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

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Laurens Benjamin Sparrius

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Faculteit der Natuurwetenschappen, Wiskunde en Informatica

Voor mijn ouders

Contents

Chapter 1

Introduction

Scope of this thesis

In this thesis field surveys and experiments are combined to explain changes in the soil and vegetation of inland dunes over the period 1950-2007. An important part of this work is devoted to the relation between nitrogen deposition and the vegetation. It builds upon previous studies on the vegetation of inland dune landscapes and the effects of nitrogen deposition in dry, acidic habitats.

Compared to the earlier studies on inland dunes, this research project had a wider geographical scope, comprising twenty inland dune areas in a gradient from low to high nitrogen deposition and differing in size and in the extent of active drift sand (bare sand).

The main research questions were:

- At what rate does succession proceed in inland dunes and what is the effect of nitrogen deposition on this succession and its rate?
- What is the impact of nitrogen deposition on species diversity in inland dunes?
- What is the impact of nitrogen deposition on soil processes and nutrient availability in the different succession stages in inland dunes?
- Does nitrogen deposition increase the rate of invasion of the bryophyte *Campylopus introflexus*?

In all chapters, results are translated into management measures, paying particular attention to mitigation of the effects of nitrogen deposition and to the conservation of relict species that occur in primary succession stages. The introductory part mainly describes previously published research. Additionally, it pays attention to topics that do not fit into one of the succeeding chapters, but are relevant for the study as a whole. These include the composition of the parent material, the flora of inland dunes, and the modelling and measuring of nitrogen deposition.

A short history of the Dutch inland dunes

Inland sand dunes occur in a narrow belt running from East England to the Baltic region (Fig. 1.1). In this belt, Weichselian cover sands abound, marked by their uniform grain size and gently sloping landscape (Koster 1995). These sands in particular acted as a source for the eolian drift sands that formed as a result of anthropogenic land degradation during the Late Holocene.

Already in the Bronze Age (3000 BC), extensive logging had resulted in removal of most of the forest on the sand belt (Hacke-Oudemans 1976). Grazing turned the area into heath and, in places, sand started to drift (Koster 2005a; Koster 2009). In the Middle Ages, sod-cutting in heath became popular, sods being used as an addition to manure and to fertilize arable land, resulting in plaggen soils where sod-based manure was applied (Pape 1970; Spek 1992). This practice, in combination with overexploitation (grazing and burning) of the heaths, reached its zenith in the 18th

Fig. 1.1. The European sand belt, the region in which inland dune systems occur (after Koster 2005).

and 19th centuries, inducing a massive degradation of the heaths and concurrent development of drift sands in that period. Radiocarbon dating showed that drift sands were formed between 600 and 1900 (Castel 1991), although recent studies revealed that luminescence dating may yield an improved accuracy (Koster 2005b). There is also some debate on the exact causes for the dramatic expansion of drift sands, with other authors ascribing this expansion to sheep grazing in combination with a relatively cold and windy climate (Heidinga 1984; Koomen *et al.* 2004; Riksen *et al.* 2006; Koster 2010).

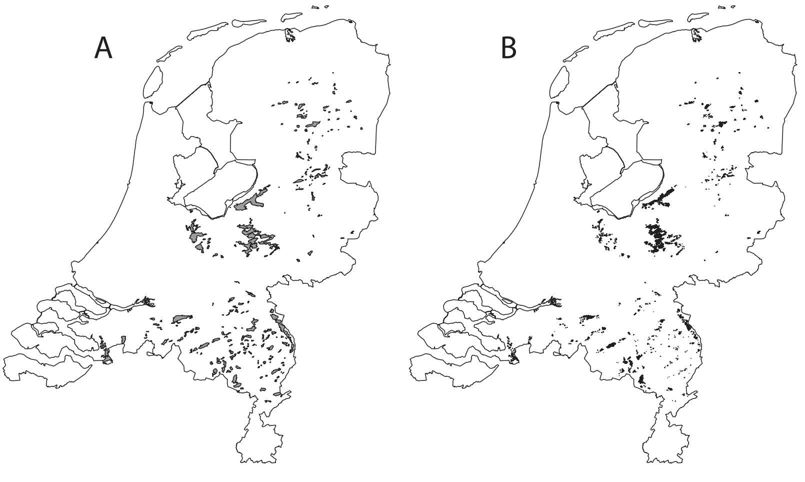

Fig. 1.2. A. The occurrence of drift sands soils in The Netherlands (Jungerius & Riksen 2010); B. Currently non-forested inland dune landscape (source: TOP10Vector 2009).

Around 1850, the industrial revolution and associated economic and technological changes, notably the introduction of chemical fertilizer, led to a decrease in sheep grazing. It also led to agricultural reclamation and afforestation of former 'waste lands'. This is particularly true for drift sands since these were unsuited for agriculture. In The Netherlands, especially between 1910 and 1950, large drift sand areas have been turned into pine forest (Koster 2009). Nowadays, around 2% of the total original drift sand area is still typical drift sand landscape, characterized by open sand and sand dune grasslands. Since 1980, in former drift sand reserves modest attempts started to maintain or even increase open drift sand landscapes through deforestation (Riksen *et al.* 2006). Stimulated by the European Habitat Directive of 1992, these attempts increased and were incorporated in the management plans of most nature conservation organizations. Fig. 1.2 shows the distribution of drift sand soils (total cover: 823 km^2) and the current distribution of non-forested inland dune landscape (total cover: 110 km^2).

Parent material

Inland dune sand largely originates from cover sands, which are Pleistocene eolian deposits (Koster 2005a; Sevink & de Waal 2010). The deflated material includes soils developed in the cover sand, notably podzols that contain fair amounts of organic matter in their various horizons. Drift sand therefore holds a small amount (approximately 0.1%) of organic matter (e.g. Riksen *et al.* 2008).

Texture

Cover sand is the main source of drift sand and the Dutch cover sands have a uniform texture. As a result, differences in soil texture between the main drift sand regions (Fig. 1.3) are small but still significant: The sands in the northern parts of the country (Drenthe), the West (Utrecht) and South (Noord-Brabant) have somewhat higher levels of loam than the other regions, but this is limited to a few mass percentages. The effect of loam is, however, clearly visible in the field, as loam stabilizes dune slopes and erosion rills, which in turn provide a habitat for species confined to vertical soil surfaces such as liverworts and many fauna species (Nijssen *et al.* 2011).

The Veluwe area shows the most uniform soil texture with a grain size of mostly 0.125-0.250 mm (see also Koster 1982). The only significant difference (chi^2: $P < 0.001$) in texture was found in old river dunes of the river Meuse. These sands are essentially cover sands mixed with coarser Holocene river deposits originating from the Ardennes (Tebbens *et al.* 2000), making the average grain size twice as large as in the other drift sands. Within a drift sand reserve, coarser sands may occur in deflation zones, where fine material has been blown away (Riksen & Goossens 2007). Such so-called desert pavements have been sampled by Hasse (2005), who found up to 8% of coarse material (> 2 mm) in some samples.

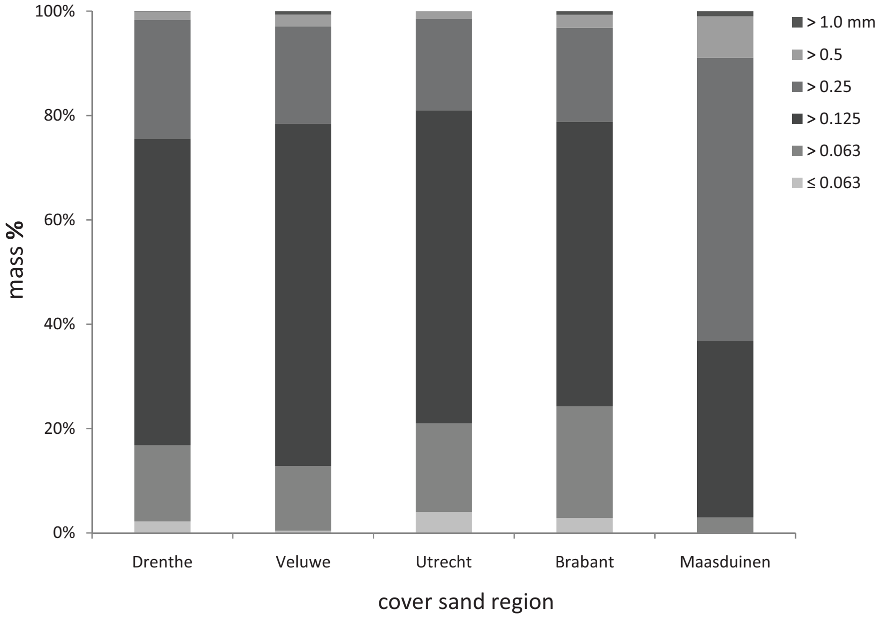

Fig. 1.3. Soil texture from bare sand for five different regions in The Netherlands (n = 31), analyzed using standard sieves varying from 0.063 to 1.0 mm in mesh size, showing minor differences except for the coarser river dune sands (Maasduinen) and absence of a loam fraction in the Veluwe.

Mineralogical composition

The mineralogy of drift sands depends on the origin of the source material and thus is strongly linked to the composition of cover sands, which exhibits regional differences (Crommelin 1964, 1965). Clearly local sources may lead to deviating composition such as the drift sand that is derived from Meuse sediment.

For the sand fractions of the drift sands, the mineralogical composition has been studied (e.g. Koster 2005a), showing that the northern sands have a lower mineral content than the southern sands. This is in line with the differences in composition of the cover sand. Drift sands may also be derived from river dunes and thus may consist of a mixture of sand from different sources. The middle to late Quaternary Meuse sediments are e.g. known to contain a relative high amount of Al and lower K and Mg (Tebbens *et al.* 1998), resulting in lower amounts of weatherable minerals (Sevink & de Waal 2010) in drift sands with a significant Meuse component.

As an example, the mineralogy of the soil of two major drift sand regions (Veluwe and Drenthe) was studied. Soil samples of drift sand deposits from the Drenthe region (Aekinge and Drouwen sites; n = 7) and Veluwe region (Kootwijk and Wekerom sites; n = 7) were finely ground, destructed in HF and the total element content measured on an inductively coupled plasma (ICP-OES) analyzer (Jackson 1985). Fig. 1.4 shows the relation between potassium and two combinations of metals occurring in pyroxene

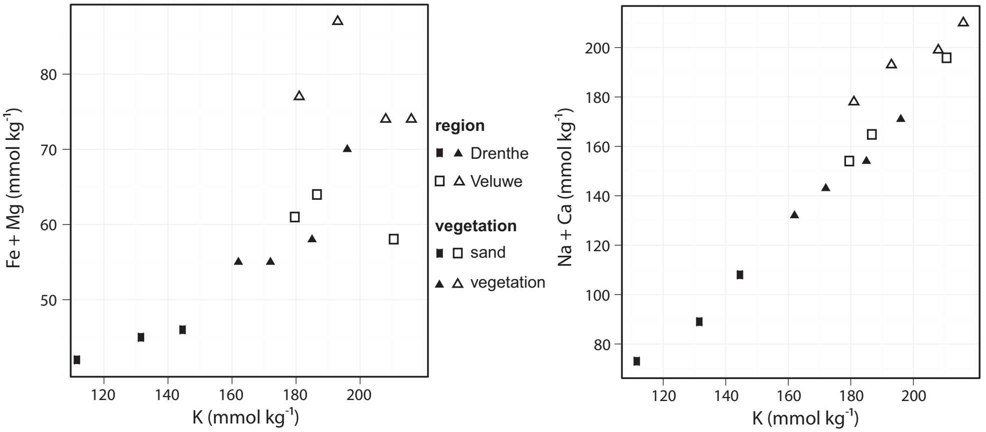

Fig. 1.4. Relations between elements in the upper 5 cm of drift sand soil in two regions for samples from bare sand and below vegetation, which include accumulated elements in the A_h horizon. Upper diagram: elements characteristic for pyroxene and biotite. Lower diagram: elements characteristic for plagioclase.

and biotite (Fe and Mg) and in plagioclase (Na and Ca). Measurements of the element content of bare and vegetated drift sand is included here, to show the effects of soil formation on the total stock of metals in the soil.

Drift sands of the Veluwe area have a significant ($P < 0.05$) higher content of Na, K, Mg, Ca, Al, Fe, Mn and Cr than Drenthe, whereas P content did not differ. The Ca:P ratio was around 10. The K:Al ratio was around 0.4. There was no significant difference in the K:Al, Ca:P and Fe:Mg ratios between the Drenthe and Veluwe regions. This shows that, except for the generally higher mineral content (other than quartz) of the Veluwe drift sands the mineral composition of both regions is more or less equal. The results also show large differences in mineral content within a region and drift sand site. If translated into percentages of non-quartz minerals, including feldspars, micas and pyroxenes, the values most probably range between 5 and 10%, with only some samples reaching values up to 15%. This is in line with the observations by Koster (2005a) and Castel (1991).

Although drift sand is largely derived from cover sand, it can be described as a matured, weathered form of cover sand, with less easily weatherable minerals. This is partly due to the intensive weathering in the Podzols developed in cover sand (see e.g. Sevink *et al.* 1970; Mokma & Buurman 1982). Thus drift sand will contain less easy weatherable minerals than cover sand and once stabilized will release lower amounts of base metals and other weathering products. Consequently, weathering will hardly contribute to the neutralization of acids (e.g. low acid neutralizing capacity of the substrate). Higher levels of base metals in the A_h horizons (Fig. 1.4: sand vs. vegetation) can most probably be attributed to the accumulation of base metals in organic matter (either in structural tissues or adsorbed) and to their supply by atmospheric deposition. The contribution of weathering is likely to be insignificant at the time scale in which these soils have developed and under these conditions.

Drift sands, if not completely derived from eluvial Podzol horizons, contain small amounts of clay minerals and sesquioxides, present as a coating on sand grains (Emmer & Verstraten 1993; Koster 2005a; Sevink & de Waal 2010). These coatings give drift sand its characteristic yellowish colour, though it is rather greyish in comparison with the truly yellow cover sand because of the presence of some organic matter. These clay minerals and sesquioxides are largely responsible for the acid neutralizing capacity of these sands, as was demonstrated by van der Salm (1999) for cover sands.

Inland dune soils

In the open inland dune landscape, the vegetation succession is highly correlated with soil development, starting with open sand, poor in nutrients and with a very low amount of organic matter, and ending in A(E)C micropodzols (van Rheenen *et al.* 1995; Koster 2005a). Further development of inland dune soil takes places below later vegetation succession stages, namely forest and heath, where a variety of well-developed soils (mainly podzols) can be formed. Emmer (1995) described the soil development and humus forms in primary *Pinus* forests and first showed the changing nutrient composition in such pine forests over time. However, soil development in the range from open sand to pioneer vegetation has not been thoroughly studied. Among the few studies are those by Paus (1997) and Hasse (2005). Paus investigated the main soil parameters (pH, loss on ignition) for most lichen species occurring in a variety of inland and coastal dunes in Northwest Europe. Hasse (2005) described vegetation classes and included the main soil parameters (pH, loss on ignition, N content, texture) for a limited number of sites where Spergulo-Corynephoretum grasslands occur. These studies showed that during primary succession the amount of organic matter in the mineral soil accumulates and pH(H_2O) decreases from about 5.5 (bare sand) to 3.8 (dry heath).

Primary succession on bare sand and the expansion of naturally established trees have been studied previously, but not on a nation-wide scale (e.g. Ketner-Oostra & Masselink 1999; Hasse 2005; Ujházy *et al.* 2011). The comparison of multiple sites is therefore one of the subjects of this thesis. Different geomorphological units can be recognized, i.e. dunes and blowouts. Due to erosion, vegetation succession may be slower on dunes and faster in blowouts. Quantifying these rates of succession can be used to estimate the efforts needed to conserve a certain area of open sand and pioneer vegetation in inland dunes.

None of the previous studies focussed on the effects of nitrogen deposition on nitrogen availability, soil acidification and succession. These effects were studied by analyzing soil and vegetation properties over gradients in N deposition, adding nutrients to measure the effect of nitrogen addition and through a mineralization experiment. The latter also helped to understand soil processes such as nitrogen mineralization, which is of importance for plant growth and therefore succession rate.

The flora of inland dunes

Inland dunes are known for their plant biodiversity. They are composed of mostly cryptogams, which are adapted to acid, semi-arid environments, the grasses *Corynephorus canescens*, *Festuca ovina* subsp. *hirtula*, *Festuca filiformis* and *Agrostis capillaris*, the bryophytes *Campylopus introflexus* (a neophyte occurring since the 1960s) and *Polytrichum piliferum* and lichens in the genera *Cladonia, Cetraria* and *Stereocaulon* (Masselink 1994; Haveman & van Ravensberg 2003; Hasse & Daniëls 2004) belong to the most common species in this habitat.

Apart from the bryophytes, most species have been the subject of recent taxonomical studies. In the latest edition of Heukels' Flora (van der Meijden 2005), the names of both inland dune *Festuca* taxa were changed following a study by Haveman & van Ravensberg (2003). In older literature, the names *Festuca ovina* and *F. tenuifolia* have been used in a wide sense including both taxa.

Name changes in *Cladonia* species make it especially difficult to compare older and recent vegetation studies in inland dunes. Names of lichens in the genus *Cladonia* have been changing constantly since the 1940s. In the 1970s many chemical varieties in the *Cladonia grayi*-group were recognized by Sipman (1973), including *Cladonia chryptochlorophaea* and *C. novochlorophaea*. Later studies have shown that these varieties reflect only genetic variation within a population of the same species (Culberson *et al.* 1988) and that there was no ecological difference between the varieties (Paus 1997). *Cladonia rei* was included in *Cladonia subulata* for practical reasons, although both species , especially *C. rei,* occur rather infrequent in drift sands. Also *Cladonia bergsohnii, C. bacillaris* and *C. pleurota* are considered chemotypes of *C. floerkeana, C. macilenta* and *C. coccifera,* respectively. The *Cladonia pyxidata*-group appeared to be composed of three distinct taxa: the base-tolerant *Cladonia pocillum*, the montane species *C. pyxidata* and the inland dune species *Cladonia monomorpha* (Aptroot *et al.* 2001). The *Cladonia cervicornis*-group was divided into three distinct species: *Cladonia cervicornis* s.s., *C. pulvinata* and *C. verticillata* (van Herk & Aptroot 2003). Since 1995, another typical inland dunes species is reported in The Netherlands: *Cladonia borealis,* which was formerly included in *Cladonia coccifera*.

Large collections of distribution data on cryptogams became available over the past ten years and can now be used in spatial analyses. Data from the Dutch Bryological and Lichenological Society and the Dutch Mycological Society were combined with the map in Fig. 1.2a. All species with a distribution pattern overlapping for 20% or more with drift sand soils are listed in Table 1.1. Surprisingly, most common, vegetation-forming species are absent in this list, e.g. all common drift sand bryophytes and most vascular plants. Apparently most of the common species of drift sands occur just in the margin of their ecological niche. Most species in the list are generally rare and are present on the Red List of endangered species in The Netherlands. Most of the listed bryophytes and the lichens *Cladonia squamosa, Cladonia sulphurina* and *Pycnothelia papillaria* are on the verge of extinction. Mycorrhizal fungi occur mostly in forest margins, where they live on young soils with a thin solum and litter layer.

Table 1.1 Species of plants, bryophytes, lichens, and macrofungi with a distribution pattern which falls for 20% or more within drift sand areas. Data is based on occurrence data in a km square grid for the period 1800-2008 (source: Dutch Bryological and Lichenological Society and Dutch Mycological Society, January 2010). * The exact number of km^2 squares is not available for *Festuca ovina* ssp. *hirtula*, and an expert guess is used instead.

Species group	**% in drift sands**	**km^2 squares in drift sands**	**km^2 squares total**
Vascular plants			
Festuca ovina ssp. hirtula	c. 50%	c. 200 *	c. 400 *
Bryophytes			
Barbilophozia floerkei	50%	3	6
Barbilophozia kunzeana	27%	49	182
Oligotrichum hercynicum	27%	59	220
Scapania compacta	20%	46	235
Tetraplodon mnioides	33%	2	6
Lichens			
Cetraria islandica	38%	17	45
Cladonia borealis	57%	43	76
Cladonia crispata	51%	163	320
Cladonia gracilis	29%	91	318
Cladonia monomorpha	58%	42	72
Cladonia phyllophora	50%	4	8
Cladonia pulvinata	47%	98	209
Cladonia squamosa	48%	11	23
Cladonia strepsilis	61%	113	185
Cladonia sulphurina	50%	4	8
Cladonia uncialis	33%	78	235
Cladonia verticillata	56%	39	70
Cladonia zopfii	52%	174	333
Micarea leprosula	50%	31	62
Micarea viridileprosa	33%	17	52
Placynthiella oligotropha	45%	36	80
Pycnothelia papillaria	33%	1	3
Stereocaulon condensatum	79%	79	100
Stereocaulon saxatile	27%	4	15
Mycorrhizal fungi			
Coltricia perennis	20%	92	455
Cortinarius fusisporus	37%	59	159

Species group	% in drift sands	km² squares in drift sands	km² squares total
Gomphidius roseus	20%	65	331
Hebeloma cylindrosporum	56%	9	16
Hygrophorus hypothejus	22%	62	276
Pseudoomphalina pachyphylla	33%	19	57
Psilocybe polytrichi	33%	16	49
Rhizopogon luteolus	33%	111	336
Rhodocybe parilis	39%	7	18
Tricholoma albobrunneum	36%	54	151
Tricholoma equestre	34%	60	176
Tricholoma portentosum	36%	38	106

Vegetation succession in inland dunes

The succession from open sand to heath and forest has been described by many authors (Fanta 1986, Prach *et al.* 1993, Hasse 2005, Ketner-Oostra & Sýkora 2008). Hasse (2005) and Ketner-Oostra & Sýkora (2008) defined several vegetation classes to which the various primary succession stages can be attributed. Fig. 1.5 shows the simplified chronosequence of the vegetation classes used in this study. The main classes were used for interpretation of aerial photographs, whereas more detailed subclasses were used for vegetation mapping.

Sand dune vegetations belong to the association of the Spergulo-Corynephoretum, which is usually subdivided into three categories, i.e. typicum, inops (species poor variant) and cladonietosum (dominated by lichens) (Weeda *et al.* 1996). More detailed descriptions of associations within the Spergulo-Corynephoretum include bryophytes, lichens and algae, and are called microsynusiae (Biermann & Daniëls 1997). These microsynusiae have been used in further studies by these authors and co-workers (Biermann & Daniëls 2001; Hasse *et al.* 2002; Hasse & Daniëls 2004; Daniëls *et al.* 2008). In this thesis, syntaxonomy is not often used. Instead, vegetation classes, combining soil, vegetation structure and geomorphological units are defined. Table 1.2 shows how vegetation classes and subclasses are related to the syntaxonomy of inland dunes vegetation types.

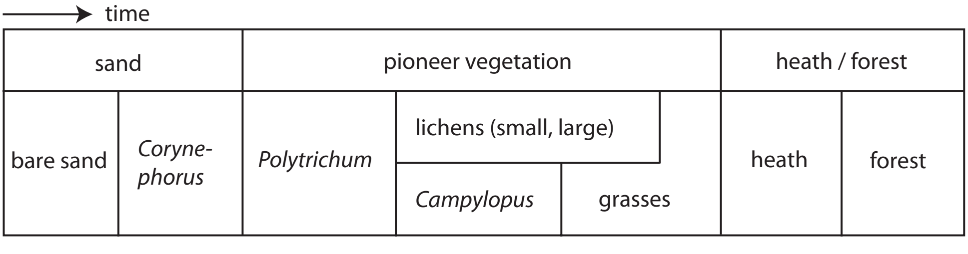

Fig. 1.5. Vegetation succession in inland dunes using a classification in main (above) and subclasses (below) based on Hasse (2005).

Table 1.2. Description of the vegetation subclasses and translation to syntaxa in Weeda *et al.* (1996) and Hasse (2005).

Vegetation subclasses	Main syntaxon (Weeda et al. 1996)
Open sand (no vegetation)	-
Corynephorus canescens and algal crusts on open sand	Spergulo-Corynephoretum inops
Polytrichum piliferum mats, few grasses present	Spergulo-Corynephoretum inops
Campylopus introflexus mats, few grasses present	Spergulo-Corynephoretum (*Campylopus introflexus* derivate association)
Small cup-lichens and grass tussocks (with abundant *Cladonia pulivinata, C. cervicornis, C. glauca, C. strepsilis, C. borealis, Festuca* spp.)	Spergulo-Corynephoretum cladonietosum
Large reindeer lichens and dense grasses (with abundant *Cladonia portentosa, C. unicialis, C. zopfii, Agrostis vinealis*)	Spergulo-Corynephoretum cladonietosum
Grasses dominant, with sparse mosses and lichens, including logging sites with stumps, *Deschampsia flexuosa* & *Carex arenaria* and forest floor bryophytes	*Carex arenaria* derivate of the Koelerio-Corynephoretea
Calluna vulgaris heath, usually with abundant lichens between the shrubs	Genisto anglicae-Callunetum (including the Juncus squarrosus-Oligotrichum hercynicum derivate of the Corynephorion canescenstis)
Pinus sylvestris and *Quercus robur* forest	Dicrano-Pinion, Betula-Quercetum

Vegetation subclasses	Microcommunity (Hasse 2005)
Open sand (no vegetation)	Grünalgen-Typ p.p. without vascular plants
Corynephorus canescens and algal crusts on open sand	Grünalgen-Typ p.p. with vascular plants
Polytrichum piliferum mats, few grasses present	*Polytrichum*-Typ
Campylopus introflexus mats, few grasses present	*Campylopus*-Typ
Small cup-lichens and grass tussocks (with abundant *Cladonia pulivinata, C. cervicornis, C. glauca, C. strepsilis, C. borealis, Festuca* spp.)	*Cladonia zopfii*-Typ + *Cladonia strepsilis*-Typ
Large reindeer lichens and dense grasses (with abundant *Cladonia portentosa, C. unicialis, C. zopfii, Agrostis vinealis*)	*Cladonia mitis*-Typ p.p. with low grass-cover
Grasses dominant, with sparse mosses and lichens, including logging sites with stumps, *Deschampsia flexuosa* & *Carex arenaria* and forest floor bryophytes	*Cladonia mitis*-Typ p.p. with high grass cover
Calluna vulgaris heath, usually with abundant lichens between the shrubs	*Cladonia mitis*-Typ p.p. as patches between heath
Pinus sylvestris and *Quercus robur* forest	-

Nitrogen deposition and its effect on soil and vegetation in dry, acid habitats

Without human activities, nitrogen deposition would be much lower in inland dunes in The Netherlands. In inland dunes, nitrogen deposition is 10 to 20 times higher than the natural background value of 1-2 kg N ha^{-1} yr^{-1}. Nitrogen is deposited in two ways: dry and wet. Dry deposition includes dust particles, such as $(NH_4)_2SO_4$ and absorbed gases (mainly NH_3) on humid surfaces. Wet deposition comprises the nitrogen dissolved in precipitation and includes NH_4^+ and NO_3^- ions. Industry and traffic are the most important sources of oxidized nitrogen, whereas livestock farms form the main source of ammonia. Livestock farms form the main source of nitrogen deposited in nature reserves and circa 60% of the wet deposition (de Haan *et al.* 2008).

Little information is available on the effect of nitrogen on soil and plants in inland dunes, but the main effects are likely to be comparable to those in *Calluna*-dominated heath on podzols and Scots pine forest on acid sandy soils, as for example described by Bobbink *et al.* (2010). The main form of nitrogen deposited in nature reserves in The Netherlands, reduced nitrogen (NH_x), can be toxic or inhibit seed germination (de Graaf *et al.* 1997, 1998; van den Berg *et al.* 2005). Nitrification of ammonium causes soil acidification (Tietema & Verstraten 1992) and leaching of metals in acid soils, such as aluminium (Al^{3+}) that can reach toxic levels for plants (Aerts & Bobbink 1999; Smit & Kooijman 2001). Addition of nitrogen changes the vegetation. In dry heaths, soil ammonium and also the Al:Ca ratio is negatively correlated with species diversity (de Graaf *et al.* 2009; Duprè *et al.* 2010; Maskell *et al.* 2010; Stevens *et al.* 2010). Lichens are widely used as highly sensitive bioindicators for ammonia, mostly by evaluating epiphytic lichen communities (e.g. van Herk 1999). Lichens are also used as a biomonitor, by analysing the nitrogen content in the lichen thallus (Hyvärinen & Crittenden 1998; Cape *et al.* 2009a; Remke *et al.* 2009; Olsen *et al.* 2010).

Local dominance of the bryophyte *Campylopus introflexus* was also related to nitrogen deposition (Ketner-Oostra & Sýkora 2008) and disturbance (Daniëls & Krüger 1996). However, strong evidence for the relation with nitrogen was lacking. As the species is a neophyte introduced during the 1960s, its expansion in Europe is still ongoing (Hassel & Söderström 2005). As the species was still expanding in the Netherlands, an increase of the species in long-term plot studies (e.g. Ketner-Oostra & Sýkora 2008) cannot be automatically attributed to atmospheric deposition. Therefore, in this thesis, sites with high and low N deposition are compared with respect to the abundance of *C. introflexus*.

Field and experimental studies

In the following chapters, field and experimental studies are described based on the research questions and hypotheses described in this introductory chapter.

In **chapter 2**, the large-scale vegetation development in the study areas is described for the period 1950-2007 in eight inland dune reserves. The effect of factors such as recreation and nitrogen deposition is studied. A prediction of the future development of the main vegetation classes (bare sand, pioneer vegetation and forest) is made.

In **chapter 3**, the soil and vegetation characteristics of the succession stages in inland dunes are presented. The influence of nitrogen deposition on soil acidification and concurrent alteration of the vegetation is discussed.

Chapter 4 zooms into the level of cryptogam mats and studies the potential mineralization of nitrogen in the soil in different succession stages in inland dunes under high and rather low N deposition. In this chapter, the authors also focus on the role of the ectorganic layer of bryophytes and lichens in N cycling.

Chapter 5 describes the results of a two-year field experiment with nitrogen and phosphorus addition that has been carried out in two inland dune sites, which particularly differ in nitrogen deposition.

Chapter 6 provides evidence for the relation between *Campylopus introflexus* dominance, soil organic matter content and nitrogen deposition.

In the synthesis, **chapter 7**, the conclusions of the chapters are briefly discussed in order to find an answer to the main research questions.

Fig. 1.6. Location of the eight main study sites.

Description of the study areas

Field studies were carried out in twenty inland dune sites, of which eight have been studied in more detail. Fig. 1.6 shows the location of the eight main study sites. Table 1.3 gives a summary of the main characteristics of the sites, including the total size of the reserve and the selected part for vegetation mapping. The sites are chosen to reflect a range in nitrogen deposition, size and geological history (northern, central and southern cover sands; former river dunes).

Table 1.3. General data on the eight main study sites.

Site name	Province	Total size (ha)	Studied area (ha)	Annual visitors x 1000	Rainfall mm a^{-1}	Geology
Aekingerzand	Friesland	100	39.6	50	750	Northern cover sands
Drouwenerzand	Drenthe	150	40.5	10	775	-
Lemelerberg	Overijssel	30	20.2	20	750	-
Loonse en Drunense duinen	Noord-Brabant	225	47.1	500	700	Southern cover sands
Kootwijkerzand	Gelderland	400	45.8	250	800	Central cover sands
Otterlosche zand	Gelderland	300	26.0	45	800	-
Wekeromse zand	Gelderland	100	86.0	50	800	-
Bergerheide	Limburg	20	15.1	0.5	700	River dunes

Nitrogen deposition: measuring, modelling and critical loads

Measuring nitrogen deposition

In this study, nitrogen deposition was measured in eight sites, using precipitation collectors and ammonia diffusion tubes. However, converting results from these measurements into actual deposition data is not as simple as it seems. There are different approaches to calculate the total nitrogen deposition, all based on the wet deposition and a contribution of dry deposition based on either terrain roughness or particle deposition velocity. High vegetation adsorbs or traps more dry deposition than low vegetation. Cape *et al.* (2009b) presented a formula to calculate the deposition of ammonia from the ammonia air concentration in combination with the particle deposition velocity, which actually reflects the adsorption of ammonia by the vegetation. High vegetation, like forest, traps more ammonia than low vegetations, such as grassland or bogs. This is supported by similar findings in inland dunes (forest versus grassland) in The Netherlands (Daniëls & Pott 2008).

