Inland dunes in the Netherlands: soil, vegetation, nitrogen deposition and invasive species

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Chapter 4

Nitrogen deposition and soil carbon content affect nitrogen mineralization during primary succession in acid inland drift sand vegetation

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Abstract

Background and Aims: Two inland dunes in the Netherlands receiving low (24) and high (41 kg N ha\(^{-1}\) yr\(^{-1}\)) nitrogen (N) deposition were compared for N dynamics and microbial activity to investigate the potential effect of N on succession rate and loss of pioneer habitats. Methods: Primary succession stages were sampled, including sand, *Polytrichum piliferum*, *Campylopus introflexus*, lichens, and grasses. This series was selected in both pristine drift sands and sites where vegetation had been removed as a restoration measure and succession started on organic matter-rich soil. Microbial characteristics and potential N mineralization were analysed in a laboratory experiment. Results: In drift sand sites, organic matter accumulated during succession, resulting in a lower pH and in higher microbial biomass, respiration and net N mineralization. The observed effects of N deposition were (1) decrease of microbial biomass, (2) higher net N mineralization per m\(^2\), (3) higher levels of free nitrogen in the soil, and (4) a higher microbial N:P ratio. In early succession stages in restored sites, organic matter contributed more to N cycling than N deposition. Conclusions: Both high levels of organic matter and elevated N deposition lead to higher N availability which may cause an increased succession rate.
Introduction

Vegetation growth in semi-arid acid sand ecosystems is, like in most pioneer environments, nitrogen limited (van Mierlo et al. 2000). During succession, N mineralization may become higher due to the increase of soil organic matter (Gerlach et al. 1994). The increase of N availability is regarded as one of the most important driving factors controlling species composition during primary succession (Crocker & Major 1955; Berendse 1990; Schulze et al. 1995; Berendse 1998). Apart from an increase of the N mineralization due to an increase in soil organic content during succession, regionally higher levels of N deposition may cause higher N availability, resulting in a higher rate of succession rate (Bobbink et al. 1998). The inland dune environment does depend much on atmospheric deposition of N as nitrogen fixation is very low or absent due the calcium-poor substrate (Nijssen et al. 2011).

In acid inland dunes, primary succession starts with bare sand, which may contain scattered Corynephorus canescens plants. In the second stage, when wind velocity has slowed down and sand has stabilized, the space between the grass tussocks is filled with Polytrichum piliferum moss mats, consisting of sparse shoots in bare sand connected with a dense rhizoid layer belowground (Bowden 1991). Lichen species start to fill up the space between the Polytrichum shoots in the subsequent stage. However, at present, P. piliferum mats are often colonized by the invasive bryophyte species Campylopus introflexus, especially in sites with high N deposition (Hassel & Söderström 2005; Sparrius & Kooijman 2011). In the last stage grasses gradually become dominant and finally Calluna vulgaris establishes (Hasse 2005). During succession on bare soil, initially a thin Ah is formed by Polytrichum rhizoids. In later stages, the topsoil is enlarged by roots of tufted and stoloniferous grasses and may become up to 12 cm thick before Calluna settles (Sparrius 2011).

The first research topic is how N mineralization and nitrification in the mineral topsoil and the ectorganic layer change during succession in drift sands. In most early succession stages, the mineral topsoil is the most important soil compartment. In Campylopus introflexus dominated vegetations, however, but also in lichens, an ectorganic layer is present, formed by the basal part of the plant. As bryophytes and lichens have no root system, the ectorganic layer could provide a substrate for mineralization and interception of atmospheric deposition, which is readily available for the upper living part of the plant (Cornelissen et al. 2001; Crittenden 1991; Sipman 1978).

The second topic is the relation between atmospheric N deposition and N dynamics. High N deposition may result in higher N availability to the vegetation directly, but also indirectly, via higher N mineralization, which can be caused by presence of organic matter with a low C:N ratio (Swift et al. 1979) or direct uptake of N by micro-organisms. This hypothesis is tested by comparing similar succession stages in inland dunes with rather low and high N deposition.
The third topic is the relation between N availability and restoration management. To preserve the inland dune habitat, forest is logged and topsoil removed to create new open sandy surfaces (Riksen et al. 2006). However, the topsoil is often not fully removed. In such cases, secondary succession occurs on topsoil high in organic matter. Such initial presence of organic matter may increase N mineralization in especially early succession stages. This could make the effect of the restoration measure less effective, as the succession rate is speeded up (Riksen & Goossens 2005). Also, high N deposition may increase N availability even more in areas with remnant stocks of soil organic matter. In both study sites, two succession seres are compared: the first starting on bare sand low in organic matter, the second on soil high in organic matter.

