Inland dunes in the Netherlands: soil, vegetation, nitrogen deposition and invasive species
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Invasiveness of *Campylopus introflexus* in drift sands depends on nitrogen deposition and soil organic matter

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**Abstract**

*Question:* Does the neophyte moss *Campylopus introflexus* invade more often in drift sand pioneer vegetations under high nitrogen (N) deposition? *Location:* Fourteen inland dune reserves in The Netherlands over a gradient of atmospheric N deposition. *Methods:* A transect study, dispersal experiment and culture experiment were carried out. In the transect study, the establishment of *C. introflexus* and lichens was measured in pure mats of *Polytrichum piliferum*, an early succession stage. The overall presence of *C. introflexus* in the area was also estimated. In the dispersal experiment, fragments of *C. introflexus* and lichen species were sown in *P. piliferum* mats at two sites with high and low N deposition. In the culture experiment *C. introflexus* fragments were grown on soil with different carbon (C) content and N dose. *Results:* The *Campylopus*:lichen ratio was positively correlated ($r^2 = 0.61$) with the atmospheric ammonia concentration. *Campylopus* began to dominate at an ammonia air concentration of 7 mg m$^{-3}$, correlated with the overall presence of the species in the sites investigated. Survival of sown *Campylopus* fragments was significantly higher and the endangered lichen *Cladonia strepsilis* significantly lower in the site with a high ammonia concentration. Survival of *Cladonia coccifera* and *Cladonia portentosa* was high at both sites. Experimental growing of *C. introflexus* showed significant responses to both C content and N, although C content showed the strongest effect. *Conclusions:* Growth of *C. introflexus* is affected by soil C content and N deposition. Inland dune reserves under high N deposition risk loss of lichen-dominated vegetations because of moss encroachment.


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**Introduction**

The neophyte moss *Campylopus introflexus* was first recorded in The Netherlands in 1961 and has expanded ever since (Fig. 6.1). The species originates from the Southern Hemisphere and is now common in most parts of Western Europe (Hassel et al. 2005 and references therein). Its strong expansion can be explained by the similar habitat and climate of its new environment to its original distribution area, and also by its strong dispersal capacity by means of spores, shoot-fragments, and larger fragments of the moss mat which can survive during dry periods (Equiha et al. 1993; Hasse 2007; van der Meulen et al. 1987). The species has a colonist life strategy, characterized by dispersal with both small sexual spores and asexual diaspores, and a rather long gametophyte lifespan (Siebel et al. 2006). The species grows on acid substrate in many different habitats, but seems to become dominant only in heaths, drift sands and superficially decalcified coastal dunes (van der Meulen et al. 1987). Previous studies hypothesized that the dominance of *C. introflexus* could be nitrogen-induced, the nitrogen being available either from the substrate or through increased atmospheric nitrogen deposition (Ketner-Oostra 1992; Ketner-Oostra et al. 2004a; Ketner-Oostra et al. 2008; Riksen et al. 2006). Many lichen species cannot compete with *C. introflexus* due to accompanying development of a strongly enhanced ectorganic layer and accelerated soil development, such that this species poses a serious threat to species characteristic of the early succession stages in the Spergulo-Corynephoretum and Violo-Corynophoretum communities (Jentsch et al. 2003; Ketner-Oostra et al. 2008). Lichens growing on acid, mineral soil rich in organic matter seem to be most vulnerable to such invasion of *C. introflexus*. Furthermore, in heathland-heaths, germination of Calluna vulgaris is inhibited when the soil is covered with *C. introflexus* (Equiha et al. 1993).

