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Article in *Applied Vegetation Science* · January 2013

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## Response of inland dune vegetation to increased nitrogen and phosphorus levels

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### Keywords

Drift sand; Nutrient limitation; Lichens; Bryophytes; *Spergulo-Corynephorum*

### Nomenclature

van der Meijden (2005) for vascular plants; Siebel & Düring (2006) for bryophytes; Aptroot et al. (2004) for lichens

Received 16 March 2011

Accepted 13 April 2012

Co-ordinating Editor: Sabine Güsewell

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### 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 and were a threat to neighbouring arable land and settlements (Fanta & Siepel 2010). Large-scale afforestation with mainly *Pinus sylvestris* took place from the 1890s to the 1940s. Some inland dune areas are protected as nature reserves, but until the 1970s no conservation management took place, resulting in a further loss of open sand and an increase of short pioneer vegetation, forest and self-sown *Pinus* trees.

Inland dune vegetation is characterized by small-scale mosaics; in general, succession of the pioneer vegetation in inland dunes is characterized as a shift from bare sand to

### Abstract

**Question:** How does pioneer vegetation of acid inland dunes respond to addition of nitrogen (N) and phosphorus (P)?

**Location:** Two inland dune reserves in The Netherlands with low and high N deposition.

**Methods:** During 2.5 yr, N and P addition (control, N, P, NP) took place in three different vegetation types of 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 × 1 m per treatment. Differences in element content of grasses and lichens were recorded, together with vegetation parameters, including cover and height of grasses, bryophytes and lichens.

**Results:** In the site with high N deposition, grasses were taller, had higher N:P ratios and 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 an increase in grass cover and size. In contrast, lichens showed a general decline in the N treatment and the lichen:grass ratio decreased. The effect of N addition was larger in the low-deposition area. The P treatment had 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 in lichen cover due to increased N deposition.

cryptogams, to grasses and further to dwarf shrubs. Over several decades, the initial pioneer vegetation (*Spergulo-Corynephorum*), with *Corynephorus canescens* growing on bare sand, develops into typical lichen-dominated grasslands, with many species that are rarely found outside inland dunes. The importance of lichen vegetation 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 because of the regional increase in nitrogen (N) deposition. N deposition leads to increased vegetation cover and grass biomass, thus negatively affecting slow-growing, smaller species such as lichens (Riksen et al. 2006). In the 1960s, 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 vegetation dominated by smaller lichens (Ketner-Oostra & Sýkora 2008), especially in areas with high N deposition (Sparrius & Kooijman 2011). Further successional stages, i.e. reindeer lichen vegetation and *Calluna vulgaris* heathland, is less affected by *Campylopus*, but is threatened by grass encroachment.

Nitrogen deposition may cause a decrease in species diversity of the pioneer vegetation of drift sands, although different species groups may be affected through different mechanisms. Bryophytes 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 N (Bowden 1991), indicating N limitation of the vegetation in this vegetation type. The bryophyte mats efficiently accumulate N 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 the death of most cryptogams, especially lichens (Hasse & Daniëls 2006) or strongly reduced lichen cover (Soudzilovskaia et al. 2005) in studies where single doses of 12–90 kg·N·ha<sup>-1</sup>·yr<sup>-1</sup> were used. However, even frequent doses of low concentrations of N caused a decline of lichen cover in alpine and arctic vegetation (Gordon et al. 2001; Britton & Fisher 2007).

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 high N use efficiency (Boot & Dubbelden 1990). Addition of N and N with P caused an increase in the N:P ratio in grasses, and increased grass biomass in a 4-yr experiment in cryptogam-dominated alpine vegetation (Soudzilovskaia et al. 2005). N addition also increased growth of *Festuca ovina* and *Deschampsia flexuosa*, grasses of acid inland dunes, although in different ways, with the latter dominating the former under high N deposition and using nitrate as the primary N source (van Mierlo et al. 2000).

