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Master thesis project

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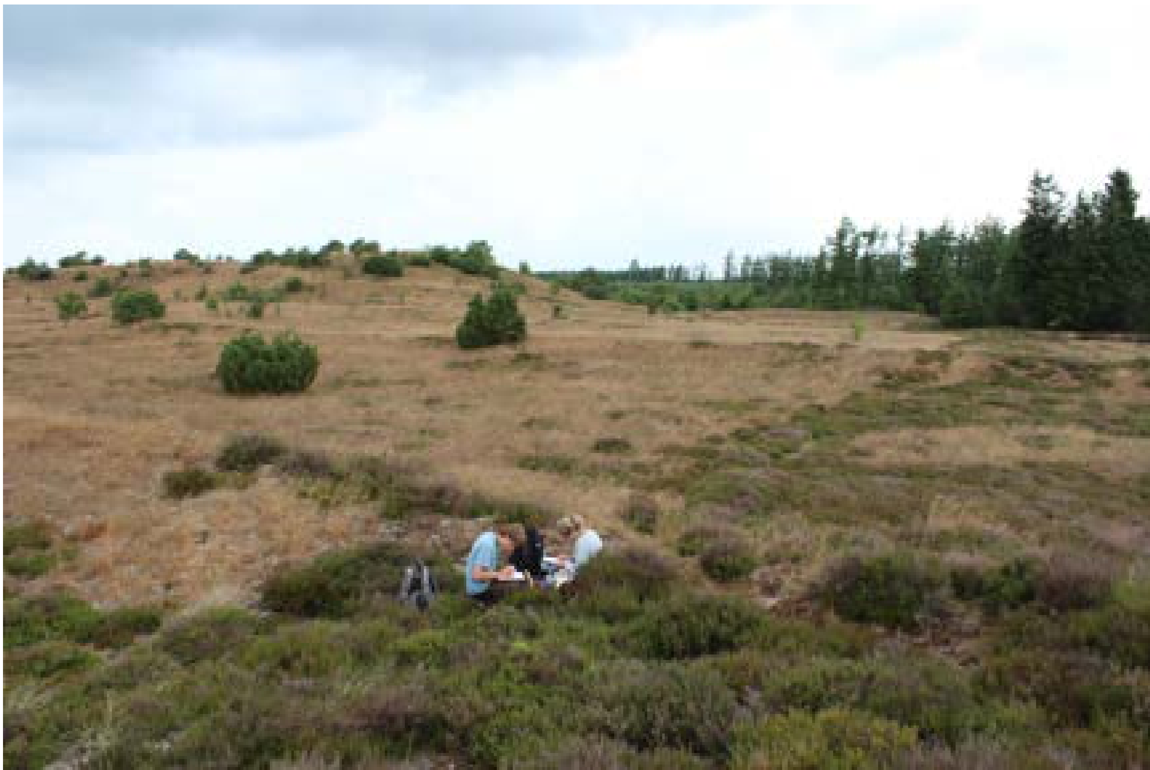
Field investigation of Danish heathlands; lichens and bryophytes as candidates for quality assessment



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DANSK RESUMÉ (DANISH SUMMARY)

Danske heder, indlands som kystnære, er i et europæisk sammenhæng kendte for deres specielle biodiversitet. Laver og bryofytter er anset som en væsentlig del af vegetationen på disse habitater og bidrager derved til den samlede europæiske artsdiversitet. På trods af dette er laver og bryofytter ikke inkluderet i de gældende vurderinger af naturkvaliteten på de danske heder, det såkaldte Naturtilsstandsindeks. Vores hypotese er, at disse to organismegrupper burde indgå i vurderingen grundet deres følsomhed over for ændringer i miljøet. Dette feltstudie af 116 felter fordelt på 12 danske hede lokaliteter sigter mod at indsamle mere viden om laver og bryofytter. Vi registrerede laver, bryofytter og karplante vegetationen for hver af de 116 registrerings cirkler men indsamlede også prøver af pH og humuslagets tykkelse. Vi præsenterer her et af de første forsøg på objektivt at bruge laver og bryofytter med gode indikator egenskaber til vurdering af danske heder. Dette sker gennem analyser med ordinationer, en indikator arts analyse samt den forholdsvis nyudviklede netværks analyse, hvor data inddeles i moduler. Vi fandt konsekvent gennem alle tre analyser at *Cladonia foliacea*, *Cladonia zopfii*, *Cladonia gracilis*, *Cladonia ciliata*, *Cladonia uncialis ssp. biuncialis* og *Cetraria aculeata* fungerede som gode indikator arter. Imidlertid kan der ikke drages nogen videre konklusion for bryofytterne grundet det lave antal arter der blev registreret. Vi bruger disse resultater og skaber et lav arts indeks for danske heder baseret på det gældende arts indeks for på bedst mulig vis at kunne sammenligne de to.

ABSTRACT

Danish coastal and inland heath habitats are notorious in for their biodiversity in a European context. Lichens and bryophytes constitute a substantial amount of the vegetation on those habitats and contribute to European species diversity. Yet, they are not included in the current assessment of nature quality (Naturtilstandsindeks) of Danish heathlands. We hypothesize that these two groups of organisms belong to such assessments due to their sensitivity to environmental changes. This field investigation of 116 plots distributed on 12 Danish heath localities aims at gathering more insights on lichens and bryophytes. We recorded the lichen, bryophyte and vascular plants vegetation but also collected pH and humus layer thickness data for each plots represented by circles of 5 meter radius. We present here, one of the first attempts at objectively identifying lichen and bryophyte species with good indicator values through analyses of ordination, indicator species analysis and the recently developed network modularity. We consistently found across the three analyses that *Cladonia foliacea*, *Cladonia zopfii*, *Cladonia gracilis*, *Cladonia ciliata*, *Cladonia uncialis ssp. biuncialis* and *Cetraria aculeata* are good indicator species. However no robust conclusion can be drawn for bryophyte species due to the low number of species recorded. We use these results and create a lichen species index for heathland based on the species index currently used in order to facilitate rapid implementation.

1. INTRODUCTION

Preventing further habitat loss and saving biodiversity are today's main goals of conservation. In Europe, almost all natural landscapes have been under high anthropogenic pressures for several millennia. As a result, habitats became fragmented and many key species were driven to extinction. It is therefor important to protect but also recreate some of those habitats. With rapid loss of biodiversity and scarce economic resources it is however becoming crucial to assess natural state of habitats and prioritize management efforts. In Denmark, heathlands are one of the remaining habitat types that harbor high biodiversity and have important educational, recreational and cultural value, which makes them important habitats to protect in a European context. Heathlands are dynamic habitats with characteristic podzolic soil, sandy and nutrient poor. They can be considered as a natural stage of a succession that eventually gives place to woodland. This stage can be maintained for as long as nutrient enrichment of the soil is prevented. Contributing to national but also European diversity, soil lichens and bryophytes thrive on these highly dynamic habitats. Still, they are almost disregarded in the current way of assessing quality of heathlands in Denmark. Indeed the present index for the state of nature (Naturtilstandsindeks) is based on a combination of a structure index and a species index that only includes vascular plants. Fueled by field observations and knowledge of the sensitive nature of lichen we decide to investigate the potential of these small organisms to be powerful indicator of heathland quality.

The goal of this master project is to get more insight about lichens and bryophytes on heathlands, pinpoint species with high indicator values through a completely subjective approach, a statistical but still naïve procedure and an analysis of modularity in the network. Also, we create a lichen species index based on our findings following the principles of the existing species index and discuss the pros and cons of implementing such an index in Denmark.

In this study, we present the result of a year project dealing with lichens and bryophytes on heathlands. We first set the scenery and go through some of the general characteristics of heath type vegetation, put them in a Danish context and mention today's threats for those habitats. We further present the twelve localities investigated and their general key characteristics. We secondly describe the current index for nature assessment and describe the method and statistical tools used for this field investigation. We then reveal the results and finally consider the impact of our findings for the future of heathland management, but also discuss several aspects that are relevant to ensure maximum diversity on heath type habitats.

2. ECOLOGY OF HEATHLAND

2.1 DEFINITION OF HEATHLAND

Defining the term "heathland" can be challenging because the vegetation and dominant species can vary from one locality to another. One heathland may be dominated by *Calluna vulgaris* and *Empetrum nigrum* while another may be overgrown with *Deschampsia flexuosa*. The two localities can look very different, but are still both defined as heathland. Symes & Day (2003) defines lowland heathlands as an area with cultural and biological importance. The cultural importance stems from the fact that this nature type has been shaped and maintained by humans for thousands of years. Furthermore, these areas are of biological importance because they harbor specialized species that are characteristic of nutrients poor and acidic soil. Dwarf shrubs from the Ericaceae family, lichens, grasses and other lower plants, are characteristic of the vegetation.

Riis-Nielsen *et al.* (1991) has a definition that fits many different heathland types and even completely overgrown vegetation can still be labeled as heath. They simply define heathland as a habitat dominated by evergreen dwarf shrubs with small leaves. This is very often one of the clearest and simplest definitions of heathland. Furthermore, Riis-Nielsen *et al.* (1991) also defines as heathlands areas that are now dominated by trees and bushes but not many years ago harbored more characteristic heathland vegetation. These two definitions clearly include areas that are in late succession stage and on the edge of becoming a different nature type (e.g. grassland or forest).

On the other hand Riis-Nielsen *et al.* (1991) also includes areas that have not yet developed into heathland by many, for example dune areas where the natural succession eventually become heathland. Stated field areas that show a succession that will cause creation of heathland are also defined as heathland.

Gimingham (1972) uses the word "heathland" to describe territories in which trees or tall shrubs are sparse or none existing, and in which the dominant life-form is the evergreen dwarf-shrub, particular represented by the Ericaceae family. Gimingham (1972) further describes the overlapping use of the word heath (German: Heide; Swedish: hed; Danish: hede). It is pointed out, that the word "heath" probably referrers to the kind of vegetation found on heathlands, 'heather' most often being the most abundant species in the heathland plant communities.

These three definitions mentioned above are slightly different, but overlap in some aspects. In all the definitions the dominant vegetation is described as being more or less dominated by evergreen dwarf shrubs. It is also pointed out by both Symes & Day (2003) and Riis-Nielsen *et al.* (1991) that heathlands are in many cases just a stage that will without management develop into other vegetation types (woodland or grassland). The main points from the different definitions above clearly reflect our perception of heathland. During sampling in the summer and fall of 2013 many different localities were investigated, all being different to some extent, but all characterized as heath type.

Inspired by all definitions mentioned above we for this master project define heathlands as:

- Open vegetation areas with acid soil and dominated by evergreen dwarf scrubs
- Areas that because of encroachment and lack of disturbances can be overgrown by woody plants or grasses
- New areas created by moving sand dunes, where the traditional heathland vegetation has not yet fully established.

What makes the definition of heathland so difficult is that areas can vary greatly The term heathland has been subdivided into smaller categories such as dune-heath, grass-heath, lichen-heath and moss-heath, also designating certain types of grassland on acid soils, alpine, arctic or coastal areas.

2.2 THE HEATHLAND VEGETATION

The open nature growing on dry soils is in many cases created by clearings of the woody vegetation. In some cases the open vegetation is in an early stage of the succession where time or the conditions have not made it possible for trees and bushes to establish (Petersen & Vestergaard 2006). The vegetation on heathland can very often be sparse compared to other nature types (Riis-Nielsen *et al.* 1991, Symes & Day 2003), because of the low level of nutrients in the soil. Only specialized plants, bryophytes and lichens that require very little from the soil are usually present.

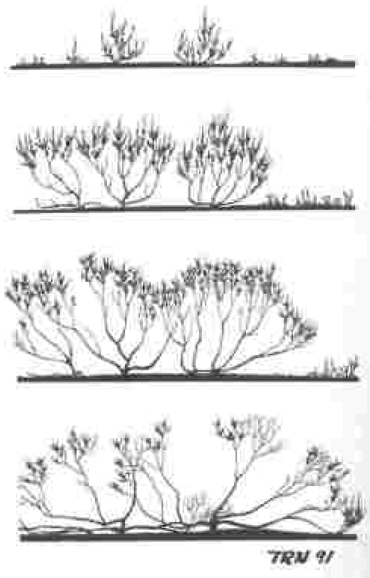
When investigating the 12 different localities around Jutland in the summer and fall of 2013 mainly lichens were registered since the vegetation on heathlands often can be sparse. Four different plants and grasses were though abundant and registered in almost all of the sampling plots. The four plants and grasses are *Calluna vulgaris, Empetrum nigrum*, *Deschampsia flexuosa* and *Molinia caerulea*. All of these species are one of the main concerns when working with restoration and conservation of heathlands. Inadequate or wrong management of the area, eutrophication or lowering of the water level can create changes in the species composition, primarily with a reduction in the coverage of dwarf shrubs and an increase in the coverage of grasses. Below is a short description of these four vascular plant species, and a description of lichens and in particular the most abundant lichen group on heathlands; the genus *Cladonia*.

2.2.1 *CALLUNA VULGARIS*

Calluna vulgaris is an evergreen dwarf shrub and is often because of its purple flowers considered as the symbol of heathland (Riis-Nielsen *et al.* 1991). The growth and structure of *Calluna vulgaris* has got a crucial effect and influence on the remaining vegetation (Symes & Day 2003).

The growth form of *Calluna vulgaris* can be divided into four different phases; the pioneer phase, the building phase, the mature phase and the degenerate phase (Symes & Day 2003). These phases represent the development of the plant from sprout to death. Below the four different phases are described (Riis-Nielsen *et al.* 1991, Petersen & Vestergaard 2006).

- The *pioneer phase* occur between the ages 0-5 years. In this phase the heather establishes itself. The emerging plants start to develop branches and start to flower after a couple of years.
- The *building phase* occur between the ages 5-15 years and is the period when most growth happens. In this the canopy grow tighter.
- The *mature phase* occur between the ages 15-25 years. Here, the growth slows down and the plants become woodier. The canopy layer less even and small gaps and openings are created.
- The *degenerate phase* in between the ages 25-40 years. Here the plants start to collapse, the canopy layer becomes even more uneven and eventually the plant dies.



These phases are arbitrary and very subjective from area to area since the development of the plant also depends on the environmental factors such as wind, water, frost and sun exposure (Barclay-Estrup & Gimingham *et al.* 1969). The environmental factors play a large role in the development of Calluna vulgaris. To ensure the water supply during drought the leaves are small with an enrolled edge and an enlarged cuticle (Petersen & Vestergaard 2006)

A mentioned earlier *Calluna vulgaris* is one of the most characteristics of heathland vegetation. Depending on the age of the Calluna a different number of other species can coexist with it (Riis-Nielsen *et al.* 1991). During the pioneer phase where there are patches with bare soil between the very young heather plants other vegetation can grow. When the heather reaches the building phase the canopy of the Calluna grow tighter and outcompete smaller species by restricting sunlight. Some shade tolerant plants and bryophytes are able to live under the Calluna canopy (e.g. *Hypnum cupresiforme*). Later in the Calluna lifecycle the canopy will open up again and make room for other species, hence the cycle of the remaining vegetation on heathland follows the cycle of the heather (Gimingham 1988). The main condition to maintain a dense cover of *Calluna vulgaris* is promoting a continuous regeneration (Petersen & Vestergaard 2006).

2.2.2 *EMPETRUM NIGRUM*

Empetrum nigrum is similar to *Calluna vulgaris* in term of ecology; it is a very common species on Danish heathlands. It is mainly found on northern heathlands located close to oceanic district because of its climatic preferences (Gimingham 1988). This evergreen dwarf shrub is up to 50 cm tall and much branched. The plant can use the new and creeping branches to climb over other vegetation and outcompete them by stealing sunlight (Bell & Tallis 1973). The new branches produce roots when they get in contact with the soil, and it is therefore not possible to calculate a maximum age for *Empetrum nigrum* (Riis Nielsen *et al.* 1991). If the conditions are good and there is a thick layer, *Empetrum nigrum* can grow so densely that it can outcompete almost all other species including Calluna vulgaris (Petersen & Vestergaard 2006). Because of it's ability to set roots from the new branches it makes it efficient and fast growing. It can grow around other vegetation and into adjacent open areas (Riis-Nielsen *et al*. 1991, Tybirke *et al*. 2000).

Empetrum nigrum is however more sensitive to drought and trampling than *Calluna*. Furthermore it is less shade tolerant than *Calluna vulgaris*; if it is not exposed to sunlight it spreads more slowly and has lower leaf density (Riis-Nielsen *et al.* 1991). *Empetrum nigrum* produces black berries and animals most often spread the seeds. The seeds are difficult to get to sprout; a cold winter period is known to be necessary for seeds germination (Bell & Tallis 1973).

2.2.3 *DESCHAMPSIA FLEXUOSA*

Deschampsia flexuosa is a common species in Denmark and is most often found on heathlands and in forests with more substrate (Bokdam & Gleichman 2000, Schou *et al.* 2009). It is easily recognized by the fresh green and narrow leaves and the wavy top branches hence its common name wavy hair grass. The grass flowers between June and July and the fruits are spread by the wind (Schou *et al.* 2009). The dominance of *Deschampsia flexuosa* on many heathlands has become an increasing problem because the grass outcompetes the heather. Especially in the north-western part of Europe the replacement of heather with grasses has been linked to the higher level of nitrogen deposition (Britton *et al.* 2003). Deschampsia flexuosa will in time if it is not disturbed, form a thick carpet that will prevent all other plants from establishing (Sand-Jensen 2007). *Deschampsia flexuosa* has because of higher levels of nitrogen been able to expand at the expense of *Calluna vulgaris* resulting in a transition from heathland into grassland type vegetation (Britton *et al.* 2003).

Heathlands are very characteristic nature type because of the acidic and nutrient poor soils, low in available nitrogen and phosphorus. Ericoid species, such as *C. vulgaris*, is a strong competitor when there is a low nutrient level but, compared with grasses, the *Calluna* appear less competitive with increase nutrients levels (Britton *et al.* 2003, Aerts 1989).

It is not only *Deschampsia flexuosa* that has become an increasing problem on heathlands, an other grass species has expanded it's cover; *Molinia caerulea* on wet heathlands. Both of these grass species are regular components of heathland vegetation (Britton *et al*. 2003) particularly following attacks from the heather beetle (*Lochmaea suturalis*) or after specific forms of management such as cutting or burning (Gimingham 1972).

2.2.4 *MOLINIA CAERULEA*

Molinia caerulea is a tussock forming grass and is easily identified by its dark purple inflorescence (Sand-Jensen 2007). Also the dark violet inflorescence is very characteristic. In the winter the withered leaves change color to a characteristic light orange that makes it possible to identify the species from a distance (Schou *et al.* 2009). The species is most often found on nutrient poor soils on heathlands but also bogs and forests (Schou *et al*. 2009).

This species was earlier associated with more wet heathlands (Britton *et al*. 2003), but today because of heavy nitrogen deposition it is found on many of the dry heats areas in Denmark too (Aerts & de Caluwe 1989). *Molinia caerulea* can grow up to 70-80 cm if the conditions are optimal. Under normal circumstances it will be around 30-50 cm. Because of its large size *Molinia caerulea* it is very good when it comes to competition for light (Aerts 1989). It is especially on areas where nitrogen deposition has increased that *Molinia caerulea* can be a problem for the heather. If the nitrogen deposition is high enough *Molinia caerulea* will outperform the heather and form large tussocks that will prevent all other vegetation in recolonize (Symes & Day 2003). It the Calluna is in the building phase it is possible for the Calluna to compete with *Molinia caerulea*.

2.2.5 LICHENS

Lichens are symbiotic organisms, where a fungal mycobiont lives in a joint relationship with an algae or cyanobacteria called photobiont. In the symbiotic organism the alga is the part that delivers the formation of nutrients because it contains chlorophyll while the mycobiont supplies the photobiont with water and minerals (Conti & Cecchetti 2001). Many refer to lichens as a case of mutualism, where all the involved parts gain positively from the relationship. The lichen symbiosis can also be seen as a controlled parasitism because benefits for the photobiont are still unclear (Nash 2010).

The degree of lichenization varies from species to species. In some cases only a few individual photobionts are present and randomly connected to the mycobiont. But in most, cases the photobiont is well integrated in the thallus with a distinct layer found just beneath the upper cortex made of fungal tissue (Nash 2010).

The symbiosis between the mycobiont and the photobiont is very successful. Lichens are found in almost all terrestrial ecosystems, from the arctic to tropics. Because of the symbiosis both photobiont and mycobiont have been able to colonize areas, where they separately would not have had the ability. Most free living algae or cyanobacteria lives in very moist area, often aquatic, but because of the lichenization the alga has now spread to areas that are frequently dry (Nash 2010). Lichens have successfully spread all over the globe. They occur commonly as epiphytes on trees and other plants. They are also frequently found colonizing bare soil where they are an important component of the cryptogram vegetation. Furthermore lichens are often found on rocks occurring as epiphytic where they grow over the surface of the rock (Nash 2010).

Lichens are a very diverse group and vary in shape and color. They can range from orange/ yellow to red colors to darker gray, brown and black. Size wise they can vary from less than an mm^2 to long forms that can hang 2 meters from tree branches (Nash 2010). The lichen biomass contribution varies from almost insignificant to extremely important component of the total ecosystem.

Lichens are poikilohydric organisms, which mean their water content is dependent and equal to moisture in the surrounding environment. Flowering plants and conifers have evolved special structures capacity to maintain the water levels and avoid desiccation and are therefore referred to as homoiohydric organisms. This lack of water retention structure and capacity to shut down cell activity allows lichen to survive fairly long period of drought and even the void of outer space (Nash 2010, De la Torre *et al.* 2010)

2.2.5.1 CLADONIA LICHENS

In this master thesis, Danish heathlands were investigated. The main species that we have worked with are species from the *Cladonia* genus. The Cladonias can be divided into two groups, the reindeer lichens (formerly known as *Cladina*) and the cup lichens.

The reindeer lichens form basal squamules as their primary thallus, which are very rarely observed. From this primary thallus podetia grow while the primary often thallus disappears. The podetia growing from the primary thallus is a hallow cylinder that varies in looks from species to species. Reindeer lichens are also known to have no upper cortex (Ahti *et al.* 2013) which differentiate them from the cup lichens.

What makes the Cladonias so interesting is the huge variation in shape and size. Not just between the different species but also within the same species. Genetic variation, the climate or the habitat can shape the species differently which can also make *Cladonia* species difficult to identify. Many of the very characteristic characters may first appear on older individuals and identification of younger individuals can be difficult (Søchting 2014 *in prep*, Ahti *et al.* 2013). The pictures below show a reindeer lichen and a cup lichen.


The most common way for lichens to spread is through vegetative reproduction; basically small pieces of the podetium will break and be dispersed by the wind. In *Cladonia* this manifest itself through the presence of structures called soredia, which are little packages of mycobiont hyphae surrounding algal cells. The soredia can be very small and fine like flower or bigger and rough. When the soredia are spread, either by animals, wind or water, the lichen start to grow and form a new thallus. In some cases it is not soredia that are formed, but small scales that easily breaks off and have the same function as the soredia. The reindeer lichens do not produce soredia but are often spread by fragments from the thallus that has been trampled while it was dry (Søchting 2014 *in prep*). These structures are very useful for identification of *Cladonia* species. The pictures below illustrate two other *Cladonia* species.


Cladonia used to be a very characteristic species on heathlands and dune heaths. Due to nutrient enrichment and the lack of management the lichens are being outcompeted by grasses and other species. There are some heathland areas left in Denmark that are still dominated by lichens and mosses. The lichen-heath on the island of Anholt is a good example (Petersen & Vestergaard 2006, Christensen 1999).

2.3 ABIOTIC FACTORS

There are other factors than soil properties and nutrient conditions that shape the vegetation composition of heathlands. Many abiotic factors such as climate, temperature, wind, water and topography have a large influence. Biotic factors refer to the living organisms and the relationship between them whereas the abiotic factors refer to the climate, geology and topography.

2.3.1 CLIMATE

Climate is often when describes divided into micro and macroclimate. They are defined as (Petersen & Vestergaard 2006);

- *Micro*climate is the climatic conditions measured on the ground level and therefore in the vegetation and the topsoil.
- *Macro*climate is the climatic conditions measured 2 meters above the ground level.

Climate is important for the formation of heathland (Symes & Day 2003). Reasonably high and regular rainfall in cool conditions encourages the formation and maintenance of the sandy and nutrient poor podzol (Symes & Day 2003). Climate also has a direct influence on the different vegetation communities of heathlands. Dwarf shrubs are not consistently tolerant to very cold conditions, plus heavy frost can cause dieback. Heather survives in colder climates only where a regular snow cover insulates it (Symes & Day 2003).

2.3.2 TEMPERATURE

The temperature has a large influence on the vegetations growth and development. The growth season is known as the part of the year where the middle temperature of the day is equal or higher than 6 degrees (Petersen & Vestergaard 2006). The temperature through a vegetation layer varies and is among other determined by the tightness of the canopy, the color of the soil surface, the thermal conductivity and heat capacity of the soil (Petersen & Vestergaard 2006).

2.3.3 WIND

In many dune heathlands, the wind has an essential role in keeping the areas open and keep the sand exposed. This makes wind a very important abiotic factor and an important disturbance source for heathland. The natural dynamics will persist and the vegetation specialized in living in habitats with a high level of dynamics will persist without anthropogenic help. Wind disperses seeds and makes pollination of the heathland vegetation possible (Petersen & Vestergaard 2006).

Wind may also result in an increase in the evapotranspiration which is the total evaporation. This and the direction of the wind can create microhabitats with very different conditions on opposite facing slopes in the dune heathland (Petersen & Vestergaard 2006).

2.3.4 WATER

Locally the water balance between precipitation, evaporation and seepage plays a huge role on the vegetation (Petersen & Vestergaard 2006).

Water is very often scarce on heathland soils because of the composition of the soil and the presence of hydrophobic organic acids released by roots and plant tissues, fungal activity, the as the main causes of soil water repellency and hydrophobicity (Petersen & Vestergaard 2006, Martínez-Zavala & Jordán-López 2009). It is established that soil hydrophobicity reduces soil infiltration rates, and enhances runoff flow and soil erosion (Martínez-Zavala & Jordán-López 2009, Petersen & Vestergaard 2006).

