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Indicator values for lichens on *Quercus* as a tool to monitor ammonia pollution in Denmark

Indikatorenwerte für Flechten auf *Quercus* für das Monitoring von Ammoniakeinträgen in Dänemark

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Key words: indicator values, eutrophication, *Xanthoria*, oak, nitrogen, Ellenberg.

Schlagwörter: Indikatorwerte, Eutrophierung, *Xanthoria*, Eichen, Stickstoff, Ellenberg.

Summary: The epiphytic lichen vegetation on solitary *Quercus* trees was investigated on 13 locations in Denmark in order to test the usability of indicator values to quantify ammonia deposition from the ambient air. Indicator values calculated for each location corresponded well with the expected ecological conditions. The indicator values for nitrogenous nutrients significantly correlated with measured nitrogen content in *Xanthoria parietina* thalli sampled from the investigated *Quercus* trees. Indicator values for some species are critically evaluated, and it is suggested that indicator values can serve as a tool for quantitative estimation of ammonia deposition in relation to sensitive Danish ecosystems.

Zusammenfassung: An 13 Orten in Dänemark wurde die epiphytische Flechtenvegetation an einzelnen *Quercus* Bäumen untersucht, um die Verwendbarkeit von Indikatorwerten für Ammoniakeinträge aus der Umgebungsluft zu quantifizieren. Die für die einzelnen Orte errechneten Indikatorenwerte entsprechen sehr gut den erwarteten ökologischen Bedingungen. Die Indikatorwerte von stickstoffhaltigen Nährstoffen entsprechen genau dem gemessenen Stickstoffgehalt in den Thalli von *Xanthoria parietina*, welche auf untersuchten *Quercus*-Bäumen gesammelt wurden. Die Indikatorenwerte von einigen Arten wurden kritisch abgeschätzt und es wird angenommen, dass diese für eine quantitative Bewertungsmöglichkeit der Stickstoffeinträge in empfindlichen dänischen Ökosystemen verwendet werden können.

Introduction

The qualitative approaches

During the last one hundred years the input of nitrogen into the global terrestrial nitrogen cycle has doubled due to human activities (VAN BREEMEN 2002) and particularly in regions of intensive farming the immission of ammonia has become significant. It is well known that ammonia mediated eutrophication of tree bark can cause a shift in epiphytic lichen composition. The classic example of this phenomenon is a shift from the generally species poor Physodion community to the rather species rich Xanthorion community, which has also been observed in Denmark (SØCHTING 1991). VAN HERK (1999), VAN DOBBEN & DE BAKKER (1996) and RUOSS (1999) have described an increase in nitrophytic lichen species (characterized by VAN HERK (2003) as species needing both a relatively high bark pH and at least some additional nitrogen) as well as a decline in the abundance of acidophytic lichen species (characterized as needing an acidic substrate and many also sensitive to increased levels of nitrogen (VAN HERK 2003)) on trees in Western Europe. The reason for this shift is attributed to a) increasing amounts of nitrogen compounds in the air, b) decreasing levels of SO_2 in the air, c) a synergistic effect of the two before mentioned. The effects are seemingly both direct by increase in nutrients and/or toxicity to some species, and indirect by increase in substrate pH (VAN HERK 2001). For some authors the increased bark pH is a more important factor than increased nitrogen availability, at least in areas close to sources (DE BAKKER 1989, FRATI et al. 2006, VAN DOBBEN & DE BAKKER 1996, VAN DOBBEN & TER BRAAK 1998). In areas of background NH_3 concentrations in the air, increased availability of nitrogen plays a larger role (VAN HERK et al. 2003). The rise in bark pH is a result of both the alkaline effects of NH_3 and the decrease in acidifying SO_2 (VAN DOBBEN & DE BAKKER 1996).

As a result of the observed shift in species, VAN HERK (1999) constructed the AIW/NIW index (Acidofiele Indicatie Waarde/Nitrofiele Indicatie Waarde). This index is based on indicator species categorized as either nitrophytes (e.g. *Xanthoria parietina* and *Physcia adscendens)* or acidophytes (e.g. *Evernia prunastri* and *Hypogymnia physodes).* These indicator species are scored on each sampled tree. The NIW is defined as the mean number of nitrophytes on a given tree. Species covering more than 1 dm^2 count double. The AIW is calculated in a similar way.

In Denmark, the best known example of development of a bioindication index is the LFI (Lav Forsurings Indeks = Lichen Acidification Index) (SØCHTING 1990). The LFI is based on 6 indicator species: *Evernia prunastri, Hypogymnia physodes, Lecanora conizaeoides, Pseudevernia furfuracea, Tuckermanopsis chlorophylla* and *Hypocenomyce scalaris*. These are assigned an indicator value based on the mean number of acidophytes found on the same trunks as each indicator species, respectively. The indicator values are multiplied with the coverage on the trunk of the given species and summed for each tree.

Some qualitative approaches focus on single species, whereas others use groups of species with similar ecological requirements. The latter method is especially useful in studies where different substrates are used or if species groups are used as substitutes for communities (VAN HALUWYN & VAN HERK 2002). It could be argued, that groups of species or communities are better indicators for the state of the environment than individual species due to their overlap in ecological properties and requirements and thereby their more holistic image of the situation (NIMIS 1991).