To test the hypothesis of the positive relation between nitrogen deposition and cover of *C. introflexus*, we conducted a transect study in sites along a nitrogen deposition gradient. In addition, two experiments were carried out. These consisted of artificial dispersal of moss and lichen fragments in the field to study the establishment of these

![Fig. 6.1. Maps showing the invasion of *Campylopus introflexus* in The Netherlands since the first record in 1961. Source: BLWG Bryophyte Database (www.verspreidingsatlas.nl), based on field records and herbarium specimens, generalized to 5 x 5 km grid cells. The species is absent or less abundant in the river and clay districts and most abundant in the Pleistocene cover sand and dune districts.](image-url)
species under different nitrogen levels, and of a growth experiment of \textit{C. introflexus} with different substrates in the University garden.

In the transect study, we measured establishment of \textit{C. introflexus} and lichens in mats of the bryophyte \textit{Polytrichum piliferum}. This is the first succession stage where drift sand becomes completely covered with vegetation, which typically consists of 0 to 10\% \textit{Corynephorus canescens} and 90 to 100\% \textit{P. piliferum} (Hasse 2005; Stoutjesdijk 1959). The space in between the moss shoots of \textit{P. piliferum} becomes filled with other species. Before \textit{C. introflexus} was introduced into the Netherlands, the moss mats were mostly colonized by lichens such as \textit{Cladonia} spp. and \textit{Cetraria aculeata}. At present, however, in many places moss mats seem colonized by \textit{C. introflexus}. The hypothesis is that the colonization of \textit{P. piliferum} mats by \textit{C. introflexus} instead of lichens is at least partly facilitated by high N deposition. Growth of \textit{C. introflexus} may increase with high N deposition. Growth of \textit{P. piliferum} mats is nitrogen limited as well, as nitrogen is taken up by an extensive, fast-growing network of below-ground moss rhizoids (Bowden 1991). \textit{Campylopus introflexus}, however, forms an ectorganic layer of dead moss, which may intercept and recycle nitrogen deposition before it reaches the soil and rhizoids of \textit{P. piliferum}. If stimulated by high N deposition, \textit{C. introflexus} may outcompete or overgrow \textit{P. piliferum} and lichen species. Also, lichens may perform less at high nitrogen deposition level. Hasse \textit{et al.} (2006) and Nijssen \textit{et al.} (2011) found that lichen cover of the reindeer lichen \textit{Cladonia portentosa} and thallus height decreased upon addition of ammonium nitrate.

In the dispersal experiment, \textit{Campylopus introflexus} and lichen fragments were sown into a pristine moss mat of \textit{Polytrichum piliferum} in two areas, one with high and one with low nitrogen deposition. If there is a positive relation between nitrogen and \textit{C. introflexus} cover, we would expect that \textit{C. introflexus} indeed invades \textit{P. piliferum} mats in the high deposition area, but not in the low deposition area. If lichens are indeed directly affected by high N deposition, establishment of lichen species would be high in the low N deposition area, but low in the high N deposition area.

Field studies (Daniëls \textit{et al.} 1996; Daniëls \textit{et al.} 2008b; Hasse 2007; Ketner-Oostra \textit{et al.} 2004b) suggested that, apart from nitrogen deposition, soil organic matter may also be important with respect to the increase of \textit{C. introflexus}. Hasse (2007), Daniëls \textit{et al.} (2008a, b) and Ketner-Oostra \textit{et al.} (2004b, 2006) have shown that disturbance (increase in game density, wildfire) of the vegetation cover in acidic cryptogam-rich grasslands, resulting in exposed organic substrate, temporarily induces the dominance of the species. In order to test whether N deposition increased growth of \textit{C. introflexus}, and to differentiate between the two factors, \textit{C. introflexus} was grown in a culture experiment under different nitrogen depositions and in soils with different organic matter content.
Materials and methods

Transect study

In the transect study, the occurrence of *Campylopus introflexus* in pristine *Polytrichum piliferum* mats was established in 14 drift sand reserves (Table 6.1), located over a gradient from low to high nitrogen deposition. Pristine *P. piliferum* mats typically occur in recently colonized sand dunes, and consist of 90-100% *P. piliferum* and 0-10% of the grass *Corynephorus canescens*. This succession stage is characterized by an AC soil profile, and an Aₖ horizon of 2 cm at most. Soil carbon content is low, approximately 0.1% in the upper 5 cm, and pH (H₂O) is about 4.8 (Nijssen *et al.* 2011). The number of 14 drift sand reserves was the maximum number of sites available, as both the studied succession stage and large drift sand sites are rare in the country.