The groundwater level has an especially large role for the dune heathland vegetation. In the taller dunes the vegetation is nondependent of the groundwater (Petersen & Vestergaard 2006). To keep the water from evaporating from the plants much of the dune heathland vegetation is specialized in transpiration retardant actions such as protective wax layers on the leaves or leaves that curls up in the sun to avoid evaporation (Petersen & Vestergaard 2006).

2.3.5 TOPOGRAPHY

The topography of an area determines aspects of the evapotranspiration together with the wind and precipitation. Slopes can create many very different microclimates according to the inclination and the direction of the slope. On the southern hemisphere the direct radiation from the sun is highest on north facing slopes. On the northern hemisphere the direct radiation from the sun is highest on southern facing slopes. A higher level of direct sunlight on the southern slopes results in a higher evaporation and I general a dryer microclimate. The reverse can be found on the northern facing slopes that are exposed to less direct sunlight and therefore are wetter than the southern slopes. All in all, this has a big influence on the vegetation, and the number of species found on dry southern facing slopes is often lower than the number of species found on a northern facing slope (Petersen & Vestergaard 2006).

3. HEATHLAND CONSERVATION

Heathland is very often found on nutrient poor sandy soils and characterized by pioneer vegetation that appears after forest clearings and nutrient depletion of the soil. These habitats are dominated by dwarf shrubs, grasses and lichens (Naturstyrelsen 2014(C)), and have been maintained this way because of different forms of management such as cutting, grazing and sod cutting. Most heathland areas in Denmark were created about 5000 years ago where forests were cut or burned to make room for farming and agriculture. The areas were grazed and the heather used as fodder for the animal over the winter. This meant that the vegetation on the heathlands were kept young, diverse and nutrients were constantly removed (Sand-Jensen 2007). After the termination of the regular heathland practices it has become necessary to “artificially” managed heathland areas if vegetation dominated by dwarf shrubs and lichens is to be maintained. This can be done using grazers, by burning, cutting the vegetation or top soil removal (Bruus *et al*. 2006).

To save this biological and cultural heritage many areas became protected during the first part of the 19th century (Dahl 1994). Heathlands are natural habitat types that have been frozen in a particular successional stage and need disturbances to avoid becoming forests. Many of the previous management plans did not allow disturbances and as a result many of the areas that used to be open and dominated by dwarf shrubs and lichens grew into forest and grassland.

3.1 BRANDMANDENS LOV, THE FIREMAN’S LAW

The fireman’s law is a guideline for prioritizing nature management projects and must in a nature management aspect be compared to the principles behind putting out a fire (Arler & Bondo-Andersen 2004). The fireman’s law is made up by 3 simple rules.

1. Preserve areas in good conditions
2. Stop any harmful effects against the area
3. Expand the area and create new areas.

The first objective is to secure and preserve valuable intact areas. Secondly, reducing the harmful effect of threats and allow recovery of recently lost parts and establishment of new nature (Bjørnsen *et al.* 2002).

3.2 THREATS AGAINST DANISH HEATHS

Because of changes in the environment and the lack of management on many of the Danish heathland habitats, this specific nature type is considered the nature type with the highest declines in size and nature quality in Denmark (Naturstyrelsen 2014(C)). When faced with lack of management heathlands are faced with several different threats.

3.2.1 OVERGROWING

Overgrowing is a natural process where heathlands are colonized by trees that outcompete the characteristic dwarf vegetation. The vegetation on heathland is specialized in living on nutrient poor and sandy soil and the overgrowing is therefore a direct threat against the heathland environment and reduces the habitats for the heathland fauna (Sand-Jensen 2007). When an area has been submitted to a non-intervention form of management then the natural succession will take place and the heathland will slowly develop towards woodland (Riis-Nielsen *et al*. 2005).

Especially fast growing species such as birch (*Betula*) and Dwarf mountain-Pine (*Pinus mugo*) will very often be the first species to invade and overgrow the open lands (Naturstyrelsen 2014(C)). *Pinus mugo* often spreads from plantations planted to lower the sand drift (Christensen & Johnsen 2001, Sand-Jensen 2007). With the overgrowing of a heathland area the lighting conditions in the area changes. The lower vegetation no longer receives enough light and in time, this result in a change of the vegetation composition and a disappearance of the original heather and lichen vegetation (Sand-Jensen 2007). Furthermore the problem with trees growing on heathlands is the litter accumulation and therefore nitrogen deposition from the canopy. Accumulation of nitrogen in the litter and soil changes the species composition even more and the nature type will shift towards forest or grassland (Riis-Nielsen *et al.* 1991, Sand-Jensen 2007).

3.2.2 EUTROPHICATION AND POLLUTION

As described earlier heathlands are nutrient poor environments and the vegetation is adapted to this. If the composition of nutrients in the environment changes, then other species are able to invade and compete with classic heath species such as heather and the Cladonias.

No direct effect of nitrogen on *Calluna vulgaris* has been proven, although side effects can be severe (Ellemann *et al.* 2001). With an increase in nitrogen deposition the level of nitrogen in the Calluna leaves increased and thereby improved the conditions for the heather beetle (Lochmaea suturalis) and thereby increased the number of beetles (Ellemann *et al.* 2001, Britton *et al.* 2000). It is therefore predicted that the number of heather beetle attacks in Denmark will increase as the nitrogen deposition increases (Ellemann *et al*. 2001).

Other abundant species on heathland are lichens. Due to their physiology, lichens are very sensitive to air pollution (Nash 2010). Because of the lichens lack of cuticle the different contaminants are absorbed over the entire surface of the organism (Conti & Cecchetti 2001). Lichens take up water and nutrients from the atmosphere, and are therefore affected by increasing levels of pollution (Nash 2010). The accumulated level of pollution inside the lichen may in the end result in a breakdown of the symbiotic balance between the mycobiont and the photobiont. There has been established a correlation between the chlorophyll damage and the concentrations of several different pollution elements such as Cr, Fe, Mn, Ni, Pb and B in the lichens (Conti & Cecchetti 2001).

Because the lichens are good bioaccumulators of specific pollution trace elements, the concentrations found in their thalli can be directly correlated with the concentration in the environment where the lichen was found (Conti & Cecchetti 2001).

3.2.3 INVASIVE SPECIES

Some invasive species can outcompete native species and thereby lower the biodiversity. They very often grow and spread fast because of the lack of competition, disease or predator that were present in their natural range (Sand-Jensen 2007). Five invasive species have been registered as the most invasive on heathland. The species are; *Pinus mugo*, Prunus serotina (glansbladet hæg), *Campylopus introflexus*, Cytisus scoparius (gyvel) and *Rosa rugosa* (Sand-Jensen 2007). Especially *Campylopus introflexus* and *Rosa rogusa* have the ability to form large mats to the point where native species are not able to grow (Ellemann *et al.* 2001, Christensen *et al.* 1998). Some native species have in later years also started to act as invasive species on coastal dune heathlands. The native grass *Deschampsia flexuosa* has invaded the costal dune heathlands in the northern part of Europe and is occasionally found as a dominant species in the area (Nielsen et al. 2011). *Deschampsia flexuosa* has not earlier been associated with costal heathland, and this change in the species composition can be explained by the elevated atmospheric nitrogen deposition (Nielsen et al. 2011), lack of management or natural succession (Degn & Søchting 2008). Due to the nitrogen deposition the cover of grasses like *Deschampsia flexuosa* and *Molinia caerulea* has become more stable and is more and more often found as dominant species on nutrient poor environments like the costal dune heathlands (Nielsen et al. 2011, Sand-Jensen 2007).

3.2.4 DYNAMICS

Dune heaths are known as highly dynamic environment were the vegetation is adapted to these tough conditions. Previous actions to lower the sand drift and dynamics on dunes were successful, but contributed to the creation of a humus layer and an accumulation of nitrogen. This combined with elevated levels of airborne nitrogen resulted in a change of vegetation away from the lichen and dwarf shrub dominated society towards an environment dominated by vascular plants, (Petersen & Vestergaard 2006).
Also, when grazing ended the dynamics in the dune heathlands has been reduced as a combined result of sand drift lowering actions and coastal protection (Sand-Jensen 2007).

3.2.5 THE HEATHER BEETLE, *LOCHMAEA SUTURALIS*

The heather beetle, *Lochmaea suturalis*, is a raising problem on Danish heathlands. The beetle attacks *Calluna Vulgaris* and eats the leaves. During the outbreak of the heather beetle, the areas covered by *Calluna vulgaris* are damaged to such an extent that the Calluna plants often die and is replaced by grasses (Brunsting & Heil 1985). Britton *et al.* 2000 observed that Calluna plants with high level of nitrogen in the leaves are more exposed to the beetle. The heather beetle has a single generation each year. When the average temperature rises over 10 degrees the heather beetle that has overwintered in the soil starts to eat the leaves and buds of the *Calluna vulgaris*. When the average temperature reaches 15 degrees the mating process begins and beetles fly around actively (Sand-Jensen 2007). If the heather is damaged from earlier attacks the beetles can fly and by the help of the wind spread to other localities. Several years ago, when heath localities were still connected, the beetles did not have problems spreading to new localities. This is not longer the case today and beetles that are unable to disperse most likely starve to death when no more food is available (Sand-Jensen 2007).

Female beetles on a good heathland locality will lie up to 175-200 eggs at the bottom of the heath. The eggs laid in May hatched in June. The larvae eat of the heather before pupation in July. In late August the newly hatched beetles will start eating the heather. Heathlands severely attacked are left with a very characteristic rusty red color (Symes & Day 2003). The beetles live of the heather as long as the weather allows it and then they crawl into their hibernation under the ground cover (Sand-Jensen 2007).

The heather beetle is both good and bad for the heather. With periodic attacks the heather is forced to regenerate and renew itself. On the other hand the attacks can become so intense that the heather dies. When the heather dies it creates an opportunity for grasses to invade the heathland (Symes & Day 2003).

3.3 MANAGEMENT OF HEATHLANDS

The goal of management is to sustain heath vegetation and to keep the areas open (Naturstyrelsen 2014(C)). Extreme conditions on dune heathlands can often naturally maintain this vegetation type. However, earlier pine plantations have created a need for management. Furthermore, increasing level of nitrogen deposition has resulted in more severe attacks by the heather beetle that contributed to grasses colonization (Naturstyrelsen 2014(C), Ellemann *et al.* 2001).

Management practices are decided based upon an evaluation of the state of the area and the result of previous management strategies (Buttenschøn 2008). Of course, it is important to have structural and species composition information of the locality for decision making (Buttenschøn 2008).

The effect of management on nutrients removal

Action	Remarks
Clearing of *Pinus mugo*	Little effect – can even have a nutrient enrichment effect if the cut down trees are left in the area.
Grazing	Can export a small level of nutrients, but can locally have an enrichment effect if substitute fodder is given
Mowing	This form of management has positive effect provided that the plant material is removed.
Burning	Tailwind burning is a quick process that is just as efficient as cutting. Headwind burning is slower, deeper and as a result even more efficient.
Top soil removal	Is very efficient because all vegetation and part of the top soil which contains nutrients is removed.

TABLE 1. MANAGEMENT TECHNIQUES FOR NUTRIENTS REMOVAL JØRGENSEN, 1999

The table 1 above describes the different forms of management of heathland starting with the actions from low to major effects. The different actions are described below.

3.3.1 CLEARING

To prevent overgrowing on heathlands with trees and woody plants, clearings take place. This removes the woody vegetation and opens the landscape. This can be used as a management form itself or as a starter for further management (Buttenschøn 2008). Trees and other vegetation that have been cut down should be removed from the area to ensure that nutrients are removed. When clearing an area it is important to consider the vegetation present in the area. Some of the woody vegetation found in overgrown heathlands will regenerate by root shots and therefore will regenerate strongly after clearing (Buttenschøn 2008). In cases like this it is important to include different forms of management to ensure that undesired species are removed and do not regenerate (Buttenschøn 2008).

3.3.2 GRAZING

Sheep, horses and cattle are responsible for most of the grazing done in Denmark. Grazing is both used as part of management and restoration strategies (Buttenschøn 2008). Grazing induces a high recovery of CALLUNA *vulgaris* on the heathlands with podzolic and peat soils (Bokdam & Gleichman 2000).

In earlier times traditional management have involved an interaction between grazing, cultivation of the land and the use of turf and plant material from the heathlands (Webb 1998) and grazing alone can therefore in some cases not be enough to keep the land open and eradicate problem species such as grasses and *Empetrum nigrum*.

Empetrum nigrum is almost never grazed by farm animals, but is affected through trampling (Buttenschøn 2008). Grazing will not stop ongoing invasion of *Deschampsia flexuosa* on Calluna heath habitats (Bokdam & Gleichman 2000).

One small challenge with grazing is that it can be difficult to organize it on relatively small patches of heathland (Webb 1998).

3.3.3 CUTTING

Cutting of the vegetation is a very common form of heathland management because it is an efficient way of renewing the heather (Buttenschøn 2008, Jørgensen 1999). Cutting of the vegetation was already done by heathland farmers who then use it as fodder, roofing ect (Riis-Nielsen *et al*. 1991). The difference with today's cutting practices is that farmers removed dead material from the heath hence removing nutrients simultaneously. Today much of the cut vegetation is left in situ, and the nutrients are therefore no longer removed (Riis-Nielsen *et al*. 1991).

Frequent cutting (every 6-10 years) will give a very uniform heathland with very young heather. If the heathland is cut less frequently in a mosaic pattern, the diversity will increase (Buttenschøn 2008). The removal of the old heather plants also generates openings with bare soil where lichens and mosses can colonize (Riis-Nielsen *et al*. 1991).

3.3.4 BURNING

As described in table 1 by Jørgensen (1999), burning can vary in efficiency. Tailwind burning is a quick process that is just as efficient as cutting. Headwind burning is slower, deeper and therefore more efficient than tailwind burning (Jørgensen 1999). Burning will very efficiently rejuvenate *Calluna vulgaris* both from vegetative shoots and germination of the seed bank present4 in the soil. At the same time it is very effective at reducing the dominance of *Empetrum nigrum* and *Deschampsia flexuosa* (Jørgensen 1999, Riis-Nielsen et al. 1999). What makes burning efficient is that it removes nutrients; especially nitrogen from the area, which is important in the long run to ensure the persistence of heathland species (Jørgensen 1999). Burning is today very often used for heathland management. Especially patch or mosaic burning becoming more popular due to success rate of this practice. Mosaic burning when compared to burning of large areas seems more appropriate for the plant and animal communities as the area become suitable for a larger range of species (Jørgensen 1999).

By choosing the mosaic burning method the fire is easier to control than traditional burnings. The smaller areas do not have as many restrictions when it comes to moist and wind conditions. Burning of smaller patches can be done in one day whereas the optimal and approved conditions for burning larger areas may only occur with years between (Jørgensen 1999).

3.3.5 TOP SOIL REMOVAL

A study by Degn (2005) has shown that topsoil removal is a valid method for recreating *Calluna* dominated heathlands. When removing the topsoil layer the entire vegetation and seed bank is removed and the bare mineral sand is exposed. Topsoil removal is expensive but also the most efficient way to remove nutrients from an area (Jørgensen 1999, Sand-Jensen 2007). By using topsoil removal an area can sustain itself naturally for a longer period (around 50 years), whereas burning for example needs to happen again after around 20 years (Sand-Jensen 2007). Heathlands that have been invaded by grasses such as *Deschampsia flexuosa* and *Molinia caerulea* can with great success be managed with topsoil removal. By removing the topsoil and thereby the total grass plant including the roots the grass will only be able to regenerate with seeds from the surroundings. Because *Calluna vulgaris* is quicker to regenerate in mineral sand the grasses should be outcompeted (Sand-Jensen 2007).

4. DESCRIPTION OF THE STUDY AREAS

4.1 SELECTION OF THE STUDY AREAS

The purpose of this study is to investigate a number of different localities and register the lichen and bryophyte vegetation. Twelve different areas were selected by different criteria. The first selection was made from the list of protected habitat heathland areas in Denmark (Naturstyrelsen 2014(E)). The next selection process was made with advices from managers and people involved with those habitats. Areas that were not on the habitat list, but known as areas with a high diversity of lichen and bryophytes were also selected. This project is supported by the Aage V. Jensen foundation and the heathlands areas owned by the foundation is therefore included as well. The last selection was made in the field simply by driving through the countryside and seek out good looking heathland localities.

The 12 selected areas are; Melby Overdrev (MO), Klosterheden Plantage(KL), Ovstrup Hede (OH), Hanstholm Reservatet (RE), Grene Sande at Store Råbjerg (GS), Harrild Hede (HA), Hulsig Hede (HH), Hvidklit (HK), Kandestederne (KS), Randbøl Hede (RH), Sønder Vosborg (SV)and Vrads Sande (VS).

The picture below shows the distribution of the selected areas. All other pictures used in the area descriptions are taken by the authors when investigating the areas.

Picture 1. The 12 localities in Denmark

4.2 DESCRIPTION OF THE STUDY AREAS

4.2.1 MELBY OVERDREV (MO)

Melby Overdrev is 130 ha. and located by Kattegat in the northern part of Zealand. Melby Overdrev is an old military area and the Danish military was active in the area for more than 100 years with training exercises (DN.dk 2014). The area is a natura2000 area and is therefore protected (Naturstyrelsen 2014(A), Naturstyrelsen 2014(D)).

Because of the history of the area the military has left traces in form of burnings and disturbance of the soil. Thanks to these disturbances the area has been left with a mosaic like vegetation with heather plants of all ages with gaps and exposed bare ground. The flora and fauna in the area is also in good condition, together making the area very special for this part of Zealand. There are clear signs of deers, which helps to keep the vegetation open by grazing and trampling the Area.

This locality is located just by the beach and close to a summerhouse area which makes the locality quite popular for the public.

4.2.2 KLOSTERHEDE PLANTAGE (KL)

Klosterhede Plantage is located in the north western part of Jutland and consists of a large pine plantation with open heathland areas. A stream called Flynder Å, where beavers (*Castor fiber*) were released in 1999 cut through the heathland area (Naturstyrelsen, 2014(B)). Both heathland and stream are protected Natura2000 areas because of the species composition.

The heathland area is mostly dominated by *Calluna vulgaris* but are in some parts completely covered by *Molinia caerulea*. Cattle and reed deer graze the entire area including the pine plantation all year round.

In spite of the very high grazing pressure the heather and different grasses are still very dominant. When the locality was visited not many other species were registreed. The lichens are most often found near smaller paths where the vegetation is open and sunlight can reach the soil.

After the sampling in the area a large part of the plantation burned down. The heathland area in Klosterheden was not touched by the fire. After the fire it has been decided to leave the burned down areas open and grazed and by time see them turn into heathland (Naturstyrelsen 2013(A)).

4.2.3 OVSTRUP HEDE (OH)

Ovstrup hede is 490 ha big and is owned by the Aage V Jensen foundation. The foundation took over the area in 2002 and started a nature recovery project to restore the vegetation and recreate the original heathland (avjf.dk 2013). Ovstrup Hede is a Natura2000 area and is thus protected. It is therefore the duty of the foundation to manage the area and keep them from growing into forest.

The area is surrounded by pine plantations and has many traces from red deer (grazing ect). The condition of the area seen in a lichen perspective is very poor. Even though the heather has been cut, the area is overgrown by *Calluna vulgaris* and different types of *Carex* species, which results in no open soil where the lichens can grow. The only places where lichens were registered were in small tracks made by the animals.

4.2.4 HANSTED RESERVATET (RE)

Hansted Reservatet is almost 4000 ha. nnd therefore the largest connected heathland area in Denmark. The area is located in the northern part of Jutland by the west coast. The landscape is a mosaic of flat open blow down surfaces and large dunes. Between the large dunes many smaller lakes are located. The area was bought by the Danish hunting society after the Second World War with the purpose of creating a free spot for the wild fauna and for keeping the area as a heathland area (Naturstyrelsen 2013(B)).

In 1972 the area was protected because of the high landscape value of the heath land and dunes and because of the many historical values in the area. The rich bird, animal and insect life was also an important factor in the protection (Naturstyrelsen 2006).

The area is dominated by *Calluna vulgaris*, *Empetrum nigrum* and *Ammophila arenaria*. In 1992 a large fire burned down 175 ha. of heathland and dune land (Naturstyrelsen 2013(C)). After that the area has been managed to protect the open land and mosaic formation. Trees are removed and the place is often burned and cut for conservation purposes. Grazing is also happening (Naturstyrelsen 2006).

Because of the closed vegetation there is little or no sand drift. There are many traces from grazers, mainly reed deer that have made small path through the vegetation. An important note is that the lichen vegetation seemed trampled down and in a poor condition

4.2.5 GRENE SANDE, STORE RÅBJERG (GS)

Grene Sande at Store Råbjerg is a 160 ha area, where the heath areas are surrounded by pine plantations. It is a Natura2000 area and is protected because of the different nature types (dry heath and wet heath) and because of the rich plant and animal life (Billund Kommune 2011). The heathland is characteristic with open windbreaks and a large parable shaped dune. Earlier ice ages and sand drifts created large slopes in the area (Naturstyrelsen 2013(D)). These slopes are today covered by the plantation, but will be cleared and turned into heath (europa.eu 2009).
Management in the area consists of promotion and renewal of the heather to maintain an open landscape, burning *Deschampsia flexuosa* dominated areas, removal of top soil and cutting in areas dominated by *Molinia caerulea*. Furthermore there are plans to remove self-sown pine trees, clearing of bushes and trees in the heath bugs followed by extensive grazing (Billund Kommune 2011).

The area is today a part of the LIFE project RAHID that has the purpose to manage inland heathlands. Because of this, large parts of the pine plantations are being cut down to create a large coherent heath area.

The area is being kept open, and this leaves open soil between the heather. The open and bare soil between the plants gives the lichens optimal conditions. Grasses today dominate extensive areas, but through the RAHID project, the overall condition of the locality will be improved (eurppa.eu 2009).

4.2.6 HARRILD HEDE (HA)

Harrild Hede is connected to Nørlund plantation, and the two areas together form a 2349 ha heathland area (Naturstyrelsen 2014(F)). The area is a Natura2000 area and has also been selected as a Natura2000 area. The area was for more than 200 years ago covered by inland dunes and shifting sands. Because of this farmers decided to plant pines to reduce the sand drift caused by sandstorms.

Because the area is very dry, it has a history of burning. In 1971 the plantation burned down and 605 ha. was lost. Some of the area was replanted with pines, but other areas have not been touched, and are today dominated by heather (Naturstyrelsen 2013(E)). Because of many unattached fires in the area, artificial lakes and dams have been created to make sure that the fires could be put out if needed.

Today, Molinia dominates large parts of the area, and gaps in the vegetation are very scarce. The area is a protected Natura-2000 area and has been selected to be part of the LIFE project RAHID that works with different ways to manage heathland. (europa.eu 2009).

4.2.7 HULSIG HEDE (HH)

Hulsig Hede is a 2170 ha. area located in the very northern tip of the Jutland peninsula. The area stretches from coast to coast over the isthmus of Skagen (Dahl 1994). The area is an EU-habitat area and is dominated by dunes and several larger moving dunes. Heather and grasses dominate the vegetation. In the 1500-1700 many pines were planted to lower the sand drift (Dahl 1994). Few of these pines are left today, and the area is being kept open.

Large parts of the area got their protected status in 1940. Today a large area containing more of our selected areas such as Hulsig Hede and Hvideklit has been pointed out as one big connected Natura2000 area (Dahl 1994). Råbjerg Mile, which is the largest moving sand dune in the northern part of Europe, is now also connected to Hulsig Hede and the protected area (Dahl 1994).

Hulsig Hede has heather of all ages and gaps are present where lichens have room to grow. The many dunes provide a varied landscape where reindeer lichens establish successfully. The very rare *Cetraria nevalis* has been found more the one time, which indicated a high nature quality.

4.2.8 HVIDKLIT (HK)

Hvidklit is located in the northern part of Jutland and is part of the large connected Natura2000 area described in the section about Hulsig Hede. The area is very open with several large dunes. Some larger patches overgrown with grasses were found, but no other signs of overgrowing were detected.

PICTURE 2. MAP OF THE NORTHERN PART OF JUTLAND (DAHL 1994)

Hvidklit is located south of Bunken Klitplantage, see picture number 2, and is therefore surrounded by more than 100 years old pine plantations (Dahl 1994).

No references from the Danish Nature Agency could be found on this specific area, thus the sparse description of the history and management of the specific area.

4.2.9 KANDESTEDERNE (KS)

Kandestederne is a dune heath area located 15 km south of Skagen in the very northern part of Jutland. Most of the dunes are covered with *Ammophila arenaria* but single dunes are open with bare sand (Dahl 1994). The area is a part of a larger connected and protected Natura2000 area that also includes Hulsig Hede, Hvideklit and Råbjerg Mile (Dahl 1994). Kandestederne is a summerhouse area with many summerhouses spread out into the area. Because of the many high dunes in the area the area seemed undisturbed and in many places untouched.

The very invasive *Rosa rugosa* is heavily present on Kandestederne, this invasive species could easily become a bigger problem if management is neglected.

4.2.10 RANDBØL HEDE (RH)

Randbøl Hede is a 750 ha. large heathland area in the middle of Jutland south east of Billund. The area is an EU-habitat area and is today protected because of the nature type and the special animal life. The area was protected for the first time in 1932, and at the time it was the biggest case of land-protected area in the history of Denmark. The purpose of the protection was to secure the open heathland areas and make sure that the heath land didn't overgrow ore were used for farming. The area was protected and any changes were prohibited. Heathlands are unstable nature types, but the lack of disturbances meant that the area overgrew and turned from a heath land into a grass heath land. Especially in the 1960´s the heath changed. Today grasses, especially Molinia, dominate large parts. In 1980 the state bought most of the area and ever since nature management has been done in the area, however without good results (Naturstyrelsen 2013(F)).

The area is quit flat, however some large open sand dunes are found. It is especially in these sand dunes that the lichens were found, since this was one of the only areas with open soil.

Untill 1992 *Lyrurus tetrix* lived and breed at Randbøl Hede and the area was therefore protected (dofbasen 2013).