Indicator values

Indicator values have been assigned to plants (ELLENBERG 1991), bryophytes (DÜLL 1991) and lichens (WIRTH 1991, NIMIS 1999, NIMIS & MARTELLOS 2001). These indicator values summarize the ecological optima for each species according to a variety of abiotic factors, e.g. light and water conditions, pH values, nitrogen compounds, and temperature. More than 450 lichen species are included by WIRTH (1991) and approximately 2200 species are included by NIMIS (1999).

If indicator values are combined with an abundance index for all taxa at a given site, the obtained average values can serve as ecological site descriptors.

Strengths and weaknesses

Indicator values have both strengths and weaknesses. The major advantage is that it is relatively simple and provides interpretations of ecological sites without the aid of instrumental measurements. Indeed, the method has the advantage that it is amenable to simplification. If only a restricted set of species is used, that can be identified by non-specialists, the index can serve to encourage a wider public appreciation of lichen monitoring (PITCAIRN et al. 2004). However, the indicator values also represent three major methodological weaknesses.

Firstly, it is hard to distinguish the categories because they affect each other to some degree. For instance, light strongly effects temperature, which in hand affects the moisture level. This makes it difficult to decide whether epiphytes react upon the factor itself or upon its secondary effects (BARKMAN 1958), and thus makes it difficult to assign indicator values in all categories to each species.

Secondly, the ecological optimum of a given taxon may differ greatly within different areas of its distribution range, rendering indicator values usable only in limited regions. Therefore it is necessary to be critical to indicator values developed in regions ecologically or climatically divergent from the ones investigated.

Thirdly, the indicator values are based on subjective perceptions of the one or few people who are assigning the values to each taxon. Indeed, it is possible to assign objective values based upon experimental field work by e.g. the use of comparable sets of relevés to calculate ecological amplitudes and optima (DIEKMANN 1995), by the use of spore germination and development optimum in response to nutrients and pH level (WAMELINK et al. 1998) and by the use of fitting log-linear models to contingency tables of presence/absence frequencies (ZOBEL 1988); this procedure would, however, be too wide-ranging to carry out on *all* species. Therefore, all presently existing values are based upon subjective judgements and subsequent validation. NIMIS & MARTELLOS (2001) state that "the practical use of expert judgements for formulating predictive hypotheses in ecology should be favoured, provided that: (a) the geographic range where they can be used with confidence is clearly specified, (b) their predictivity is tested against real data, and (c) that a procedure for correcting the values when necessary is made explicit"

Objective

The objective of the present study was to test the usability of lichen indicator values in Denmark. To facilitate this investigation, Wirth's indicator values were utilized on the epiphytic lichens occurring on solitary *Quercus* trees at 13 Danish locations. It was the purpose that these studies should establish a tool for future monitoring and adjustment of the existing critical loads for nitrogen deposition to Danish ecosystems.

This paper is a tribute to Volkmar WIRTH in appreciation of his contribution to understanding of lichen autecology, and without whom this paper had not been possible.

Materials and methods

Locations

Thirteen sites were chosen, mainly on Zealand, but also on Lolland and Møn (Fig. 1). The thirteen locations can broadly be divided into four groups, shown in table 1.

Selection of solitary *Quercus* trunks and analysis of lichen vegetation

At each site, five suitable *Quercus* trees were chosen randomly according to the guidelines proposed by ASTA et al. (2002). Only trees with a girth between 100 and 350 cm were chosen. In addition, the trees should be solitary and free of low-hanging branches, which could create unequal light conditions.

Two rubber bands, each with eight pre-made marks, were secured around the trunk at heights of 60 and 200 centimetres, respectively. Subsequently, pin-point analyses were carried out at each of the 8 marks, starting at 200 centimetres and moving down the trunk with 10-centimeter intervals, thus resulting in 120 pin-points. The analyses were always initiated on the west side of the trunk and carried out clockwise around the trunk. At each pin-point, the lichen species present (if any) was noted. Each tree was also subjected to a total species analysis. The trunk was thoroughly examined and all lichen species present were noted. Critical species were collected for further microscopic investigation. The nomenclature follows SØCHTING & ALSTRUP (2007).

Finally, thalli of *Xanthoria parietina* were collected whenever present for analysis of total nitrogen content. *X. parietina* is not expected to occur on bark of *Quercus* due to the fairly low primary pH of the bark in an unpolluted environment. Accordingly, *X. parietina* was only collected at eight of the thirteen sites.

The sampling took place October and November 2006.

Indicator value calculations for lichens

The importance value expressed as percentage cover was calculated for each species using the pin-point analysis. The importance value of each species on each location was multiplied with the indicator values of the particular species (if any were available) in the categories: light (L), temperature (T), continentality (K), moisture (F), reaction (R) and nutrients (N) following WIRTH (1991).

The sums of the multiplications were subsequently divided by the sum of importance values in each category yielding an indicator value for each location.

