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Iron-rich dune grasslands: Relations between soil organic matter and sorption of Fe and P

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Iron-rich dune grasslands may be protected from high N-deposition and grass-encroachment only when SOM is low, because only then P-fixation in iron phosphates occurs.

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A B S T R A C T

Effects of high atmospheric nitrogen-deposition partly depend on availability of phosphate. Lime-poor, but iron-rich dune grasslands are supposedly protected from grass-encroachment, due to P-fixation in iron phosphate. However, in iron-rich Dutch hinterdunes, dunes have low, but dry former beach plains high grass-encroachment. To test whether these zones differ in nutrient availability, and whether this changed with duration of grass-encroachment, we measured net N-mineralization, microbial characteristics and different fractions of P and Fe from pioneer and shortgrass to tallgrass stages approximately 10, 20 and >25 years old. N-mineralization did not differ between zones, but increased in older tallgrass stages in the organic layer. P-availability was significantly lower in the low grass-encroachment zone, with SOM values below 3% and mineral Fe above 40% allowing for P-fixation in iron phosphates. In the high grass-encroachment zone, however, P-availability increased, because SOM increased and Fe became incorporated in organic matter complexes, with more reversible P-sorption.

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1. Introduction

Grey Dunes, consisting of species-rich dune grasslands, belong to the priority habitats of the EU-Habitat Directive. In NW-Europe, dune grasslands are threatened by grass-encroachment, and many of them have become dominated by tallgrass (Veer and Kooijman, 1997; Kooijman and Besse, 2002; Jones et al., 2004; Plassmann et al., 2009; Remke et al., 2009;). Grass-encroachment is generally attributed to increased atmospheric nitrogen-deposition. In the early 1990s, N-deposition in the coastal dunes was estimated as 27–30 kg N ha^{-1} yr^{-1} (Dopheide and Verstraten, 1995). N-deposition has decreased since then, but is probably still above the critical load of 10–20 kg N ha^{-1} yr^{-1} for Grey Dunes (Achermann and Bobbink, 2003; van Dobben and van Hinsberg, 2008). Grass-encroachment may even start at much lower levels, such as N-deposition of 5–8 kg N ha^{-1} yr^{-1} (Remke et al., 2009). According to the Dutch government, 75% of the coastal dunes are no longer threatened by N-deposition (Natuurbalans, 2006), but this number is probably too optimistic for dune grasslands, which are more sensitive than most other dune habitats (Achermann and Bobbink, 2003; van Dobben and van Hinsberg, 2008).

The impact of high N-deposition partly depends on the availability of phosphate (Kooijman et al., 1998; Kooijman and Besse, 2002). In calcareous dunes, P is mainly present in calcium phosphate (Lindsay and Moreno, 1966; Syers and Walker, 1969), and relatively unavailable to plant roots. In calcareous dunes, high N-deposition may lead to grass-encroachment (Jones et al., 2004; Rhind et al., 2007). However, in Dutch dunes, grass-encroachment was lower in calcareous than in partly decalcified dunes (Kooijman and Besse, 2002). If P-availability is limited, high N-deposition may not be such a problem, and grass-encroachment then probably is relatively low. In partly decalcified soil, however, P-availability may increase with the dissolution of calcium phosphate, which may account for 60 g m^{-2} P per mm soil depth (Kooijman et al., 1998), and lead to high grass-encroachment. In acid soils, P-availability is mainly regulated by iron, which is a strong P-adsorbent (Hamad et al., 1992; Golterman, 1997). In iron-poor dunes, such as around the Wadden Sea in the Netherlands, Germany and Denmark (Eisma, 1968), P-availability and grass-encroachment are high, because iron levels are too low for P-fixation in iron phosphate

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(Kooijman et al., 1998; Kooijman and Besse, 2002). In iron-rich dunes, however, located in west and south-west NL (Eisma, 1968), P-availability and grass-encroachment may be relatively low. Similar to iron-rich wetlands (Lamers et al., 1998), phosphate concentrations may be reduced due to P-fixation in iron phosphate.

