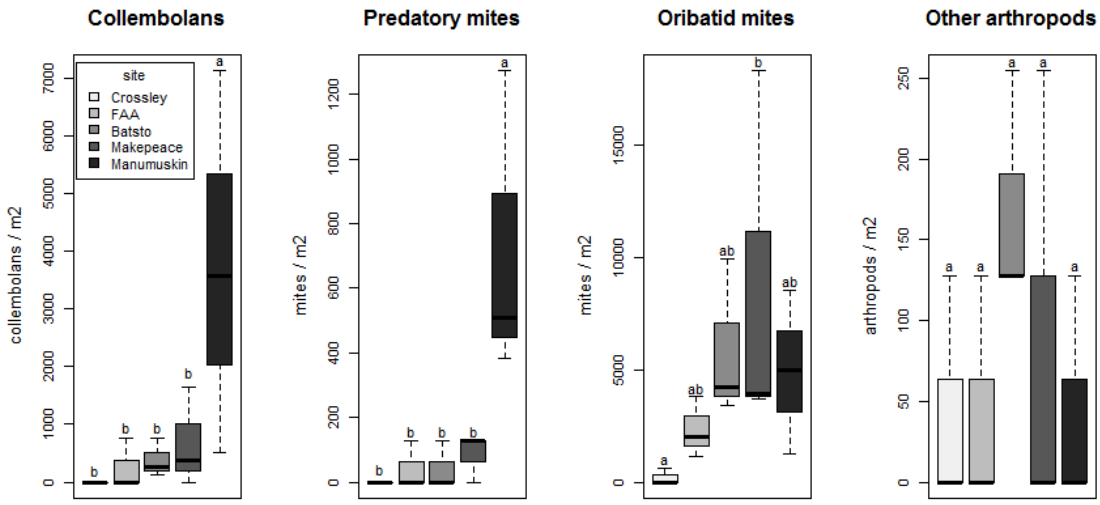


Figure 5. Arthropod community sampling at each site, summer 2013. 5(a) collembolans / m^2 in top 5cm of soil. 5(b) predatory mites / m^2 in top 5cm of soil. 5(c) oribatid mites / m^2 in top 5cm of soil. 5(d) other organisms/ m^2 in top 5cm of soil. Horizontal lines in the center of the boxplots represent the mean value (n=5), and the horizontal lines represent the standard error. Values with different letters represent significantly different quantities as determined by the Tukey HSD test.



3 Ecological function of soil lichens in the NJ Pinelands

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Abstract.

Lichens are drivers of ecosystem patterns where there are few other producers in a landscape; our work addresses the less well understood role of soil lichens in forests, when they are in direct contact with the soils but represent a small portion of total productivity. In the NJ Pinelands, lichens are abundant, their communities are well characterized, and the soils have low nutrient availability so any lichen-induced effects will be important for the whole forest. Our study asks whether the lichens altered soil nutrient cycling patterns, and whether the lichens have top-down influence on soil micro-arthropod communities. We prepared a transplant grid with different aboveground material (lichens, pine needles, oak leaves, and bare ground) on two sites in the Pinelands in January 2013 and monitored these sites seasonally for 2 years. We found that the influence of lichens on soils varies with soil conditions and with climate conditions. In summer, when soils have low water content (< 10% water), lichens help them retain significantly more moisture, and when soils have higher inorganic phosphorus availability (> 3ug/g PO_4-P), lichens significantly reduce extractable phosphorus concentrations. In our study the lichens did not have significant effects on soil ammonium or nitrate levels, on soil enzyme activity or decomposition rates. Lichens did not influence density or diversity of arthropods overall, although predatory arthropod density was higher underneath lichen. Stumbling blocks to gaining more funding for

lichen conservation include lack of public knowledge of lichens and lack of scientific evidence for how lichens contribute to ecosystem functioning; our work contributes to building our understanding of how lichens influence soil function.

Introduction.

Understanding soils is vital in order for humans to responsibly manage planetary biological and chemical cycles since belowground soil processes drive the productivity and diversity of aboveground systems. Soils play a direct role in ecosystem services including provisioning services (production of food and fuel), regulating services (regulation of water, climate, floods, pest populations), cultural services, and supporting services (nutrient cycling, habitat and biodiversity), as summarized by Adhikari & Hartemink (2016). Decomposition is a critical process for providing these services because decomposition produces soil organic matter that in turn promotes both soil water retention and soil nutrient retention, thereby controlling plant growth and aboveground productivity. The decomposers that process soil organic matter are the fungi, bacteria, invertebrates that live in the soils. The soil invertebrates break down material through ingestion/ excretion, and active fungi and bacteria create soil enzymes that chemically break down material. Therefore, the chemistry and biology of the belowground habitats where decomposers live and act has large implications for the aboveground ecosystem function. This study investigates how aboveground lichens regulate the abiotic conditions and biological communities in the soil, which helps us understand the role that lichens play in the temperate forests where these studies take place.

The aboveground producers are often important regulators of soil processes. The chemistry of the aboveground litter (particularly the C:N and the presence of phenolic compounds) influence which decomposers will thrive in the belowground habitat (fungi vs. bacteria), and the decomposer communities dictate the speed of nutrient turnover and the quantity of C-sequestration (Wardle et al. 2004). Different soil invertebrate communities also thrive in different litter types (Moço et al. 2010). These findings suggest that organisms aboveground, like lichens, may change the belowground conditions that then drive large scale patters in ecosystem productivity and diversity. The purpose of this study is to describe how lichens exert these influences on soils.

When lichens are growing on soils, the conditions they produce below them alter fundamental soil properties through the same mechanisms that allow lichens to physically and chemically degrade their substrate when they are growing on rocks (reviewed by Chen et al., 2000). The presence of lichens changes erosion rates, water retention, temperature, and habitat quality in the soils below them. Lichen rhizomorphs (belowground ropes of fungal hyphae) can serve as soil-anchoring structures in biological soil crusts, preventing erosion (Belnap et al. 2003). During extended dry periods lichen-covered soils retain water for longer than bare areas (Cantón et al. 2004; Porter & Woollett, 1929). Lichen influence on soil moisture may be linked to the lichens' ability to absorb up to 300% (chlorolichens) or 1000% (cyanolichens) of their dry weight in water (Kranner et al. 2008). Lichen covered soils also have lower soil temperatures than soils that are bare (Gold et al. 2001). The more stable, cooler and moister conditions under the lichens mean that lichen-dominated soils will provide different habitat than areas where lichens are absent. Lichens are able to alter the soil habitat in these ways through their

ability to retain moisture in their fungal component, and they also alter belowground habitats through their ability to create and leach organic acids and other secondary compounds.

Lichen-induced abiotic changes in soils are intimately intertwined with changes to soil biotic communities, including microbial communities, and soil animal communities. Lichens produce secondary compounds that inhibit the growth of fungi and cyanobacteria (Gazzano et al., 2013), prevent bacterial (reviewed by Shrestha & St. Clair, 2013) and viral (reviewed by Odimegwu et al., 2015) invasions, and prevent the growth of potential competitors (reviewed by Huneck, 1999). With decreases in fungal density, and colder, wetter, conditions, one might expect that decomposition rates would be lower under lichens, and Sedia & Ehrenfeld (2006) found this to be the case. With lower decomposition rates in soils below the lichens, organic matter may accumulate there, promoting better habitat conditions for detritivores. Soil animals are able to use lichens as a food source; Chahartaghi et al. (2005) used isotope analysis to determine that collembolans feed on lichens; Erdmann et al. (2007), Ruiter-Djkmann (1993), and Schneider et al. (2004) all documented microarthropods feeding on lichens. Taken together, these soil responses to aboveground lichens mean that lichens may be important drivers of soil conditions (soil chemistry and biology) in forests.

Our study aimed to determine how and whether lichens influenced belowground abiotic and biotic processes in soils. We expected they did have important influence on soil conditions because of the evidence discussed above, that shows the many ways lichens make abiotic and biotic changes to soils in lab contexts and in other ecosystems. One way we could quantify exactly how much difference lichens were making on the

soils in the NJ pinelands was to create a transplant study. When soils cleared and homogenized had lichens placed above them, any significant differences that developed had to be due only to the presence of lichens. The NJ pinelands is an ideal candidate for studying the lichen effects because the lichen flora has recently been extensively characterized here (Lendemer 2006), and because the lichen biomass is so high (Wright et al., 2003).

Methods

Study Sites:

The New Jersey Pine barrens are located on the southeastern coastal plain in New Jersey, USA. We chose sites that were representative of areas across the Pinelands with high-densities of lichens in on the forest floor; chose these sites in particular because their lichen cover was robust, because they were not high traffic areas, and because we had permission by the landowners to conduct the experiments. We chose sites in the Brendan Byrne State Forest in Woodlands, Burlington County, NJ (39.840798 N, -74.520503 E), and in Wharton State Forest in Hammonton, Atlantic County, NJ (39.644956 N, -74.663316 E), and obtained permission to carry out the experiments from the NJ State Parks and Forests. The plant communities at both sites are Atlantic Coastal Plain pine-oak forests, with an open canopy of pitch pine (*Pinus rigida*) with few blackjack oaks, (*Quercus marilandica*) in the understory, and a sparse herbaceous layer of huckleberry (*Gaylussacia baccata*), greenbriar (*Smilax rotundifolia*), and sedges (*Carex pensylvanicum*). Both of these sites occur on paleodune geological formations, though on different types of sand. The soils at Byrne State forest are Lakehurst Sands, soils with

sandy horizons to 40 inches deep, where there is sandy clay loam; the slopes are 0-5% slopes and are moderately well drained (Soil Survey Staff, Natural Resources Conservation Service, USDA). The soils at Wharton State Forest were also Lakehurst sands, with slightly decomposed plant material in the top horizon and sand in the rest of the soil horizons (Soil Survey Staff, Natural Resources Conservation Service, USDA). The mean annual precipitation at both forests is 28-59 inches, with mean annual air temperature 46-79°F (Soil Survey Staff, Natural Resources Conservation Service, USDA). The general NJ Pine Barrens landscape is described extensively by Forman (1998), and we consider these two sites to be representative of many other sites in the Pinelands with extensive lichen mats.

Transplant Establishment:

In January of 2013 we established a transplant grid at each site; in an area of 2.5 m x 3.4 m, we removed the vascular plants, and homogenized the soil by raking until the area had visual uniformity. We divided the plot into 12 plots, each 0.5 m x 1 m with a 10cm buffer around it. Each plot received one of 4 different aboveground covers: pine needles, mixed leaf litter, lichens, and bare ground. The biomass of litter added is equal for each treatment type (500g / 0.5m^2), which represents the average dry biomass when clumps of dried lichens are assembled as a uniform mat of *Cladonia submitis*, the most common lichen in both sites. The mixed litter treatment roughly reflects the observed composition of leaf litter in local forests (huckleberry : pine : oak 3:11:11). The pine only litter treatment was included to represent low diversity leaf litter (since lichen cover represents only one genus: *Cladonia*). This transplant system allowed for comparison of how lichens influenced soils differently than other potential aboveground covers. We

chose not to include plants in this comparison because their roots would be influencing soils directly, not from aboveground. We expected some tree roots to be present in the plots, but they were not in the top 5cm that we were measuring, and since they would be widely distributed under the plots regardless of treatment, we assumed that their effects would be equal between plots. Since our major interest is in how aboveground lichens influence belowground processes, it made sense to restrict the comparison to aboveground-only material.

Soil sampling:

We conducted soil sampling for two growing seasons starting 6 months after transplant establishment (July 8th and 15th, 2013), 10 months (November 20th/27th 2013), 14 months (March 6th and 9th, 2014), 17 months (June 9th and 16th, 2014), 19 months (August 28th and September 1st, 2014), and 21 months (October 20th and 27th, 2014). To monitor decomposition, we set out 5cm x 10cm litterbags with 1mm mesh that were filled with 1g of pine needles. There were 196 bags total, 4 treatments x 3 replicate cover types x 2 sites x 5 time periods. The litterbags were collected 4 times: July 2013, January 2014, June 2014, October 2014; the first measurement, January 2013, comes from the initial mass of the litterbag, before being placed outside. The open ends of the bags were secured with dental floss, and placed on the soil surface. The litterbags on the bare plots were exposed, and on the plots with mixed leaf cover, pine cover, and lichen cover, the litterbags were just underneath the aboveground cover. On one plot (3rd bare plot, Wharton State forest) the litterbags were removed by an unknown person, so they were not available for analysis. After collecting the litterbags, we quantified decomposition rates by measuring mass loss at each time interval.

On each of the soil sampling days, we collected 6 cores at each plot: three 5cm soil cores for invertebrate sampling and 3 cores (which were pooled) to assess the following parameters: soil moisture, loss on ignition, inorganic N, inorganic soil available P, soil microbial biomass N and C and enzyme activity for 5 enzymes as described below. We determined the moisture content of the soil samples by drying at 70°C. We measured the loss on ignition by burning the samples in a muffle furnace overnight at 500°C.

Chemical analyses:

Soil chemistry samples were taken on field moist soils within 6 hours of sampling in the field. Ammonium and nitrate were extracted into solution by shaking 10g of sample with 2M KCl for one hour at 200 rpm and then vacuum filtering the material on a Whatman #1 filter. The filtrate was frozen and subsequently analyzed on an Astoria Pacific autoanalyzer. Soil phosphorus was extracted by shaking 5g for 1 hour with bray solution (ammonium fluoride); this solution was then mixed with solutions of ammonium molybdate and potassium antimonyl tartarate, and color changes proportional to phosphorus concentrates were measured on a spectrophotometer at 800 nm (Bray and Kurtz 1945). Total soil C and N was measured by shaking 10g for one hour with Potassium sulfate solution; we froze a filtrate sample from this solution and analyzed this sample on a Shimadzu C/N autoanalyzer. Microbial biomass C and N were determined using the chloroform fumigation extraction (Brookes et al. 1985, and Vance et al., 1987). Briefly, samples were incubated with chloroform for 24 hours, and were extracted with 10 mL of 0.5 mol L^{-1} K_2SO_4. These samples were shaken for 1 hour at 200rpm and vacuum filtered on Whatman #1 filter paper. The samples were frozen until analysis.

Microbial activity:

In order to tell how microbial communities are functioning, we assayed the activity of enzymes that break down compounds of carbon and nitrogen: these enzymes are well correlated with decomposition and nutrient availability in soil (Sinsabaugh 1999). We conducted assays for the activity of 5 representative enzymes in the soil. β-glucosidase (BG; which reflects decomposition of simple C compounds), β-glucosamidase (NAG: chitinase; related to organic N breakdown to constituent amino acids) and acid phosphatase(AP), were measured using the p-nitrophenol (pNP) method. Phenol oxidase and peroxidase acitivity were also measured (related to degradation of complex organic substrates). We followed the procedures described by Sinsabaugh (1999). Briefly, 1 g of soil was mixed with 2mL 50 mmol/L acetate buffer (pH 5). The substrates added were pNP-b-D-glucopyranoside (to measure b-glucosidase activity), 4-Nitrophenyl-N-acetyl-β-D-glucosaminide (b-glucosamidase), and pNP-phosphate (acid phosphatase). For phenol oxidase and peroxidase, the substrate was L-DOPA, (L-3,4-dihydroxyphenylalanine), which was incubated with hydrogen peroxide for the peroxidase activity assay. The soil slurry was incubated with 2 mL of the corresponding substrate at 20C 1 hr with constant mixing. After incubation, the samples were centrifuged, and 2 mL of supernatant was transferred to a tube, .2 mL of 1 M NaOH was added. These samples were diluted with water to 4 mL, and their absorbances were measured at 410nm for the p-NP substrates, and at 460 for the l-DOPA substrates, and were compared with standards for quantification of enzyme activity, which we expressed as umol/L substrate converted per gram of dry soil per hour.

We also conducted an accessory study to determine whether different lichen species produced different microbial activity profiles. We used a spoon to collect 3 samples each from soils directly underneath different specimens of *Cladonia uncialis*, *Cladonia rappii*, and *Cladonia subtenuis*, moss mats (*Polytrichum commune*) and pine needles. We then created a soil slurry which we applied to 32 different substrates in a 96 well ECOLOG plate (BIOLOG, CA, USA); we analyzed substrate utilization through color change in the substrate as described by Garland and Mills (1991).

Soil invertebrate sampling:

We collected soil invertebrates from the organic soil horizon (0-5cm); we extracted the arthropods into 70% ethanol w/ glycerol by dynamic extraction from soil using a Tullgren extractor. We identified the organisms to morphogroups for mites and collembolans. Oribatid mites were characterized as being in one of the following groups: Eulohmannoidea, Phthiracaroidea, Oppiodea, Bellboidea, Nanhermanoidea, Tectocephus, Galuminoidea, Brachythonoidea, other dark brown round mites (Ceratizoidea, Oribatuloidea, Pelopoidea, Licaroidea, Caraboidea), juvenile mites, other oribatids. Predatory mites were grouped as Mesostigmata, Prostigmata, and Astigmata. Collembolans were grouped as Symphyolena, Onychiuridae, Poduridae/Hypogasturidae/Neanuridae, Entomobryidae, Isotomidae, Tomoceridae. Our final group, other arthropods included: Ixodida, Pseudoscorpionida, Arachnida, Coleoptera larvae, other insect larvae, Ants, Protura/Diplura/Myriopoda. With these groups, density and Shannon-Weiner diversity index values were calculated for each site; though density changes in soil animal communities more closely follow changes in soil function than species richness values do (Nahmani and Lavelle 2002).