Materials and Methods

Study sites

Succession gradients were compared in two inland dune reserves: Aekinge (52.924° N, 6.294° E), a site with relatively low N deposition, located in the North of The Netherlands, and Wekerom (52.100° N, 5.679° E), a site with high N deposition 150 kilometres South, in the central part of the country. Both areas are similar in climate and size and contain active drift sands, but also restored drift sands where trees and undergrowth vegetation were removed.

The sites mainly differ in the amount of atmospheric nitrogen deposition, predominantly ammonia from agricultural sources. Nitrogen deposition has been measured from June 2008 till May 2009, by sampling with monthly replaced ammonia diffusion tubes (Sparrius 2011). Total deposition can be calculated from the ammonia air concentration with a formula developed by Cape et al. (2009) assuming a low particle deposition velocity of 15 mm s⁻¹. This resulted in an average deposition of 24 kg N ha⁻¹ yr⁻¹ in Aekinge (5.8 µg NH₃ m⁻³) and 41 kg N ha⁻¹ yr⁻¹ in Wekerom (9.7 µg NH₃ m⁻³), which agrees with modelled deposition values (Eerens & van Dam 2001; van Jaarsveld 2004; de Haan et al. 2008). Precipitation in both sites is around 800 mm yr⁻¹ (Sluijter and Nellestijn 2009).

In both areas, a succession sere on formerly active drift sand dunes was sampled. We selected 1 x 1 m plots over a gradient of succession stages varying from 0 to c. 20 years in age since vegetation development started (Sparrius 2011): (1) bare sand, (2) mats of Polytrichum piliferum, (3) mats of Campylopus introflexus, (4) pioneer vegetation dominated by lichens (Cladonia spp.) and (5) pioneer vegetation dominated by grasses, which are described in Table 1. The classification of succession stages is adapted from Hasse (2005). For each succession stage in a particular area, four replicate plots were selected according to a stratified random procedure. All plots were similar with respect to slope and orientation. Within a particular succession stage, soil depth was similar between replicates. Nomenclature or species follow Aptroot & van Herk (2004) for lichens, Siebel & During (2006) for bryophytes and van der Meijden (2005) for vascular plants.
Table 4.1. Description of the five succession stages analyzed in this study. The description is valid for both study areas, and for succession series in both drift sand and restored sites.

| Succession stage | Description                                                                 | $A_h$ (cm) |
|------------------|-----------------------------------------------------------------------------|------------|
| Bare sand        | Bare sand, occasionally with a c. 0.1 mm thick algal crust, between scattered plants of Corynephorus canescens | 0          |
| Polytrichum      | 90-100% Polytrichum piliferum mats with scarce Corynephorus canescens tufts | 1-3        |
| Campylopus       | 80-100% Campylopus introflexus mats, accompanied with Corynephorus canescens, Polytrichum piliferum | 2-4        |
| Lichens          | Dominant small Cladonia spp. (e.g. C. glauca, C. macilenta, C. ramulosa, C. strepsilis) on Polytrichum piliferum mats | 4-8        |
| Grasses          | 80-90% Agrostis vinealis and Festuca filiformis accompanied with larger Cladonia species | 6-12       |

In both study areas, also restored drift sand sites were sampled, where Scots pine stands and undergrowth vegetation have been removed in the early 1990s. In Aekinge, after forest removal, the soil consisted of a rather thick, 4-5 cm A horizon containing 2.0% carbon, and covered with a 2-3 mm thick F layer of poorly fermented litter of bark flakes and branches left after logging. The pioneer stage with Polytrichum piliferum was not present in restored sites, probably due to the high soil organic matter content. In Wekerom, after forest removal, the soil consisted of a 2-3 cm thick Ah horizon containing 0.4% carbon without a litter layer. These differences in soil organic matter are far from ideal, especially because they potentially counteract or mask the effects of high atmospheric N deposition. Nevertheless, we included them in the sampling scheme, because, over the gradient in N deposition, sites with more similar soil conditions were absent.