Today Randbøl Hede is part of the Life project RAHID, and large parts of the area will be exposed to different form of management to try to recreate the original heathland (europa.eu 2009). In 1996 a trial was made at Randbøl Hede where the top soil was removed in an area and the open soil was left untouched. In 2005 HJ Degn could describe the effects of the trial (Degn 2005).

Because of the very good results the RAHID project will among other use top soil removal to stop the invasion of Molinia, (europa.eu 2009).

4.2.11 SØNDER VOSBORG (SV)

Sønder Vosborg Hede is a 170 ha. large area located in the western part of Jutland. The area is filled with sand dunes and open windbreaks. Between the dunes bushes of *Juniperus communis* grow. Because of earlier burnings the heather is found in all ages, which also leaves openings for the lichens to grow (Naturstyrelsen 2013(G)).

Until resent time the area have been completely closed to the public because the black grouse (LYRURUS TETRIX) USED TO breed there. When LYRURUS TETRIX WENT EXTINCT at Sønder Vosborg in the 1980´ths (Naturstyrelsen 2013(H)) the area was opened up for the public that today uses it for hiking, biking ect. In the area there are signs of grazing from a large population of reddeer.

Today the area has been selected to be part of the Life project RAHID and will until 2015 have a higher level of management than normal. The different form of management that will be implemented at Sønder Vosborg Hede is among other top soil removal and burning (europa.eu 2009)

4.2.12 VRADS SANDE (VS)

Vrads Sande is located in the middle of Jutland and is a special area because the land never has been used as farmland, (Naturstyrelsen 2013(I)). Because of the very well developed dunes in the area it has not been possible to use it for anything than grazing, and therefore the land today is still untouched. The management in the area today contains of keeping the area open so that the dunes appear clearly and visible, (Naturstyrelsen 2013(I)).

The area is overgrown with heather that, because of the very hard winter, has died. Before this the heather has been so dominant, that almost no other species were found in the area (see picture). Around the area a large pine plantation have been planted.

5. METHOD

5.1 FIELD WORK

The fieldwork was realized between May and September 2013, which is in Denmark the logical choice due to pleasant weather conditions. Prior to any work, a list of all the protected Danish heathland localities was made and from this list the localities were prioritized for being in good conditions. Focusing on good heaths was important, as the aim was to find characteristics responsible for rich and diverse lichen and bryophyte flora.

The designed for data collection was heavily influenced by the method used for the species index and NOVANA surveillance used for land monitoring. The goal was therefore to sample plots represented by 5-meter radius circles and as many plots as necessary to capture diversity with the maximum number of plots being 10. It was also decided to sample an equal number of random and selected plots at each location.

When arriving at a new locality the first step of the registration was a long walk around the area. This allowed us to make a mental representation of the area and pick out some of the better places for the hotspot circles. The hotspots were laid out in areas where lichen diversity seemed above average, with either many species or few but rare species, growing on optimal heathland conditions. When the hotspot had been defined the registration would begin and from there, we selected a random circle.

The procedure for random circles was to walk in a random direction given by a compass on an Iphone and for a random number of steps (ca. 1 meter steps) represented by the number of seconds that a digital watch was displaying at the time (therefore between 10 and 60). To make sure the circles did not overlap the minimum of seconds that could be used was 10.

For each circle was registered presence/absence of species on sheets prepared beforehand. Registration includes vascular plants, bryophytes and lichens. All species that were not identified in the field were collected in paper bags and brought back to the University of Copenhagen for further identification. On the bags was written a code for the specific heath locality and the corresponding circle from which the sample was taken.

Additionally, for each circle a soil sample was collected and placed into paper bags labeled similarly with the addition of 'soil sample'. The soil was usually collected close to the center of the circle and humus layer thickness was measured with a small ruler. Additionally, 100 Raunkjær circles were thrown on each locality to study the extent coverage of reindeer lichens.

We further noted GPS coordinate of each circle and the date at which data was collected. Also to be mentioned, a short description of the heathland was written upon arrival on heathland sites. Description includes some of the key structures, such as the presence of dunes and bare soil but also dominating species, presence/absence of trees and invasive species. To document the state of the visited locality pictures were taken as proof.

All samples brought back were further identified and registered on excel sheets.

5.2 SPECIES IDENTIFICATION

The further identification of species was done at the University of Copenhagen were literature and necessary equipment is available.

Because many lichen species were unknown to us it was at the beginning necessary to key almost every single one met in the field and in lichenology this process often require the use of good hand lenses or binocular microscopes and chemicals.

Three major chemicals must be listed here as they greatly helped in the identification of Cladonias; Potassium hydroxide (K), bleach (C), and *para*-phenyldiamine (pD). These chemicals are used for spot tests on the lichen thallus and do or do not react with secondary components present in the thallus.

Another common characteristics for the identification of lichens are the reaction under UV light and therefore UV lamps were used.

A species checklist for bryophytes of Danish heathland greatly facilitated the identification of species as it limited the number of possibilities. We hardly keyed any of the bryophyte species we collected but used the knowledge of people who could help.

Most vascular plants were identified *in situ* and the remaining ones were keyed back at the university.

After the identification process of lichens, all data will be registered on Svampeatlas.dk

5.3 LABORATORY WORK

Work in the lab was necessary for the identification of lichen that could not be identified with the traditional use of keys and spot tests.

5.3.1 THIN LAYER CHROMATOGRAPHY (TLC) & HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

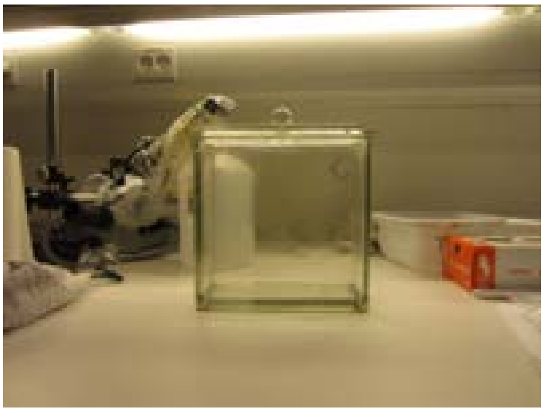
The identification of Cladonia species from the so-called “Chlorophaea group” is often difficult when using only morphological characteristics due to the huge variability in shape and size of the different species. It is therefore often necessary to turn towards chemical analysis, especially “Thin Layer Chromatography” (TLC) since spot tests give similar results for the different species.

Thin Layer Chromatography is a chemical technique allowing the separation of different chemical compounds in a mixed solution. In the case of lichens we study the second chemistry present in the lichen thallus, which most often differ from species to species. Two types of procedures were used, the more common TLC approach based on (White & James 1985) and a more recent and advanced procedure called High Performance Thin Layer Chromatography (HPTLC) based on (Arup et al. 1993).

The exact procedures found in references cited above were followed and the solvent used was always C (Toluene : acetic acid; 200 : 30 ml (T.A.))

For the TLC development a vertical twin through chamber and 20x20 cm silicate plates were used. HPTLC was however performed with a camag horizontal developing chamber that only allows 10x10 cm silicate plates.

Nanomat III



Classic TLC chamber

In order to apply chemical mixtures on plates, a Nanomat III with a holder for capillary tubes (picture above).

Possible candidates for our samples comprised: *Cladonia chlorophaea*, *Cladonia merochlorophaea*, *Cladonia novochlorophaea*, *Cladonia cryptochlorophaea*, *Cladonia grayi* and *Cladonia pyxidata*.

Examination of plates and identification of chemical spot is done by looking at Rf values of the different spots.

$$Rf = \frac{Migration\ distance\ of\ subtance}{Migration\ distance\ of\ solvent}$$

White & James (1985) offer nice charts for identification of common lichen products by TLC.

We used atranorin and norstictic acid as chemical references.

We also used vouchers, courtesy of Teuvo Ahti who has worked extensively with the genus *Cladonia*.

Another group that has proven to be most difficult is the "Coccifera group" with mainly four different species: *Cladonia deformis*, *Cladonia coccifera*, *Cladonia pleurota* and *Cladonia diversa*. However, chemical analysis has not proven to be successful since different species can have same chemical profile but also variation in chemical profile within a single species. The topic of Zeorin-containing red fruited *Cladonia* species is discussed extensively by Steinova et al. 2013, and concludes that chemical analysis is obsolete on the group due to genetic incongruence and problem of species delimitation. The best way to identify the currently accepted species is therefore still size, shape, character of podetium and soredia.

5.3.2 ANALYSIS OF SOIL SAMPLES

All soil samples were brought back and analyzed in the laboratory at the University of Copenhagen. From the 112 plot circles investigated in Jutland, soil sample from 8 of them were somehow lost.

5.3.2.1 PH

For the pH 30 g. of the collected soil and 50 ml. of distilled water were mixed and shaken using a clean spoon for 10 min. Samples were measured for pH using an Accumet® model 15 pH meter which had been calibrated using pre-prepared solutions at pH 4 and 7.

5.4 THE SATE ASSESSMENT INDEX

The state assessment index can be used to assess the quality of an area. The state of a nature type is assessed by different structure indicators combined with a registration of the vascular plants in a five meter documentation circle. The circle is selected as the spot in the area that is the least affected by impacts and is as a result sometime not representative of the entire area. The documentation circle expresses the best biological value in the area. The biological value is important to know when it comes to management both for concrete management efforts but also for the prioritization of different areas, Naturstyrelsen, 2010.

Both the species and the structure index vary between 0-1, where 1 is the best and highest possible score and 0 is the poorest and lowest score. The overall condition of the nature is further subdivided into five different assessment classes (Fredshavn et. al. 2010).

1. High state is characterized by little or no human created changes in the physical, chemical and hydrological elements in the area. The values for the different biological conditions are what normally characterize a similar untouched nature type. Furthermore there are little or no signs of changes in the area.
2. Good state is characterized by the fact that biological conditions show very little changes as a consequence of human activity. The nature type only differs a little bit from similar nature types that are untouched.
3. Moderate state is characterized by a moderate difference from similar untouched nature types.
4. Poor state is characterized by larger changes in the values for the different biological indicators. The relevant biological composition differs greatly from a similar but untouched nature type.
5. Bad state is characterized by severe changes in the values for the different biological indicators. Large parts of the biological composition that will be found in a similar but untouched nature type do not appear.

The structure and species index combined will give the nature state assessment index that on a scale between 0-1 will show the condition of the nature type. This index gives for the first time a national method to standardize and compare the quality of the nature in different areas (Fredshavn et. al. 2010).

5.4.1 THE STRUCTURE INDEX

With a basic registration in the area, a structure index can be calculated that also vary on a scale from 0-1 and describes the current state and the management needs of the area relative to threats such as draining, overgrowing ect. (Naturstyrelsen 2010).

Different parameters of the nature types characterize the structure index. These parameters are:

- Vegetation structure
- Hydrology and costal security
- Soil
- Characteristic structures for the specific nature type.

An improvement of the structure index will lead to an improvement of the species composition and deterioration in the effects in the nature will most often lead to a deterioration of the species composition (Fredshavn *et. al.* 2010).

The parameters reflect the pressure from different threats on the area and thus express the need (or not) for management. It is important that the different indicators are registered the way they look the day the area is investigated (Naturstyrelsen 2010).

5.4.2 THE SPECIES INDEX

With an expanded registration in the area it is possible to calculate a species index. The species index *A* describes on a scale from 0-1 the currently best biological state in the area (Naturstyrelsen 2010). The species index is build from two smaller indexes; the species score index A_s, and the species diversity index A_d. By combining these two indexes the species index can be calculated (Fredshavn *et al.* 2008).

The species index *A* indicates if the species normally found in similar nature types have been able to colonize and survive in the investigated area. This index therefore also reflects the historical development of the area. The species index is composed by all the species registered inside a 5-meter documentation circle.

For different nature types different indicator species has been selected. There indicator species represent the most common found species in earlier registrations. All species, not only the 20-selected indicator species has been given a score between -1 and 7. Species with -1 are the problem species, species with 0 are the cero-species that does not contribute with anything in the area and species with a score between 1-7 are the species that contribute positively.

- Species that contribute are species that positively contributes to the calculation of the species index.
- Problem species are species that under normal conditions would not occur on the specific nature type. Species that are highly foreign and creates a high level of disturbance. This is also species that rarely are found in areas in a good condition. This could for example be invasive species.
- Cero-species are species that has been brought ore introduced including many farm plants and garden plants that under natural conditions would not be found. Because they are seen as random “visitors” they are given the value 0, so they have no influence on the species index.

The calculation of the species score index A_s is made with the following formula:

$$A_s = 1\Big/\Big(1 + \exp_e(m_a)\exp_e\big(1{,}60(1-m)\big)\Big)$$

Where m is the middlescore for the specific area, m_a is the average value of the areas middle scores and $\exp_e$ is the natural exponential function. In this index the species number is included but the positive and negative species are not differentiated.

The calculation of the species diversity index A_d is calculated with the following formula:

$$A_d = (a_b/a_t)\Big(1-\big(1/\exp_e(s/d)\big)\Big)$$

Where s is the sum from the specific area and d is a diversity parameter that depends on the average number of species for the nature type. a_b/a_t is the relative part of the species that contribute positively a_b relative to the total number of species included the cero and negative species a_t.

The species index *A* is in the end calculated as follow:

$$A = 0,75A_s + 0,25A_d$$

The species score index is weighted higher because it expresses a more general and independent information than the species diversity index that is weighted lower (Fredshavn *et al.* 2008).

5.5 DATA ANALYSIS

In order to explore data and get more insight on community ecology of the different heath localities we visited, we decided to perform many different types of statistical analysis, where the general concepts will be described below. The software used were Rstudio, Modular, and PAST statistics.

THE NAÏVE APPROACH

This approach is our first attempt at making sense of the data. All statistics used evolve around our exploratory NMDS ordination.

5.5.1 NONMETRIC MULTIDIMENSIONAL SCALING (NMS)

Nonmetric Multidimensional Scaling (NMS) is an unconstrained ordination technique that has been increasingly used in community ecology. NMS is able to measure distances in communities with the advantage of not making many assumptions about the nature of the data such as linear relationships in Principal Component Analysis for example (Holland 2008).

The goal in this thesis was to explore patterns in ecological communities and investigate correlations with environmental variables such as humus layer thickness and pH value.

By using ordination the data is geometrically arranged and the distances between the sites represent their ecological distance. Sites placed close to each other on the ordination plot are similar in term species composition. Conversely sites located far from each other have a more different species composition. The ordination can also show the ecological preferences for individual species. The closer two species are located; the more similar are their ecological preferences (Borcard et al. 2011).

5.5.2 INDICATOR SPECIES ANALYSIS

The Indicator species analysis (ISA) is a statistical test that aims at determining the occurrence of a set of indicator species that reflect either biotic or abiotic state of the environment (De Caceres 2013). We performed two different indicator species analysis using two different classifications of sites into groups.

For the first ISA the aim was to find characteristic species for different thickness of humus layer. Plots were divided into four groups based on the quartiles of the humus layer thickness dataset, which 0, 1, 3.5 and 7. Plots were then grouped as follow:

- Group 1: Species associated with circles with Humus layer = 0cm
- Group 2: Species associated with circles with Humus layer] 0; 1] cm
- Group 3: Species associated with circles with Humus layer] 1; 3.5] cm
- Group 4: Species associated with circles with Humus layer] 3.5; 7] cm

For the second analysis we grouped the sites according to their NMDS1 scores. The grouping was done subjectively following this order:

- Group 1: Minimum NMSD1 score to -0.5
- Group 2:]-0.5;0]
- Group 3:]0;0.5]
- Group 4:]0.5 to maximum NMSD1 score

This allowed to identify indicator species for all environmental parameters including humus layer thickness but also not measured ones affecting scores on the NMDS1 axis.

The indicator species analysis was carried out in statistical program R with “indicspecies” package.

THE NETWORK APPROACH

This approach is completely different and much more objective, we followed the procedure for modularity used by Olesen 2007 and used the software MODULAR.

5.5.3 MODULARITY

Ecological systems can be seen as a network of interactions and a key feature of these networks is there organization into connected elements called modules (Marquitti et al. 2013). Analysis of modularity is used to identify those modules and in ecology can allow the identification of species or sites important for maintaining cohesion in the network (Marquitti et al. 2013). Areas that are link-dense are often described as compartments or modules, and species inside a module are more tightly linked to species within their own module than to the species in other modules (Olesen et al. 2007, Marquitti et al. 2013). Because the different modules contain areas or species connected to each other, modularity may reflect habitat heterogeneity and phylogenetic clustering of related species (Olesen et al. 2007). To calculate the different modules in our datasheet the program MODULAR was used. MODULAR is a software designed to detect modules in unipartite or bipartite networks such as ours. The software is user friendly and allows autonomous computation of modularity. The software gives the choice between two metrics extensively used in ecology; Newman and Girvan's - Q(Newman and Girvan 2004) and the Barber's - Q_B (Barber 2007) which we used as it is optimal for bipartite networks. The software also includes two null models that validate the degree of modularity from two theoretical benchmarks (Marquitti et al. 2013). We ran 100 null models.

Degree of modularity is given by the following formula:

$$M = \sum_{s=1}^{N_M} \left(\frac{I_s}{I} - \left(\frac{k_s}{2I} \right)^2 \right)$$

Where N_M is the number of modules in the network, I_s is the number of links between all the species within the module *s*, I is the number of links in the network and k_s is the sum of degrees of all species in *s*, Olesen et al. 2007.

With the result and partition of sites and species into modules *z* and *c* scores can be calculated:

$$z = \frac{k_{is} - \overline{k_s}}{SD_{ks}}$$

and,

$$c = 1 - \sum_{t=1}^{N_M} \left(\frac{k_{it}}{k_i} \right)^2$$

Where *z* is the standardized within module degree, *c* is the among-module connectivity k_{is} is the number of links of *i* to other species in its own module *s*, $\overline{k_s}$ and SD_{ks} are averages and standard deviation of within module *k* of all species in *s*, k_i is the degree of species *i*, and k_{it} is the number of links for *i* to species in module *t* (Olesen et al. 2007, Barber 2007).

5.5.4 ANOVA

ANOVA is an Analysis of variance, and was in this case used to compare the means between the different modules (Fowler *et al.* 2008). The means of the pH value, the thickness of the humus layer and the distance to the coast was investigated for three of the five modules. Module four and five was not used in the ANOVA analysis because these modules were too small and insignificant to show anything (appendix 5 and 6).

We used the same procedure to determine differences between the groups in second Indicator species analysis using NMDS scores.

5.5.5 MULTI LINEAR REGRESSION

To investigate the some of the parameter that could explain high z and c scores for site we used a simple multi linear regression analysis. We used pH, Humus layer thickness, distance to coastline and total number of species predictor variables of separately z and c.

6. RESULTS

6.1 RESULT FROM SPECIES IDENTIFICATION BY THE USE OF TLC AND HPTLC

Some species proved to be very difficult to identify just by looking at the morphological characters. It is therefore necessary to turn towards two similar chemical tests for the final lichen identification; Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC). Species from the *chlorophaea* species group are particularly challenging to identify (e.g. *Cladonia novochlorophaea*, *merochlorophaea*, *cryptochlorophaea* and *grayi)* because of their similar morphologies.

In picture number 3 shown below an example of TLC plate is presented. On the TLC plate 17 species from Klosterheden has been tested with TLC. In this plate all four of the different species from the *chlorophaea* group were found. Because of the good state of this plate, it was used as a reference in the rest of the TLC identification process, which was made with use of the description from White *et al.* 1985.

Picture 3. TLC plate from Klorsterheden showing four chemical profiles of interest to differentiate between *Cladonia cryptochlorophaea*, *Cladonia merochlorophaea*, *Cladonia novochlorophaea* and *Cladonia grayi*.

Chemical compounds found in the lichens are shown very clearly and are distinct from one species to another. Below we review chemical profiles of the four different species from the *Chlorophaea* group is shown.

C. cryptochlophaea contains fumarprotocetraric acid in *Rf* class 2 and cryptochlorophaeic acid n Rf class 5. Both chemical compounds are very visible on the TLC plate, and the sample is therefore identified as *Cladonia cryptochlorophaea*.

C. merochlorophaea contains one of two different chemo types; 1) merochlorophaeic acid in Rf class 5-6 and fumarprotocetraric acid in Rf class 2 or 2) merochlorophaeic acid in Rf class 5-6. On the TLC plate from Klosterheden only merochlorophaeic acid is visible, and the sample has been identified as *Cladonia merochlorophaea* chemo type 2.

C. novochlorophaea contains one of two different chemo types; 1) homosekikaic acid in Rf class 5-6, sekikaic acid in Rf class 5-6 or 2) homosekikaic acid in Rf class 5-6, sekikaic acid in Rf class 5-6 and fumarprotocetraric acid in Rf class 2. Since all chemical compounds are visible on the TLC plate the sample is identified as *Cladonia novochlorophaea* chemo type 2.

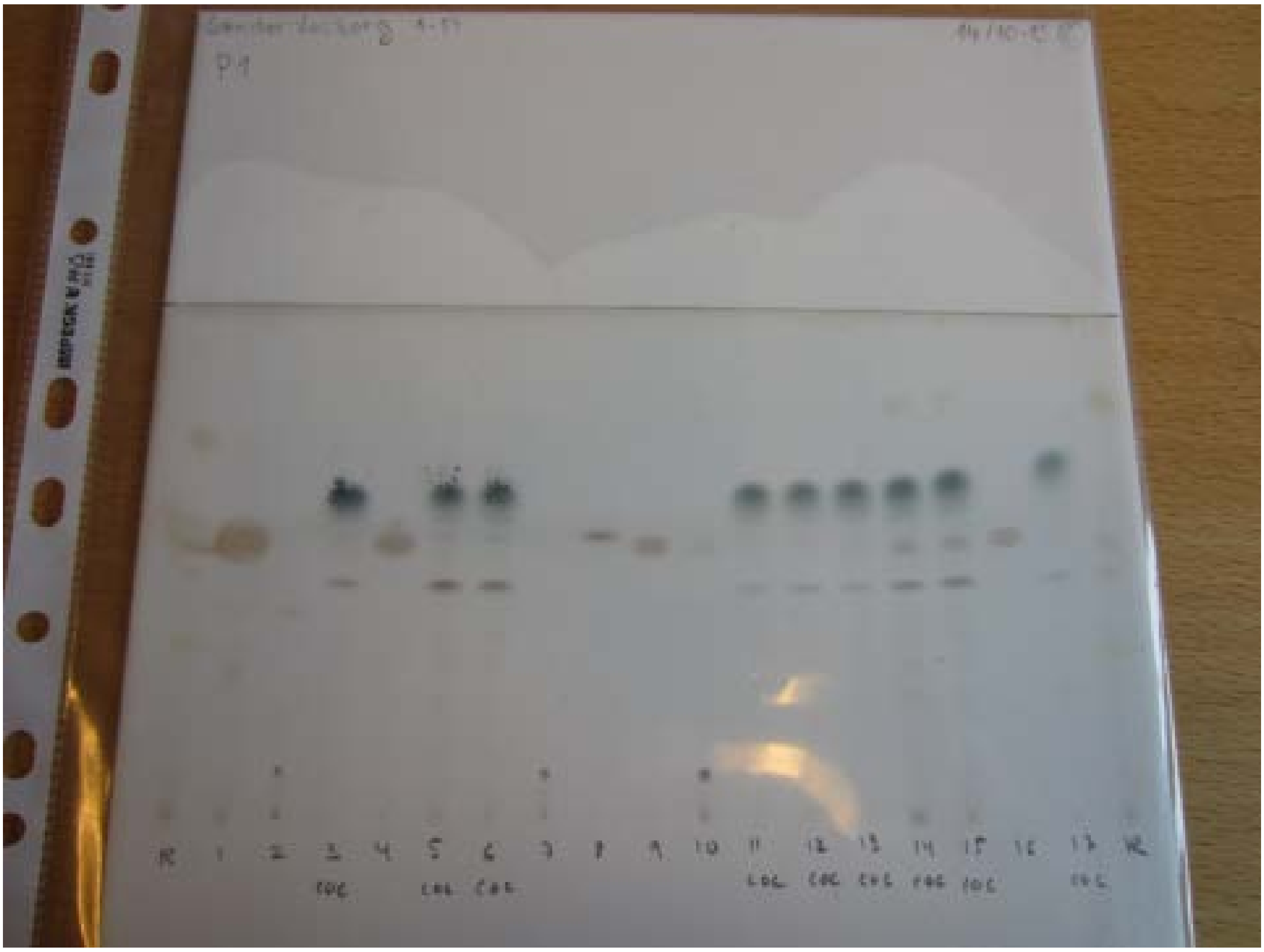
C. grayi contains one of two different chemo types; 1) grayanic acid in Rf class 5-6 or 2) grayanic acid in Rf class 5-6 and fumarprotocetraric acid in Rf class 2. The sample contains all the chemical compounds and is therefore identifies as *Cladonia grayi* chemo type 2.

For the rest of the TLC identification process this specific plate from Klosterheden (Picture 3) was used as a benchmark for the identification of *C. novochlorophaea, C. merochlorophaea, C. cryptochlorophaea* and *C. grayi.*

The same procedure was carried out for the HPTLC plates, see appendix 3.

In some cases the sample that was tested as a member of the *chlorophaea* group turned out to be a member of another group, the *coccifera* group. This was easily spotted by the presence of usnic acid in Rf class 6, see picture 4 below. This mistake was often made with samples from the *coccifera* group that had not yet made the conspicuous red fruits and therefore looked like a young member from the *chlorophaea* group. One of the problems encountered during the TLC identification process was the quality of our plates. Even though the method used when preparing the plates was very standardized the outcome varied greatly, see picture 4 and appendix 2. Picture 4 shows the first TLC plate from Sønder Vosborg Hede. It is clear that the solvent ran faster on the sides of the plate hence the curved shape. In these cases the identification was made more difficult because the different chemical compounds are no longer found in their regular Rf class.

Because of this some of the TLC plates were remade with HPTLC to get the identification of the different species as precise as possible.



Picture 4. TLC plate from Sønder Vosborg Hede showing the problems experienced with the solvent.

We only used solvent C for all TLC procedure because of the toxic nature of other solvent (A, B and G) (White & James 1985) and our lack of experience in the lab. With other solvents some of the chemical compounds in the lichen samples may have been more visible and the identification could have been made with more precision but we feel confident that the vast majority of our species have been identified correctly.