However, even iron-rich dunes have zones with low and high grass-encroachment. In a typical dry dune area such as the Amsterdamse Waterleiding Duinen (AWD), with groundwater tables several metres below the surface (van Til and Mourik, 1999), former beach plains have become completely covered with tall-grass vegetation, e.g., with *Calamagrostis epigejos* (L.) Roth, while adjacent dune zones for a large part still consist of shortgrass, with e.g., *Corynephorus canescens* (L.) P. Beauv. Grass-encroachment may partly be a self-reinforcing process, which increases biomass production, root uptake capacity and N-mineralization once started (Veer and Kooijman, 1997). Grass-encroachment may be further stimulated by atmospheric N-deposition, especially because acid soils have more efficient N-cycling than calcareous soils (Kooijman and Besse, 2002; Kooijman et al., 2008). However, both zones have dry, acid soils, so this cannot explain differences in grass-encroachment between them. Possibly, although they have similar pH, dunes and former beach plain differ in soil organic matter (SOM), which may affect N-mineralization (Gerlach et al., 1994; Kowalchuk et al., 1997; Booth et al., 2005), but also P-availability via changes in sorption of Fe and P (Koenin and Hooper, 1976; Borggaard et al., 2006).

The objective of this paper is to test differences between iron-rich and decalcified dune zones with low and high grass-encroachment, in order to improve understanding of the impact of high N-deposition on acid dune grasslands. In both zones, we selected pioneer vegetation (if present), shortgrass and different tallgrass stages approximately 10, 20 and more than 25 years old from aerial photographs. We measured N-dynamics and microbial characteristics in a laboratory incubation experiment, and P-availability with differential fractionation of P, Fe (and Al).

2. Materials and methods

2.1. Study sites

Study sites were selected in the lime-poor, but iron-rich hinterdunes of the Amsterdam Water supply Dunes (AWD), the Netherlands, formed in the 9th and 10th century. The zone with high grass-encroachment was located in a former beach plain area of approximately 2 km length (Beach Plain), covered with a thin layer of drift sand, varying from 1–3 metres. Soils generally have a Ah-AC-C soil profile. In this zone, almost all grasslands have become covered with tallgrass (van Til and Mourik, 1999). The zone with low grass-encroachment was located in the adjacent dune zone (Kopjesduinen). Soils generally have a Ah-AC-C soil profile. In this zone, pioneer and shortgrass vegetation is still common. In both areas, groundwater levels have dropped considerably since 1850, due to drinking water mining and lowered water tables in adjacent polder areas, and are outside reach of the vegetation (van Til and Mourik, 1999). Before the 19th century, however, the beach plain has probably been wetter, especially in winter.

In both zones, different stages of grass-encroachment were selected in unmanaged areas with false colour photographs (1:5000) of 1979/1980, 1990 and 2001. Pioneer stages, around active blowouts, and shortgrass were characterized as such on all photographs. Tallgrass stages were classified as approximately 10, 20 and more than 25 years old. Ten year old tallgrass stages appeared as tallgrass on the 2001 photographs, but still existed of shortgrass in 1990. Twenty year old tallgrass stages appeared as tallgrass on the 1990 photographs, but was shortgrass earlier, and thirty year old tallgrass was already classified as such on the 1979/1980 photographs. In Beach Plain, where grass-encroachment was generally much higher, pioneer stages were absent, and shortgrass rare. In Kopjesduinen, with relatively low grass-encroachment, pioneer vegetation and shortgrass were still abundant, and older tallgrass stages were rare, and mainly found on soils with higher pH.

Tallgrass could be dominated by *Carex arenaria* L. or *C. epigejos* . If present, the two species were sampled separately. However, in Kopjesduinen, the oldest tallgrass stage, which was rare anyway, only consisted of *C. arenaria*. In contrast, in Beach Plain, where grass-encroachment was much more extensive, the youngest tallgrass stage only consisted of *C. epigejos*. It is possible that grass-encroachment starts with *C. arenaria*, but is replaced to some extent by *C. epigejos*, which may be a better competitor (van den Berg et al., 2005). However, as soil characteristics and nutrient availability did not differ between the two species, values of similar tallgrass stages were combined.

In both Kopjesduinen and Beach Plain, for each grass-encroachment stage present, and for *C. arenaria* and *C. epigejos* separately, five replicate patches were randomly selected, comprising a total of 65 samples. In each patch, aboveground biomass, bryophyte layer and litter layer were collected in a 25 × 25 cm plot in March. Mineral topsoil (0–10 cm) was sampled with a rectangular sampler called ‘humushapper’ (Wardenaar, 1987). Fresh weight and gravimetric moisture content of litter layer and mineral topsoil were determined, and dry weight and bulk density calculated. After homogenization by hand, pH values were determined in water, using a 1:2.5 weight:volume ratio for mineral samples and 1:10 weight:volume ratio for litter. After drying (48 h at 70 °C for organic and 105 °C for mineral samples) and grinding of the subsamples, C and N-contents were determined with a CNS analyzer (Westerman, 1990).