**Sampling**

Fresh, slightly humid samples were collected in the last week of October 2007 during dry weather and maximum temperatures around 10 °C. The upper 5 cm of the mineral topsoil was collected by combining three subsamples taken with metal cylinders of 100 cm³ and stored for four to six days at 4 °C before further analysis.

In case of the Campylopus and lichen-dominated stages, in each plot the ectorganic layer was also sampled. This was done by collecting the moss or lichen mats in 25 x 25 cm squares and removing the living parts from the basal ectorganic layer in the laboratory immediately after collection. In the case of lichens, dry weight of the ectorganic layer was determined for all four replicates separately, but because little material was available, this was combined into one sample in the incubation experiment.

**Laboratory analysis**

Fresh samples were gently homogenized and weighed. A subsample was dried at 60 °C for 48 hours to determine moisture content. Total C and N were measured in a Fisher EA1110 CNS analyzer (Westerman 1990). Nitrate, ammonium and phosphate were
measured in extracts of fresh soil in 50 ml of 0.5 M K₂SO₄ solution (1:2.5), using an Auto Analyzer III (Bran + Luebbe). In these extracts, element concentrations (Ca, Fe, Na, Mg, and Al) were measured with an inductively coupled plasma analyzer (ICP-OES, Fisher Iris Intrepid II).

To estimate microbial biomass and element content in fresh samples, the chloroform fumigation and extraction method was used (Brookes et al. 1985; Brookes et al. 1982). Immediately after 24 h of fumigation with chloroform, element concentrations of water-soluble substances were analyzed as fresh samples. The concentration of elements in the microbial biomass was calculated by subtracting the concentrations of the non-fumigated samples from the fumigated samples. A few obvious outliers were omitted for N:P and C:N ratios at very low P (three times) or N (one time) values respectively.

To estimate potential nitrogen mineralization and nitrification, c. 50 g of mineral topsoil or organic layer were kept for 40 days in petri dishes in the dark at room temperature. The moisture content of the samples was retained at 20-30%, checked weekly and replenished with demineralized water when necessary. Ammonium and nitrate concentrations were measured in fresh and incubated samples by K₂SO₄ extraction as described for fresh samples. Net N mineralization and nitrification were calculated, based on differences between incubated and fresh samples. N mineralization per unit microbial C was calculated using microbial C values at the start of the incubation experiment.

To estimate microbial activity, CO₂ production of both fresh and incubated samples was measured at room temperature at 30 min. intervals using gas chromatography (Kooijman et al. 2008; Tietema 1992). Mean values of respiration before and after incubation were used as an estimate for microbial activity.

Statistical analysis

Values in mg kg⁻¹ were transformed into stock values per m² to account for decrease in bulk density during succession. N:P and C:N ratios were calculated in g g⁻¹, NH₄⁺:NO₃⁻ ratios are molar ratios. Because of the large differences in soil organic matter in the restored drift sands with forest removal, which potentially masked effects of atmospheric N deposition, actual drift sands and restored sites were analyzed separately. In each series, a two-way ANOVA was applied, with stage of succession (open, Polytrichum, Campylopus, lichen-rich vegetation and grassland) and N deposition (low and high) as independent factors. Two-way ANOVA was applied on measured values, but due to high variation between early and later succession stages, also on log-transformed values. Differences between individual mean values were tested with lsmeans tests. Different letters in graphs indicate significant differences (P < 0.05) between means.
Results

Soil characteristics per succession stage

The two study areas showed similar developments during succession. In actual drift sand, organic matter built up during succession, resulting in an increase in solum depth (Table 4.1). As a result of soil formation, pH(H₂O) decreased from 5.4 to 4.3 (Table 4.2). The soil C:N ratio varied between succession stages. Bare sand had relatively low values of 7-13, but C:N ratios significantly increased to 17-18 in stages with *Polytrichum piliferum* and values around 21 in *Campylopus introflexus*. In lichens, however, C:N ratios decreased again to values of 13-15. Although there was a strong difference in N deposition, total soil N content and C:N ratio and extractable cation concentrations (Ca, Fe, Na, Mg, and Al) did not show differences between the low and high N deposition site and these data were not used in further analyses.