6.2 THE EXPERT VALUATION OF THE DIFFERENT LICHEN SPECIES

The purpose of selecting indicator species is to simplify quality assessment of an area by using the vegetation (Fredshavn *et al.* 2010). In order to work as an indicator species, at least in the case of the species index, different values are given to different species according to how they represent a certain habitat type or other character such as sensitivity to changes in the environment, rarity and conservation value (Fredshavn *et al.* 2010). Because of the combination with the structure index it is possible to have areas with a low nature quality but where one or more indicator species occur and areas with high nature quality but where no indicator species occur (Fredshavn *et al.* 2010).

To make the lichen species index comparable to the original index by Fredshavn *et al*. 2010, the lichen species index that we are creating is build upon the exact same principles, and we therefore used the same formulas to calculate the species index.

The valuation and the selection of the lichen scores were made together with our supervisor Proff. Ulrik Søchting. This was done to stress the point that it had to be an expert valuation. Our own experience was made during the summer and fall of 2013 where all our data was collected from the 12 different areas located around Denmark. Species from the *Cladonia* genus that earlier had been found in Denmark, but not during our sampling, automatically received the highest possible score (7) because of their rarity.

Table 2 below shows the different species found in the registration circles, the percentage of times it has been found in the 116 registration circles and the given score that has been determent for them by the expert valuation.

Art	Pct	Score		Art	Pct	Score
C. arbuscula	16,4	5		*C. phyllophora*	0,9	7
C. cervicornis	5,2	7		*C. pleurota*	27,6	5
C. chlorophaea	29,3			*C. polydactyla*	0,9	
C. cenotea	0,9			*C. portentosa*	91,4	4
C. ciliata	16,4	5		*C. ramulosa*	41,4	5
C. coccifera	46,6			*C. rangiferina*	1,7	6
C. cornuta	6,0	7		*C. rangiformis*	5,2	6
C. crispata	14,7	5		*C. scabriuscula*	13,8	5
C. cryptochlorophaea	12,9	5		*C. squamosa*	0,9	7
C. diversa	3,4	5		*C. subulata*	31,9	5
C. deformis	1,7	6		*C. sulphurina*	6,9	6
C. fimbriata	6,9	4		*C. symphycarpa*	0,9	7
C. floerkeana	64,7	4		*C. uncialis ssp. biuncialis*	33,6	5
C. foliacea	31,0	5		*C. uncialis ssp. uncialis*	2,6	6
C. furcata	22,4	5		*C. zopfii*	23,3	6
C. glauca	52,6	4		*Cetraria aculeata*	42,2	5
C. gracilis	25,9	5		*Cetraria islandica*	3,4	7
C. grayi	0,9	6		*Cetraria nivalis*	1,7	7
C. humilis	0,9	6		*Hypogymnia physodes*	14,7	
C. macilenta	42,2	5		*Peltigera membranacea*	3,4	5
C. meroclorophea	40,5	4		*Placimatia glauca*	6,0	
C. mitis	0,9	5		*Stereocaulon condensatum*	5,2	6
C. novochlorophaea	12,1	4		*Stereocaulon saxatile*	6,0	6

Table 2. Lichen scores based on expert valuation.

Each species was valuated and a score was determined. Different factors were included in the process such as the number of times a species had been found in the registration circles, if the species is known to be indicator for bare sand and also the scarcity of the species. The tricky part when selecting scores for the indicator lichen species is to optimize the chances that the area gets a high nature quality but minimize the risk that the state of the nature is overrated.

To keep the lichen species index as close and as similar as the original species index described by Fredshavn *et al.* 2010 the first lichen species that were given a score were the most common one in the registration circles e.g. *Cladonia portentosa*. *C. portentosa* is in our circles just as common as *Calluna Vulgaris* and *C. portentosa* is a known and very common heathland species. Because *Calluna vulgaris* in the original index has been given the score 4, it was decided to give *C. portentosa* the same. By using the *C. portentosa* score as a benchmark together with the factors described above the rest of the *Cladonia* species scores were determined.

In the lichen species index there are no problem species (score -1) because none of the lichens are categorized as invasive or in any way harmful for the rest of the vegetation. All the *Cladonia* species are contributing species and have therefore been given a score between 4 and 7. Of the registered species there are 6 species with a score of 4, 17 species with a score of 5, 10 species with a score of 6 and 7 species with a score of 7.

Cladonia species that have been registered in Denmark, but not found in any of the registration circles are given a score of 7 based on their general rarity. These species can be seen in table 3 below (Søchting & Alstrup 2008).

Name	Score	Status
C. amaurocraea	7	Rare (R)
C. bellidiflora	7	Rare (R)
C. borealis	7	
C. botrytes	7	Rare (R)
C. caespiticia	7	Common
C. callosa	7	Rare (R)
C. carneola	7	Rare (R)
C. coniocraea	7	Common
C. incrassata	7	Common
C. macrophylla	7	Rare (R)
C. monomorpha	7	
C. ochrochlora	7	Common
C. parasitica	7	Rare (R)
C. phyllophora	7	Rare (R)
C. pocillum	7	Rare (R)
C. pulvinata	7	Rare (R)
C. pyxidata	7	Common
C. rei	7	Rare (R)
C. stellaris	7	Rare (R)
C. strepsilis	7	Vulnerable (V)
C. stygia	7	Rare (R)
C. symphycarpa	7	Rare (R)
C. verticillata	7	Common

Table 3. Lichens from the *Cladonia* genus registered in Denmark, but not found in any of the 116 registration circles (Søchting & Alstrup 2008).

The expert valuations described and viewed above will be combined with the statistical analysis. These two factors will produce the scores and indicator species that will be used in the final lichen species index.

By using the expert valuation above the lichen species index is calculated for each of the circles registered. The expert based results are displayed together with the final species index results in appendix 4.

6.3 NMS ORDINATION

The figure below displays the NMS ordination of 102 plots collected around Denmark during the summer and fall of 1013. It shows the patterns of similarity and species composition expressed on continuous scales (Borcard *et al.* 2011). Only 102 plots are displayed because of missing values for pH or humus layer thickness in 14 plots. The pH is an environmental variable shown as a blue vector on the plot and the thickness of the humus layer is represented with a red fitted surface.

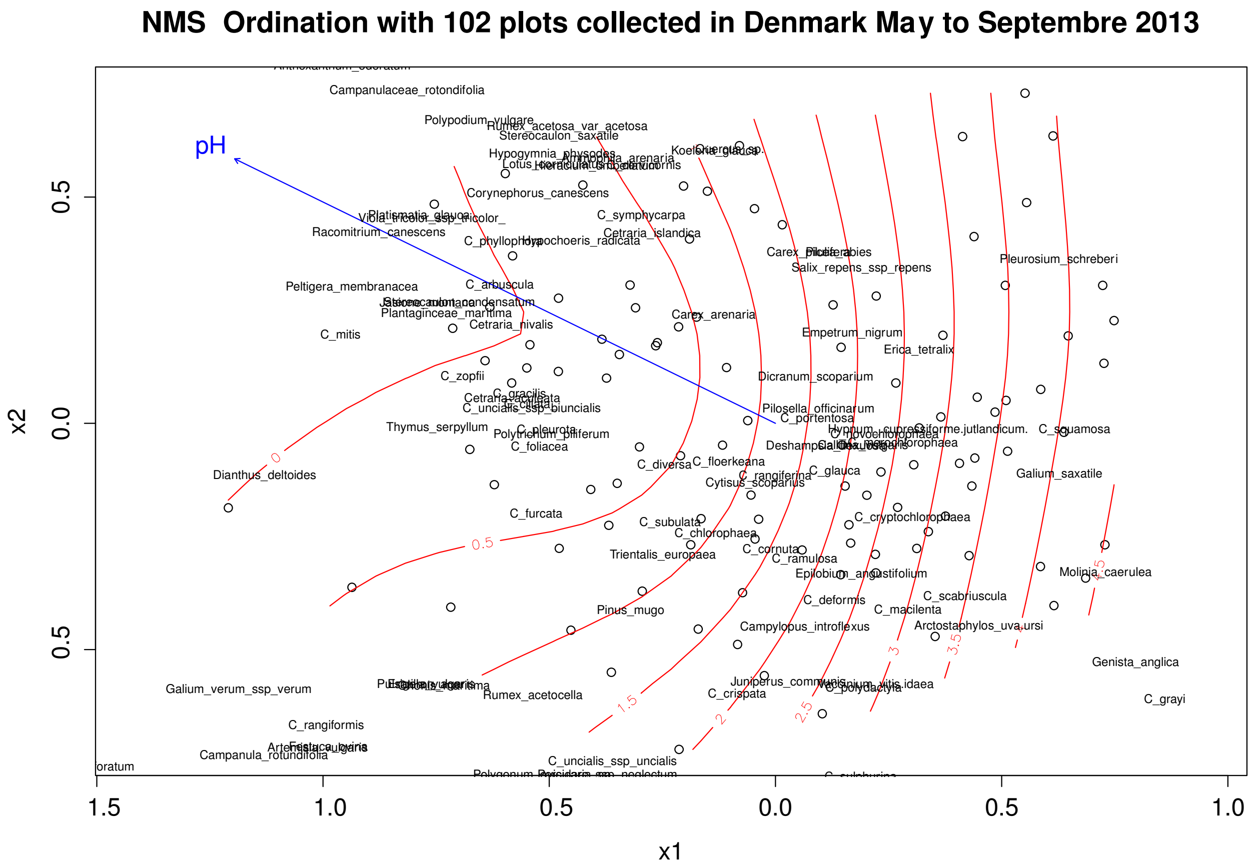


Figure 1 NMS ordination with 102 plots collected in Denmark May to September 2013

The closer the sites a located in the ordination plots the more similar a species composition and the lower is the ecological distance. Also species wise the ordination tells which species have similar ecological preferences, the closer the species are to one another, the more similar ecological preferences they have. It is visible that *Cladonia mitis* and *Cladonia zopfii* (seen in the left side of the ordination plot) have more similar ecological preferences than for example *Cladonia mitis* and *Cladonia ramulosa* (*C. ramulosa* seen in the right side of the ordination plot) (figure 1).

We found that pH and Humus layer thickness are mostly correlated with NMDS1 axis. The species found in plots with a thinner humus layer and higher pH are located in the left side of the ordination while other species that are found in plots with a thicker humus layer and lower pH are located on the right side of the ordination. The species located were humus layer between is 0-1 cm are also the species that, in the field have been associated with none or thin humus layer. The vector arrow shows the direction of the gradient and the length of the arrow is proportional to the coalition between the variable and the ordination. In figure 1 the vector represents the pH.

	pH	HL	NMDS1
pH	1	-0,6020719	-0,7303889
HL	-0,6020719	1	0,6733693
NMDS1	-0,7303889	0,6733693	1

Cor NMDS1 pH	p value
-0,7303889	<2.2e-16

Cor NMDS1 HL	p value
0,6733693	8,88E-15

Table 4. Correlation between NMDS1 scores, pH and humus layer thickness with their respective *p* values.

6.4 INDICATOR SPECIES ANALYSIS (ISA)

The indicator species analysis shows lichens, bryophytes and plants that have been selected as indicators for humus layer thickness. The dataset was divided into four different groups indicating different thickness of humus layer:

- Group 1: Species associated with circles with Humus layer = 0cm
- Group 2: Species associated with circles with Humus layer] 0; 1] cm
- Group 3: Species associated with circles with Humus layer] 1; 3.5] cm
- Group 4: Species associated with circles with Humus layer] 3.5; 7] cm

The selected species are shown below in table 5. Group 1 shows the species associated with no humus layer, group 2 the species associated with a humus layer between 0-1 cm and so on.

Below in table 4 and 5 the indicator species analysis. In table 4 the indicator species analysis shows the indicator species for each group. In table 5 the indicator species analysis is shown for the groups combined.

Group 1, HL = 0	**Stat**	**p-value**
Corynephorus canescens	0,637	0.001 ***
Viola tricolor ssp. tricolor	0,546	0.001 ***
Jasione montana	0,53	0.001 ***
Cladonia gracilis	0,502	0.001 ***
Cladonia zopfii	0,501	0.001 ***
Hypochoeris radicata	0,459	0.003 **
Cladonia pleurota	0,448	0.001 ***
Platismatia glauca	0,341	0.007 **
Stereocaulon condensatum	0,314	0.026 *
Racomitrium canescens	0,314	0.031 *
Polypodium vulgare	0,297	0.040 *
Group 2, HL]0;1]	**Stat**	**p-value**
Achillea milleforlium	0,361	0.006**
Trifolium arvense	0,361	0.006**
Cladonia cervicornis	0,353	0.008**
Cladonia rangiformis	0,335	0.014*
Epilobium angustifolium	0,283	0.033*
Group 4, HL]3,5;7]	**Stat**	**p-value**
Cladonia fimbriata	0,32	0.020*
Pilosella officinarum	0,288	0.034*
Pleurosium schreberi	0,282	0.046*

Group 1+2, HL [0;1]	**Stat**	**p-value**
Cetraria aculeata	0,582	0.001 ***
Polytrichum piliferum	0,493	0.001 ***
Cladonia uncialis ssp. biuncialis	0,462	0.002 **
Cladonia diversa	0,437	0.002 **
Cladonia arbuscula	0,368	0.009 **
Cladonia foliacea	0,362	0.008 **
Cladonia ciliata	0,329	0.012 *
Group 1+3, HL=0 and HL]1;3.5]	Stat	p-value
Carex arenaria	0,343	0.014*
Group4 , HL]1;7]	Stat	p-value
Molinia caerulea	0,476	0.001***
Group 1+2+3, HL [0;3.5]	Stat	p-value
Cladonia floerkeana	0,292	0.044*
Group 2+3+4, HL]1;7]	Stat	p-value
Calluna vulgaris	0,36	0.002**

Table 6. Species indicator analysis for combined groups.

Table 5. Species indicator analysis for the different groups.

By using the species indicator analysis the species indicating none or thin humus layer is selected for groups 1 and 2 and in the combined group for 1 and 2. This analysis will therefore among other provide a list of species that indicates none or thin humus layer and can be used if only species lists from a locality is available. This way it is possible to estimate the state of the soil with a species list from the area.

6.5 INDICATOR SPECIES ANALYSIS USING SCORES FROM THE NMS-ORDINATION

For the next analysis, which is still considered as a naïve approach towards the finding of indicator species, an indicator species analysis using the scores from the NMA-ordination has been made. Another Indicator species analysis was performed and this time, sites were grouped according to the scores they received on the first axis (NMDS1) of the NMS-ordination. This axis is chosen because it is the one that explains most of the variation in our dataset.

Group 1 , # sps. 12	**stat**	**p.value**
Plantaginceae maritima	0.473	0.001 ***
Galium verum ssp. verum	0.466	0.001 ***
Thymus serpyllum	0.436	0.003 **
Artemisia vulgaris	0.411	0.003 **
Pinus mugo	0.397	0.006 **
Ononis maritima	0.397	0.004 **
Achillea milleforlium	0.361	0.015 *
Trifolium arvense	0.361	0.015 *
Cladonia rangiformis	0.357	0.008 **
Peltigera membranacea	0.321	0.009 **
Campanula rotundifolia	0.287	0.041 *
Campanulaceae rotondifolia	0.287	0.046 *
Group 2, # sps. 1	**stat**	**p.value**
Carex arenaria	0.362	0.018 *
Group 3, # sps. 1	**stat**	**p.value**
Cladonia fimbriata	0.315	0.021 *
Group 4, # sps. 2	**stat**	**p.value**
Galium saxatile	0.365	0.013 *
Luzula sp.	0.346	0.026 *
Group 1+2, # sps. 17	**stat**	**p.value**
Cetraria aculeata	0.796	0.001 ***
Polytrichum piliferum	0.694	0.001 ***
Jasione montana	0.646	0.001 ***
Cladonia zopfii	0.613	0.001 ***
Cladonia foliacea	0.586	0.001 ***
Cladonia uncialis ssp. biuncialis	0.579	0.001 ***
Corynephorus canescens	0.507	0.003 **
Cladonia furcata	0.5	0.002 **
Cladonia gracilis	0.483	0.002 **
Hypochoeris radicata	0.442	0.002 **
Cladonia pleurota	0.429	0.003 **
Hypogymnia physodes	0.424	0.002 **
Cladonia ciliata	0.422	0.003 **
Viola tricolor ssp. Tricolor	0.412	0.004 **
Ammophila arenaria	0.399	0.008 **
Cladonia arbuscula	0.335	0.020 *
Polypodium vulgare	0.327	0.020 *
Group 2+3, # sps. 6	**stat**	**p.value**
Cladonia macilenta	0.462	0.001 ***
Cladonia glauca	0.46	0.003 **
Campylopus introflexus	0.45	0.003 **
Cladonia ramulosa	0.449	0.001 ***
Cladonia merochlorophaea	0.417	0.005 **
Deshampsia flexuosa	0.404	0.005 **
Group 3+4, # sps. 4	**stat**	**p.value**
Hypnum cupressiform jutlandicum	0.373	0.008 **
Molinia caerulea	0.352	0.017 *
Calluna vulgaris	0.333	0.022 *
Pleurosium schreberi	0.29	0.049 *
Group 1+2+3, # sps. 3	**stat**	**p.value**
Cladonia floerkeana	0.519	0.001 ***
Cladonia diversa	0.485	0.001 ***
Cladonia subulata	0.372	0.010 **

Table 7. Results from the second indicator species analysis. Sites were grouped according to NMDS1 coordinates from the ordination.

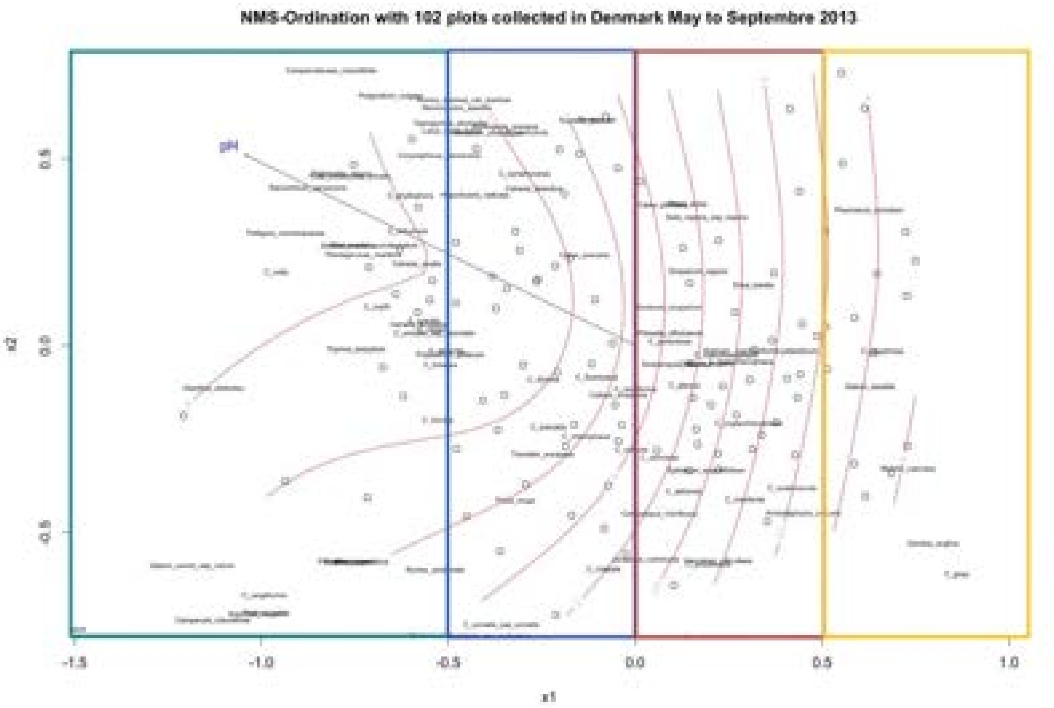


Figure 2. Group 1: lowest score to -0.5 (included), Group 2:]-0.5 ; 0], Group 3:]0 ; 0.5], Group 4: 0.5(excluded to highest score)

6.6 ANOVA WITH NMDS GROUPS

We investigate differences between the four selected group for the indicator species analyses that used NMDS1 scores. We present the result below:

6.6.1 DIFFERENCE IN TERMS OF PH

pH	1	2	3	4
1		0,2029	0,00014	0,00014
2	2,799		0,00014	0,00014
3	10,86	8,062		0,8189
4	12,1	9,297	1,234	

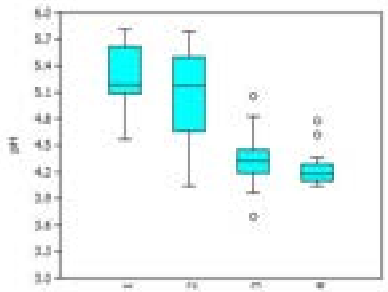


Table 8. ANOVA groups in term of pH together with boxplot.

6.6.2 DIFFERENCE IN TERMS OF HUMUS LAYER THICKNESS (IN CM)

HL (cm)	1	2	3	4
1		0,8232	0,00014	0,00014
2	1,222		0,00016	0,00014
3	8,106	6,884		0,1978
4	10,92	9,702	2,818	

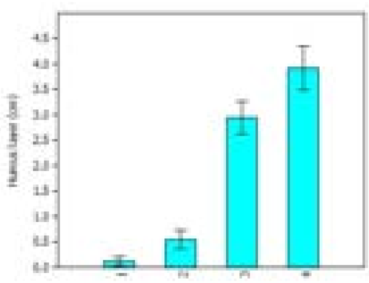


Table 9. ANOVA groups in term of humus layer thickness (cm) together with bar chart.

6.6.3 DIFFERENCE IN TERMS OF DISTANCE TO COASTLINE (IN KM)

Dist to coast	1	2	3	4
1		0,259	0,000604	0,000141
2	2,609		0,1162	0,002232
3	5,797	3,188		0,4876
4	7,811	5,202	2,014	

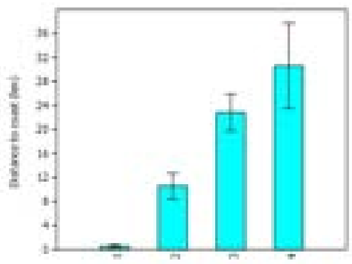


Table 10. ANOVA groups in term of distance to coastline (km) together with bar chart.

THE NETWORK APPROACH

6.7 MODULARITY ANALYSIS

As an attempt to analyze the data in a perhaps more objective way and investigate congruence in results, a modularity analysis was performed on the dataset including all sites and all recorded species. The software “Modular” was used as it is both user friendly and allows calculations of modularity in big network in very short time. To check for eventual randomness in the dataset and confirm significance, 100 null models were ran and following results were obtained:

Nr. modules	Modularity	P.Null1	P.null2
5	0.242239	0.000000	0.000000

Table 11. Modularity

From this, it is possible to reject the null hypothesis (i.e. randomness) and hence confirm the presence of 5 significant modules in the dataset.

The figure bellow illustrates the 5 modules and connection within and among modules.

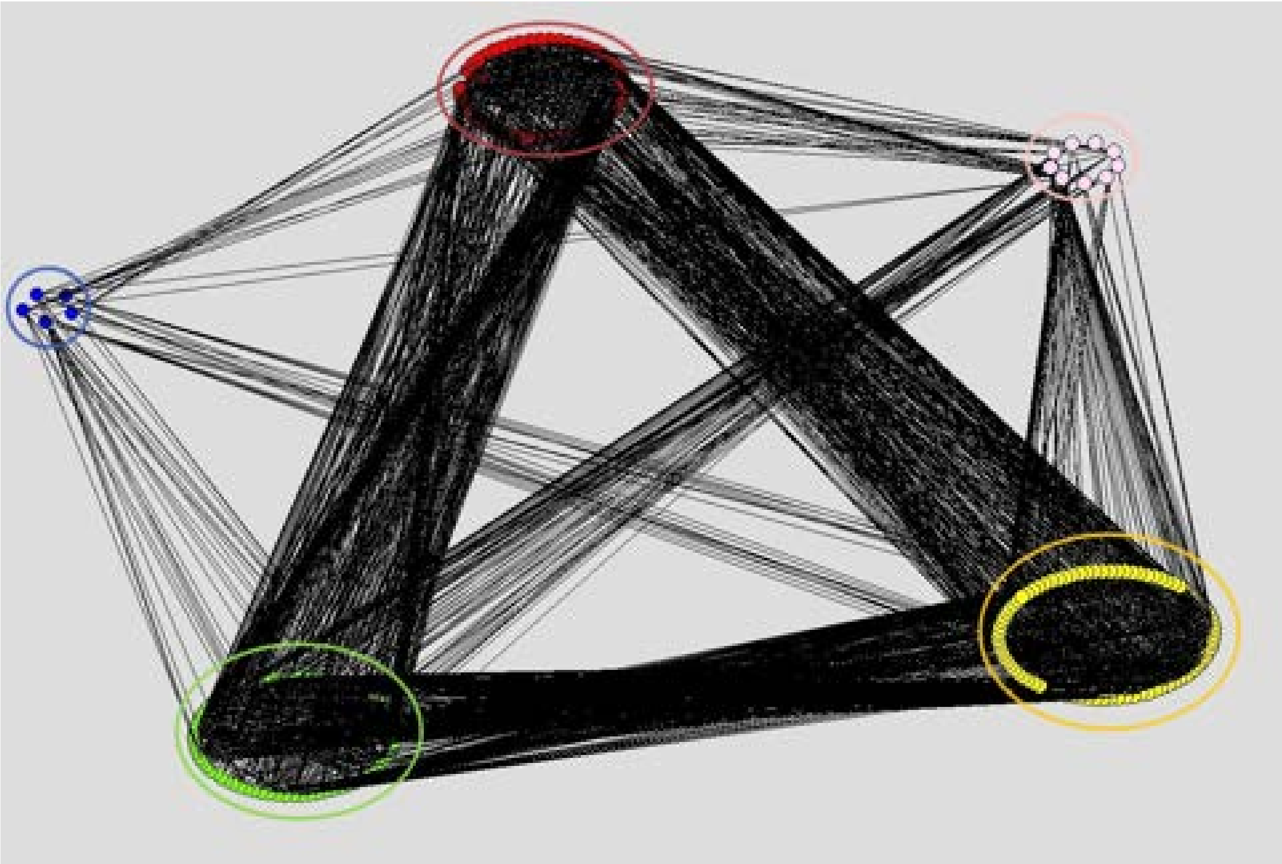


Figure 3. Modules and their connections. Module 0 is red, module 1 is green, module 2 is yellow, module 3 is blue and module 4 is pink. See appendix for more details about modules.