The hypothesis that mosses, grasses and lichens respond in different ways to N addition was tested in a field experiment. To explore potential long-term effects of N deposition, the experiment was performed in inland dunes with low and high N deposition levels. The field experiment included three distinct, major vegetation types within the inland dune pioneer vegetation: (1) mats of *Polytrichum piliferum*, (2) vegetation dominated by *Campylopus introflexus* and (3) vegetation dominated by reindeer lichens (*Cladonia* subgen. *Cladina*). The three stages are ordered by increasing soil development and cover of grasses such as *Corynephorus canescens*, *Festuca ovina* subsp.

*hirtula*, *F. filiformis* and *Agrostis vinealis*. Other main inland dune vegetation types that are dominated by *Corynephorus canescens*, smaller lichens and *Calluna vulgaris* were not included in this study.

Increased N deposition could lead to P-limitation of vegetation growth. Compared to coastal dunes, inland dune sand contains extremely low amounts of P (Sparrius 2011). P-limitation is relevant for nature conservation, as N deposition has a lower impact when vegetation growth is limited by nutrients other than N (Kooijman & Besse 2002). To test whether the vegetation in areas with high N deposition had become P-limited, additional P (and N + P) was applied. Moreover, cryptogams may be more P-limited than other plants, because they do not possess below-ground parts to acquire nutrients and atmospheric supply of P is very low (Sparrius 2011). P limitation in the cryptogam layer was suggested by Daniëls et al. (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 Benner & Vitousek (2007) in other epiphytic lichens, where experiments with P addition led to a significant increase in lichen growth.

The research questions addressed 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 N and P addition 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 northeast of The Netherlands, at Drouwen (52.955° N, 6.800° E), a site with relatively low N deposition (25 kg·ha<sup>-1</sup>·yr<sup>-1</sup>), and one at Kootwijk (52.163° N, 5.767° E), located in the central part of the country, with average N deposition (34 kg·ha<sup>-1</sup>·yr<sup>-1</sup>). Total N deposition was obtained from the OPS model (van Jaarsveld 2004) using 2006 emission data (RIVM 2010). Even in the low-deposition site, N deposition is above the critical load of 10–20 kg·N·ha<sup>-1</sup>·yr<sup>-1</sup> (van Dobben et al. 2006).

In both sites, plots were selected in three different vegetation types, which can be compared with those of Hasse (2005): (1) *Polytrichum piliferum* mats that had 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, which 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 & Kooijman 2011); and (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, which may develop from *Campylopus* mats and vegetation with small lichen species. This last vegetation type 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 in each vegetation type, 12 plots of 1 m × 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 (C) content. Plots indeed did not differ significantly from each other in these parameters.

Experimental addition of nutrients was carried out with four treatments: control, N (N, as  $\text{NH}_4\text{NO}_3$  solution), P (P, as  $\text{Na}_2\text{HPO}_3$  solution) and N + P (NP, both treatments), in three replicate plots per treatment. Treatment N is equal to a dose of  $42.9 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ , and treatment P to  $1.9 \text{ kg}\cdot\text{P}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ . The N dose is equivalent to about 2.5 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 N forms occur in rainwater (Nijssen et al. 2011). Plots were sprayed six times per year with 500 ml demineralized water with added nutrients to avoid toxic effects and excessive leaching of the added nutrients. For N, this resulted in a treatment with single doses of  $7.2 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ . Based on 800 mm annual rainfall in both sites, spraying added only ca. 1% to the precipitation intercepted by the vegetation annually. The experiment started in November 2006 and lasted till July 2009 (2 yr and 8 mo).

### Sampling

Soil samples of 5 cm topsoil (for each replicate plot mixed from three subsamples) were collected in 100-cm<sup>3</sup> soil sampling rings before and after the experiment. The sam-

ples were dried at 60 °C for 24 h, and weighed to estimate soil bulk density. Ground samples were analysed with a CNS analyser (Fisher EA1110) to estimate the C and N content. Sieved (2 mm) samples were shaken in purified water at a soil:water ratio of 1:2.5 and pH and conductivity ( $\text{EC}_{25}$ ) were then measured. The water extracts were filtered through a 0.2- $\mu\text{m}$  membrane. Dissolved organic carbon (DOC), water-soluble phosphate and N compounds ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ) were measured using a continuous flow analyser (AutoAnalyzer 3, Bran+Luebbe GmbH). Element concentrations were measured with an inductively-coupled plasma analyser (IRIS Intrepid II, Thermo Fisher Scientific). 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.