Data analysis:

We conducted repeated measures ANOVA analyses in SAS to determine whether lichen cover influenced the measured variables and described the interactions between the variables and the treatments. We used repeated measures ANOVA to test treatment differences for specific soil parameters and soil animal groups. We then conducted power analysis using the pwr package in R (R Core Team, 2015) to determine whether our sample sizes were appropriate to determine differences in the measured treatments.

Results:

Aboveground treatment influenced decomposition throughout the experiment, influenced soil moisture at the end of the experiment, and had no effect on soil organic matter content. Repeated measures ANOVA of soil moisture data showed that lichens did not overall create significantly moister conditions ($p = 0.1852$). Soil moisture was significantly influenced by time ($P < 0.0001$) and there was a time * treatment effect (using Wilkes' Lambda statistic, $p = 0.0080$). The influence of the treatment on soil moisture was only significant ($p = 0.0279$) in the October 2014 sampling; the Wharton State Forest site had the most difference between lichens and other treatment (Figure 1).

The organic material in the soil, proportional to the % of dry mass lost on ignition (LOI), increased along the course of the experiment so time significantly influenced LOI ($p < 0.0001$), and there was a significant difference in LOI between the two sites studied ($p = 0.0110$), but there was no significant difference in LOI with aboveground treatments (Figure 2; $p = 0.2694$). A power analysis suggested that one additional replicate per site would have provided the replication we needed to capture the between-treatment differences, if they were present.

Repeated measures ANOVA clarified that the state of decomposition (mass remaining in litterbags) was significantly affected by time ($p < 0.0001$), forest ($p = 0.0007$), and treatment ($p = 0.0010$). The decomposition was slowest underneath the lichens, and most rapid on the bare soil (Figure 3), but this difference was only significant in the last measurement, after 18 months and only in Brendan Byrne State Forest, where decomposition was generally slower than it was at Wharton State Forest.

In the analysis of soil chemistry, the only significant response we found was soil phosphorus. Soil phosphorous response to aboveground litter was significant ($p = 0.0342$), soil P also changed significantly over time ($P < 0.0001$) and there was a significant time * treatment effect (using Wilkes' Lambda statistic, $p = 0.0475$). The % change in soil P with lichens was the greatest in fall at Wharton, the site with higher background soil P (Figure 4). There was no significant change in the other measured parameters of soil chemistry with aboveground treatment: ammonium ($p = 0.4294$), nitrate ($p = 0.7422$), microbial biomass nitrogen ($p = 0.3216$) or microbial biomass carbon ($p = 0.2747$) . The power analysis suggested that our replication was suitable for capturing potential differences in ammonium between treatments, but we would have need 4 replicates per site to detect treatment differences in microbial biomass nitrogen, and 7 replicates for nitrate and microbial biomass carbon.

Repeated measures ANOVA of enzyme activity showed that aboveground treatment did not affect belowground enzyme activity. None of the enzymes significantly responded to treatment (β-glucosidase, $p = 0.7574$; chitinase, $p = 0.9753$; peroxidase, $p = 0.2675$; phenol oxidase, $p = 0.5715$; acid phosphatase, $p = 0.2167$; figure 5). Although we sampled the enzyme activity 4 times during 2014, we expected the enzyme activity to

be greatest at the latest sampling date, when the interaction between the lichens and the soil was the most developed. However, there were still no measurable differences in aboveground treatments (Figure 5). In a power analysis of the sampling on that date (October 2014) we determined that 8 replicates would have been necessary to capture true differences in β-Glucosidase and N-Acetyl Glucosaminidase activity; for phenol oxidase activity, 4 replicates; for peroxidase, 9 replicates per site, and for acid phosphatase, 6 replicates per site.

MANOVA of effects of aboveground cover on substrate utilization in the biolog activity assay revealed that aboveground cover did not correspond to differential use of substrates in most cases (Figure 6). In 3 substrates, N-acetyl-D-glucosamine (F-value: 3.7804; p = 0.04007) , D-glusaminic acid (F-value: 10.719; p = 0.001223) α-Lactose (F-value: 3.7191; p = 0.04188), aboveground material did have an effect on substrate utilization, but the lichens did not have consistently different effects on enzyme activity compared with the other ground covers. There was more utilization of D-glucosaminic acid which is a carboxylic acid, by enzymes from the soils under pine needles than under any other cover. The soils under *Cladonia rappii* and *Polytrichum commune* had low utilization rates for N-acetyl-D-glucosamine, which is a carbohydrate. The second carbohydrate α-Lactose, demonstrated low rates of enzymes activity under mosses with higher activity under pines, a pattern that was consistent, though not significant, in many of the other substrate utilization profiles.

In a repeated measures ANOVA of total arthropod abundance with different aboveground treatments, the treatment type was not significant (p = 0.08; see Figure 7). However, the density of predators did respond significantly to aboveground treatment (p

= 0.046). The lichens generally had higher predator densities, and this trend was especially pronounced in the August and October measurements and at Wharton state forest (figure 8). The other arthropod groups did not show significant responses to the aboveground treatment; collembolans ($p = 0.6615$) and oribatid mites ($p = 0.3235$). Our power analysis indicated that 32 replicates per site would have been necessary to capture any treatment-related differences in collembolan density. Arthropod morphogroup diversity did not significantly change with soil cover (Figure 9).

Discussion:

In previous studies (Sedia and Ehrenfeld, 2006), many factors of soil biology were found to be significantly different under naturally occurring lichen and moss mats than under other ground covers. Our work found fewer significant differences. This can be explained because our transplant study profoundly disturbed the ground beneath the lichens and was of short duration. If lichen influence on soils is exerted over a scale of years, not months, as the lichens release secondary compounds and cover the ground with the mat of dead fungal matter, it makes sense that our study would not have captured these differences until the later sampling times. Other studies of the effects of lichens on soils involved simple removal of the lichens (Sendstad, 1981) or removal of lichens plus the top 1cm of soil (Barger et al., 2006); however in those cases, in Svalbard and Canyonlands National Park, there is no mention vascular plants growing at the site that would confound the analysis of the direct effects of lichens on soils. Our soils had to be more disturbed in order to remove the *Smilax*, *Gaylusacia*, *Vaccinium*, and *Carex* plants that were growing among the lichens. In future transplant studies with lichens, lichen effects on soils may be more obvious if the top layer is left more intact, so that the

processes that established lichens create can be discretely measured and directly compared to the effects of the aboveground litter.

The lichen influence at Brendan Byrne was different from the lichen influence at Wharton two sites was different. Since aboveground lichens increase soil moisture in dry conditions (Chamizo et al., 2013; Porter & Woollett, 1929) it makes sense that lichen effect on moisture was significant only when the disturbance was distant in time, and when the soils below other ground covers were relatively dry. The effect of lichens on phosphorus depletion, was significant at Wharton and not significant at Brendan Byrne. Lichens are known to recycle nitrogen within their tissues, preventing the nitrogen from dead parts of the lichen from leaching into the ground (Ellis et al., 2005), so phosphorus recycling has also been suggested (Hyvärinen and Crittenden, 2000). This means that compared with soils below decomposing leaves, the soils beneath the lichens would be depleted in phosphorous. When the whole lichen dies, phosphorous may leach from the thallus, enriching soil phosphate levels (Knops et al., 1996), but with lichens that still contain any living material, as the lichens in our study did, available phosphorus would be used by the lichen. To avoid confounding effects of plant growth among the lichens, we removed sprouting *Carex* and *Smilax* species that we found growing in the transplant area. Allowing continued growth of plants in the plots would presumably further deplete soil P, but we did not address how lichen mat associated soil P-depletion compares in magnitude to P-depletion in soils below plants.

Soil enzyme activity was not affected at by the aboveground cover. Lichens themselves produce laccases and peroxidases (Beckett et al., 2015); and β-glucosidase (Yague and Estevez, 1988). But since my samples were in the soil below them, these

enzymes might have been too diluted in the soil, or have passed through the soils earlier. When *Cladonia* leaches compounds into the soil, the concentrations are negligible one hour later (Dudley and Lechowicz, 1987). Kourtev et al. (2002) found that aboveground plants had significant effects on enzyme activity of bulk soil; however, the fact that the plants' root systems provide an extensive surface area through which they interact with the soil as compared with the area of the lichen surface in physical contact with the soil may explain why their influence on soil enzyme activity is more significant. The results of the negligible impact of lichen presence on enzyme activity make sense since the organic matter availability, soil moisture, and soil nitrogen, three factors important for bacterial and fungal activity and enzyme production, were not found to be different beneath the lichens. The enzyme activity in our samples was low compared to activities measured in similar sandy soils of the NJ pinelands beneath plants (Kourtev et al., 2002; Geng et al., 2012).

Since the lichen cover retained more soil moisture during dry periods at Brendan Byrne state forest, and since moisture is an important environmental factor influencing faunal abundance, we expected that the soils beneath lichens would harbor higher arthropod densities. In particular, we expected that lichens create better habitat for collembolans, soft-bodied arthropods that are more prone to desiccation than the hard-bodied mites. However, there was no significant increase in general arthropod density, or collembolan density below the lichens. This may be because protection again drying may be important for these organisms in the short term, but in the long term the food base would be more important. Additionally, collembolans may travel up to 16m in search of food sources (S. Smith, unpublished data) so the small patch size we used in this study

might have been small enough that it only captured movement of collembolans through it, and did not harbor resident organism.

Some collembolans specialize on algae as their food resource, and eat the fungal material around the algae in lichens (Chahartaghi et al. 2005) but many collembola are deterred from feeding on lichens by the secondary compounds the lichens produce (Asplund et al., 2015). Bokhorst et al. (2015) found that invertebrate communities of soil-dwelling lichens reflect the community composition of the soil invertebrate pool; lichens do not provide a unique habitat for them. Further, Bokhorst et al. (2015) found that collembolans were more abundant on foliose than fruiticose lichens (the growth form involved in this study) and more abundant on N-fixing lichens compared to non N-fixing lichens (the type involved in this study). If there are few collembola associating directly with the lichens we studied, the collembolan assemblages in the soils beneath those lichens should not be much different than the soils elsewhere, as we found.

Additionally, because mites living on bark can be characterized as members of a lichen-feeding guild, using lichens as the major part of their diet (Erdmann et al., 2007; Meier et al., 2002; Fischer et al., 2014), we expected that there might be some soil mites that were restricted to the lichen-covered plots. However our resolution (to order) was not sufficient to distinguish these differences: for example, of the Carabodes mites that Erdmann et al., (2007) found, were divided into oak specialists, algae specialists, and lichen specialists, so the taxonomic resolution we used would have lost this subtlety by grouping all of them as Carbodes. Erdmann et al. (2007) also found that the mites in the lichen feeding guild were lower in abundance than the mites in the bark or algal feeding guilds. Therefore, in this study, the decreases in density of mites in other feeding guilds

may have swamped the signal of increases in density of lichen feeding mites. Dighton et al. (2012) found no trend in soil animal abundance even with a 6-fold increase in aboveground woody debris, and suggested that mites may respond more to belowground disturbance than to aboveground processes.

The predatory mites, however, did respond significantly to the presence of lichens. Asplund et al. (2015) found that mesostigmatid mite abundance and species richness was not significantly influenced by removal of secondary compounds from lichens, which makes sense since they are predators and not directly feeding on the lichens.

Conclusion:

In general, our hypothesis that the lichens create unique habitat conditions in the soils below them was not supported, with two exceptions. Where soils were dry, lichen cover led to higher soil moisture, and where soils contained greater than 0.2 ug PO_4-P, lichens depleted belowground phosphorus levels. We attribute this lack of capture of a lichen effect to the fact that the soils were disturbed to >1cm deep, the spatial range in which the effects of lichens have otherwise been detected, and to the lack of time since disturbance (22 months for the final measurement). Also power analyses indicated several portions of the study in which our replication was not high enough to capture variation in the properties we studied; this problem was especially pronounced in the enzyme study and in the the soil microbial biomass sampling. Had this study involved less aboveground disturbance, or had it been carried out for a longer time period, our results may have more closely aligned with the findings of Sedia & Ehrenfeld (2006) of changes to soil chemistry and enzyme activity with aboveground cover of lichen mats.

The effect of lichens on soil phosphate levels could be important for vascular plant growth in areas receiving high quantities of nitrogen deposition, in which production may be phosphate-limited, and lichen presence may therefore influence vascular plant growth indirectly, through moderation of available soil nutrient levels. Our results of lichen importance in dry conditions are in agreement with findings from soil lichen research in desert habitats; if moderation of soil moisture is critical mechanism for lichen effects on those habitats, the fact that temperate forest organisms and abiotic processes experience regular precipitation events helps to explain why the importance of lichens in deserts is not reflected in our studies in these Pinelands Forests.

The results of this study lend support to the idea that lichen's abiotic influence on soils is more meaningful than their impacts on biological activity (enzyme activity, soil animal communities).

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Figure 1: Soil moisture beneath lichens vs. soil moisture beneath other ground covers. Ground covers are designated as: Bare (■) = ground cover removed, Lichen (●) = 100% cover of lichen, Mixed (▲)= mixed leaf litter (mixture of *Vaccinium*, *Quercus* and *Pinus*) Pine (◆) = pine needles. Symbols represent the mean of 3 replicate samples and bars represent standard deviation. Sample time is indicated as two digit year and three letter month abreviation after initial transplant (January 2013), and soil moisture is derived from comparison of weight of field moist soils with the weight of oven-dried soils.

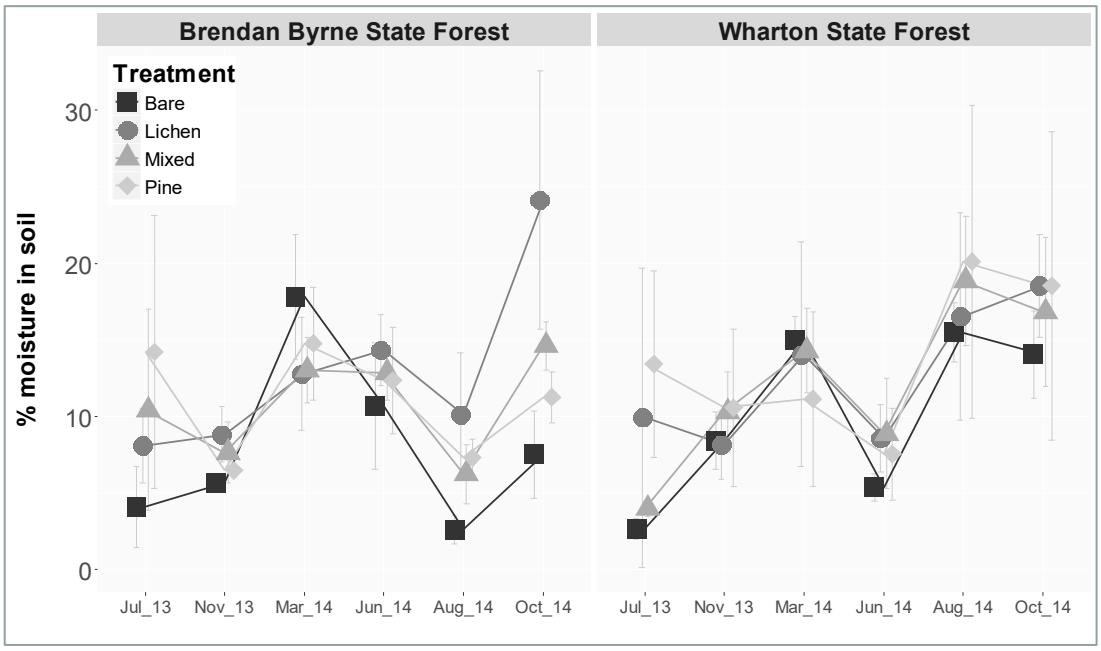


Figure 2: Loss on ignition under lichens vs. other ground covers. Ground covers are designated as: Bare (■) = ground cover removed, Lichen (●) = 100% cover of lichen, Mixed (▲)= mixed leaf litter (mixture of *Vaccinium*, *Quercus* and *Pinus*) Pine (◆) = pine needles. Symbols represent the mean of 3 replicate samples and bars represent standard deviation. Sample time is indicated as two digit year and three letter month abreviation after initial transplant (January 2013), and % mass lost when the dried soil was ignited at 500°C.