Results

Indicator values

71 lichen species were recorded on the examined trees: *Amandinea punctata, Anaptychia ciliaris, Anisomeridium polypori, Arthonia didyma, A. radiata, A. spadicea, A. vinosa, Calicium glaucellum, C. salicinum, C. viride, Caloplaca obscurella, Candelaria concolor, Chaenotheca chlorella, C. chrysocephala, C. ferruginea, C. phaeocephala, C. stemonea, C. trichialis, Chrysothrix candelaris, Cladonia coniocrea, Cliostomum griffithii, Dimerella pineti, Diploicia canescens, Evernia prunastri, Haematomma ochroleucum, Hypocenomyce scalaris, Hypogymnia physodes, H. tubulosa, Lecanactis abietina, Lecanora allophana, L. argentata, L. carpinea, L. chlarotera, L. conizaeoides, L. expallens, L. persimilis, L. saligna, Lecidella elaeochroma, Lepraria incana, Melanelixia fuliginosa, M. subaurifera, Microcalicium disseminatum, Opegrapha varia, O. vermicellifera, Parmelia saxatilis, P. sulcata, Pertusaria albescens, P. albescens var. corallina, P. amara, P. coccodes, P. hymenea, P. pertusa, Phaeophyscia orbicularis, Phlyctis argena, Physcia adscendens, P. tenella, Physconia distorta, P. enteroxantha, P. grisea,*

Placynthiella icmalea, P. uliginosa, Pleurosticta acetabulum, Pyrrhospora quernea, Ramalina farinacea, R. fastigiata, Schismatomma cretaceum, S. decolorans, Strangospora pinicola, Xanthoria candelaria, X. parietina, X. polycarpa.

The indicator values for each location are shown in fig. 2.

<u>Temperature and continentality</u>

The indicator values for temperature and continentality show no differences between locations (figs. 2b and 2c).

<u>Nutrients and reaction</u>

Reaction and nutrient values are shown in figs. 2e and 2f. Two locations, Dedelev and Sydhavsgrisen, stand out in having remarkably higher values than all other locations. Albeit not reaching the level of Dedelev and Sydhavsgrisen, the locations Mørkøv and Kattrup show higher reaction and nutrient values than the remaining locations. In addition the woodland localities were the only ones getting a nutrient score less than 3 with Ulvshale having the lowest value of 2.1. The roadside localities get intermediate nutrient values but somewhat low reaction values along with the woodland localities.

<u>Light</u>

Light values are shown in fig. 2a. Dedelev and Sydhavsgrisen again share high scores, whereas the two woodland locations, Ulvshale and Krenkerup, show remarkably low light scores. Correspondingly all light values show a negative correlation with the average tree girth of the localities with an r^2 of 0.57 (fig. 3).

<u>Moisture</u>

Moisture values are shown in fig. 2d. Woodland locality Ulvshale stands out in having a high moisture value compared to all remaining localities, and no differences amongst these can be accounted for.

Nitrogen content in in situ *Xanthoria parieti*

The graphs for nutrient values and for thallus nitrogen contents (fig. 4) show that livestock locations have the highest values (ca. 3.1%), the agricultural location is intermediate (2.7%) and that woodland locations have the lowest scores (ca. 2.3%). No *Xanthoria parietina* occurred at either of the roadside locations. It is seen in fig. 5 that the actual nitrogen content measured in in situ *X. parietina* thalli strongly correlates with the nutrient indicator values (r^2=0.88).

Discussion

Temperature and continentality

For the temperature and continentality values no differences were observed. This coincides well with the expectation that temperature or continentality differences are only detectable on a larger geographical scale than the one investigated here (figs. 2b and 2c).

Nutrients and reaction

Because of the poikilohydric nature of lichens, which makes their response towards air gasses and aerosols very high, we expected to observe differences between localities according to nutrient values. This was expected primarily between woodland localities and the ones in near vicinity to live stockhold and agricultural fields (figs. 2e and 2f).

At the stockhold localities, the *Quercus* trees were dominated by nitrophytic species, such as *Lecidella elaeochroma*, *Physcia adscendens*, *P. tenella*, *Xanthoria parietina* and *X. polycarpa*, thus giving a high nutrient score. The reaction score was also expected to be high due to the alkaline effect of ammonia deposition on *Quercus* trunks. The results turned out to correspond well with our expectations in that Dedelev and Sydhavsgrisen, the two localities close to live stockholds, had the highest scores in both categories. The same was the case for Mørkøv and Kattrup, although their scores did not reach the levels of Dedelev and Sydhavsgrisen.

Light

Even though the investigation was carried out on solitary standing *Quercus* trees mainly in order to uniformize the light conditions for the corticolous lichens, the light values showed clear differences between localities (fig. 2a). In this case, the two localities Dedelev and Sydhavsgrisen once again got the highest scores, but these results are most likely due to tree girth since there is a clear negative correlation (r^2=0.55) between the light values and the mean tree trunk girth of the localities (fig. 3). A tree with a small trunk most likely also has a small canopy which causes more light to reach the trunk. Correspondingly the localities with the largest average trunk girths, Ulvshale and Krenkerup, also have the lowest light values.