At each site, the presence of *Campylopus*-dominated vegetations was classified in three categories: low (absent or presence distinctly related to e.g. logging sites), local (small moss mats present within otherwise lichen-dominated vegetations), and high (several hectares present).

<table>
<thead>
<tr>
<th>Site name</th>
<th>Ammonia air concentration</th>
<th>Modeled N deposition</th>
<th>Co-ordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg NH₃ m⁻³</td>
<td>kg ha⁻¹ yr⁻¹</td>
<td>Decimal degrees (WGS 84)</td>
</tr>
<tr>
<td>Aekingerzand ¹</td>
<td>5.8</td>
<td>28.9</td>
<td>52.924° N, 6.294° E</td>
</tr>
<tr>
<td>Beekhuizerzand</td>
<td>7.0</td>
<td>32.5</td>
<td>52.334° N, 5.665° E</td>
</tr>
<tr>
<td>Bergerheide ¹</td>
<td>10.0</td>
<td>39.2</td>
<td>51.595° N, 6.079° E</td>
</tr>
<tr>
<td>Drouwenerzand ¹</td>
<td>6.6</td>
<td>25.3</td>
<td>52.955° N, 6.800° E</td>
</tr>
<tr>
<td>Hulshorsterzand</td>
<td>7.0</td>
<td>32.2</td>
<td>52.334° N, 5.724° E</td>
</tr>
<tr>
<td>Kootwijkerzand ¹</td>
<td>7.5</td>
<td>34.3</td>
<td>52.163° N, 5.767° E</td>
</tr>
<tr>
<td>Lange Duinen Soest</td>
<td>6.0</td>
<td>17.4</td>
<td>52.146° N, 5.284° E</td>
</tr>
<tr>
<td>Lemelerberg ¹</td>
<td>7.6</td>
<td>36.7</td>
<td>52.447° N, 6.387° E</td>
</tr>
<tr>
<td>Loonse en Drunense Duinen</td>
<td>7.5</td>
<td>42.7</td>
<td>51.642° N, 5.112° E</td>
</tr>
<tr>
<td>Maashorst</td>
<td>12.0</td>
<td>50.4</td>
<td>51.687° N, 5.604° E</td>
</tr>
<tr>
<td>Otterlosche Zand</td>
<td>7.0</td>
<td>35.2</td>
<td>52.109° N, 5.795° E</td>
</tr>
<tr>
<td>Strabrechtse Heide</td>
<td>12.0</td>
<td>49.0</td>
<td>51.408° N, 5.617° E</td>
</tr>
<tr>
<td>Weerterbergen</td>
<td>7.0</td>
<td>32.5</td>
<td>51.256° N, 5.616° E</td>
</tr>
<tr>
<td>Wekeromse Zand ¹</td>
<td>9.7</td>
<td>50.3</td>
<td>52.100° N, 5.679° E</td>
</tr>
</tbody>
</table>

Table 6.1. The 14 sites were field data were collected for the transect study in *Polytrichum piliferum* mats. Drouwen and Kootwijk are the sites where experimental dispersal of lichen and bryophyte fragments took place. ¹locations were ammonia air concentrations were measured.
In each of the 14 drift sand areas, one or two transects of 100 m length were randomly selected (n = 21). Possible source populations of both lichens and \textit{C. introflexus} were present in all sites within a distance of 30 to 50 m. Transects were subdivided into 1 m segments of 0.2 m width (Fig. 6.2). For each 1 m segment, the presence of \textit{C. introflexus} and/or lichens (\textit{Cladonia} spp. or \textit{Cetraria aculeata}) was noted if their cover exceeded 1 cm$^2$. Transects were used only if a minimum of 20% and a maximum of 80% of the segments contained lichens and/or \textit{Campylopus}. The \textit{Campylopus}:lichen ratio was calculated by dividing the number of segments per transect containing \textit{Campylopus} by the number of segments where lichens were present.