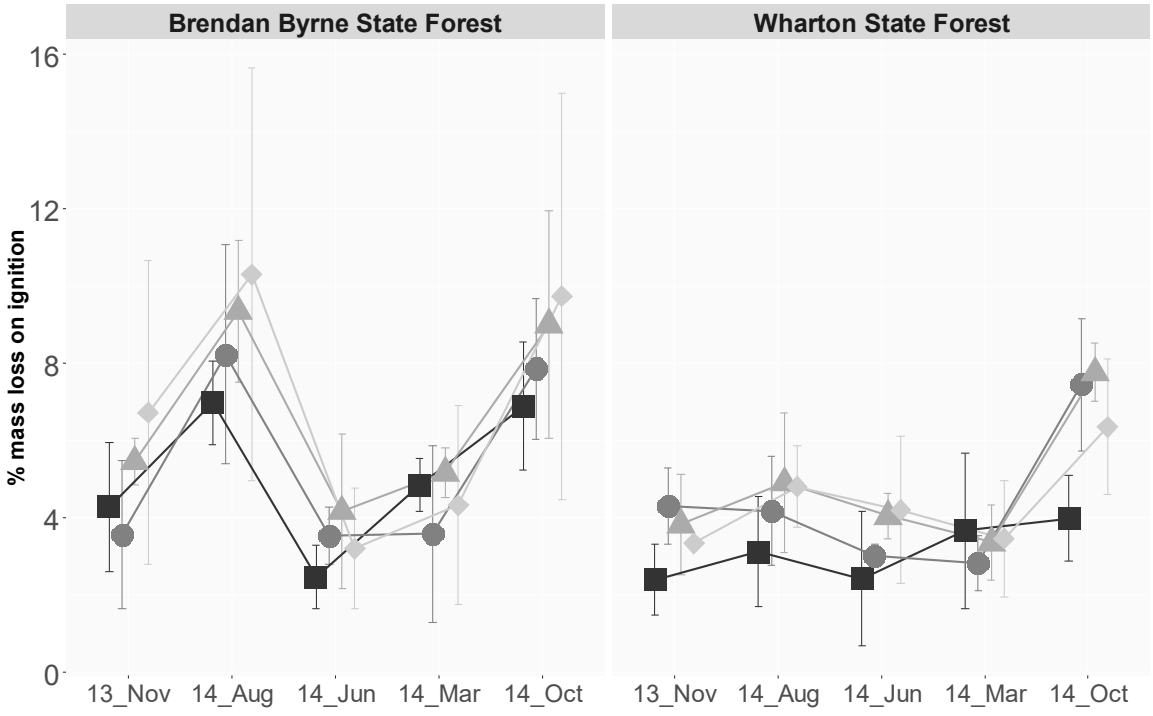


Figure 3: Decomposition under lichens vs. under other ground covers. Ground covers are designated as: Bare (■) = ground cover removed, Lichen (●) = 100% cover of lichen, Mixed (▲)= mixed leaf litter (mixture of *Vaccinium*, *Quercus* and *Pinus*) Pine (◆) = pine needles. Data represents % mass loss of litter from litterbags collected in indicated season and year (Litterbags were placed in winter (January) of 2013). Points represent mean of 3 samples each, bars represent standard deviation. Lines connecting the points are meant to estimate the decomposition trajectory since the time of collection.

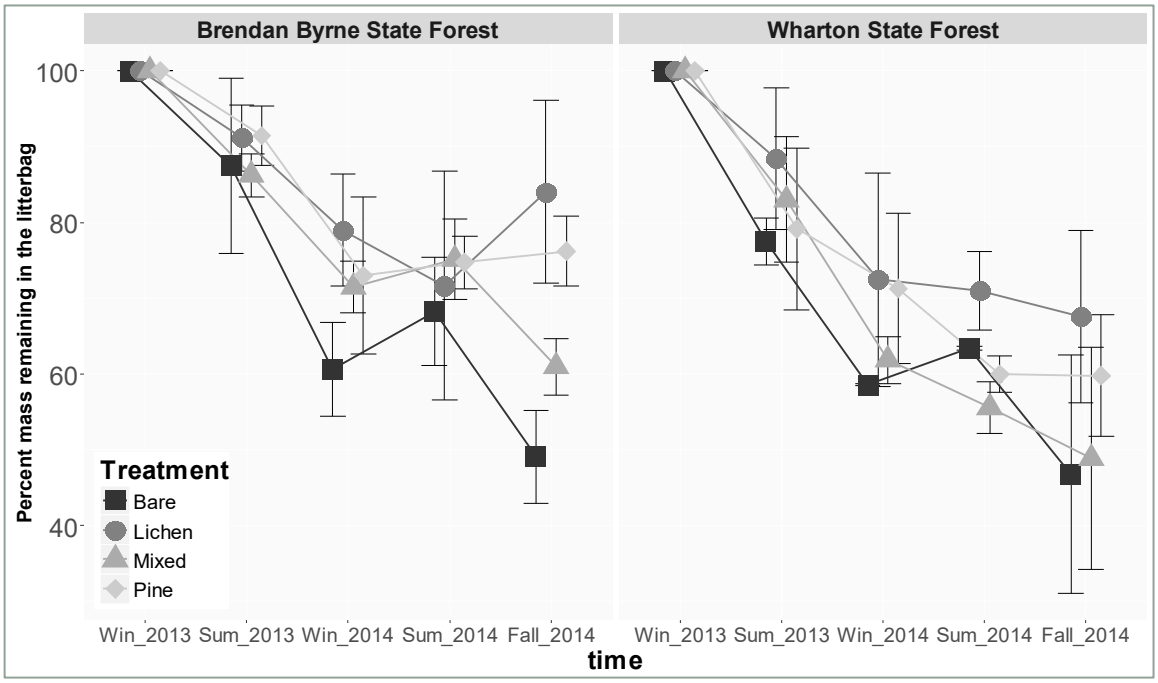


Figure 4: Soil Phosphorus values at Brendan Byrne State Forest and Wharton State Forest in 2014, under different litter covers, expressed as ug PO_4-P per gram of soil. Ground covers are designated as: Bare (■) = ground cover removed, Lichen (●) = 100% cover of lichen, Mixed (▲)= mixed leaf litter (mixture of *Vaccinium*, *Quercus* and *Pinus*) and Pine (◆) = pine needles,. Points represent mean of 3 samples each, bars represent standard deviation. The only significant difference in soil chemistry is in soil phosphorus, and only in the fall, and only at Wharton State Forest.

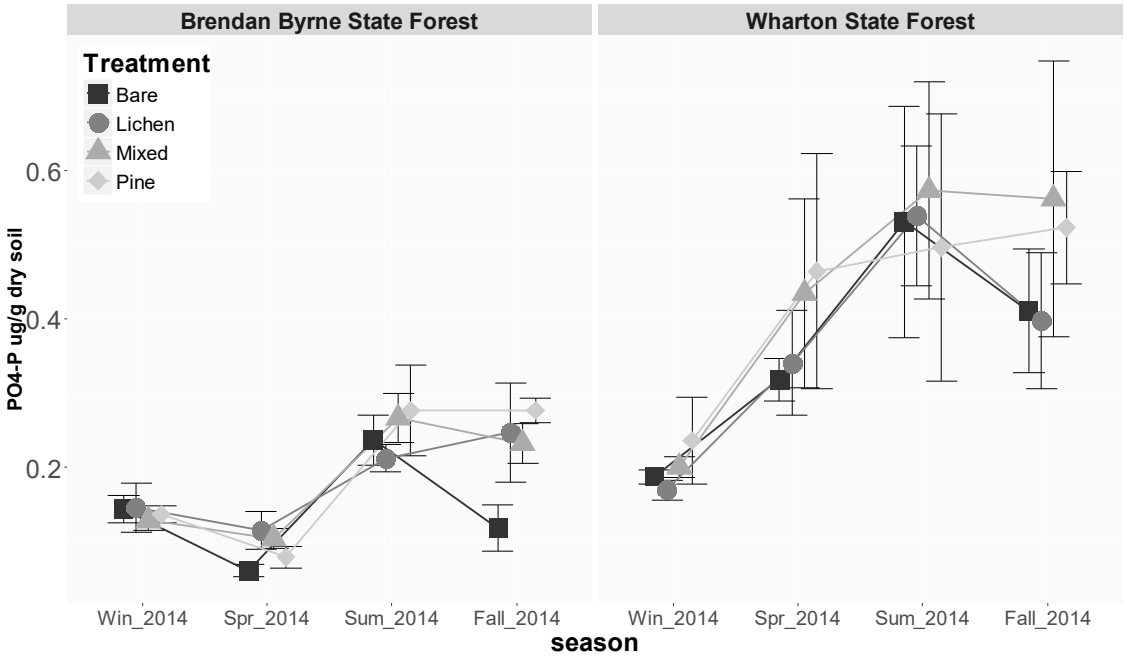


Figure 5: Comparison of enzyme activity as expressed in umols of substrate transformed for different aboveground treatments, from final sampling on October 2014 sampling. Activities from 5 enzymes are displayed: Acid Phosphatase, B-glucosidase, N-acetyl-glucosaminidase, Peroxidase, and Phenol oxidase. There is no significant difference in enzyme activity due to aboveground cover. Cover types are designated as follows: light grey = bare; black=lichen; dark grey=mixed leaf litter; light grey = pine.

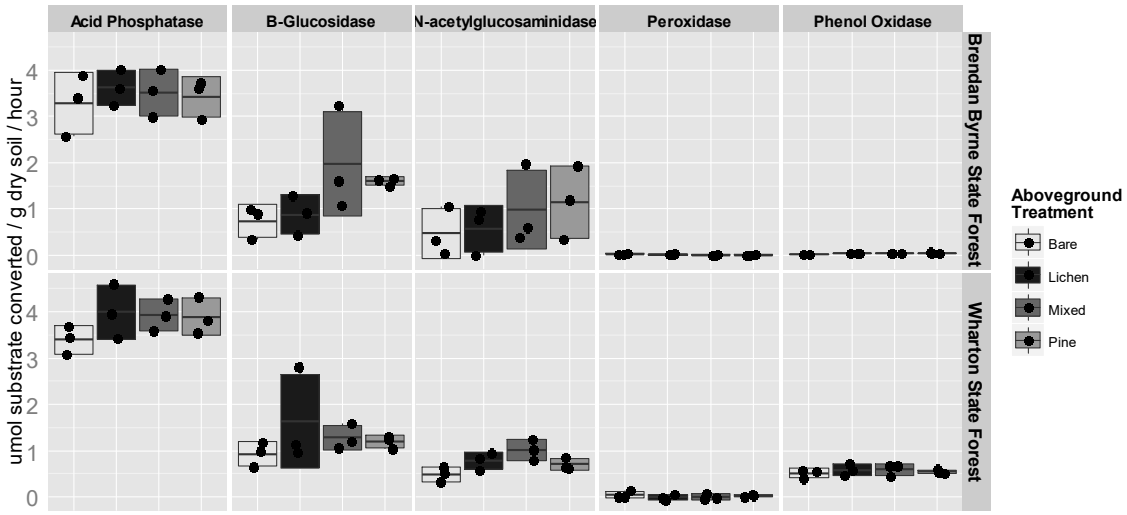


Figure 6. Results from biolog activity assay. The measured quantities represent the difference in colorimetric readings between the final and initial readings. Each substrate is listed in the grey tab above its graph, and the aboveground covers are indicated as follows: Cladonia rappii (■); Cladonia subenuis (●) Cladonia uncialis (▲); Polytrichum commune (moss, ◆); Pine needles (●). In 3 substrates, N-acetyl-D-glucosamine (F-value: 3.7804; p = 0.04007) , D-glusaminic acid (F-value: 10.719; p = 0.001223) and A-D-lactose (F-value: 3.7191; p = 0.04188), aboveground material did have an effect on substrate utilization; these are highlighted in grey.

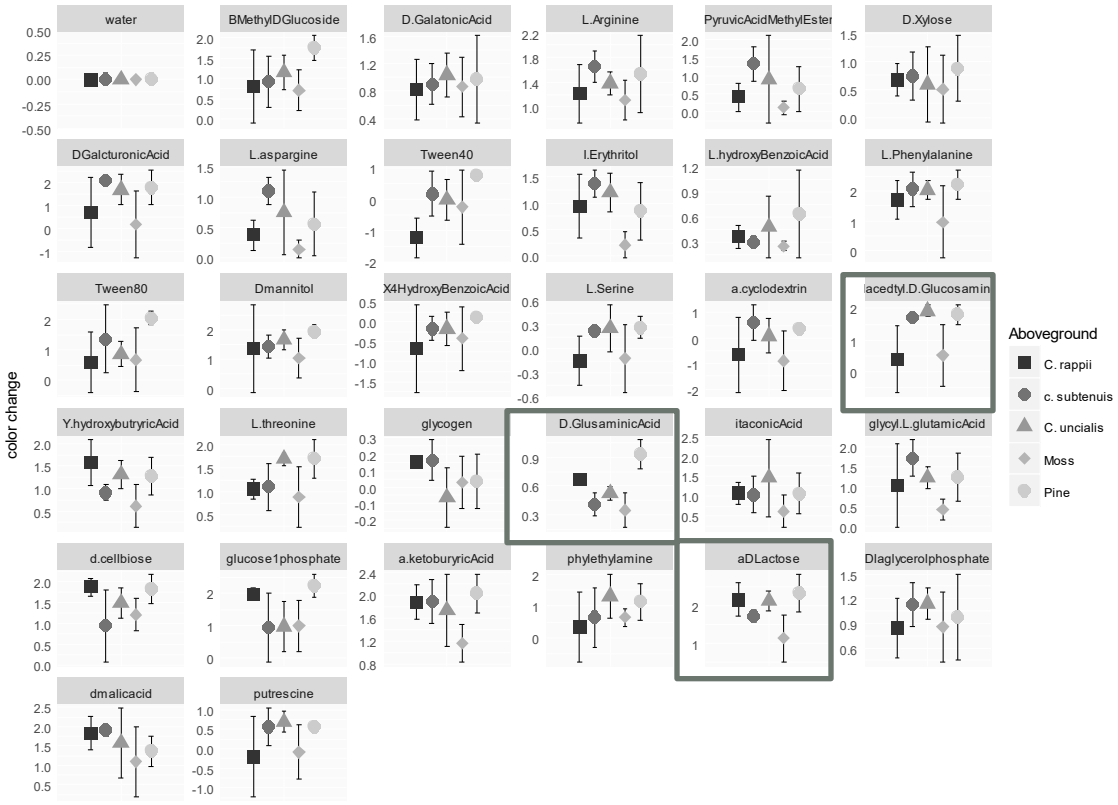


Figure 7. Arthropod density beneath different soil covers. Ground covers are designated as: Bare (■) = ground cover removed, Lichen (●) = 100% cover of lichen, Mixed (▲)= mixed leaf litter (mixture of *Vaccinium*, *Quercus* and *Pinus*) and Pine (◆) = pine needles. Points represent mean of 3 samples each, bars represent standard deviation. Arthropods extracted from 3 soil samples from cores 5cm wide x 5cm deep were pooled for each data point, and these values were converted to individuals per square meter. Sample times are indicated by two digit year followed by three letter month. In the fall, lichens may promote higher arthropod densities than other cover types, but this difference is non-significant

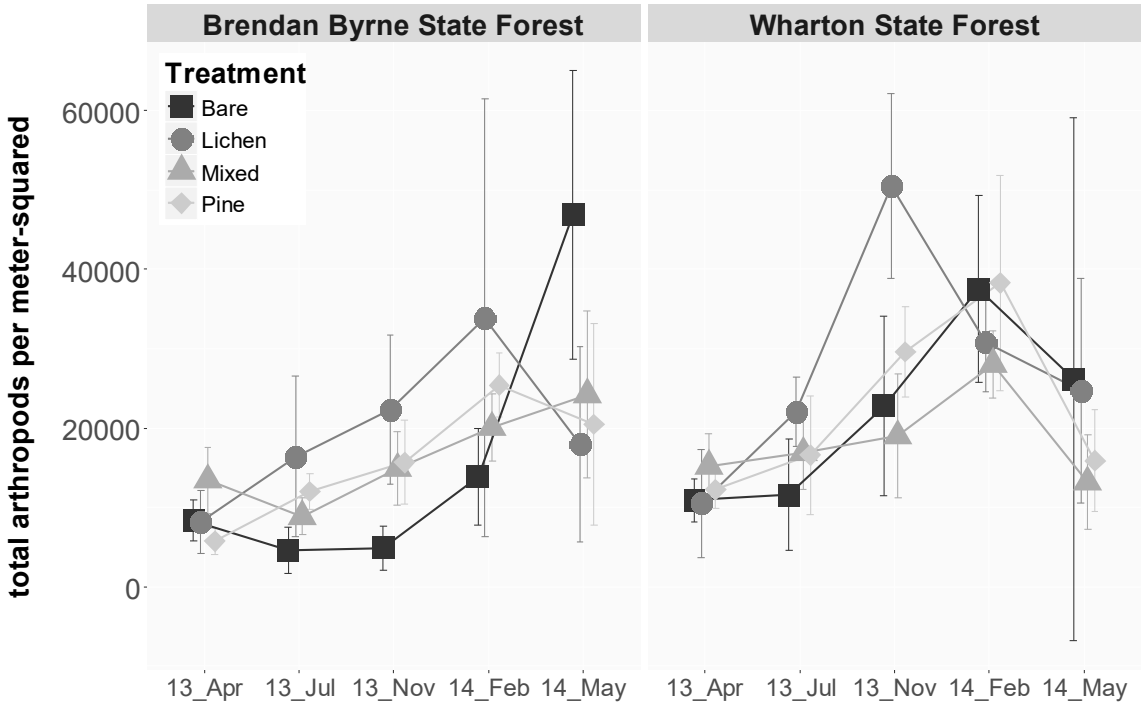


Figure 8. In total arthropod sampling over 1 year, including 4 sampling periods, average arthropod density was higher below lichens than it was below other cover types, but not significantly higher (p=.08); predator density was significantly higher (p=.0003). Bars are color coded according to arthropod presence: oribatid mites (including juveniles)= light grey; predatory mites (including mesostigmatids, prostigmatids and astigmatids)=medium grey; collembolans=dark grey; other organisms=black.