Moisture

Only one locality, Ulvshale, differs from the others (fig. 2d), which can be explained by the fact that the solitary *Quercus* trees on this locality were growing in a forest opening. The surrounding trees most likely hold back sufficient amounts of evaporation to influence the lichen species composition in the forest opening. Moreover, it is reasonable to assume that a high moisture score is related to the tree girth, which on average was largest in Ulvshale. An old tree has a heterogenous bark structure that can host many different moisture containing microclimatic habitats for epiphytic lichens.

It should also be remembered that light strongly influence moisture and evaporation (BARKMAN 1958). Ulvshale (along with Krenkerup) is the locality with lowest light values, thus giving a good explanation for high moisture values. However, there is no clear correlation between light and moisture for the remaining localities.

Indicator values for lichens

The use of indicator values has some major weaknesses as mentioned in the introduction. It is therefore necessary to be critical to the values of each species and the calculated results for the locations. Below follows a brief discussion of suggested modifications of indicator values based on observations from the present study.

Anaptychia ciliaris has a relatively small absorption capacity for water vapour, but a large capacity for liquid water. As an epiphyte it is therefore restricted to growth on the western part and south-western part of the trunk where the prevailing wind carries rain water to the trunk (BARKMAN 1958). This statement responds well with our observations at the Kattrup locality where it strictly grew on the western part of the trunks. It is given an intermediate F-value of 5, but we suggest assigning this species a somewhat higher F-value.

Arthonia radiata was found only on the livestock locality Sydhavsgrisen. Likewise VAN HERK (2001) found this species on *Quercus* only at localities categorized as "intensively used agricultural areas" It is hard to separate environmental effects determining occurrence of a single species, and care should be taken when trying to impose improved indicator values. However, these observations point to *Arthonia radiata* being somewhat nitrophytic, and thus we suggest assigning this species a higher N-value than the one of 3 given by WIRTH (1991).

Evernia prunastri was found on various locations mainly occurring with rather low frequencies. However three exceptions were observed. At Ølsmosevej, Sæby and Nødebo the percentage cover was 6.4, 8.1 and 15.2, respectively. These locations were the only ones placed right next to a lake, suggesting that *E. prunastri* is highly affected by the presence of higher F and N-values assigned to *E. prunastri* than the ones of 3.

Physcia adscendens is only found on Sydhavsgrisen, where it is extremely abundant (average coverage of 42.4%). Even though this species is already considered to be a distinct nitrophyte by VAN HERK (2002), this result suggests an even higher N-value than 6.

Notable indicator species

Calicium viride was found on all six woodland localities and not at any of the remaining localities. This observation suggests *C. viride* as a good indicator of woodland localities. Besides *C. viride* a number of Caliciales species were found on one or more woodland locations including *Calicium glaucellum*, *C. salicinum*, *Chaenotheca chrysocephala*, *C. ferruginea*, *C. stemonea* and *C. trichalis*. *C. ferruginea* is an exception in that it is also found on Ølsmosevej, which is a partly roadside and partly woodland location. The fact that the Caliciales species, when fertile, are easily recognisable as a group and (apart from one locality) have not been found except in more or less undisturbed woodland locations make them good indicators of old *Quercus* woodlands. At Ulvshale the *Chaenotheca* species *C. chrysocephala* and *C. stemonea* were extremely abundant, which correspond to Ulvshale being the most undisturbed woodland of the investigated locations.

Lepraria incana was the most frequent species found on 11 of 13 locations. Van Herk (1999) regards this species as acidophytic concurring with our observations that the two locations lacking *L. incana* are the livestock locations indicating high proportions of ammonia being poisonous to this otherwise ubiquious species.

The crustose genus *Opegrapha* grows with thallus inside the bark making it vulnerable to algal overgrowth, and is suggested to be, at least on beech, an indicator of a humid forest environment and a certain degree of ecological continuity by SØCHTING & CHRISTENSEN (1997). In this investigation two different species of *Opegrapha* were found on three woodland locations. It was most abundant in Ulvshale, again indicating this location to be the most undisturbed.

Pyrrhospora quernea was found on five of six woodland locations also suggesting this species a good indicator of unmanaged woodland.

Nitrogen content in in situ *Xanthoria parietina*

The measured *X. parietina* nitrogen contents were highest at the stockhold locations Dedelev and Sydhavsgrisen, intermediate at the agricultural location Kattrup, and lowest at woodland locations (fig. 4).

The thallus nitrogen content of *X. parietina* shows a fine regression with $r^2=0.88$ when correlated with the nutrient values (fig. 5). This supports the use of indicator values as a quantitative tool for monitoring eutrophication, and for determining critical loads of nitrogen to Danish ecosystems. Critical loads are set to assure that an ecosystem is not severely affected and changed by pollutants both at species level as well as on ecosystem level. But determining these limits is a delicate balance. If they are set too high, it is risked that an ecosystem is not properly protected due to insufficient limitations. Conversely, if they are set too low, it is risked that protection of some ecosystems is forfeited due to the cost of pollution reductions. Lichen community composition, indicator values and *X. parietina* nitrogen content could be used when determining proper limits since differences in these variables are shown to reflect small scale ammonia air concentration differences. For this initiative to be taken into action, existing tolerance limit definitions should be redefined, and new political agreements would be necessary. It is a complicated matter and reaches beyond the scope of this investigation.