In eight of the 14 sites, atmospheric ammonia concentration values were measured at monthly intervals from May 2008 until April 2009, employing passive samplers (model DIF400RTU obtained from and analyzed by Gradko Ltd., Winchester, U.K.) (Sutton \textit{et al.} 2001). The atmospheric ammonia concentration is the average of all measurements. To estimate the ammonia air concentration for other sites, modeled data from measurements dating from 2000-2001 are used (Velders \textit{et al.} 2002), which are currently the best available data and agree with our own ammonia measurements. The sites where ammonia measurements were carried out and for which sites modeled
data was used is shown in Table 1. The nitrogen deposition (wet + dry) was obtained from modeled data using the OPS model (van Jaarsveld 2004) and emission data of the year 2006.

Experimental dispersal

In the field experiment, experimental dispersal of *Campylopus introflexus* and lichens was carried out in pristine *Polytrichum piliferum* mats in the Wekeromse Zand (an area with high nitrogen deposition: 50.3 kg ha\(^{-1}\) yr\(^{-1}\), and 9.7 µg NH\(_3\) m\(^{-3}\)) and Drouwenzerzand (an area with rather low nitrogen deposition: 25.3 kg ha\(^{-1}\) yr\(^{-1}\), and 6.6 µg NH\(_3\) m\(^{-3}\)). The original vegetation and soil are similar. Vegetation consists of 90% *P. piliferum* moss mats with 1 or 2 tufts of *Corynephorus canescens* per m\(^2\). The soil consists of at least 0.3 m drift sand, and has A\(_h\) of 2 cm, mainly consisting of dense *P. piliferum* rhizomes. The following species were sown: the invasive moss species *C. introflexus*, and the characteristic lichen species *Stereocaulon condensatum* (a species of an earlier succession stage), *Cladonia coccifera* (both typical colonizers of moss mats), *Cladonia strepsilis* (a small species of older succession stages) and *Cladonia portentosa* (a large species of older succession stages). For each species, including a control, four replicate plots of 50 x 50 cm were used. On average, 35 fragments of 2 to 3 mm diameter were sown. The number of sown fragments was counted at the start (3 April 2008) and end (30 March 2009) of the experiment. The control plots were used to measure spontaneous establishment of these species. Lichens and bryophytes had been collected from the same site and only some hours before the start of the experiment. Species were fragmented by gentle crushing or with the use of scissors.

Culture experiment

In the culture experiment, *Campylopus introflexus* was grown with different nitrogen supply and different soil carbon content in the Amsterdam University Garden. The sand was collected from the Kootwijkerzand drift sand reserve, which contained 0.1% carbon. To obtain a soil gradient from low (0.1%), intermediate (0.75%) to high (1.5%) carbon content, the Kootwijk soil was mixed with strongly organic topsoil material collected in the Aekingerzand, an area with low nitrogen deposition. The upper green part of freshly collected *C. introflexus* plants was fragmented with scissors and knife into < 1 mm pieces and sown by hand with a density of approximately five fragments per cm\(^2\) in well-drained pots of about 8 cm diameter. Pots were set up in the open air in the period from 19 June 2008 to 23 April 2009. To obtain a gradient of low- intermediate-high N deposition, 0, 5 and 10 ml of a 25.1 mM ammonium nitrate solution (0, 5 or 10 µmol N cm\(^{-2}\)) was added to the pots bimonthly, which is the equivalent of 0, 42 and 84 kg N ha\(^{-1}\) yr\(^{-1}\). Because the background deposition in Amsterdam is 23 kg N ha\(^{-1}\) yr\(^{-1}\), the actual range was 23, 65, and 107 kg ha\(^{-1}\) yr\(^{-1}\). Each treatment, that is any of the nine combinations of N dose and organic matter content, had six replicates. Controls with non-sown pots did not show any moss cover (Fig. 3). After one year some of the pots were just fully covered in moss, and moss cover was assumed to be a good estimate for actual biomass. Cover of *C. introflexus* was visually
estimated. For an additional quantitative digital estimate, all pots were photographed under equal light conditions, and relative cover was estimated by the green value of the digital images (the colour intensity ranging from 0 to 255), minus the green value of bare sand. The outcome was similar compared to the visual estimate.