From this test, each species and sites were given a number corresponding to the module in which they belong. From this was then calculated *z* and *c* scores representing respectively within and among module degree or connectivity (see Methods for formulas). For *z*, the higher the score, the higher is the species or site connected to species (or site) in its own module. Same for *c* scores except that now connection are between species (or sites) in other modules. Furthermore, these scores allow the creation of scattered plots, which are presented below:

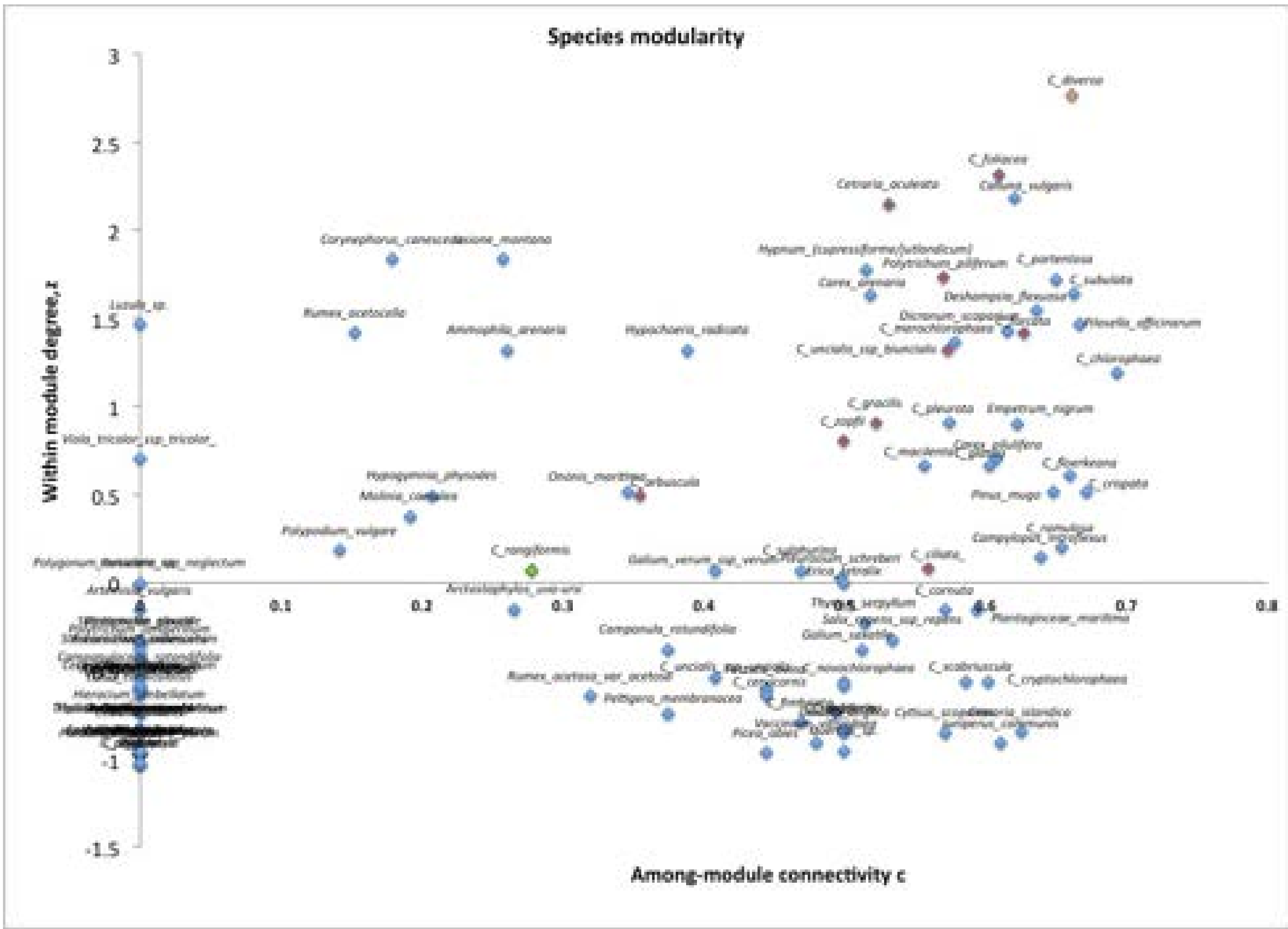


Figure 4. Scatter plot for species modularity with z scores on the y-axis and c score on the x-axis.

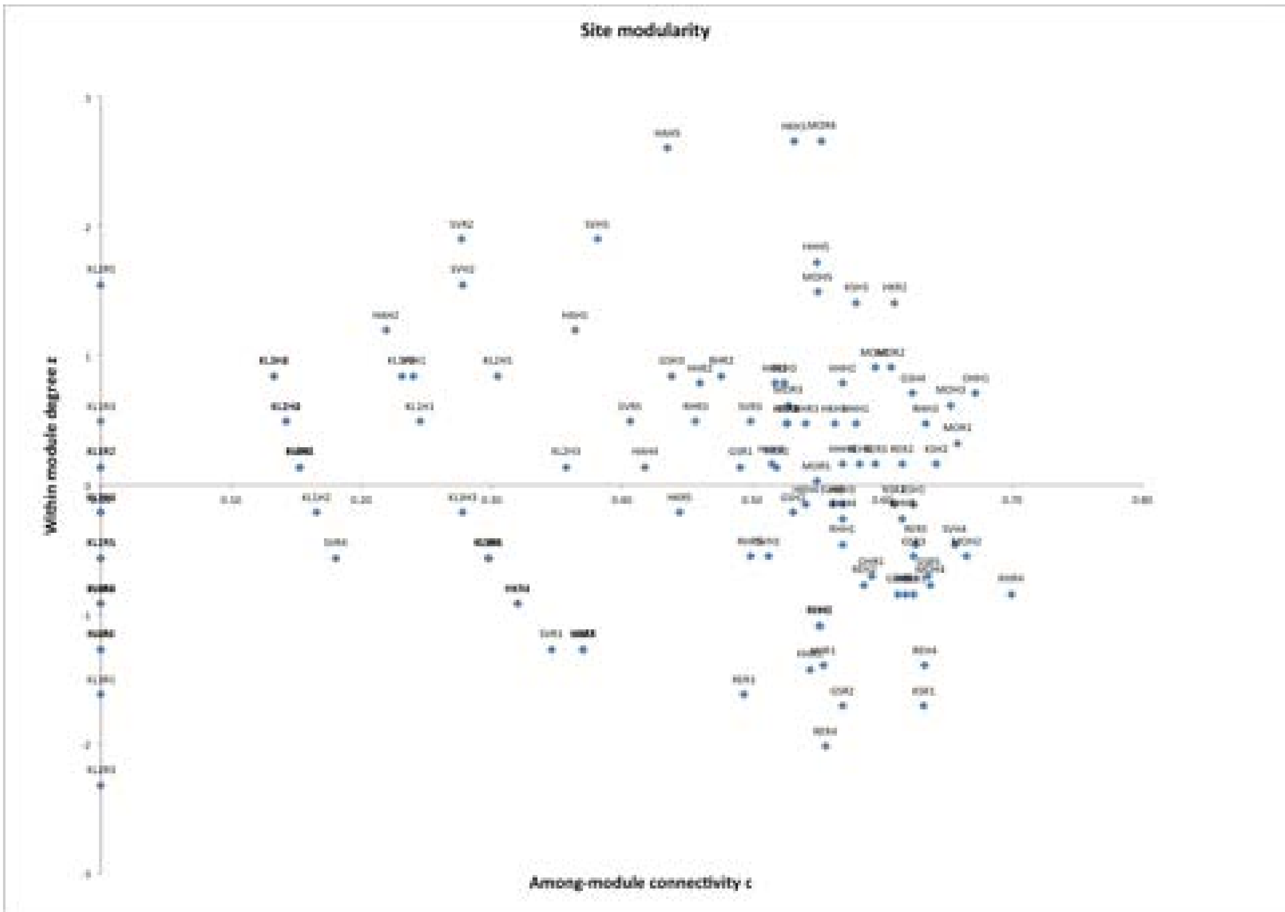


Figure 5. Scatter plot of site modularity with z scores on the y-axis and c score on the x-axis.

6.8 ANOVA MODULES

ANOVA stands for analysis of variance and is a test that allows comparison between any number of sample-means to be done, Fowler et al. 2008.

Below the Tukey test has been made for the different modules to compare the means of the humus layer thickness (cm), the pH and the distance to the coast (km). Only modules 1, 2 and 3 has been used because module 3 and 4 only consists of 2 circles each and therefore is too small to deliver any relevant conclusions.

Tukey´s test is used when the F-test in the ANOVA analysis indicates, that there is a significant difference of the means of the groups. In all the ANOVA tests, the F-test has indicated significant differences and the Tukey test have therefore been made.

The Tukey test is shown above the diagonal and the tests that show significantly differences between the modules are colored pink.

6.8.1 ANOVA PH

The table 12 the Tukey test shows the means of pH from module 1, 2 and 3. The test shows that the means from the tree modules all are significantly different because the p-value is below 0,05.

pH	1	2	3
1		0,000105	0,000603
2	10,39		0,000105
3	5,519	15,91	

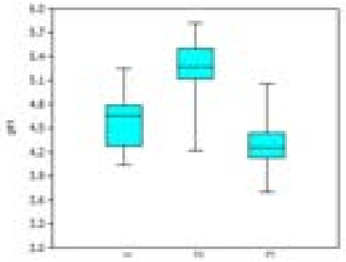


Table 12. ANOVA modules 1, 2 and 3 showing the pH.

6.8.2 ANOVA HUMUS LAYER THICKNESS

Tukey´s Q below the diagonal, p(same) above the diagonal. Significant comparisons are pink.

HL (cm)	1	2	3
1		0,00449	0,006291
2	4,596		0,000104
3	4,439	9,035	

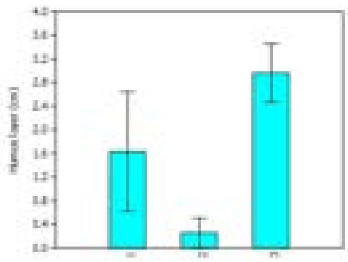


Table 13. ANOVA modules 1, 2 and 3 showing the humus layer thickness (cm).

The table above shows that all the tree different modules are significantly different.

6.8.3 ANOVA DISTANCE TO COAST

Tukey´s Q below the diagonal, p(same) above the diagonal. Significant comparisons are pink.

Dist to	1	2	3
1		0,1317	0,003802
2	2,748		0,000116
3	4,668	7,416	

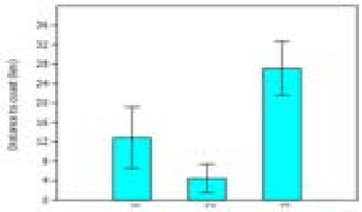


Table 14. ANOVA modules 1, 2 and 3 showing the distance to coastline (km) displayed with bar chart.

The table above shows that module 1 and 3 are significantly different. Module 2 and 3 is significantly differently, but module 1 and 2 is not significantly different.

6.9 MULTI LINEAR REGRESSION, WHAT INFLUENCES *Z* AND *C* SCORES FOR SITES?

As an attempt to push the study a little further we used our available data to investigate what parameters influence *z* and *c* scores for sites. We therefore ran a multi linear regression using pH, humus layer thickness, distance to nearest coastline and species richness as predictor variables for separately *z* and *c* scores.

With Z scores as response variables:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.862252	1.019506	-0.846	0.4
Distcoast	0.003943	0.004328	0.911	0.364
pH	-0.322893	0.214787	-1.503	0.136
HL	0.073311	0.051962	1.411	0.161
Totsp	0.123738	0.016774	7.377	4.27e-11 ***

Table 15. Multilinear regression with *z* scores.

With R-squarred: 0.3536

Total number of species is the only significant predicator variable for within module connectivity.

With *c* scores as response variables:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-3.79E-01	1.82E-01	-2.079	0.04012 *
Distcoast	2.86E-05	7.73E-04	0.037	0.97054
pH	1.07E-01	3.84E-02	2.791	0.00626 **
HL	-7.55E-03	9.29E-03	-0.813	0.41828
Totsp	1.78E-02	3.00E-03	5.922	4.23e-08 ***

Table 16. Multi linear regression with *c* scores.

With R-squarred: 0.5568

In this case we found that both pH and total number of species influences among modules connectivity. An analysis of residuals was carried to make sure that no rules were violated for the regression as pH and total species richness are strongly correlated with each other.

As a final statement it can be concluded that the main predictor variable for among modules connectivity in this analysis is species richness R^2 = 05349 and to a small extent pH which was still significant but only explains 5% (R^2 = 0.04774) of the model.

6.10 THE FINAL LICHEN SPECIES INDEX

The species that have been selected as good indicators are displayed below. The indicator species has been given a higher score than the original expert valuation. The expert valuation and the new and final score for each of the registered species can be seen in table 15 below.

- Species that found in two tests are given a score that is one point higher than the original expert valuation. The species in this category are; *Cladonia diversa*, *Cladonia arbuscula* and *Cladonia rangiformis*.
- Species that have shown to present indicator value in all three statistical tests have automatically been given the highest possible score of 7. The species in this category are; *Cladonia foliacea, Cladonia uncialis ssp. biuncialis*, *Cladinia zopfii*, *Cladonia ciliata* and *Cladonia gracilis* and *Cetraria aculeata*.

Species	Pct.	Expert valuation	Final score	Species	Pct.	Expert valuation	Final score
C. arbuscula	16,4	5	**6**	*C. pleurota*	27,6	5	5
C. cervicornis	5,2	7	7	*C. polydactyla*	0,9		
C. chlorophaea	26,7			*C. portentosa*	91,4	4	4
C. cenotea	0,9			*C. ramulosa*	41,4	5	5
C. ciliata	16,4	5	**7**	*C. rangiferina*	1,7	6	6
C. cornuta	6	7	7	*C. rangiformis*	5,2	6	**7**
C. crispata	14,7	5	5	*C. scabriuscula*	13,8	5	5
C. cryptochlorophaea	13,8	5	5	*C. squamosa*	0,9	7	7
C. diversa	48,3	5	**6**	*C. subulata*	31,9	5	5
C. deformis	1,7	6	6	*C. sulphurina*	6,9	6	6
C. fimbriata	6,9	4	4	*C. symphycarpa*	0,9	7	7
C. floerkeana	64,7	4	4	*C. uncialis ssp. biuncialis*	33,6	5	**7**
C. foliacea	31	5	**7**	*C. uncialis ssp. uncialis*	2,6	6	6
C. furcata	22,4	5	5	*C. zopfii*	23,3	6	**7**
C. glauca	52,6	4	4	*Cetraria aculeata*	42,2	5	**7**
C. gracilis	25,9	5	**7**	*Cetraria islandica*	3,4	7	7
C. grayi	0,9	6	6	*Cetraria nivalis*	1,7	7	7
C. humilis	0,9	6	6	*Hypogymnia physodes*	14,7		
C. macilenta	42,2	5	5	*Peltigera membranacea*	3,4	5	5
C. meroclorophea	62,9	4	4	*Placimatia glauca*	6		
C. mitis	0,9	5	5	*Stereocaulon condensatum*	5,2	6	6
C. novochlorophaea	12,1	4	4	*Stereocaulon saxatile*	6	6	6
C. phyllophora	0,9	7	7				

Table 17. Scores for the lichen species index

The species that have been selected as indicator species are the only species for which scores were modified. These are the final scores that can be used to calculate a species index based on the lichen vegetation in a five-meter circle. Table 15 seen above only contains the species that has been registered in the 116 circles. The species from the genus *Cladonia* that have not been registered in the circles, but were registered earlier are seen in table 3 and the scores for these species will not change.

6.11 CALCULATED SPECIES INDEXES

Table 16 below displays the calculated indexes for all the random registration circles. We chose to use results from random circles to avoid biases. It must be remembered that Vrads Sande and Ovstrup Hede only have one random circle each, so the localities in table 16 do not display an average, but the true index from the only random circle made at the locality (hence the lichen species index average at 0,000 for Vrads Sande).

The calculated indexes are seen for the plants, the lichens and the plants and lichens combined.

Locality	Plant index	Lichen index	Total vegetation index
Harrild Hede	0,486	0,330	0,568
Hulsig Hede	0,588	0,909	0,802
Hvidklit	0,752	0,839	0,865
Hansted Reservatet	0,748	0,911	0,882
Kandestederne	0,694	0,636	0,810
Randbøl Hede	0,621	0,942	0,889
Vrads Sande	0,192	0,000	0,110
Sønder Vosborg Hede	0,471	0,829	0,715
Grene Sande, Store Råbjerg	0,628	0,924	0,899
Ovstrup Hede	0,755	0,644	0,769
Klosterheden	0,435	0,689	0,689

Table 18. Species index displayed for the random circles for plants and the final lichen species index.

In table 16 the lichen species index is in 7 out of 12 casess higher than the plant species index. If Ovstrup Hede and Vrads Sande are excluded because of low data value the lichen species index is higher than the plant species index in 7 out of 10 cases. In the species index for the total vegetation were both lichens and plants are included the index is affected by often lower plant species index and the value is therefore pulled down. This explains the result, where the species index for the total vegetation often is insignificantly different from the highest species index, but still lower than the highest species index.

To display the significant or insignificant differences in the tree indexes figure 6 below shows the indexes as bars with standard deviations.

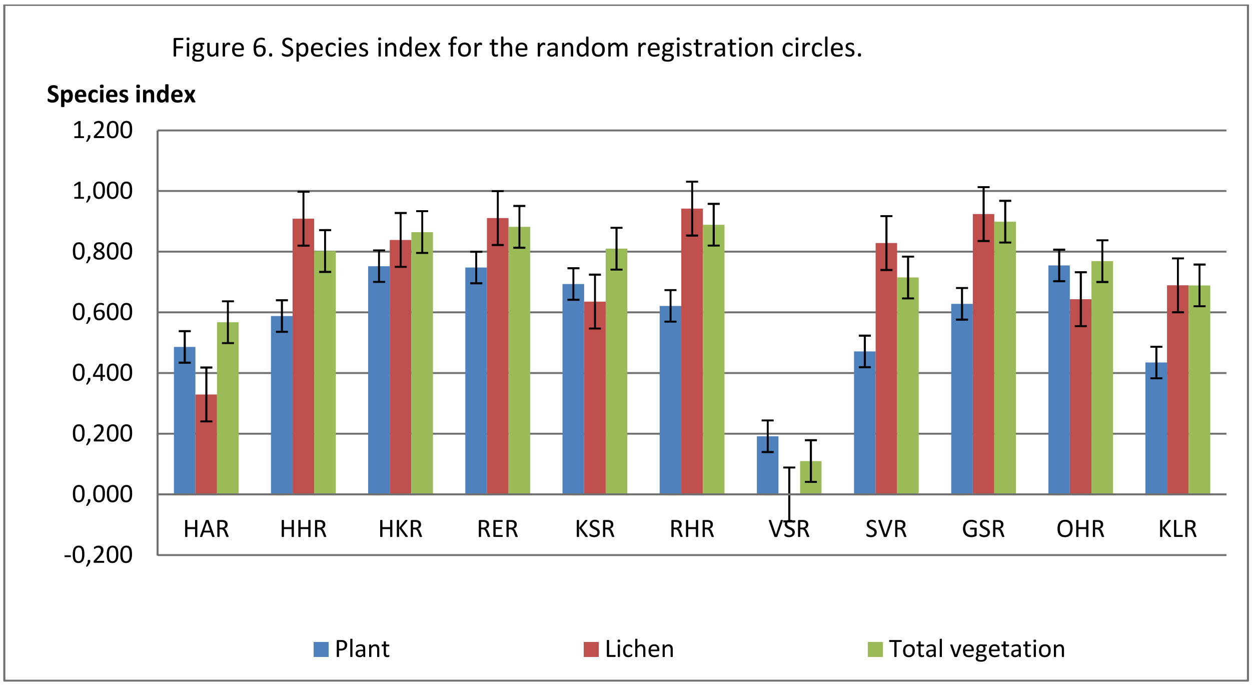


Figure 6. Species index for the random registration circles. HA= Harrild Hede, HH= Hulsig Hede, HK= Hvidklit, RE= Hansted reservatet, KS= Kandestederne, RH= Randbøl hede, VS= Vrads Sande, SV= Sønder Vosborg, GS=Grene sande, OH= Ovstrup Hede, KL= KLosterheden plantage.

From figure 6 it is clear that the red lichen species index in 7 out of 10 cases is significantly higher than the blue plant species index. In 8 out of 10 times (Ovstrup Hede and Vrads Sande still excluded) the total species index is not significantly different from the lichen species index.

7. DISCUSSION

7.1 THE QUEST TO FIND INDICATOR SPECIES

Habitats and ecosystems loss is nowadays greater than ever, we must therefore overcome great challenges to avoid reaching a tipping point and maintaining a healthy state of ecosystems. One of the challenges we face is the correct state assessment of natural habitats, monitoring changes and making relevant decision for the future. As human we often have to rely on visual cues and historical information to judge the state of the nature of an area. Of course human are very subjective creatures and what defines good quality and poor quality habitat fluctuates from person to person and is also influence by today's goal of conservation. A first subjective approach is always good but science provides many tools that we can now use to confirm or disproof some hypotheses. A part of this project focused on finding lichens and bryophytes indicator species to assess the quality of Danish heathland habitats. This investigation was fuelled by the fact that lichens and bryophytes are major components of heathlands but often overlooked during the process of assessing the quality of those habitats.

The first approach used to identify lichens and bryophytes that reflect the quality of Danish heaths was an expert evaluation. This approach is probably the cheapest and quickest way of establishing rankings among species based on observations in the field, see part 6.2. This approach is of course highly subjective and trying to think back on it, has a tendency to rank highly species found on sites presenting valuable assets for conservationist, such as gaps in the vegetation were lichen can thrive an often do. Furthermore, we had a tendency to put more value on rare species compared to common ones. Common species tend to usually be used to name a stage of the succession process or a habitat type (e,g. "heathland" name after the heather plant) (Gimingham 1973). In this instance we gave high scores to species such as *Cetraria nivalis*, *Cladonia cervicornis* and *Cladonia phyllophora* which were found extremely rarely in our study. The fairly common *Cladonia portentosa* however only received 4 because it was considered to indicate as much as *Calluna vulgaris* (which received 4 in current species index (Fredshavn & Ejrnæs 2009)).

In an attempt to be more objective we decided to conduct a more statistical approach to strengthen eventual conclusions. The second approach consists purely of explorative statistics, which are presented in the results and evolved around the use of ordination and species indicator analyses. We considered this approach as a naïve one because it was the first attempt at making sense of the dataset with statistical tools.

7.2 ORDINATION

Ordination techniques are widely used in community ecology as it has proven to be a great tool to make sense of complex ecological communities and the possible relationship with biotic and abiotic factors, but also for illustrative purposes. As seen previously Non Metric multidimensional Scaling (NMDS) is an unconstrained analysis and therefore more suitable for data exploration. The ordination was not aimed at testing any analysis but merely to investigate eventual patterns in the dataset and correlations between pH and humus layer thickness. From the results can be concluded that pH and humus layer thickness are tightly correlated, which make sense biologically (table 4). It was found that pH and humus layer can explain some of the variation in term of ecological distance along the NMDS1 axis, respectively-0,73 and 0,67 but less clearly on the second axis. What this allowed, is the visualization of sites and species along this gradient by pH and thickness of the humus. From there it can be observed that some of the more appreciated species were found (e.g. *Cetraria nivalis* and *Cladonia arbuscula* which were found on sites with low humus layer (<1) and higher pH)

7.3 SPECIES INDICATOR ANALYSIS USING ONE ENVIRONMENTAL VARIABLE

From there on it was decided to run out an indicator species analysis that is a statistical tool used to identify species that can be used as indicators of environmental conditions, environmental change or as indicator of certain habitat type (De Cáceres *et al.* 2010). This test requires the data matrix and the classification of sites into groups. First, it was investigated which species reflect the humus layer thickness state of an heathland knowing that this would also reflect pH as these two parameter are strongly correlated. From table 5 and 6 can be observed characteristic species for sites with humus layer being equal to 0, comprise into intervals]0;1],]1;3.5] ,]3.5;7] cm and eventual combinations. These numbers represent the four quartiles of the humus layer dataset. The characteristic species for shallow humus layer and no humus layer (Group 1, 2 and 1+2) are particularly interesting because they reflect a state of the habitat that are thought to be important for maintaining the biodiversity of heathlands; bare ground. This gives us a good idea of what species have potentially good indicator values and it is also good to notice that compared to the expert evaluation, are found here species that are not necessarily extremely rare but still were known to grow on heathlands in good condition. *Cladonia foliacea*, *Cladonia gracilis* and *Cetraria aculeata* are all good examples as theses species were always sampled on heathlands presenting conditions for optimal lichen biodiversity (e.g. bare ground). However, these species with these test only reflects one aspect which is a biotic factor that can easily be measured in the field without identifying any species; the humus layer. Even though it is possible to extrapolate some crucial information from this list of indicator species it was decided to push the analysis further.