Relevés of all 72 replicate plots were made in November 2006 and July 2009. Cover values of mosses, lichens and vascular plants were estimated in July 2009 for both the groups of species and individual species. Height of the three groups was measured in subplots of 50 cm × 50 cm. In six randomly selected individuals, ramet length was measured for two cup-lichen species (podetia of *Cladonia coccifera*, *C. grayi* and *C. portentosa*) and two bryophyte species (shoots of *Polytrichum piliferum* and the upper green parts of *Campylopus introflexus*). The selected lichens were absent in the *Polytrichum* stage in both study sites, but otherwise all species were present. Vascular plant shoots and entire lichen thalli of *Cladonia portentosa* were collected in the 50 cm × 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. The above-ground parts, including attached decaying parts, were collected. Biomass of bryophytes and lichens other than *Cladonia portentosa* was not measured, as the sampling of hundreds of individuals per subplot would take too long. Grasses, including *C. canescens*, *Agrostis vinealis*, *Festuca ovina*, *F. filiformis*, were dried at 60 °C for 24 h and weighed. Plant material was milled to <0.2-mm fragments and dried at 110 °C for 4 h. The milled samples were digested in concentrated  $\text{HNO}_3$  and HCl in a microwave for 25 min (Jackson 1985). Cations, S and P were measured with an inductively coupled plasma analyser. Additionally, milled grass fragments were analysed in a CNS analyser to estimate C and N content. Grass species were sampled separately, resulting in one to three 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 vegetation type as independent factors. Differences between individual

mean values were tested with LSMEANS tests ( $P < 0.05$ ) using SAS Analytics (SAS Institute Inc.). In order to test for potential effects of nutrient treatments, a three-way ANOVA was applied, with treatment, vegetation type and site (differing in N deposition) as independent factors. Differences between individual mean values were tested with LSMEANS tests ( $P < 0.05$ ). Data were randomly selected and independent, and groups had more or less normal distributions and equal variances.

To estimate whether limitation of N, P and/or K occurred, grass nutrient content was compared to critical levels provided in fig. 1 of Olde Venterink et al. (2003). Nutrient content was therefore calculated in  $\text{mg}\cdot\text{g}^{-1}$ , P being multiplied by ten (10P) for better data visualization because a N:P ratio of ten to 15 is common under low-N conditions. Although the data of critical levels for nutrient limitation were originally developed for aquatic plants, the authors assume that they can also be used in other habitats.

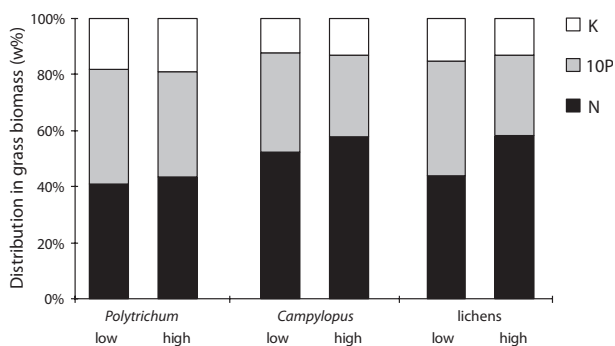
Mean grass content was calculated from individual grass samples, depending on the amount of grass species (one to three) in a plot. The *Polytrichum* and *Campylopus* plots typically contain one or two grass species, whereas the lichen plots contain two to three grass species. In one site, the three replicates per treatment may yield four to 12 grass samples.

## Results

### Differences between control plots in low- and high-deposition sites

Comparison of soil data (Appendix S1) shows that the C:N ratio of the soil is slightly (but not significantly) lower in the high-deposition site (13.3 vs 14.9). Also, pH, individual water-extractable N compounds and base cations did not differ significantly between the sites.