**Statistical analysis**

All statistical tests were performed in R. Boxplots show median, upper and lower quartiles, minimum and maximum values. In the field survey the relation between ammonia air concentrations and the *Campylopus*:lichen ratio was calculated with linear regression. Differences in dominance of *C. introflexus* at site level were tested with a t-test. In the dispersal experiment, differences in fragment survival between sites were tested using a t-test. Differences in cover of *C. introflexus* in the culture experiment were tested with two-way ANOVA, with nitrogen deposition and soil organic matter as independent variables. Differences between individual mean values were tested with least square means tests.

![Fig. 6.4. Scatterplot (n = 21) showing the relation between the ammonia air concentration and the *Campylopus*:lichens ratio in 100 m transects.](image-url)
Results

Transect study

The *Campylopus*-lichen ratio increased with the atmospheric ammonia concentration ($r^2 = 0.61$, $P < 0.001$; Fig. 6.4). *Campylopus introflexus* showed a positive ($r^2 = 0.35$; $P < 0.001$) and lichen presence a negative ($r^2 = 0.21$; $P < 0.001$) correlation with ammonia (both with $n = 21$). Due to a limited availability of sites, no data points around 9 or 11 µg NH$_3$ m$^-3$ were available. If the two upper right data points in Fig. 6.4 are omitted as outliers, the fit is still good with $r^2 = 0.54$ ($P < 0.001$). At levels of around 7 µg NH$_3$ m$^-3$, lichens and *C. introflexus* were present at equal amounts (a ratio of 1).

The estimate of the presence of *Campylopus*-dominated vegetation at site level also showed a relation with the atmospheric ammonia concentration, with the classes for local and abundant dominance starting around 7 µg NH$_3$ m$^-3$, and no dominance of *C. introflexus* in sites with lower concentrations (Fig. 6.5).

![Fig. 6.5. Boxplot showing the ammonia air concentration for inland dune sites classified by the presence of large-scale *Campylopus*-dominated vegetations ($n = 4$ to 7 for each category). All groups differ significantly from each other ($P < 0.05$).](image)

Experimental dispersal of *Campylopus* and lichens

Lichen fragments sown in *Polytrichum piliferum* mats showed different rates of survival per species (Table 6.2). Survival of *Campylopus introflexus* was significantly higher in the high N deposition site, which is in accord with the results from the transect study. *Cladonia strepsilis* showed a significant lower survival rate at the high N deposition site. Low to medium survival at both sites was observed for the other species: survival of
common species *Cladonia portentosa* and *Cladonia coccifera* was high, whereas survival of *Stereocaulon condensatum* was low.

**Table 6.2.** Survival as the percentage of the original number of fragments sown in pristine moss mats of *Polytrichum piliferum* after one year. Survival percentages are shown as mean values with standard error in parentheses. Differences between sites are shown as level of significance (n.s. = not significant)

<table>
<thead>
<tr>
<th>Species</th>
<th>Fragment survival (%) per site</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wekerom (high N deposition site)</td>
<td>Drouwen (low N deposition site)</td>
</tr>
<tr>
<td>Campylopus introflexus</td>
<td>25 (12)</td>
<td>0 (1)</td>
</tr>
<tr>
<td>Cladonia coccifera</td>
<td>50 (20)</td>
<td>57 (36)</td>
</tr>
<tr>
<td>Cladonia portentosa</td>
<td>58 (23)</td>
<td>45 (3)</td>
</tr>
<tr>
<td>Cladonia strepsilis</td>
<td>10 (3)</td>
<td>32 (4)</td>
</tr>
<tr>
<td>Stereocaulon condensatum</td>
<td>6 (2)</td>
<td>5 (6)</td>
</tr>
</tbody>
</table>