2.2. Net mineralization and microbial characteristics

Potential net mineralization of N and P and microbial characteristics were measured in a two-month laboratory incubation experiment. Fresh, homogenized samples were put into large petri dishes and brought to optimal gravimetric moisture levels (300% for litter layer and 50% for mineral topsoil; Tietema, 1992). Some litter samples did not contain enough material for all analyses. When litter mass was below approximately 500 g m^{-2}, samples were combined with others of the same group, to analyze mineralization rates and microbial characteristics per kg material. Values per m^2 were based on amounts per kg of composite samples, and actual litter mass of each individual replicate.

The petri dishes were stored in slightly open polyethylene bags with moist paper, at 20 °C in the dark. Moisture content was checked and replenished when necessary. Ammonium, nitrate and phosphate concentrations of fresh and incubated samples were extracted with 50 ml 0.5M K_2SO_4 solution, and measured on a continuous-flow analyzer (Westerman, 1990). Net mineralization of N and P was calculated for the litter layer from differences between incubated and fresh samples. For the mineral topsoil, net mineralization could be calculated only for N, because in the iron-rich soils, P-sorption was too high to release any P during the incubation period. Instead, mean P-concentrations in fresh and incubated K_2SO_4 extractions, which clearly differed between sites and stages of grass-encroachment, were used as estimate for plant-available P.

Microbial C, N and P were measured with the chloroform fumigation and extraction procedure (Brooks et al., 1985). Fumigated samples were flushed for 24 h with chloroform and extracted with 0.5 M K_2SO_4 immediately afterwards, to prevent microbial regrowth. In addition to ammonium, nitrate and phosphate, DON and DOC were measured in fumigated and non-fumigated samples using a continuous-flow analyzer. Microbial C, N and P concentrations were calculated as differences between fumigated and non-fumigated samples. Incubated samples were not included in further analyses, since they generally did not differ from fresh ones, indicating that microbial populations remained more or less stable.

Respiration was measured at the start and end of the incubation experiment. Fresh or incubated material was placed in open glass jars during one night, with the equivalent of 5 g dry for organic and 10–15 g dry for mineral samples. During measurements, the jars were closed and air samples were extracted by needle. CO_2-concentrations were measured three times, at intervals of approximately 2 h, by injecting the air sample into a Carlo Erba Varian gas chromatograph (Tietema, 1992). CO_2-production rates were calculated from the increase in CO_2-concentration during the day, the volume of the headspace and sample dry weight. Total CO_2-production over the incubation period was calculated, based on its duration and CO_2-production at start and end of the experiment.

2.3. Fractionation of P and Fe

Selective extractions of P, Fe (and Al) were applied to samples of the mineral topsoil (Westerman, 1990; Kooijman et al., 1998). Total P was determined after heating 1 g of ground soil sample for 6 h at 500 °C in order to digest organic matter. Samples were subsequently extracted with 50 ml 0.5 M H_2SO_4 by shaking for 16 h. Samples were centrifuged and filtered over a 0.2 mm filter. P was measured spectrophotometrically with a sulphuric acid/ammonium molybdate/ascorbic acid/potassium antimonyl tartrate solution at 880 nm. Mineral P was extracted in the same manner, but with non-heated samples. Organic P was calculated as the difference between total P and mineral P. P bound to amorphous iron-organic matter complexes and/or iron (hydr)oxides was determined by extraction in the dark with 0.073 M ammonium oxalate/0.05 M oxalic acid at pH 3 (P-oxalate); 10 g soil was used, weight/volume ratio was 1:5 and shaking time 2 h.

Total Fe (and Al) were determined in the oxalate extracts described above. Organic Fe (and Al) were determined by Na-pyrophosphate/NaOH extraction at pH 9.8, with weight/volume ratio 1:5. Aluminium, or Fe and Al combined, generally showed the same behaviour as Fe, and was left out of further discussion. Organic Fe is incorporated in iron-organic matter complexes which contribute to reversible P-adsorption. Inorganic, mineral Fe, calculated as the difference between total and organic Fe, is present in amorphous oxides and may form insoluble iron phosphates at low pH. It should be stressed that the data in this study give only a rough indication of the bio-availability of P. The extraction methods used always yield more than one P-form and it is not really possible to separate all of them. Also, because the laboratory samples are ground and reactive surfaces are much increased, the data should be treated as potential rather than actual amounts of 'available' P.