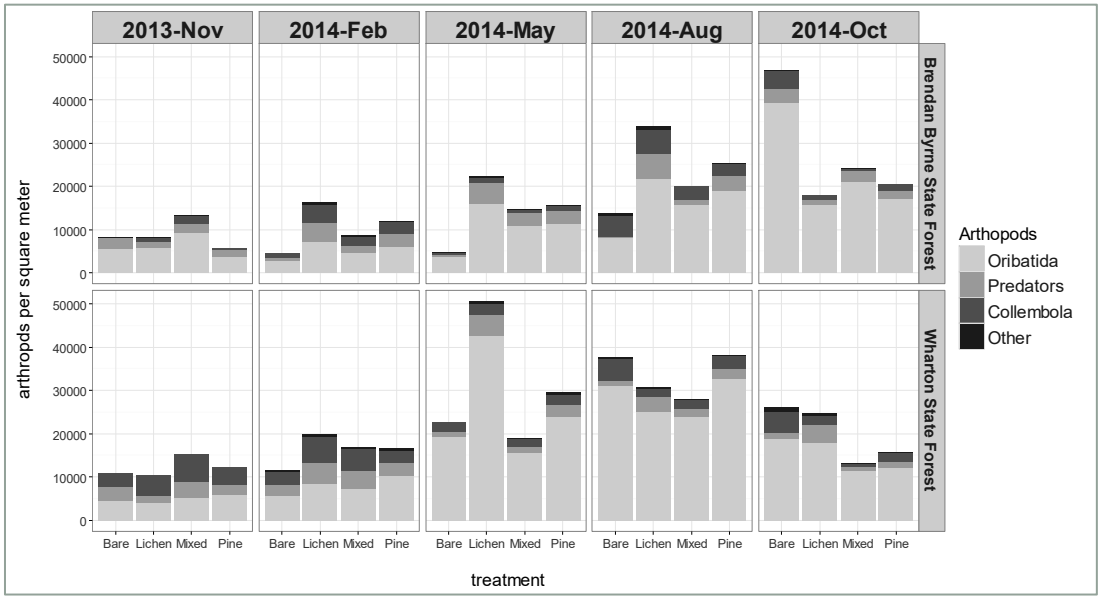
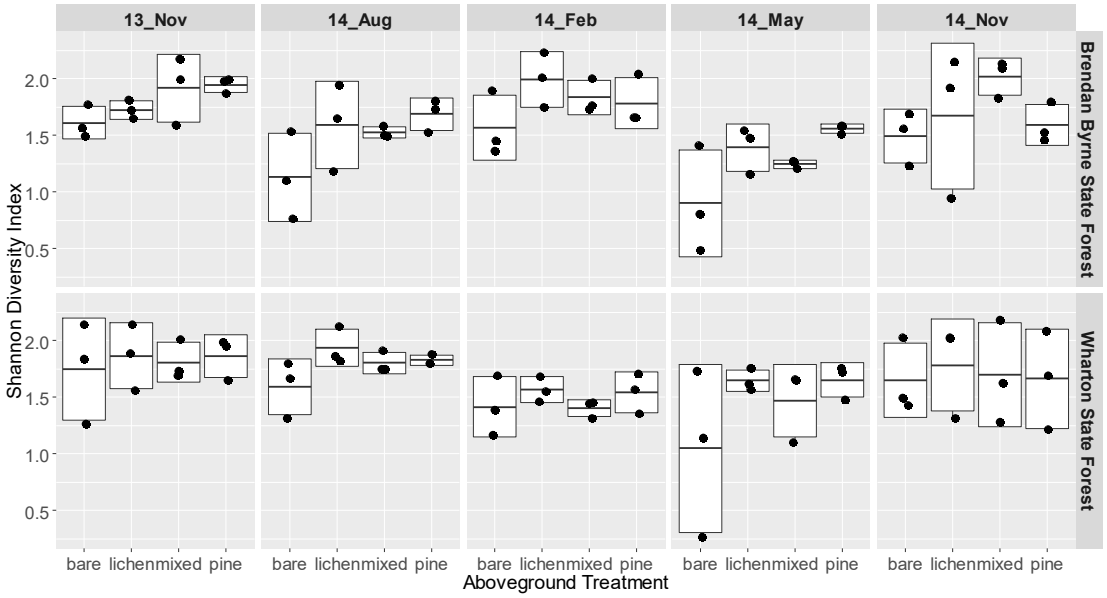


Figure 9. Arthropod diversity did not respond to aboveground cover. Diversity values are calculated using the Shannon-Weiner diversity Index: H'=-$\sum_i^R pi * \ln(pi)$, where H' is the diversity value for a site, R is the total species list for the site and p_i is the proportion of the ith species (number of individuals of species i divided by total number of individuals at the site). Data is displayed for November 2013, February 2014, May 2014, August 2014, and November 2014, and data for both Brendan Byrne State Forest and Wharton State Forest are presented.



4 Lichen interception of nitrogen pollution

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Abstract.

We were interested in determining how soil lichen mats influence nitrogen cycles in the NJ Pinelands (USA). We applied nitrogen (ammonium nitrate; 0, 5, 50 kg N Ha^{-1} yr^{-1} equivalent) to mesocosms that included 5cm deep of native homogenized soil with a cover of either: nothing, lichens (*Cladonia subtenuis*), or false lichens (polyester mesh). Ground cover had significant influence on leaching of ammonium and nitrate and, at high levels of N deposition, on soil accumulation of ammonium and nitrate. There were no significant differences between ammonium and nitrate content of soils covered with lichens and with the fabric lichens. Lichens were able to accumulate some of the excess nitrogen, especially in their growing tips, but lichen accumulation of N did not increase linearly with N added, suggesting potential N saturation of lichens or potential P limitation of those sites. These results demonstrate that lichens are able to mitigate the effects of nitrogen pollution in soils when the N load is moderate, but that their capacity to perform this function is overwhelmed at the highest levels of N deposition, and that the mechanism of action of lichens in N protection of soils is physical rather than biochemical.

Introduction

Anthropogenic emissions of airborne nitrogen have profound effects on the plants and soils where they land (Matson et al., 2002). Most anthropogenic nitrogen pollution worldwide is in the form of ammonia (Krupa 2003) and most of this is produced by animal wastes, chemical fertilizers and biomass burning, (Bouwman et al. 1997); nitrate, which is the dominant form of N pollution in the United States (USEPA, 2002) comes from burning fossil fuels (Lee et al. 1997). Most NO_x (NO, NO_2) and NH_x (NH_3 and NH_4^+) emitted to the atmosphere is transferred back to the surface of the Earth within hours or days (Galloway et al. 2004).

N addition may be a principal driver of ecosystem change in temperate and boreal forest and grassland landscapes (Magnani et al., 2007), and acidic soils are particularly vulnerable (Simkin et al 2016). N addition drives changes in plant communities by changing competitive dynamics, herbivory, symbioses and disease processes (reviewed by Gilliam et al. 2006). N addition can also have widespread ecosystem impacts via soils; ammonium deposition can lead to soil acidification (Nilsson et al. 2006), base cation depletion (Horswill et al. 2008), and solubilization of toxic metals (Stevens et al. 2009), and nitrate leaching can contaminate groundwater and lead to eutrophication of surface waters (Galloway et al., 2004) and can also contribute to soil acidification (Ruess and Johnson, 1986).

For lichens (fungi that derive their C from a symbiotic alga or cyanolichen), N addition drives changes in their communities (Davies et al. 2007; Wolseley et al. 2006) and in their chemical composition (Hyvärinen and Crittenden 1998c). Lichens may also mediate the release of excess N to other components of the ecosystem, as Knops et al. (1996) found; in their study epiphytic lichens in an oak woodland captured N from the

atmosphere, and when they became part of the soil litter, they slowly released that N, increasing soil N concentrations. We are interested in how lichens interact with soil N dynamics as the Knops et al. (1996) study suggests lichens may play an important role in N cycling that has been underinvestigated. To determine lichen influence on N movement through the system we used N-15 labelling, as Nordbakken et al. (2003) did to trace N deposition impacts on boreal bog plant communities, and as Blodeau et al. (2006) did to quantify N–retention by *Sphagnum*.

Accumulation of N compounds can be toxic to lichens, but many lichens have mechanisms to tolerate high levels of ambient N (Gaio-Oliveira et al. 2001). Lichens can take up nitrogen in the forms of ammonium or nitrate (Crittenden 1998) though at high concentrations, ammonium uptake is preferred (Dahlman et al. 2004; Ellis et al. 2005). Ammonium can be held in cation exchange sites in the extracellular space of the lichen's thallus, but those ammonium ions are easily lost to the environment (Miller and Brown 1999). Inside the cell, carbon skeletons can prevent cyctotoxic effects of intracellular ammonium (Hauck, 2010), and the ammonium can then be converted to amino acids (Cruz et al. 2006). For lichens to assimilate nitrate, the ion must first be reduced to nitrite and then to ammonium (Shapiro 1983), so the energetic costs of nitrate assimilation are higher than for assimilation of ammonium. Lichens do increase the concentration of N in their tissues as anthropogenic N deposition increases (Hyvärinen and Crittenden 1998a). N deposition can also change production of pigments including chlorophyll and β-carotene, and xanthins (Ochoa-Hueso and Manrique 2011). Our study clarifies where in the lichen the N is accumulated; if the N is accumulated in the upper edges it is likely incorporated as part of new growth into the tissues, whereas equal distribution of N

throughout the lichen suggests lichen use of N in protective compounds that are needed throughout the organism, or storage of N for future use.

The differential capabilities of lichen species to tolerate excess N means that nitrogen deposition also leads to changes in lichen communities. Some epiphytic lichens are recognized nitrophiles (described by Wolseley et al. 2008; Sutton et al. 2008). In contrast, few members of soil communities have been characterized as nitrophiles but Ochoa-Hueso and Manrique (2011) found increases in cover of *Cladonia folicacea* in the desert of Southern Spain with N deposition, and suggest that changes in cover and physiology of the lichen could be useful for biomonitoring N deposition. Some soil lichens have also been found to be sensitive to N deposition; Britton and Fisher (2007) found decreases in cover of *Cladonia* after 5 years at N deposition levels of 10, 20, and 50 kg N ha^{-1} y^{-1}; and Tomassen et al. (2004) found *Cladonia* cover decreases after just 3 years of lower N deposition levels (2, 4, and 8 kg N ha^{-1} y^{-1}) in ombotrophic bogs in Ireland. Our study involved *Cladonia subtenuis*, a lichen that is tolerant of N deposition, and survived the experiment even at the highest level of N-deposition, 50 kg N ha^{-1} y^{-1}.

Here we employ a manipulative experiment and stable isotopic labeling to examine how terrestrial lichens can moderate the effects of nitrogen deposition. As vascular plants usually invade areas where soil nitrogen is high enough, except where the soil is otherwise unsuitable for plant growth (Hauck 2010), we expect that lichen depression of soil N levels could be a mechanism for long-term persistence of soil lichen mats. The *Cladonia* lichens we studied depend on airborne N sources (Crittenden 1989) and are able to scavenge NH_4^+,NO_3^- and PO_4^{2-} from rainwater (Hyvärinen and Crittenden 1998a; Hyvärinen and Crittenden 1998b) and therefore we predict that the presence of

lichens will reduce nitrogen concentrations on the soils and water below them and that lichens use that nitrogen for growth of their own tissues.

Our goal was to see how much lichens influence soil nutrient cycles. We added 15-N labelled nitrogen to follow the fate of N deposition into lichen cover or the supporting soil. Further, due to the labelling approach, we were able to determine where in the lichen the nitrogen is retained and incorporated. Dahlman et al. (2002) carried out such analysis on N-location in two foliose arctic lichens after treatment with N-15 under controlled lab conditions, and found that lichens accumulated N in their growing tissues; similarly (Hogan et al., 2010) found N accumulation in *Cladonia portentosa* tips in their lab study so we expected that *Cladonia subtenuis* would similarly accumulate N in its growing tips. If there were no difference in N accumulation with high levels of N deposition, we would assume that the lichens were N-saturated. Since our study was conducted with N-additions in mesocosms outside, under the biological and biophysical conditions that exist in the field, we verify that processes previously studied in lab settings are occurring in lichen mats in the field.

1. Methods

The study was conducted in the growing season of 2014 at the Rutgers Pinelands Field Station, the U.S.F.S. Silas Little Experimental Forest, at 39°54’58.9 N, 74°35’52.4 W, in New Lisbon, Burlington County, NJ, United States. This forest is part of the Pinelands National Reserve and the forest there is dominated by pitch pine (*Pinus rigida* Mill) and several oak species, with an understory composed primarily of ericaceous shrubs. The soils are sandy with thin organic horizons; these soils are described by the USDA as Evesboro Sand, which are excessively drained soils, with sand overlying loamy

sand to 80 inches deep with 0-5% slopes (Web Soil Survey, 2016). During this period, the temperature ranged from a minimum of -17.6°C to a maximum of 33.5°C, with a total precipitation of 1.11 m (Clark, 2016). Soil available N is primarily in form of ammonium, and NH4-N per gram of dry soil range from 6 ug g^{-1} in the spring to 15 ug N g^{-1} dry soil in the fall (Dighton et al., 2004). Forman (1998) describes the landscape in more detail.

Cladonia subtenuis was the aboveground lichen used for this experiment, as it is one of most common lichens in the Pinelands of New Jersey and is abundant in the vicinity of the Pinelands field station. The family Cladoniaceae (Ascomycotina: Lecanorales) includes over 500 described species (Pino-Bodas et al. 2013), and new species continue to be found, especially in the Andes, in the Brazilian Highlands, and in the Guayana Highlands (Ahti, 2000). The genus *Cladonia* is ecologically diverse, and is widespread in the boreal forest and along the Atlantic coastal plain, most prominent in areas with acidic, sandy soils, an overstory of conifers, and an understory of ericaceous shrubs (Ahti 2000). Lichen-woodland forests may represent a stage along a post-fire or post-logging successional sequence, in which *Cladonia* species are common in the 40–100 years following the disturbance but mosses become more dominant as the canopy closes (summarized by Haughian and Burton 2015). Lichens may also be a final successional stage in some sites, where low soil nutrient availability prevents shrub invasion of lichen woodlands (Crittenden 1989). Many studies support the idea that fires are important for maintenance of *Cladonia* dominance in the understory (Zouaoui et al. 2014) but *Cladonia* also colonize other types of disturbed areas including metal contaminated areas (Howe and Lendemer, 2010), slag dumps (Osyczka and Rola 2013)

and reclaimed mine areas (Duncan 2015). The substrate preferences are also wide, and *Cladonia* may grow on rocks, sand, bark, and decorticate logs (Brodo et al., 2001).

To study how aboveground lichen cover influenced soil processes, we used small, controlled mesocosms (Figure 1). The mesocosms consisted of lysimeters, pans of soil with drainage tubes to trap and collect leachates in belowground bottles and some lysimeters included aboveground cover (lichens or plastic mesh). The plastic pans were 10.2 cm wide x 20.3 cm long x 10cm deep, with a mesh-covered drainage hole in the bottom that was attached to a drainage tube leading to a 1 L collection bottle. Collection bottles were fitted with caps with openings just large enough for the drainage tube to fit in with space for air to escape, but there was little space in the top of the bottle for contaminants to enter. Each lysimeter pan was filled with 5 cm deep of pinelands soil. Holes were dug for the assembly so that the soil in the lysimeters was at ground level. This was done to minimize effects of warming and drying of the lysimeters that would happen were the pans exposed to sunlight. We started the study on 1 May 2014, and finished it on 3 December 2014, with a run time of 216 days. We ran the collectors for 1 month before we started N additions, to make sure they were not leaky, and continued collecting for 1 month after the last N-15 addition to be sure to capture all the added nitrogen.

We created 36 of these mesocosms, and placed them in groups of 9 at different locations within 100m of the Rutgers Pinelands Field Station. We chose sites based two features: (1) there was a canopy opening at the site with no understory shrubs present, so nitrogen levels in our mesocosms would not be heavily influenced by throughfall from overhead pines (as needles and twigs both may leach nitrate (Lang, Reiners, and Pike

1980); (2) *Cladonia* species were already growing at that microhabitat, so we knew the environmental conditions were suitable for the season-long persistence of the lichens involved in our experiment.

To test the effects of lichens on passage of N through the soil we used 3 aboveground treatments in the mesocosms: lichens, false lichens, or bare soil. The false lichens were polyester mesh sewn with polyester thread to cover approximately the same dry mass and volume as the lichens (13g / 206cm^2). The polyester lichens were designed to reproduce the abiotic conditions the lichens created (surface shading, and moisture retention) without the biology involved in lichens (lichen associated microbiome, and lichen-associated secondary compounds, photosynthesis and respiration).

We treated the mesocosms with one of three levels of nitrogen deposition to determine if the soil nitrogen regulation depended on nitrogen quantity present. We used 3 treatment levels: addition of 0, 10, or 50 kg N · ha $^{-1}$ yr^{-1} of ammonium nitrate in which 10% of it is $^{15}NH_4{}^{15}NO_3$. 10% labeled N-additions were used by Nordbakken et al. (2003) and Xing et al. (2010) in studies of peatland ecology, and that concentration led to detectable levels of N-15 in their samples. The control mesocosms received only water, with no labeled N, and only the ambient levels of nitrogen deposition, 5 kg N · ha $^{-1}$ yr^{-1} (Dighton et al. 2003). The 10 kg N · a $^{-1}$ yr^{-1} mesocosms would be receiving 2 times the ambient nitrogen load, and the 50 kg N · ha $^{-1}$ yr^{-1} mesocosms would be receiving 10 times ambient conditions, which represents the N deposition at the most polluted sites recorded in Europe (Emmett et al. 1998).

The nitrogen was divided among 5 additions, once a month June-October in 2014; several other studies have also divided the additions across the growing season

(Nordbakken et al., 2003; Xing et al. 2010), as that will allow the organism to assimilate the N in a more representative way than the addition of all the n in one pulse would be. The nitrogen was added in 125 mL increments, which is comparable to a rain event of .6cm over the 206 cm^2 mesocosm area; this represents our system since, when it did rain over this period, the average rainfall was 0.8 cm.

This arrangement of 36 mesocosms included 4 replicates x 3 aboveground treatments x 3 N addition levels. We collected the rain from the bottles when they were over half full, and froze a 5% subsample from each bottle. At the end of the experiment, we analyzed the nitrogen concentration in this total water sample and in the soils.