7.4 SPECIES INDICATOR ANALYSIS USING NMDS SCORES

The next step makes use of the same statistical analysis. However, the classification of sites into groups was made differently. Here, we used the scores from the NMDS ordination and more particularly the coordinate each sites obtained on the NMDS1 axis as it is the axis were most of the variation is observed. The division is here however subjective, it was chosen to divide the axis into four zones, minimum score to -0.5,]-0.5; 0],]0;0.5] and 0.5 to maximum score. There is no particularly good explanation for this way of dividing the data apart from the fact that it was easy and seemed to divide the dataset into 4 groups of roughly the same amount of sites. It was later tested with an ANOVA, differences in terms of pH, Humus layer thickness and distance to coastline among these 4 groups (table 8, 9 and 10). As a general trend was found that group 1 and 2 were never statistically different and same for group 3 and 4. However, both 1 and 2 are in most cases significantly different from 3 and 4. From this can be concluded that group 1 and 2 represent sites with higher pH, shallow humus layer and are located closer to the coast than group 3 and 4. Coming back to the indicator species analysis, indicator species for group 1 and 2 seem to present the most interesting results in terms of lichens. It is interesting to notice that there are again found species from the previous analysis such as *Cladonia zopfii*, *Cladonia foliacea*, *Cladonia gracilis* and *Cetraria aculeata*. We therefore observe some congruence in the results but can we conclude more about those species than previously? Well, from previous results it has been observed that pH and Humus layer explain a good amount of the variation on the NMDS1 axis but not everything. It can therefore be argued that using NMDS scores enclose other variables that have not been measured and therefore the species found are indicators of several characteristics of the environment.

7.5 THE NETWORK APPROACH

To push the study even further it was chosen to analyse the dataset using a network approach. A recent technique that show promising results is the analysis of modularity which aim at identifying subset of tightly connected elements. Inspiration was taken from Olesen *et al.* (2007) that investigates the modularity of pollination network. The idea was to identify species in our dataset that are more susceptible to be connected to other species within their module but also to species in other modules which can be important to protect as they connect sites together. Five significant modules were found in the data and we illustrate them with the Pajek software on figure 3. An ANOVA analysis was performed on the modules to try to make sense of why these sites were grouped together. We only used the 3 biggest modules; the 2 other were disregarded for the ANOVA analysis, as they are both composed of only 2 sites, which cannot tell us very much. All three modules showed significant differences in terms of pH, Humus layer thickness and distance to coastline. With the classification of species and sites it was then possible to calculate z and c scores for each species and each sites. These scores represent respectively the within and among module connectivity. By then plotting these scores on a simple scatter plot it is easy to visualize species that are important for the network. Typically, this graph can be divided into roles (sections) following Guimera & Nunes Amaral's (2005) guidelines however this partition of the graph is still very subjective. By looking at species with high z or high c or both, it can be deduced which species have more indicator values. We show below the same scatter plot figure with rectangles showing species with values within the 10% of highest values (both for *z* and *c*). To this date, analysis of modularity of a heathland networks to find important lichen species for the network is probably unique. We coloured the dots for the species found in our previous analyses figure 7 below and found that they are indeed important species for the network and therefore allow more robust conclusions.

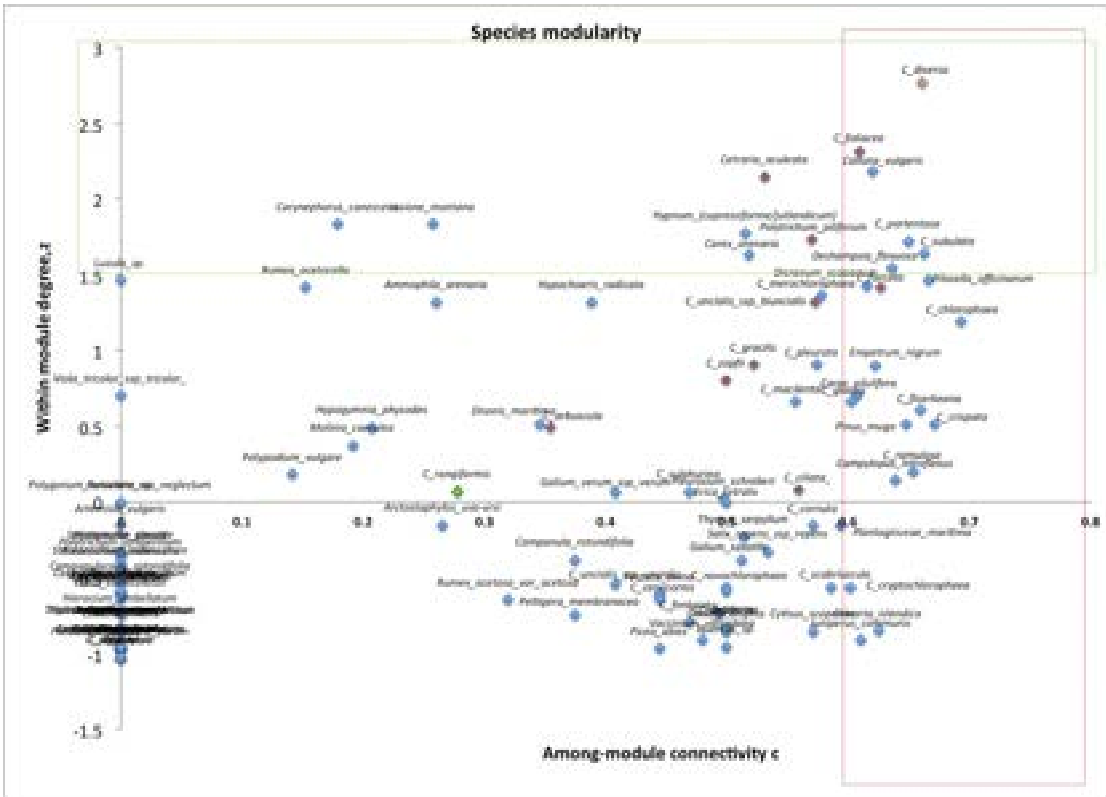


Figure 7. Scatter plot of species modularity, the coloured rectangles roughly represent 10% of higher *z* and *c* scores. Coloured dots are lichen and bryophyte species that were found as indicator of good quality with Indicator species analysis.

Of course species with high z and c score are not necessarily indicator species, but are species important for the cohesion of the network. *Deschampsia flexuosa* has obtained high *z* and *c* score showing just how much this species is connected to not only its own but other module which confirm observation about spreading of this species to almost all heath type and may reflect a general degradation of all heath habitats.

7.6 BRYOPHYTES AS INDICATOR SPECIES

When the total statistical analysis was done it was clear, that no bryophytes had been selected as indicator species. This was not surprising to us because of the very low number of bryophyte species registered in the 116 circles. After this was made clear we chose to focus completely on the lichens and their properties for quality assessment.

7.7 DESCRIPTION OF SELECTED INDICATOR SPECIES.

We propose to describe below the species found in the three statistical tests to be indicators of good heaths properties and to be important in the network we investigated.

7.7.1 CLADONIA FOLIACEA

Cladonia foliacea is dominated by the primary thallus and is in Denmark the most common of the species dominated by primary thallus. It differs from other species by the white/light yellow underside of the primary thallus that gets exposed in dry periods where the lichen roles up and exposes the underside of the thallus (Søchting 2014 *in prep*, Smith *et al.* 2009). *Cladonia foliacea* is often found in unstable environments such as sand dunes. It tolerates heavy sun exposure, drought and sand coverage (Søchting 2014 *in prep*).

The thallus has a spotted surface and the small wholes seen as spots are used to transfer oxygen to the photobiont (Hørnell *et al.* 2004).

7.7.2 CLADONIA ZOPFII

Cladonia zopfii may at first sight resemble a reindeer lichen, but is not. Podetia are smoothly corticated becoming more rugulose with time and are usually dichotomously branched (Ahti *et al.* 2013). *Cladonia zopfii* can also be confused with *Cladonia uncialis* (both *ssp. uncialis* and *ssp. biuncialis*) but they differ in size and in *Cladonia zopfiis* lack of inflated tips*. Cladonia zopfii* is one of the first species to appear on open sand and is therefore a good indicator for open soil and a high dynamic level.

7.7.3 CLADONIA UNCIALIS SSP. BIUNCIALIS

Cladonia uncialis ssp. biuncialis is easily identified because of the inflated tips and the open angles on the pointy ending branches (Ahti *et al.* 2013). Especially when wet, *Cladonia uncialis ssp. biuncialis* look very characteristic and large compared to other lichens. The podetia dies from behind as the thallus grow from the tips (Søchting 2014 *in prep*). As mentioned above *Cladonia uncialis ssp. biuncialis* is sometimes be confused with *Cladonia zopfii*, but with a closer look the two species are easy to tell apart. *Cladonia uncialis* has two sub species; *ssp. uncialis* and *ssp. biuncialis*. *ssp. biuncialis* is far the most common one in Denmark and is often found on open sandy soils, common on heathland (Hørnell *et al*. 2004).

7.7.4 CLADONIA GRACILIS

Cladonia gracilis is common in Denmark and is often found on heathlands and dune and is difficult to confuse with other species because of the very characteristic shape. The podetium is long and slender often fertile and producing narrow scyphi. The cups can have new shots growing from the sides (Søchting 2014 *in prep*, Hørnell *et al*. 2008). The bark is always smooth and often olive green or brown dependent on the exposure to sunlight.

7.7.5 CLADONIA CILIATA

Cladonia ciliata is a reindeer lichen and is characteristic because of the more tenuous podetia and the brown tips which are clearly bent to one side (Hørnell *et al.* 2008, Søchting 2014 *in prep*). *Cladonia ciliata* is common on heathlands and in nutrient poor dunes, but not as common as *C. portentosa* (Smith *et al.* 2009). At first gland *Cladonia ciliata* may look very similar to *Cladonia portentosa*, but with a closer look the two species differ from one another a great deal (e.g. the colored tips and the finer structure at *Cladonia ciliata*).

7.7.6 CETRARIA ACULEATA

Cetraria aculeata is easily recognized by the dark brown color. This lichen is often found close to the sea and on open sandy soils where there is a high degree of sun exposure (Wirth *et al.* 2013, Hørnell *et al.* 2004). The species is frequently found on heathlands and often together with the family of Cladonia where it can form bigger or smaller mats (Smith *et al.* 2009).

7.8 HEATHLANDS AND LICHENS, THE IMPORTANCE OF BARE GROUND

During the study, it became very clear that the number and abundance of lichen species tend to increase considerably within gaps in the vegetation where the bare ground is exposed. On localities with extensive cover by dwarf shrubs (e.g. Ovstrup Hede and Vrads Sande) and no apparent gaps, lichens where always found in paths, tracks and also close to nearest road.

Results from ordination plot and correlation (figure 1 and table 4) between circles with high species richness and thin or non-existent humus layer also support this field observation. Chytry *et al.* (2001) also showed that lichen bryophyte diversity increased the most after humus layer was removed and bare ground exposed. It is therefore of huge importance for future managements that we take this into consideration. Sparrius (2011), who investigated the importance of bare sand in the Netherlands, bring empirical support for the rapid decrease of sand drift and the need for conservation and restoration, due to its importance for the pioneer vegetation and preventive role in term of dune stabilization. Current views on heathland management support the idea of mosaic conservation in order to optimize biodiversity on heathland.

Bare ground is not only beneficial for lichens; it also provide habitats for other plants that are poor competitors and as a result insects that depends on those plants but also offer nesting opportunities for reptiles [which ones and how] (Symes & Day 2003).

Patches of bare ground are therefore of high value and interest in term of conservation. However, opening the vegetation also mean opening the door for undesired and invasive species. During the study it was observed that grasses such as *Molinea caerulea* and *Dechanpsia flexuosa* are quick to colonize and form dense mat that undoubtedly deny any future lichen colonization. Another species that seem to pose problem is the moss *Campylopus introflexus* that is invasive and was introduce in Denmark in the 60's. Campylopus is much better at dispersal and quicker at colonizing gaps than lichen and other local bryophyte species especially when under heavy nitrogen deposition (Sparrius 2011). Danëls *et al.* (2008) showed that few lichen species can however establish (e.g: *Cladonia portentosa*) even in the presence of the Campylopus moss, and that it the neophyte moss is not as adapted as lichen to disturbances caused by sand drift or sand coverage. This is the only comforting thought, as no real solution yet exists to solve this mossy problem.

7.9 THE NITROGEN FACTOR ON HEATHLAND

Nitrogen pollution linked to anthropogenic activities has become one of the greatest sources of pollution that has been increasing considerably during past decades with the massive development of industries. Nitrogen is of course a macronutrient necessary for life but is now excessively release in the environment and N saturation on natural habitats has been the focus of many studies which have proven to perturb normal nitrogen cycle with deep implication for ecosystems, and their community structure (Southon *et al.* 2012). It is therefore necessary to touch upon the subject in this project and discuss the implication of nitrogen on the plant and lichen communities and the future management of Danish heathlands. As seen before, most remaining Danish heathlands are located on the peninsula of Jutland were the agricultural pressure is high and land of the renowned Danish pig meat industry. When talking about Nitrogen pollution one often come across two types, dry and wet deposition. Will be particularly considered here wet deposition, which includes ammonium (NH_4^+) and ammonia (NO_3^-). Traffic and industries are mainly responsible for ammonium emissions and farms for the ammonia.

On several heathlands included in the study, the abundant presence of Klebsormidium algae and nitrophylous lichens such as *Hypogymnia physodes* showed clear signs of nitrogen deposition.

What could this mean for the community structure of heathland and especially the lichen vegetation? Sparrius (2011) investigated extensively the effect of nitrogen deposition on 8 stages of Dutch inland heaths and gives us several insights on the matter. The 8 stages were named according to dominant species at each stage and are hence defined as bare sand, corynephorus, polytrichum, smaller lichens, campylopus, reindeer lichens, dense grassland and heath stage.

The study reveals that lichen species diversity is affected negatively by high levels of N deposition and especially during the "smaller lichens" stage of heathlands. In the "smaller lichens stage" is found mainly pioneer crustose lichens such as *Trapeliopsis granulosa* but also pioneer fruticose lichens (e.g *Cladonia coccifera*, *Cetraria aculeata*).

Southon *et al.* (2012) observed rapid growth of Calluna shoots after 2 years of treatments and each year twice as many buds were observed on plants within the N addition plots. They as a result argue that decline in lichen vegetation is affected by N addition but most likely driven by higher and faster canopy cover of the dwarf shrub vegetation than direct effect by Nitrogen. Other species that are found to respond positively to N fertilization are mosses such as *Polytrichum piliferum* and grasses (e.g. *Corynephorus canescens*) thus presenting more competition for lichens (Sparrius 2011).

7.9.1 NITROGEN AND THE INVASION OF *CAMPYLOPUS INTROFLEXUS*

Since the 1960s Danish heaths have been afflicted by the invasion of a newcomer much smaller that traditional invaders, the moss *Campylopus introflexus*. The Campylopus moss establishes during the Polytrichum phase does not seem to be affected or is even boosted (Southon *et al.* 2012, Sparrius 2011) by N addition and is a much better competitor than both *Polytrichum pilliferum* and pioneer lichens (Sparrius 2011). The Campylopus moss was observed on several heaths localities during our study always forming characteristic dense mat. The formation of this thick ectorganic layer also improves the mineralization of Nitrogen and nutrient cycling (Sparrius 2011). However, during their very detailed study of a lichen-rich corynephorus grassland, Daniels *et al.* (2008) observed that *Campylopus introflexus* do affect negatively the lichen biodiversity at certain stages of the vegetation succession, but does not seem to have a drastic negative impact on the long term as the Campylopus moss does not cope well with stress and disturbances such as sand coverage compared to species such as *Cladonia portentosa* and *Polytrichum piliferum*.

It is though obvious that more insights is needed on the ecology and impact of this bryophyte and even though it may not have a direct damaging effect on future succession of heathland vegetation it stills presents a danger for lichen diversity if smaller lichen are constantly out competed. It is also fair to assume that with time, mat of the Campylopus moss and further fragmentation of heaths habitats may greatly reduce propagule size of pioneer lichens beyond a certain critical point. More studies should focus on the effect of this bryophyte in the future.

7.9.2 NITROGEN AND POST FIRE REGENERATION

Burning is a very common practice for the management of heathland (Buttenschøn 2008). It helps with opening the vegetation, suppressing humus layer hence resetting the accumulation of nutrients and allows the creation of gaps. However during an extensive study realized on a British lowland heathland, Southon *et al.* (2012) warn about the post fire regeneration of heathlands subject to high nitrogen deposition. Indeed their result show that the lichen vegetation was significantly affected by nitrogen and did not recover after fire even with removal of canopy cover.

Instead N addition to soil promotes the rapid colonization of nitrophilous grasses and bryophytes. Furthermore as seen earlier, the invasive *Campylopus introflexus* is faster at colonizing and out compete typical heathland bryophytes such as *Polytrichum juniperinum.* It is clear that Nitrogen deposition has an impact on vegetation structure, therefore managers should be aware of this and consider with care the use of fire in their management strategy if the goal is to maintain classic heath vegetation and improve habitat quality for lichens and other species dependent on heathlands.

7.10 MISSING SPECIES AND MISIDENTIFICATION

It is without doubt that some species in this study were misidentified, although we are confident that the number of errors is low and that getting those identification correctly would not have change significantly the results we propose here. Main causes for misidentifications are probably lack of experience with this group of organism, which probably caused some errors at the beginning of identification and sampling effort, but for which we try to correct eventual mistakes. But also species for which genetic analyses is just starting to clear some problems with species delimitation (e.g *coccifera* group) and species with huge phenotypic variations that often need advance chemical analyses (*Chlorophaea* group).

Some groups (e.g. *diversa* and *coccifera*) gave extra problems because new research results appeared during our identification process. The research concluded that it is not possible chemically to tell these species groups apart. The research made by Jana Steinova referes to the more commonness of *Cladonia diversa* than *Cladonia coccifera* (Stenova *et al.* 2013). Because of this all lichens from these groups were registered as *Cladonia diversa*. This of course means that some potential *C. cocciferas* may have been registered as *C. diversa*.

Two species that proved difficult to distinguish between is *Cladonia arbuscula* and *Cladonia mitis*. These two species are both reindeer lichens and have very much the same morphological features. Chemically they differ from one another by the presence/absence of fumarprotecetraic acid and the reaction to paraphenylendiamin (P) (Søchting 2014 *in prep*). *Cladonia arbuscula* is P+ red and contains fumarprotecetraic acid and it should be possible to taste this. Conversely *Cladonia mitis* is P- and does not contain fumarprotecetraic acid and will therefore have a more mild taste. In the field it proved to be most difficult to distinguish between these two species since no chemical tests with P was made in the field and the taste of fumarprotecetraic acid was hard to recognize.

Furthermore, it is important to mention some lichens species that are unfortunately missing from this study. No crustose lichens such as *Pycnothelia papillaria* or *Trapeliopsis granulosa* that are pioneer lichens on heathland and known to be present in Denmark (Søchting & Alstrup 2008) Two possible explanations for the absence of those species in our plots could be that we missed those small species while in the field and the other is that they might not have been present all together in our plots which could also tell something about general lack of disturbance on Danish heath.

7.11 DISCUSSION OF THE NATURE INDEX

The state assessment index has been developed to assess the state of different nature types in Denmark. The index is used by the municipalities in their registrations of nature and is widely accepted. However, there are certain points in the state assessment index that have been criticized.

The species score was at first based on an expert evaluation and was dependent on the specific nature type. Today, the species score is still based on an expert valuation, but is now equal in all nature types no matter how different they may be (Bak *et al*. 2013). The species score used to express the rarity of a species but now expresses the species sensitivity to unspecified effects (Bak *et al*. 2013). The species *Deschampsia flexuosa* can be used as an example; in the species index this species has been given a score of 3. Even though *Deschampsia flexuosa* is found on dune heathlands, where it used to be rare this species is now becoming dominant and problematic for the original heathland vegetation but is still counts positively in the calculation of the species index (Bak *et al.* 2013). This is questionable because the presences of *Deschampsia flexuosa* on the other hand will suppress the common dune heathland vegetation with a higher score and therefore lowers the total species index (Fredshavn *et al.* 2013).

The species index can give a wrong impression in cases where the index get a higher value from new species, at the expense of a decline in the native vegetation. As an example; a heathland that has been exposed to a raising level of nitrogen will in most cases show a decline in the coverage of *Calluna vulgaris* to the point where this species is no longer is dominant. This can result in higher species richness and thus a higher species index value even though the locality is affected negatively (Bak *et al.* 2013). On the other hand, many of the new species that colonize the area have a species score lower than the score for *Calluna vulgaris* and the species index is unlikely to increase (Fredshavn *et al.* 2013).

The state assessment index is at present only used in Denmark and is therefore not comparable with the nature quality assessment done in other countries (Bak *et al.* 2013). This becomes problematic when it comes to the exchange of data between countries and therefore does not facilitate exchange of knowledge.

When registering a locality using the NOVANA method other factors are also included such as the degree of coverage, carbon/nitrogen ratio and pH (DMU 2009). These physical and chemical parameters are collected to get early cues of environmental changes and are therefore a more accurate assessment of the state of the specific nature type (Bak *et al.* 2013).

The structure index that is part of the nature index has as for weakness the fact that it is based on a pure visual assessment. This makes the process impossible to reproduce. It further makes the index incapable of detecting effects and changes in the investigated area (Bak *et al.* 2013). On the other hand; if the visual assessments had to be replaced by detailed parameters from the monitoring program then the current economical frame would not hold. Today the economical frame allows a visit of 1-1½ hour pr. nature area. If the monitoring parameters from the NOVANA program had to be included, time spend per area would increase by a factor 8 (Fredshavn *et al.* 2013). The biggest advantage of the index is that it can be done quickly and cheaply.

Another problem about the state assessment index that has been emphasized is the fact that the species index is that sensitive species have to disappear completely before the final score obtained by a locality changes, thus making the index indifferent to species abundances. These species have to disappear totally from the registration circle before it has any effect on the species index. The species index is furthermore based only on the observation of vascular plants, which means that lichens and bryophytes are not included. The species index will therefore not be able to detect changes in areas dominated by lichens and bryophytes (Bak *et al.* 2013).

As discussed earlier the registration of nature has to be done quickly, because of limited economic resources in nature monitoring. This means that species that requires more time and perhaps laboratory work to identify are probably not good candidates in this framework.

7.12 THE GENERAL USE OF LICHENS FOR STATE ASSESSMENT

The final calculations of the different species indexes showed us, that the lichen index is most cases higher than the plant species index even if the calculation is made only from the random registration circles to make sure no species was favoured. This supports our original hypothesis that lichens can be used for state assessment of heathland in Denmark.

From figure 6 it is clear that the lichens species index in most cases is significantly higher than the plant species index. The lichens are often part of the dominant vegetation in heathlands and must therefore also be able to express the state of the area where they grow. Because of the lichens have a higher sensitivity and a quicker response to air pollution and nitrogen deposition; the changes in the environment may be reflected at an earlier stage by the lichens than by the plants.

By using the lichen vegetation for assessment of the state of an area it is avoided that large areas dominated by very few species is used. We generally had a higher number of lichens in our random plots than plants and by using lichens more species would provide data for the species index.

Some of the localities were, when visited, not seen as being in a good state, but still, they scored a high value in the species index (e.g. Ovstrup Hede and Hansted Reservatet). This could indicate that the lichen species index has the tendency to assess the state of an area better than the actual state. This must be taken into account and the index will need to be tested thoroughly and the species scores will have to be revaluated. With this index the statistical part was carried out to select the good indicator species, it would therefore be interesting to try to identify indicator species for poor conditions and re-evaluate scores for some species.

All in all is it clear that the lichen species index is merely a first attempt at including new organism groups in the quality assessment of heathlands and needs more work to function well on its own.

By including both the plants and the lichens in one big joint species index the picture may become more accurate. But since one of the main ideas with the original index was to make it quick to use, this may increase workload. More data is always good, but it is important to keep focusing on the fact, that the index is something that should be possible to do in situ. Although we argue that the species we found as being good indicators are easy to identify with only morphological characteristics. The difficulties with the identification of other lichen species will not disappear but with training the vast majority heathland lichens can be identified in situ and the lichens could be included in the assessment of heathlands in Denmark.

8. CONCLUSION

Assessing accurately the quality of a nature type, on top of it being rapid and cheap is a real challenge. The Danish index for the assessment of nature quality (Naturtilstandsideks) aims at fulfilling this goal but include a very limited and amount of data based on a subjective approach. For this master thesis we aimed at investigating the potential of lichens and bryophytes to be good indicator of heathland quality due to their sensitive nature to environmental conditions. We present a more objective way of identifying indicator species and for the first time investigate the modularity of the network. We conclude that nothing can be deduced regarding bryophytes due to low number of species sampled. However, *Cladonia ciliata, Cladonia foliacea, Cladonia gracilis, Cladonia uncialis ssp biuncialis, Cladonia zopfii and Cetraria aculeata* consistently appeared in all statistical approaches. We are therefore confident that these species have great indicator value for heathlands. Not only do they seem to be good indicator, they are also very easy to identify in the field with some practice and could as a result be rapidly implemented into the current index.

Nevertheless, attributing scores to species is still very subjective, how should scores be influence by statistical analysis would require further thinking. We here propose a way of attributing scores that is open for discussion and calculate index value based only on lichens. These values appear to be different from the ones given by vascular plants but more congruent with an index that uses both. We therefore argue that there is good potential for lichens to be more precise indicators but do not fit in this framework as they require more identification work and thus time.