Element content in grasses, where all species responded in the same way and did not differ in element content, also



**Figure 1.** Distribution of N, 10P and K content (wt% as in Olde Venterink et al. 2003) in grass biomass in areas with low and high N deposition in control plots in different vegetation types.

differed between the two sites (Table 1). Grass P content increased with vegetation type in the low-deposition site, but decreased in the high-deposition site. In both sites, the N:P ratio in grasses was initially around ten to 12 in *Polytrichum* mats. In the low-deposition site, N:P ratios remained relatively low. In the high-deposition site, however, N:P ratios increased with vegetation type to values above 20, which indicates a relative shortage of P. The N, K, Ca and Mg content of grasses increased with vegetation type in the low-deposition site, but not in the high-deposition site; grass K, Ca and Mg content was significantly lower in the high-deposition site. The N:P ratio increased with vegetation type in the high-deposition site. Moreover, the K content of grasses decreased in the high-deposition site (Fig. 1). Differences in grass N, P and K content between the low- and high-deposition sites became more pronounced, especially in the reindeer lichen stage (Fig. 2).

The *Polytrichum* stage seemed co-limited by N, P and K in both sites, with an N:P ratio around ten and a relatively high K content (Table 1). The *Campylopus* stage was P-limited, with relatively high amounts of N and a N:P ratio between 15 and 20. 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 last two vegetation types (Appendix S1). 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%). There was little difference in pH between treatments, with a generally slight acidification after N addition. pH was higher in the (N)P-treated plots, where Na salts were applied. P addition also led to significantly lower amounts of some base cations (K, Ca and Mg) in the soil, probably due to the application of Na salts.

The element content (C, N, P and base metals) of grasses differed between treatments, vegetation types and/or sites (Table 2). In all sites and vegetation types, N and P content and C:N and N:P ratios differed significantly from control treatments according to the nutrients added (Table 3). 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



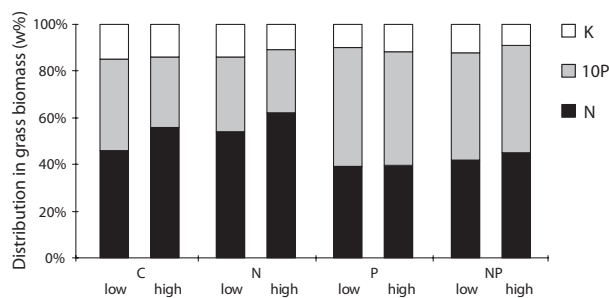
**Table 1.** Characteristics of the vegetation in the control plots for three different vegetation types in a low and high N deposition inland dune site. Vegetation types are: pristine *Polytrichum* mats, lichen-dominated vegetation and *Campylopus*-dominated vegetation.

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

<sup>1</sup>Significant difference between sites.

<sup>2</sup>Significant difference between succession stages (two-way ANOVA).

Different letters denote significant differences ( $P < 0.05$ ) between vegetation types within a site. Values are presented as means and SD based on four replicates ( $n = 4$ ).

**Figure 2.** Distribution of N, 10P and K content (wt% as in Olde Venterink et al. 2003) in grass biomass in sites with low and high N deposition in plots with different treatments.

high-deposition site, and also of K. With N addition, the N:P ratio in all vegetation types decreased (Fig. 2). In the P and NP treatments, P content increased, N decreased and differences between the high- and low-deposition sites became smaller. Addition of P and NP also resulted in different ratios between N, P and K content in grasses. Na content was affected by treatment, but probably only due to the use of a Na salt in the P treatment.

In approximately half of the plots, interactions between nutrient treatment, vegetation type and/or site were significant. For site x vegetation type, for example, Ca and Mg levels in grasses were higher in the site with low deposition, but mainly in stages with *Campylopus introflexus* and lichens, and not in mats with *Polytrichum piliferum*. In vegetation type x nutrient treatment, stages with *Campylopus* and lichens showed slightly stronger responses to application of nutrients than the site with *Polytrichum*. In site x

nutrient treatment, responses to nutrient application were generally slightly higher in the low-deposition site. However, these interactions did not change the overall response patterns and were not included in the ANOVA table.