Soil chemistry: Soil chemistry samples were taken on field moist soils within 24 hours of sampling in the field. A subsample was weighed, then dried at 70°C to constant weight and re-weighed to calculate the percent moisture. The subsample was then incinerated in a muffle furnace at 250°C to calculate the loss on ignition. Ammonium and nitrate concentrations were determined by extracting the ions into solution by shaking 10g of field moist sample with KCl for one hour, vacuum filtering the material, then freezing the filtrate, which was analyzed later on an Astoria Pacific autoanalyzer. Total Soil C and N were determined by shaking 10g of field moist soil for one hour with potassium sulfate solution; we froze a filtrate sample from this solution and analyzed this sample on a Shimadzu C/N autoanalyzer.

Isotope analysis: We collected samples of the soils, the lichens, and the false lichens for analysis of the N-15 enrichment over the course of the experiment. On each lichen, samples were taken from the growing apices (top 25mm) and basal portions (bottom 25mm); fabric samples were taken from the top 25mm only. Samples were

ground in a Wiley mill that was ethanol rinsed between samples, were packaged into tin cylinders, and were analyzed for atmospheric % of N-15 at the University of California at Davis Stable Isotope Facility. We calculated N enrichment in samples using the formula used by Dahlman et al., (2002) and Nordbakken et al., (2003): Relative N uptake = $[(15N_s\text{-}15N_c) \times (totN/.1)]$, where $15N_s$ is the %15N of the sample , $15N_c$ is the %15N in the control, totN is the total N concentration (sum of both N isotopes; g^{-1} dw), and 0.1 corrects for the fraction of labeled N in the N additions (10%). We compared these N enrichment values for lichen, fabric and soil for each treatment type using MANOVA in R (R Core Team, 2015).

Results

Overall results: The recurring theme of this study was that aboveground lichens had effects on the soil that were statistically indistinguishable from the effects of fabric cover.

Influence of lichens on soil moisture and water leaching. Our results demonstrate that lichens contributed to temporary retention of soil moisture (Figure 2), but that lichens contribute no more than any other kind of shade to increasing the total amount of water that passes through the soil (Figure 3a). The presence of aboveground lichens did mean there was less leaching of ammonium to the soil water below compared with fabric cover (Figure 3b) but aboveground cover had no significant on leaching of nitrate (Figure 3c).

Influence of lichens on soil chemistry: Our analysis of soil chemical properties (Table 1) demonstrates that aboveground cover is important in soil processing of ammonium and total N. Aboveground cover has no effect on soil nitrate levels (Fig 4b). Lichens or any soil shading prevent buildup of ammonium in the soils; this influence is

only significant at very high levels of N deposition (Figure 4a). Lichens and fabric also have the same effect on organic N buildup in the soils (Figure 5a). This organic N is not associated with increases in nitrogen in microbial biomass N or C (Figures 5b, 5c). Aboveground cover also made no significant difference to total soil carbon, or loss on ignition.

Lichen use of added nitrogen. Lichens were able to use added nitrogen, even at the highest doses, since their tissues were enriched in N-15, and the growing tips of the lichens were more enriched than the basal sections (Figure 6a). Aboveground fabric, and any microbiota associated with them did not retain added N. Even though the fabric did not retain any of the added N, the N content of the soils below them was comparable to the N-enrichment of soils below the lichens (Figure 6b). ANOVA of lichen biomass change demonstrated that lichens w/ high N additions did not accumulate significantly more biomass (mean 14.7% increase in dry mass, standard deviation 10.4) than lichens without N additions (mean 12.5% increase in dry mass; standard deviation 5.6).

Model of Effects of N deposition on Pinelands Soils. Comparison of the total N accumulated in the mesocosms before vs after the different N additions generates the data portrayed in Table 2 and Figure 7, showing percent changes in Total N. There are no significant effects of aboveground cover on % changes in total soil N, but fabric and lichens were significantly different in their % change in Total N. Increasing the N addition lead to increasing total N leaching into groundwater, but fabric cover led to a smaller % change in N leaching than bare or lichen cover did.

Discussion:

Summary: Our hypothesis that the presence of aboveground lichens leads to changes in soil nutrient cycling that are distinct from other types of ground cover was not supported by our findings Overall, lichen effects on N retention in soils were statistically indistinguishable from to the effects of fabric cover. This comparison suggests that the main mechanism through which lichens reduce N buildup in soils is a physical one. However, since this study took place over only 6 months, lichens may have chemical or biological influences on soils that build up over larger time periods.

Soil moisture: Our findings were consistent with results of other studies (Chamizo et al. 2013; Porter and Woollett 1929) that show that lichens can contribute to retention of soil moisture. The fact that there were no significant differences in soil moisture retention under lichens and under fabric lichens suggests that the shading aspect alone of the lichen may be more important than the ability of the lichen thallus to retain water above the soil. Lichens were shown to be important in capture of moisture from dew, fog, and high humidity (Lange et al. 2007; Stanton et al. 2014) in deserts, but in our temperate system, the moisture absorbing capability of lichens may be less important, since soils directly receive so much water here. In Stanton et al.'s (2014) study of water interception by epiphytes, the presence of epiphytes on vascular plants aboveground increased soil moisture significantly during dry periods, but did not have significant effects during moist periods, supporting the idea that soil lichens might also be less important for soil moisture during moist periods in our ecosystem. Our soil moisture study captured only the effects of the lichen on soil moisture on the day of the harvest: the results of a separate long term lichen transplant study we performed indicate that lichens have more importance for soil moisture retention during drier periods. Since the

relative humidity of the harvest day was 98%, and since it had rained 4.2 cm the day before and 2.5 cm the night before the harvest (Clark, 2016), this harvest day represented a wetter than usual period, so the yearly importance of lichens for soil moisture retention are underrepresented in our measurements.

Soil nutrient cycling: In high N conditions, lichens and fabric suppressed total soil N (Figure 6b), driven mostly by the fact that both lichens and fabric cover lowered soil ammonium concentrations compared with bare soils (Figure 4a). The presence of the lichens did not affect the soil nitrate concentrations (Figure 4b); nitrate concentrations in these soils are very low and ammonium is the dominant form of available N in these soils (Dighton et al. 2004). Additionally, Lewis and Kaye (2012) have found that abiotic nitrate retention is important in forests, so the fact that biological activity of the lichens did not change belowground nitrate concentrations should not be surprising.

This suppression of soil N is in contrast to previous work on of the effects of epiphytic lichens on soil N. Cyanolichens, which affiliate with cyanobacteria as their photosynthetic partners, are able to fix nitrogen and Antoine (2004) found that in the forests of Washington State , Pacific NW, USA that she studied, the cyanolichen *Lobaria pulmonaria* could fix 2.6-16.5 kg N ha^{-1} y^{-1}, depending on biomass. This N may be retained in the system and made available for plant use as the lichens decompose (Pike 1978). Even lichens that are not fixing nitrogen still enhance the receipt of N from the atmosphere where they were growing as Knops et al. (1996) found in their study of fruiticose epiphytes in California, USA. In their study, the N additions to ecosystem did not change tree growth or soil nutrient status since the pool of soil N was so large. In lower nutrient systems like ours, the lichens' interception of N and delivery to soils

would be more important, relative to soil N concentrations, but we found no increases in soil N with lichen cover. This might be because our study (6 months) was not on the time scale at which lichen decomposition would contribute meaningfully to soil N status.

The lichens and fabric cover promoted transformation of inorganic N to organic N compounds in soils (Figure 5a), but these changes were not represented as significant increases in microbial biomass N below the lichens and fabric lichens (Figure 5b), or as significant increases in aboveground biomass. Lichens produce many organic compounds, and since the lichens were not using the N to build more biomass, they may have been using the N for production of defensive compounds. Microbes growing on the lichen or the fabric at the interface of the soil and aboveground substrate may also have created these organic N compounds that were then leached into the soil.

When Knops et al. (1998) considered the epiphytic lichen contribution to soil nutrient cycling, they included lichen decomposition, and decomposing lichens added organic N to soils. The decomposition of the lichens could have added some organic N to the soils, but the organic N below the fabric cannot be explained in this way since the fabrics did not lose mass over the course of the experiment. The fabric led to slightly more leaching of ammonium into the groundwater (figure 3b) and the lichens led to aboveground retention of N (fig 6a); these combined effects could explain why lichens and fabric had similar effects on soil ammonium concentrations.

That the lichens' effect on ammonium was consistently stronger than the effect on nitrate ions in the soil (Figure 4a vs. 4b) or in the water leachate (Figure 3b vs. 3c) is consistent with previous findings that at high concentrations of ammonium and nitrate, lichens preferentially uptake ammonium (Dahlman et al. 2004; Ellis et al. 2005).

Additionally, Lewis and Kaye (2012) found abiotic nitrate retention taking place in abiotic matrices in the soil, so it makes sense that soil nitrate patterns are consistent across both the biotic (lichen) and abiotic (fabric) aboveground treatments.

Crittenden (1989) found that for small rain events, 100% of the nutrients were intercepted by the lichens. Since all of our rain events were larger (0.6 cm), our calculations of lichen interception of nutrients were smaller. We chose this amount of rainfall, since it represented an average, but lichens would likely intercept more nutrients on smaller rainfall events and fewer on large rainfall events.

Crittenden (1989) argues that mat-forming lichens possess mechanisms for tight nutrient cycling. Our prediction was that the lichen would use the added N and reduce N enrichment in the soils below them, and our evidence corroborates this claim. Even at high levels of N deposition, soils under lichens receive much less ammonium than soils without ground cover. But this tight cycling may have less to do with the lichen itself than with the presence of any type of ground cover, a claim that is supported by the fact that lichens and fabric have similar effects on patterns in total N in the soils (Table 2).

N use in the lichen: Our N-15 approach allowed us to conclude that lichens accumulate N in their growing tips, and thus are actively taking up added N in the field, even when (as in our study), these N additions are at higher concentrations than the lichen usually experiences in the field. These findings align with the results of Hogan et al. 's (2010) lab study of *Cladonia portentosa* in Finland, in which the N concentration in lichen apices increased with total wet deposition of nitrogen. Hogan et al. (2010) suggested that at low levels of ambient N deposition, the concentration of N at the apices of the lichens are sensitive to changes in N deposition. Our study suggests that even with

the 5 kg N Ha^{-1} yr^{-1} of N deposition present at this site in the Pinelands, (Dighton et al., 2004) the *Cladonia submitis* still uses the N it encounters for apical growth.

Implications: The decreases in soil N concentrations below the lichens and fabric in conditions of high N deposition could have important implications for the rest of the ecosystem. Johnson et al. (1998) found that N addition had different effects on microbial biomass in different soils: in a P-limited acid grassland, it decreased microbial biomass, and in an N-limited peatland, it increased microbial biomass, and in a calcareous grassland, it had no effect on microbial biomass. Our findings of no changes to microbial biomass carbon (Figure 4c) are aligned with their findings from the calcareous grassland, which is counter-intuitive since the pinelands soils are so acidic and oligotrophic. However, both of the calcareous grasslands in Johnson et al.'s (1998) study and our soils were sandy soils that had low values for % mass loss on ignition, which is proportional to soil carbon. In these systems it seems, with low levels of soil organic matter as a carbon source for microbial biomass, other organisms (in our case, lichens) may regulate N cycling to a larger degree than the microbes do.

Another explanation for the lack of a microbial biomass C or N response to N addition may be that the soils were not N limited. Since our experiments tested mesocosms in which no plants were growing and only aboveground non-plant cover existed, the lack of demand for nitrogen from plant roots may have meant that there was more N than the microbial communities could use, even in the control plots. Indeed, Dighton et al. (2004) concluded that NJ Pinelands sites they studied were not N-limited, but rather P-limited.

The similar patterns in soils and water below lichens and fabric suggest that the physical structure of the lichen may be more important than the biological activity in regulation of belowground processes in temperate forests. This is a markedly different conclusion from research conducted on primary substrates, where chemical excretions of lichens contribute to primary weathering and soil formation (Asta et al., 2001; Jackson, 2015). However the importance of biological and chemical activities of lichens on soil nutrient cycling may simply take longer to develop than the time period of this study allowed.

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Figure 1. Arrangement of Mesocosms. Plastic lysimeter trays were filled with soil and an aboveground cover and connected to a drainage pipe and collection bottle. The arrangement was placed outdoors in holes so that the soil in the lysimeter was at the same level as the surrounding soil.

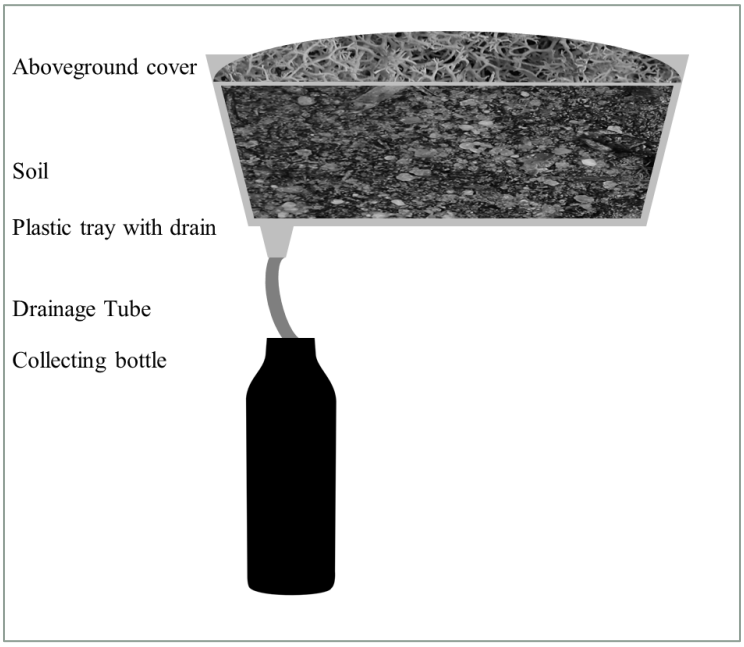


Figure 2. Lichen influence on soil moisture. Boxplots include a horizontal line to represent mean values, and vertical lines to represent standard deviation. Groups with different letters are significantly different from each other using a Tukey HSD test. Soil moisture on the day of harvest (3 December 2015). More water is temporarily retained in soils under lichens.

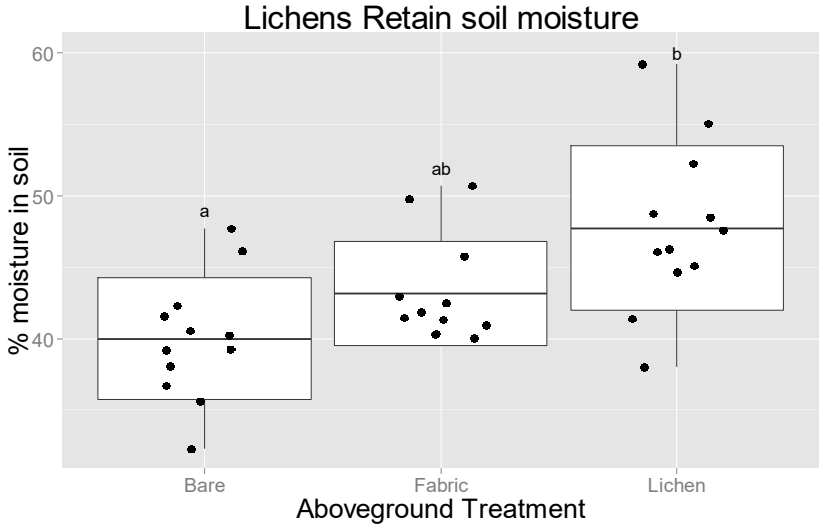
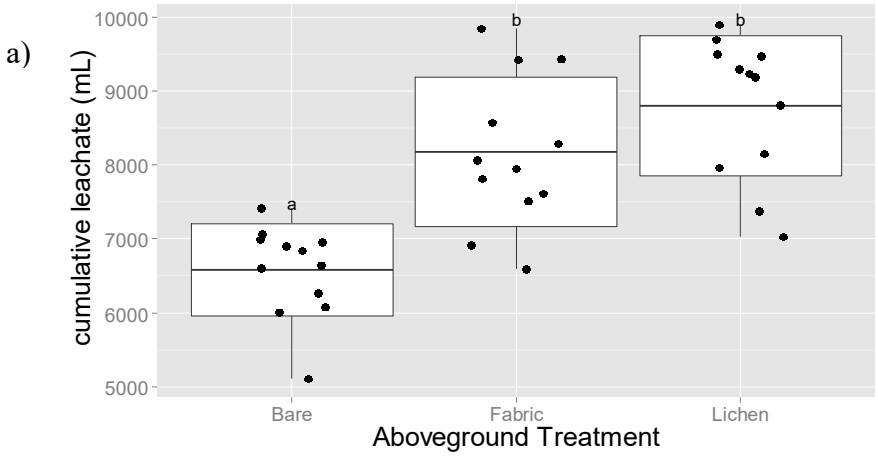
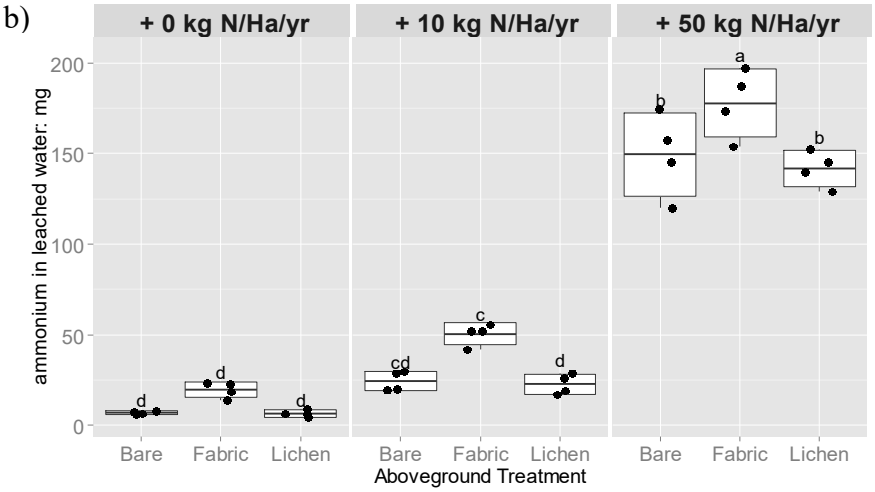


Figure 3. Lichen influence on water leached through soil. Boxplots include a horizontal line to represent mean values, and vertical lines to represent standard deviation. Groups with different letters are significantly different from each other using a Tukey HSD test. (a) Cumulative water leachate volumes throughout the experiment. Leachate values were indistinguishable between lichens and fabric treatments. (b) Total ammonium leached to groundwater throughout the treatment. The most ammonium was leached under fabric treatments. (c) Total nitrate leached to groundwater.