The ectorganic layer was only present in plots with *Campylopus introflexus* and lichens. This layer is c. four times higher in weight in *Campylopus* than in lichens, and had a significantly higher C:N ratio of 28 ± 3, compared to 19 ± 0 in lichens. C:N ratios of the two stages were however not different between the high and low N deposition area, nor between drift sand and restored sites. Carbon content in the mineral top soil varied from c. 0.02 kg C m⁻² in bare sand to 0.5 kg m⁻² in lichen and grass stages. The ectorganic layer added c. 1 kg C m⁻² in the *Campylopus* stage and 0.5 kg m⁻² in the lichen stage.

Drift sand sites

Soil microbial C and respiration

Statistical analysis showed that microbial C was affected by both succession and N deposition. During succession the amount of microbial C increased (Fig. 4.1). The ectorganic layer of *Campylopus introflexus* and lichens strongly contributed to the microbial C stocks in the *Campylopus* and lichen succession stages. In *Campylopus*, this layer was two to four times higher in weight than in lichens, which is also reflected in the observed values for microbial C. Soil microbial C was significantly higher in Aekinge, the low N deposition area, than in Wekerom, especially in later stages of succession. Microbial activity is reflected in respiration (Fig. 4.1). Like microbial C, respiration generally increased during succession. Respiration did however not differ between Aekinge and Wekerom, which suggests that micro-organisms were actually more active in the high-deposition area.

Nitrogen mineralization

Net nitrogen mineralization was significantly affected by vegetation stage, and increased during succession (Fig. 4.2). Net nitrogen mineralization was low in open sand and stages with *Polytrichum*. In the latter, net N mineralization per unit microbe was lower than in all other stages. Nitrification in *Polytrichum* was only 24% of total N mineralization, which further points to N stress. In open sand, nitrification amounted
Nitrogen mineralization in acid inland drift sand vegetation

Table 4.2. Characteristics of the mineral top soil of the succession stages in the two study sites, for both succession series in a drift sand site (poor in soil organic matter) and restored sites (rich in soil organic matter).

|                     | Aekinge (low-deposition site) | Wekerom (high-deposition site) |
|---------------------|------------------------------|--------------------------------|
|                     | pH(K₂SO₄) | C (g m⁻²) | N (g m⁻²) | C:N ratio | pH(K₂SO₄) | C (g m⁻²) | N (g m⁻²) | C:N ratio |
| Drift sand          |            |            |            |           |            |            |            |           |
| Bare sand           | 5.4 (0.0)  | 29 (5)     | 2 (1)      | 12.8 (5.0)| 5.3 (0.1)  | 14 (0)     | 2 (1)      | 6.7 (2.4) |
| Polytrichum         | 4.8 (0.0)  | 279 (23)   | 15 (1)     | 17.6 (1.2)| 4.5 (0.0)  | 263 (44)   | 15 (3)     | 18.0 (3.9)|
| Campylopus          | 4.7 (0.1)  | 259 (20)   | 14 (1)     | 21.2 (3.0)| 4.5 (0.0)  | 184 (26)   | 14 (1)     | 21.5 (3.0)|
| Lichens             | 4.3 (0.2)  | 502 (300)  | 37 (17)    | 13.2 (1.1)| 4.2 (0.0)  | 434 (48)   | 31 (3)     | 14.9 (1.2)|
| Grasses             | 4.8 (0.0)  | 166 (25)   | 10 (1)     | 16.2 (1.6)| 4.0 (0.2)  | 578 (293)  | 31 (10)    | 17.8 (3.2)|
| Restored site       |            |            |            |           |            |            |            |           |
| Bare sand           | 3.9 (0.2)  | 1054 (580) | 50 (20)    | 20.2 (2.4)| 4.6 (0.0)  | 248 (49)   | 19 (2)     | 12.5 (1.3)|
| Polytrichum         | -          | -          | -          | -         | 4.2 (0.0)  | 312 (23)   | 21 (1)     | 14.6 (1.2)|
| Campylopus          | 3.6 (0.0)  | 1334 (347) | 61 (13)    | 22.3 (1.4)| 4.2 (0.1)  | 317 (177)  | 23 (9)     | 16.7 (1.7)|
| Lichens             | 3.2 (0.1)  | 1773 (965) | 73 (40)    | 23.9 (2.1)| 4.0 (0.1)  | 422 (80)   | 29 (6)     | 16.5 (0.5)|
| Grasses             | 3.7 (0.1)  | 1171 (134) | 56 (5)     | 20.6 (1.0)| 3.9 (0.0)  | 658 (127)  | 40 (7)     | 16.4 (0.9)|