Conclusion

Correlations between indicator values of the epiphytic lichen flora on *Quercus*, and measured ammonia air concentrations showed significant regressions. This indicates a basis for the usability of this method in future evaluations of tolerance levels of Danish ecosystems.

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Table 1: Grouping of *Quercus* locations

In near vicinity of large stock holds	Dedelev Sydhavsgrisen
Old woodlands with isolated old trees	Ulvshale Krenkerup Store Frederikslund Dyrehave Asserbo Slotsruin Nødebo Jægersborg Dyrehave
Rural areas in near vicinity of agricultural fields	Mørkøv Kattrup
Close to roads with dense traffic	Sæby Strandvejen
Both road side and old woodland	Ølsmosevej

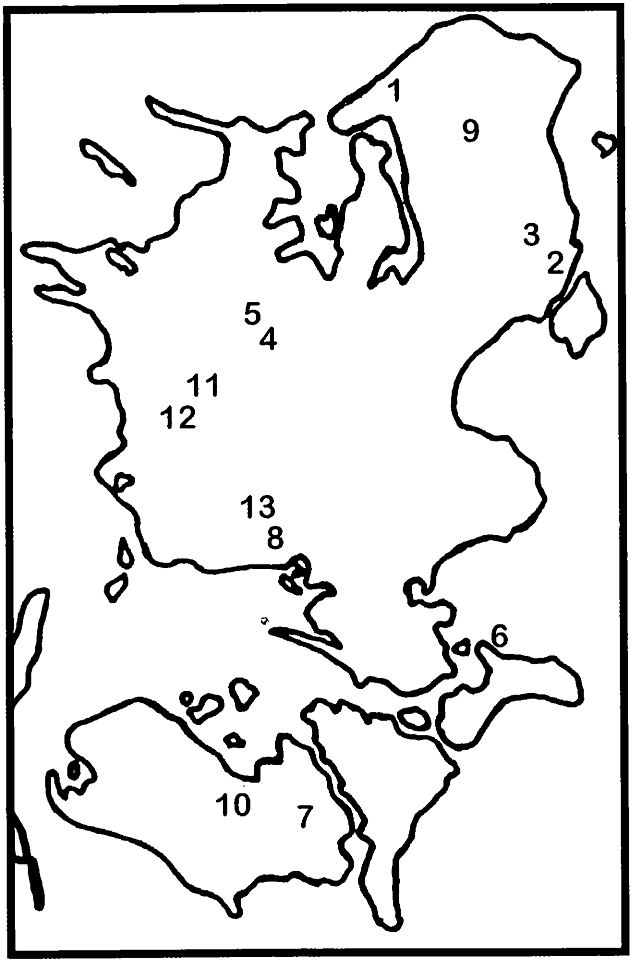


Fig. 1: Location of 13 investigated sites on Zealand. 1. Asserbo Slotsruin, 2. Strandvejen, 3. Dyrehaven, 4. Mørkøv, 5. Dedelev, 6. Ulvshale, 7. Krenkerup, 8. St. Frederikslund, 9. Nødebo, 10. Sydhavsgrisen, 11. Kattrup, 12. Sæby, 13. Ølsmosevej.

Fig. 2 a-f: Indicator values for epiphytic lichens growing on *Quercus* calculated for 13 locations in Denmark. 2a) light; 2b) temperatures; 2c) continentality; 2d) moisture; 2e) reaction; 2f) nutrients. Black bars indicate stockhold localities. Dark grey bars indicate woodland localities. Light gray bars indicate road-side localities. White bars indicate agricultural field localities.