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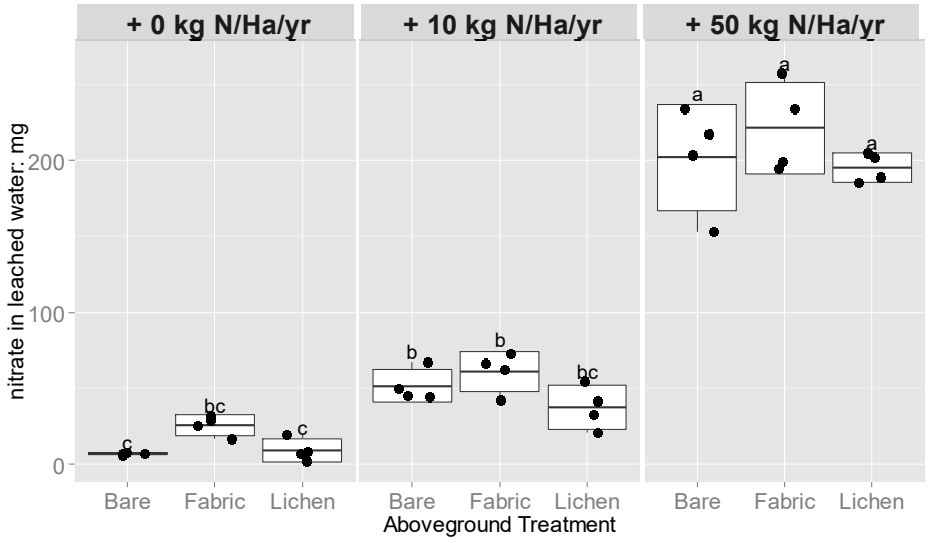

+ 0 kg N/Ha/yr
+ 10 kg N/Ha/yr
+ 50 kg N/Ha/yr
nitrate in leached water: mg
0
100
200
c
bc
c
b
b
bc
a
a
a
Bare
Fabric
Lichen
Bare
Fabric
Lichen
Bare
Fabric
Lichen
Aboveground Treatment

Table 1. Results of one way Analysis of Variance of effects of aboveground treatment on selected parameters, for 3 levels of N additions. F-values and p-values are indicated. Significant responses are the 95% confidence level are in bold.

	0 kg N ha^{-1} yr^{-1}		5 kg N ha^{-1} yr^{-1}		50 kg N ha^{-1} yr^{-1}	
soil moisture	F_0.145	p_0.867	F_5.068	**p_0.034**	F_8.266	**p_0.009**
loss on ignition	F_0.543	p_0.599	F_0.476	p_0.636	F_1.128	p_0.366
total water leached	F_8.742	**p_0.008**	F_5.322	**p_0.029**	F_4.517	**p_0.044**
total ammonium leached	F_18.02	**p<0 .001**	F_31.93	**p<0 .001**	F_6.653	**p_0.017**
total nitrate leached	F_10.83	**p_0.004**	F_5.714	**p_.025**	F_50.63	**p_0.034**
final soil ammonium	F_1.047	p_0.390	F_4.589	**p_0.042**	F_27.63	**p<0 .001**
final soil nitrate	F_2.282	p_0.158	F_1.5	p_0.274	F_3.395	p_0.080
final soil total carbon	F_3.051	p_0.097	F_0.366	p_0.704	F_0.144	p_0.867
final soil total nitrogen	F_11.85	**p_0.003**	F_24.76	**p<0 .001**	F_6.413	**p_0.019**
final soil microbial biomass C	F_2.294	p_0.157	F_1.666	p_0.242	F_0.524	p_0.609
final soil microbial biomassN	F_1.075	p_0.382	F_0.387	p_0.690	F_1.05	p_0.389

Figure 4. Effect of cover on soil inorganic nitrogen concentrations at different N addition loads. (a) Soil ammonium. Any aboveground cover kept soil ammonium low compared to the no cover treatment, in conditions of high N deposition. Concentrations are displayed as ug-NH^{4+}-N per gram dry soil, n=4 replicates. (b) Soil nitrate + nitrite. There was no influence of aboveground cover on soil nitrate + nitrite, and these levels were very low compared to the concentrations of ammonium in the soil. Concentrations are displayed as ug-NO_3^- + NO_2^--N per gram dry soil, n=4 replicates.

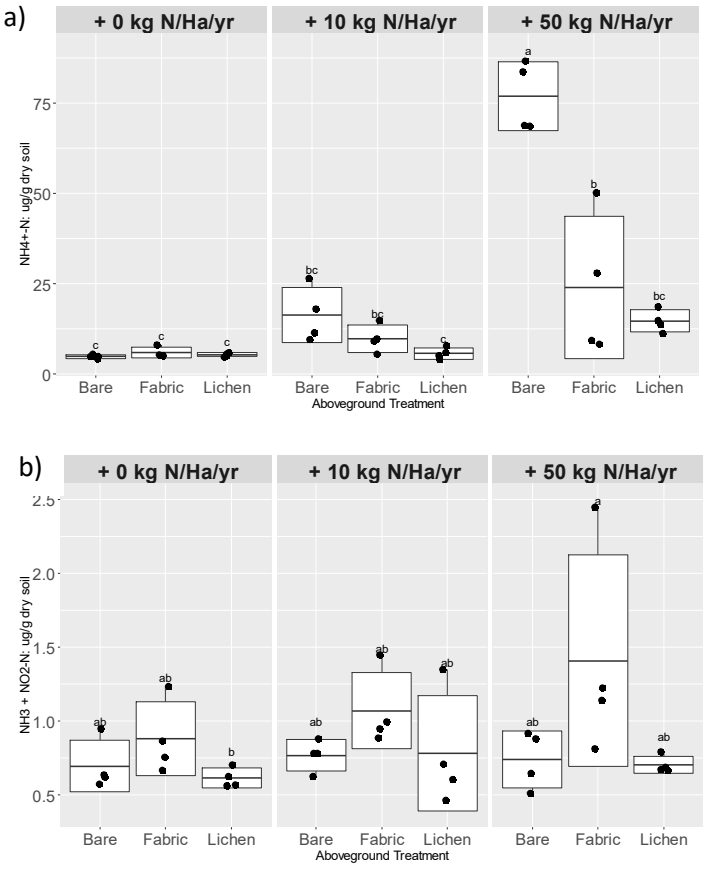
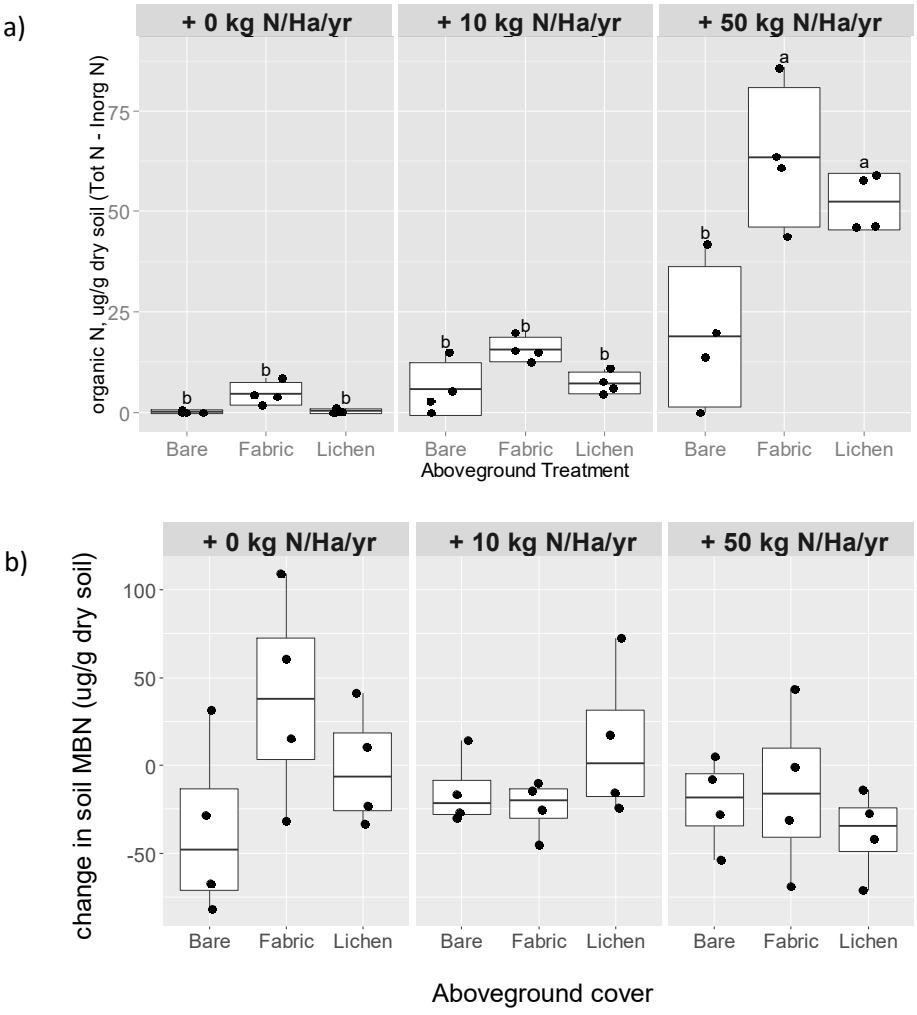


Figure 5. Effect of cover on soil organic matter. (a) Effect of cover on organic N in soils (ug N/g dry soil; calculated from soil total N – soil ammonium - soil nitrate) at different N addition loads. With the highest N-deposition scenario, lichens kept soil organic nitrogen higher, but this effect was only significant from fabric cover. (b) Effect of cover and N addition on microbial biomass nitrogen in soils. N treatment and aboveground cover had no significant impact on microbial biomass nitrogen. (c) Effect of cover and N addition on microbial biomass carbon in soils. N treatment and aboveground cover had no significant impact on microbial biomass carbon.



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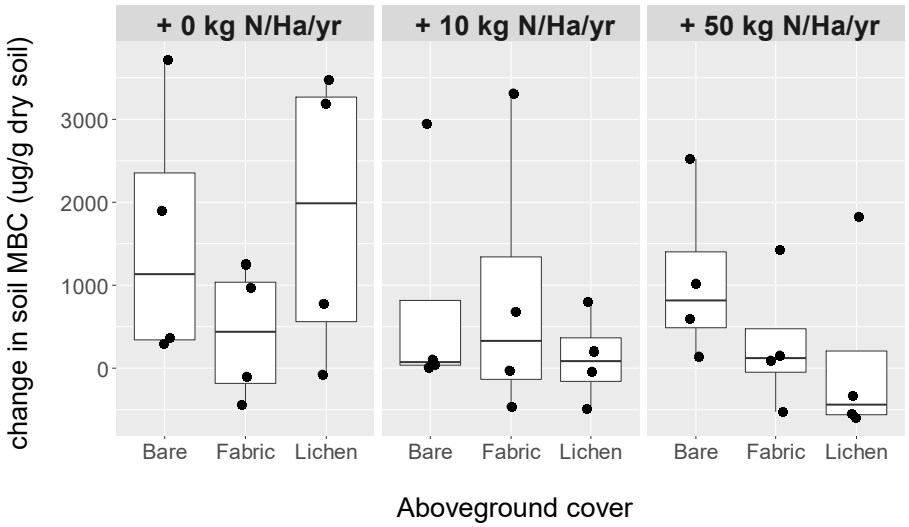

+ 0 kg N/Ha/yr
+ 10 kg N/Ha/yr
+ 50 kg N/Ha/yr
change in soil MBC (ug/g dry soil)
3000
2000
1000
0
Bare
Fabric
Lichen
Aboveground cover

Figure 6. Relative N uptake of lichens and soils in mesocosms. (a) Relative N uptake (gN / g dry weight) of lichen samples. Apices of lichens (top 25mm) took up more N than the bases of the lichens, and all lichen parts took up more N than the fabric did. (b) Relative N uptake (gN / g dry weight) of soil samples. Soils below lichen and fabric covers retained less of the added N-15 than the soil in bare areas did, and this effect was more pronounced at the 50 kg / N / Ha / year addition level.

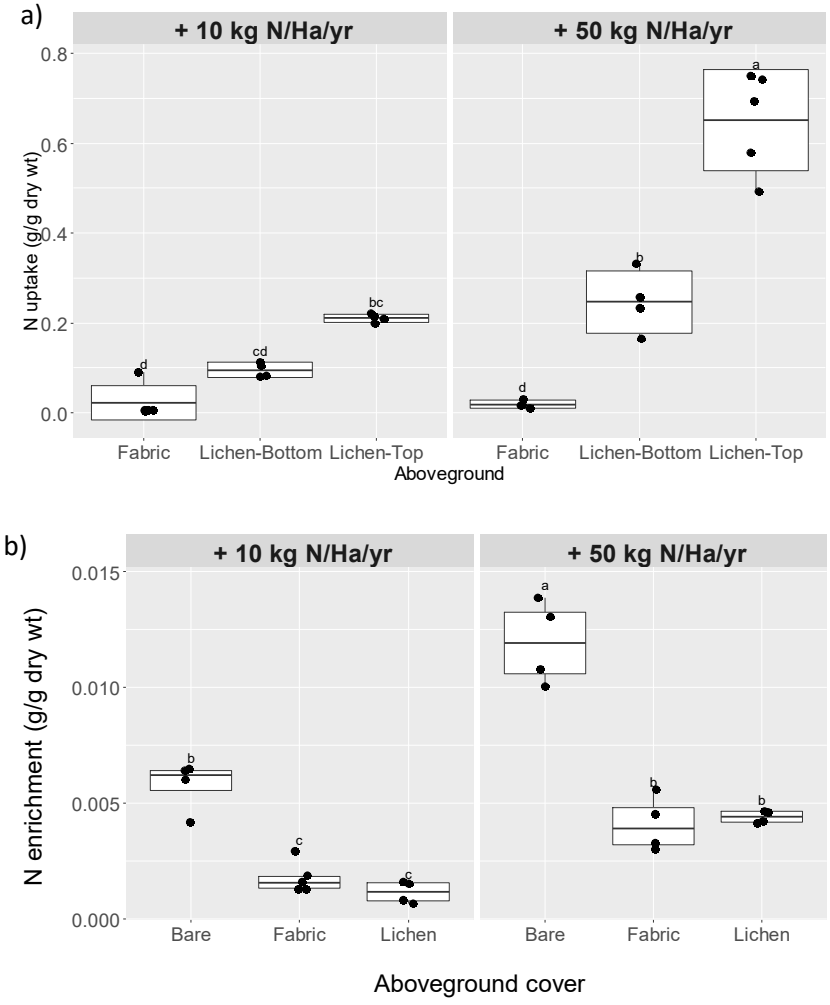
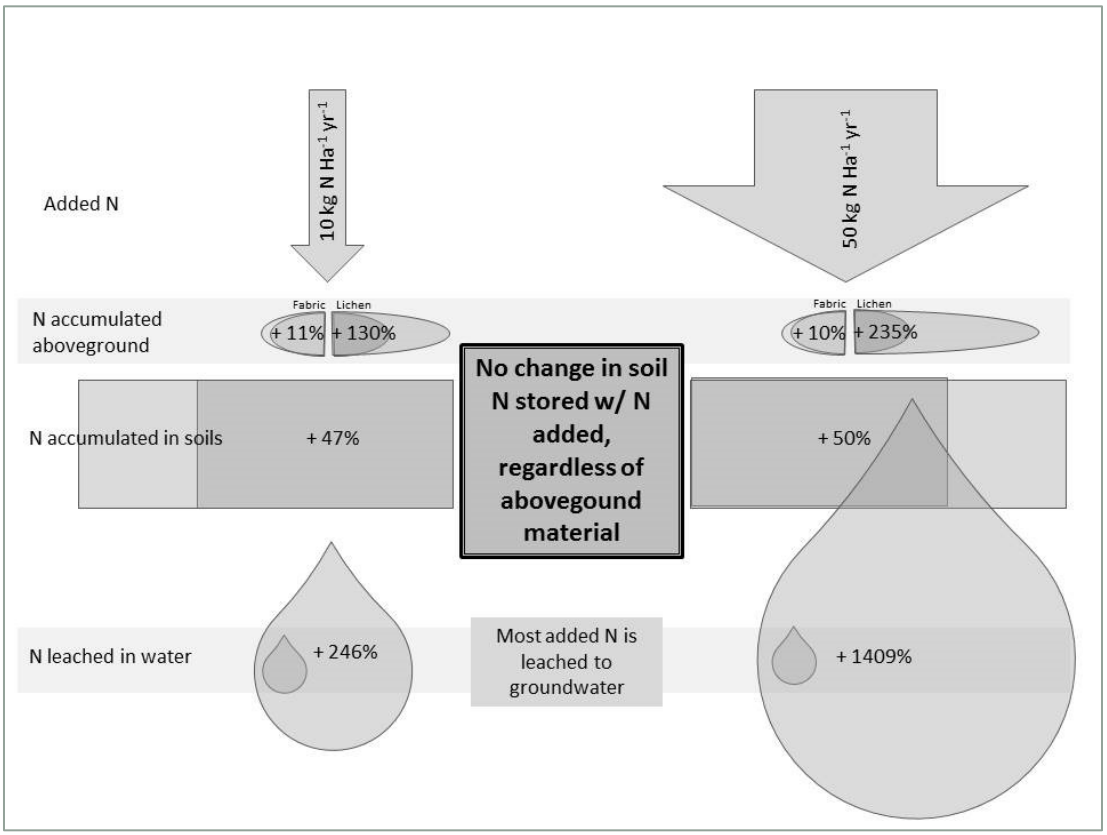


Table 2. % change in N storage in ecosystem compartments before and after N addition. Each value represents mean of 4 replicates with standard deviation. Significantly different values ($p < .05$) are indicated by subscript letters.