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APPENDIX 1

All collected data registrations, lichens, mosses, plants, pH values and humus layer thickness are available on dropbox through the link below:

https://www.dropbox.com/s/mexg9ctg50vfkb8/All%20lichens%20in%20one%20sheet.xlsx

APPENDIX 2

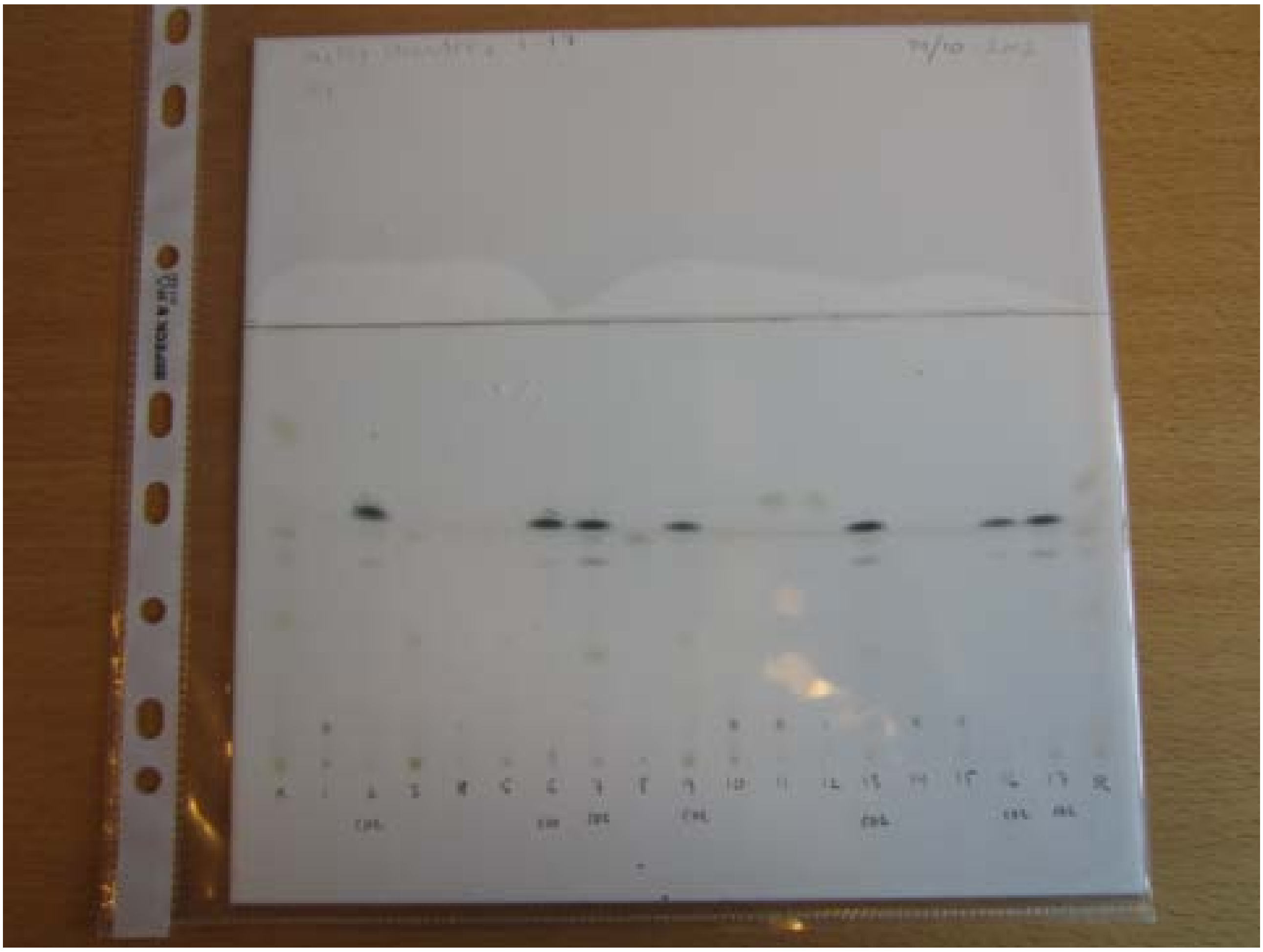
TLC plates

Below is shown all the produced TLC plates.

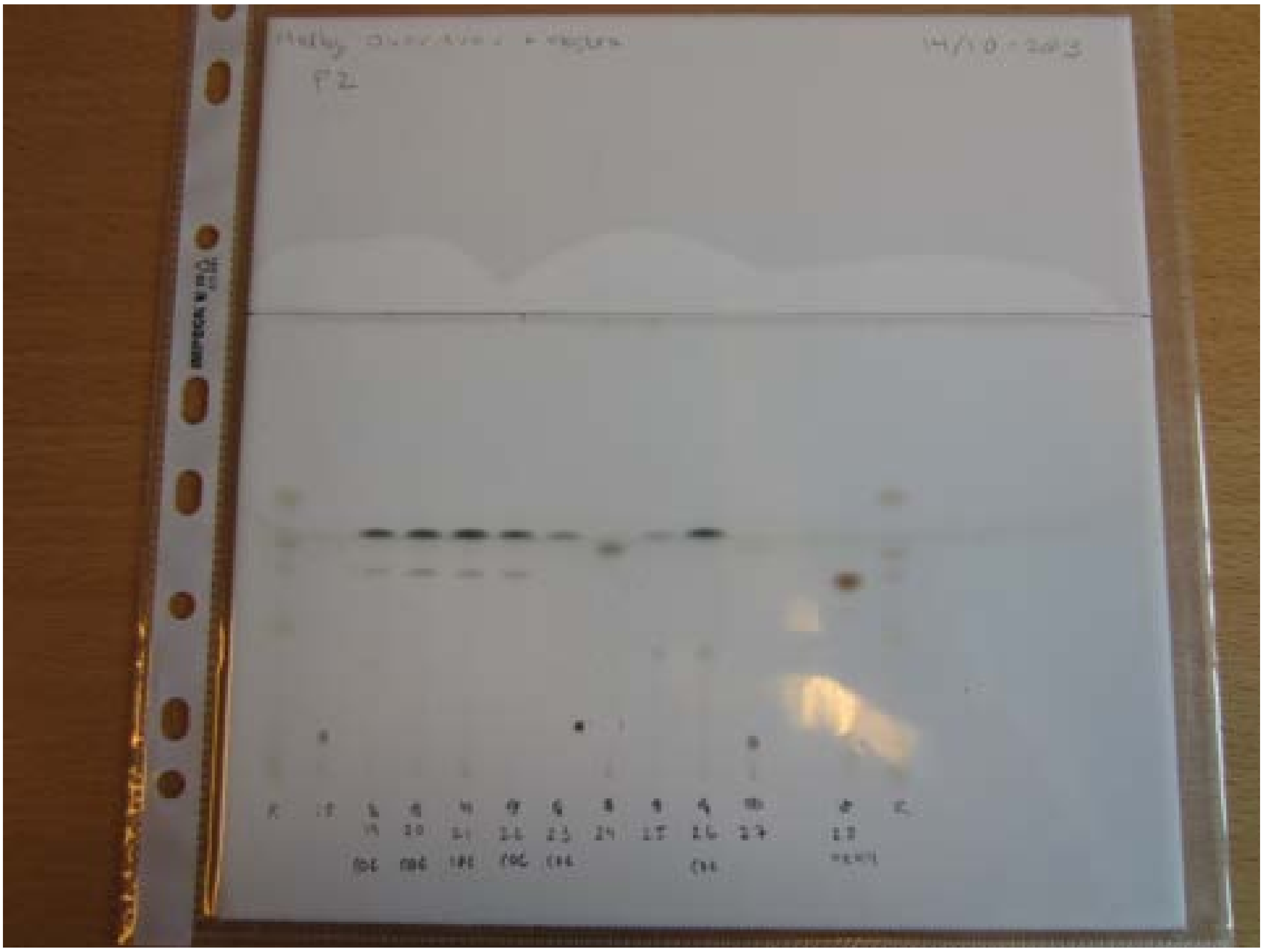
Klosterheden plate 1 (1-17) solvent C

Klosterheden plate 2 (18-25), solvent C

Melby Overdrev plate 1 (1-17) solvent C

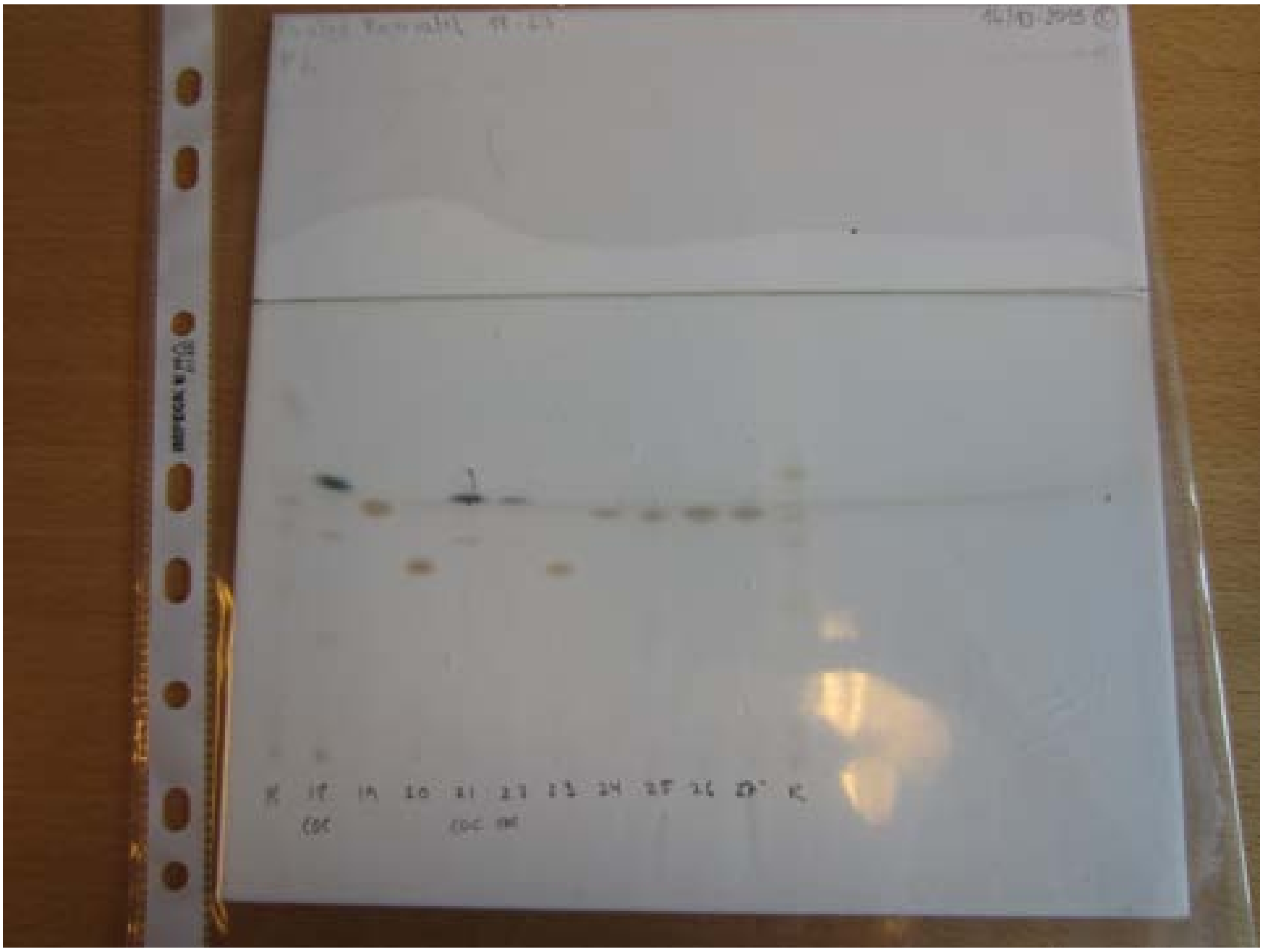


Melby Overdrev plate 2 (18-27) Hvidklit (28) solvent C

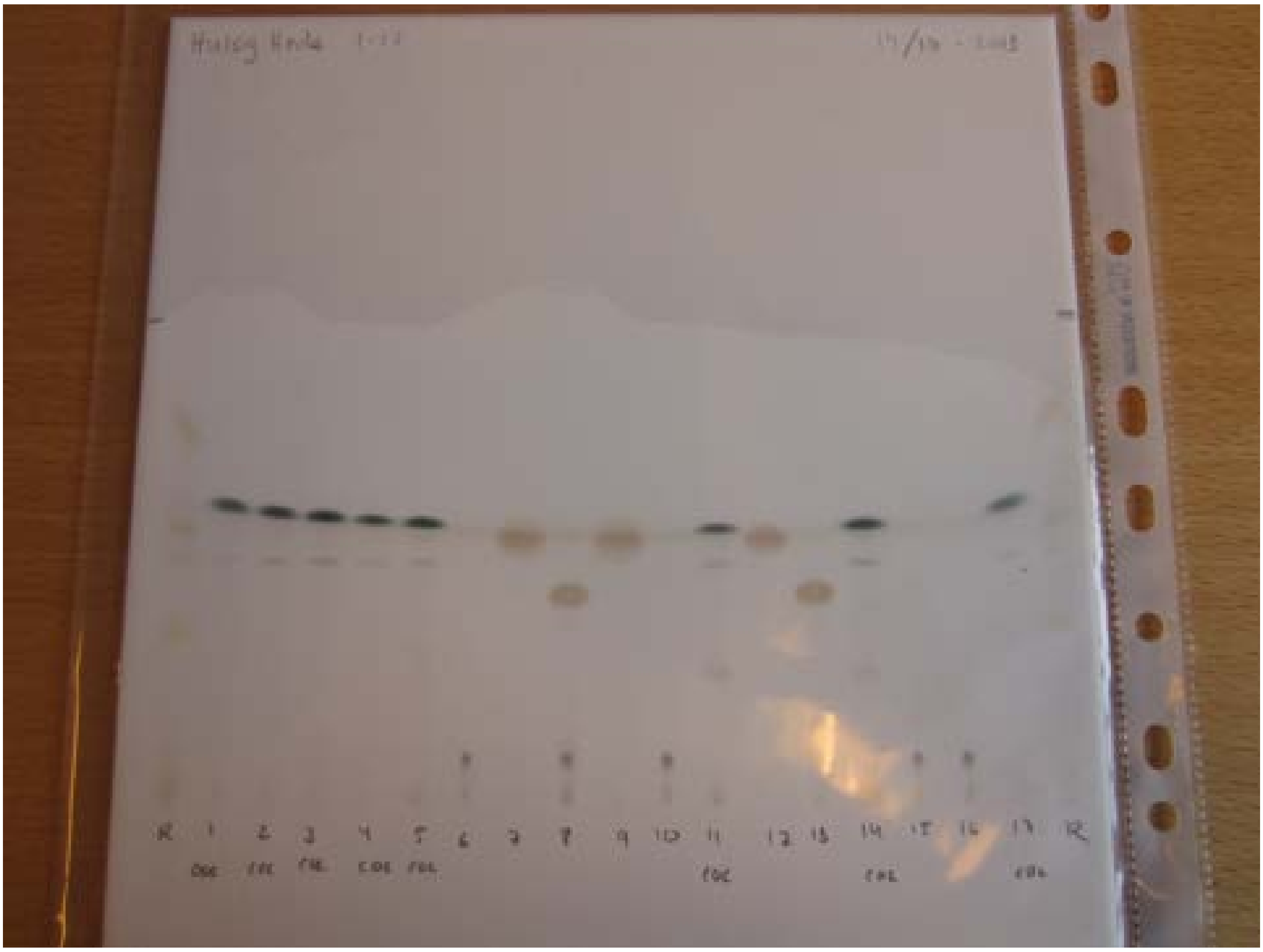


Hansted Reservatet plate 1 (1-17) solvent C

Hansted reservatet plate 2 (18-27) solvent C

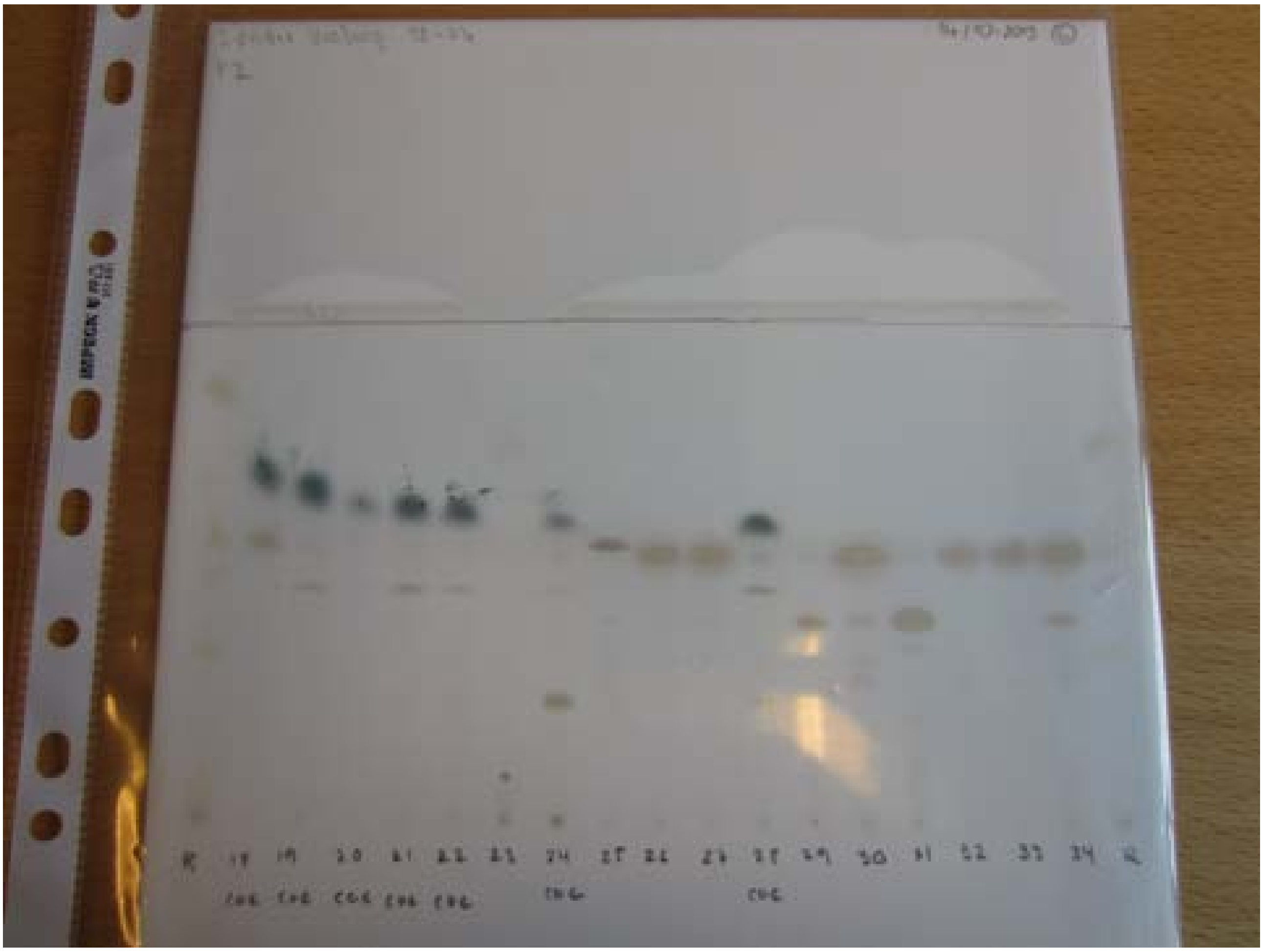


Hulsig Hede plate 1 (1-17) solvent C

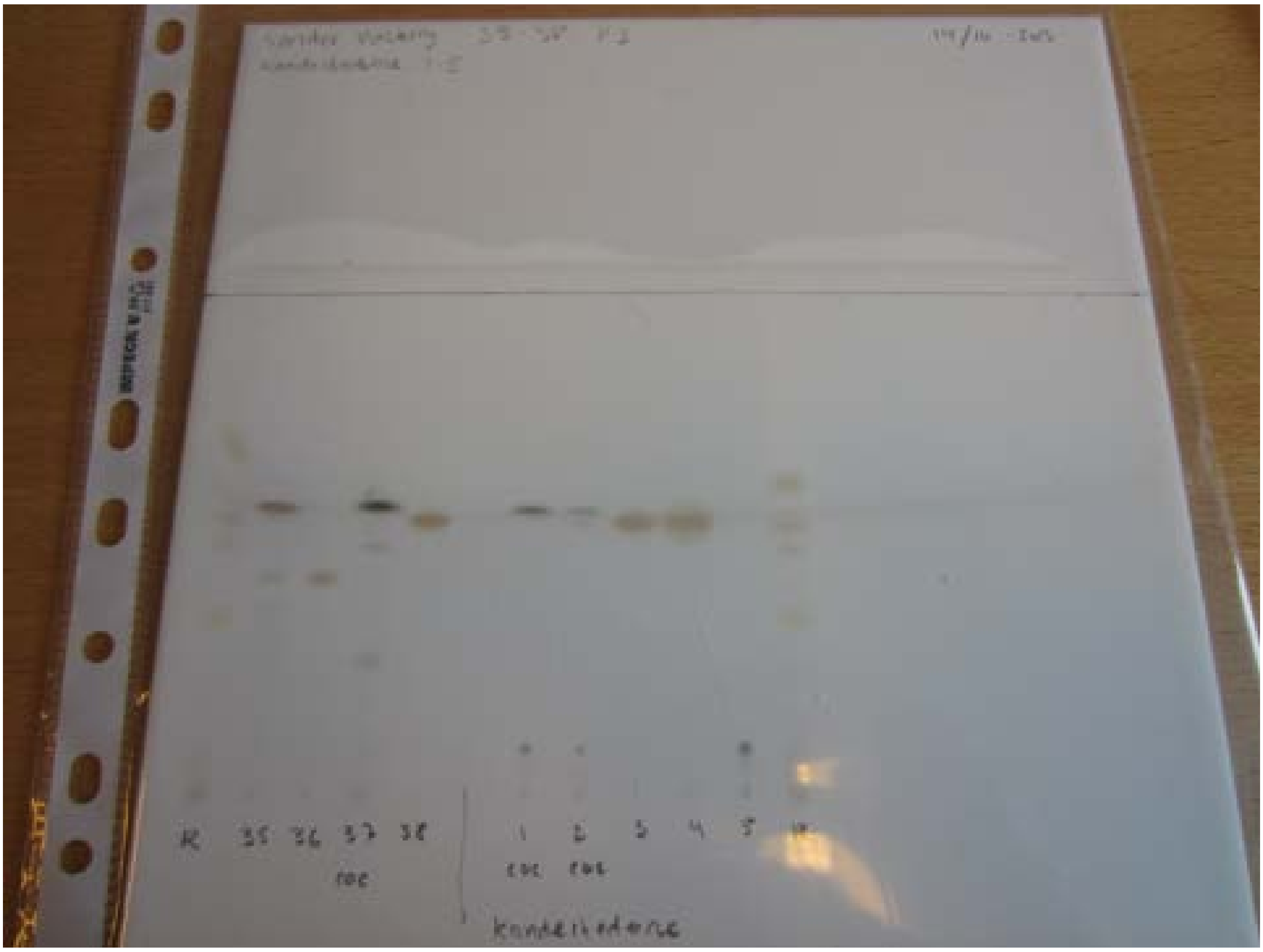


Sønder Vosborg Hede plate 1 (1-17) solvent C

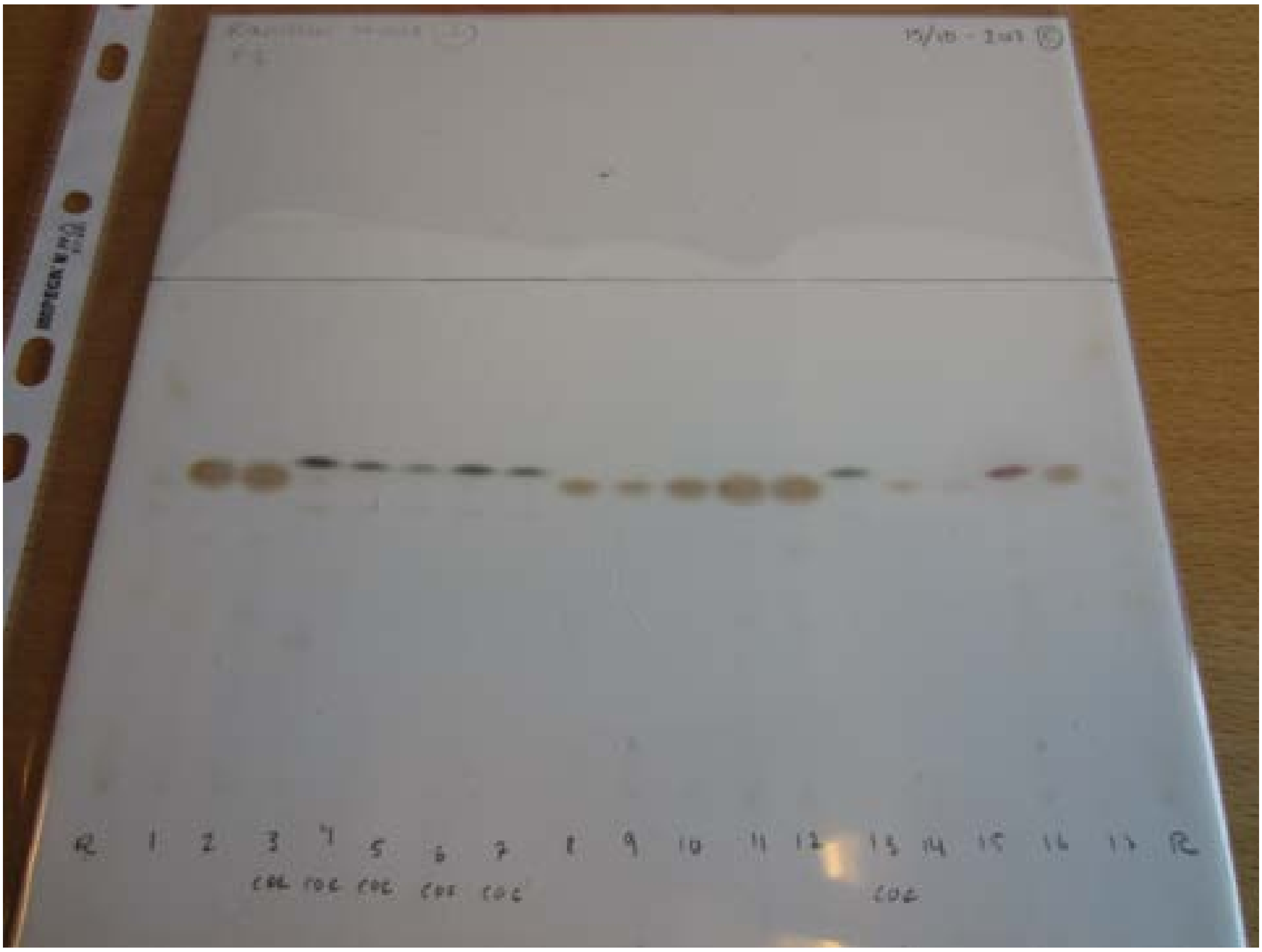
Sønder Vosborg Hede plate 2 (18-34) solvent C