Modelled nitrogen deposition

The so-called OPS (Operationeel model Prioritaire Stoffen) model in The Netherlands is based on emission data and terrain roughness and has been developed to predict the nitrogen deposition for any location (van Jaarsveld 2004) in a 1 x 1 km grid cell. Many inland dunes are situated within forested areas and the predicted deposition can be too high when a grid cell contains both forest and pioneer vegetation. The air concentrations of nitrogen compounds are calculated at 5 km resolution and downscaled to 1 km resolution, based on average terrain roughness. This data set is the most complete data source for nitrogen deposition, although little difference can be made between low vegetation and adjacent forest sites. Also errors in modelled deposition values can reach 20-30% (van Jaarsveld 2004).

Deposition data used in this study

In this study, nitrogen deposition has been measured during 2008-2009 by sampling wet deposition with monthly replaced precipitation traps, and dry deposition with monthly replaced ammonia diffusion tubes (Nijssen *et al.* 2011). The results are presented in Table 1.4, showing total wet deposition and the average ammonia concentration. The wet deposition consisted for 60-80% of NH_4^+. The total nitrogen deposition could then be calculated in two ways. First, assuming that terrain roughness is so low that almost no dry deposition occurs; the measured wet deposition can then be regarded as the lowest estimate for N deposition. Second, the total deposition can be calculated from the ammonia air concentration with a formula by Cape *et al.* (2009b) assuming a low particle deposition velocity of 15 mm s^{-1}. The latter is regarded here as an average estimate. OPS data, which are modelled from nitrogen emissions, are regarded as a high estimate.

Table 1.4. Different approaches to estimate the nitrogen deposition in pioneer vegetation in inland dunes. The measured data (2008-2009) can be regarded as a low estimate. An average estimate is based only on the atmospheric ammonia concentration (Cape *et al.* 2009b) for a particle deposition velocity of 15 mm s^{-1}. Modelled data (here for 2006) can be regarded as a high estimate. * source: Velders *et al.* (2002).

Site name	**Measurements**		**Calculated deposition**	**Modelled data**
	N_{wet}	NH_3	N_{tot} (Cape *et al.*)	Ntot (OPS)*
	kg (mol) ha^{-1} yr^{-1}	µg m^{-3}	kg (mol) ha^{-1} yr^{-1}	kg ha^{-1} yr^{-1}
Aekingerzand	11.0	5.8	24	28.9
Drouwenerzand	11.1	6.6	26	25.3
Lemelerberg	12.7	7.6	30	36.7
Loonse en Drunense duinen	-	7.5*	29	42.7
Kootwijkerzand	12.3	7.9	30	34.3
Otterlosche zand	-	7.0*	28	35.2
Wekeromse zand	13.9	9.7	41	50.3
Bergerheide	12.8	10.0	42	39.2

Modelled OPS data are used for ranking, describing or grouping sites, i.e. in chapter 2, 3 and 5. In chapters 4 and 6, the air concentration itself or deposition values calculated from ammonia air concentrations are used, by using the formula presented by Cape *et al.* (2009b). In order to estimate the ammonia air concentration for other sites, measurements dating from 2000-2001 are used (Velders *et al.* 2002). These are currently the best available data. The ammonia air concentration has dropped by 10-20% since 2001. However, the error in the measurements is of a similar order and differences in atmospheric ammonia concentration between sites are relatively large.

Critical load and critical level

A critical load for atmospheric deposition is the highest deposition that will not cause chemical changes leading to long term harmful effects on ecosystem structure and function (Nilsson & Grennfelt 1988). The critical load of nitrogen deposition for inland dune vegetation has been modelled by van Dobben *et al.* (2006), based on historical relevees and nitrogen deposition data. Their calculations resulted in a critical load of 10.4 kg N ha^{-1} yr^{-1}, a value which is below even the lowest estimate of N deposition (wet deposition only) in the studied sites (Table 1.4).

The critical level for ammonia was defined as "the concentration in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to present knowledge" and has been estimated at 8 µg NH_3 m^{-3} (Posthumus 1988). This value is around the median value of the studied sites (Table 1.4).

Other studies have shown that for cryptogams, based on the exact definition of the critical load, this load is already met at very low N deposition and atmospheric ammonia concentrations. For example, chemical changes in bryophytes were already detected at values as low as 1 µg NH_3 m^{-3} in a study on the response of tissue N content to elevated N levels in bryophytes (Cape *et al.* 2009a). This shows that the critical load values must be interpreted with care and that they are usually within a range of gradual changes to the ecosystem.

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Chapter 2

Vegetation succession in eight inland drift sands in relation to geomorphology and nitrogen deposition

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Abstract

Questions: (1) At what rate does succession take place in active and more stabilized drift sands in regions with low and high N deposition in The Netherlands? (2) What is the present composition of the pioneer vegetation in active and more stabilized drift sands in regions with low and high N deposition? (3) What efforts are necessary to conserve bare sand in drift sands? *Location*: Eight inland dunes in The Netherlands, which represent four active and four more stabilized drift sands in regions with low and high N deposition. *Methods*: Aerial photographs from 1950, 1981, 1995 and 2007 were used to compare rates of succession from bare sand towards forest. For each site, a detailed vegetation map was made in 2007. *Results*: In all studied sites, bare sand decreased, especially in regions with high N deposition. This decline was significantly higher between 1981 and 2007 than in the other periods. The loss of bare sand did not lead to increase of pioneer vegetation, which remained more or less stable. It led, however, to an increase of heath and forest. In active drift sands, pioneer vegetation was characterized by *Corynephorus canescens* and *Polytrichum piliferum*, while lichens, lichen-rich grassland and *Campylopus introflexus* were more common in stabilized drift sands. In regions with high N deposition, the proportion of *Campylopus introflexus* to older pioneer stages was significantly higher. In regions with high N deposition, the contribution of forest to the 'heath/forest' vegetation class was also higher. The calculated lifespan of bare sand decreased in all sites. Extrapolation of the results suggests that in the absence of restoration measures, bare sand will vanish in 2035. *Conclusions*: In both active and more stabilized drift sands, a generic loss of bare sand and an increase in forest area was found. The increase was higher in regions with high N deposition. Conservation management should include the annual transformation of 43 ha of forest into bare sand in order to stop the loss of bare sand.

Nomenclature source: van der Meijden, R. (2005) for vascular plants; Siebel & During (2006) for bryophytes; Aptroot *et al.* (2004) for lichens.

Introduction

Since the Middle Ages, until about 1850, intensified land use, mainly sheep grazing and sod cutting, led to the degradation of heaths on light sandy soils in Western Europe. This resulted in drift sands. Since the 16th century, local governments took measures to control further expansion of these drift sands with no substantial results (Tesch *et al.* 1926; Riksen *et al.* 2005). With the collapse of the wool industry and introduction of fertilizers, the heaths lost their economic value. With the increasing demand for wood for the mines in the 19th century it became economically feasible for the Dutch government to afforest the heaths and drift sands. Scots pine was planted on a large scale between 1898 and 1940. Only a few large (> 200 ha) drift sand areas were conserved as nature reserves or used for military purposes.

Inland drift sands in Western Europe form a semi-arid landscape and a protected habitat type (H2330) in the European Habitat Directive (EC 2007). Although active inland drift sands occurred in the entire European sand belt (Koster 2005), today they can almost only be found in The Netherlands. Most of the drift sand reserves that remain today are still scarcely vegetated, harbouring bare sand and pioneer vegetation (Riksen *et al.* 2006). Increase of vegetation cover forms the main threat to the drift sand habitat. The further transformation from pioneer vegetation to forest results in a decline in biodiversity. Research showed that succession rates might be further increased by high levels of nitrogen deposition (Pluis *et al.* 1993; Riksen *et al.* 2006; Daniëls *et al.* 2008b; Remke *et al.* 2009). Although the lichen-rich grasslands have the highest biodiversity of flora and fauna, bare sand is an essential feature of inland drift sands as it provides a future habitat for pioneer vegetation (Nijssen *et al.* 2010).

To some extent, drift sands may be compared with sand deserts. They are both habitats under influence of wind erosion. However, while deserts remain open due to the extremely dry climate, inland drift sands without human influence show a succession towards grassland, and eventually heath and forest. In the first stages of succession, grasses such as *Corynephorus canescens* and mosses such as *Polytrichum piliferum* dominate the vegetation (Hasse, 2005). In the moss mats, lichen communities establish, followed by lichen-rich grasslands. At present, however, the *Polytrichum* moss mats may also be colonized by *Campylopus introflexus*, an exotic moss species, which has arrived in the 1960s (van der Meulen *et al.*, 1987). In pioneer vegetation, seedlings of *Pinus sylvestris* may take root and eventually transform pioneer vegetation into forest.

Geomorphological differences within the inland drift sands seemed to be an important factor with respect to succession. Inland drift sands are largely formed by local reworking of terrestrial (mainly aeolian) deposits by wind (Koster 2005). This took place mainly in areas with young cover sand or river dune sand at the surface.

The open character of these areas in combination with (high) local disturbance by intensive land use like sheep herding, heath burning and sod cutting, made this landscape extremely vulnerable to wind erosion. In periods with major storms, drift sands developed and expanded quickly, due to deflation and burying of vegetation. The development of a drift sand area depends on the size, the local weather conditions, the availability of erodible soil material (total area times the thickness of the high erodible sand layer), the presence of less erodible soil layers, the presence of a water table near the soil surface, the position in the landscape, and the land use. Within a drift sand area this can result in a number of geomorphologic units with their own characteristics and thus different conditions for vegetation and fauna (Castel *et al.* 1987; Jones *et al.* 2008; Jungerius *et al.* 2010). Specifically, two geomorphological units can be recognized: active drift sand dunes and stabilized blowouts.

Rates of succession differ between active and more stabilized drift sand areas. In active drift sands, continuous movement of sand slows succession. In less dynamic areas, such as blowouts, succession is no longer hindered by aeolian activity. In the blowouts, fluvio-periglacial and other compact non-aeolian deposits become exposed. These fluvio-periglacial deposits are more variable in grain size, may contain pebbles, are usually densely packed and are much less sensitive to wind erosion (Riksen *et al.* 2006). The lack of erosion activity gives room to the settlement of pioneer vegetation.

Among the factors known to increase the natural succession rates, a high level of N deposition has shown to play an important role. The Netherlands is among the European countries with the highest level of nitrogen deposition. The amount of nitrogen deposition doubled over the period 1950-1981, remained high and was eventually reduced by approximately 30% over the period 1990-2007 (de Haan *et al.* 2008). Within The Netherlands, nitrogen sources are concentrated in a few areas in the southeast, leaving the northern and western part of the country relatively devoid of high levels of atmospheric deposition. This spatial pattern in nitrogen deposition exists since around 1960 (de Haan *et al.* 2008). In 2004, nitrogen deposition in inland drift sands varied from 22 to 45 kg ha^{-1} yr^{-1} (de Haan *et al.* 2008). All these values are far above the modelled critical load for inland dunes of 9.8 kg ha^{-1} yr^{-1} (van Dobben *et al.* 2006). In the present study, one of the main questions is whether drift sand areas with low and high N deposition differ in the rate of succession.

Under present conditions conservation measures are necessary to prevent the drift sand landscape from turning into forest (Ketner-Oostra *et al.* 1998; Riksen *et al.* 2006). For a long time, removal of young tree seedlings was the only management measure in drift sands. In some areas where drift sand is part of a bigger nature reserve with heath, grazing is seen as a measure to reduce the succession rate. Since the 1970s, conservation measures also include tree and topsoil removal to set back the vegetation succession to bare sand. This decision was in most cases taken on an ad hoc basis by the managers. Since the 1990s, experts more carefully plan the selection of measures and locations for drift sand restoration. However, the scale and frequency at which these measures should take place is still a point of discussion. Drift sand areas in which all succession stages are present are seen as the optimal situation. Therefore, an important goal of drift sand management is to create and maintain diversity in succession stages.

In this study, rates of succession were compared in eight inland drift sand nature reserves within The Netherlands. The three research questions in this study are:

1. At what rate does succession from bare sand to pioneer vegetation take place in active and more stabilized drift sands in regions with low and with high N deposition, respectively?
2. What is the present composition of the pioneer vegetation in active and more stabilized drift sands in regions with low and high N deposition?
3. What is the life span of bare sand and further succession stages in drift sands, and which efforts are necessary to conserve the characteristic primary succession stages?

Succession rates in inland drift sands are usually studied at site level, and cover only a short period (Daniëls 1990; van Rheenen *et al.* 1995; Biermann *et al.* 1997; Biermann *et al.* 1998; Ketner-Oostra *et al.* 1999; Ketner-Oostra 2004; Nijssen *et al.* 2007; Daniëls *et al.* 2008a; Ketner-Oostra *et al.* 2008). However, by combining data from aerial photographs of different periods with a current vegetation map, it was possible to study the rates of succession at a larger time and spatial scale. Four active and four more stabilized drift sands were selected, evenly distributed over regions with low and high N deposition.

Based on calculated succession rates, the future cover of the main vegetation classes (bare sand, pioneer grassland and heath/forest) can be predicted. The outcome may help to improve measures to conserve the area of bare drift sand and support management of similar types of dune systems, e.g. coastal dunes, which exhibit a similar decline of the area of bare sand (Provoost *et al.* 2011).

Methods

Study sites

Eight inland drift sand areas were selected in the Netherlands (Fig. 2.1; Table 2.1). They are well distributed over the potential drift sand area (Koster 1978) and include the main drift sands left in the country. Four of the sites (Aekingerzand, Loonse en Drunense Duinen, Kootwijkerzand and Wekeromse Zand) still contain active drift sand and four are stabilized (Drouwenerzand, Lemelerberg, Otterlosche Zand, Bergerheide). Stabilized sites typically consisted for a large proportion of blowouts. The two groups, active and stabilized drift sands, were subdivided in two regions (north and southwest versus southeast), which are characterized by relatively low and high atmospheric N deposition (Velders *et al.* 2002; van Jaarsveld 2004; de Haan *et al.* 2008). Differences in N deposition between sites, based on modelled values of 2004, are sometimes small and the uncertainty may be up to 20-30% (van Jaarsveld 2004).

Other site properties that may affect succession were not used as a site selection criterion and include recreation pressure, which was quantified as the annual number of visitors (provided by site managers) and the total length of paths and hiking trails. Mean annual rainfall was derived from Sluijter & Nellestijn (2009) (Table 2.1). Past rainfall is not taken into account, but the weather station in De Bilt, which is generally used as a reference for Dutch weather data, was 9% higher in the period 1981-2007 (although not significant) compared to 1940-1981.

Fig. 2.1. Location of the study sites within The Netherlands.

Table 2.1. Site characteristics of study sites. Area size is the section of the site that was mapped in this study.

Site name	**Drift sand type**	**N deposition**	**Modelled N deposition**	**Area size**	**Visitors**	**Trail length**	**Rainfall**
			kg ha^{-1} yr^{-1}	ha	10^3 yr^1	10^3 m	mm yr^{-1}
Aekingerzand	active	low	22.6	39.6	50	25.3	750
Loonse en Drunense Duinen	active	low	28.3	47.1	500	17.6	700
Kootwijkerzand	active	high	29.0	45.8	250	6.4	800
Wekeromse Zand	active	high	44.8	86.0	50	18.3	800
Drouwenerzand	stable	low	24.4	40.5	10	6.8	775
Lemelerberg	stable	low	33.7	20.2	20	3.2	750
Otterlosche Zand	stable	high	34.0	26.0	45	5.3	800
Bergerheide	stable	high	37.1	15.1	0.5	5.5	700

Aerial photo interpretation

For each site, we created maps of the vegetation in several years based on photo interpretation. Characteristic sections with the open inland drift sand landscape (bare sand and pioneer vegetation) surrounded by a fringe of predominantly self-sown trees were selected. These sections, of 15 to 86 ha each, represent about 3% of all non-forested drift sand habitat in The Netherlands and about 30% of the most undisturbed (i.e. not used for military purposes) drift ‘sand reserves. Digital high resolution aerial photographs, all with < 1 x 1 m pixel size, made in 1950, 1981, 1995 (black and white imagery) and 2007 (RGB+NIR channels) (source: Kadaster) were georeferenced at 1 m resolution and automatically classified in 3 main vegetation classes (Fig. 2.2): 1. sand, 2. pioneer vegetation and 3. heath / forest. Classification was done using image segmentation in Definiens Developer 7 (Definiens AG. 2007) and ArcGIS 9.3. Additional to reflection, pattern recognition in Definiens Developer was used to classify tree canopies using contrast split segmentation with settings depending on the image quality (Fig. 2.2). Unpaved paths and hiking trails were digitized as line features. The years were chosen due to the fact that high-quality photographs were available for all sites. Heath and forest could be distinguished with recent colour photographs; this was not possible with older material.

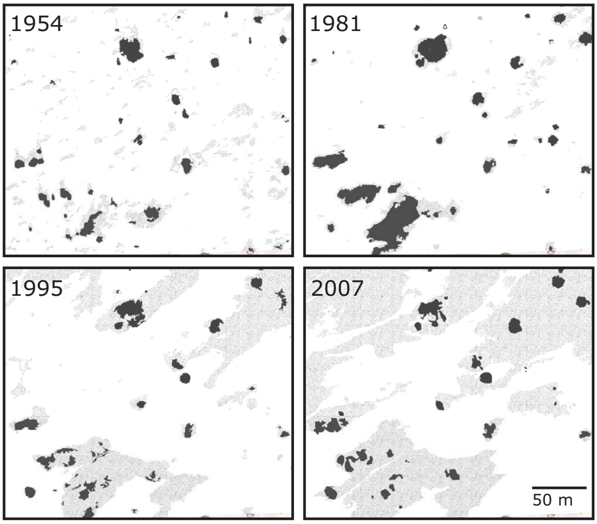

Fig. 2.2. Example of digitized aerial photographs (site: Kootwijkerzand) showing loss of bare sand (white space) and changes in pioneer vegetation (grey) and forest (black). The decrease of forest between 1981 and 1995 is due to conservation management.

Field survey

For the most recent map in our series, present-day vegetation in 2007, we produced a more detailed vegetation classification by relying on field surveys. These vegetation subclasses (Fig. 2.3) are based on the vegetation structure and derived from Hasse (2005), although the subclasses are here named after the dominant species or species group. Compared to the classes used in photograph interpretation, the main class sand was subdivided into the subclasses ‘bare sand’ and ‘bare sand with *Corynephorus canescens* tussocks’. Pioneer vegetation was subdivided into ‘*Polytrichum piliferum*

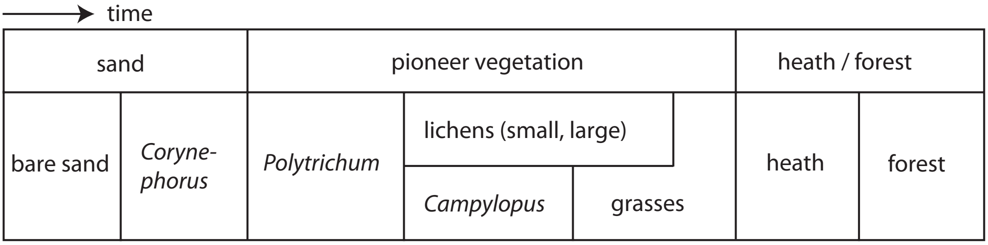

Fig. 2.3. Vegetation succession in inland drift sands using a classification in main vegetation classes (above) and subclasses (below).

mats', '*Campylopus introflexus* mats', '*Cladonia* spp. lichen dominated vegetation', and 'lichen-rich grassland on shallow soil dominated by *Agrostis vinealis* and *Festuca* sp.' The 'heath/forest' vegetation class was subdivided into 'heath' and 'forest' subclasses. All maps were digitized at about 1 m resolution.

Spatial data processing

All layers (classified photographs of 1950, 1981, 1995 and 2007, and the detailed vegetation map of 2007) were overlaid in ArcGIS 9.3 (using "union") resulting in a fine pattern of $7.9\ 10^5$ segments covering 3.5 km^2, each having a combination of vegetation (sub)classes for all years, additional to environmental properties at site level. From the original dataset, areas in which large-scale measures in the drift sands of Aekinge and Wekerom (Nijssen *et al.* 2007) were removed, were subtracted to differentiate between autonomous processes in vegetation succession and conservation measures (tree logging and top soil removal). In the case of drift sands, retrogression of the vegetation occurs rarely (Walker *et al.* 2010) and is mainly the result of conservation measures, such as logging and topsoil removal. The selection of this dataset (further called "succession only") thus only contained segments with stable or progressive vegetation classes over time. In this way, effects of large-scale conservation measures could be eliminated, which made the datasets of the four different years more comparable. However, the dataset still includes small-scale measures, especially tree removal in pioneer vegetation. Both the original and the "succession only" datasets are used in the analyses.

Statistical analysis

The results of the field survey and image data were analyzed using R for Windows 2.11 (R development core team 2010) and Microsoft Office Excel and Access 2007. Annual loss of bare sand was calculated for two periods separately, 1950-1981, and 1981-2007, in order to differentiate between periods with lower and higher atmospheric N deposition. In the 1950s, N deposition had only slightly increased with respect to natural background levels, but annual N deposition reached its peak in the 1990s (de Haan *et al.* 2008). The annual loss of bare sand was calculated as the annual percentage loss of the original cover of bare sand in 1950. Differences in loss of bare sand were tested with three-way ANOVA, with drift sand type (active and more stabilized), N deposition region (low and high), and period (before and after 1981) as factors. Rates of changes in pioneer vegetation or heath/forest did not differ between the periods before and after 1981 and are not further treated.

Differences in the area of the pioneer vegetation from the field survey in 2007 were tested with two-way ANOVA, with drift sand type and N deposition region as independent variables. Additionally, ratios of vegetation types indicating habitat quality were calculated and tested in a similar way. The bare sand ratio was calculated as the cover of bare sand divided by the cover of bare sand including the stage with *Corynephorus canescens*, indicating the early settlement of vegetation. The *Campylopus* ratio is indicative of the contribution of *Campylopus introflexus* to older pioneer stages, which developed from open mats of *Polytrichum piliferum*. This ratio was calculated as the cover of *Campylopus* divided by the combined cover of lichens, lichen-grassland and *Campylopus*. The forest ratio, indicative of the relative contribution of forest and heath to the heath/forest class, was calculated as the cover of forest divided by the total cover of forest and heath combined.

In order to model the succession rate, a transition matrix was made based on the succession only dataset and represented in a Markov diagram representing the annual percentage of change of the original cover of the vegetation class for two periods (e.g. Tang *et al.*, 2007): 1950-1981 and 1995-2007, as differences in succession rates between those periods differed significantly. The transition from sand directly to forest did not occur. Forest represents an end state. To illustrate the transition matrix, the estimated development time of each vegetation subclass is given. This estimate is based on results from long-term monitoring of permanent plots in inland drift sands (Ketner-Oostra *et al.* 2008) and detailed studies of vegetation development in drift sand landscapes (Ujházy *et al.* 2011).

In order to predict the future cover of the three main vegetation classes, the succession rates in the periods 1950-1981 and 1995-2007 were extrapolated into the future and used as minimum and maximum scenarios respectively. This results in a minimum and maximum value for the year in which no bare sand is expected to remain, and the percentage of bare sand that will be lost annually starting from 2007. This percentage is multiplied by the total area of bare sand currently present in The Netherlands (Riksen & Jungerius, 2010) to estimate future efforts necessary to preserve bare sand surfaces.

2.4 Results

2.4.1 Changes in main vegetation classes

In the ‘succession only’ dataset, the area of bare sand decreased in all sites over the period 1950-2007 (Fig. 2.4). The actual loss of bare sand was higher in active drift sands than in more stabilized drift sands, which already had a low sand cover in 1950. However, in all sites the decrease was about 50%. In the dataset with all

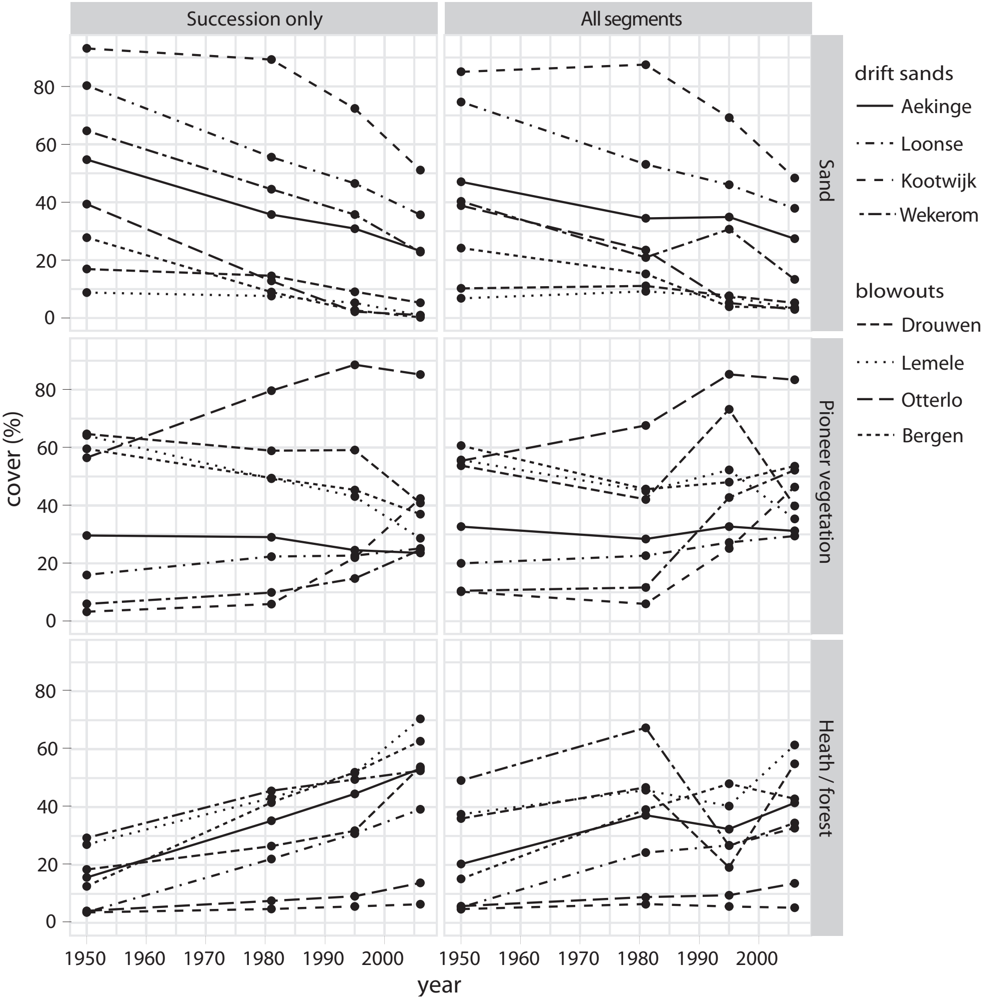

Fig. 2.4. Change in cover of three vegetation classes for each site based on succession only and all polygons (including retrogression and conservation measures).

polygons, including those showing retrogression of the vegetation due to management measures, the cover of bare sand decreased in all sites as well, with more or less similar overall rates of loss of bare sand. The restored drift sands of Aekinge and Wekerom temporarily showed an increase in cover of bare sand and decrease of pioneer vegetation in 1995, after large-scale restoration efforts to transform forest and pioneer vegetation back into bare sand. However, restoration measures had only temporary effect and could not prevent the loss of bare sand over the entire period.

In both datasets, the loss of bare sand was accompanied by a net increase in heath/ forest, rather than an increase in pioneer vegetation. In the period 1950-1981 and again between 1981-1995, most of the bare sand was transformed into pioneer vegetation. However, much of the pioneer vegetation transformed to heath/forest. Especially after 1995, heath/forest was formed at a higher rate than before. As a result, after slightly more than 50 years, the net area of pioneer vegetation remained more or less the same.

While actual loss of bare sand expressed as surface area was higher in active drift sands than in more stabilized ones, the annual loss as a percentage of the area in 1950 did not differ between drift sand types (Figs 2.4, 2.5). However, regions with high N deposition had significantly higher losses of bare sand. Also, the loss of bare sand in all sites together was significantly higher in the period of 1981-2007 than in 1950-1981.

Local differences in present-day vegetation

The decrease of bare sand may be further reflected in the even lower amount of bare sand, compared to initial stages of succession with *Corynephorus canescens*, which are both included in the main class of sand in the analysis of aerial photographs (Table

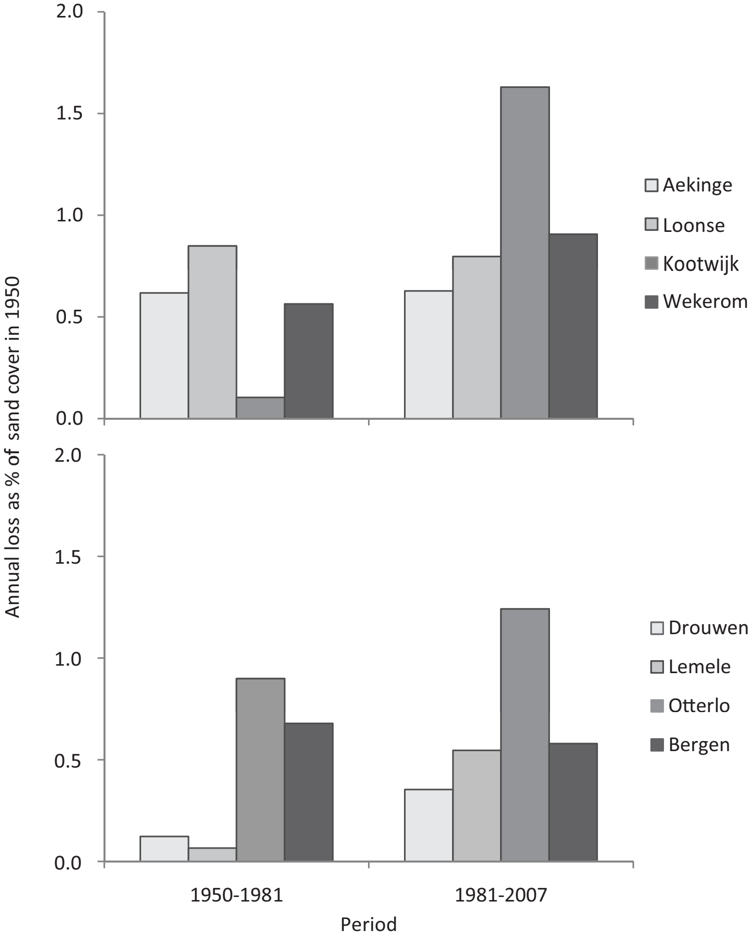

Fig. 2.5. The annual loss of bare sand as percentage of the original cover of bare sand in 1950 for the periods 1950-1981 and 1981-2007, using the succession only data set. Sites are grouped by the dominant geomorphological unit and ranked by increasing N deposition.

2.2). The contribution of bare sand without *C. canescens* individuals is reflected in the bare sand ratio, which is usually lower than 65%. However, the bare sand ratio did not differ between active drift sands and blowouts, or between low and high N deposition regions.

Table 2.2. The distribution of bare sand, pioneer stages, heath and forest in 2007 in active and more stabilized drift sands in regions with low and high N deposition (in % cover), based on the forward succession data set. Bare sand ratio = cover of bare sand divided by cover of bare sand including the *Corynephorus*-stage. *Campylopus* ratio = cover of *Campylopus introflexus* divided by the cover of late pioneer stages (lichens, grasses and *Campylopus*). Forest ratio = cover of forest divided by the cover of heath + forest. [1] significant difference between active and more stabilized drift sands; [2] significant difference between N deposition regions ($P < 0.05$).