		10 Kg N Ha^{-1} yr^{-1}				50 Kg N Ha^{-1} yr^{-1}			
		Mean		st dev		Mean		st dev	
soils	bare	56.87%		14.17%		47.50%		10.13%	
	lichen cover	26.48%		10.27%		60.33%		25.58%	
	fabric cover	52.45%		17.73%		41.99%		20.56%	
aboveground	lichen	129.5%	b	27.28%		234.5%	a	84.93%	
	fabric	11.89%	c	13.69%		10.60%	c	21.87%	
leachate water	bare	330.2%	m	65.06%		1849%	n	420.7%	
	lichen cover	269.3%	m	145.4%		1737%	n	994.2%	
	fabric cover	137.5%	m	49.96%		640.5%	m	216.2%	

Figure 7. Schematic model of % changes in N storage in different ecosystem components over the course of the experiment. N addition is represented as arrows, aboveground material are represented as half ovals: Fabric (left) and Lichens (right), soil N is represented as a rectangle, and water N as a droplet shape. Darker patches represent control conditions, when no N is added, and light patches represent increase with the stated additions of N. Since there is no significant difference in N accumulation in soils or N leaching in water under lichens vs. under fabric, mean values for lichens and fabric treatments combined are represented in the soil and water fractions.



5 Appendix: Effects of lichens on nutrient capture in stemflow water

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Abstract:

An early study we conducted in 2011 contributed to our overall goal of investigating lichen contribution to forest function (nutrient flows, carbon cycling, and water dynamics) in the NJ Pinelands. As opposed to the other studies, which focused on the soil lichens, this initial study investigated the epiphytic lichens on the bark of oak trees in the Pinelands. We monitored how epiphytic lichens on the bark of *Quercus prinus* mediated nitrogen and carbon concentrations in water flowing down bark (stemflow). We found that though lichens did reduce total water leaching through trees, their effects on nutrients were minimal, and we attribute some of this lack of effect size to problems with the maintenance of structural integrity of our stemflow collectors.

Introduction:

Plant tissues have long been known both to leach nutrients and chemicals into precipitation (enriching throughfall and stemflow water) and to absorb ions from water. In areas where epiphytic lichens represent high cover, they can also influence precipitation chemistry. In lab studies (Lang et al., 1976) and field studies (Levia, 2002) bark lichens absorbed ammonium and nitrate from rainwater, and Dahlman et al. (2004) found that several different lichens are also able to take up organic nitrogen (Dahlman et al., 2004; Kielland, 1997). Additionally, bark dwelling lichens are able to sequester

phosphorus from stemflow (Zhang and Mitchell, 1995). The Pine Barrens of New Jersey represent a low nutrient system (Forman, 1998), so even small lichen influences on N and P fluxes could have important implications for forest growth.

A factor that we expected would influence nutrient uptake/release patterns in lichens is N supply rate. N deposition is an important factor in lichen ecology because atmospheric N deposition is increasing globally (reviewed in Galloway et al, 2008), and though many lichens flourish under conditions of increased nitrogen deposition, other species are sensitive and disappear in those conditions (Larsen-Visholm et al., 2009). N and light availability appear to be co-limiting factors for lichen growth (Palmqvist et al., 2008), but N toxicity may occur. Though many lichens may lack mechanisms to downregulate N uptake in N-enriched conditions (Hyvarinen & Crittenden, 1998), N tolerance may be linked to the ability to maintain the C:N balance between the photobiont and mycobiont in the lichen symbiosis (Palmkvist et al., 2008). So lichens may respond to N deposition with a decrease in the release of C-rich compounds, or with an increase in carbon sequestration (by allocation more N to photobiont growth; Palmqvist and Dahlman, 2006). With simulated N deposition in field conditions, the lichens Johansson et al., (2010) studied were assimilating the extra N available, even after several years of continuing deposition.

In our experiment, we subjected trees to varying levels of simulated N deposition (0,10 and 50 kg N Ha^{-1} yr^{-1}) to see whether N concentration influenced leaching of N, P, and C compounds from the lichens. We expected that at low levels, N would be retained by the lichens, but at higher deposition rates, the N would wash out of the system, as lichen growth would be limited by other nutrients, perhaps phosphorus as was found by

Hogan et al. (2010). We tested both lichen retention of inorganic N, representing anthropogenic pollution, and organic N, which is present in stemflow, particularly in the fall as deciduous leaves are senescing.

We also expected that lichens would lead to lower volumes of stemflow on trees, as lichens can absorb water. We thought initial water status could influence lichen compound leakage. Although they are poikilohydric organisms that can tolerate dry conditions, when lichens experience prolonged drought, they suffer many negative physical effects including membrane leakage (Kranner et al., 2008). When lichens are desiccated for extended periods, pigments may break down and reactive oxygen species may accumulate, leading to changes in photosynthetic activity and membrane integrity. Further, lichens are physically affected by the severity and length of precipitation events: Levia and Frost (2003) reviewed several studies that found less leaching of lichen compounds in heavy rainfall events, where water washes over tree surfaces more quickly.

Methods:

Replication and Tree selection: For this study we selected 24 trees from the area of the Rutgers Pinelands Research Station (Figure 1) in Burlington County, NJ, in an Atlantic Coast Plain pine-oak forest on Evesboro soils (mesic, coated lamellic quartzipsamments; USGS Soil Survey, 2012); this landscape is described extensively by Forman (1998). 12 of these trees had high lichen cover (> 50%) and 12 had low lichen cover (<10%) so the effect of lichen presence on leachate chemistry could be separated from reactions taking place in the bark alone. The 12 trees were divided into 4 treatments

with 3 replicates each: no nitrogen added, inorganic N (low concentration), inorganic N (high concentration), and organic N (low concentration).

Collection of leachates: On each tree, an aluminum gutter collected water flow across the trunk, attached to the trunk with silicone caulk so that no water would leach except through a plastic outflow collection hose. During natural rainfall, water was allowed to leak out the hose, but during collection periods, the hose was connected to 1L Nalgene collection bottles, held to the tree with an aluminum wire (Figure 2). All trees were living chestnut oak, *Quercus prinus*, canopy trees with diameter at breast height of 15-30 cm. All trunks were shaded by the leaves of canopy representing >70 % cover (approx). All lichen communities were relatively similar, dominated by *Flavoparmelia caperata, Parmotrema spp.*, and *Physcia millegrana*.

Nitrogen addition: Nitrogen was sprayed on tree bark in the form of NH_4NO_3 in quantities representing deposition rates of 0,10 and 50 kg N Ha^{-1} yr^{-1} which covers the ranges of actual nitrogen deposition in forests (Dighton et al., 2004). Johnsson et al. (2010) found no significant difference in uptake of nitrate and ammonium in field conditions, though lab studies showed a range of species took up ammonium more readily than nitrate (Dahlman et al. 2004). Organic N was added at 10 kg N Ha^{-1} yr^{-1} because this was found to be in the range of stemflow concentrations in oak forests (Carlisle et al., 1967). We used glycine as an indicator of total organic lichen N uptake because in a study of organic N uptake using several amino acids, Dahlman et al. (2004) found that glycine consistently represented about 10-20% of total amino acid uptake for lichens of different fungal families, growth forms, and photobionts. So each tree received 0, 0.02, or 0.10 g NH_4NO_3-N or 0.02 g glycine-N on the .25 m treatment area each

month. This was dissolved in 250 mL water. The following day, we washed the area with 250mL water. We applied treatments in the morning once a month for a year, from July 2012-July 2013; since lichen metabolism will vary seasonally, we wanted to capture 3 leachate snapshots per season.

Chemical analysis: In the leachates, we measured the total N, (divided into organic N, NH^{4+} and NO_2^- plus NO_3^-) and total C in leachates from lichen-covered bark (12 trees; 3 replicates x 4 treatments) vs. non-lichen covered bark (12 trees). The samples were frozen for storage and subsequently and analyzed for on ammonium and nitrate on an Astoria Pacific autoanalyzer and analyzed for total C and N on a Shimadzu C/N autoanalyzer

Statistical analysis: To analyze the data, we converted values from concentrations in collected water to total mg collected by multiplying volume collected x concentration. We present data for Total C as mg C leached from the tree. For N calculations, we subtracted mg N collected as leachate from the mg N added to determine the quantity of N retained on the tree. We conducted repeated measured ANOVA in SAS to determine the effects of lichen presence on N and C leaching and on total water volumes leached.

Results:

Repeated measures ANOVAs on the various measured parameters demonstrated that lichen cover did affect the volume of leachate water (F-value = 20.80, $p < 0.0003$; figure 3), and total nitrogen (F-value= 5.46, $p < .0328$; figure 4), but did not have significant effects on the leaching of carbon (F-value = 4.14, $p < 0.0587$; figure 5)

ammonium (F-value = 0.50, $p < 0.4882$, figure 6), or nitrate (F-value = 0.14, $p< 0.7124$, figure 7).

Figure 3 displays the consistent finding that bark with more lichen leached less water. The exception to this trend were the sampling dates on November 2013 and June 2014.

In the 50 kg N Ha^{-1} yr^{-1} inorganic N addition treatments, total N was retained on tree bark regardless of lichen cover at the beginning of the experiment during the September and October sampling dates (Figure 4). In September, this pattern was likely driven by bark retention of ammonium in the high N treatment (Figure 6). In the low N treatment, bark retained ammonium. Then in November, those trees leached more N than was added. Total N was retained on the tree in the glycine treatment May. Carbon was leached more from low cover trees in October and November (Figure 5), but there was no difference in C leaching throughout the rest of year.

Discussion.

That higher lichen cover led to more capture of stemflow water (Figure 1) is not surprising given the large literature about the ability of lichens to absorb water (reviewed by Beckett et al, 2008) The fact that lichen cover did not make any difference in water capture in November 2012 and June 2013 might be explained if the lichens had been already saturated and not able to capture any more moisture. On the June sampling date, there were 4.82 cm of rain before the sampling was finished at 4pm, but in November, there was no precipitation occurring that day (Clark, 2016). It had been freezing earlier in the morning, and PAR was low that day, so potentially there was little evaporation from the lichen.

N retention. The pattern of no response to N addition after December 2013 was consistent throughout the experiment (Figs 4, 6, and 7). This may be because the lichens had already been saturated after the initial additions. If this had been the case however, we would have expected to see the low N treatment (10 kg N Ha^{-1} yr^{-1}) trees continue to retain N in the months after the initial addition, while the high N treatment trees would be more quickly saturated. Additionally, one would expect the lichens to return to some degree of N retention in the summer, when they would be growing more. This did seem to occur in May of 2014, when lichens retained more organic-N than they had before (Figure 4), but inorganic N retention did not spike during that period.

We suggest that we saw no pattern in N retention because of the leakiness of the stemflow collectors. In the area where the aluminum collector hit the bark and the silicone caulk healed the cracks between the aluminum and the bark, the local drainage was poor, and the collector shaded the bark, so the bark in that immediate area retained moisture for longer than it usually would. This led to rotting of the bark in some places, so the caulk no longer sealed the collector, and leaking was observed after the treatment on many occasions. Since the leaked water was not collected, it would be counted as retained water, and the N dissolved in that water would be counted as N retained. Even if the volume leaked out of the collectors were small, if it was more than the volume of water used by the lichens, the differences in retention between lichen and non-lichen covered trees would not be observed, which was the case as the months progressed. We see this trend even in the water collections, in which total water retrieved from the collectors displays a decreasing trend across the period of the experiment, and the difference between trees with and without high lichen cover also decreases (Figure 3).

Likens and Eaton (1976) used polyurethane stemflow collectors in their study of stemflow chemistry at Hubbard brook. These collectors had a larger area of contact with the bark, so if the moisture were retained between the collector and the bark, it would be less likely to leach out of the collection apparatus altogether. Another solution to the leaching problem would be to plan a shorter study, although seasonality influences throughfall chemistry because of the growth and senescence patterns of leaves (Hamburg et al., 1998), so a seasonal study may not be scalable to reflect long-term patterns. This leaching of nutrients in stemflow might be hinted at by our October results from Figure 7, in which all trees, regardless of lichen cover and N treatment, leached more nitrate than was added.

The fact that bark with and without lichens did retain much of the added N at the beginning of the experiment (Figure 4), even in the treatment level at which we expected them to be overwhelmed by the high N concentration, suggests that bark conditions may play an interesting role in capture of nutrients from stemflow, but our study was not able to capture any long-term or large-scale implications of lichens in this process.

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Rutgers provided access to statistical tools and the online R community provided assistance with the statistical analyses.

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Figure 1. Location of tree sampling at the Rutgers Pinelands Field Station, Silas Little Experimental Forest, New Lisbon, Burlington County, NJ, United States. Yellow indicates low lichen cover >10% of .25m2 area of bark to which the water or N-treatment was added, and blue indicates high lichen cover <50%.

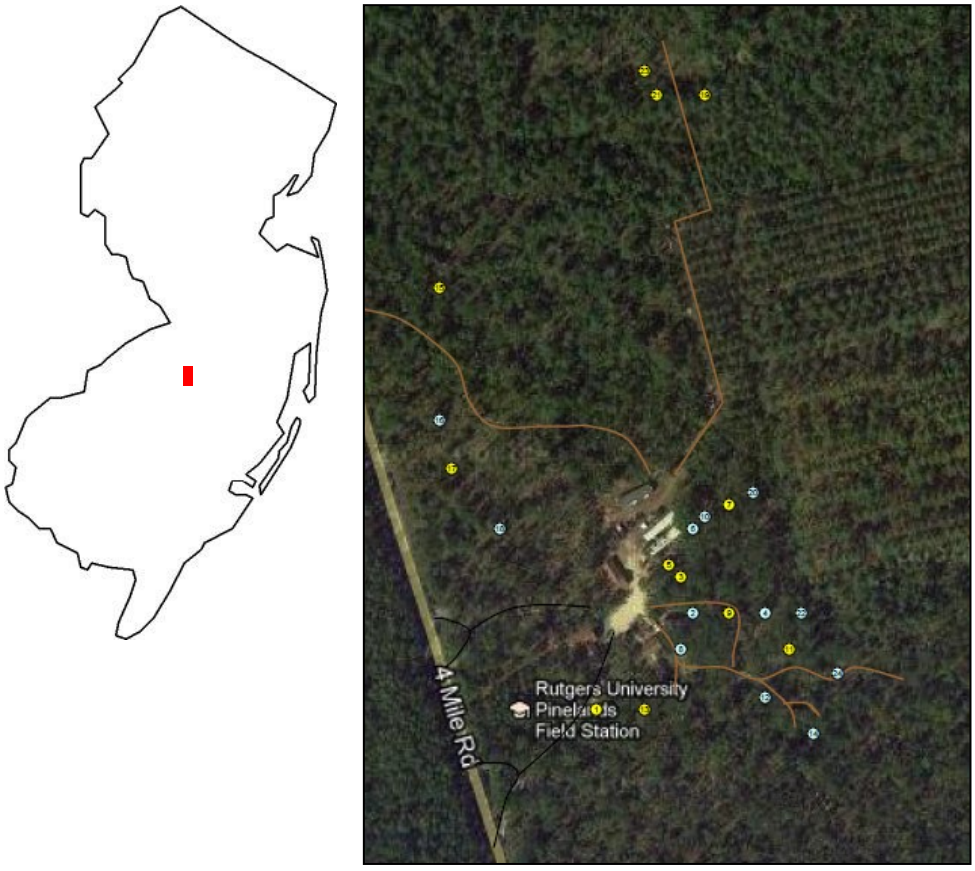


Figure 2. Lichen collection apparatus, including aluminum gutter connected to the tree by silicone caulk, with plastic tube for collecting leachates and aluminum wire to hold collection bottle.

Figure 3. Water collected from trees. In a repeated measures ANOVA, trees with high lichen cover leached significantly less water than trees with low lichen cover.