In later stages of succession, values still accounted for 41-64% of total net N mineralization. In later stages of succession, the contribution of the organic layer to the total N mineralization also became more important. In addition to succession stage, net N mineralization was also affected by N deposition. Net nitrogen mineralization was slightly higher in Wekerom to 82%, and in later stages of succession, values still accounted for 41-64% of total net N mineralization. In later stages of succession, the contribution of the organic layer increased to 9-17% and in Campylopus plots even to 33-42% of the total N mineralization. In addition to succession stage, net N mineralization was also affected by N deposition. Net nitrogen mineralization was slightly higher in Wekerom to 82%.
than in Aekinge and this difference was significant when based on log-transformed values. Net N mineralization per unit microbial C showed even larger differences between sites with high and low N deposition. Net N mineralization per unit microbe was generally 2-3 times higher in Wekerom than in Aekinge.

The amount of inorganic N (nitrate and ammonium) in fresh samples did not differ between vegetation types, but was significantly affected by N deposition (Fig. 4.2). Especially ammonium concentrations were higher in Wekerom than in Aekinge, resulting in a higher ammonium:nitrate ratio, which is, averaged for all succession stages, $2.0 \pm 1.1$ in Aekinge, and $6.0 \pm 5.7$ in Wekerom.

![Microbial biomass and respiration](image)

**Fig. 4.1.** Microbial biomass and respiration under lab conditions measured in fresh soil samples in different succession stages in drift sand in both study sites. Means ($n = 4$) and standard deviations (error bars) of the soil and ectorganic layer. Names of succession stages are abbreviated to the first four or five letters. Different letters mean significant differences between succession stages (lsmeans test performed on the sum of mineral soil and ectorganic layer). Significant differences between sites were found for soil microbial C only.
Fig. 4.2. Net N mineralization and N mineralization per unit microbial C (soil + ectorganic layer) under lab conditions and amounts of K₂SO₄-extractable ammonium and nitrate in fresh samples of soil and ectorganic layer (Campylocus and lichen stages only) in different succession stages in drift sand sites in Aekinge and Wekerom. Means (n = 4) and standard deviations (error bars) of the soil and ectorganic layer. Different letters are significant differences between succession stages. Names of succession stages are abbreviated to the first four or five letters (lsmeans test performed on the sum of mineral soil and ectorganic layer). All parameters showed significant differences between sites.
Restored sites

Soil microbial C and respiration

In the restored sites, where forest has been removed, Aekinge had up to twice the amount of microbial C than Wekerom (Fig. 4.3), which can be related to the two to five times higher soil C content. Respiration (Fig. 4.3) did not differ between both study areas, in spite of the higher microbial C content.

Fig. 4.3. Microbial biomass and respiration under lab conditions measured in fresh soil samples in different succession stages in restored sites in both study sites. Means (n = 4) and standard deviations (error bars) of the soil and ectorganic layer. Names of succession stages are abbreviated to the first four or five letters. Different letters mean significant differences between succession stages (lsmeans test performed on the sum of mineral soil and ectorganic layer). Significant differences between sites were found for soil microbial C only.
Fig. 4.4. Net N mineralization and N mineralization per unit microbial C (soil + ectorganic layer) under lab conditions and amounts of K$_2$SO$_4$-extractable ammonium and nitrate in fresh samples of soil and ectorganic layer (Campylopus and lichen stages only) in different succession stages in restored sites in Aekinge and Wekerom. Means (n = 4) and standard deviations (error bars) of the soil and ectorganic layer. Different letters are significant differences between succession stages. Names of succession stages are abbreviated to the first four or five letters (lsmeans test performed on the sum of mineral soil and ectorganic layer). All parameters showed significant differences between sites.
Nitrogen mineralization

In restored sites, net nitrogen mineralization was significantly affected by study area and succession stage (Fig. 4.4). However, differences between succession stages are not as clear as in drift sands. Early stages of succession already had a high net nitrogen mineralization, due to the higher soil organic matter content. Also, in the site rich in organic matter, Aekinge, the most N limited stage (*Polytrichum*) was not present under restored conditions at all.