2.4. Statistical analysis

Differences between Kopjesduinen and the former Beach Plain, as well as differences between shortgrass and tallgrass of 10, 20 and more than 25 years old were analyzed with two-way ANOVA, with the two sites and different stages of grass-encroachment as independent factors. Pioneer stages were excluded from this analysis, because they were only found in Kopjesduinen. Also, in the oldest tallgrass stages of Beach Plain, one of the ten replicates had very high soil organic matter content, and was excluded from further analysis. Differences between individual mean values were tested with least square means tests.

Relationships between soil organic matter and different fractions of P and Fe were tested with linear regression. In this analysis, pioneer vegetation was included, except for one sample with high pH, which suggest regulation of P-availability by calcium rather than iron. The five replicates of the oldest tallgrass stages in Kopjesduinen were excluded for the same reason.

3. Results

3.1. Differences in site characteristics

Both Kopjesduinen and former Beach Plain were located in decalcified hinterdunes, and pH of the mineral topsoil was low accordingly, except for the oldest tallgrass stages in Kopjesduinen (Table 1). Kopjesduinen had slightly, but in ANOVA significantly higher overall pH values than Beach Plain. Soil organic matter (SOM) clearly differed between the two zones, with significantly lower values for Kopjesduinen. In Beach Plain, SOM especially increased in older stages of grass-encroachment. C:N ratio of the mineral topsoil did however not show consistent differences between sites or stages of grass-encroachment.

In both Kopjesduinen and Beach Plain, aboveground phanerogam biomass significantly increased with time of grass-encroachment (Table 1), while mass and cover of the bryophyte layer decreased (data not shown). In Kopjesduinen, mass of the litter layer was not affected by grass-encroachment, but in the former Beach Plain, litter significantly increased from younger to older tallgrass stages. C:N ratio of the litter layer was higher in Kopjesduinen, but here values did not differ with stage of grass-encroachment. In the former Beach Plain, however, litter C:N ratio was lower in the oldest tallgrass vegetation.

3.2. Differences in N-dynamics

Net N-mineralization mainly consisted of nitrification, which was generally above 90% in both litter layer and mineral topsoil, in both Kopjesduinen and Beach Plain. In the litter layer, potential net N-mineralization significantly increased with stage of grass-encroachment in both Kopjesduinen and Beach Plain (Fig. 1). In later stages, net N-mineralization was significantly higher in Beach Plain than in Kopjesduinen. In Kopjesduinen, the litter layer contributed approximately 22% to the total of litter layer and mineral topsoil combined, and in Beach Plain approximately 35%. However, in the mineral topsoil, net N-mineralization did not differ between Kopjesduinen and Beach Plain at all, despite the clear increase in soil organic matter (Table 1). Also, net N-mineralization of the mineral topsoil remained the same in all stages of grass-encroachment.

In Kopjesduinen, the increase in net N-mineralization in the litter layer with age of grass-encroachment was not due to higher microbial mass or respiration (Table 2). However, efficiency of N-mineralization per unit microbe clearly increased in later stages of grass-encroachment. In Beach Plain, however, the increase in net N-mineralization in the litter layer with stage of grass-encroachment was not only associated with higher efficiency of N-mineralization, but also with higher microbial mass and respiration. In the mineral topsoil, net N-mineralization of Kopjesduinen and Beach Plain was similar, but probably derived from different mineralization dynamics. In Beach Plain, microbial mass and respiration were generally higher than in Kopjesduinen, but efficiency of N-mineralization per unit microbe generally lower, especially in later stages of grass-encroachment. This lower efficiency was probably due to lower microbial activity, as indicated by significantly lower respiration quotients per unit microbe. High amounts of soil organic matter and microbes in Beach Plain tallgrass stages may thus be compensated for by low microbial activity, and lead to similar soil respiration and net N-mineralization as in Dune soils with lower soil organic matter.

3.3. Differences in P-availability

Similar to N, net P-mineralization in the litter layer significantly increased from shortgrass to older tallgrass stages in both Kopjesduinen and Beach Plain (Fig. 2). Also, net P-mineralization was higher in Beach Plain than in Kopjesduinen, especially in older stages. In the iron-rich mineral topsoil, net P-mineralization could not be measured, due to chemical sorption of phosphate ions released during decomposition. However, plant-available P clearly increased in older tallgrass stages, and was significantly higher in Beach Plain than in Kopjesduinen.