Lichens were only analysed in the lichen-dominated vegetation in the low-deposition site and responded similarly to grasses, although changes as a result of treatments were generally larger. The C:N ratio in *Cladonia portentosa* (Appendix S2) was low after N treatment, high in the NP treatment and did not differ from the control in the P treatment. N and P content 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 vegetation types, 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, N 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 vegetation type. Grass cover was significantly higher in the N treatment than in control and P treatments. In both sites, grass height did not increase after N treatment, but was negatively affected after P treatment.

Lichen cover differed significantly between treatments, vegetation types and sites. Compared to the control,

**Table 2.** Analysis (three-way ANOVA) of differences in vegetation responses between nutrient treatments, succession stage and site (N deposition).

	Nutrient treatment	Vegetation type	Site
Grass C content (mg·g <sup>-1</sup> )	n.s.	0.0001	0.04
Grass N content (mg·g <sup>-1</sup> )	0.0001	0.0001	n.s.
Grass P content (mg·g <sup>-1</sup> )	0.0001	0.04	0.0001
Grass C:N ratio	0.0001	0.03	n.s.
Grass N:P ratio	0.0001	0.0001	0.0001
Grass K Content (mg·g <sup>-1</sup> )	n.s.	0.0001	0.0001
Grass Na Content (mg·g <sup>-1</sup> )	0.0001	n.s.	n.s.
Grass Ca Content (mg·g <sup>-1</sup> )	0.0001	0.0003	0.0001
Grass Mg Content (mg·g <sup>-1</sup> )	0.0001	0.0001	0.0001
Grass biomass (g·m <sup>-2</sup> )	n.s.	0.0001	0.01
Grass cover (%)	0.004	0.0001	n.s.
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	n.s.
Height <i>Polytrichum</i> (cm)	0.0001	0.0001	0.0001
Height <i>Campylopus</i> (cm)	n.s.	0.0001	n.s.
Height <i>Cladonia portentosa</i>	0.0001	0.0001	0.0001
Height <i>C. coccifera</i>	0.0001	0.0001	0.0001
Height <i>C. grayi</i>	n.s.	0.03	0.0001

Significant effects are given as *P* values; n.s. = not significant (*P* > 0.05).

application of N had a negative impact on lichens (Fig. 3) and lichen cover was reduced by about 50% at both site types. P addition resulted in a strong increase in lichens, resulting in a high lichen:grass ratio. Species with 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 *Cladonia portentosa* differed significantly between sites, vegetation types and treatments, similar to the observed differences in lichen cover, and increased in the P treatments. The ramet length of two lichens with low cover, *Cladonia coccifera* and *C. grayi*, however, tended to be 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, vegetation type and site. Bryophyte height was affected by treatment and vegetation type, but did not differ between sites. Bryophytes did not respond 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, vegetation type and treatment. P and NP addition resulted in significantly longer shoots compared to the control plots. *Campylopus introflexus* ramet length was only affected by vegetation type and was highest in the *Campylopus* stage.

**Table 3.** Element content, N:P and C:N ratios of grasses (*Corynephorus canescens*, *Festuca ovina*, *F. filiformis* and *Agrostis vinealis*) for control plots and three treatments in inland dune sites with low and high N deposition. Figures are given as mean values with SD between brackets. N = 20–30 for each given value, depending on the number of grass species occurring in the plot.