In contrast to actual drift sands, differences between the study areas do not reflect differences in N deposition, but predominantly differences in soil organic matter content. Net N mineralization is significantly higher in Aekinge, where N deposition is lower, but soil organic matter content is higher than in Wekerom. Potential effects of high N deposition in restored sites are thus counteracted by high soil organic matter content. Even in the case of net N mineralization per unit microbial C, the effects of high N deposition may still be obscured by high soil organic matter content: in contrast to actual drift sands, where net N mineralization per unit microbe was two to three times higher in Wekerom than in Aekinge, differences between Aekinge and Wekerom were not significant for restored sites.

The amount of inorganic N in fresh samples may clearly reflect differences in N deposition. Hardly any ammonium was detected in the mineral soil in the restored site in Aekinge, the site with low N deposition, in spite of the high N mineralization. Nitrate concentrations were also relatively low. In Wekerom, however, especially

### Table 4.3. Microbial C:N and N:P ratios in the soil in different succession stages in drift sand and restored sites of both study sites with low (Aekinge) and high (Wekerom) N deposition. Mean values and standard deviations (n = 4). The difference between the two study sites was significant for both drift sand and restored sites (P < 0.05), except for the C:N ratio in the restored site. Values for bare sand could not be calculated due to phosphorus values below the detection limit.

| Site type       | Site  | Polytrichum | Campylopus | Lichens | Grasses |
|-----------------|-------|-------------|------------|---------|---------|
| **Microbial C:N ratio** |       |             |            |         |         |
| Drift sand site | Aekinge | 20 (3)      | 19 (1)     | 13 (2)  | 13 (2)  |
|                 | Wekerom | 10 (2)      | 8 (2)      | 9 (4)   | 9 (2)   |
| Restored site   | Aekinge | -           | 6 (2)      | 7 (1)   | 12 (2)  |
|                 | Wekerom | 11 (2)      | 8 (2)      | 11 (8)  | 8 (0)   |
| **Microbial N:P ratio** |       |             |            |         |         |
| Drift sand site | Aekinge | 4 (1)       | 9 (2)      | 23 (3)  | 10 (2)  |
|                 | Wekerom | 35 (7)      | 35 (10)    | 25 (10) | 16 (9)  |
| Restored site   | Aekinge | -           | 13 (6)     | 4 (2)   | 6 (2)   |
|                 | Wekerom | 30 (12)     | 35 (17)    | 18 (6)  | 8 (1)   |
ammonium concentrations were high in all stages of succession. Ammonium values were approximately ten times higher than in Aekinge. As a result, the average ammonium:nitrate ratio in all succession stages together was 1.8 ± 0.6 in Aekinge and 3.0 ± 2.0 in Wekerom.

**Microbial N:P and C:N ratios**

Differences in N deposition may also be reflected in microbial C:N and N:P ratios (Table 4.3). In actual drift sands, the microbial C:N ratio had values of 13 to 20 in Aekinge and 8 to 10 in Wekerom. In restored sites, the C:N ratio did not differ between the two areas. However, microbial N:P ratios were significantly higher in Wekerom than in Aekinge in both pristine drift sand sites and in restored sites, pointing to excess N.

**Discussion**

**N mineralization and soil organic matter**

This study focuses on changes in microbial characteristics and availability of nitrogen in the course of succession, under different regimes of atmospheric N deposition and restoration measures. Along with the succession and build-up of soil organic matter, N mineralization increased in both drift sand sites. The N mineralization was low or even negative (Aekinge) in *Polytrichum piliferum* mats, despite the increase in soil organic matter and microbial C compared to bare sand. This is reflected in the low net N mineralization per unit microbe, and low nitrification, which suggests that N is a limiting factor in this stage of succession, even in areas with high atmospheric N deposition. This could perhaps be explained by the composition of the rhizoids of *Polytrichum piliferum*, which are the primary source of carbon in the Ah horizon (Bowden 1991) and have a relatively high C:N ratio. Decomposition of organic matter with a high C:N ratio could therefore result in lower release of N by microbes (Swift et al. 1979), as compared to a substrate with a lower C:N ratio.