	Aekinge	**Loon**	**Kootwijk**	**Wekerom**	**Drouwen**	**Lemele**	**Otterlo**	**Bergen**
drift sand type	active	active	active	active	stable	stable	stable	stable
N deposition region	low	low	high	high	low	low	high	high
Bare sand and *Corynephorus*[1]	29.0	39.7	49.8	23.8	7.1	2.1	2.0	0.3
Bare sand[1]	17.4	27.0	19.4	18.2	4.5	1.3	0.6	0.0
Corynephorus	11.6	12.7	30.4	5.6	2.6	0.8	1.4	0.3
Bare sand ratio	0.60	0.68	0.39	0.76	0.63	0.62	0.30	0.00
Pioneer vegetation	23.4	24.7	45.4	25.6	40.4	29.4	82.2	39.8
Polytrichum	15.1	14.3	42.7	14.1	5.7	10.8	5.4	0.0
Lichens	0.4	1.4	0.0	1.5	8.3	8.1	7.4	0.0
Grasses	7.1	8.9	1.4	6.1	26.4	8.8	0.0	1.4
Campylopus[1,2]	0.8	0.1	1.3	3.9	0.0	1.7	69.4	38.4
Campylopus ratio[1,2]	0.10	0.01	0.48	0.34	0.00	0.09	0.90	0.96
Heath and forest	47.6	35.6	4.8	50.7	52.5	68.5	13.2	59.8
Heath[2]	45.9	14.3	0.0	0.9	28.6	24.5	0.0	6.1
Forest	1.7	21.3	4.8	49.8	23.9	44.0	13.2	53.7
Forest ratio[2]	0.04	0.60	1.00	0.98	0.46	0.64	1.00	0.91

In pioneer vegetation, early pioneer communities with *Corynephorus canescens* and *Polytrichum piliferum* were more prominent in active drift sands than in more stabilized ones. In the latter, older stages with lichen-rich vegetation, but also mats with *Campylopus introflexus* were more abundant. Except for *Campylopus introflexus*, the cover of particular early pioneer stages was not significantly affected by region of N deposition. In regions with high N deposition, however, *Campylopus introflexus* had a significantly higher cover, or formed a higher percentage of older pioneer stages. In low N deposition regions, the *Campylopus* ratio, calculated as the percentage of *Campylopus* *introflexus* of all older pioneer stages combined, did not exceed values of 10%. In regions with high N deposition, the *Campylopus* ratio increased to 37-42% in active drift sands and to 90-96% in more stabilized areas.

Development to heath or forest also seemed to be affected by N deposition. The total amount did not differ between active and more stabilized drift sands or regions with low and high N deposition. In regions with high N deposition, the forest ratio (i.e. the contribution of forest to heath and forest combined) was significantly higher. In contrast, presence or development of heath mainly occurred in regions with low N deposition.

Predicting future development

For each vegetation subclass, the cover of the main vegetation classes in previous years was calculated and presented as a transition matrix (Table 2.3) and Markov chain (Fig. 6). The future cover of the three main vegetation types can be modelled by extrapolating the changes in cover of bare sand and forest/heath (Fig. 2.7). The model assumes unidirectional succession without conservation measures and is based on the observed succession rate in the last period (1995-2007). This model predicts that at the present rates of loss of bare sand, without management, in 2035 no bare sand will be left. If we use the lower succession rates of the period 1951-1981, the year in which bare sand has completely disappeared and transformed into the next succession stage would be 2050. The difference between the 2035 and 2050 scenarios is shown in grey in Fig. 2.7. Together with the vanishing bare sand, the cover of pioneer vegetation and forest will increase. As soon as no bare sand is left, the area of pioneer vegetation will start to decline and gradually transform into heath or forest.

Discussion

Loss of the open inland dune habitat

Our data show that the surface of bare sand in selected inland dune sites declined by 50% between 1950 and 2007 and that the rate of decline increased in the recent times. There are differences between individual sites, mainly connected to geomorphology (drift sand dunes or blowouts) and the percentage cover of bare sand in 1950. Most authors consider the abandonment of the original land use to be the main factor in the decline of bare sand (Koster 2005; Riksen *et al.* 2005). As to the overall increase in succession rate, it is clear that the recent climate change has led to a prolonged growing season and increased precipitation, which must have enhanced the settlement of pioneer vegetation over the past decades. However, the spatial and temporal differentiation in this loss of inland dune habitats, as observed in earlier studies on the development of drift sand landscapes (Riksen & Jungerius 2010) as well as in our study must be attributed to other factors.

Table 2.3. Transition matrix with the percentage of cover of vegetation classes in previous periods for each vegetation subclass in 2007, based on the succession only data set. The column on the right-hand side shows an estimation of the development time.

Vegetation class	**bare sand**			**pioneer vegetation**			**forest or heath**			**development time**
Subclass in 2007	1950	1981	1995	1950	1981	1995	1950	1981	1995	yr
Corynephorus	100	100	100	-	-	-	-	-	-	1
Polytrichum	96	86	53	5	14	47	-	-	-	5
Campylopus dominant	25	6	1	75	94	99	-	-	-	10
Lichens dominant	34	17	2	66	83	98	-	-	-	15
Mosses, lichens, grasses	30	15	6	70	85	94	-	-	-	10-20
Psammophilous heath	15	2	1	57	38	74	29	60	74	20
Forest	27	5	1	34	26	17	39	70	82	30

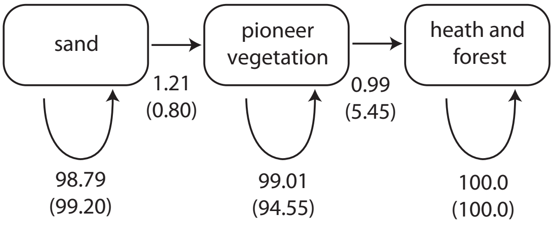

Fig. 2.6. Markov chain representing the succession rate between three vegetation classes for the periods 1950-1981 (between brackets) and 1995-2007. Values represent the percentage of cover in the starting year (1950 or 1995). Figures are based on the succession only dataset.

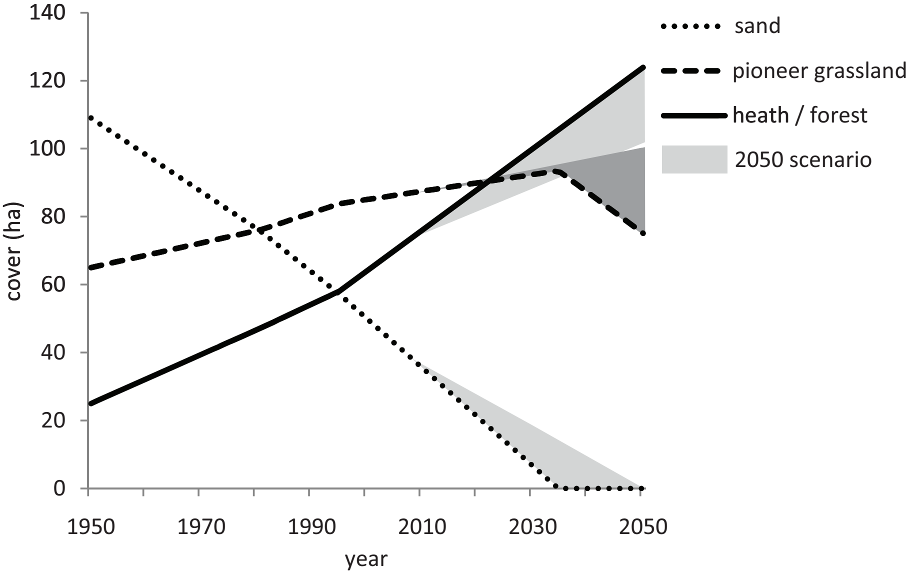

Fig. 2.7. Past changes and a prediction of the amount of the vegetation classes in the eight studied drift sands together, Predictions are based on succession rates in the Markov diagram (Fig. 2.6). From 2007 onwards, solid lines were calculated using the highest succession rates (1995-2007). The difference with the prediction based on the lowest succession rates between 1950 and 1981 is shown in grey (pioneer grassland in darker grey). This figure is based on the assumption of autonomous development without retrogression due to restoration management.

In accordance with earlier studies (Riksen & Goossens 2007; Jones *et al.* 2008), we find that geomorphology is an important factor, blowouts and dunes clearly differing in succession rate. Wind and water erosion cause active dunes to become covered by vegetation at a much lower rate than the relatively stable blowouts.

Vegetation growth in the nutrient-poor inland dune landscape is N limited, at least in low N deposition sites within The Netherlands (Sparrius 2011). Especially in early succession stages, in which plants highly depend on nutrients from atmospheric deposition, the succession rate is positively influenced by extra nitrogen input (Sparrius 2011). The results presented here show that the decline of bare sand is indeed higher where N deposition is high.

Loss of bare sand may be counteracted by local recreation pressure on hiking trails and large sandy surfaces, which causes disturbance of bare sand and disables the settlement of pioneer vegetation (Riksen 2005). A comparable process is the disturbance observed in military training areas, which often have relatively large areas of well-preserved bare sand. Our results show that active drift sands had a general higher number of visitors and more hiking trails than sites dominated by blowouts. However, the total impact is of a minor order as for example evidenced by the data in Table 2.1.

Composition and quality of pioneer vegetation

From the biodiversity perspective, pioneer vegetation is considered the most valuable habitat type within the drift sand landscape, as many species are limited to this habitat type, including many lichens. Results show that in 1950 the highest cover of pioneer vegetation occurs in the more stabilized drift sands, which can be explained by the lower wind erosion and disturbance, resulting in an earlier establishment of the vegetation compared to active drift sands. Consequently, valuable lichen-rich pioneer vegetation, which includes the subclasses lichens and grassland, is found more often on blowouts than on dunes.

Little is known about the development time of older stages of pioneer vegetation. Young stages of pioneer vegetation (e.g. *Polytrichum*) obviously have a higher percentage of bare sand cover in earlier years than late succession stages. However, the rather high values around 50% bare sand cover in 1950 for late stages (*Campylopus*, lichens) suggest that these vegetation types might be locally ancient, probably up to a century old, although permanent plot studies (Ketner-Oostra 2007) and our own observations show that they might develop within a much shorter period of time of c. 15 yr. Longer permanent plot studies are required to make a more precise estimate of the development time of especially lichen-rich vegetations.

N deposition was already found to cause faster growth and early settlement of thick mats of the invasive bryophyte *Campylopus introflexus*, leaving less space for lichen vegetations (Sparrius & Kooijman 2011). Their study included several sites on which results are reported here. We indeed observe that the relative cover of the *Campylopus* vegetation class is much higher in regions with high N deposition in both active and more stabilized drift sand. The quality of pioneer vegetation thus is indeed strongly influenced by the atmospheric input of N.

Also, further development of the vegetation towards *Calluna*-heath seems to be negatively affected by N deposition, in favour of Scots pine forest. Earlier studies have shown that moss mats of *Campylopus introflexus* suppress the germination of *Calluna* (Equiha & Usher 1993), whereas our observations showed that germination of Scots pine does not seem to be affected.

Relevance for the conservation management of drift sand areas

The results of this study show that bare sand in inland dunes is disappearing at an alarming rate. Measures are necessary to keep the drift sand areas open to prevent the open drift sand landscape from becoming covered in forest. At the relatively low rates of succession in the period 1950-1981, without management, bare sand would have disappeared around 2050. At the present higher rates of succession, bare sand will disappear by 2035. Based on the current data, we can describe what management is required to maintain a steady state regarding the relative distribution of succession stages.

Predicted life spans for bare sand of 43 (2007-2035) or 28 (2007-2050) years mean that 1/43th to 1/28th or 2.3% to 3.6 % of the current area of bare sand should be newly created annually to conserve the current area of bare sand. Based on a total area of *c.* 12 km^2 of bare sand in the drift sand habitat left in The Netherlands in 2006 (Riksen & Jungerius 2010), this means a nationwide area of *c.* 28 to 43 ha yr^{-1}. Taking into account that past management measures in Aekingerzand and Wekeromse Zand only led to temporary increase of the area of bare sand, it is strongly advised to use the latter, higher estimate. Currently, the restoration activities in inland dunes in the Netherlands are somewhere in between these figures, with c. 40 ha of restored drift sand annually over the past ten years. Riksen & Jungerius (2010) estimated a net increase of 109 ha of bare sand in the period 2000-2006, meaning that current restoration efforts are sufficient and should be continued.

The foregoing implies that for a full range of succession stages to occur in a balanced series, continuous creation of bare sand is required that subsequently will transform into a later succession stage. In terms of effectiveness and sustainability, restoration of locations with drift sand dunes rather than blowouts is to be preferred, since pioneer vegetation establishes faster in the latter. Similar remarks can be made regarding N deposition, with low deposition providing better conditions for restoration because of the slower succession. Evidently, restoration of drift sand areas with less favourable conditions requires adapted management, mitigating the faster succession. However, such management cannot compensate for the lesser quality of the habitat. Examples from coastal dunes of such reactivation of wind erosion in dunes versus blowouts, including management measures and subsequent vegetation succession has been given by e.g. Arens *et al.* (2004) and Arens & Geelen (2006).

Conservation and related management of the various succession stages depend on the geomorphology and main vegetation type. Active drift sands can be kept open by recreation though its impact in terms of area affected is relatively small. Clear impacts are only likely to occur in inland dune sites with over 1000 visitors ha^{-1} yr^{-1}. Riksen & Goossens (2005) studied conservation measures for large bare sand surfaces, i.e. the removal of *Corynephorus canescens* and small patches of *Polytrichum piliferum* with tillage machinery, such as a beach sand cleaner and rotary cultivator. The management of blowouts could focus on the conservation of lichen-rich pioneer vegetation, by means of young tree-removal and low-density grazing mainly to avoid the settlement of trees. Development of psammophilic *Calluna*-heath does not harm the pioneer vegetation as much as the transformation to forest, as the shrubs are relatively slow-growing, eaten by deer and sheep, and usually form a mosaic with the lichen vegetation (van der Bilt & Nijland 1993).

Conclusions

Windblown bare sand and pioneer vegetation in inland drift sands in The Netherlands are in danger of vanishing and transforming into a forest of self-sown trees. An analysis of eight inland drift sands reserves suggests that geomorphology and N deposition region are driving factors influencing succession rate and habitat quality respectively. The results predict that without conservation measures, bare sand will be lost by around 2035, or then only occur in association with small-scale disturbances, e.g. hiking trails. Bare sand that declined during 1950-2007 did not contribute to a higher cover of species-rich pioneer vegetation, as a similar area of forest or mainly self-sown Scots pine developed. In order to maintain the current area of bare sand, forest on drift sand soils should be removed including their topsoil at a nation-wide total area of between 28 and 43 ha per year.

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Chapter 3

Effects of nitrogen deposition on soil and vegetation in primary succession stages in inland drift sands

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Abstract

Background and Aims: Primary succession was studied in acid inland drift sands. Main research questions were: 1) How do vegetation and soil change during succession? 2) How are soil parameters and species abundance affected by atmospheric nitrogen deposition? *Methods*: 165 plots were selected in 21 drift sands throughout The Netherlands, divided over eight succession stages from bare sand to dry heath and within a gradient in nitrogen deposition. Vegetation development and soil parameters were described and water-extractable elements measured and differences between high (> 30 kg N ha^{-1} yr^{-1}) and low nitrogen deposition sites calculated. *Results*: Vegetation cover and height increased during succession. Lichens contributed most to plant species diversity. Thickness of A_h horizon increased and pH decreased and concentrations of Fe, Al, S increased. Base cations increased as well, despite the drop in pH. Also, water-extractable ammonium, nitrate and phosphate increased, along with the NH_4:NO_3 ratio. Sites with high nitrogen deposition had higher NH_4:NO_3 and Al:Ca ratios, lower pH, higher cover of algae, lower lichen and total species diversity, more *Pinus sylvestris* seedlings and more species of late succession stages. *Conclusions*: Drift sand succession seems to be mainly driven by an increase in organic matter, but is accelerated by nitrogen deposition.

Introduction

Many studies on soils and vegetation of Western European inland dune habitats focused on floristic and syntaxonomical aspects of the mainly cryptogam-dominated vegetations (Daniëls *et al.* 1993; Masselink 1994; Paus 1997). The study by Hasse (2005) has a broader scope, also paying attention to the soil, and provided a generic classification based on species and soil data, such as pH, soil texture, carbon and nitrogen content. The classification starts with bare sand, followed by vegetation dominated by *Corynephorus canescens*, *Polytrichum piliferum*, cup-lichens (and other smaller *Cladonia* spp.), *Campylopus introflexus*, reindeer lichens (and other larger *Cladonia* spp.), grass-dominated vegetation and heath (Fig. 1). Hasse (2005) showed that accumulation of soil organic matter was the main factor driving succession, covariating with pH and nitrogen (N) content.

Apart from the total N stocks, no extensive data exist on the plant-availability of nutrients in drift sands. Elements, such as P, base cations or Al are important factors for plant growth. P is a major plant nutrient and, like N, affected by cycling of organic matter and soil chemical conditions (Kooijman *et al.* 1998; Kooijman *et al.* 2009). Base cations (Ca^{2+}, K^{+}, Mg^{2+} and Na^{+}) are also important, as they may selectively be taken up by the vegetation (Moszynska 1991; Smit *et al.* 2002) and decrease with the drop in pH during succession. A lower soil pH may also lead to an increase in Al^{3+} concentrations and Al:Ca ratios causing potentially toxic conditions to the vegetation (Tietema 1992; de Graaf *et al.* 1997; van den Berg *et al.* 2005a; Bowman *et al.* 2008).

Acidification may reflect a loss of acid-neutralizing capacity of the soil, due to exchange of base cations (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}) for protons from plant roots and microorganisms. Other causes are atmospheric deposition of protons and proton producing substances, such as sulphur in the past (Bleeker and Erisman 1996; Eerens & van Dam 2001) and nitrogen compounds in past and present, particularly ammonium, which causes soil acidification after nitrification (van Breemen *et al.* 1983). Elevated nitrogen deposition may thus cause a lower soil pH, displacement and leaching of base cations and an increase in acid cations, such as Al^{3+}.

The aim of this paper was to further explore the development of nutrient and base status during succession and the impact of atmospheric deposition in inland dunes. The research questions were: 1) How do vegetation and soil change during succession? 2) How are soil parameters and species abundance affected by atmospheric nitrogen deposition? To answer these questions, vegetation relevees and soil samples were collected throughout The Netherlands, divided over several succession stages and within a pronounced gradient in atmospheric nitrogen deposition, which allowed testing for both succession stage and nitrogen deposition.

Materials and Methods

General survey

A field survey was conducted in 21 drift sand areas, distributed over the main cover sand regions (Drenthe, Veluwe, Utrecht, Brabant and river dunes along the Meuse; Fig. 3.1). In the 21 drift sand areas, a set of 165 relevees of random 1 m^2 plots has been selected, stratified over eight different succession stages (Fig. 3.2) representing the chronosequence from bare sand to dry heath. The succession stages are derived from

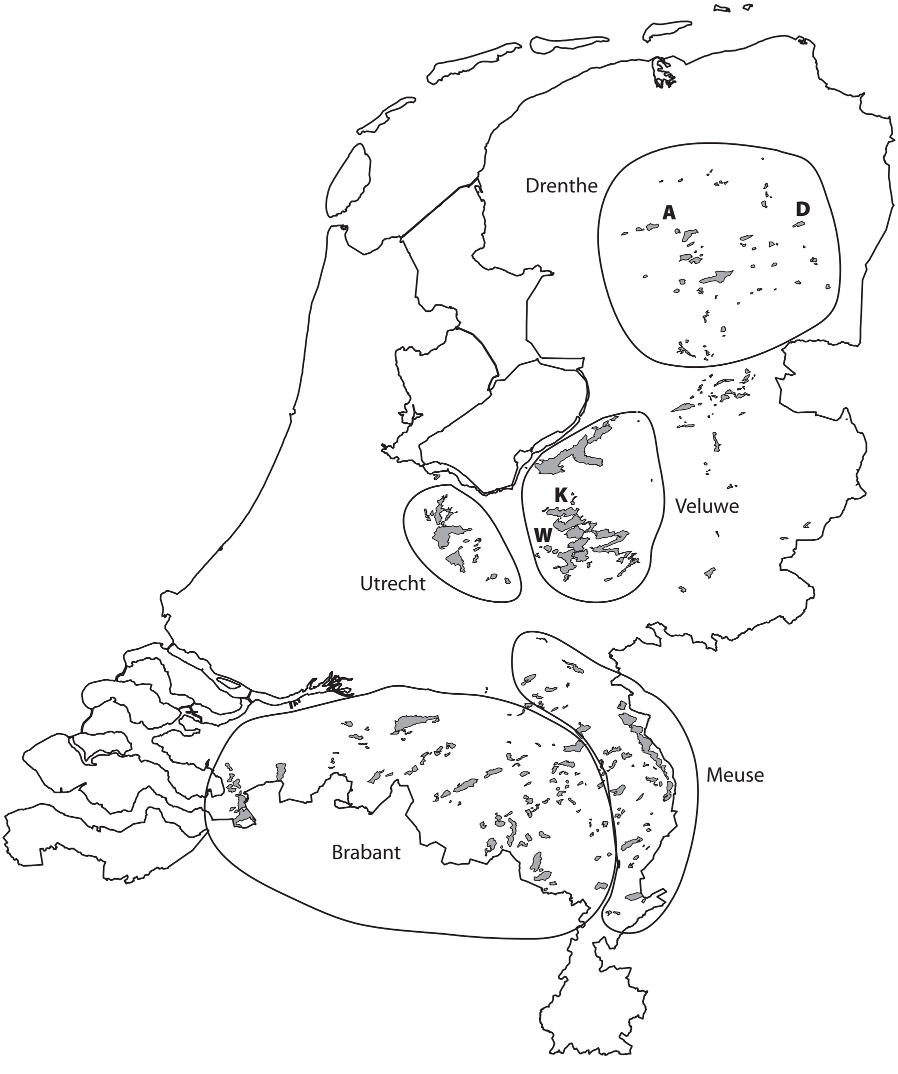

Fig. 3.1. Map of the Netherlands showing the five main cover sand areas, inland dunes and investigated sites (A=Aekinge, D=Drouwen, K=Kootwijk, W=Wekerom) and regions, that are mentioned in the text.

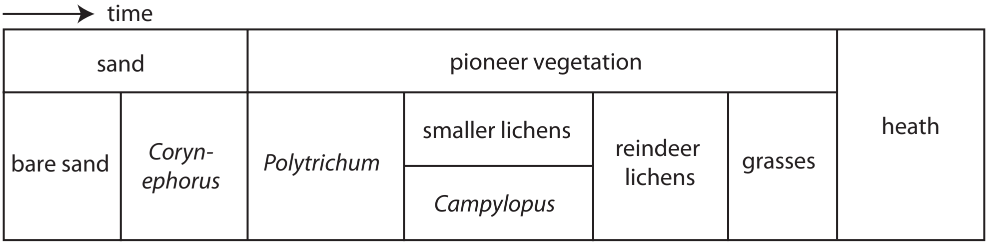

Fig. 3.2. Vegetation succession in inland dunes using a classification in succession stages derived from Hasse (2005). Primary succession includes sand, pioneer vegetation and heath.

Hasse (2005) but are named after the dominant species: 1 = bare sand (n = 24); 2 = bare sand with tufts of *Corynephorus canescens* (n=5); 3 = mats of *Polytrichum piliferum* (n = 22), 4 = vegetation dominated by small lichens (n = 38); 5 = mats of *Campylopus introflexus* (n = 24) and 6 = vegetation with reindeer lichens (n = 28). These six succession stages were extended with 7 = grasses, i.e. vegetations with > 25% grasses, also containing some lichens and bryophytes (n = 9) and 8 = dry heath with > 25% cover of *Calluna vulgaris* (n = 15).

For each of the 165 relevees, the vegetation structure was described: height and cover of the different life forms (vascular plants, cryptogams), bare sand and litter. Names of taxa used in this paper are derived from van der Meijden (2005) for vascular plants, Aptroot *et al.* (2004) for lichens, and Siebel & During (2006) for bryophytes. On mineral soil, in the *Corynephorus* stage, over 22 algal taxa can potentially be found (Prach *et al.* 1993), but in this study only the dominant species were surveyed, i.e. *Klebsormidium* spp (on bare sand poor in organic matter), *Zygogonium ericetorum* (on bare mineral soil rich in organic matter) and *Palmogloea protuberans* (on litter).

In each of the 165 relevees, thickness of the Ah was measured, and relative root biomass estimated in three classes (1 = low, 2 = intermediate and 3 = high). Also, the upper 5 cm of the mineral soil was sampled, combining three subsamples of 100 cm^3 for analysis of water-extractable nutrients and base cations, which is considered to be a good measure for plant-availability. Soil samples were dried at 70 °C for 48 h and weighted to estimate the bulk density. Sieved and homogenized samples were shaken in purified water using a soil:water ratio of 1:2.5. The water extract was filtered over a 0.2 μm membrane. Dissolved organic carbon (DOC), water-soluble phosphate and nitrogen compounds (NH_4^+, NO_3^-) were measured using an Auto Analyzer III (Bran+Luebbe). Cation concentrations were measured with an inductively coupled plasma analyzer (Fisher Iris Intrepid II). Additionally the pH(H_2O) and conductivity (EC_{25}) were measured.

The 165 soil samples were also used to analyze the effect of atmospheric deposition on soil parameters. Atmospheric N deposition was derived from modeled data, based on emissions in 2004 (de Haan *et al.* 2008), and ranged from 21 to 47 kg ha^{-1} yr^{-1}. The median value, based on the average N deposition per site was 30 kg ha^{-1} yr^{-1}, which was also found to be a critical level for effects on the vegetation by

Sparrius & Kooijman (2011). The dataset was split into 69 samples in sites with low deposition, i.e. values below the median value of N deposition, and 96 samples with high deposition. In low and high N deposition areas, distribution of samples over succession stages was more or less the same (chi-square test; $P > 0.05$). However, vegetation with small lichens occurred more often in low-deposition sites, whereas large lichen vegetations were more common in high-deposition sites. In order to test whether soil parameters were affected by high atmospheric deposition of ammonium or, in the past, SO_x, values for each stage of succession were calculated for sites with high and lower N deposition values. High N deposition may affect NH_4:NO_3 and Al:Ca ratios. Al:Ca ratios above 2 are regarded as a critical level for aluminium toxicity for some plants (de Graaf *et al.* 1997). Apart from the Al:Ca ratio, the Al:(Al+Ca) ratio was used, which reduces the variation in the Al:Ca ratio when dividing by relative low values of Ca.

Statistical analysis

A two-way ANOVA was used to test for differences between succession stages, and between high and low N deposition sites, with stage of succession and N deposition as independent parameters. Significant effects ($P < 0.05$) of N deposition on occurrence of particular plant species were tested with a chi-square test for species having at least 15 occurrences in the data set and being 25% more abundant in one group compared to the other.

Results

Species composition

Vegetation cover and height increased during succession (Table 3.1). In the first stage, vegetation cover was only 9%, but cover gradually increased to 99% during succession. Algae and bryophytes dominated in early succession stages, whereas vascular plants, shrubs and lichens had the highest cover in older stages. The tall grass *Ammophila arenaria* is sometimes present in the *Corynephorus* stage as a relic from earlier times, when the species was planted as an erosion prevention measure. Vegetation height increased from 4 up to 27 cm. In early stages, cover of algae was higher than in later stages. Cover of bryophytes and lichens gradually increased, but cryptogam cover was at its highest in the two lichen-dominated stages.

Total species density increased during succession, but some of the species groups showed distinct optima. Algae showed their optimum cover in the *Corynephorus* stage. Highest bryophyte diversity was found in the last stages and includes most liverworts, which were present in shaded niches within *Agrostis vinealis*-dominated grassland and sparse *Calluna* vegetation. Lichen diversity had its optimum in lichen vegetations with small lichen species. Nevertheless, lichens formed an important part of plant diversity in almost all stages. In lichen-dominated stages, lichens contributed to 52-62% of species richness, but in other stages, contribution of lichens still ranged from 23-44%. Many species depend on the presence of patches of bare mineral soil. Total species density was also highest in the stage with smaller *Cladonia* species. Larger reindeer lichens occurred in older, taller pioneer vegetation that was already somewhat encroached by grasses. Vascular plant density was highest in the oldest succession stages. In the first succession stages, diversity was mainly due to grasses. In older stages, more and more herbs were present. The species recorded in the relevees are presented in Table 3.2, ordered by their optimum succession stage. A separate group of indicator species was made for taxa occurring in a maximum of three stages.

Table 3.1. The eight succession stages and vegetation characteristics showing means and standard deviations. A two-way ANOVA was performed to test for differences between succession stages (* = $P < 0.05$).

	Bare sand	**Corynephorus**	**Polytrichum**	**Smaller lichens**	**Campylopus**	**Reindeer lichens**	**Dense grassland**	**Heath**
Number of plots	24	5	22	38	24	28	9	15
Vegetation structure (height in cm, cover in %)								
Height shrubs*	0.0 (0.0)	0.0 (0.0)	0.5 (2.5)	1.3 (4.6)	6.3 (15)	8.2 (25)	2.2 (6.6)	21 (13)
Height vascular plants*	4.0 (12)	14 (8.0)	18 (12)	17 (9.0)	18 (9.0)	26 (10)	27 (11)	26 (14)
Height bryophytes*	0.0 (0.0)	0.2 (0.4)	1.9 (1.6)	2.6 (0.9)	2.7 (0.9)	4.4 (1.2)	2.8 (1.2)	3.1 (1.0)
Cover bare sand*	91 (22)	76 (11)	15 (15)	6.0 (8.0)	4.0 (6.0)	2.0 (4.0)	1.0 (1.0)	7.0 (8.0)
Cover litter*	0.1 (0.3)	1.6 (1.9)	0.9 (1.4)	3.3 (6.8)	5.0 (6.0)	12 (16)	18 (31)	14 (21)
Cover vascular plants*	3.0 (7.4)	18 (12)	14 (8.0)	23 (14)	16 (12)	27 (17)	57 (29)	18 (16)
Cover bryophytes*	0.0 (0.0)	4.2 (8.8)	60 (30)	47 (31)	76 (16)	25 (27)	32 (26)	51 (30)
Cover lichens*	0.0 (0.0)	0.0 (0.0)	7.0 (18)	31(20)	7.7 (11)	43 (27)	22 (29)	13 (10)
Cover algae*	23 (38)	62 (21)	19 (23)	14 (20)	8.0 (20)	3.0 (7)	10 (19)	12 (18)
Species densities ($n\ m^{-2}$)								
All species*	0.6 (0.4)	3.7 (0.4)	6.0 (2.4)	15 (4.5)	10 (3.5)	10 (3.2)	9.8 (3.1)	11 (3.2)
Algae*	0.4 (0.5)	0.7 (0.0)	1.0 (0.5)	1.1 (0.6)	0.6 (0.6)	0.4 (0.6)	0.6 (0.5)	0.9 (0.7)
Bryophytes*	-	0.3 (0.5)	1.4 (0.6)	1.6 (1.3)	1.8 (0.6)	1.7 (0.7)	2.0 (1.3)	1.8 (1.2)
Lichens*	-	0.9 (0.0)	1.4 (0.0)	9.3 (3.8)	4.4 (3.1)	5.2 (2.4)	3.0 (2.3)	4.1 (0.9)
Macrofungi	-	-	0.0 (0.2)	0.0 (0.2)	-	0.0 (0.2)	-	0.1 (0.3)
Vascular plants*	0.3 (0.5)	1.9 (0.5)	2.0 (1.1)	3.2 (1.6)	3.3 (1.5)	3.0 (1.4)	4.2 (1.6)	4.2 (1.9)

Table 3.2. The eight succession stages and their species composition. Species order reflects their appearance during succession. Indicator species are species restricted to a maximum of three succession stages. A two-way ANOVA was performed to test for differences between succession stages (* = P < 0.05).