**Experimental growing of *Campylopus introflexus***

After one year, moss cover showed a significant increase in relation to soil carbon content (Fig. 6.6; Table 6.3). Cover values were significantly higher at intermediate N deposition, especially at high soil organic matter content. However, the effect of nitrogen was not significant as moss cover was lower at a higher N dose. An ANOVA
model without the highest N dose resulted in a significant effect of both N and soil carbon content (Table 6.3). In both models, the combined effect of organic matter and nitrogen was not significant.

Table 6.3. Results of a two-way ANOVA of the factors nitrogen addition and carbon (organic matter) content on the growth response of *Campylopus introflexus*. Two ANOVA models were used: with all N treatments, and excluding the highest N dose. DF: degrees of freedom, P: *P* < 0.05, **P** < 0.001, n.s. not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DF</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Including all N treatments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nitrogen</td>
<td>2</td>
<td>2.22</td>
<td>n.s.</td>
</tr>
<tr>
<td>carbon content</td>
<td>2</td>
<td>19.36</td>
<td><strong>P</strong>&lt; 0.001</td>
</tr>
<tr>
<td>nitrogen x carbon content</td>
<td>4</td>
<td>1.44</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excluding the highest N dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nitrogen</td>
<td>1</td>
<td>5.31</td>
<td>*</td>
</tr>
<tr>
<td>carbon content</td>
<td>2</td>
<td>17.41</td>
<td><strong>P</strong>&lt; 0.001</td>
</tr>
<tr>
<td>nitrogen x carbon content</td>
<td>2</td>
<td>3.27</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

*Campylopus introflexus* dominance and nitrogen deposition

As hypothesized by Riksen *et al.* (2006) and Daniëls *et al.* (2008b), dominance of *Campylopus introflexus* occurs primarily in sites with high N deposition. The results of this study show that the colonization of *C. introflexus*, compared to lichens, is positively correlated with nitrogen input over the long term resulting in dominance of this species at site level, at the cost of lichen-cover. Survival of sown fragments of *C. introflexus* is also higher in sites with elevated N levels.

The effect of nitrogen addition was less visible in the growing experiment, where soil organic matter was found to be the dominant factor. The interaction of organic matter and nitrogen was not significant, although some effect of the enhanced retention of especially ammonium in organic matter was expected.

The highest nitrogen dose (107 kg ha⁻¹ yr⁻¹) resulted in a lower moss cover. This is possibly caused by a toxic effect of the nitrogen dose of a single treatment (10 µmol cm⁻²) or exposure to the highest NH₄⁺ concentration (25 mM). A similar die-off of mosses and other cryptogams in fertilization experiments has been observed by Hasse et al. (2006), although the experiment was carried out with a higher NH₄⁺ concentration (100 mM) and a N dose of 20 µmol cm⁻² per treatment. Soudzilovskaia *et al.* (2005) found die-off of lichens using urea as a fertilizer at a dose of 30 µmol
cm². Future fertilization experiments in cryptogam vegetations using ammonium compounds should therefore be carried out with multiple single doses of up to c. 5 µmol N cm⁻² in order to avoid an adverse effect of ammonium or nitrate toxicity.