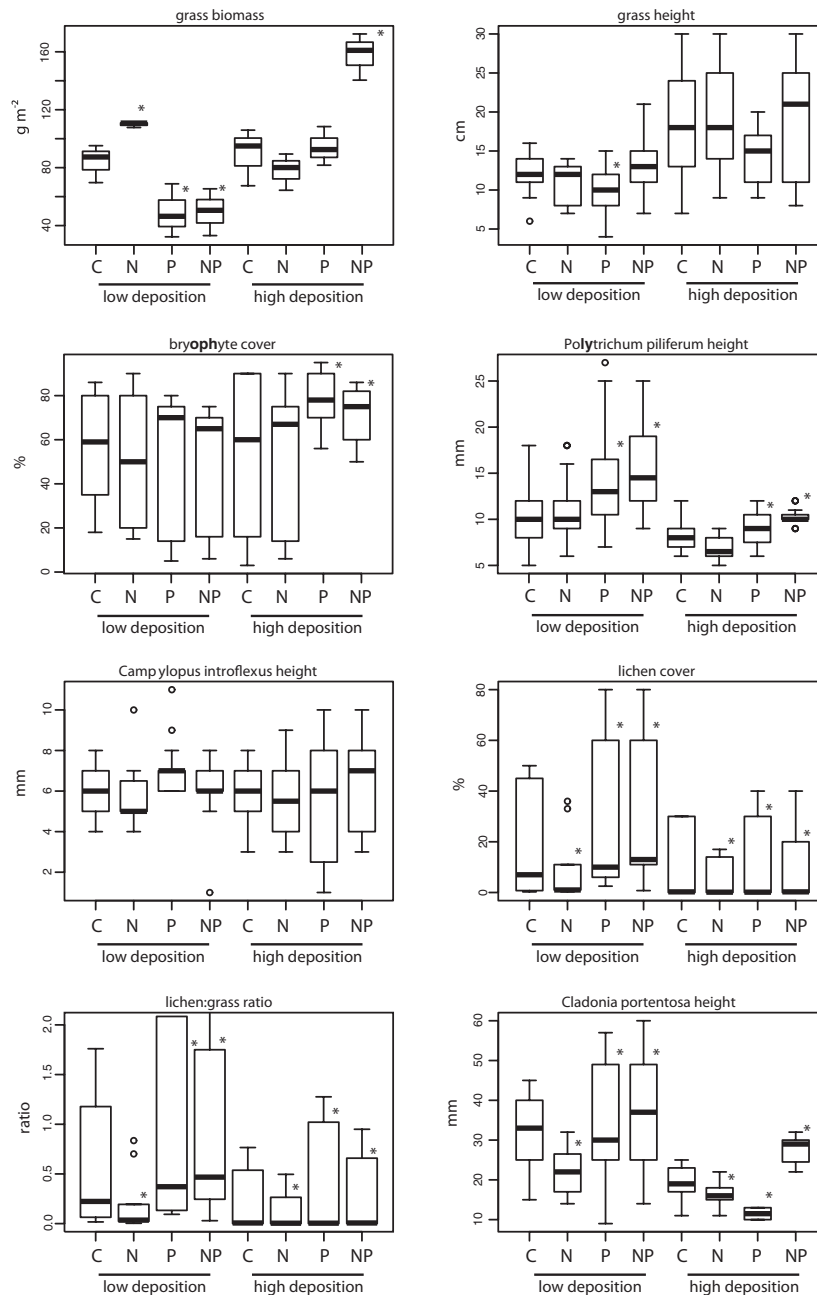
	Treatment			
	Control	N	P	NP
Low N deposition site, mg·g <sup>-1</sup>				
N	10 (1.4)	11.8 (1.9)**	10.5 (1.5)	11.3 (1.9)*
C	421 (36)	438 (26)	415 (45)	440 (28)
P	0.8 (0.2)	0.7 (0.1)*	1.3 (0.2)***	1.2 (0.3)***
C:N ratio	42.8 (7)	37.6 (5.6)**	39.7 (4.3)	39.8 (7)
N:P ratio	12.5 (3.5)	17.3 (3.9)***	7.8 (1.3)***	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 <sup>-1</sup>				
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)

Significance compared to control: \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

## 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 of 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 vegetation types in the high-deposition site. Furthermore, differences between the low and high N deposition sites were found in all three vegetation types with respect to grass P content. The grass N:P ratio in the high-deposition site was around 20 in the two older vegetation types, which indicates the absence of N limitation (Koerselman & Meuleman 1996; Güsewell & Koerselman 2002). In all plots in the low-deposition site, the grass N:P ratio was around ten to 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 of Olde Venterink et al. (2003), the *Polytrichum* stage seemed to be 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



**Figure 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.

*Campylopus* stage, however, was only P-limited, not N- and K-limited, which further confirms that this species prefers relatively N-rich conditions (Sparrius & Kooijman 2011). In the older lichen-rich vegetation, grass growth was still limited by K + N + P in the low-deposition site, confirming that nutrient availability in drift sands is low. However, in the high-deposition site, P seemed to be the main limiting factor, due to increased N availability.