The mineral soil characteristics below *Campylopus introflexus* mats were more or less similar to *P. piliferum*. Also, net N mineralization in the mineral soil was only slightly higher than in *P. piliferum*. The main difference is that in *C. introflexus*, N cycling and C storage for a large part take place aboveground in the moss mat itself. Due to the development of an ectorganic layer of dead moss, two times the amount of C and N can be stored aboveground as compared to the mineral soil below. The microbial biomass, respiration and N mineralization in the ectorganic layer were of the same order as in the mineral soil. *Campylopus introflexus* therefore added a successful way of nutrient cycling to the inland dune habitat, by creating a thick ectorganic layer of moss necromass lying on top of the mineral soil, functioning as a sponge, potentially preventing leaching of water and nutrients and therefore supporting the internal nutrient cycling. This way of nutrient cycling is similar to the compact, vertical structure of e.g. *Sphagnum* bogs (Lang et al. 2009) and reindeer lichens (Cornelissen...
et al. 2007; Crittenden 1991). Especially the much thicker ectorganic layer in *Campylopus* is a competitive advantage over the thin layer in most lichen species.

Lichen and grass dominated stages are usually the oldest stages of pioneer vegetation in inland dunes (Sparrius 2011). In lichens, an important part of the N cycling takes place in a very thin ectorganic basal layer of the thalli (Crittenden 1991; Ellis et al. 2005). However, in grass-dominated plots, N mineralization in the mineral soil is generally lower than in lichen-rich vegetation. This can be explained by the composition of the soil, mainly consisting of fine roots, leading to an increase of the C:N ratio (Table 2). Although the soil C:N ratio in the grass stage is similar to the *Polytrichum* stage, stocks of soil organic matter are much larger due to the deeper solum and plant growth increased, resulting in a higher N mineralisation in the grass stage.

**N mineralization and N deposition**

Atmospheric N deposition may elevate levels of plant-available N and N content of micro-organisms in a direct way. However, N deposition may also increase N availability indirectly by creating a more efficient N mineralization in high N deposition areas, i.e. by making the microbial community more C limited. In sites with elevated N deposition, N availability in the soil is higher, causing a change in the N concentration, N:P and C:N ratios in the vegetation. This change has been demonstrated in a fertilization experiment in inland dune vegetations (Sparrius 2011). Decomposition of litter, moss rhizoids and grass roots with low C:N ratios may thus yield higher amounts of N for each decomposing unit of C, making the N mineralization more efficient and also affect the C:N and N:P ratios of soil microbial biomass (Swift et al. 1979).

A paradox was found for the amount of microbial biomass, which surprisingly decreased with increasing N deposition, although net N mineralisation was higher. As a result, in Wekerom, N mineralization per unit microbial C was twice as high as in Aekinge. This suggests a surplus in microbial N, demonstrated by the lower microbial C:N ratio, and saturation of the soil with N compounds in the high N deposition site, even though the C:N ratio of the soil was not different between the high and low N deposition sites. Similar results, i.e. low amounts of micro-organisms in soils with high N availability, have been found in previous studies in forest ecosystems (Berg 1988; Wallenstein 2003; Wallenstein et al. 2006). The mechanism behind these observations is unclear, but it shows that elevated levels of N deposition may lead to deviating microbial communities adapted to a high N environment.

**The ectorganic layer of *Campylopus introflexus***

Apart from a generic increase in succession rate, the moss species *Campylopus introflexus* is invasive in sites with high N deposition or on bare soil rich in organic matter, outcompeting the original lichen vegetation (Sparrius & Kooijman 2011). The formation of a thick ectorganic layer in the moss carpets facilitates the water
Nitrogen mineralization in acid inland drift sand vegetation holding capacity, interception of deposited nutrients, N mineralization and uptake of mineralized compounds in the upper, living parts of the moss mat (Sparrius & Kooijman 2011). It is not completely clear why *C. introflexus* is unsuccessful in low N deposition sites. However, it is possible that in the absence of excess nitrogen, basal decomposition of the ectorganic layer is faster than apical growth, making the moss mat thinner. The apical, living part of the moss mat is only a few mm thick. Without a robust ectorganic layer, the moss mat is easily overgrown by other species, including lichens.