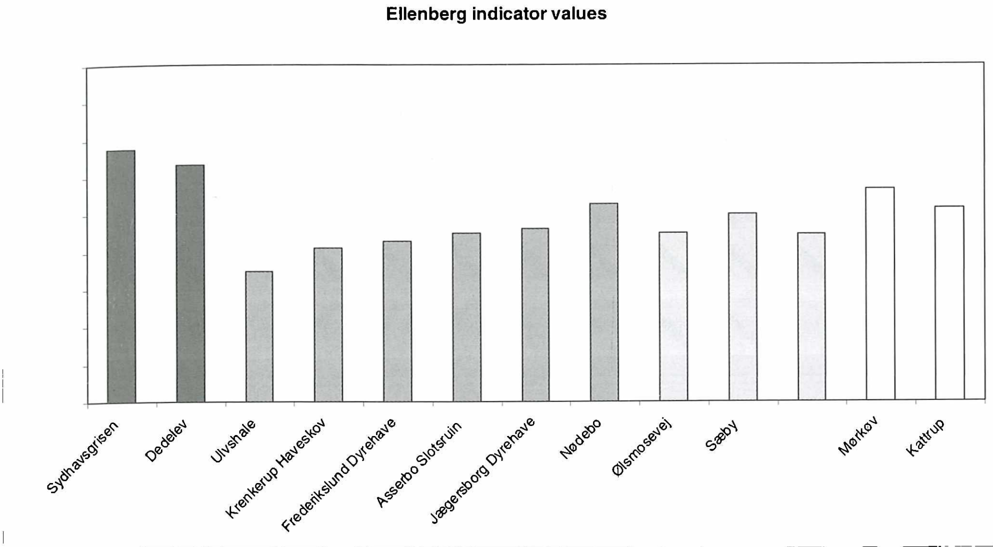


Fig. 2a

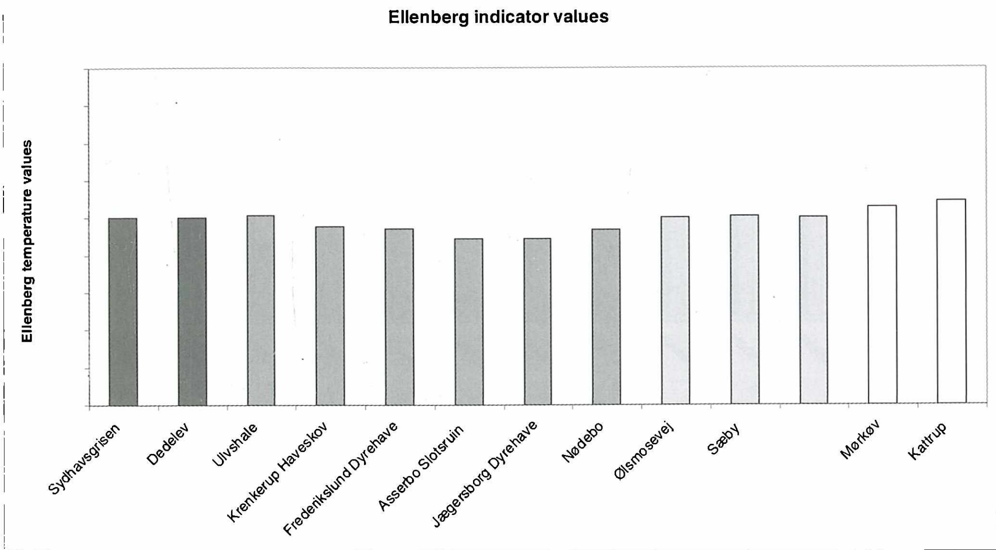
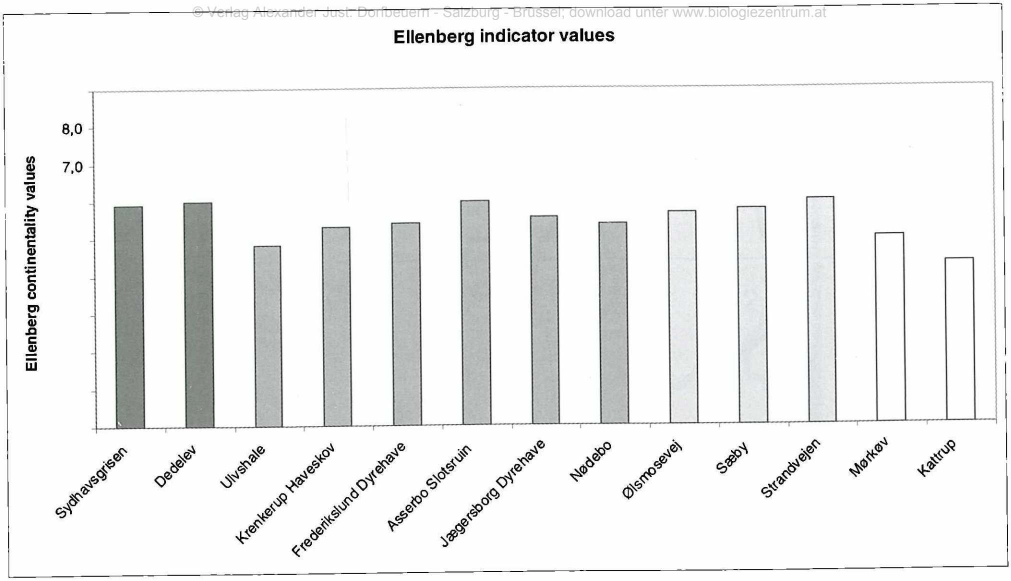


Fig. 2b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Ellenberg continentality values
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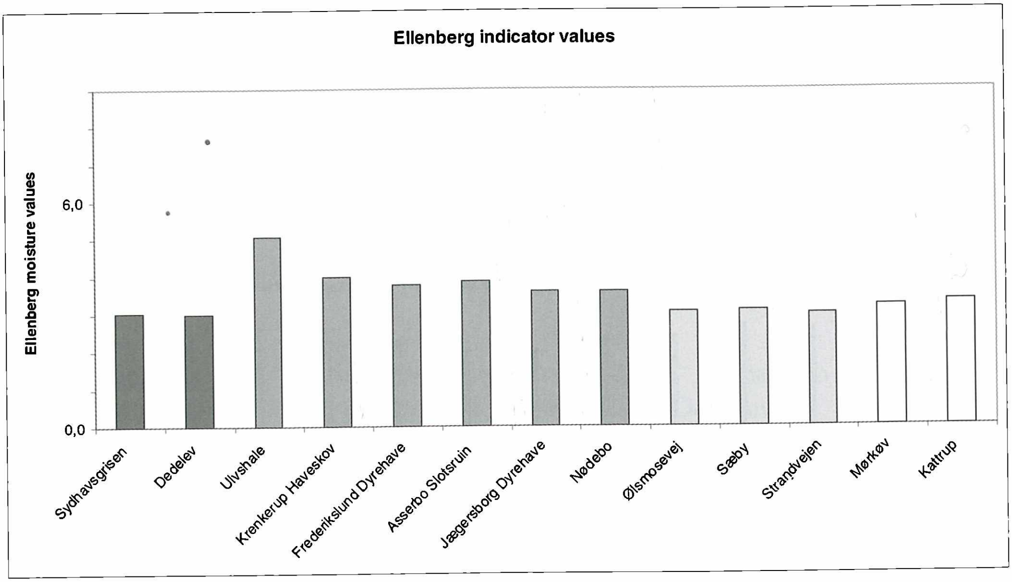
7,0
Sydhavsgrisen
Dedelev
Ulvshale
Krenkerup Haveskov
Frederikslund Dyrehave
Asserbo Slotsruin
Jægersborg Dyrehave
Nødebo
Ølsmosevej
Sæby
Strandvejen
Mørkøv
Kattrup