Decrease of lichens under high N deposition

The older pioneer vegetation in some inland dune reserves in The Netherlands is already completely dominated by mats of *C. introflexus*. This is the case in Bergen (Province of Limburg) and Slabroek (Province of Noord-Brabant). Although lichen species have not yet become locally extinct at these sites, the population size of especially smaller lichen species (cup-shaped and squamulose lichens), such as *Cladonia cervicornis* s.l., *Cladonia monomorpha*, and *Cladonia strepsilis* has strongly declined and these species are on the verge of extinction. Most lichen species in inland dunes disperse using fragments of 0.1 to 5.0 mm in size, although some species have fine soredia (Hasse 2005). Once species are locally extinct, the recolonization of a site may therefore be a long-term process and depend on vectors such as deer and sheep. Most sensitive lichens are confined to surfaces with exposed mineral soil with an organic matter content of 0.5-1.0 % (Nijssen *et al.* 2010). Lichen species which are able to grow on the litter layer of older *C. introflexus* mats can survive in inland dunes, e.g. *Cladonia macilenta* and *Cladonia floerkeana* (Ketner-Oostra *et al.* 2008) and the larger reindeer lichens and *Cladonia gracilis* and *Cladonia uncialis* (Daniëls *et al.* 2008b; Hasse 2007). However, those species are usually generalists, not typically confined to this habitat.

The dispersal experiment also demonstrated that lichen species are more of less confined to a certain succession stage, independent of their dispersal strategy. Both *Cladonia coccifera* and *Cladonia portentosa* had a high survival rate at both sites. *Cladonia coccifera* normally invades fresh *Polytrichum* mats and it is dispersed both as fungal ascospores and vegetative diaspores (fine powder-like soredia). *Cladonia portentosa*, however, mostly grows in late succession stages in short-grass dominated vegetations. Therefore, the high survival of fragments was surprising. This survival rate was similar to studies where fragments were sown on mineral soil in a clear-cut boreal forest (Christensen 1988; Roturier *et al.* 2007). A possible explanation for the high survival of fragments for a species normally absent in this early succession stage is its main dispersal mode, namely by means of rather large fragments (the largest of all drift sand inhabiting lichens) which do not easily spread, even over small distances. The absence of a relation between occurrence of *C. portentosa* and nitrogen deposition was also found in earlier studies (Hyvärinen *et al.* 1998). Nitrogen deposition, however, can lead to smaller individuals and lower cover values (Nijssen *et al.* 2010). In the present study, lichen cover was clearly reduced in areas with high N deposition. Also, in an N fertilization experiment in drift sands with low and high N deposition, lichen cover and height of *C. portentosa* were negatively affected by excess N (Nijssen *et al.* 2011). A lower survival of lichen fragments in the high N deposition site was found for *Cladonia strepsilis*, a species that is known to occur mainly in relic populations and is rarely discovered at new locations, which might indicate a dispersal-survival
bottleneck. The dispersal experiment showed that survival in the high-deposition site was significantly lower than in the low-deposition site. It requires further study to test whether *C. strepsilis* is a true nitrogen-sensitive species.

**Soil organic matter**

Apart from nitrogen, soil organic matter content played an important role in the establishment of *C. introflexus*. It was the most important factor in the experimental growing of *C. introflexus*. This result is in accord with field observations of its preferential niche. Increased organic matter content leads to a more favorable growing condition for the species, such as higher nitrogen mineralization and water retention capacity, extending the growing time. The acidity of the substrate is also higher. *Campylopus introflexus* is known to favor low pH soils and the pH (H$_2$O) of bare sand in inland dunes is generally around 5.0, whereas the soil pH in *Campylopus*-dominated vegetation is around 4.5 (Nijssen et al. 2011). Soil inoculation can be a third factor, adding both micro-organisms and nutrients that may not occur in the soil with 0.1 % carbon content. Larger organic particles may also function as a place where moss fragments initially find a holdfast.

Although soil organic matter content is rather constant in pristine *Polytrichum piliferum* mats, soil rich in organic matter may become exposed due to disturbance caused by recreation or grazing. Such patches of exposed mineral soil provide an

**Fig. 6.7.** Vertical section through a young moss mat of *Campylopus introflexus* on sandy soil showing A. *Campylopus introflexus*, B. the lichen *Cladonia coccifera*, C. the bryophyte *Polytrichum piliferum*. 
excellent opportunity for invasion by *Campylopus introflexus* and mats can eventually lose their original lichen-rich vegetation cover. Management to improve the vitality of *Corynophorus canescens* in pioneer grasslands in sites with high N deposition (Tschöpe *et al.* 2010) could therefore result in loss of species diversity.