### Effects of fertilization on soil and plant element content

The N and P content in grasses changed according to the nutrients added. Addition of N led to a lower P content in the site with low N deposition. In the site with high N deposition, N addition led to a 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 declined in the N-treated plots, especially in the site with high N deposition, which suggests that ammonium also displaced some of the bivalent cations. Comparison with data of Olde Venterink et al. (2003) suggests 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 a shift from co-limitation to P-limitation when sites with low and high N deposition are compared. Experimental N addition and comparison of sites with different N deposition thus show similar responses of the vegetation.

The P and NP treatments, however, resulted in K limitation. K content did not actually decrease, but content of P increased. In contrast to grasses, N addition did not lead to lower Mg and Ca content in the lichen *Cladonia portentosa*, probably because of the strong decrease in size of lichen thalli when N is applied. The size of a lichen is related to both basal decay and apical growth (Sipman 1978). Basal decay may not be affected by N fertilization, but apical growth is much reduced; thus lichen growth requires less nutrients. Also, a single dose of ammonium might have displaced cations such as Mg and Ca from binding sites shortly after the treatment (Miller & Brown 1999), but Mg and Ca from precipitation may compensate this. Analysis of precipitation collected in these sites showed that 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 responded positively to increased N availability. Higher grass cover, height and biomass with higher N availability was found as both a short-term (fertilization) and long-term response (high N deposition) to high N supply. P and NP addition resulted in lower grass biomass in the low-deposition site, mainly as a result of grasses being overgrown by lichens, particularly in the lichen vegetation type. In the high-deposition site, a significant positive response of grass biomass was only found in the NP treatment, probably because of P limitation in grasses in the *Campylopus* and lichen vegetation type. A similar response would be expected in P treatment, but these plots had an initial higher lichen:grass ratio, causing lichens to expand at the expense of grasses.

Lichens showed a different response to that of grasses. In accord with other studies (Gordon et al. 2001; Soudzilovskaia et al. 2005; Hasse & Daniels 2006; Makkonen et al. 2007; Britton & Fisher 2010), there was a clear decrease in lichen cover with high N supply or high N deposition. The reduction in vitality of lichens could be a result of ammonium toxicity, as demonstrated in studies in inland dunes (Hasse & Daniels 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* was reduced in size in the N treatment, as also 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, decreased in cover after N application, but there was no response in podetium size. Cup-lichens consist of a primary thallus of squamules lying on the soil surface, with vertical podetia (cups) of rather constant size (Sipman 1978), but where cup height may be less dependent on nutrient availability.

In contrast to results of 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 addition. Within the short time span of this experiment, no evidence for a positive effect of N and P on growth of *Campylopus introflexus* was found, even though grasses in the *Campylopus* stage seemed P-limited in both the low and high N deposition sites. The *Campylopus* mats used in this experiment already had a very high cover, which was unlikely to increase significantly. N addition did not cause a decrease in *Campylopus* height, which can be attributed to tolerance to relatively high levels of N (Sparrius & Kooijman 2011) and efficient interception of nutrients by the thick moss mat (see e.g. Cornelissen et al. 2001), thus avoiding N uptake by grass roots. N addition also did not affect the thickness of the upper green part of the *Campylopus* shoots. This green layer is more or less constant in size, as ramets stand close together in the moss mat and light intensity rapidly falls towards the base of the layer, leading to inhibition of photosynthesis (Cornelissen et al. 2001). The effects of N deposition on *Campylopus introflexus* could therefore be better studied in a dedicated experiment with spores or fragments cultivated under different nutrient conditions (Sparrius & Kooijman 2011).