Fig. 2c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Ellenberg moisture values
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0,0
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Dedelev
Ulvshale
Krenkerup Haveskov
Frederikslund Dyrehave
Asserbo Slotsruin
Jægersborg Dyrehave
Nødebo
Ølsmosevej
Sæby
Strandvejen
Mørkøv
Kattrup

Fig. 2d

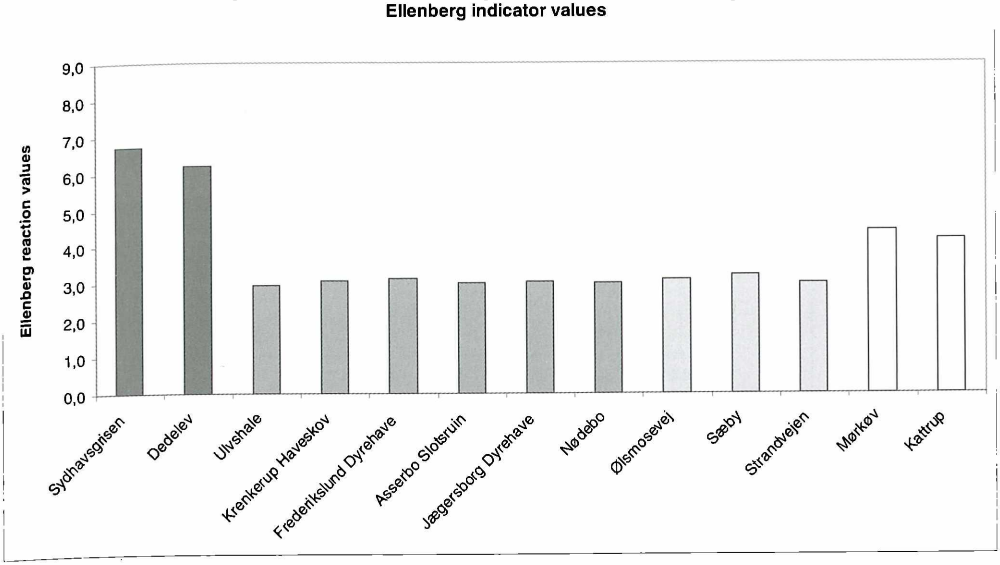
Ellenberg indicator values
Ellenberg reaction values
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Sydhavsgrisen
Dedelev
Ulvshale
Krenkerup Haveskov
Frederikslund Dyrehave
Asserbo Slotsruin
Jægersborg Dyrehave
Nødebo
Ølsmosevej
Sæby
Strandvejen
Mørkøv
Kattrup

Fig. 2e

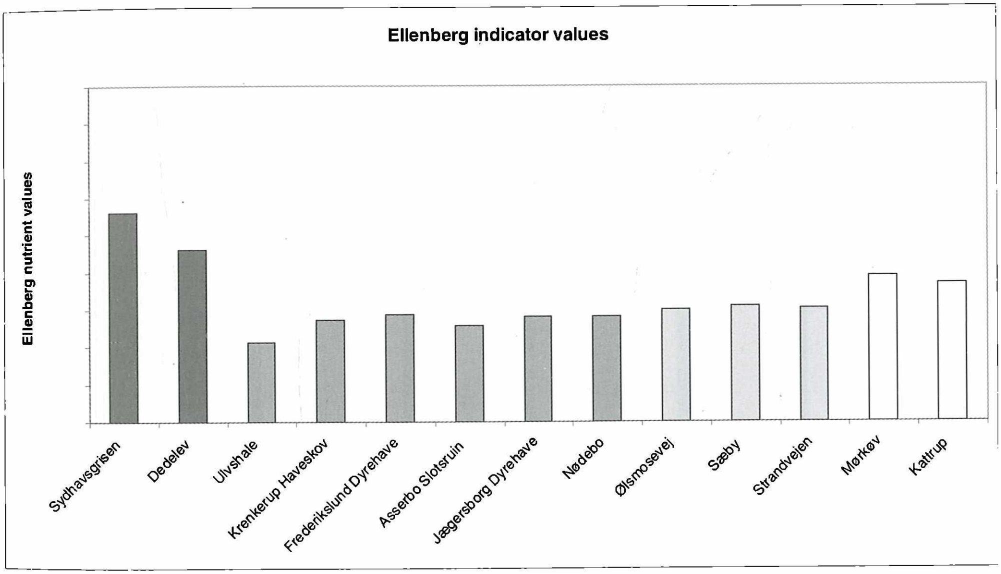
Ellenberg indicator values
Ellenberg nutrient values
Sydhavsgrisen
Dedelev
Ulvshale
Krenkerup Haveskov
Frederikslund Dyrehave
Asserbo Slotsruin
Jægersborg Dyrehave
Nødebo
Ølsmosevej
Sæby
Strandvejen
Mørkøv
Kattrup