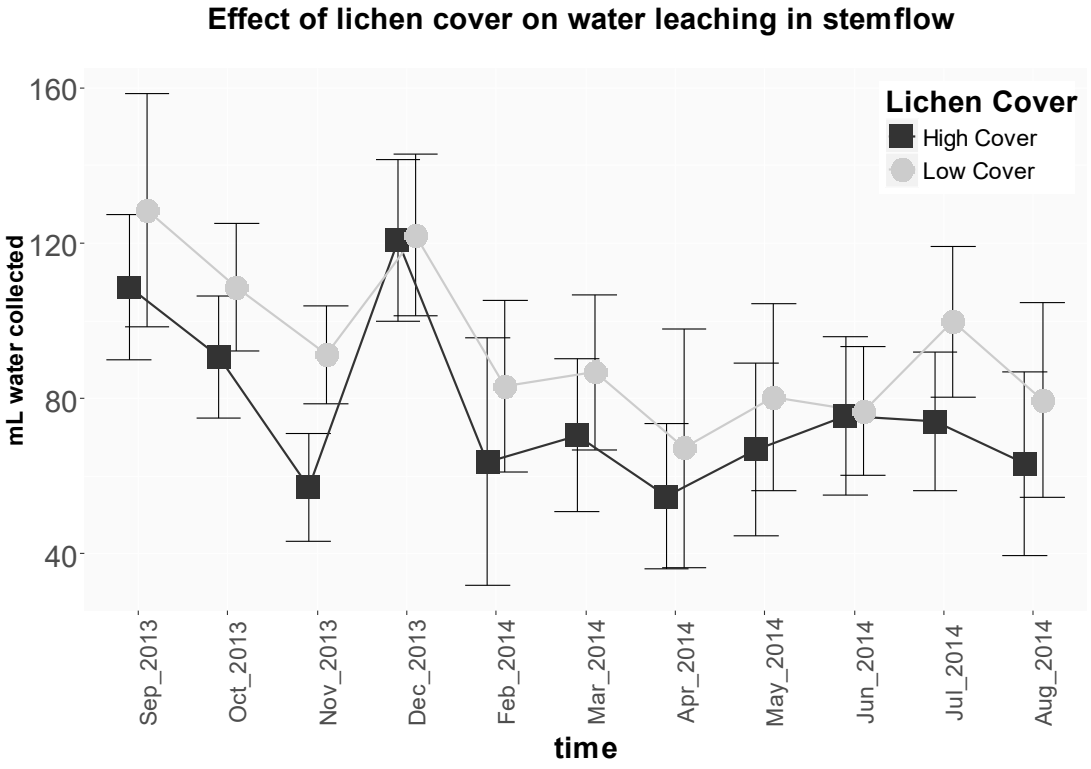


Figure 4. Total N collected from trees. The graph is divided into four rows by treatment: CON = control treatment, no added N; LOW = 10 kg N Ha^{-1} yr^{-1} added as NH_4NO_3; HIGH = 50 N Ha^{-1} yr^{-1} added as NH_4NO_3; ORG = 10 kg N Ha^{-1} yr^{-1} added as glycine. N retained is calculated as total N added minus total N retrieved in leachate; negative values indicate net leaching of N. In a repeated measures ANOVA, trees with high lichen cover did not leach a significantly different amount of N than trees with low lichen cover.

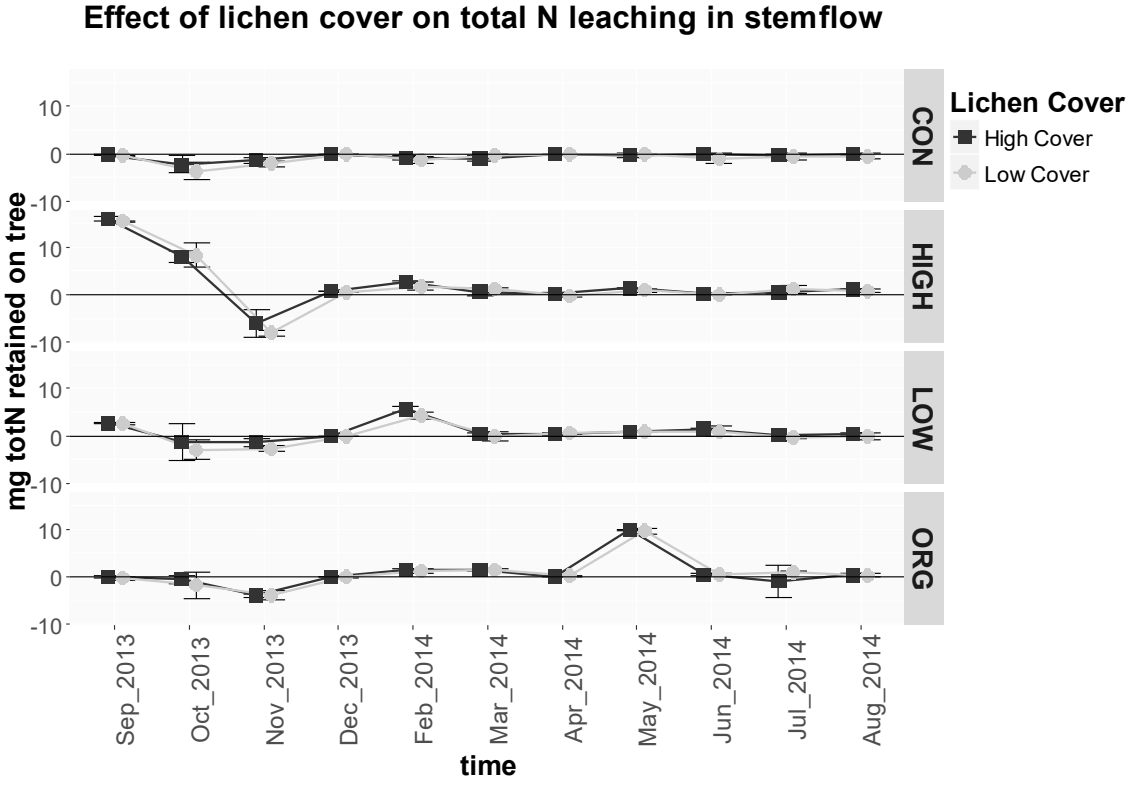


Figure 5. Total carbon collected from trees. In a repeated measures ANOVA, trees with high lichen cover did not leach significantly different quantities of N than trees with low lichen cover. Values are presented as total C collected as leachate from the sprayed areas of the tree.

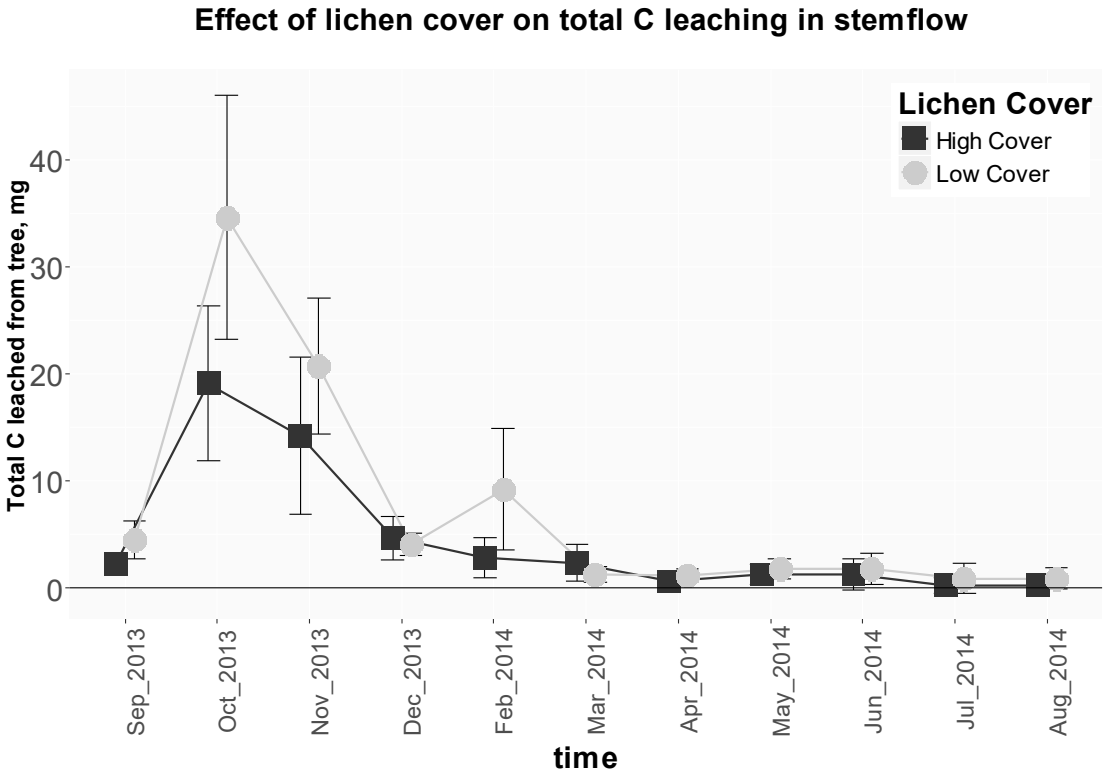


Figure 6. Ammonium collected from trees. The graph is divided into four rows by treatment: CON = control treatment, no added N; LOW = 10 kg N Ha^{-1} yr^{-1} added as NH_4NO_3; HIGH = 50 N Ha^{-1} yr^{-1} added as NH_4NO_3; ORG = 10 kg N Ha^{-1} yr^{-1} added as glycine. N retained is calculated as total ammonium-N added minus total ammonium-N retrieved in leachate; negative values indicate net leaching of ammonium. In a repeated measures ANOVA, trees with high lichen cover did not leach a significantly different amount of ammonium than trees with low lichen cover.

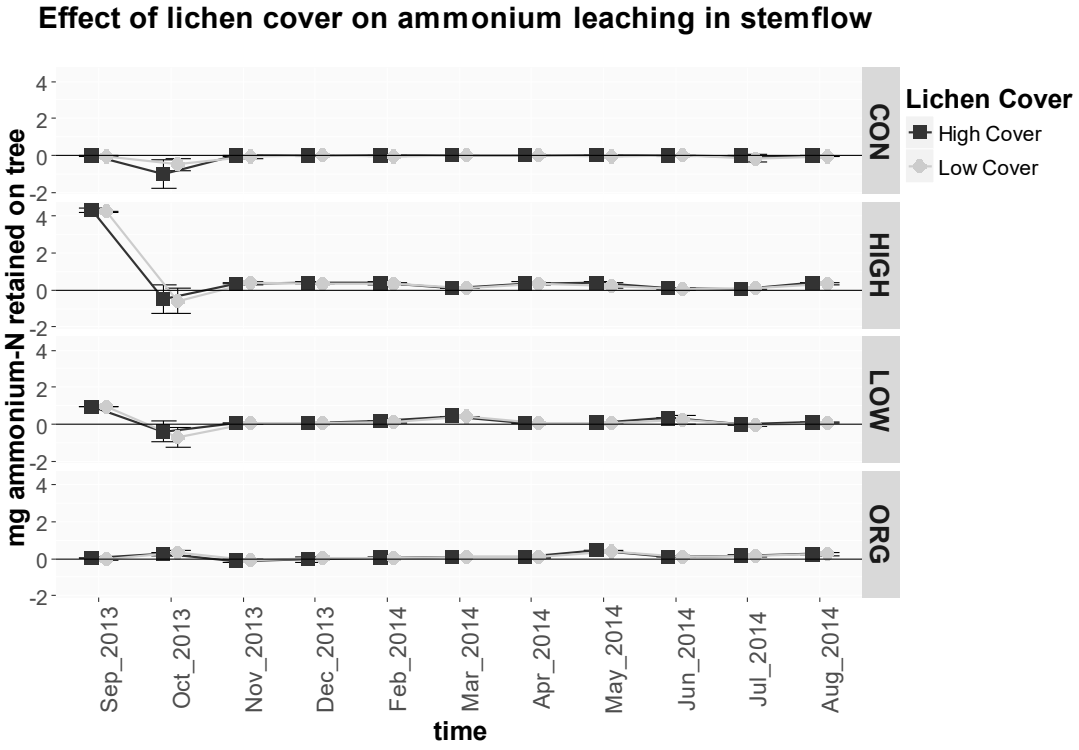
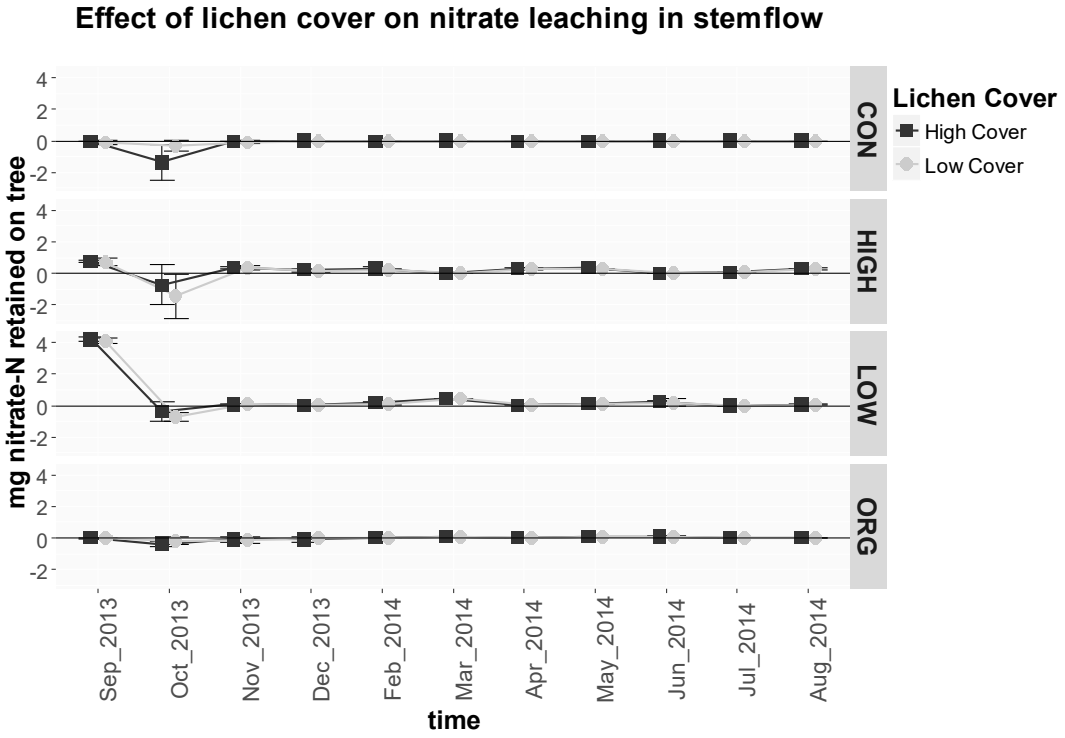


Figure 7. Nitrate collected from trees. The graph is divided into four rows by treatment: CON = control treatment, no added N; LOW = 10 kg N Ha^{-1} yr^{-1} added as NH_4NO_3; HIGH = 50 N Ha^{-1} yr^{-1} added as NH_4NO_3; ORG = 10 kg N Ha^{-1} yr^{-1} added as glycine. N retained is calculated as total nitrate-N added minus total nitrate-N retrieved in leachate; negative values indicate net leaching of nitrate. In a repeated measures ANOVA, trees with high lichen cover did not leach a significantly different amount of nitrate than trees with low lichen cover.



6 Summary: Significance of this research

This work contributes to our understanding of how lichens change forest soils and therefore why the maintenance of lichen-rich forests is important. Recent exciting biogeography research has targeted parts of the mid-Atlantic coastal plain as regional lichen biodiversity hotspots; the most lichenologically diverse areas also the most vulnerable to habitat loss through sea level rise. But stumbling blocks to gaining more funding for lichen conservation include lack of public knowledge of lichens and lack of scientific evidence for how lichens contribute to ecosystem functioning. We are able to assist those conservation efforts both by finding that evidence of functional contributions of lichens, and by conducting outreach related to our research.

A recurrent theme in this research is that short term surveys of lichens find no difference in the influence of aboveground lichens compared with the influence of other aboveground material (leaves or fabric); this means that the unique features of lichen interaction with the soil emerge only after several years. This finding suggests that development of the lichen necromass, a slime layer that forms at the base of the lichen patch, may be key to mediating the lichen's chemical and physical influence on the soils. Since this necromass does not build up quickly, it takes time for the lichen to exert a more powerful impact on the soils than the other aboveground covers can. A related theme in this research is that the lichens influence on soils in the short term is strong abiotic. That they influence soil moisture means that they are able to moderate a key feature of soil environment that many other soil processes depend on.

Because our research involves the very charismatic “reindeer lichens’ we are able to use our research to engage students and the public in discovering more about these overlooked organisms. Knowledge of this ‘hidden biodiversity’ can help people connect more meaningfully with their landscapes and potentially become more committed to helping their societies live more sustainably in those landscapes. In fact, the research has already led to my leading several outreach activities in the Pinelands, including assisting in organizing a lichen workshop for the New Jersey Mycological Society and assisting in organizing the Andrews Foray, a lichen and moss foray in the Pinelands, open to the public. I also conducted several lectures on lichen ecology, for the public at the Pinelands Short Course held at Stockton University, and for Rutgers University students in the Plant Ecology course, in the Fungi in Ecosystems course, and in the Plant Diversity and Evolution course.

Our work is building our understanding of what lichens do in temperate forests, why their presence is important, why their absence is alarming, and why we should put effort into lichen conservation. Our work provides new insights on how lichens influence soil function (and therefore, how they influence productivity and function of whole forests). Extensive research exists concerning the influence of soil lichens on boreal forests, where they are critical for survival of caribou, and on the influence of soil lichens in arid lands, where they mediate limiting soil moisture. However, the influence of soil lichens in the extensive area of temperate forests, where they occur on acidic, sandy soils, is less well understood, and we hope this research will inspire further investigation in this field.