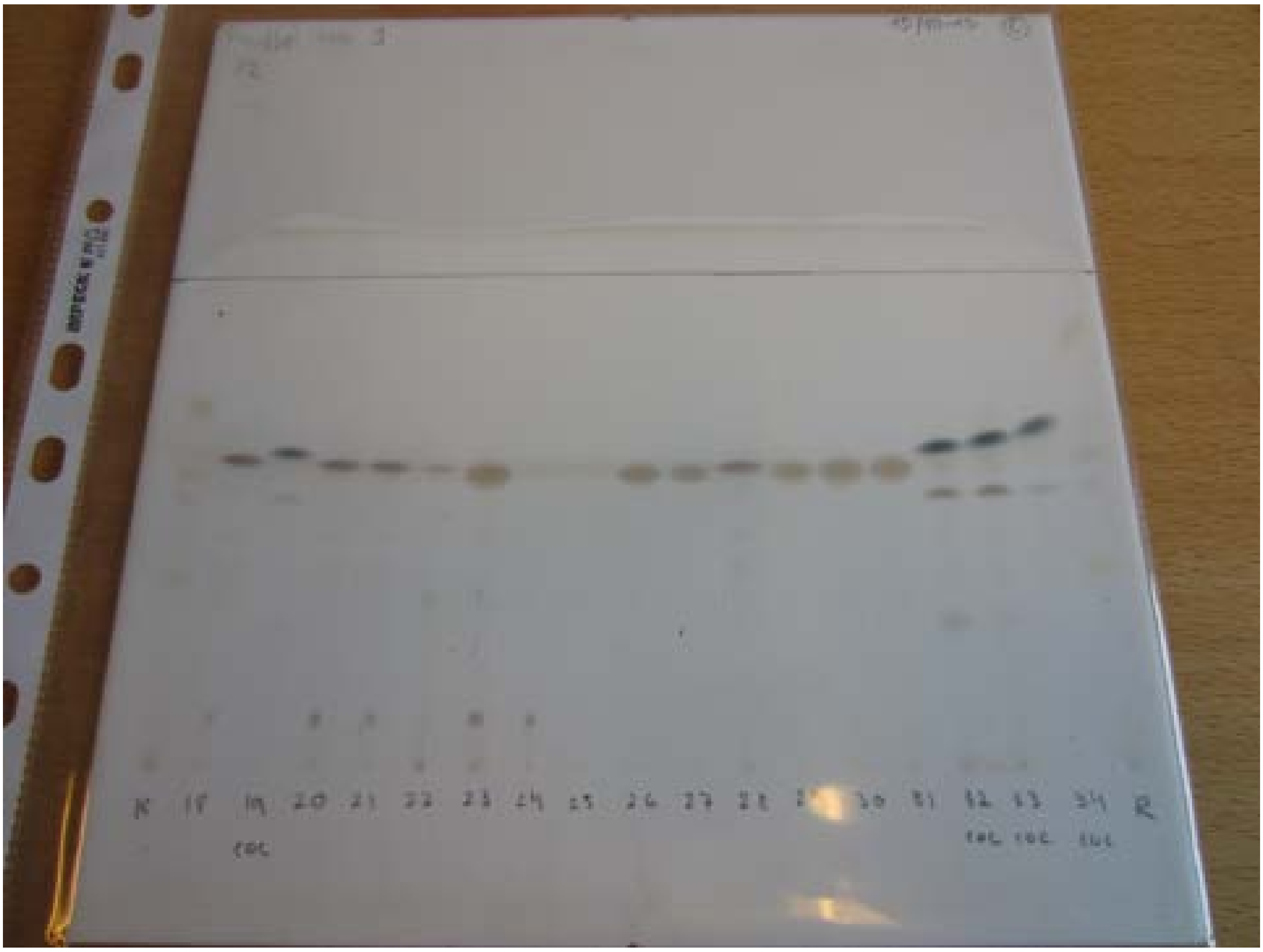
Sønder Vosborg Hede plate 3 (35-38) Kandestederne (1-5) solvent C



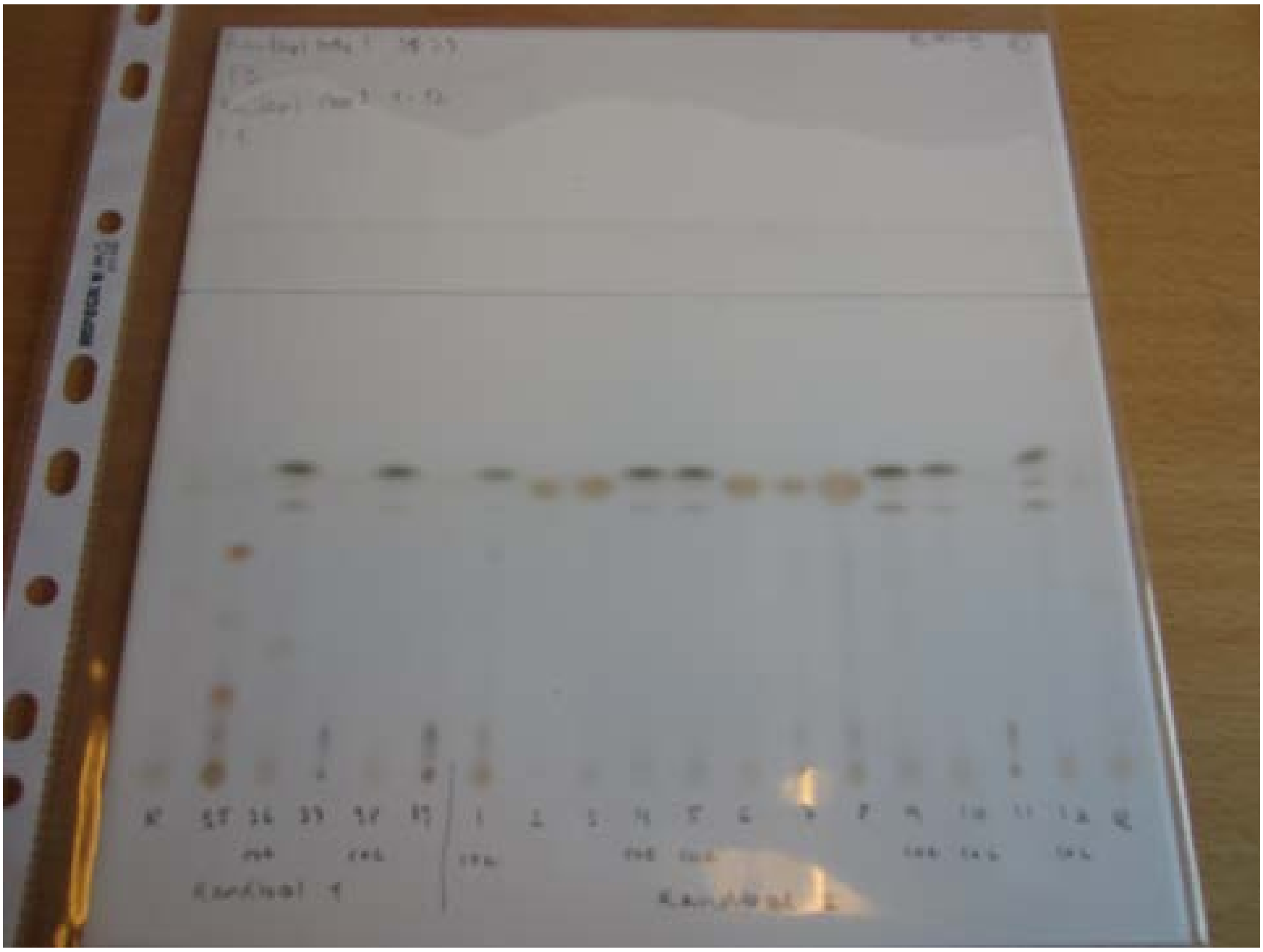
Randbøl Hede 1.1 (1-17) solvent C



Randbøl Hede 1.2 (18-34) solvent C



Randbøl Hede 1.3 (35-39) Randbøl Hede 2.1 (1-12) solvent C



Randbøl Hede 2.2 (13-29) solvent C

APPENDIX 3

Appendix 2. HPTLC plates

Harrild Hede (1-24) Vrads Sande (25-29) solvent C

Hvidklit (1-32) solvent C

Store Råbjerg, Grene Sande (1-12) solvent C

APPENDIX 4

The table below shows the different species scores for each of the 116 investigated circles. First the original species index based on the vascular vegetation, then the species index based on the expert valuations is displayed and after that the final species index based on both the statistical tests and the expert valuation.

Locality	Plant	Expert	Final	Locality	Plant	Expert	Final
HAH1	0,557	0,953	0,955	**HAR1**	0,643	0,000	0,000
HAH2	0,602	0,855	0,855	**HAR2**	0,561	0,831	0,831
HAH3	0,496	0,836	0,836	**HAR3**	0,561	0,817	0,817
HAH4	0,446	0,841	0,859	**HAR4**	0,496	0,000	0,000
HAH5	0,321	0,944	0,952	**HAR5**	0,170	0,000	0,000
HHH1	0,703	0,931	0,964	**HHR1**	0,597	0,859	0,897
HHH2	0,719	0,925	0,963	**HHR2**	0,650	0,881	0,943
HHH3	0,692	0,920	0,948	**HHR3**	0,565	0,858	0,920
HHH4	0,630	0,905	0,947	**HHR4**	0,677	0,885	0,948
HHH5	0,706	0,919	0,964	**HHR5**	0,451	0,814	0,836
HKH1	0,725	0,948	0,974	**HKR1**	0,827	0,841	0,874
HKH2	0,790	0,883	0,927	**HKR2**	0,802	0,956	0,978
HKH3	0,792	0,873	0,920	**HKR3**	0,677	0,644	0,644
HKH4	0,755	0,933	0,956	**HKR4**	0,814	0,750	0,750
HKH5	0,757	0,816	0,885	**HKR5**	0,643	0,939	0,949
REH1	0,851	0,932	0,960	**RER1**	0,770	0,750	0,793
REH2	0,677	0,894	0,939	**RER2**	0,843	0,920	0,948
REH3	0,814	0,939	0,959	**RER3**	0,799	0,923	0,946

REH4	0,738	0,874	0,897	**RER4**	0,596	0,899	0,934
REH5	0,814	0,787	0,814	**RER5**	0,732	0,893	0,932
KSH1	0,650	0,895	0,939	**KSR1**	0,572	0,000	0,000
KSH2	0,776	0,919	0,946	**KSR2**	0,814	0,899	0,939
KSH3	0,769	0,894	0,948	**KSR3**	0,695	0,949	0,968
RHH1	0,802	0,891	0,937	**RHR1**	0,658	0,858	0,920
RHH2	0,695	0,969	0,978	**RHR2**	0,419	0,889	0,899
RHH3	0,792	0,946	0,975	**RHR3**	0,739	0,971	0,972
RHH4	0,520	0,955	0,962	**RHR4**	0,596	0,948	0,970
RHH5	0,520	0,926	0,946	**RHR5**	0,695	0,933	0,948
VSH1	0,482	0,831	0,831	**VSR1**	0,192	0,000	0,000
SVH1	0,631	0,790	0,827	**SVR1**	0,800	0,755	0,822
SVH2	0,643	0,889	0,909	**SVR2**	0,381	0,879	0,891
SVH3	0,542	0,854	0,893	**SVR3**	0,402	0,770	0,797
SVH4	0,569	0,936	0,957	**SVR4**	0,269	0,765	0,800
SVH5	0,435	0,912	0,920	**SVR5**	0,505	0,814	0,833
GSH1	0,417	0,942	0,969	**GSR1**	0,765	0,932	0,954
GSH2	0,552	0,854	0,903	**GSR2**	0,552	0,939	0,952
GSH3	0,765	0,894	0,933	**GSR3**	0,552	0,945	0,966
GSH4	0,596	0,833	0,891	**GSR4**	0,552	0,738	0,770
GSH5	0,703	0,939	0,966	**GSR5**	0,719	0,953	0,978
OHH1	0,811	0,750	0,750	**OHR1**	0,755	0,644	0,644
MOH1	0,435	0,960	0,976	**MOR1**	0,599	0,956	0,971
MOH2	0,520	0,944	0,973	**MOR2**	0,635	0,948	0,972

MOH3	0,352	0,920	0,959	**MOR3**	0,799	0,910	0,937
MOH4	0,559	0,879	0,925	**MOR4**	0,654	0,944	0,966
MOH5	0,516	0,981	0,984	**MOR5**	0,300	0,933	0,939
KL1H1	0,435	0,821	0,821	**KL1R1**	0,372	0,765	0,765
KL1H2	0,340	0,831	0,831	**KL1R2**	0,435	0,765	0,765
KL1H3	0,146	0,776	0,776	**KL1R3**	0,340	0,807	0,807
KL1H4	0,557	0,874	0,874	**KL1R4**	0,340	0,673	0,673
KL1H5	0,159	0,889	0,889	**KL1R5**	0,269	0,755	0,755
KL2H1	0,720	0,745	0,745	**KL2R1**	0,269	0,755	0,755
KL2H2	0,552	0,847	0,847	**KL2R2**	0,698	0,673	0,673
KL2H3	0,698	0,792	0,814	**KL2R3**	0,146	0,000	0,000
KL2H4	0,552	0,866	0,866	**KL2R4**	0,241	0,673	0,673
KL2H5	0,755	0,903	0,903	**KL2R5**	0,739	0,859	0,859
KL3H1	0,552	0,841	0,859	**KL3R1**	0,552	0,673	0,673
KL3H2	0,552	0,866	0,866	**KL3R2**	0,552	0,807	0,807
KL3H3	0,765	0,851	0,851	**KL3R3**	0,698	0,755	0,755

APPENDIX 5

Site modules

Sites	module	Z	C
RHH4	0	-0.260843121	0.57
RHH5	0	-0.260843121	0.615702479
RHR1	0	-0.843904214	0.618343195
RHR4	0	-0.843904214	0.698961938
RHR5	0	-0.552373667	0.498614958
HHR5	0	-1.426965307	0.544378698
MOH1	0	0.905279066	0.594674556
MOH2	0	-0.552373667	0.664819945
MOH3	0	0.613748519	0.652777778
MOH5	0	1.488340159	0.550347222
MOR1	0	0.322217973	0.657844991
MOR2	0	0.905279066	0.6064
MOR3	0	0.613748519	0.527777778
MOR4	0	2.654462346	0.553571429
MOR5	0	0.030687426	0.549382716
SVH1	0	-0.552373667	0.512396694

GSH1	0	-0.843904214	0.623966942
GSH5	0	-0.843904214	0.612244898
GSR3	0	-0.552373667	0.623966942
RHH1	1	-0.462895476	0.569444444
RHH2	1	-1.085700299	0.551984877
RHH3	1	0.471311758	0.633333333
REH1	1	0.159909346	0.582644628
REH2	1	-0.774297888	0.586419753
REH3	1	0.782714169	0.524793388
REH4	1	-1.397102711	0.6328125
REH5	1	-1.085700299	0.551111111
RER2	1	0.159909346	0.615384615
RER3	1	0.159909346	0.595041322
RER4	1	-2.019907534	0.556213018
RER5	1	-0.462895476	0.62585034
HHH1	1	0.471311758	0.579861111
HHH2	1	0.782714169	0.569444444
HHH3	1	0.159909346	0.515

HHH4	1	0.159909346	0.569444444
HHH5	1	1.716921404	0.549346017
HHR1	1	-1.397102711	0.5546875
HHR2	1	0.782714169	0.46
HHR3	1	0.471311758	0.541322314
HHR4	1	0.471311758	0.526077098
HKH1	1	2.651128638	0.532598714
HKH2	1	0.782714169	0.517361111
HKH3	1	-0.151493065	0.5696
HKH4	1	-0.151493065	0.541322314
HKH5	1	0.471311758	0.5632
HKR2	1	1.405518992	0.609733701
KSH1	1	-0.151493065	0.623966942
KSH2	1	0.159909346	0.6416
KSH3	1	1.405518992	0.580357143
KSR1	1	-1.708505122	0.631944444
KSR2	1	-0.151493065	0.609504132
KSR3	1	0.471311758	0.5275
MOH4	1	-0.774297888	0.637119114
SVH3	1	-	0.5616

		0.151493065	
SVH4	1	-0.462895476	0.655612245
GSR2	1	-1.708505122	0.569444444
RHR2	2	0.840899134	0.476454294
RHR3	2	0.4894786	0.456747405
RER1	2	-1.619044601	0.49382716
HAH1	2	1.192319667	0.364197531
HAH2	2	1.192319667	0.21875
HAH3	2	0.138058067	0.152777778
HAH4	2	0.138058067	0.417777778
HAH5	2	2.598001801	0.4352
HAR1	2	-0.916203534	0
HAR2	2	0.138058067	0.152777778
HAR3	2	-1.267624067	0.37037037
HAR4	2	-1.267624067	0
HAR5	2	-1.267624067	0.37037037
HKR1	2	0.138058067	0.519031142
HKR3	2	-0.916203534	0.32
HKR4	2	-0.916203534	0.32

HKR5	2	-0.213362467	0.444444444
KL1H1	2	0.4894786	0.244897959
KL1H2	2	-0.213362467	0.165289256
KL1H3	2	-0.213362467	0
KL1H4	2	0.840899134	0.132653061
KL1H5	2	0.840899134	0.231111111
KL1R1	2	-0.564783	0.297520661
KL1R2	2	0.138058067	0
KL1R3	2	0.4894786	0
KL1R4	2	-0.916203534	0
KL1R5	2	-0.564783	0
KL2H1	2	0.138058067	0.152777778
KL2H2	2	0.4894786	0.142011834
KL2H3	2	0.138058067	0.357142857
KL2H4	2	0.4894786	0.142011834
KL2H5	2	0.840899134	0.3046875
KL2R1	2	-0.564783	0
KL2R2	2	-1.267624067	0
KL2R3	2	-2.321885668	0
KL2R4	2	-0.213362467	0

KL2R5	2	1.543740201	0
KL3H1	2	-0.564783	0.297520661
KL3H2	2	0.840899134	0.132653061
KL3H3	2	-0.213362467	0.277777778
KL3R1	2	-1.619044601	0
KL3R2	2	0.138058067	0
KL3R3	2	-0.916203534	0
SVH2	2	1.543740201	0.277777778
SVH5	2	1.895160734	0.380952381
SVR1	2	-1.267624067	0.345679012
SVR2	2	1.895160734	0.27700831
SVR3	2	0.4894786	0.498614958
SVR4	2	-0.564783	0.18
SVR5	2	0.4894786	0.40625
GSH2	2	-0.213362467	0.53125
GSH3	2	0.840899134	0.438271605
GSR1	2	0.138058067	0.491349481
GSR4	2	-0.564783	0.297520661
VSH1	2	0.840899134	0.24
VSR1	2	-1.267624067	0.37037037

GSH4	3	0.707106781	0.62345679
GSR5	3	- 0.707106781	0.635416667
OHH1	4	0.707106781	0.671875
OHR1	4	- 0.707106781	0.591715976

APPENDIX 6

Species modules

species	module	Z	C
C_chlorophaea	0	1.192851476	-0.192851476
C_cornuta	0	-0.154369015	1.154369015
C_crispata	0	0.519241231	0.480758769
C_diversa	0	2.764608714	-1.764608714
C_deformis	0	-0.82797926	1.82797926
C_foliacea	0	2.315535218	-1.315535218
C_furcata	0	1.417388224	-0.417388224
C_rangiferina	0	-0.82797926	1.82797926
C_rangiformis	0	0.070167734	0.929832266
C_subulata	0	1.641924973	-0.641924973
C_sulphurina	0	0.070167734	0.929832266
C_uncialis_ssp_uncialis	0	-0.603442511	1.603442511
Achillea_milleforlium	0	-0.603442511	1.603442511
Anagallis_arvensis	0	-0.82797926	1.82797926
Artemisia_vulgaris	0	-0.154369015	1.154369015

Campanula_rotundifolia	0	-0.378905763	1.378905763
Dactylis_glomerata	0	-0.82797926	1.82797926
Erigeron_acer	0	-0.82797926	1.82797926
Festuca_ovina	0	-0.603442511	1.603442511
Galium_verum_ssp_verum	0	0.070167734	0.929832266
Ononis_maritima	0	0.519241231	0.480758769
Pinus_mugo	0	0.519241231	0.480758769
Plantaginceae_maritima	0	-0.154369015	1.154369015
Pulsatilla_vulgaris	0	-0.82797926	1.82797926
Rumex_acetocella	0	1.417388224	-0.417388224
Sedum_acre	0	-0.82797926	1.82797926
Thalictrum_minus_ssp_Minus	0	-0.82797926	1.82797926
Trientalis_europaea	0	-0.603442511	1.603442511
Trifolium_arvense	0	-0.603442511	1.603442511
Tripleurospermum_perforatum	0	-0.82797926	1.82797926
Vicia_sativa	0	-0.82797926	1.82797926
Polytrichum_juniperinum	0	-0.378905763	1.378905763
C_arbuscula	1	0.494053034	0.505946966
C_cervicornis	1	-0.641531551	1.641531551

*C_ciliata_*	1	0.081113185	0.918886815
C_gracilis	1	0.906992883	0.093007117
C_humilis	1	-0.951236438	1.951236438
C_mitis	1	-0.951236438	1.951236438
C_phyllophora	1	-0.951236438	1.951236438
C_pleurota	1	0.906992883	0.093007117
C_symphycarpa	1	-0.951236438	1.951236438
C_uncialis_ssp_biuncialis	1	1.319932732	-0.319932732
C_zopfii	1	0.803757921	0.196242079
Cetraria_aculeata	1	2.14581243	-1.14581243
Cetraria_islandica	1	-0.848001476	1.848001476
Cetraria_nivalis	1	-0.848001476	1.848001476
Hypogymnia_physodes	1	0.494053034	0.505946966
Peltigera_membranacea	1	-0.744766514	1.744766514
Platismatia_glauca	1	-0.331826664	1.331826664
Stereocaulon_condensatum	1	-0.435061627	1.435061627
Stereocaulon_saxatile	1	-0.331826664	1.331826664

Ammophila_arenaria	1	1.319932732	-0.319932732
Anthoxanthum_odoratum	1	-0.951236438	1.951236438
Campanulaceae_rotondifolia	1	-0.538296589	1.538296589
Carex_arenaria	1	1.629637619	-0.629637619
Carex_pilulifera	1	0.700522958	0.299477042
Corynephorus_canescens	1	1.836107543	-0.836107543
Dianthus_deltoides	1	-0.951236438	1.951236438
Hieracium_umbellatum	1	-0.744766514	1.744766514
Hypochoeris_radicata	1	1.319932732	-0.319932732
Jasione_montana	1	1.836107543	-0.836107543
Koeleria_glauca	1	-0.538296589	1.538296589
Lotus_corniculatus	1	-0.641531551	1.641531551
Polypodium_vulgare	1	0.184348147	0.815651853
Quercus_sp.	1	-0.951236438	1.951236438
Rosa_rogusa	1	-0.951236438	1.951236438
Rosa_sp.	1	-	1.951236438

		0.951236438	
Rumex_acetosa_var_acetosa	1	-0.641531551	1.641531551
Thymus_serpyllum	1	-0.228591702	1.228591702
Vaccinium_myrtillus	1	-0.951236438	1.951236438
*Viola_tricolor_ssp_tricolor_*	1	0.700522958	0.299477042
Camptothecium_lutescens	1	-0.951236438	1.951236438
Polytrichum_piliferum	1	1.732872581	-0.732872581
Racomitrium_canescens	1	-0.435061627	1.435061627
C_cryptochlorophaea	2	-0.558251699	1.558251699
C_fimbriata	2	-0.791768749	1.791768749
C_floerkeana	2	0.609333554	0.390666446
C_glauca	2	0.667712816	0.332287184
C_grayi	2	-1.0252858	2.0252858
C_macilenta	2	0.667712816	0.332287184
C_merochlorophaea	2	1.368263968	-0.368263968
C_novochlorophaea	2	-0.558251699	1.558251699
C_polydactyla	2	-1.0252858	2.0252858
C_portentosa	2	1.718539543	-

			0.718539543
C_ramulosa	2	0.200678715	0.799321285
C_scabriuscula	2	-0.558251699	1.558251699
C_squamosa	2	-1.0252858	2.0252858
Arctostaphylos_uva-ursi	2	-0.14959686	1.14959686
Betula	2	-1.0252858	2.0252858
Calluna_vulgaris	2	2.185573644	-1.185573644
Cytisus_scoparius	2	-0.850148012	1.850148012
Deshampsia_flexuosa	2	1.543401756	-0.543401756
Empetrum_nigrum	2	0.901229867	0.098770133
Epilobium_angustifolium	2	-0.733389487	1.733389487
Galium_saxatile	2	-0.383113911	1.383113911
Genista_anglica	2	-0.850148012	1.850148012
Juniperus_communis	2	-0.908527275	1.908527275
Molinia_caerulea	2	0.375816503	0.624183497
Picea_abies	2	-0.966906537	1.966906537
Salix_repens_ssp_repens	2	-0.324734648	1.324734648
Vaccinium_vitis-idaea	2	-	1.908527275

		0.908527275	
Campylopus_introflexus	2	0.142299453	0.857700547
Dicranum_scoparium	2	1.42664323	-0.42664323
Hypnum_(cupressiforme/jutlandicum)	2	1.776918806	-0.776918806
Pleurosium_schreberi	2	0.025540927	0.974459073
Pseudoscleropodium_purum	2	-0.966906537	1.966906537
Erica_tetralix	3	0	1
Persicaria_sp.	3	0	1
Polygonum_aviculare_ssp_neglectum	3	0	1
Carex_flacca	4	-0.585540044	1.585540044
Carex_panicea	4	-0.585540044	1.585540044
Cerastium_semidecandrum	4	-0.585540044	1.585540044
Luzula_sp.	4	1.463850109	-0.463850109
Pilosella_officinarum	4	1.463850109	-0.463850109
Trichophorum_caespitosum	4	-0.585540044	1.585540044
Leucobryum_glaucum	4	-0.585540044	1.585540044