Fig. 2f

Fig. 3

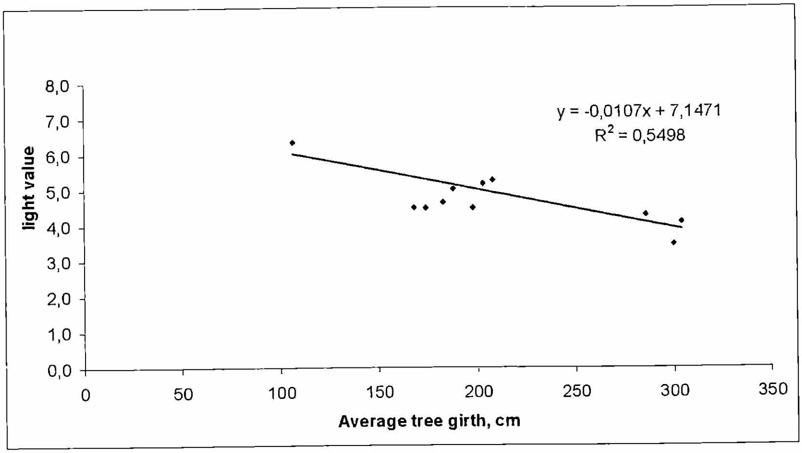

Fig. 3: Average *Quercus* tree girth as a function of calculated light values for epiphytic lichens at 13 locations in Denmark. Black line represents linear regression.

Fig. 4

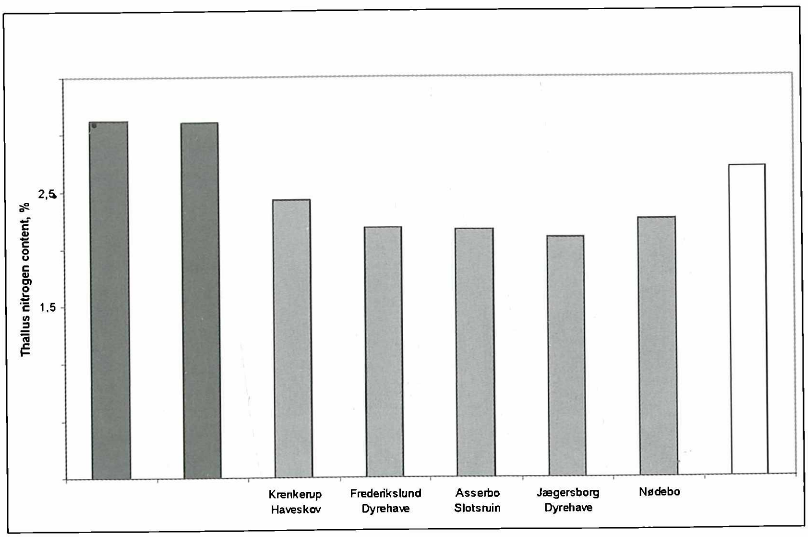

Fig. 4: Percentage of total nitrogen in *Xanthoria parietina* thalli collected on 7 locations in Denmark. Error bars indicate the 95% confidence intervals.

Fig. 5

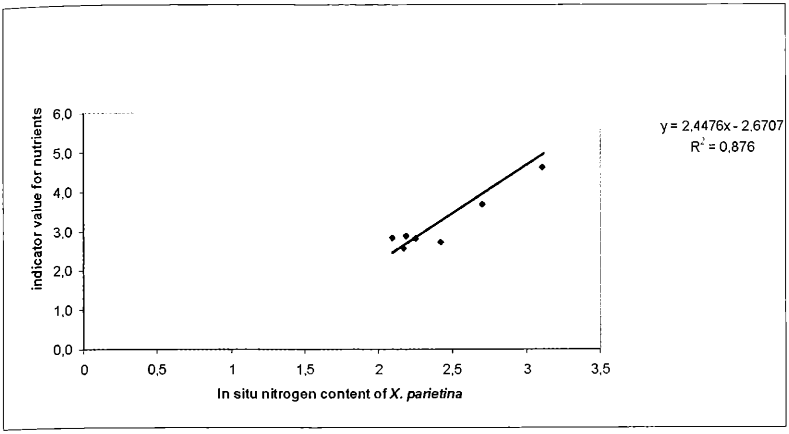

Fig. 5: Nutrient values calculated for the epiphytic lichen community growing on *Quercus* as a function of the percentage of total nitrogen in *Xanthoria parietina* thalli at 7 locations in Denmark. Black line represents linear regression.