	Bare sand	Corynephorus	Polytrichum	Smaller lichens	Campylopus	Reindeer lichens	Dense grassland	Heath
Number of plots	25	7	23	39	26	28	10	15
Common species	*Occurrence in plots (%)*							
Corynephorus canescens	16	86	83	85	77	39	50	20
Klebsormidium spp.	36	71	35	31	12	7		33
Carex arenaria	8		9	5	15	18	20	20
Polytrichum piliferum		29	96	87	69	54	30	40
Rumex acetosella			4	23	31	43	30	27
Spergula morisonii			26	31	42	7	20	13
Zygogonium ericetorum			13	5	4	4		13
Ceratodon purpureus			4	3	4		10	7
Palmogloea protuberans			52	77	46	32	60	40
Hypochaeris radicata			9	10	8	7	20	13
Campylopus introflexus			30	46	96	32	20	40
Cladonia coccifera			57	92	73	50	40	73
Agrostis vinealis		14	39	62	42	46	40	40
Cladonia ramulosa			13	69	35	57	30	53
Cladonia subulata			9	18	23	21		20
Cladonia floerkeana			4	36	15	7	10	13
Cladonia glauca			4	41	15	7	20	13
Cladonia arbuscula		14		28	8	25	10	7
Cladonia crispata				46	15	18	10	7
Cladonia uncialis		14		51	19	29		7
Cladonia zopfii				41	19	14	10	7

Cladonia furcata			4	10		29	40	13
Cladonia macilenta			4	38	31	14	20	20
Cladonia portentosa			4	46	23	93	40	27
Cladonia grayi			13	51	42	43	50	40
Placynthiella icmalea				5	4	7	20	20
Pinus sylvestris (seedlings)				13		21	10	20
Cladonia pulvinata				21	4	4		7
Festuca filiformis			9	49	38	32	70	13
Calluna vulgaris			4	10	19	18	10	93
Deschampsia flexuosa			4	18	19	46	50	47
Hypnum jutlandicum			4	3	4	21	20	27
Dicranum scoparium				18	8	50	60	33
Indicator species								
Ammophila arenaria	4		9					
Placynthiella uliginosa		14		10				13
Salix repens			4					
Stereocaulon condensatum			13	8	4			
Diploschistes muscorum				10	4			
Cetraria aculeata		14		46	12	4		7
Cladonia borealis				31	12			7
Cladonia cervicornis		14		41	19	11		
Cladonia foliacea			4	21	8	7		
Cladonia gracilis				46	8	43		7
Cladonia monomorpha				18	8			7
Cladonia phyllophora				5				
Placynthiella oligotropha				3				
Pycnothelia papillaria				3				

Cephaloziella divaricata			3				
Micarea lignaria			3				
Cladonia strepsilis			31	8	7		
Cladonia verticillata			13				
Micarea leprosula			13	4			7
Stereocaulon saxatile			5	8			
Trapeliopsis granulosa	14		8	8			
Cetraria islandica			15		11		
Cladonia ciliata			3		11		
Festuca ovina subsp. hirtula				4		10	
Ptilidium ciliare						10	
Juncus squarrosus	14						20
Filago minima		4					7
Gnaphalium sylvaticum			3				7
Teesdalia nudicaulis			3				7
Jasione montana					4		7
Nardus stricta			3		11		13
Galium saxatile							7
Baeomyces rufus		9	3		4		13
Molinea coerulea			3	4			13
Polytrichum juniperinum					4	10	13
Empetrum nigrum							7
Juniperus communis							7
Lophozia ventricosa							7
Pohlia nutans							7
Scapania compacta							7
Thymus serpyllum							7

In the bare sand and *Corynephorus* stages, the vegetation consisted of single tussocks of *C. canescens* in bare sand, with the sand grains often stuck together by algal filaments of *Klebsormidium* sp., an indicator species present on patches of bare sand was the lichen *Stereocaulon condensatum*. Over time, the space between the tussocks of *Corynephorus canescens* became filled up by the bryophyte *Polytrichum piliferum*. The moss mat still had an open structure, with sand visible between the individual shoots. Eventually, most of the space became filled up with sparse grasses like *Agrostis vinealis*, but especially with small lichen species, such as *Cladonia coccifera*, *C. ramulosa* and *C. grayi*. In the stage with *Campylopus introflexus*, which also established in the *Polytrichum* moss mat, many lichen species may still occur, even though lichen diversity had clearly dropped and smaller species were generally lacking.

Most vegetation parameters clearly differed between succession stages, but not between areas with low and high N deposition. However, significant effects of atmospheric N deposition were found for cover of algae, total number of species and number of lichen species (Table 3.3). Cover of algae was generally two times higher in areas with high N deposition. At high N deposition, total species number was lower in almost all succession stages than at low N deposition. Number of lichen species also significantly decreased at high N deposition, especially in the stage with small lichens.

Table 3.3. Significant effects of N deposition on vegetation characteristics in sites with relatively low (< 30 kg N ha^{-1} yr^{-1}) and high atmospheric N deposition. Means (n = 2-22) and standard deviations. * = significant difference between high and low deposition sites (two-way ANOVA).

	N deposition	Corynephorus	Polytrichum	Small lichens	Campylopus	Reindeer lichens	Dense grassland	Heath
Cover of algae*	low	17 (31)	15 (25)	7 (7)	2 (2)	4 (6)	5 (7)	6 (7)
(%)	high	39 (42)	23 (23)	25 (27)	13 (26)	4 (8)	12 (22)	20 (25)
Total plant species *	low	1 (1)	7 (3)	17 (5)	11 (4)	11 (5)	10 (4)	10 (4)
	high	1 (1)	6 (2)	14 (3)	10 (3)	10 (3)	10 (3)	12 (2)
Lichen species *	low	0 (0)	2 (2)	10 (4)	5 (3)	5 (3)	4 (1)	3 (2)
	high	0 (0)	1 (1)	8 (3)	4 (3)	5 (2)	3 (3)	5 (3)

Few individual species showed a response to N deposition (Table 3.4). The lichens *Cetraria aculeata* and *Cladonia floerkeana*, species characteristic for early succession stages, and the perannual herb *Hypochaeris radicata*, characteristic for late succession stages were species that had significantly higher frequency in low-deposition sites. Several species were more abundant in high-deposition sites, including species from late succession stages (*Cladonia gracilis*, *C. zopfii*), species from substrates rich in organic matter (*C. macilenta*, *C. ramulosa, Dicranum scoparium*), an annual herb from early succession stages (*Spergula morisonii*), a graminoid (*Carex arenaria*) and seedlings of trees (*Pinus sylvestris*).

Table 3.4. Significant effects of N deposition on individual species presence as % of sites with relatively low (< 30 kg N ha^{-1} yr^{-1}) and high atmospheric N deposition.

Species	Low N	High N
Cetraria aculeata	20	7
Cladonia floerkeana	20	10
Hypochaeris radicata	17	4
Carex arenaria	3	21
Cladonia gracilis	13	24
Cladonia macilenta	16	23
Cladonia ramulosa	29	47
Cladonia zopfii	13	19
Dicranum scoparium	17	24
Pinus sylvestris (seedlings)	7	10
Spergula morisonii	17	26

Soil characteristics

During succession, thickness of the A_h increased from zero to approximately 7 cm, and bulk density and pH decreased (Table 3.5). Along with the decrease in pH, the amounts of water-extractable Fe, Al and S in the topsoil increased, as well as Al:Ca ratio. Base cation concentrations were two to three times higher in late succession stages compared to open sand and *Corynephorus*, despite the decrease in pH. Divalent cations such as Ca and Mg occurred in lower quantities in water extracts than monovalent cations like Na and K, which can be regarded as a side effect of the use of water instead of extraction with a salt solution. Also water-extractable ammonium, nitrate and phosphate both increased during succession, as well as the NH_4:NO_3 ratio.

Most soil parameters clearly changed during succession, but did not differ between areas with low and high atmospheric N deposition. However, NH_4:NO_3 ratio, pH and Al:(Al+Ca) ratio were indeed affected by N deposition (Table 3.6). NH_4:NO_3 ratio was significantly higher at high N deposition, especially in later stages of succession. Soil pH decreased with 0.1-0.3 units in sites with high atmospheric deposition. Also, Al:Al+Ca ratio was significantly higher at high N deposition. The Al:Ca ratio was consistently 0.2-0.9 units lower in the low-deposition sites, but the difference was not significant.

Table 3.5. The eight succession stages and their soil characteristics. A two-way ANOVA was performed to test for differences between succession stages (* = $P < 0.05$).

	Bare sand	**Corynephorus**	**Polytrichum**	**Small lichens**	**Campylopus**	**Reindeer lichens**	**Dense grassland**	**Heath**
Number of plots	24	5	22	38	24	28	9	15
Soil parameters	*means and standard deviations*							
Ah solum depth (cm) *	0.2 (1.0)	0.0 (0.0)	1.0 (1.0)	5.9 (3.5)	4.0 (2.1)	6.8 (4.1)	7.0 (4.7)	6.4 (4.4)
Soil bulk density (g cm_{-3}) *	1.3 (0.1)	1.2 (0.1)	1.1 (0.2)	0.9 (0.2)	1.0 (0.2)	0.8 (0.1)	0.6 (0.2)	0.8 (0.2)
Relative root biomass in A_h (1..3) *	0.0 (0.2)	0.4 (0.5)	0.6 (0.7)	1.3 (0.6)	0.8 (0.6)	1.6 (0.6)	1.5 (0.7)	1.5 (0.5)
pH(H_2O) *	4.9 (0.4)	5.2 (0.5)	4.9 (0.3)	4.5 (0.3)	4.5 (0.3)	4.3 (0.3)	4.2 (0.5)	4.6 (0.5)
Conductivity EC_{25} *	16 (4.0)	14 (2.0)	21 (7.0)	35 (15)	44 (40)	54 (15)	96 (46)	54 (33)
Soil chemistry (water-extractable)								
Acid cations (mmol m^{-2})								
Al *	1.3 (1.1)	0.5 (0.2)	2.0 (0.9)	2.8 (1.4)	3.4 (1.6)	4.2 (1.7)	4.5 (1.8)	3.8 (2.6)
Fe *	0.2 (0.2)	0.0 (0.0)	0.3 (0.2)	0.8 (0.4)	0.9 (0.6)	1.1 (0.5)	1.0 (0.9)	1.0 (0.7)
S^{tot} *	4.2 (1.5)	4.1 (1.4)	4.7 (1.5)	5.2 (2.7)	6.1 (3.6)	7.0 (2.8)	9.7 (4.5)	6.8 (4.4)
AL:CA ratio *	1.8 (1.4)	0.7 (0.3)	2.0 (0.9)	2.2 (0.9)	2.5 (0.9)	2.4 (1.0)	2.1 (1.8)	2.3 (2.2)
Base cations (mmol m^{-2})								
Ca *	0.8 (0.2)	0.8 (0.2)	1.1 (0.3)	1.3 (0.8)	1.5 (1.1)	1.9 (0.7)	2.5 (1.1)	2.3 (2.5)
K *	2.3 (1.5)	3.7 (2.5)	4.9 (2.1)	4.8 (2.4)	5.0 (2.8)	7.1 (2.2)	8.9 (2.3)	7.1 (3.9)
Mg *	0.7 (0.4)	0.8 (0.1)	1.2 (0.4)	1.5 (1.0)	1.6 (0.9)	2.0 (0.7)	3.1 (1.2)	2.1 (1.1)
Na *	3.8 (2.0)	3.0 (1.2)	6.5 (3.0)	6.7 (3.2)	7.9 (3.9)	7.2 (2.0)	8.7 (3.1)	8.5 (3.6)

Sum of base cations *	7.7 (3.6)	8.5 (3.6)	14 (4.7)	15 (5.8)	16 (7.4)	18 (4.4)	23 (6.4)	20 (8.1)
Nutrients (mmol m^{-2})								
NH_4^+ *	1.0 (1.4)	0.1 (0.2)	0.8 (0.9)	2.2 (2.4)	3 (4.6)	6.1 (5.2)	12 (17)	9.1 (20)
NO_3^- *	3.9 (3.9)	1.7 (1.0)	2.3 (1.7)	4.8 (4.7)	7.9 (13)	5.3 (4.9)	7.9 (7.0)	5.9 (12)
NH_4 as % of total N *	20 (21)	6.0 (14)	23 (24)	28 (23)	22 (20)	52 (28)	54 (21)	42 (39)
NH_4:NO_3 ratio *	0.4 (0.7)	0.1 (0.2)	0.5 (0.6)	0.7 (1.2)	0.4 (0.5)	2.7 (3.9)	2.3 (3.2)	2.4 (3.2)
PO_4^{3-} *	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (0.5)	0.6 (1.4)	1.3 (1.4)	1.8 (1.9)	1.4 (1.8)

Table 3.6. Significant effects of N deposition on soil characteristics in sites with relatively low (< 30 kg N ha^{-1} yr^{-1}) and higher atmospheric N deposition. Means (n = 2-22) and standard deviations. * = significant difference between high and low deposition sites (two-way ANOVA).

	N deposition	**Corynephorus**	**Polytrichum**	**Small lichens**	**Campylopus**	**Reinder lichens**	**Dense grassland**	**Heat**
NH_4:NO_3 ratio*	low	0.4 (0.5)	0.8 (0.7)	0.5 (0.7)	0.4 (0.6)	0.8 (0.7)	0.6 (0.1)	2.1 (2.0)
(mmol $mmol^{-1}$)	high	0.4 (0.7)	0.3 (0.5)	1.0 (1.6)	0.5 (0.4)	3.2 (4.2)	2.8 (3.6)	2.7 (4.0)
pH *	low	5.0 (0.4)	5.0 (0.1)	4.7 (0.4)	4.5 (0.5)	4.6 (0.6)	4.3 (0.3)	4.7 (0.7)
	high	5.0 (0.5)	4.9 (0.4)	4.4 (0.2)	4.5 (0.3)	4.3 (0.2)	4.3 (0.6)	4.5 (0.4)
Al:Al+Ca ratio *	low	48 (22)	63 (11)	66 (11)	66 (10)	64 (6)	58 (4)	60 (20)
(mmol $mmol^{-1}$)	high	57 (15)	65 (10)	68 (6)	70 (8)	70 (8)	65 (13)	67 (11)
Al:Ca ratio	low	1.4 (1.4)	1.9 (0.8)	2.3 (1.2)	2.2 (0.9)	1.8 (0.4)	1.4 (0.2)	2.0 (1.0)
(mmol $mmol^{-1}$)	high	1.7 (1.4)	2.1 (1.0)	2.2 (0.5)	2.6 (1.0)	2.5 (1.0)	2.3 (1.2)	2.3 (1.0)

Discussion

Vegetation succession

The clear increase in vegetation cover and changes in species composition during succession were in accord with Hasse (2005). Succession started with bare sand, which is colonized by *Corynephorus canescens*, in active drift sands usually only after mild winters and wet summers (Riksen *et al.* 2008). Cover of algae was especially high in the *Corynephorus* succession stage, which agrees with previous studies (van den Ancker *et al.* 1985; Hasse 2005; Guo *et al.* 2008). In this stage, where the vegetation consists of single tussocks of *C. canescens* in bare sand, with the grains stuck together by algal filaments of *Klebsormidium* sp. Indicator species may be used for the assessment of habitat quality and site history (Ott 1978). The tall grass *Ammophila arenaria* may be seen as a relic from earlier times (c. 1850 to 1930) when the species was planted as an erosion prevention measure (van der Meijden 2005). As establishment of *A. arenaria* is restricted to bare sand (van der Putten *et al.* 1993), it is now a good indicator of sites that have not been covered in vegetation over the past 80-160 years. Another indicator species present on bare sand is *Stereocaulon condensatum*, a lichen containing cyanobacteria in addition to green algae, which enable nitrogen fixation, supporting findings by Sparrius (2011) that the early stages of pioneer vegetation are strongly N limited.

Over time, the space between the tussocks of *Corynephorus canescens* becomes filled up by the bryophyte *Polytrichum piliferum*, which creates a uniform A_h horizon consisting of moss rhizoids (Bowden 1991). The moss mat has an open structure, with sand still visible between the individual shoots. Splash erosion is an important factor to maintain open sand, especially on slopes (Riksen & Goossens 2007), which reduces colonization by other species and may favour the development of highly adapted species characteristic of the smaller lichen vegetation. Eventually, most of the space between the individual *P. piliferum* shoots becomes filled up with small lichen species and sparse grasses, especially tussocks of *Festuca* species in addition to *Corynephorus canescens*. In accord with Hasse (2005), the first lichens in this stage are primarily species that usually produce fungal spores (*Cladonia coccifera* and *C. grayi*) and fine soralia or fragments (*Cladonia glauca* and *C. macilenta*). Observations show that species with larger diaspores settle later, e.g. *Cladonia borealis*, *C. strepsilis* and species with a shrub-like, reindeer lichen habit (*C. arbuscula*, *C. portentosa* and *C. zopfii*). This general increase in size in individual lichen thalli may be related to water retention in the topsoil and transpiration of the vegetation, causing a milder microclimate.

Since its introduction in the 1960s, the exotic, invasive bryophyte *Campylopus introflexus* establishes between *Polytrichum piliferum* shoots as well (Sparrius & Kooijman 2011). In contrast to the smaller lichens, *C. introflexus* is a strong competitor, especially as it forms an ectorganic layer of several cm. This ectorganic layer improves N mineralization and nutrient cycling (Sparrius 2011). Conversely, most lichens form only a thin mat, incapable of competing with taller species. *Campylopus introflexus* is predominantly present in sites with high N deposition (Sparrius and Kooijman 2011) and usually forms a uniform moss mat up to 5 cm high. However, under low N deposition, reindeer lichen species may become dominant, and form a succession stage that may last for several decades, before turning into heath and forest (Sparrius 2011).

Both *Campylopus* and lichen vegetations are eventually invaded from below by stoloniferous grasses, mostly *Agrostis vinealis* and sometimes *Carex arenaria*. The vegetation then usually transforms into grassland, with a shorter or longer period in which reindeer lichen species dominate. In older stages, more and more herbs were present, many of which are indicators of well-buffered soils and primary occur in the Genista anglicae-Callunetum community (Weeda 1996).

At some sites, especially on gravel-rich blowouts and sometimes on sites with buried profiles *Calluna vulgaris* settles to form a mosaic with lichen vegetations. Apart from lower aeolian dynamics and more stable conditions for soil development, which allows the vegetation to grow faster, sites with a buried soil or a vertical transition of drift sand to more compact (peri)glacial deposits may also have improved growth conditions via higher water retention capacity as found by Bijlsma (2010) in forested drift sands. Such more humid places can be recognized in earlier succession stages by the presence of *Juncus squarrosus*, *Molinea caerulea* and *Salix repens*. The lifespan of species-rich lichen vegetations may be prolonged if *Calluna* growth and forest development is controlled by frequent grazing and tree removal (Daniëls *et al.* 2008; Sparrius 2011).

Soil formation and nutrient availability during succession

In accord with Hasse (2005), soil organic matter increased during succession and the pH became lower as a result of the accumulation of soil organic matter. However, in spite of the drop in pH, base cations also increased during succession. This can be explained by the increase in soil organic matter and cation exchange sites and concurrent enhanced capacity of the soil to retain cycled base cations (see e.g. Berendse 1998). Also, the abundance of living grass roots in the topsoil increase with succession, resulting in especially higher levels of phosphate and potassium (Nye 1968). Plant available ammonium, nitrate, potassium and phosphate increased during succession as well, nutrients promoting biomass production and accumulation of soil organic matter. Of both N forms, ammonium becomes the dominant N form in later succession stages, probably as a result of better adsorption of ammonium from atmospheric deposition to soil organic matter.

Effect of N deposition on the vegetation

High N deposition seemed to affect the composition of the vegetation. The total number of species was lower in high-deposition areas. For vascular plants, field surveys in the same sites by Nijssen *et al.* (2011) have shown that herbs like *Jasione montana* and *Filago minima*. occurred more frequently in low-deposition sites. However, those species are always present at very low densities, which made it impossible to detect differences in abundance with the method used in this paper.

Pinus sylvestris and *Spergula morisonii* showed a strong affinity with high-deposition sites. These species usually settle in early succession stages with low N availability (Sparrius 2011), and may thus profit from higher N. Although survival of young trees strongly depends on weather conditions, a higher abundance of seedlings can be seen as an indicator for permanent settlement of trees, which is a problem in inland dunes, as removal of young trees is the most frequently applied conservation measure to maintain the open landscape (Riksen *et al.* 2008; Nijssen *et al.* 2011).

Higher algal cover was also found by van den Berg *et al.* (2005b), who found a three-fold increase of algal biomass after fertilization with a dose equivalent to 80 kg N ha^{-1} yr^{-1} on calcareous dune sand at a site with 1 kg N ha^{-1} yr^{-1} background deposition.

The decrease in number of lichen species at high N deposition corresponds with the general decrease of lichens with application of N (Gordon *et al.* 2001; Soudzilovskaia *et al.* 2005; Hasse & Daniëls 2006; Britton & Fisher 2010; Sparrius 2011). The decrease in lichen species was most prominent in vegetation dominated by small cup-lichens. Effects for individual species were more difficult to detect, as they were mainly present in low densities. Two lichens *Cetraria aculeata* and *Cladonia floerkeana* of early succession stages with patches of bare soil and a perennial herb showed a clear preference for low-deposition sites. Common species of later succession stages, such as *Cladonia gracilis, C. macilenta, C. ramulosa, C. zopfii* and *Dicranum scoparium* even showed a preference for high-deposition areas, which suggests that they are less sensitive to N deposition than smaller species. Apart from *C. zopfii*, all these species have a much wider distribution than typical inland dune species and also occur on decomposing litter (Hasse 2005). *C. macilenta* and *Dicranum scoparium* also occur widely on wood and epiphytic (van Herk & Aptroot 2004; Siebel & During 2006). This suggests that in sites with high N deposition, typical inland dune lichens seem to be replaced by more ubiquitous species. The grass *Carex arenaria* was mainly found in sites with high N deposition. In lime-poor coastal dunes, *C. areneria* is the main threat to species-rich pioneer vegetation as its leaf litter decomposes slowly (Kooijman & Besse 2002).

Effect of N deposition on the soil

Within the range of succession stages, soil carbon content is the most important factor explaining pH and N availability (Berendse 1998, Hasse 2005, Ketner-Oostra & Sýkora 2008, Sparrius 2011). However, N deposition seemed to play a role as well. Differences between low and high deposition sites were found with respect to indicators of soil acidification, such as pH and the ratio between Al and Ca. This can be explained by atmospheric deposition of protons and proton producing substances, such as sulphur in the past (Bleeker & Erisman 1996; Eerens & van Dam 2001). As atmospheric N deposition mainly originates from lifestock breeding, high-deposition sites have elevated ammonium levels whereas deposition of nitrate is more or less equal in high and low-deposition sites. This difference in atmospheric deposition is reflected in a higher ammonium:nitrate ratio in the soil. Ammonium causes displacement of especially the less bound Ca^{2+} ions, compared to the stronger bound Al^{3+} on cation exchanges sites (van Breemen *et al.* 1983; Bowman *et al.* 2008). Ammonium may also stimulate nitrification by the increased amount of substrate for nitrifying bacteria (van Breemen *et al.* 1983; Sparrius 2011), which is also responsible for soil acidification The observed increase in the Al:Ca ratio could be due to increased release of Al from fines, which are sequioxides and clay coatings on soil particles. Al dissolution is considerably enhanced by the drop in pH (Sevink & de Waal 2010).

Conclusions

Drift sand succession seems to be mainly driven by an increase in organic matter, but is accelerated by nitrogen deposition. Solum depth, nutrient availability and amount of base cations of the soil increased during succession, along with the increase in soil organic matter, and despite the decrease in pH. Increased N deposition cause cation displacement from cation exchange sites and leaching of base cations from the top soil resulting in a lower pH and higher Al:Ca and NH_4:NO_3 ratios. The vegetation is affected by nitrogen deposition, promoting growth of algae, seedlings of *Pinus sylvestris* and some other species of late succession stages, but reducing diversity in lichens

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Chapter 4

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

L.B. Sparrius & A.M. Kooijman

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 A_h 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 NH_3 m^{-3}) and 41 kg N ha^{-1} yr^{-1} in Wekerom (9.7 µg NH_3 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 seres 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 cm^3 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 K_2SO_4 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 K_2SO_4 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, CO_2 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 m^2 to account for decrease in bulk density during succession. N:P and C:N ratios were calculated in g g^{-1}, NH_4^+:NO_3^- 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(H_2O) 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

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(K_2SO_4)	C (g m^{-2})	N (g m^{-2})	C:N ratio	pH(K_2SO_4)	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)

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 ectorganic layers to the N mineralization also became more important. In lichens, contribution of the ectorganic 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 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 ± 1.1 in Aekinge, and 6.0 ± 5.7 in Wekerom.

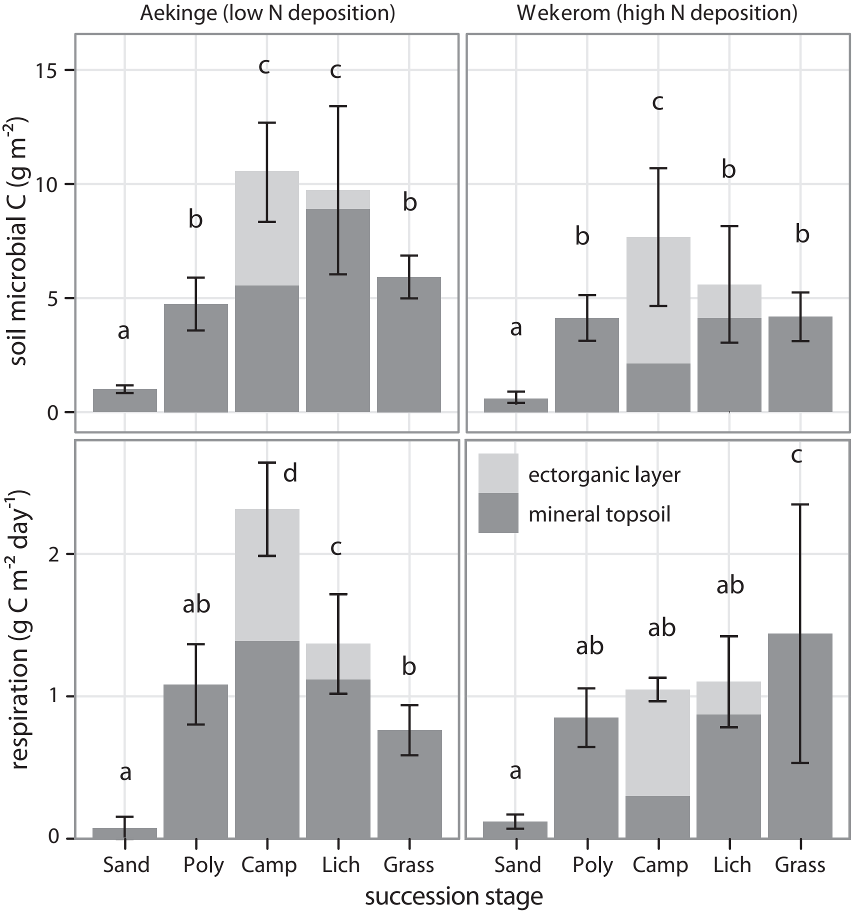

Fig. 4.1. Microbial biomass and respiration under lab conditions measured in fresh soil samples in different succession stages in drift sand in both study sites. Means (n = 4) and standard deviations (error bars) of the soil and ectorganic layer. Names of succession stages are abbreviated to the first 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.

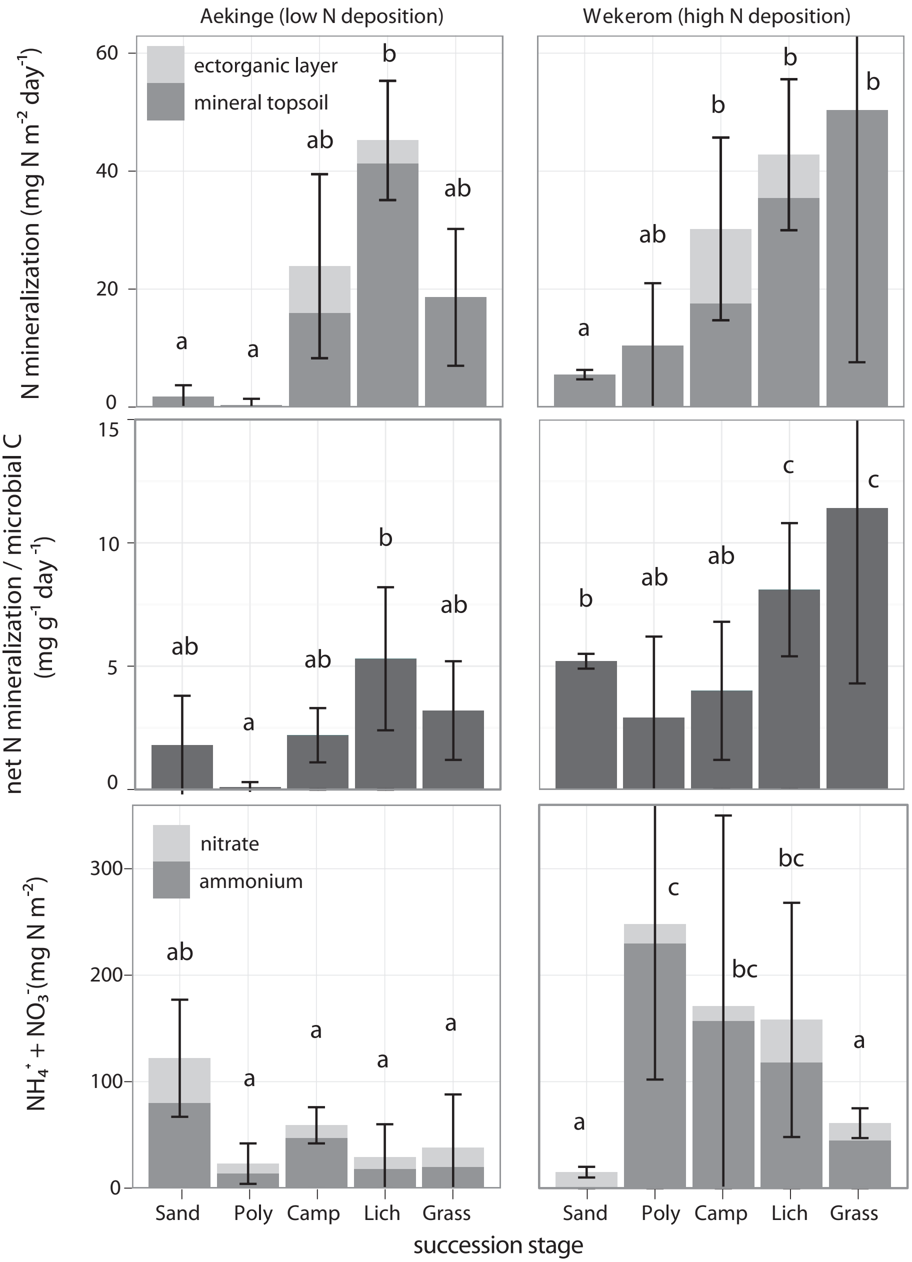

Fig. 4.2. Net N mineralization and N mineralization per unit microbial C (soil + ectorganic layer) under lab conditions and amounts of K_2SO_4-extractable ammonium and nitrate in fresh samples of soil and ectorganic layer (*Campylopus* and lichen stages only) in different succession stages in drift sand 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.

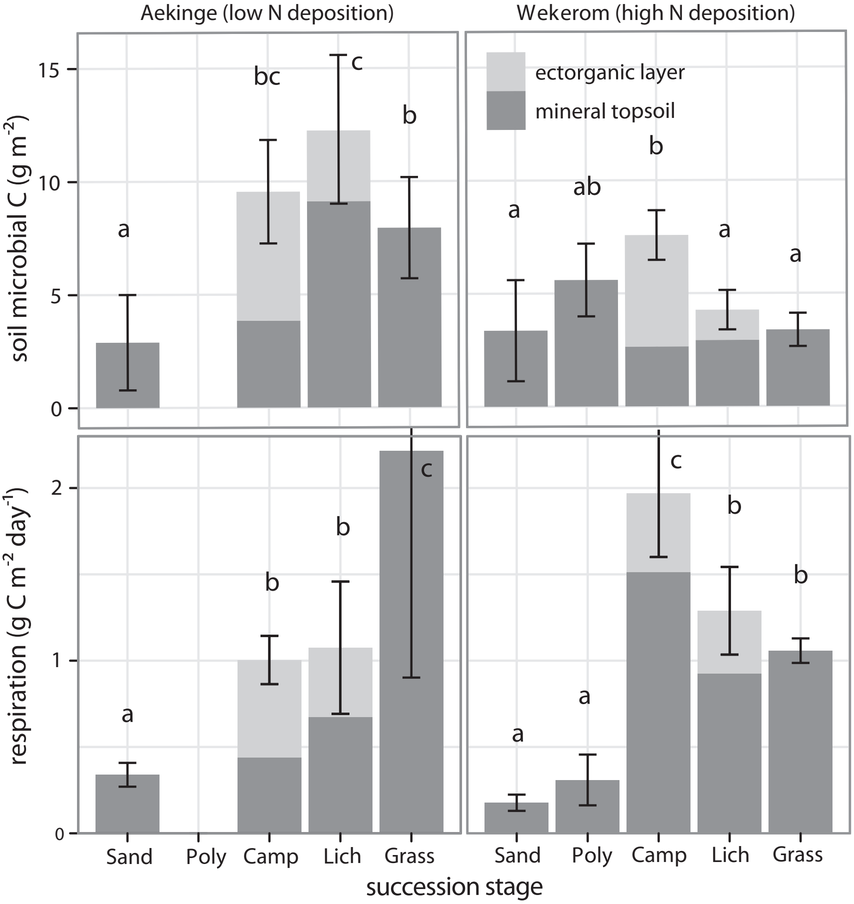

Fig. 4.3. Microbial biomass and respiration under lab conditions measured in fresh soil samples in different succession stages in restored sites in both study sites. Means (n = 4) and standard deviations (error bars) of the soil and ectorganic layer. Names of succession stages are abbreviated to the 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.