**Drift sand versus restored sites**

In restored sites, the succession started on sandy soil with a high amount of organic matter. Nevertheless, the observed differences between sites with high and low N deposition is similar to trends in actual drift sand sites with respect to the amounts of microbial C, ammonium concentrations in fresh samples, and the microbial N:P ratio. The microbial C:N ratio in restored sites was generally higher in the low-deposition site, which is probably caused by a combination of low N deposition and a much higher soil carbon content in the low deposition site as compared to the high deposition site. However, N mineralization was not lower in the low N deposition site, but higher, due to the relative large amount of soil organic matter, providing N to micro-organisms (Swift *et al.* 1979). As expected, the higher soil C content led to N limitation in the microbial biomass, even in the high-deposition site.

Restored sites with increased organic matter showed especially large differences with pristine drift sands in early succession stages (*sand, Polytrichum* and *Campylopus*). Instead of a linear increase during succession, all succession stages had a similarly high microbial biomass, respiration, N mineralization and N availability. This may cause an increased succession rate as species that are adapted to soils with higher N availability may replace pioneer species at an earlier stage (e.g. Berendse 1998). The presence of high amounts of organic matter left after restoration of drift sands may thus cause similar effects as increased N deposition, as both situations result in higher N availability.

**Relevance for nature conservation**

Nitrogen deposition is one of the major threats to nutrient-poor habitats such as drift sands (e.g. Bobbink *et al.* 2010). The results showed that in all succession stages N mineralization and N availability significantly increased in the high N deposition site. As vegetation growth is mostly N limited, N deposition is likely to cause an increase in succession rate. Reduction of N deposition should therefore be one of the most important measures to conserve the drift sand habitat.

In sites where restoration management has taken place by topsoil removal, care should be taken that litter layers and the entire Ah horizon are removed. Bare mineral soil high in organic matter adsorbs ammonium in high N deposition areas, replacing base cations (Aerts & Bobbink 1999; de Graaf *et al.* 2009; Sevink & de Waal 2011).
The vegetation succession rate is also affected by the higher N mineralization and N availability (Berendse et al. 1998). The abiotic conditions between the succession stages in both restored sites are not very different, and bare sand already has higher soil organic matter content than pristine drift sands. This may not only explain why the pioneer moss *Polytrichum piliferum* is not very common, but also why some species settle earlier than in a succession series starting on nutrient poor sand. In the high N deposition area where N availability is high, competitive species, initially *C. introflexus* and later grasses are likely to start dominating the vegetation on such soils (Sparrius & Kooijman 2011).

**Conclusions**

During drift sand succession, soil organic matter accumulates, accompanied by a lower pH, higher microbial biomass, respiration, net N mineralization and nitrification. Mats of *Polytrichum piliferum* formed an exception, as this was the only succession stage where net N mineralization was low or even negative, which supports that early succession in drift sands is N limited, even in areas with high N deposition. In later succession stages, the ectorganic layer of *Campylopus introflexus* and lichens played an important role in N cycling, presumably facilitating uptake of the mineralized compounds by the living part of the plant above.

Atmospheric N deposition (mainly as ammonium) caused a decrease in microbial biomass, but led to higher net N mineralization, especially per unit microbial C. Differences between microbial C:N and N:P ratios supported that micro-organisms in sites with high N deposition were not N limited. As a result, N deposition leads to higher N availability in the form of ammonium and nitrate for plants in both direct and indirect ways.

In restored drift sands, where forest has been removed, succession starts on soil with much higher organic matter content. The soil characteristics and N dynamics are therefore rather similar between succession stages, and stages characteristic for open, nutrient-poor sand were rare. The effect of N deposition on the soil in restored sites was masked by high soil organic matter, but similar as in the drift sand sites. Both high levels of organic matter and elevated N deposition lead to higher N availability which may cause an increased succession rate.

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