**Competition between lichens and Campylopus**

A hypothesized invasion strategy of *Campylopus introflexus* (Fig. 6.7) suggests that as *C. introflexus* cover increases, lichens and the old moss mat of *Polytrichum piliferum* are both enclosed and strangled. Remnants of lichens can be found by breaking up those *C. introflexus* moss mats. The enclosed species may act as a temporary nutrient source for a growing moss mat. As soon as the enclosed plants are mineralized, the moss mat depends on nutrients dissolved in rain water and dry deposition.

**Critical load**

The equilibrium point above which *C. introflexus* starts dominating was found to be about 7 µg NH₃ m⁻³, equivalent to modeled deposition values of about 30 kg N ha⁻¹ yr⁻¹. This value is much higher than the 10 kg ha⁻¹ yr⁻¹ calculated in a study to estimated critical loads for Natura 2000 habitat types (van Dobben *et al.* 2006). There is evidence that N-induced accelerated succession is already taking place at lower deposition rates, as the vegetation is N limited in especially early succession stages (Nijssen *et al.* 2011). The value of 30 kg ha⁻¹ yr⁻¹ can been regarded as a as level above which inland dunes vegetations are seriously threatened, as the typical lichen-rich vegetations do not develop anymore. Even with decreasing N deposition levels, the future prospects for *C. introflexus*-dominated inland dune vegetations are poor, as the ectorganic layer contributes to the soil formation and therefore accelerates succession and N availability. Such a case was described by Daniëls *et al.* (2008b), where soil disturbance in vegetation with small cup-lichens resulted in temporary *Campylopus*-dominance. The thick moss mat eventually changed back into a reindeer lichen and grass-dominated vegetation.

**Conclusions**

The results from the field survey demonstrate that nitrogen deposition contributes to the growth of the moss *Campylopus introflexus* and inhibits lichen species. This is supported by experimental dispersal of moss and lichens fragments, where *C. introflexus* showed a higher survival rate in the high nitrogen deposition site. The lichen *Cladonia strepsilis* showed an opposite effect and other lichens species had a low to medium survival rate. The high survival rate of *C. portentosa* might be explained by the distribution of this species, which is strongly dispersal-limited.

The results from experimental growing of *C. introflexus* further support the previous findings. However, the highest nitrogen dose led to a decrease in moss cover, probably due to toxic effects of the ammonium nitrate solution. Apart from nitrogen deposition, soil carbon content was found to be an important factor in the
establishment of moss on bare sand. Evidently this may be related to effects of organic matter content on nitrogen mineralization, water retention capacity, pH, and soil microbial inoculation. Two additional proxies demonstrate that the settlement of *C. introflexus* in a young succession stage has an effect on the species diversity in later stages.

The outcome of this study is relevant for the conservation of inland dune reserves. The positive relation between *C. introflexus* and nitrogen deposition means that for the conservation of lichen dominated vegetations, the atmospheric ammonia concentration in such sites needs to be lowered to a level of 7 µg m\(^{-3}\) or lower, which is the point where equal amounts of lichens and *C. introflexus* are present (Fig. 4). The observed relation between moss growth and organic matter content implies that for successful restoration of inland dune habitats, topsoil must be completely removed in order to prevent *C. introflexus* starting to grow directly on bare sand with high carbon content. Disturbance of the soil and vegetation may also lead to an invasion of *C. introflexus* when mineral soil becomes exposed. Although inland dunes may seem to be a dynamic habitat, protection against disturbance of older pioneer vegetation may be advisable to protect species-rich lichen vegetations from being invaded by *C. introflexus*.

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