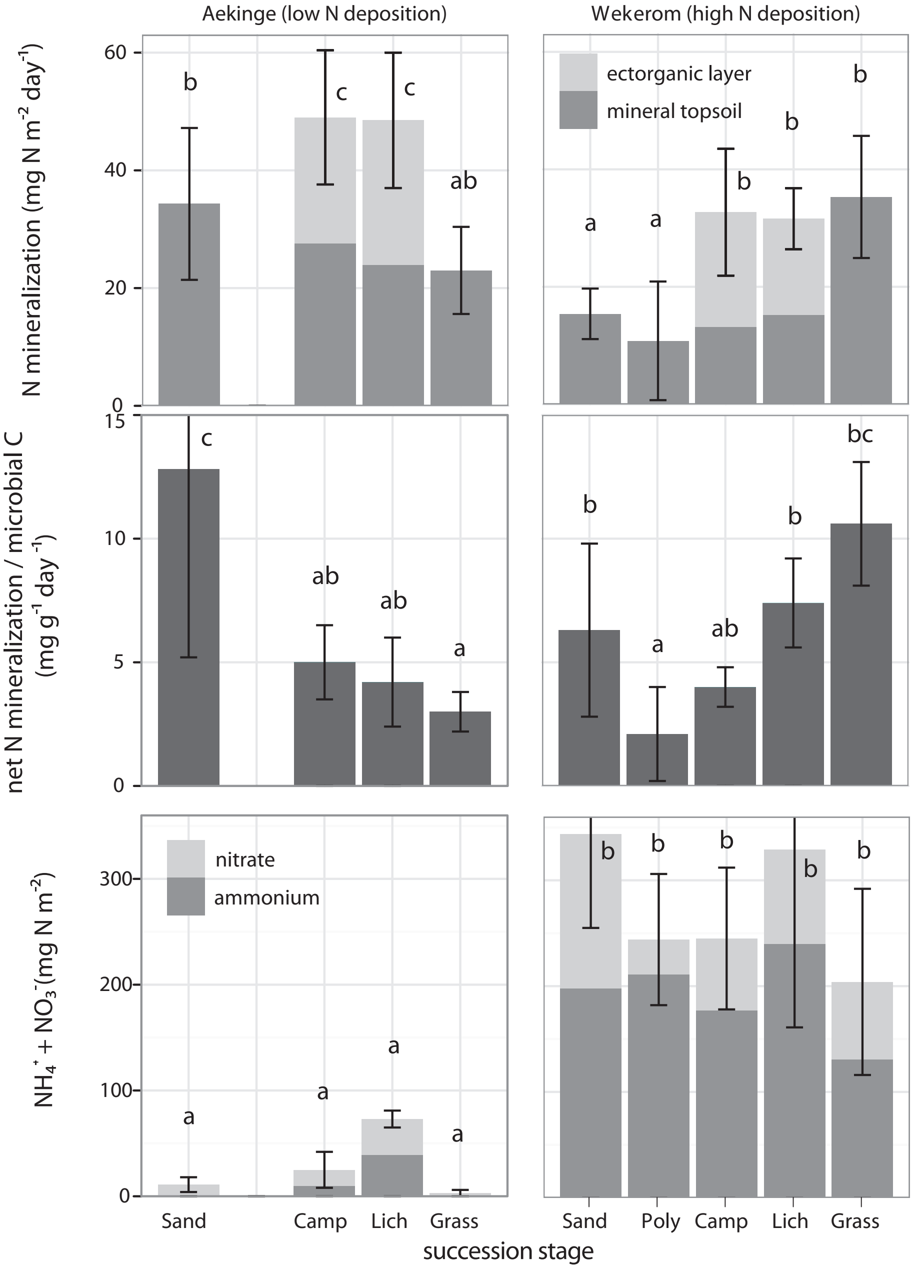

Fig. 4.4. Net N mineralization and N mineralization per unit microbial C (soil + ectorganic layer) under lab conditions and amounts of K_2SO_4-extractable ammonium and nitrate in fresh samples of soil and ectorganic layer (*Campylopus* and lichen stages only) in different succession stages in restored 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 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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Chapter 5

Response of inland dune vegetation to increased nitrogen and phosphorus levels

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Abstract

Question: How does pioneer vegetation of acid inland dunes respond to addition of nitrogen and phosphorus? *Location*: Two inland dune reserves in The Netherlands with low and high N deposition. *Methods*: During 2.5 years, nitrogen and phosphorus addition (control, N, P, NP) took place in three different succession stages in inland dunes (*Polytrichum piliferum* mats, *Campylopus introflexus* mats and lichen-dominated vegetation). In each site, changes in the vegetation were recorded in three replicate quadrats of 1 m^2 per treatment. Differences in element content of grasses and lichens were recorded along with vegetation parameters, including cover and height of grasses, bryophytes and lichens. *Results*: In the site with high N deposition grasses were taller and had higher N:P ratios and there was a generally lower lichen cover than in the low-deposition area. Experimental N application resulted in higher N and lower base metal concentrations in grasses and increase in grass cover and size. In contrast, lichens showed a general decline in the N treatment and lichen:grass ratio decreased. The effect of N addition was larger in the low-deposition area. The P treatment showed an opposite effect: lichens increased in size and cover and overgrew grasses especially when reindeer lichens were present. This suggests that the cryptogam layer was P-limited even in the low N deposition site. *Conclusions*: Pioneer vegetation in inland dunes is susceptible to N and P addition. The results provide evidence for a decrease of lichen cover due to increased N deposition.

Introduction

Drift sands in The Netherlands are semi-arid, semi-natural landscapes with wind-blown, nutrient-poor, acid sandy soils. Until about 1850, most drift sands had no vegetation cover at all and were a threat to neighboring arable land and settlements (Fanta & Siepel 2010). Large-scale afforestation with mainly *Pinus sylvestris* took place in the 1890s until the 1940s. Some inland dune areas were protected as nature reserves, but until the 1970s no conservation management took place, resulting in a further loss of open sand and increase of short pioneer vegetation, forest and self-sown *Pinus* trees.

Inland dune vegetations are characterized by small-scale mosaic vegetation. Over decades, the initial pioneer vegetation (Spergulo-Corynephoretum) develops into typical lichen-dominated grasslands, with many species that are rarely found outside inland dunes. The importance of lichen vegetations was already known in the 1950s (Stoutjesdijk 1959). Succession may have accelerated since the 1960s, due to a slight increase in precipitation over the past century, but especially to the regional increase in nitrogen (N) deposition, increasing vegetation cover and negatively affecting slow-growing species such as lichens (Riksen *et al.* 2006). In the 1960s, the introduction of the invasive bryophyte *Campylopus introflexus* (Hassel & Söderström 2005) added a new threat to the lichen-dominated grassland communities of the inland dunes, as this species partially replaced the lichen vegetation (Ketner-Oostra & Sýkora 2008), especially in areas with high N deposition (Sparrius & Kooijman 2011).

Potential impact of nitrogen deposition on the vegetation

The decrease in species diversity in drift sands may thus partly be caused by increased N deposition, although different species groups may be affected by different mechanisms.

Bryophyte and lichens are in general sensitive to N fertilizers, as nutrients are adsorbed and taken up by all parts of the plant (Cape *et al.* 2009). In *Polytrichum piliferum* mats, one of the first stages of succession, N addition caused an increase in growth under low background deposition of nitrogen (Bowden 1991), indicating N limitation of the vegetation in this early succession stage. The bryophyte mat efficiently accumulates nitrogen over time, storing it in both shoots and rhizoids.

Lichen-rich vegetation, however, seems to be mostly negatively affected by N supply. N addition resulted in death of most cryptogams, especially lichens (Hasse & Daniëls 2006) or strongly reduced lichen cover (Soudzilovskaia *et al.* 2005). Even frequent doses of low concentrations of N caused a decline of lichen cover in alpine and arctic vegetation (Britton & Fisher 2007; Gordon *et al.* 2001).

Grasses may positively respond to N fertilization as their relative growth rate is positively correlated with N content (Boot & Dubbelden 1990). The common inland dune species *Corynephorus canescens* and *Agrostis vinealis* have a high nitrogen use efficiency (Boot & Dubbelden 1990; Kytoviita & Crittenden 2007). Addition of N and NP caused an increase in the N:P ratio in grasses, and increased grass biomass in a four-year experiment in a cryptogam-dominated alpine vegetation (Soudzilovskaia *et al.* 2005). N addition also increased growth in *Festuca ovina* and *Deschampsia flexuosa*, grasses of acid inland dunes, although differently, with the latter dominating the first under high N deposition and using nitrate as the primary nitrogen source (van Mierlo *et al.* 2000).

In general, increased nitrogen deposition causes soil acidification (van Breemen *et al.* 1983) and increases N availability, which may lead to the disappearence of sensitive species (e.g. Dupré *et al.* 2010). Part of these responses may be due to more advanced soil development, as soil organic matter content increases during succession (Hasse 2005; Sparrius 2011) and older succession stages may retain more of the nutrients added. Also, addition of ammonium could lead to displacement of base cations, especially K^+, which is an important nutrient for grasses (Smit *et al.* 2002).

Experimental addition of N

The hypothesis that mosses, grasses and lichens respond in a different way to nitrogen addition was tested in a field experiment. In contrast to earlier studies (e.g. Hasse & Daniëls 2006), short-term effects of nitrogen are simulated by adding nutrients more frequently and in smaller doses. To explore potential long-term effects of nitrogen deposition, the experiment was performed in inland dunes with low and high deposition levels.

The fertilization experiment included three distinct succession stages: 1) mats of *Polytrichum piliferum*, 2) vegetation dominated by *Campylopus introflexus* and 3) vegetation dominated by lichens. All stages include small amounts of grasses such as *Corynephorus canescens*, *Festuca ovina* subsp. *hirtula, F. filiformis* and *Agrostis vinealis.*

Addition of phosphorus (and nitrogen + phosphorus) was also applied in order to test whether the vegetation in areas with high N deposition had become P-limited. Also, lichens may be more P-limited than other plants. P limitation in the cryptogam layer has been suggested by Daniëls (1990) who found that lichen cover quickly improved after burning of the vegetation, resulting in temporary high P and K values in the topsoil. Strong P limitation was also found by McCune & Caldwell (2009) in lichens containing cyanobacteria and by Brenner *et al.* (2007) on other epiphytic lichens as well, experiments in which P addition led to a significant increase in lichen growth.

The research questions to be answered in this paper are:

- Do sites with low and high N deposition differ in vegetation parameters such as biomass, cover, height, N and P concentrations of vascular plants, and cover of mosses and lichens?
- What are the effects of nitrogen and phosphorus additions in different succession stages in inland dunes on biomass, cover, height and element concentrations of vascular plants and cover and height of mosses and lichens?

Methods

Sites and plots

The experiment was carried out in two inland dune reserves, one in the Northeastern part of the Netherlands, Drouwen (52.955° N, 6.800° E), a site with relatively low nitrogen deposition (25 kg ha^{-1} yr^{-1}), and one in Kootwijk (52.163° N, 5.767° E), located in the central part of the country, with average nitrogen deposition (34 kg ha^{-1} yr^{-1}). Total N deposition was obtained from the OPS model (van Jaarsveld 2004) using 2006 emission data (RIVM 2010). In both sites, plots were selected in three different succession stages, which can be compared with Hasse (2005): 1) *Polytrichum piliferum* mats that recently developed on bare sand with a minimum of 90% moss cover and a maximum of 10% grasses (mostly tussocks of *Corynephorus canescens*) on a 2-3 cm thick A_h horizon; 2) *Campylopus introflexus*-dominated vegetation with over 90% moss cover and sparse presence of grasses and lichens on a 3-4 cm thick A_h horizon. This succession stage usually develops on older *Polytrichum piliferum* mats and may be interpreted as an alternative stable state for young lichen vegetation under high N deposition or after disturbance (Sparrius and Kooijman 2011); 3) species-rich *Cladonia*-dominated vegetation (mainly *Cladonia portentosa*, *C. cervicornis, C. gracilis* and *C. furcata*) with over 30% lichen cover on a 6-7 cm thick A_h horizon. This succession stages also develops on *Polytrichum piliferum* mats, but is generally older than the *Campylopus* type, which is reflected in the thicker A_h horizon.

Addition of nutrients

In each site and succession stages, 12 plots of 1 x 1 m with similar soil and vegetation were selected according to stratified random sampling procedures within a dune zone with larger areas of the required species. At the start of the experiment in 2006, we tested whether the 12 plots had similar starting conditions with respect to vegetation cover, pH, cation concentrations and soil carbon content. Plots indeed did not differ significantly from each other in these parameters.

Experimental addition of nutrients was carried out with four treatments: control, nitrogen (N, as NH_4NO_3 solution), phosphorus (P, as Na_2HPO_3 solution) and nitrogen + phosphorus (NP, both treatments), in four replicate plots per treatment. Treatment N is equal to a dose of 42.9 kg N ha^{-1} yr^{-1}, and treatment P to 1.9 kg P ha^{-1} yr^{-1}. The N dose is equivalent to about two and a half times the mean annual N deposition in the high-deposition site and comparable to the highest N deposition levels in dry sand habitats in the country. N was applied as ammonium nitrate, as both nitrogen forms occur in rainwater (Nijssen *et al.* 2011). Plots were sprayed 6 times per year with 500 ml demineralized water with nutrients to avoid toxic effects and excessive leaching of the added nutrients. Based on 800 mm annual rainfall in both sites, spraying added only c. 1% to the precipitation intercepted by the vegetation annually. The experiment started in November 2006 and lasted till July 2009 (2 years and 8 months).

Sampling

Soil samples of 5 cm topsoil (for each replicate plot mixed from 3 subsamples) were collected in 100 cm^3 soil sampling rings before and after the experiment. The samples were dried at 60 °C for 24 hours, and weighted to estimate soil bulk density. Ground samples were analyzed with a CNS analyzer to estimate the carbon and nitrogen content. Sieved (2 mm) samples were shaken in purified water using a soil:water ratio of 1:2.5 and the pH and conductivity (EC_{25}) were then measured. The water extracts were filtered over a 0.2 µm membrane. Dissolved organic carbon (DOC), water soluble phosphate and nitrogen compounds (NH_4^+, NO_3^-) were measured using an Auto Analyzer III (Bran + Luebbe). Element concentrations were measured with an inductively-coupled plasma analyzer (ICP-OES, Fisher Iris Intrepid II). Although water does not completely extract all metals bound to cation exchange sites, the results can be used for comparison within the scope of this experiment.

Relevees of all 72 replicate plots have been made in November 2006 and July 2009. Cover values of mosses, lichens and vascular plants were estimated in July 2009 for both groups and individual species. Height of the three groups was measured in subplots of 50 x 50 cm. Ramet length of particular lichens (podetia of *Cladonia coccifera, C. grayi* and *C. portentosa*) and bryophytes (the upper green parts of *Campylopus introflexus* and *Polytrichum piliferum*) was also measured, on six randomly picked individuals. The selected lichens were absent in the *Polytrichum* stage in both study sites, but otherwise all species were present. Vascular plant shoots were collected in the 50 x 50 cm subplots, to estimate biomass and element concentrations. Sampling took place in early July 2009, in the middle of the flowering season of the grasses, when grass biomass reached its maximum. Biomass of bryophytes and lichens other than *Cladonia portentosa* was not measured, as the sampling of hundreds of individuals per subplot would take excessively much time. Grasses, including *C. canescens, Agrostis vinealis, Festuca ovina, F. filiformis*, were dried at 60 °C for 24 hours and weighed. Plant material was milled to < 0.2 mm fragments, and dried at 110 °C for 4 hours. The milled samples were digested in concentrated HNO_3 and HCl in a microwave for 25 minutes (Jackson 1985). Cations, S and P were measured by an inductively coupled plasma analyzer. Additionally, milled grass fragments were analyzed in a CNS analyzer to estimate C and N content. Grass species were sampled separately, resulting in 1-3 samples per plot.

Statistical analysis

In order to test for differences in vegetation characteristics between areas with low and high N deposition, two-way ANOVA was applied, with N deposition and succession stage as independent factors. Differences between individual mean values were tested with LSMEANS tests ($P < 0.05$) using SAS. In order to test for potential effects of nutrient treatments, a three-way ANOVA was applied, with treatment, succession stage and site (differing in N deposition) as independent factors. Differences between individual mean values were tested with LSMEANS tests ($P < 0.05$).

To estimate whether limitation of N, P and/or K occurred, grass nutrient content was compared to critical levels visualized in Fig. 1 in Olde Venterink *et al.* (2003). Nutrient content was therefore calculated in mg g^{-1}, P being multiplied by ten (10P). Although data of critial levels for nutrient limitation were originally developed for aquatic plants, the authors assume that they can be used in other habitats as well.

Results

Differences between control plots in low and high-deposition sites

Comparison of soil data (Table 5.1) shows that C:N ratio of the soil is slightly lower in the high-deposition site. However, pH, individual water-extractable nitrogen compounds and base cations did not show a significant difference between the sites.

Element content in grasses, of which all species responded in the same way, also showed differences between the two sites (Table 5.2). Grass P content decreased with succession stage in both sites, but generally had much higher values in the low-deposition site. In both sites, the N:P ratio in grasses started around 10-12 in the first succession stage (*Polytrichum* mats). In the low-deposition site, N:P ratios remained relatively low. In the high-deposition site, however, N:P ratios increased in later succession stages to values above 20, which indicate a relative shortage of P. The N, K, Ca and Mg content of grasses, increased with succession stage in the low-deposition site, but not in the high-deposition site. Grass K, Ca and Mg content were significantly lower in the high-deposition site. During succession, N:P ratio increased in the high-deposition site. Also, relative K content of grasses decreased (Fig. 5.1). Differences in grass N, P and K contents between the low- and high-deposition sites became more pronounced, especially in later stages of succession (Fig. 2). The *Polytrichum* stage seemed co-limited by N, P and K in both sites and *Campylopus* P-limited. The lichen stage showed a clear shift from co-limitation of all three nutrients at low N deposition to P limitation at high N deposition.

The vegetation structure differed between sites with low and high N deposition (Table 1). Grass cover did not differ between sites, but grasses were taller in the high-deposition site. In contrast, the low-deposition site had higher lichen cover especially in the lichen-rich plots, resulting in a higher lichen:grass ratio.

Effects of fertilization on soil and plant element content

In accord with the treatment, N and P addition resulted in higher water-extractable N and P concentrations in the soil, especially in the older succession stages (Table 5.1). Soil C:N ratio did not change during the experiment, which can be explained by the relatively low amount of N added compared to the total soil stock (1-2%). The pH showed only little difference between treatments, generally showing slight acidification after N addition. A higher pH was found in the (N)P treated plots, where Na salts were applied. P addition also led to significant lower amounts of some base cations (K, Ca and Mg) in the soil, probably due to the application of a sodium salt.

Table 5.1. Soil characteristics (pH, C:N ratio and water-extractable compounds) of control and treated plots at the end of the experiment showing mean values (n = 3) and standard deviations between brackets. * = significant difference between control and treated plot ($P < 0.05$).

		Low N deposition site			**High N deposition site**		
	treatment	**Polytrichum**	**Campylopus**	**Lichens**	**Polytrichum**	**Campylopus**	**Lichens**
pH (H_2O)	C	5.0 (0.2)	4.1 (0.1)	4.4 (0.2)	5.0 (0.2)	4.4 (0.1)	4.3 (0.1)
	N	4.9 (0.1)	4.0 (0.2)	4.5 (0.1)	5.6 (0.9)	4.0 (0.1) *	4.1 (0.1) *
	P	5.5 (0.0) *	4.4 (0.1) *	4.7 (0.1)	5.6 (0.0) *	4.5 (0.1)	4.6 (0.4)
	NP	5.4 (0.1)	4.3 (0.1) *	4.7 (0.2)	5.5 (0.1) *	5.0 (0.4)	4.3 (0.3)
C:N ratio	C	15.2 (0.8)	17.0(1.6)	12.6 (0.4)	12.5 (2.7)	12.9 (0.8)	14.6 (1.1)
	N	15.1 (0.5)	16.8 (1.6)	13.1 (0.7)	11.0 (1.0)	12.9 (0.6)	14.1 (0.7)
	P	14.5 (0.7)	14.9 (0.1)	13.4 (0.4)	16.9 (4.1)	14.0 (1.3)	13.8 (0.0)
	NP	15.1 (0.2)	15.7 (0.4)	13.7 (0.3) *	14.5 (2.4)	11.9 (0.5)	14.3 (0.7)
NH_4	C	28 (29)	405 (212)	40 (37)	24 (42)	12 (20)	81 (7)
µmol kg^{-1}	N	35 (34)	755 (447)	163 (58) *	195 (89) *	99 (103)	224 (195)
	P	0.0 (0.0)	202 (75)	86 (21)	0.0 (0.0)	15 (25)	0.0 (0.0) *
	NP	0.0 (0.0)	232 (69)	100 (23)	0.0 (0.0)	25 (26)	144 (54)
$NO_3 + NO_2$	C	0.0 (0.0)	213 (74)	25 (6)	11 (20)	10 (17)	10 (9)
µmol kg^{-1}	N	0.0 (0.0)	353 (194)	96 (29)	216 (164)	238 (1) *	165 (192)
	P	0.0 (0.0)	137 (54)	31.6 (29)	0.0 (0.0)	38 (54)	6.9 (6.1)
	NP	0.0 (0.0)	273(134)	7.6 (7.4)	0.0 (0.0)	17 (21)	128 (90)
PO_4	C	2.3 (1.8)	61.3 (44)	3.5 (2)	0.6 (0.5)	0.8 (0.6)	1.9 (1.2)
µmol kg^{-1}	N	1.3 (0.2)	52.2 (43.5)	3.8 (0.1)	34.6 (56.6)	11 (19)	1.6 (1)
	P	0.6 (1.1)	13.7 (10)	36 (12) *	9.9 (6.4)	3.9 (6.7)	2.3 (2)
	NP	3.9 (3.4)	7 (5.6)	10.1 (9.8)	1.7 (0.1)	12 (13)	6.3 (6.6)
K	C	66 (16)	204 (78)	89 (27)	79 (18)	56 (9)	82 (6)
µmol kg^{-1}	N	114 (23) *	279 (88) *	116 (8)	86 (33)	86 (21)	135 (54)
	P	74 (4)	101 (23)	64(13)	35 (4)	43 (7)	26 (1) *
	NP	86 (88)	110 (9)	71 (12)	43 (4)	49 (21)	89 (8)
Na	C	114 (17)	317 (76)	158 (20)	124 (19)	124 (17)	148 (34)
µmol kg^{-1}	N	151 (19)	260 (69)	171 (20)	209 (137)	160 (44)	144 (33)
	P	226 (12) *	382 (49)	326 (23) *	227 (146)	239 (38) *	318 (16) *
	NP	232 (13) *	389 (34)	302 (61)	285 (33) *	279 (39) *	318 (101)
Ca	C	7.7 (2.6)	58.9 (27.3)	21.8 (1.8)	10.1 (2)	11.2 (2.4)	17.5 (5.5)
µmol kg^{-1}	N	15.3 (5.7)	59.3 (33.4)	20.3 (12.4)	10.2 (5.6)	18.8 (6.3)	12.8 (3.1)
	P	7.6 (1.1)	26.3 (3.5)	10.3 (4.1)	4.8 (2.2)	10.1 (2.5)	9.7 (2.9)
	NP	5.8 (1.7)	34.7 (5.6)	12 (2.5) *	4.6 (0.7) *	8.6 (4.6)	16.8 (8.5)
Mg	C	10.1 (1.6)	50.5 (12.8)	28.4 (2)	13.2 (2.2)	16.4 (4.3)	25.1 (6.2)
µmol kg^{-1}	N	22.4 (7.2)	52.7 (6.4)	27.1 (19.3)	18.7 (13.5)	26.4 (3.4)	22.8 (2.9)
	P	10.3 (1.3)	24.8 (3.9) *	9.7 (3.7) *	4.7 (2.4) *	14.6 (3)	10.9 (1.7)
	NP	8.6 (3.1)	27.8 (3.9)	12.3 (7.1)	3.8 (1.1) *	17.3 (12.7)	18.4 (6.7)

Table 5.2. Characteristics of the vegetation in the control plots for three different succession stages in a low and high N deposition inland dune site. Succession stages are pristine *Polytrichum* mats, lichen-dominated vegetation, and *Campylopus*-dominated vegetation; 1 = significant difference between sites; 2 = significant difference between succession stages (two-way ANOVA). Different letters mean significant differences ($P < 0.05$) between succession stages within a site. Values are represented as means and standard deviations based on four replicates ($n = 4$).

	Low N deposition site			**High N deposition site**		
	Polytrichum	**Campylopus**	**Lichens**	**Polytrichum**	**Campylopus**	**Lichens**
Grass N content (mg g^{-1}) ^{2}	8.6 (1.2) a	8.6 (1.2) a	10.1 (1.3) b	8.3 (0.5) a	9.1(1.6) ab	9.9 (0.9) b
Grass P content (mg g^{-1}) ^{1,2}	0.8 (0.2) a	0.8 (0.2) a	1.0 (0.3) b	0.7 (0.0) a	0.5 (0.1) b	0.5 (0.1) b
Grass K content (mg g^{-1}) ^{1,2}	3.9 (1.7) b	2.6 (0.6) ab	3.3 (0.7) b	3.6 (0.3) b	2.0 (0.4) a	2.2 (0.4) a
Grass Na content (mg g^{-1})	0.1 (0.0) a	0.2 (0.0) a	0.4 (0.3) a	0.2 (0.0) a	0.4 (0.3) a	0.2 (0.1) a
Grass Ca content (mg g^{-1}) ^{1}	0.8 (0.2) a	1.2 (0.2) b	1.1 (0.2) b	1.1 (0.2) b	0.8 (0.1) a	0.9 (0.2) ab
Grass Mg content (mg g^{-1}) ^{1,2}	0.5 (0.1) a	0.7 (0.1) ab	0.8 (0.2) b	0.5 (0.0) a	0.5 (0.1) a	0.6 (0.2) a
Grass N:P ratio ^{1,2}	10.0 (2.6) a	15.2 (2.4) a	10.9 (2.2) a	11.7 (1.3) a	20.0 (3.5) b	20.9 (3.8) b
Grass biomass (g m^{-2}) ^{2}	25 (13) a	175 (33) b	136 (30) b	61 (13) a	92 (31) a	164 (46) b
Grass cover (%) ^{2}	16 (4) a	37 (11) b	35 (5) b	17 (2) a	22 (3) a	47 (9) b
Grass height (cm) ^{1}	13 (3) a	9 (3) a	14 (1) a	11 (3) a	26 (4) c	18 (6) b
Bryophyte cover (%) ^{2}	84 (3) a	61 (18) c	25 (9) b	65 (9) a	90 (0) c	9 (7) b
Lichen cover (%) ^{1,2}	1 (0) a	8 (4) c	48 (3) b	0 (0) a	0 (0) a	30 (0) b
Lichen:grass ratio (%) ^{1,2}	4 (2) a	19 (11) c	58 (5) b	0 (0) a	1 (1) a	39 (4) b

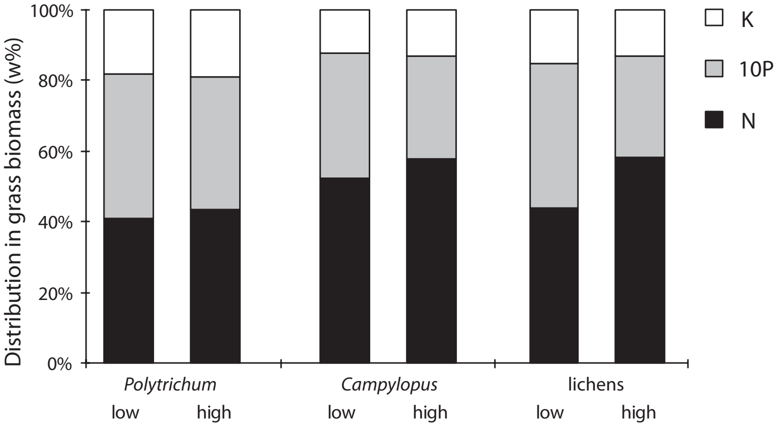

Fig. 5.1. Distribution of N, 10P and K contents (wt%) in grass biomass in areas with low and high N deposition in control plots in different succession stages.

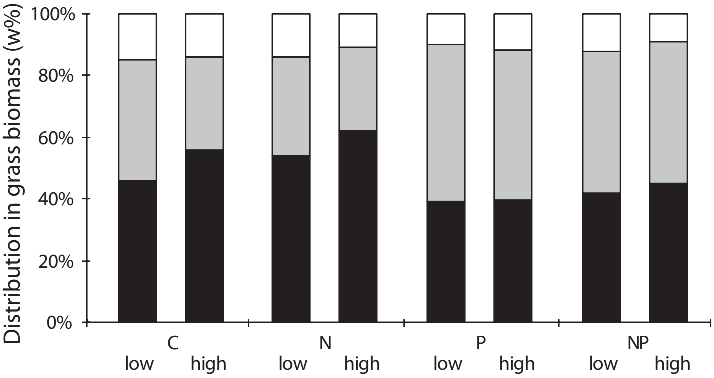

Fig. 5.2. Distribution of N, 10P and K contents (wt%) in grass biomass in sites with low and high N deposition in plots with different treatments.

The element content (C, N, P and base metals) of grasses differed between treatments, succession stages and/or sites (Table 5.3). In all sites and succession stages, N and P content and C:N and N:P ratios significantly differed from control treatments according to the nutrients added (Table 5.4). N addition resulted in a higher plant N content and N:P ratio, but a lower C:N ratio. N addition also led to a significant decrease of Mg and Ca and in the high-deposition site also of K. With N addition, all succession stages became P-limited (Fig. 5.2). In the P and NP treatments, P content increased, N decreased and differences between the high- and low-deposition site became smaller. Addition of P and NP also resulted in different ratios between N, P and K content in grasses, which suggested a shift towards K limitation. Na content was affected by treatment, but probably only due to the use of a sodium salt in the P treatment.

Table 5.3. Analysis (three-way ANOVA) of differences in vegetation responses between nutrient treatments, succession stageand site (N deposition). Significant effects are given as P values; ns = not significant (P > 0.05).

	Nutrient treatment	Succession stage	Site
Grass C content (mg g^{-1})	ns	0.0001	0.04
Grass N content (mg g^{-1})	0.0001	0.0001	ns
Grass P content (mg g^{-1})	0.0001	0.04	0.0001
Grass C:N ratio	0.0001	0.03	ns
Grass N:P ratio	0.0001	0.0001	0.0001
Grass K content (mg g^{-1})	ns	0.0001	0.0001
Grass Na content (mg g^{-1})	0.0001	ns	ns
Grass Ca content (mg g^{-1})	0.0001	0.0003	0.0001
Grass Mg content (mg g^{-1})	0.0001	0.0001	0.0001
Grass biomass (g m^{-2})	ns	0.0001	0.01
Grass cover (%)	0.004	0.0001	ns
bryophyte cover (%)	0.001	0.0001	0.0001
Lichen cover (%)	0.0001	0.0001	0.0001
Lichen:grass ratio	0.0001	0.0001	0.0001
Grass height (cm)	0.0001	0.0001	0.0001
Bryophyte height (cm)	0.0001	0.0001	ns
Height *Polytrichum* (cm)	0.0001	0.0001	0.0001
Height *Campylopus* (cm)	ns	0.0001	ns
Height *Cladonia portentosa* (cm)	0.0001	0.0001	0.0001
Height *C. coccifera* (cm)	0.0001	0.0001	0.0001
Height *C. grayi* (cm)	ns	0.03	0.0001

Lichens were only analyzed in the lichen-dominated vegetation in the low-deposition site and responded similar to grasses, although the changes as a result of the treatments were generally larger. The C:N ratio in *Cladonia portentosa* (Table 5.5) was low in the N treatment, high in the NP treatment and not different from the control in the P treatment. N and P contents and N:P ratio changed in a similar way as observed in grasses. Mg and Ca, however, showed only small differences between control and N treatment in contrast to the significantly lower values found in grasses.