#### Effects of P addition on 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), a difference in the N:P ratio of rainwater may directly influence the tissue N:P ratio. Under P-limited conditions, lichens are highly efficient in phosphate uptake (Hyvärinen & Crittenden 1998b; Hogan et al. 2010) and it is likely that in the high N deposition site P addition will increase lichen growth, in agreement with the present results. In the low-deposition site, however, the response of lichens was similar to that of *Polytrichum piliferum* in height. This indicates that even in the low-deposition site, lichen growth is already N-saturated, resulting in P-limitation.

*Cladonia* N content in both sites agreed with the maximum levels for *Cladonia* N of 10 mg·g<sup>-1</sup> (average of apical and basal parts) in Hyvärinen & Crittenden (1998a). In the low-deposition sites, grasses do not (yet) show P-limitation, as they can take up additional P from the soil. This may explain why grasses and cryptogams respond differently to P addition even when growing close together.

The addition of P in inland dune systems resulted in opposite effects to those of N addition with respect to the vegetation structure, as also found in related ecosystems. In a 4-yr 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*, while N addition led to a decrease (Pilkington et al. 2007). Furthermore, Vagts & Kinder (1999) observed a decline in ramet length of terricolous lichen species (*Cladonia furcata* and *C. portentosa*) after N addition, but an increase after NPK addition. Similar to the present study, nutrient application to smaller lichens such as *C. floerkeana*, *C. coccifera* and *C. grayi* had no effect. P addition may lead to a strong growth response through effects of P on cell division of the algal partner, but this does not seem to damage the lichen symbiosis over the longer term (Makkonen et al. 2007).

#### Differences in lichen:grass ratio

The lichen:grass ratio may be used as indicator value for the vitality of cryptogam vegetation (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 after application of N, and increased after application of P and NP. Furthermore, the lichen:grass ratio was lower in the site with high N deposition, in accord with Remke et al. (2009), who found a negative effect of N deposition on the lichen:grass ratio in the Baltic Sea region.

#### Nutrient limitation and species responses

In this experiment, there was an increase in biomass and vegetation cover as a result of nutrient addition. Moreover, differences in nutrient limitation were found between sites that had been exposed to high and low levels of N deposition over a long period. Both short- 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 of the stoloniferous *Agrostis vinealis*. Long-term high N deposition may even lead to P limitation of grasses in later vegetation stages with N:P ratios 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 had deposition above modelled and empirical critical loads for inland dune pioneer grasslands of 10–20 kg·N·ha<sup>-1</sup>·yr<sup>-1</sup> (van Dobben et al. 2006). P limitation caused by high N deposition may thus explain the strong, positive response of cryptogam growth after P treatments, especially for species without rhizomes or a relatively large contact surface with the soil, such as the reindeer lichen *Cladonia portentosa*. Lower availability of P, and perhaps also K, for grasses in the high N deposition sites, however, may prevent the complete loss of cryptogam vegetation. This may explain why strong grass encroachment observed in some acidic coastal dunes (Kooijman & Besse 2002; Remke et al. 2009) does not occur in the low vegetation of inland dunes.

#### Conclusions

Nitrogen addition in inland dunes generally caused an increase in grasses and a decrease in lichen cover. This is supported by the observation that effects on the vegetation, especially grasses, are more significant in the low N deposition site. P addition had opposite effects: of the main two cryptogam types, lichens showed a stronger response to nutrient addition than bryophytes; N addition caused no significant change in bryophyte cover or height of individual species. The two life forms, cryptogams and grasses, responded differently to addition of N and P. Cryptogams primarily use nutrients deposited in precipitation, which often contains surplus N compared to P, even in the study site with lower N deposition.

#### Acknowledgements

We thank L. Hoitinga, P. Wartenbergh and A.J. van Wijk for laboratory assistance and Prof. L. Stroosnijder and two anonymous reviewers for comments on the manuscript. Staatsbosbeheer and Stichting Het Drentse Landschap allowed us to perform experiments in their nature reserves. This research was financially supported by the OBN program of the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

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## Supporting Information

Additional supporting information may be found in the online version of this article:

**Appendix S1.** 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.

**Appendix S2.** Element content and ratios of the lichen *Cladonia portentosa* for the control plots and three different treatments in a lichen-rich vegetation in the low N deposition site.

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