Effects of fertilization on vegetation structure

Grass biomass differed between succession stages, but was not affected by treatment, because responses to the treatments differed between sites with low and high N deposition. In the low-deposition site, nitrogen addition had a positive effect on grass biomass (Fig. 3). In the high-deposition site, however, increased grass biomass was only found in the NP treated plots. Grass cover was significantly affected by treatment and succession stage. Grass cover was significantly higher in the N treatment than in control and P treatments. In both sites, grass height did not increase in the N treatment, but was negatively affected in the P treatment.

Lichen cover significantly differed between treatments, succession stages and sites. Compared to the control, application of N had a negative impact on lichens (Fig. 5.3) and lichen cover was reduced by about 50% at both sites. P addition resulted in a strong increase in lichens, resulting in a high lichen:grass ratio. Species that showed the largest increase in cover were the reindeer lichens *Cladonia arbuscula* and *C. portentosa* (in the lichen-dominated plots) and *Cetraria aculeata* (in the *Polytrichum*-dominated plots). The height of the lichen *Cladonia portentosa* differed significantly between sites,

Table 5.4. Element content, N:P and C:N ratios of grasses (*Corynephorus canescens, Festuca ovina, F. filiformis* and *Agrostis vinealis* together) for control plots and three treatments in inland dune sites with low and high N deposition. Figures are given as mean values and standard deviations between brackets (n = 20-30 for each given value). Significance compared to control: * P < 0.05, ** P < 0.01, *** P < 0.001.

	Treatment			
	control	**N**	**P**	**NP**
Low N deposition site				
mg g^{-1}				
N	10 (1.4)	11.8 (1.9) **	10.5 (1.5)	11.3 (1.9) *
C	421 (36)	438 (26)	415 (45)	440 (28)
P	0.8 (0.2)	0.7 (0.1) *	1.3 (0.2) ***	1.2 (0.3) ***
C:N ratio	42.8 (7)	37.6 (5.6) **	39.7 (4.3)	39.8 (7)
N:P ratio	12.5 (3.5)	17.3 (3.9) ***	7.8 (1.3) ***	9.4 (2.1) **
K	3.12 (1.12)	3.04 (1.22)	2.85 (0.82)	3.12 (1.42)
Na	0.24 (0.20)	0.23 (0.23)	0.61 (0.38) ***	0.57 (0.45) ***
Ca	1.05 (0.22)	0.90 (0.24) *	1.18 (0.32)	1.01 (0.18)
Mg	0.69 (0.21)	0.58 (0.17)	0.79 (0.23)	0.73 (0.18)
High N deposition site				
mg g^{-1}				
N	8.9 (1.3)	10.7 (2.2) **	10.7 (2.3) **	11.6 (3.4) **
C	416 (57)	417 (37)	429 (32)	422 (35)
P	0.5 (0.1)	0.4 (0.1)	1.2 (0.2) ***	1.1 (0.3) ***
C:N ratio	46.5 (4.8)	39.8 (6) ***	41.5 (8.4) *	38.9 (9.9) **
N:P ratio	18.5 (4.3)	23.9 (4.3) ***	9.2 (2.5) ***	10.5 (2.7) ***
K	2.35 (0.69)	1.73 (0.89) **	2.70 (1.23)	2.30 (0.66)
Na	0.23 (0.18)	0.19 (0.08)	0.64 (0.48) ***	0.67 (0.48) ***
Ca	0.92 (0.15)	0.76 (0.14) **	0.92 (0.18)	0.86 (0.15)
Mg	0.56 (0.13)	0.39 (0.08) ***	0.62 (0.17)	0.57 (0.12)

Table 5.5. Element content of the lichen *Cladonia portentosa* for the control plots and three different treatments in a lichen-rich vegetation in the low N deposition site. Figures are given as mean values and standard deviations between brackets (n = 3 for each treatment). Significance in t-test: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

		treatment		
	control	N	NP	P
mg g^{-1}				
N	8.2 (1.4)	14.7 (0.6) **	6.2 (0.3)	9.8 (0.7)
C	397 (8)	427 (9)	432 (1)	43 (1)
P	0.43 (0.03)	0.53 (0.06)	1.1 (0.1) ***	1.5 (0.19) **
C:N ratio	46.9 (5.2)	29 (1.1) **	69.3 (3.3) **	44 (3.6)
N:P ratio	18.3 (2.3)	26.9 (3.1) *	5.6 (0.3) **	6.4 (0.3) **
K	1.39 (0.15)	1.81 (0.53)	1.57 (0.01)	1.84 (0.21) *
Na	0.17 (0.02)	0.14 (0.01)	0.40 (0.05) **	0.57 (0.09) **
Ca	0.36 (0.02)	0.30 (0.04)	0.44 (0.06)	0.46 (0.10)
Mg	0.30 (0.04)	0.32 (0.02)	0.42 (0.00) *	0.46 (0.03) *

succession stages and treatments, similar to the observed differences in lichen cover and increased in the P treatments. The ramet length of two cup-lichens with low cover, *Cladonia coccifera* and *C. grayi*, however tended to become slightly shorter with P addition, although this was only significant for *C. coccifera*. The length of both species was negatively affected by N deposition.

Bryophyte cover was significantly affected by treatment, succession stage and site. Bryophyte height was affected by treatment and succession stage, but not different between sites. Bryophytes did not show a response to N addition. However, bryophyte cover increased in P treatments, especially in the site with high N deposition. *Polytrichum piliferum* ramet length was affected by site, succession stage and treatment. P and NP addition resulted in significantly longer shoots compared to the control plots. *Campylopus introflexus* ramet length was only affected by succession stage and highest in the *Campylopus* stage.

Discussion

Differences between control plots in low and high-deposition sites

Although only two inland dune reserves were part of this study, they are representative for larger parts of the drift sand area. In general, N deposition resulted in a lower lichen:grass ratio, lower lichen cover, taller grasses and higher grass biomass in all succession stages in the high-deposition site. Also, differences between the low and high N deposition site were found in all three succession stages with respect to grass P content. The grass N:P ratio in the high-deposition site was around 20 in the two older succession stages, which indicates the absence of N limitation (Koerselman &

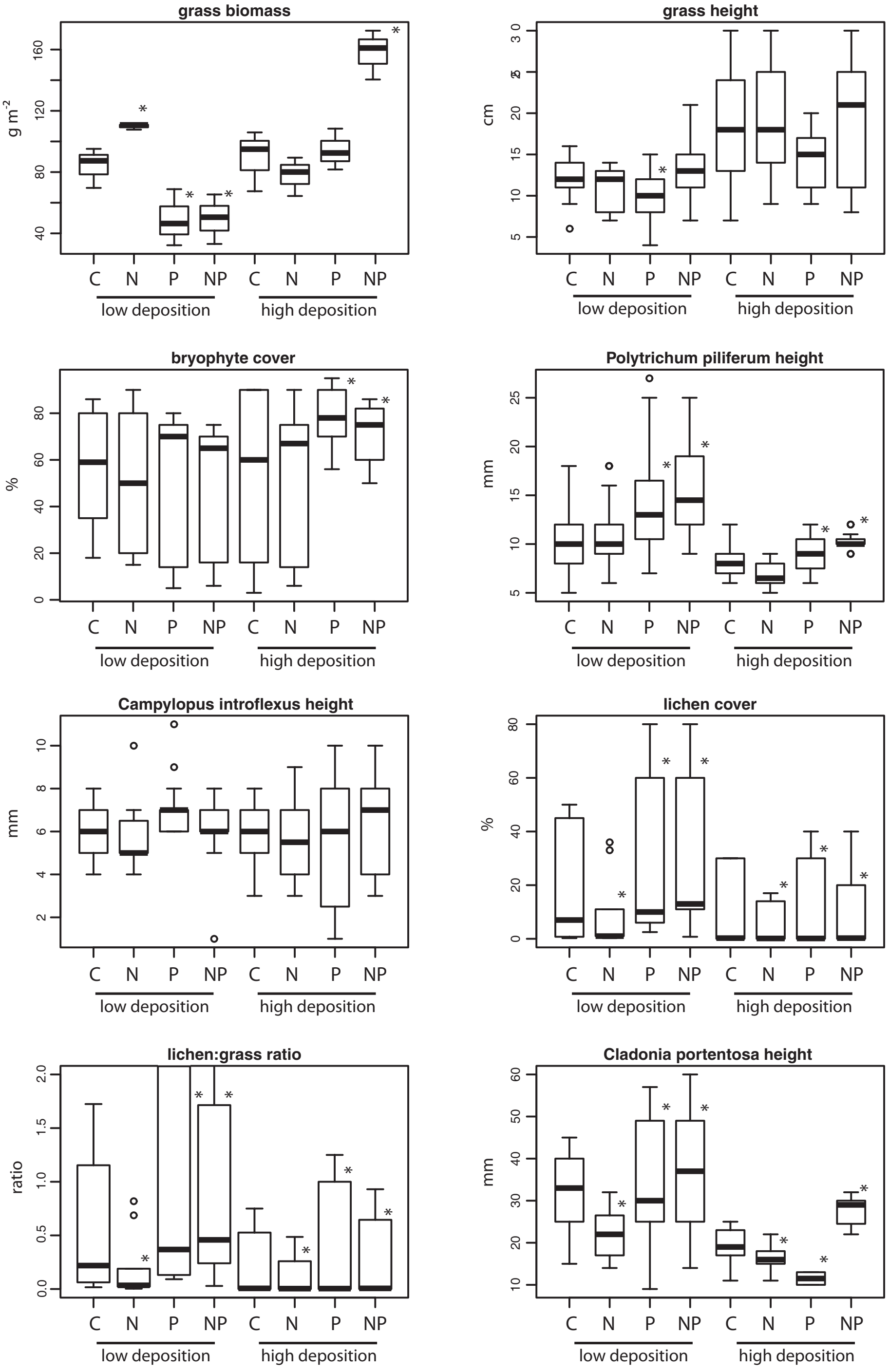

Fig. 5.3. Comparison of vegetation and species responses to different treatments (n = 9 for each treatment per site). Asterisks indicate significant differences ($P < 0.05$) between treatments and the control within one site. Boxplots, showing median, upper and lower quartiles, minimum and maximum values were used as further illustration.

Meuleman 1996; Güsewell & Koerselman 2002). In all plots in the low-deposition site grass N:P ratio was around 10-15, which may reflect the difference in N deposition. In the high deposition site, the *Polytrichum* stage still had low N:P ratios, which may reflect low N availability in very early stages of succession (Sparrius & Kooijman 2011). When the results are compared to values by Olde Venterink *et al.* (2003), the *Polytrichum* stage seemed actually co-limited by N, P and K in both sites, which further supports low nutrient availability in early stages of drift sand succession (Sparrius 2011). The *Campylopus* stage, however, was only limited by P, and not by N and K anymore, which further supports that this species prefers relatively N-rich conditions (Sparrius & Kooijman 2011). In the older lichen-rich vegetation, grass growth was still limited by N+P+K in the low-deposition site, which again suggests that nutrient availability in drift sands is low. However, in the high-deposition site, P seemed to have become the main limiting factor, due to increase in N availability.

Effects of fertilization on soil and plant element content

N and P contents in grasses changed according to the nutrients added. Addition of N led to lower P content in the site with low N deposition. In the site with high N deposition, N addition led to lower K-content in grasses, which may be caused by displacement of K^+ by NH_4^+ in the soil resulting in lower K availability for plants (van Breemen *et al.* 1983). Ca and Mg also showed a decline in the N treated plots, especially in the site with high N deposition, which suggests that ammonium has displaced some of the bivalent cations as well. Comparison with data by Olde Venterink *et al.* (2003) suggested that N treatment resulted in P limitation in both sites, despite the decrease in K content in the high-deposition site. This shift to P limitation was more or less in accord with the shift from co-limitation to P limitation when sites with low and high N deposition are compared. Experimental N addition and comparison of site with different N deposition thus show similar responses to the vegetation

The P and NP treatments, however, resulted in K limitation. K contents did not actually decrease, but P increased due to P supply. In contrast to grasses, N addition did not lead to lower Mg and Ca contents in the lichen *Cladonia portentosa*. An explanation can be found in the strong decrease in size of the lichen thalli when N was applied. The size of the lichen is dependent on both basal decay and apical growth (Sipman 1978). Basal decay may not be affected by N fertilization, but apical growth is much reduced in any case, requiring less base metals. Also, a single dose of ammonium might displace cations from the Mg and Ca bounding sites shortly after the treatment (Miller & Brown 1999), but repletion from precipitation may compensate this. Analysis of precipitation collected in these sites showed that the annual deposition of base metals is sufficient (Nijssen *et al.* 2011).

Effects of fertilization on vegetation structure and biomass

In accord with other studies (Boot & Dubbelden 1990; van Mierlo *et al.* 2000), grasses were positively affected by increased N availability. Higher grass cover, height and biomass with higher nitrogen availability was found as both short-term (fertilization) and long-term response (high N deposition) to high N supply. P and NP addition resulted in a lower grass biomass in the low-deposition site mainly as a result of grasses being overgrown by lichens, in particular in the lichen succession stage. In the high-deposition site, a significant positive response of grass biomass was only found in the NP treatment. This can be explained by P limitation in grasses in the *Campylopus* and lichen succession stages. A similar response would be expected in P treatment, but those plots had an initial higher lichen:grass ratio causing lichens to expand at the cost of grasses.

Lichens showed a different response than grasses. In accord with other studies (Gordon *et al.* 2001; Soudzilovskaia *et al.* 2005; Hasse & Daniëls 2006; Makkonen et al 2007; Britton & Fisher 2010), lichens showed a clear decrease in cover with high N supply or in the site with high N deposition. The vitality of lichens could be reduced as a result of ammonium toxicity, which has been demonstrated in studies in inland dunes (Hasse & Daniëls 2006), arctic and boreal conditions (Mols *et al.* 2000; Nilsson *et al.* 2002; Fremstad *et al.* 2005) and Atlantic heaths (Hyvärinen & Crittenden 1998a). The effect of N was also reflected in the ramet height of individual species. The large fruticose lichen *C. portentosa* became smaller in the N treatment. This has also been observed in some individual plots for *Cetraria aculeata* and *Cladonia uncialis*, species with a similar growth type. The cup-lichens *C. coccifera* and *C. grayi*, however, showed a decrease in cover with N application, but no response in podetium size. Cup-lichens consist of a primary thallus of squamules lying on the soil, and vertical podetia (cups), which seem to be rather constant in size (Sipman 1978), and their height may be less dependent on nutrient availability.

In contrast to Bowden (1991), bryophytes showed little response to N addition. Height of *Polytrichum* shoots increased with application of P and NP, but did not respond to N. Within the short time span of this experiment, no evidence for a positive effect of N and P on the growth of *Campylopus introflexus* has been found, even though grasses in the *Campylopus* stage seemed P-limited in both low and high N deposition sites. The *Campylopus* mats used in this experiment had already a very high cover, which was unlikely to increase significantly. Nitrogen also did not affect the thickness of the upper green part of the *Campylopus* shoots. This green layer could be of more or less constant size, as ramets stand close together in the moss mat and sunlight intensity fades quickly towards the bottom of the layer, inhibiting photosynthesis (Cornelissen *et al.* 2001). The effect of nitrogen deposition on *Campylopus introflexus* can therefore be studied better in a dedicated experiment where spores or fragments are cultivated under different nutrient conditions (Sparrius & Kooijman 2011).

Effects of phosphorus addition to lichens

Lichens have little contact with the soil and showed a positive response to P under both low and high N deposition. As lichens are largely dependent on nutrients in precipitation (Ellis *et al.* 2004), the difference the N:P ratio of the rainwater may be of direct influence on the N:P ratio of the plant tissue. Under P-limited conditions, lichens are highly efficient in phosphate uptake (Hogan *et al.* 2010, Hyvärinen & Crittenden 1998b) and it is likely that in the high N deposition site P addition will lead to an increase in lichen growth, which agrees with the results. The low-deposition site, however, responded similarly with respect to lichen cover and also *Polytrichum piliferum* height. This indicates that even in the low-deposition site, the growth of lichens is already saturated with N, resulting in P limitation. This is supported by the fact that *Cladonia* N content in both sites agreed with maximum levels of *Cladonia* N content of 10 mg g^{-1} (average of apical and basal parts) found by Hyvärinen & Crittenden (1998a). In the low-deposition area, grasses do not (yet) show P limitation, as they may take up additional P from the soil. This may explain why grasses and cryptogams respond differently to P addition even when growing close together.

P addition in inland dunes resulted in opposite effects compared to N addition with respect to the vegetation structure. These results support experiments in related ecosystems. In a four-year field study in *Calluna* heath with pleurocarpous mosses and reindeer lichens, with atmospheric N deposition comparable to sites in this study, P addition led to an increase in cryptogams and a reduction of *Calluna vulgaris*, but N addition to a decrease (Pilkington *et al.* 2007). Also, Vagts & Kinder (1999) observed a decline in the ramet length of terricolous lichen species (*Cladonia furcata* and *C. portentosa*) with N addition, but increase with NPK addition. Like in the present study, application on smaller lichens such as *C. floerkeana, C. coccifera* and *C. grayi* had no effect. P addition may lead to a strong growth response, due to a toxic effect of P on algal cell division, but this does not seem to harm the lichen symbiosis over a longer period (Makkonen *et al.* 2007).

Differences in lichen:grass ratio

The lichen:grass ratio may be used as indicator value for the vitality of cryptogam vegetations (Fremstad *et al.* 2005). Rootless cryptogams and rooted vascular plants showed opposite responses to fertilization with N and P. As a result, the lichen:grass ratio strongly decreased with application of N, and increased with application of P and NP. Also, the lichen:grass ratio was lower in the site with high N deposition, which is in accord with Remke *et al.* (2009), who found a negative effect of nitrogen deposition on the lichen:grass ratio in the Baltic Sea region.

Evidence for accelerated succession

Increased N deposition may lead to accelerated succession in drift sands (Sparrius 2011). In this experiment, an increase of biomass and vegetation cover as a result of nutrient addition has indeed been observed. Also, differences in nutrient limitations were found between sites that had been exposed to high and low levels of N deposition over a long-term period.

Both short-term and long-term addition of N led to a shift from lichens to grasses due to higher N availability. In the low-deposition area, N addition led to an increase in grass cover, mainly with the stoloniferous Agrostis vinealis. Long-term high N deposition may even lead to P limitation for grasses in later succession stages, which had N:P ratio values around 20 (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003). In contrast to vascular plants, the cryptogam layer seemed to be P-limited even at low levels of N deposition, probably as a result of N deposition above critical levels, in combination with high N uptake efficacy and internal N cycling (Cornelissen *et al.* 2007).

Both sites have deposition values above modeled and empirical critical loads for inland dune pioneer grasslands of 10-20 kg N ha^{-1} yr^{-1} (van Dobben *et al.* 2006). P limitation caused by high N deposition may thus explain the strong, positive response of cryptogam growth in P treatments, especially for species without rhizomes or relatively large contact surface with the soil, such as the reindeer lichen *Cladonia portentosa*. A lower availability of P, and perhaps also K, for grasses in high N deposition sites, however, may prevent the complete loss of cryptogam vegetations. This may explain that strong grass encroachment observed in certain acid coastal dunes (Kooijman & Besse 2002; Remke *et al.* 2009) does not occur in low vegetation in inland dunes.

Conclusions

N addition in inland dunes generally caused an increase in grasses and a decrease in lichen cover. This is supported by the observation that the effects on the vegetation, especially grasses, are more significant in the low N deposition site. P addition led to opposite effects.

Two life forms, cryptogams and grasses, responded differently to addition of N and P. Cryptogams primarily use nutrients deposited by precipitation, which apparently contains a surplus of N compared to P, even in the study site with low N deposition.

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Chapter 6

Invasiveness of *Campylopus introflexus* in drift sands depends on nitrogen deposition and soil organic matter

L.B. Sparrius & A.M. Kooijman

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.

Nomenclature source: van der Meijden, R. (2005) for vascular plants; Siebel & During (2006) for bryophytes; Aptroot *et al.* (2004) for lichens.

Applied Vegetation Science 14: 221-229 (2011)

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 heathlandheaths, 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

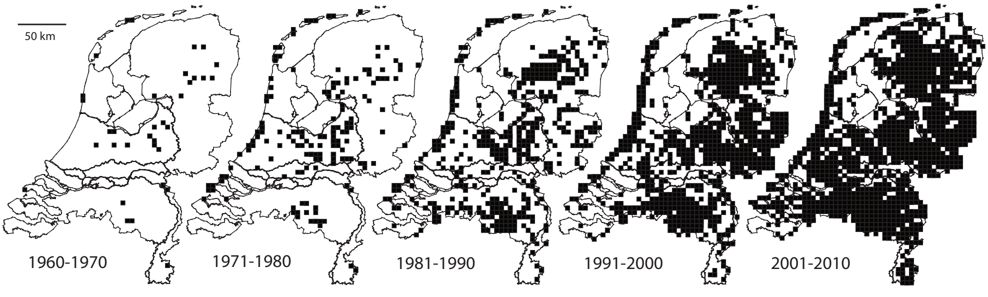

Fig. 6.1. Maps showing the invasion of *Campylopus introflexus* in The Netherlands since the first record in 1961. Source: BLWG Bryophyte Database (www.verspreidingsatlas.nl), based on field records and herbarium specimens, generalized to 5 x 5 km grid cells. The species is absent or less abundant in the river and clay districts and most abundant in the Pleistocene cover sand and dune districts.

species under different nitrogen levels, and of a growth experiment of *C. introflexus* with different substrates in the University garden.

In the transect study, we measured establishment of *C. introflexus* and lichens in mats of the bryophyte *Polytrichum piliferum*. This is the first succession stage where drift sand becomes completely covered with vegetation, which typically consists of 0 to 10% *Corynephorus canescens* and 90 to 100% *P. piliferum* (Hasse 2005; Stoutjesdijk 1959). The space in between the moss shoots of *P. piliferum* becomes filled with other species. Before *C. introflexus* was introduced into the Netherlands, the moss mats were mostly colonized by lichens such as *Cladonia* spp. and *Cetraria aculeata*. At present, however, in many places moss mats seem colonized by *C. introflexus*. The hypothesis is that the colonization of *P. piliferum* mats by *C. introflexus* instead of lichens is at least partly facilitated by high N deposition. Growth of *C. introflexus* may increase with high N deposition. Growth of *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). *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 *P. piliferum*. If stimulated by high N deposition, *C. introflexus* may outcompete or overgrow *P. piliferum* and lichen species. Also, lichens may perform less at high nitrogen deposition level. Hasse *et al.* (2006) and Nijssen *et al.* (2011) found that lichen cover of the reindeer lichen *Cladonia portentosa* and thallus height decreased upon addition of ammonium nitrate.

In the dispersal experiment, *Campylopus introflexus* and lichen fragments were sown into a pristine moss mat of *Polytrichum piliferum* in two areas, one with high and one with low nitrogen deposition. If there is a positive relation between nitrogen and *C. introflexus* cover, we would expect that *C. introflexus* indeed invades *P. piliferu*m 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 *et al.* 1996; Daniëls *et al.* 2008b; Hasse 2007; Ketner-Oostra *et al.* 2004b) suggested that, apart from nitrogen deposition, soil organic matter may also be important with respect to the increase of *C. introflexus.* Hasse (2007), Daniëls *et al.* (2008a, b) and Ketner-Oostra *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 *C. introflexus*, and to differentiate between the two factors, *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 A_h horizon of 2 cm at most. Soil carbon content is low, approximately 0.1% in the upper 5 cm, and pH (H_2O) 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 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. [1] locations were ammonia air concentrations were measured.

Site name	Ammonia air concentration	Modeled N deposition	Co-ordinates
	µg NH_3 m^{-3}	kg ha^{-1} yr^{-1}	Decimal degrees (WGS 84)
Aekingerzand [1]	5.8	28.9	52.924° N, 6.294° E
Beekhuizerzand	7.0	32.5	52.334° N, 5.665° E
Bergerheide [1]	10.0	39.2	51.595° N, 6.079° E
Drouwenerzand [1]	6.6	25.3	52.955° N, 6.800° E
Hulshorsterzand	7.0	32.2	52.334° N, 5.724° E
Kootwijkerzand [1]	7.5	34.3	52.163° N, 5.767° E
Lange Duinen Soest	6.0	17.4	52.146° N, 5.284° E
Lemelerberg [1]	7.6	36.7	52.447° N, 6.387° E
Loonse en Drunense Duinen	7.5	42.7	51.642° N, 5.112° E
Maashorst	12.0	50.4	51.687° N, 5.604° E
Otterlosche Zand	7.0	35.2	52.109° N, 5.795° E
Strabrechtse Heide	12.0	49.0	51.408° N, 5.617° E
Weerterbergen	7.0	32.5	51.256° N, 5.616° E
Wekeromse Zand [1]	9.7	50.3	52.100° N, 5.679° E

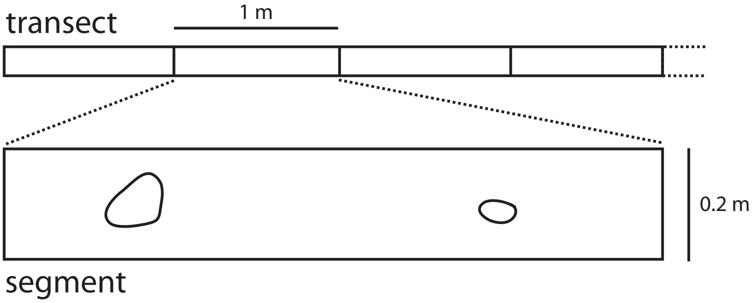

Fig. 6.2. A transect with a 1 m segment (invaded by with two small individuals of a species) as used to measure the presence of young individuals of *Campylopus introflexus* and lichens invading pristine moss mat of *Polytrichum piliferum*.

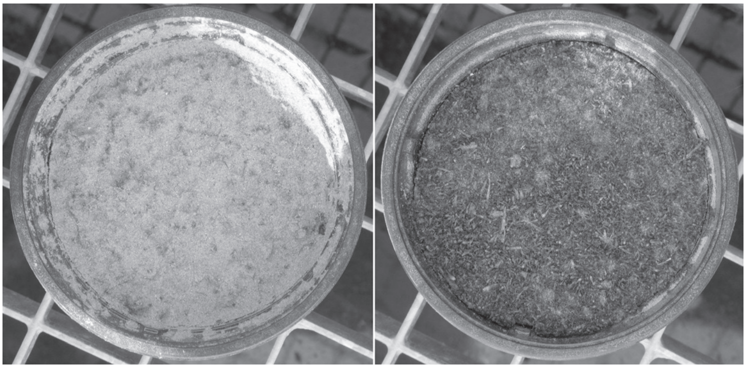

Fig. 6.3. Sample of pots used for growing *Campylopus introflexus* after one year. Left a pot with sand showing no moss growth, on the right a pot completely covered in moss. Most pots show a moss cover in between those shown here.

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 *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 *C. introflexus* and/or lichens (*Cladonia* spp. or *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 *Campylopus*. The *Campylopus*:lichen ratio was calculated by dividing the number of segments per transect containing *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 *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 *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 Drouwenerzand (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 cm2 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 quantitive 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.

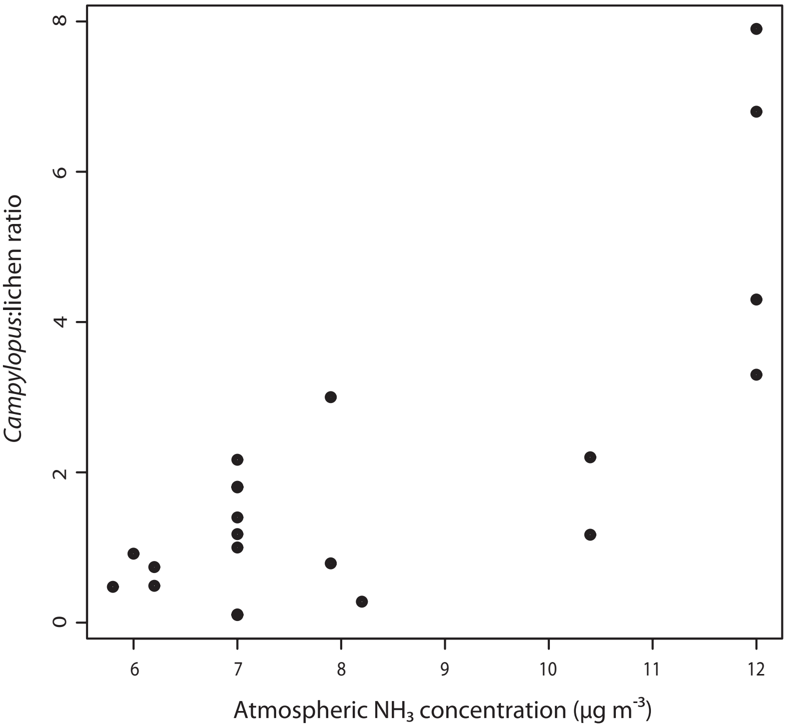

Fig. 6.4. Scatterplot (n = 21) showing the relation between the ammonia air concentration and the *Campylopus*:lichens ratio in 100 m transects.

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).

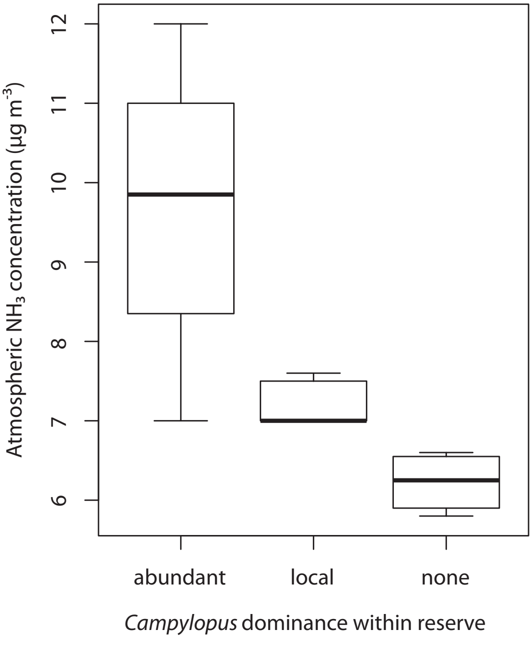

Fig. 6.5. Boxplot showing the ammonia air concentration for inland dune sites classified by the presence of large-scale *Campylopus*-dominated vegetations ($n = 4$ to 7 for each catagory). All groups differ significantly from each other ($P < 0.05$).

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)

Species	**Fragment survival (%) per site**		**P value**
	Wekerom	**Drouwen**	
	(high N deposition site)	(low N deposition site)	
Campylopus introflexus	25 (12)	0 (1)	0.0059
Cladonia coccifera	50 (20)	57 (36)	n.s.
Cladonia portentosa	58 (23)	45 (3)	n.s.
Cladonia strepsilis	10 (3)	32 (4)	0.0001
Stereocaulon condensatum	6 (2)	5 (6)	n.s.

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

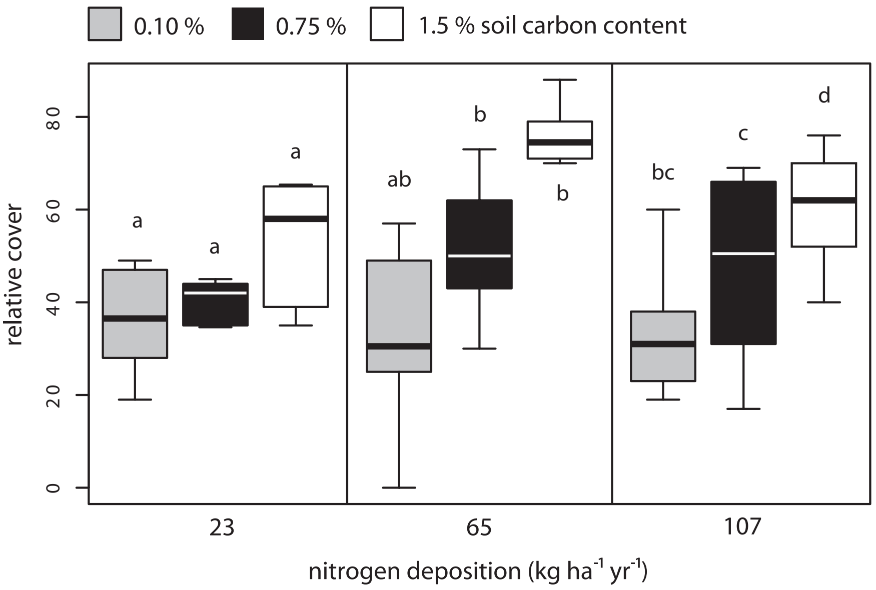

Fig. 6.6. Growth reponse of *Campylopus introflexus* grown on sand with different organic matter content and nitrogen addition, grouped by nitrogen deposition. Different letters indicate a significant difference ($P < 0.05$) between treatments.

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.

Variable	DF	F	P
Including all N treatments			
nitrogen	2	2.22	n.s.
carbon content	2	19.36	***
nitrogen x carbon content	4	1.44	n.s.
Total	45		
Excluding the highest N dose			
nitrogen	1	5.31	*
carbon content	2	17.41	***
nitrogen x carbon content	2	3.27	n.s.
Total	30		

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 NH_4^+ 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 NH_4^+ concentration (100 mM) and a N dose of 20 µmol cm^{-2} per treatment. Soudzilocskaia *et al.* (2005) found die-off of lichens using urea as a fertilizer at a dose of 30 µmol

cm^{-2}. 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_2O) 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

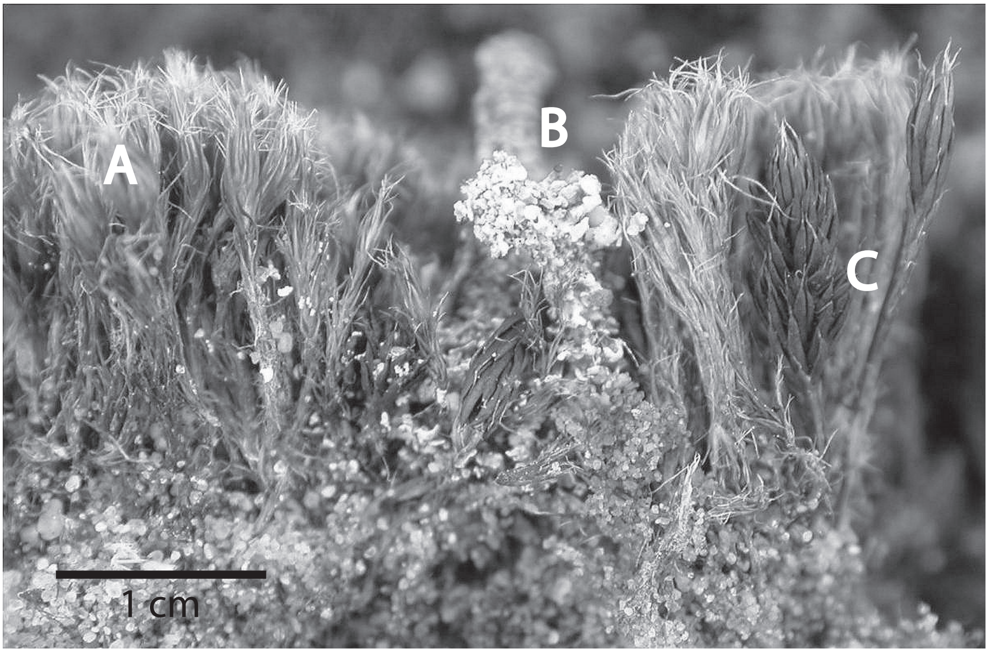

Fig. 6.7. Vertical section through a young moss mat of *Campylopus introflexus* on sandy soil showing A. *Campylopus introflexus*, B. the lichen *Cladonia coccifera*, C. the bryophyte *Polytrichum piliferum*.

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 NH_3 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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Chapter 7

Synthesis

Inland dunes, a rapidly declining semi-natural landscape

Bare sand

The inland dune landscape has always been changing. From the Late Middle Ages until about 1850, the area of bare sand steadily increased, but bare sand almost vanished in recent times due to afforestation and abandonment of pre-industrial agricultural practices (Koster 2010).

The causes of ongoing loss of bare sand area have been studied in eight inland drift sands, four of which were stabilized and four had larger areas of active drift sand (chapter 2). These eight sites were chosen within a gradient of nitrogen deposition, mineralogy, recreation intensity and precipitation. In all study sites about half of all bare sand has become covered in vegetation since 1950. In the period 1981-2007, the rate of decline was 1.5 to 2 times higher than in the previous period (1950-1981). Geomorphology and recreation were important factors explaining succession speed at a local scale. The main effect of geomorphology was the more rapid succession on blowouts than on drift sand dunes, which is explained by the stronger effect of wind erosion and water erosion on dune slopes (Riksen & Goossens 2007). Recreation causes bare sand to be maintained on trails, yet this forms an insignificant small part of drift sand areas. Other factors were hypothesized, including generic changes amongst which elevated nitrogen deposition and a prolonged growing season. Increased nitrogen deposition may have stimulated plant growth, and thus increased rate of succession. Also, vegetation growth in the semi-arid bare sand habitat is likely to be strongly limited by drought. Therefore, increased rainfall and warmer winters may have led to a more rapid succession.

Pioneer vegetation

Loss of bare sand area is not only the loss of shifting sands as a geomorphological phenomenon, but also a threat to future development of pioneer vegetation with its highly adapted species, that are confined to this habitat. During the past decades, the area of pioneer vegetation created upon loss of bare sand balanced the loss of this pioneer vegetation by its succession to forest (**chapter 2**). This means that if no bare sand is left, the area of pioneer vegetation will start to reduce. At present, the ongoing succession towards forest therefore is the main threat to old, species-rich vegetation in drift sand reserves and will remain so in the future. This process is also stimulated by high levels of N deposition. The effect of N deposition on the vegetation cover was especially visible in blowouts, which had a significantly higher forest cover in sites with high N deposition.

Although some species are truly confined to bare sand, such as the lichen *Stereocaulon condensatum*, most other species have their optimum in older succession stages. The pioneer stages of drift sands are renowned for their lichen vegetations (Ketner-Oostra *et al.* 2010), which are different from the more buffered soils in the coastal dunes (Paus 1997). High diversity occurs mainly in the stage in which small *Cladonia* species dominate. The development time of such lichen vegetation is estimated to be 15-20 years. However, the average age appeared to be more than 40 years, which is due to several factors including low-intensity grazing, tree-removal and perhaps microclimate.

Although effects of N deposition on the loss of bare sand in inland dunes were partly masked by differences in geomorphology, it has been shown that -on a local scale- primary succession was affected by atmospheric nitrogen deposition, either through an increased succession rate, species replacement, or changes in the performance of species. These findings are discussed in the paragraphs hereafter.

Soil and vegetation development

During succession from bare sand to heath and forest, organic matter, base cations and nutrients accumulate in the topsoil (**chapter 3**). Early succession stages largely depend on atmospheric deposition for their growth. Nutrient cycling becomes important in later stages: in early succession stages, the cryptogam mat largely depends on interception of atmospheric deposition, while in later stages, nutrient retention in the topsoil and nutrient cycling by vascular plants become more important (Table 7.1). This is supported by the finding that nitrogen mineralization increases during succession.

As succession takes place, a vegetation of mainly cryptogams is formed, which are then becoming replaced by grasses. In the first succession stages, a bryophyte mat of *Polytrichum piliferum* is formed. This mat becomes colonized by small lichen species. This results in species-rich lichen vegetation which becomes gradually replaced by vegetation dominated by grasses and fewer, larger lichen species. Eventually, dwarf shrubs (*Calluna vulgaris* and *Empetrum nigrum*) and forest appear. Succession in cryptogam species also includes a change from species that spread with small diaspores to species that disperse with larger fragments (Bültmann 2005).

Table 7.1. Dominant processes of nutrient accumulation and change in cycling during primary succession in inland dunes.

<table>
<tr><th>Open sand</th><th>Corynephorus in sand</th><th>Polytrichum piliferum mats</th><th>Smaller Cladonia species</th><th>Campylopus introflexus</th><th>Larger Cladonia species</th><th>Dense grassland</th><th>Calluna vulgaris heath</th></tr>
<tr><td></td><td colspan="5">interception of atmospheric deposition</td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td colspan="3">nutrient cycling in the mineral soil</td></tr>
<tr><td colspan="3">N limitation in grasses</td><td colspan="5">N+P+K limitation in grasses
(N+P limitation in high N deposition sites)</td></tr>
</table>

Some authors concluded that lichen vegetations mainly develop on soil high in base cations and phosphate (Daniëls 1990, Ketner-Oostra & Sýkora 2008). Based on the results in this thesis, this seems to be partly true: lichen-rich vegetations have a relatively well-developed soil, in which those elements accumulated in organic matter over time. Growth of lichens, however, may be enhanced by elevated phosphate levels in the topsoil, which may occur e.g. after wildfire (Daniëls 1990).

Apart from nutrient availability, drought stress is a growth-limiting factor in inland dunes, which is particularly effective during the first succession stages when the soil is still thin. Algal growth, and germination and survival of *Corynephorus canescens* seeds depend on wet summers, autumns and mild winters (Pluis & van Boxel 1993, Hasse 2005, Riksen & Goossens 2005). Due to the thicker organic soil layer in later succession stages, water retention is increased and drought stress becomes less important, resulting in a higher biomass production.

Effects of increased N deposition on soil and vegetation

In drift sand soils, the nutrient status depends largely on atmospheric deposition of nutrients, since the release of P and base cations by weathering of the parent material is low (van der Salm 1999, Kooijman *et al.* 2010, Sevink & de Waal 2010). During succession elements accumulate and are adsorbed to organic matter. In precipitation, ammonium was found to be the most abundant cation and showed large differences between sites. Concentrations of most other elements did not vary significantly. Nutrients and base cations are sufficiently available in precipitation to keep pace with vegetation growth (Table 7.2).

Ammonium likely competes with other base cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) for cation exchange sites in soil organic matter as in other dry sand habitats. This leads to lower and often limiting amounts of those base cations in high N deposition sites (chapter 3). In the past, deposition of oxidized sulphur compounds, “acid rain”, may also have contributed to enhanced leaching of base cations. Ammonium-rich soil is further acidified by increased nitrification, due to higher availability of substrate for nitrifying bacteria. Finally, increased uptake of ammonium by plant roots will increase proton concentrations in the soil solution (van Breemen *et al.* 1983). Due to soil acidification, aluminium may also become more soluble in acid soil. Earlier studies have shown that both ammonium and aluminium have a negative influence on the survival of lichens and plants (van Breemen *et al.* 1983, Skrindo & Okland 2002, Hasse & Daniëls 2006, de Graaf *et al.* 2009). Indeed, a higher soil Al:Ca ratio and lower soil pH was found in drift sand sites with high N deposition compared to low-deposition sites, indicating displacement of Ca^{2+} and a higher solubility of Al^{3+}. These high-deposition sites also had a significantly higher ammonium:nitrate ratio in the soil. In the Veluwe area, changes in the Al:Ca ratio may be somewhat more pronounced as the soil is richer in weatherable minerals as discussed in the introduction (chapter 1). The possible contribution of weathering could be part of future studies.

Table 7.2. Atmospheric input (precipitation; n = 4) and approximate aboveground vegetation stocks (n = 6 - 20) for several elements in pioneer vegetation in inland dunes. Atmospheric input (measured in 2008-2009) is given for regions with high (Kootwijkerzand, Wekeromse Zand) and low (Aekingerzand, Drouwenerzand) N deposition separately, but in aboveground biomass, stocks did not differ significantly between regions, and are combined. Mean values are given with standard deviations between brackets. Significant differences in atmospheric input between regions are marked with asterisks (* $P < 0.05$; ** $P < 0.01$). A range of the minimum development time and average age are given below the names of the succession stages. Methods and results are derived from Nijssen *et al.* (2011).

	Atmospheric input		**Stocks in aboveground vegetation**			
	mmol m^{-2} yr^{-1}		**mmol m^{-2}**			
Element	**Drenthe**	**Veluwe**	**Polytrichum**	**Smaller lichens**	**Campylopus**	**Reindeer lichens**
			5-9 yr	*15-53 yr*	*10-53 yr*	*20-49 yr*
Ca	9.5 (3.7)	8.8 (2.6)	5.8 (0.2)	6.1 (0.5)	16 (1.1)	9.7 (0.6)
K	8.5 (1.5)	8.1 (2.1)	17 (1.2)	18 (2.6)	47 (5.1)	28 (2.8)
Mg	6.1 (0.3)	5.5 (1.1)	6.2 (0.4)	6.4 (0.8)	17 (1.5)	10 (0.8)
Na	46 (38)	38 (6)	2.5 (0.4)	2.6 (1.0)	7.0 (1.9)	4.2 (1.1)
Al	0.4 (0.1)	0.2 (0.1) *	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
C	0 (0)	0 (0)	8404 (181)	8708 (394)	23449 (783)	13945 (429)
N	49 (10)	109 (7) *	155 (5)	160 (10)	431 (21)	257 (11)
NH_4	26 (9)	65 (5) **	-	-	-	-
P	2.0 (0.4)	2.6 (0.4)	4.7 (0.3)	4.8 (0.6)	13 (1.1)	7.7 (0.6)

The effects of N deposition were also visible in the microbial biomass in different succession stages (**chapter 4**). Microbial biomass was lower in sites with high N deposition, but the higher N:P and lower C:N ratios of soil microbes clearly indicated effects of higher N availability. Soil acidification and high levels of ammonium also have their effect on the vegetation. In a fertilization experiment in three different succession stages in sites with high and low N deposition, grasses contained more N and less base metals after N fertilization and also N:P and C:N ratios changed significantly (**chapter 5**). There were also significant differences in the grass N:P ratio between low- and high-deposition sites, with values around 10 at low, and around 20 at high N deposition levels. N deposition could also lead to P and K limitation of vegetation growth. P was limiting vegetation growth of the cryptogam layer in both high and low N deposition sites. Especially lichens showed a strong growth response to P addition, whereas vascular plants and moss species with root-like structures (*Polytrichum piliferum*) in the mineral soil or below a thick ectorganic horizon (*Campylopus introflexus*) did not respond. At low N deposition, grass growth was co-limited by N, P and K. In high N deposition sites, however, only P and K limitation was found, which was also the case and in low deposition sites after N fertilization. As expected, P addition resulted in an opposite effect: lower N:P ratios and even lower plant N concentrations. Absence of a growth response of grasses to P addition suggests that K limitation, and possibly also limitation by other base cations, may be a reason why strong grass encroachment does not occur in inland drift sands, as compared to observations in some coastal dunes (Kooijman & Besse 2002),whose soils are often much richer in minerals and receive base cations from salt spray. This may explain why lichen vegetation, although impoverished, is still present in inland dunes with very high levels of N deposition (e.g. 40 kg ha^{-1} yr^{-1}), whereas they largely disappeared from coastal areas.

Sites with high N deposition had a lower species diversity, especially of lichens, which form the most diverse group in drift sands. Experimental fertilization of three succession stages showed a decrease of lichen cover and increase of grass cover after N fertilization (**chapter 5**). The effects of N fertilization were larger in a low-deposition site, than in a high-deposition site. P fertilization gave an opposite effect: lichens increased, resulting in a higher lichen:grass ratio. High nitrogen deposition sites had a higher cover of algae, higher settlement of Scots pine and species of late succession stages. Increase of algae on bare sand reduces wind erosion and accelerates the settlement of *Corynephorus canescens*, i.e. speeding up succession. Scots pine forms an ongoing threat to the pioneer vegetation and is costly to remove. Thus, sites with high N deposition also require more frequent management to retain the area of bare sand and keep vegetation low.

Nitrogen sensitivity of cryptogams

Little literature exists on the nitrate versus ammonium use of bryophytes, but most studies find relationships with ammonium (Glime 2007). Ammonium toxicity is a common problem in cryptogams, especially lichens. There are several mechanisms that could make species tolerant to ammonium. Earlier studies on lichens have shown that species insensitive to ammonium toxicity tend to have less cation-exchange sites and are more capable of transforming intracellular ammonium into nitrate (Gaio-Oliveira *et al.* 2001). The rather ammonium-tolerant moss from dry acid soils, *Polytrichum formosum*, was found to have a low cation-exchange capacity of between 50 and 100 mmol kg^{-1} dry weight (Büscher *et al.* 1990), whereas values between 650 and 700 are reported for the N sensitive reindeer lichen *Cladonia rangiferina* (Meychik *et al.* 2010). Ammonium does not seem to harm the invasive moss species *C. introflexus* under ambient conditions, although at extreme deposition values around 100 kg N ha^{-1} yr^{-1} slower growth, not die-off, was found (**chapter 6**). Whether *C. introflexus* has high or low amounts of cation-exchange sites is unclear and open to future research.

Replacement of lichens by *Campylopus introflexus*

Campylopus introflexus, an invasive bryophyte

Many authors have described the invasion of the exotic bryophyte *Campylopus introflexus* in sandy soil in Western Europe (van der Meulen *et al.* 1987, Equiha & Usher 1993, Biermann & Daniëls 1995, Hassel & Söderström 2005, Hasse 2007, Ketner-Oostra & Sýkora 2008). Some authors supposed that nitrogen deposition may have stimulated its expansion in inland dunes, although no data supporting this hypothesis was provided (e.g. Ketner-Oostra & Sýkora 2008). **Chapter 6** of this thesis showed that settlement of *C. introflexus* is favored by both nitrogen deposition and presence of mineral soil rich in organic matter.

Replacement of lichens by *Campylopus*

The mechanism of the initial settlement and replacement of lichens by *Campylopus introflexus* is shown in Fig. 7.1. The first succession stage in which drift sand becomes fully covered with vegetation is the stage in which *Polytrichum piliferum* forms a belowground mat of rhizoids (Bowden 1991). The space between the shoots can then be filled up by lichens or *Campylopus introflexus*. In high N deposition and/or organic matter-rich sites, *C. introflexus* is favoured and starts to dominate the vegetation, forming a thick mat enclosing the existing vegetation, like a strangler fig. Local dominance of *C. introflexus* was found to occur at N deposition levels of 30-32 kg N ha^{-1} yr^{-1} or atmospheric ammonia concentrations of 7 µg NH_3 m^{-3}.

Campylopus introflexus forms mats of densely packed vertically orientated shoots that grow apically and die-off a few mm below. It may build a thick ectorganic layer, which acts as a sponge absorbing precipitation and elements contained in it. As the ectorganic layer grows, nutrients released by its decomposition are retained and easily taken up by the upper living part of the moss mat. Within the set of plant species in inland dunes, only *C. introflexus* possesses such a thick ectorganic layer. Lichens do have a similar structure, a basal layer of necromass (Crittenden 1991), but this layer is much thinner, especially in smaller species, such as cup-lichens and crustose lichens. The thick ectorganic layer makes *C. introflexus* a powerful competitor to the characteristic small lichen vegetations. Large lichens, such as reindeer lichens, can survive in cracks in older moss carpets. The latter has also been found by Daniëls *et al.* (2008) in a long-term monitoring study on *C. introflexus* encroached plots. The shift from smaller to larger lichen species as a result of elevated N deposition has also been found in dunes along the Baltic coast (Remke *et al.* 2009).

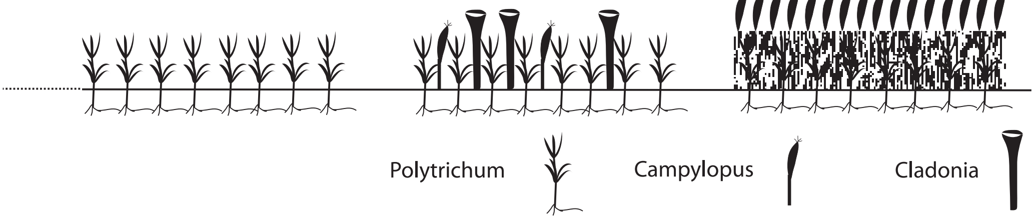

Fig. 7.1. Left to right: The invasion of *Polytrichum piliferum* mats (left) by lichens (middle) and *Campylopus introflexus* (right) under high N deposition, eventually resulting in dominance of *Campylopus introflexus* (right).

Decrease of lichen vegetation

The competition between lichens and *Campylopus introflexus* is supposed to be the main threat for future development of lichen vegetations. Older lichen vegetations may be threatened as well, due to the increase of the grass:lichen ratio of the vegetation caused by the combination of a possibly toxic effect of ammonium on lichens and increase of grass biomass production and settlement of trees. Although nitrogen deposition was seen as the main cause for a generic increase in grass cover in long-term monitoring plots in the Veluwe area (Ketner-Oostra & Sýkora 2008), this thesis provides the first evidence of increase of grasses in inland dunes based on experimental nutrient addition and a comparison of low and high-deposition sites.

Nitrogen stress in lichens has been further demonstrated by the dramatic increase of lichen biomass after P addition, showing an imbalance between N and P. Also, the decline of lichens after N addition showed that these species are extremely sensitive to nitrogen. Existing lichen vegetation may also become more vulnerable to damage by trampling, as exposed organic matter-rich mineral soil may be invaded faster by *C. introflexus* than by lichens. The same holds true for the replacement by *C. introflexus* of the generally short-lived crustose lichens (e.g. *Micarea leprosula, Placynthiella* sp., *Pycnothelia papillaria*), which grow directly on organic matter-rich mineral soil. Vegetation maps of eight inland dune sites showed that sites with N deposition levels above 30-32 kg ha^{-1} yr^{-1} had a lower cover of vegetation classes dominated by lichens relative to the *Campylopus* vegetation class.

The dispersal of lichens, measured as the survival of vegetative propagules, was negatively affected by nitrogen deposition and the invasion of *C. introflexus* as well. At least one species (*Cladonia strepsilis*) performed less under high N deposition in a dispersal experiment carried out in sites at high and low N deposition. Also, the cover of suitable substrate for the species, patches of bare mineral soil rich in organic matter, decreases as *C. introflexus* rapidly colonizes such patches.

Evidence for the decline of lichen vegetation due to high N deposition is further supported by comparing the age of the lichen vegetation inferred by soil depth (Fig. 7.2). In high N deposition areas, the mean thickness of the Ah horizon below the N sensitive small lichen vegetations is much larger than in low-deposition areas. The thicker A_h horizon suggests that the lichen vegetation in high-deposition areas are on average older as less new lichen vegetations are formed than in the past. An analysis of historical aerial photographs has shown that species-rich lichen vegetation may be several decades old. This means that part of this vegetation has been formed before the introduction of *Campylopus introflexus*. In low-deposition sites, lichen vegetations are formed at a much higher rate, resulting in a relatively younger soil, characterized by a thinner Ah horizon. This can be explained by lesser competition with *C. introflexus* and grasses. Apart from the soil age, the number of lichen species in randomly selected plots of 1 m^2 is also negatively affected by N deposition (Fig. 7.3).

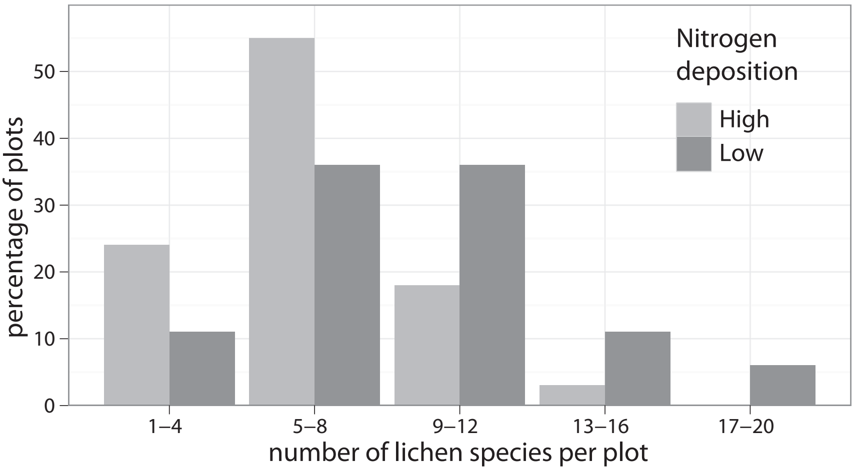

Fig. 7.2. Average A_h horizon depth below small lichen dominated vegetations (lichen cover 25% or more) of 20 plots in sites with high and low nitrogen deposition divided by the median value of nitrogen deposition (32 kg ha^{-1} yr^{-1}). (level of significance: $P < 0.05$). Based on data from Nijssen *et al.* (2011).

Although lichen diversity in 1 m^2 plots is about 20% lower in high deposition sites than in low-deposition sites within The Netherlands (chapter 3), species diversity at site level is probably not lower than under pristine conditions. Average species densities of 8 - 10 lichens per m^2, about 30% of all terricolous lichen species found in inland dunes, also compare well to a study along the Baltic coast (Remke *et al.* 2009) and Anholt (Christensen & Johnsen 2001). The effect of N deposition is therefore difficult to infer from large-scale distribution maps (e.g. www.verspreidingsatlas.nl). An exception forms the current distribution of some rare and sensitive lichen and bryophyte species, such as *Cetraria islandica* and the liverwort *Scapania compacta*. The current distribution of these species match low N deposition areas (van Tooren & Sparrius 2007). Rare sensitive species also disappeared from high-deposition sites.

Recommendations for conservation and restoration

Inland dunes are currently protected under the EU Habitat Directive. Recently, conservation targets have

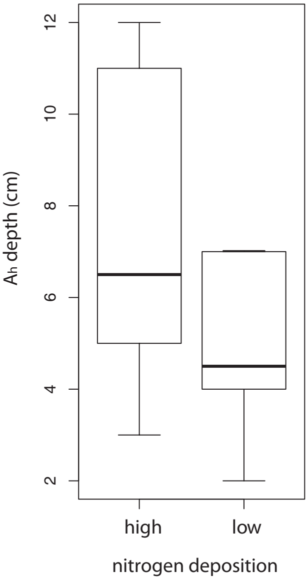

Fig. 7.3. Histogram with the distribution of the number of lichen species found in 172 randomly stratified plots of 1 m^2 in The Netherlands over a gradient of N deposition. High and low N deposition are separated by the median value of 30 kg N ha^{-1} yr$^{-1.}$ Only plots containing lichen species were selected. Based on the dataset used in Chapter 3.

been formulated and translated into restoration measures to stabilize or increase the area of bare sand and pioneer vegetation (Londo 1997, Bal *et al.* 2001, Riksen *et al.* 2006). Although the aim of this thesis was not specifically to evaluate restoration measures, the results of the field surveys and experiments confirm that conservation and restoration management is necessary in order to preserve bare sand and lichen-rich pioneer vegetation in inland dunes. Several common measures for the restoration and conservation of inland are discussed in this paragraph.

Creating bare sand

The most commonly applied measure to restore a drift sand site is to create bare sand by removal of forest and topsoil (Bal *et al.* 2001, Riksen *et al.* 2006, Riksen *et al.* 2008). Without this restoration management all bare sand would be lost in the period 2035-2050. In order to keep up with the ongoing loss of bare sand, forest removal needs to be carried out at a large scale, comprising around 43 ha per year for the entire country. This value is based on the succession rate in the period 1950-2006 and almost equivalent to restoration measures carried out within the period 2000-2006 (Riksen & Jungerius 2010). This means that current efforts are sufficient, but must be continued at the same rate to preserve the drift sand landscape. Target areas for restoration have been identified by Nijssen *et al.* (2011).

After topsoil removal, the succession rate depends on colonization and erosion. For example, active drift sand dunes can remain bare for a long time due to wind erosion, whereas blowouts become vegetated within a short period of time. It may take two or more decades before a closed, species-rich vegetation is formed. This slow development makes it difficult to rapidly evaluate restoration measures. Long-term monitoring is therefore required to get insight in the vegetation development of inland dunes.

The results of **chapters 4** and **6** showed that removal of topsoil, which is rich in organic matter, is also a critical factor in the restoration of drift sands. N mineralization due to the presence of higher levels of organic matter in bare soil contributed more to N availability than N deposition when a high- and low-deposition site in The Netherlands are compared. Also, in the *Campylopus* growth experiment, soil organic matter contributed more to *Campylopus* growth than nitrogen deposition. The results of this study implies that when after a restoration measure organic matter is left in the forms of wood chips, living vegetation or a partial Ah horizon, vegetation succession rate will increase, *Campylopus introflexus* may settle even before any other species and species confined to the earliest succession stages, such as *Stereocaulon condensatum*, will not return after restoration. Leaving organic matter after restoration will also result in higher management costs as the measures have to be more frequently repeated (Riksen *et al.* 2008).

The relative cover of bare sand versus vegetated parts in inland dunes remains a problem for conservation management (Castel & Koster 1987, Riksen & Goossens 2005, Riksen *et al.* 2006), in which a trade-off between current biodiversity and future development of pioneer vegetation must be made. The results of chapter 2 indicate that geomorphology plays an important role in the sustainability of newly created bare sand surfaces. In blowouts, wind erosion is low and pioneer vegetation will settle soon. In inland dune sites characterised by a large cover of blowouts, the management could therefore focus on maintaining pioneer vegetation instead of bare sand. To provide a habitat for species of the earliest stages, small-scale topsoil removal could be advised. Inland dune sites with a relatively large cover of drift sand dunes are more suited for restoration of bare sand surfaces of which the lifespan is prolonged by wind erosion processes (Castel 1991, Riksen *et al.* 2008).

Tree removal and grazing

Common measures to protect species-rich vegetation are removal of tree seedlings and isolated trees, especially Scots pine (Londo 1997, Bal *et al.* 2001, Riksen *et al.* 2006). Settlement of small Scots pine trees in the pioneer vegetation was found more frequently in high N deposition sites. Removal of small trees is one of the main conservation practices in inland dunes (Siepel *et al.* 2010) and high levels of N deposition may make drift sand management therefore more expensive.

Grazing by sheep and deer occurs in most inland dunes and helps to preserve the diverse mosaic vegetation of lichen-rich vegetation and dwarf-shrubs in dry heath (van der Bilt & Nijland 1993).

Protection of lichen-rich vegetations

Lichen vegetation is usually rather old and, once gone, may take several decades to develop on freshly created bare sand surfaces. Currently existing lichen vegetation must therefore be carefully protected. They may act as a source population from which earlier succession stages can be colonized. Some lichen species, such as reindeer lichens and several small species without frequent fruiting bodies or fine soredia (e.g. *Cladonia borealis, C. cervicornis, C. monomorpha, C. phyllophora, C. strepsilis* and *Stereocaulon condensatum*) are supposed to have a limited dispersal capacity. They are able to colonize younger succession stages, but only within a short distance of an existing population. Observations showed that sites with a high density of potential vectors (e.g. sheep, deer and rabbit) usually have a more even distribution of those species, although this should be a subject for future study (Nijssen *et al.* 2011).

In general, the oldest lichens vegetations can be found in blowouts, which by their stable nature, become earlier vegetated than drift sand dunes. However, blowouts are also prone to transform to forest earlier. Species-rich vegetation on blowouts therefore require more management in terms of tree-removal and low-intensity grazing than drift sands, in which erosion plays an important role in slowing down the succession rate.

Disturbance and vegetation being blown-over by sand are promoted as management tools by some authors. Riksen *et al.* (2006) postulated that pioneer vegetation must be covered with sand regularly. This is not supported with data and contradictory to measurements of sedimentation in closed pioneer vegetation, which is almost zero and primarily caused by local splash erosion instead of long-distance wind transport (Riksen & Goossens 2007). An exception must be made for plants of *Corynephorus canescens* and *Polytrichum piliferum*, which are more vital and have a better nutritional value for fauna species when they grow on a bare sand surface instead of a closed vegetation (Marshall 1965, Martínez & Maun 1999, Nijssen & Siepel 2010). Disturbance as a measure to promote the vitality of inland dune plants as suggested by Tschöpe & Tielbörger (2010) is likely to have an adverse effect, as disturbance results in litter and bare mineral soil rich in organic matter, which is the primary substrate for *Campylopus introflexus*. It can be concluded for the results in this thesis and other studies (Biermann & Daniëls 1997, Hasse & Daniëls 2006) that disturbance stimulates moss-encroachment, which may persist at least in sites with high nitrogen deposition. The use of tillage techniques in closed inland dune vegetation was also discouraged by Riksen & Goossens (2005) and Ketner-Oostra *et al.* (2008).

Post-*Campylopus* stages

There are only few studies on the development of a vegetation dominated by *Campylopus introflexus* and the possible measure to get a lichen-rich vegetation back in such sites. In some cases, mats of *C. introflexus* disintegrated after five to ten years (Daniëls *et al.* 2008) and transformed into a reindeer lichen dominated vegetation, in which most of the smaller lichen species are lacking. Other observations have shown that *C. introflexus* may be a forerunner of grass encroachment on drift sands developed on river dunes (Nijssen *et al.* 2011) or that the species thrives for a much longer period. Factors that might be of influence of the development of older *C. introflexus* mats are currently under investigation by the author.

Potential effects of N deposition reduction and resilience

Although restoration measures can preserve inland dunes by mitigating the enhanced succession rate caused by atmospheric deposition, they will have no or limited effects on soil acidification and changes in nutrient ratios and N availability caused by nitrogen deposition. Therefore, the ultimate way to restore N affected drift sands is to reduce N deposition. Levels of N deposition are currently decreasing and it is likely that the vegetation in inland dunes responds to this. N mineralization and plant-available nitrogen in the aboveground vegetation is likely to become lower when N deposition levels fall. Eventually, excess N in biomass could be completely lost, although this may take long, as N is efficiently recycled in acid habitats. Loss of N may result in less nitrification, less displacement and repletion of base cations and thus a higher soil pH (van Breemen *et al.* 1983). A lower availability of N will also have a direct effect on habitat quality. Settlement of pine trees will decrease and growth of grasses and *Campylopus introflexus* will be reduced in favour of lichens.

Main conclusions

Previous studies were largely focused on descriptive, often sequential studies of plots and, if experimental, on the effects of classic management measures on vegetation composition through sequential observations on plots. Though providing interesting results, these studies did not lead to fundamental insight into the impacts of nitrogen deposition on the performance of the various major plant species and their mitigation. It is only through the combination of extensive field surveys, field experiments on the effects of N and P fertilization and survival of lichen and bryophyte fragments under low and high N deposition, and laboratory experiments on N mineralization and growth of *Campylopus introflexus* under different conditions, that more basic information on the functioning and management of pioneer vegetation in inland dunes could be obtained.

Bare sand and pioneer vegetation (chapter 2)

An analysis of a sequence of aerial photographs revealed that the area of bare sand in land dunes decreased with 50% over the period 1950-2007. Between 1981 and 2007, the decline was significantly higher than in the previous period. Although bare sand was converted into pioneer vegetation, the area of pioneer vegetation did not change as an equal area was transformed into forest. A field study showed that the succession rate was higher in blowouts than on drift sand dunes. In blowouts, effects of nitrogen deposition were visible as a relatively higher cover of heath and forest compared to pioneer vegetation. Also *Campylopus*-dominated vegetation occurred relatively more than lichen-dominated vegetation. It is expected that between 2035 and 2050 most bare sand has been lost if no restoration management is carried out. The area of bare sand created in the period 2000-2006 was just enough to compensate the loss of bare sand. This means that the area of bare sand and pioneer vegetation will remain more or less equal if restoration is performed at the same rate in future.

Soil and vegetation (chapter 3)

During succession, soil organic matter accumulates and the pH drops. During this process, the availability of base cations and nutrients increases. Nitrogen deposition causes soil acidification which leads to a lower pH, higher Al:Ca ratio and higher ammonium:nitrate ratio in the soil. Nitrogen deposition also leads to a lower species density of especially lichens, a higher cover of algae and more seedlings of Scots pine.

Nitrogen dynamics (chapter 4)

With the increase of organic matter during succession, the microbial biomass, microbial activity (respiration) and nitrogen mineralization increase. Nitrogen deposition caused a decrease in microbial biomass, higher net nitrogen mineralization and nitrogen availability and a higher microbial N:P ratio. An important part of N cycling took place in the ectorganic layer of lichens and especially *Campylopus introflexus*. When the succession sere started on sand that was already high in organic matter content, e.g. after restoration measures where some topsoil or litter was left, presence of organic matter contributed more to nitrogen availability than differences in nitrogen deposition. This means that restoration measures must be performed carefully, removing all topsoil and leaving no litter, roots or wood chips.

Fertilization experiment (chapter 5)

When comparing two drift sands at low and high N deposition, the vegetation in the high-deposition site had taller grasses with a high N:P ratio and lower lichen cover. Experimental fertilization with ammonium nitrate caused higher N content and lower K, Mg, Ca and Na content in grasses. The grass:lichen ratio increased, mainly due to a decrease in lichen cover. The effect of N fertilization was larger in a site with low N deposition than in a high-deposition site. Phosphate addition caused an opposite effect, resulting in a lower grass:lichen ratio.

Cryptogams were P-limited in both sites, which can be concluded from the responses to N and P addition. Grasses were limited by N, P and K in the low N deposition site, but K+P-limited in the high-deposition site, or became so after experimental N addition. The shift from lichens to grasses indicates that N deposition leads to accelerated succession as grasses strongly contribute to soil development.

Campylopus introflexus (chapter 6)

The invasive species *Campylopus introflexus* invaded most parts of The Netherlands, including all inland dunes, since its introduction in the 1960s. However, its growth and invasiveness in inland dunes depends on both soil organic matter content and nitrogen deposition. This species was especially dominant in sites with nitrogen deposition higher than 30-32 kg N ha^{-1} yr^{-1} or an average atmospheric ammonia concentration of 7 µg m^{-3}. At low N deposition, survival of experimentally sown *Campylopus introflexus* fragments was very low, whereas nitrogen-sensitive lichens partially showed opposite results.

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Summary

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

In The Netherlands, over the past decades not only the total area of inland drift sand significantly declined, also in the remaining inland dunes the area of bare sand decreased and habitat quality became lower. Loss of the open inland dune habitat is mainly caused by the increase of forest starting as self-sown *Pinus sylvestris* trees. A lower habitat quality is largely due to encroachment by an invasive moss species, *Campylopus introflexus*, which competes with the characteristic lichen vegetation, but also loss of patches of bare sand and increase of algae. The main objectives of this thesis are to explain habitat loss and decreasing habitat quality. This is achieved through both field and laboratory studies, building upon previous studies that hypothesized the most important factors affecting the inland dune habitat. These factors mainly concern nitrogen deposition and restoration and conservation management.

Between 1950 and 2007, the area of bare sand declined by about 50% in all reserves in the country. The transformation did not lead to a larger area of pioneer vegetation, as an equal amount of pioneer vegetation had been transformed into forest of self-sown trees or closed heathland vegetation. Based on past transformation rates, bare sand will have vanished between 2035 and 2050 if no restoration management is carried out.

Habitat quality is clearly influenced by nitrogen deposition. This was visible in the vegetation. Sites with high nitrogen deposition had more *Corynephorus canescens* and algae growing on bare sand, taller vegetation in later succession stages, more grass compared to lichens, more *Campylopus introflexus* moss carpets compared to lichens, a higher settlement of *C. introflexus* compared to lichens, higher establishment of Scots pine, and more forest compared to heathland.

Soil conditions were significantly related to nitrogen deposition. During succession, soil organic matter increased, resulting in higher nitrogen mineralization, higher N availability and lower soil pH. In sites with high N deposition, nitrogen mineralization was even higher, and soil pH even lower. Also, both soil organisms and vascular plants showed a higher N:P ratio under high N deposition. In moss mats of *Campylopus introflexus*, half of the nitrogen mineralization took place in an ectorganic layer, suggesting efficient cycling of nutrients by this species, which may explain its success.

Experimental addition of nitrogen confirmed these effects. In plots where N was applied, the plant N:P ratio became higher and plant C:N ratio lower, lichens declined and grasses increased in cover. In a site with low N deposition, grass growth was found to be N limited, whereas in a high N deposition site, grass growth was limited by N, P and K. Experimental growing of *Campylopus introflexus* showed that its growth was promoted by higher soil organic matter content, but also by higher N deposition. Survival of *C. introflexus* and lichens partially showed opposite results. *Campylopus introflexus* only survived in a high deposition site, which agreed with the field survey.

Survival of lichen fragments under high nitrogen deposition was only lower for *Cladonia strepsilis*.

The results of this research can be used to enhance nature conservation. Regarding nitrogen deposition, a level of 30-32 kg N ha^{-1} yr^{-1} or 7 µg m^{-3} ammonia was found to be a critical level for *Campylopus introflexus* encroachment, which could be used in prioritizing emission reduction. At site level, restoration measures are required to compensate the loss of the inland dune landscape by forest and topsoil removal. As soil organic matter content promotes nitrogen mineralization and *C. introflexus* settlement, it is advised that after restoration measures by which bare sand area is created, the soil carbon content is as low as possible. This can be done by complete topsoil removal including litter and wood chips.

Samenvatting

Stuifzanden in Nederland: bodem, vegetatie, stikstofdepositie en invasieve soorten

In dit proefschrift staan de vragen centraal hoe de vegetatie in stuifzanden de afgelopen decennia is veranderd en wat de rol van stikstofdepositie daarbij is. Stuifzanden kenden hun maximale omvang halverwege de negentiende eeuw. Het ontstaan van stuifzanden moet worden gezocht in het plaatselijk intensieve en tot degradatie leidende landgebruik, een combinatie van eeuwenlang begrazen en plaggen (potstalcultuur) van arme heidegronden. Later werden maatregelen genomen om het stuivende zand vast te leggen, in het bijzonder door aanplant van Grove den, waarvan het hout vooral werd gebruikt in de mijnen. Ondertussen nam het oppervlak stuifzand aanzienlijk af. De laatste tijd wordt het stuifzand juist beschermd, onder meer vanwege de vermelding als beschermd habitattype op de Europese Habitatrichtlijn. Binnen het ecosysteem zandverstuiving zijn de soortenrijke pioniervegetaties van Buntgras, mossen en korstmossen van Europees belang.

In **hoofdstuk 2** is onderzocht hoe de vegetatiebedekking in acht grote stuifzandgebieden is veranderd over de periode van 1950 tot 2007 en welke factoren daaraan ten grondslag liggen. In die periode is de helft van het kale stuifzand begroeid geraakt. De afname was 1,5 tot 2 maal zo hoog tussen 1981 en 2007 als in de periode daarvoor. De verwachte toename van de soortenrijke pioniervegetaties bleef echter uit, omdat een gelijk oppervlak van met name naaldbos en heide ontstond. Al eerder was bekend dat Grove den, die van oorsprong niet of weinig in Nederland voorkwam, zich in stuifzanden gemakkelijk uitbreidt. Al met al wordt verwacht dat het kale zand in zandverstuivingen rond 2035, en in het meest gunstige scenario rond 2050, verdwenen is als er geen grootschalig herstelbeheer zou worden gevoerd. De recent waargenomen versnelde afname van kaal zand kan voor een deel worden toegeschreven aan de verlenging van het groeiseizoen en een toename van de stikstofdepositie op landelijke schaal. Daarnaast speelt atmosferische stikstofdepositie een rol, hoewel het effect hiervan wordt gemaskeerd door de op zichzelf al hogere succsiesnelheden in uitgestoven laagten, vergeleken met de stuifzandduinen. Door erosieprocessen raken stuifzandduinen minder snel begroeid dan uitgestoven laagten. Het effect van stikstofdepositie was vooral zichtbaar in de huidige kwaliteit van de verschillende successiestadia. In gebieden met hoge stikstofdepositie groeide meer buntgras op het kale zand. Ook was het aandeel soortenrijke korstmossenvegetaties geringer ten opzichte van vegetaties met de invasieve exoot Grijs kronkelsteeltje (*Campylopus introflexus*) en was er meer bos ten opzichte van heide.

In **hoofdstuk 3** worden de verschillende successiestadia in stuifzanden beschreven, voortbouwend op eerder onderzoek. Gedurende de successie neemt de hoeveelheid organische stof in de bodem toe en daalt de pH. Tegelijk met organische stof nemen ook de voor de vegetatie beschikbare concentraties van basische kationen (calcium, natrium, kalium en magnesium) toe. Ook worden tijdens de successie de milieuomstandigheden steeds minder extreem, waarbij droogtestress en erosie geleidelijk afnemen. Elk successiestadium heeft hierdoor zijn eigen kenmerkende plantengroei en de dominante groeivormen gaan van algen, mossen en korstmossen naar grassen en dwergstruiken, voordat de successie naar bos wordt ingezet.

Vervolgens werd onderzocht wat de effecten van stikstofdepositie op de bodem waren. In gebieden met een hoge stikstofdepositie was de pH lager en de verhouding aluminium:calcium hoger. De verlaging van de pH is te o.a. verklaren doordat er meer nitrificatie optreedt. Ook nemen planten ammonium op, waarbij een proton wordt afgestaan. Door de hogere concentraties aan H^+ en NH_4^+ wordt calcium van kationbindingsplaatsen in organische stof verdrongen. Tegelijk neemt de oplosbaarheid van Al^{3+} iets toe. Al met al kan een hogere aluminiumconcentratie leiden tot vergiftiging en verminderde kieming van basentolerante vaatplanten. Een hoge ammoniumconcentratie is mogelijk schadelijk voor korstmossen.

Stikstofdepositie had ook effecten op de vegetatie. Gebieden met hoge stikstofdepositie worden gekenmerkt door een hogere bedekking aan algen, een lager aantal korstmossoorten, een hogere kieming van grove den en minder kenmerkende soorten van vroege successiestadia.

De stikstofcyclus in stuifzanden wordt besproken in **hoofdstuk 4**. Hierbij is de netto stikstofmineralisatie onderzocht, de som van afbraak en vastlegging van stikstof door micro-organismen. Gedurende de successie in stuifzanden neemt het organische stofgehalte in de bodem toe en daarmee komt ook de stikstofcyclus steeds verder op gang. Een uitzondering hierop vormt één van de eerste stadia, matten van Ruig haarmos (*Polytrichum piliferum*), waar de netto mineralisatie van stikstof zeer gering of negatief was. In de volgende stadia, matten van Grijs kronkelsteeltje en korstmossen, is een ectorganische laag van afgestorven mos of korstmos aanwezig, die nog aan de plant erboven vastzit. Een belangrijk deel, tot zo'n 50% van de totale stikstofmineralisatie vond plaats in deze laag. Zo kunnen mossen en korstmossen, die zelf geen wortels hebben, de vrijgekomen stikstof en andere afbraakproducten gemakkelijk opnemen in de levende plantendelen erboven. Als we een vergelijking maken tussen gebieden met een hoge (Wekeromse Zand) en relatief lage stikstofdepositie (Aekingerzand), dan blijkt dat de invloed van stikstofdepositie op de hoeveelheid ammonium en nitraat in verse bodemmonsters significant is. Ook de stikstofmineralisatie nam sterk toe door stikstofdepositie. Een nog onverklaard effect dat al eerder in bossen was waargenomen, werd hier ook gevonden: de afname van de microbiële massa in de bodem in gebieden met een hoge stikstofdepositie, wat mogelijk samenhangt met stikstofverzadiging van microbiële massa. De hoogte van de stikstofmineralisatie per eenheid micro-organisme was dus veel hoger in het stuifzandgebied met hoge stikstofdepositie. In het onderzoek werden ook terreindelen in stuifzand vergelijken met terreindelen waarin bos is gekapt en de bodem ondiep is afgeplagd, zodanig dat er nog een klein deel van de voedselrijke bovenste bodemlaag (A-horizont) overbleef. Op zulke plekken verloopt de vegetatiesuccessie snel en is de stikstofmineralisatie al vanaf het begin af aan hoog. Vanuit beheersoogpunt is dit ongewenst omdat het de duurzaamheid van de herstelmaatregel verlaagt en ruimte schept voor de vestiging van het invasieve mos grijs kronkelsteeltje.

Het effect van bemesting van stuifzandvegetaties met stikstof en fosfaat staat beschreven in **hoofdstuk 5**. Hierbij zijn ook weer gebieden met hoge (Kootwijkerzand) en lage stikstofdepositie (Drouwenerzand) met elkaar vergeleken in plots in verschillende successiestadia. Door onbemeste plots tussen beide gebieden te vergelijken, kan inzicht worden verkregen over de langetermijneffecten van stikstofdepositie op de vegetatie. Hierbij wordt aangenomen dat de gebieden niet in andere factoren verschillen. Dit is in de vorige hoofdstukken al min of meer uitgesloten voor factoren als jaarlijkse neerslag, verwering van mineralen en depositie in neerslag van andere stoffen dan stikstofverbindingen. Wanneer we de plots vergelijken, dat blijkt in het hoge depositiegebied de vegetatie iets hoger te zijn, evenals de verhouding in de bedekking grassen:korstmossen. Ook hebben de grassen een hogere N:P verhouding. Bemesting met stikstof leidde in twee jaar tijd tot ongeveer dezelfde resultaten: grassen namen toe en korstmossen af. Dit bevestigt eerder onderzoek in andere habitattypen dat korstmossen gevoelig zijn voor stikstofdepositie en dat grassen kennelijk profiteren van de stikstofgift. Het effect van stikstofbemesting was tevens het grootst in het gebied met lage stikstofdepositie. Ook de chemische samenstelling van de bovengrondse delen van planten en korstmossen veranderde door stikstofbemesting. Niet alleen het stikstofgehalte nam toe, andere elementen namen met name in grassen namen af. Dit kan leiden tot tekorten aan essentiële bouwstoffen zoals kalium. Bemesting met natriumwaterstoffosfaat maakte ook deel uit van het experiment. Het leidde voor de vegetatiebedekking tot omgekeerde resultaten: een toename van korstmossen en afname van grassen. Op het Drouwenerzand leidde dit zelfs tot een zeer sterke groei en uitbreiding van twee soorten rendiermossen (*Cladonia arbuscula* en *C. portentosa*) in korstmossenvegetaties en snelle vestiging en uitbreiding van kraakloof (*Cetraria aculeata*) in haarmosmatten, ten opzichte van de niet- of met ammoniumnitraat behandelde plots. Ook op het Kootwijkerzand namen korstmossen toe ten opzichte van grassen. Dit experiment toont aan dat de verhouding tussen de belangrijkste nutriënten in de vegetatie, stikstof en fosfor, duidelijk uit balans zijn, vooral in gebieden met hoge stikstofdepositie. Grassen bleken in gebieden met hoge stikstofdepositie in hun groei beperkt te zijn door zowel fosfor als kalium.

Tot slot wordt in **hoofdstuk 6** de relatie tussen de invasieve exoot Grijs kronkelsteeltje en de stikstofdepositie onderzocht. Deze relatie werd al door eerdere onderzoekers vermoed, maar is nooit goed onderzocht. Dit mos is in de jaren 1960 in Nederland aangevoerd en heeft zich sindsdien sterk uitgebreid. Op alle geschikte groeiplaatsen komt de soort nu in meer of mindere mate voor. Stuifzanden, met name op de Veluwe en in de Maasduinen zijn er soms helemaal mee begroeid en de soort verdringt daar de oorspronkelijke korstmossenvegetatie. In twintig gebieden in heel Nederland is de vestiging van korstmossen en grijs kronkelsteeltje onderzocht in matten van ruig haarmos met slechts enkele pollen buntgras als enige andere aanwezig soort. Het bleek dat een correlatie bestaat tussen de aanwezigheid van grijs kronkelsteeltje (positief) en korstmossen (negatief) met de stikstofdepositie. De verhouding Grijs kronkelsteeltje/

korstmossen gaf de beste correlatie en kan ook door beheerders worden gebruikt voor de evaluatie van beheermaatregelen. Om meer inzicht in de achtergronden van deze correlatie te verkrijgen, is Grijs kronkelsteeltje samen met enkele korstmossoorten uitgezaaid in een gebied met hoge en lage stikstofdepositie om zo het effect van stikstofdepositie op de overlevingskans van fragmenten te onderzoeken. Grijs kronkelsteeltje en de meeste korstmossoorten in stuifzanden verspreiden zich hoofdzakelijk door fragmentatie over korte afstanden van bronpopulaties. Het bleek dat de overleving van Grijs kronkelsteeltje in het gebied met hoge stikstofdepositie veel hoger was dan in het gebied met lage depositie. Het omgekeerde gold voor het voor stuifzanden kenmerkende korstmos Hamerblaadje (*Cladonia strepsilis*). Andere soorten korstmossen met een wat bredere ecologie vertoonden geen duidelijk verschil tussen de gebieden. Tenslotte werd nog onderzocht hoe Grijs kronkelsteeltje reageert op experimentele toediening van stikstof op zand met verschillend organische stofgehalte. Het bleek dat organische stof de belangrijkste factor voor de overleving van ingezaaide fragmenten was. Ook was de invloed van stikstoftoediening merkbaar, zij het dat de soort bij een zeer hoge dosis minder goed presteert.

Dit onderzoek, deels gebaseerd op hypothesen uit eerdere studies, heeft veel nieuwe kennis over stuifzandvegetaties opgeleverd. Het is duidelijk dat hoge stikstofdepositie tot sterke achteruitgang van het stuifzandecosysteem heeft geleid. Sommige conclusies kunnen worden toegepast in het natuurbeheer. Zo blijkt stuifzandherstel vooral kansrijk in gebieden met een relatief lage stikstofdepositie. De dalende stikstofdepositiewaarden zorgen er voor dat in de toekomst soortenrijke korstmossenvegetaties gevormd kunnen blijven worden zonder dat deze door het mos Grijs kronkelsteeltje overgroeid raken. Een afname van de stikstofdepositie kan ook leiden tot een vertraging van de successie waardoor minder beheerinspanningen nodig zijn. De verbossing van oude successiestadia in de pioniervegetaties blijft een punt van zorg. Deze kan worden tegengegaan door opslag van bomen in een vroegtijdig stadium te verwijderen, zodat soortenrijke vegetaties beschermd worden tegen overgroeien. Bij grootschalige herstelmaatregelen waarbij bos gekapt wordt en de voedselrijke bovenlaag van de bodem wordt verwijderd, is het belangrijk dat tot op het blonde zand wordt afgegraven. Als strooisel of voedselrijke bodem overblijft, wordt de vestiging van planten en het mos Grijs kronkelsteeltje versneld, wat de duurzaamheid van de maatregelen niet ten goede komt.

Dankwoord

Na vijf jaren onderzoek is dit proefschrift dan eindelijk af! Een hoop werk, waar naast een dienstverband van vier jaar ook veel vrije tijd in is gaan zitten. Uiteraard heb ik al die tijd niet kunnen werken zonder de hulp van velen. Mijn promotiebaan heb ik vooral te danken aan Hans Esselink (Stichting Bargerveen), die mij daarvoor benaderde toen ik in 2005 in Nijmegen werkte. Zijn stichting zat twee verdiepingen lager in hetzelfde gebouw. Hans had gehoord dat ik iets van korstmossen wist en vroeg me om mee te werken aan een projectvoorstel. Vanwege het meetnet korstmossen in stuifzanden in het Netwerk Ecologische Monitoring dat ik bij de Bryologische en Lichenologische Werkgroep coördineer, had ik de meeste stuifzanden in Nederland al wel gezien en wilde daarom graag meewerken. Bovendien was ik op zoek naar een promotieonderzoek waarin mossen en korstmossen een rol spelen. Hans bracht me in contact met Annemieke Kooijman van de Universiteit van Amsterdam. Voor mij geen onbekende naam, omdat haar promotieonderzoek over laagveenmossen ging. Er was direct een klik en ik ben Annemieke dankbaar dat ik in Amsterdam aan de slag kon bij IBED-vakgroep Paleo-ecologie en Landschapsecologie, na een korte kennismaking met mijn promotoren Jan Sevink (Universiteit van Amsterdam) en Leo Stroosnijder (Wageningen Universiteit). De discussies en gezamenlijke veldbezoeken met Annemieke en later ook de discussies over de resultaten met Jan hebben de artikelen in dit proefschrift sterk verbeterd. Dank voor jullie geduld en vertrouwen!

Het onderzoeksproject dat leidde tot dit proefschrift omvatte meerdere projecten met onderzoekers van diverse onderzoeksinstellingen. Met Marijn Nijssen (Stichting Bargerveen) en Michel Riksen (Wageningen Universiteit) heb ik vele plezierige dagen in het veld doorgebracht, zowel in Nederland als tijdens korte buitenlandse reizen in Denemarken, Oost-Duitsland en Vlaanderen. Het was een voorrecht om ook met eerdere vegetatieonderzoekers in stuifzanden samen te werken: Rita Ketner-Oostra promoveerde op de vegetatieontwikkeling en beheer van stuifzanden en Waddenduinen op basis van jarenlang pq-onderzoek. Dankzij haar kennis, contacten en grote collectie overdrukken en rapporten kon ik mij gemakkelijk inlezen in het onderwerp. Ook organiseerde Rita een excursie met Fred Daniëls (Universiteit van Münster) op het Oud-Reemsterzand waar al 25 jaar een serie pq's werd gevolgd. De onderzoekers uit de vakgroep van Fred Daniëls kende ik al: Thilo Hasse en Helga Bültmann. Hen wil ik ook hartelijk danken voor het toesturen van overdrukken en onderzoeksgegevens. Andere leden van het onderzoeksteam waren Pim Jungerius (Stichting Geomorfologie en Landschap), Rienk-Jan Bijlsma, Han van Dobben, Rein de Waal (Alterra), Christiaan Both (SOVON Vogelonderzoek) en Chris van Swaaij (De Vlinderstichting). Een bijzonder droevig moment was het overlijden van Hans Esselink in de zomer van 2008. Hans speelde in het project een cruciale rol om de integratie van het werk van alle onderzoekers te waarborgen. Later werd deze rol door Arnold van den Burg overgenomen.

Tijdens het eerste jaar van het onderzoek, werd ik lid van het OBN-deskundigenteam Droog Zandlandschap, het programma Onderzoek en Beheer Natuurkwaliteit van het ministerie van Economische Zaken, Landbouw en Innovatie, waaruit dit onderzoek gefinancierd is. In het bijzonder Roland Bobbink, Ido Borkent, Han van Dobben, Wouter van Heusden, Jan den Ouden, Henk Siebel, Henk Siepel en Theo Verstrael hebben een bijdrage aan de discussie geleverd met betrekking tot het nut van dit onderzoek voor het natuurbeheer. Naast dit proefschrift is een rapport verschenen met een veelheid aan informatie voor beheerders van natuurgebieden, waaronder een stuifzandherstelkaart, een gedetailleerde landsdekkende kaart waarop beheerders kunnen zien waar boskap voor het vergroten van stuifzanden mogelijk is, een idee dat tijdens een vergadering van het deskundigenteam is ontstaan.

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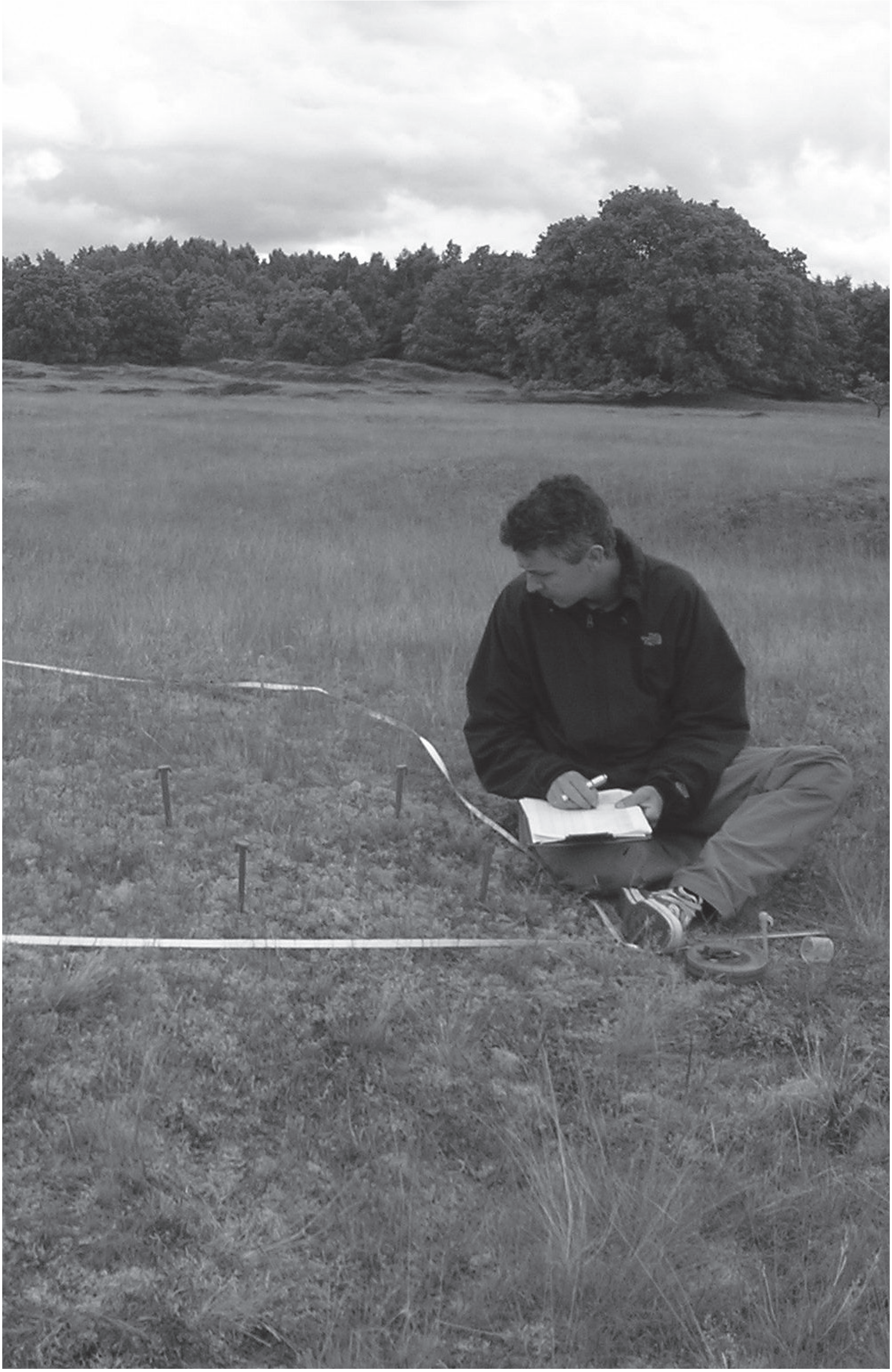

Curriculum vitae

Laurens Benjamin Sparrius was born on the 5th of December 1976 in Gouda, where he grew up and attended grammar school at the Coornhert Gymnasium. In 1995 he started studying Chemistry at Leiden University, but after some years switched to Biology, and graduated in 2004 with a combination of both studies. His Master's thesis dealt with lichen taxonomy carried out at the Centraalbureau voor Schimmelcultures under supervision of lichenologist André Aptroot and the Nationaal Herbarium Nederland. This resulted in a publication called "A monograph of *Enterographa* and *Sclerophyton*" describing 14 new lichen taxa, which has been published just before his graduation. During and after his study Laurens worked a few months ad interim as project leader at the Vereniging Onderzoek Flora en Fauna and Het Natuurloket. In 2003, he started his business BIO.DIV, specialized in both lichen monitoring and web application development, which was his primary work between 2003 and 2005. Beside these jobs he worked already since 2002 as a manager for the Dutch Bryological and Lichenological Society (BLWG). At first, this job focussed on database management for a mapping scheme carried out by volunteers, later Laurens co-ordinated many biodiversity and nature conservation projects dealing with lichens and bryophytes. In 2006 Laurens accepted a PhD research project at the University of Amsterdam, which resulted in this thesis. Beside research, he devoted a few months to teaching, including a fieldwork course in landscape ecology in Luxembourg and a botanical course for BSc students. Laurens currently works fulltime for the Dutch Bryological and Lichenological Society.

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Since 1997, Laurens devoted much of his time to lichenology and travelled throughout the Netherlands, Europe, Southeast Asia and North America together with lichenologists André Aptroot, Kok van Herk and Leo Spier his main lichenology teachers and co-workers. He published over 160 papers and book chapters in both Dutch and English. The lichen genus *Sparria* Ertz & Tehler (2011) and the species *Epigloea sparrii* Aptroot (2003) were named after him.

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