Higher plant-available P in Beach Plain than in Kopjesduinen is probably associated with higher SOM, and associated changes in Fe and P-sorption (Fig. 3A). Kopjesduinen and Beach Plain did not differ in total amorphous Fe, which ranged between 11.6 mmol kg^{-1} in Kopjesduinen and 12.8 mmol kg^{-1} in Beach Plain. However, percentage of mineral and organic forms clearly

Table 1

Characteristics of coastal dune grasslands in Kopjesduinen (K) and former beach plain (BP) in different stages of grass-encroachment (0 = shortgrass; 10 = tallgrass vegetation for 5–15 yr; 20 = tallgrass vegetation for 15–25 yr; >25 = tallgrass vegetation for >25 yr). Mean values (n = 5–10) and standard errors.

	pH topsoil[1]	SOM topsoil[1] (%)	C:N topsoil[2]	Biomass[2] (g m^{-2})	Litter mass (g m^{-2})	C:N litter[1]
K0	4.6 (0.0) a	3.0 (0.4) a	14.4 (0.3) a	145 (16) a	864 (137) bc	25.2 (1.0) b
K10	4.8 (0.1) a	3.0 (0.3) a	15.8 (0.7) ab	264 (23) b	741 (97) ab	24.9 (1.0) b
K20	4.8 (0.1) a	2.6 (0.3) a	15.3 (0.6) a	299 (28) b	661 (46) ab	24.0 (0.6) b
K > 25	5.7 (0.5) b	3.1 (0.7) a	13.7 (0.2) a	398 (63) c	826 (314) b	25.7 (1.6) b
BP0	4.5 (0.0) a	3.1 (0.3) a	14.6 (0.3) a	133 (29) a	302 (55) a	25.4 (1.8) b
BP10	4.5 (0.1) a	4.0 (0.7) ab	16.9 (0.6) b	187 (26) a	766 (171) ab	21.7 (0.3) a
BP20	4.5 (0.1) a	4.3 (0.4) ab	14.9 (0.4) a	305 (46) bc	997 (174) bc	22.6 (0.6) ab
BP > 25	4.5 (0.1) a	6.8 (0.9) b	14.9 (0.4) a	402 (44) c	1500 (355) c	21.4 (0.4) a

Different letters indicate significant differences between particular stages or sites for a particular parameter.

[1] Significant differences in two-way ANOVA between the two sites.

[2] Significant differences in two-way ANOVA between stages of grass-encroachment ($p < 0.05$).

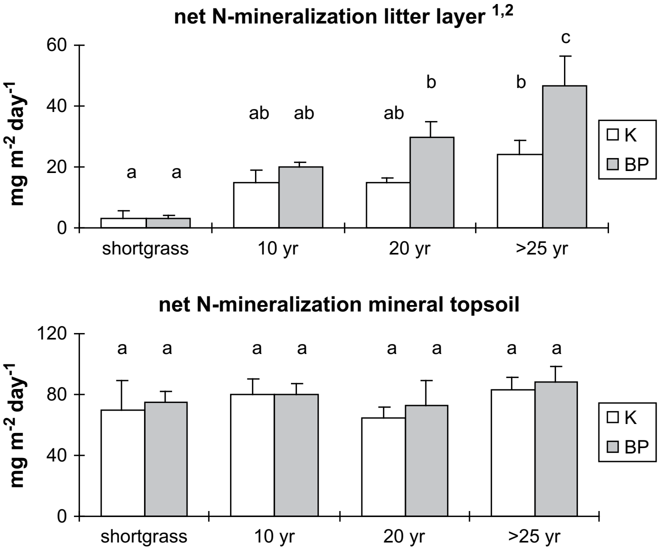

Fig. 1. Net N-mineralization in litter layer and mineral topsoil (0–10 cm) of coastal dune grasslands in Kopjesduinen (K) and former beach plain (B) in different stages of grass-encroachment. 10 yr = tallgrass vegetation for 5–15 yr; 20 yr = tallgrass vegetation for 15–25 yr; >25 yr = tallgrass vegetation for >25 yr. Mean values ($n = 5$–10) and standard errors. ^{1} = significant differences in two-way ANOVA between the two sites; ^{2} = significant differences in two-way ANOVA between stages of grass-encroachment ($p < 0.05$). Different letters indicate significant differences between particular stages or sites for a particular parameter.

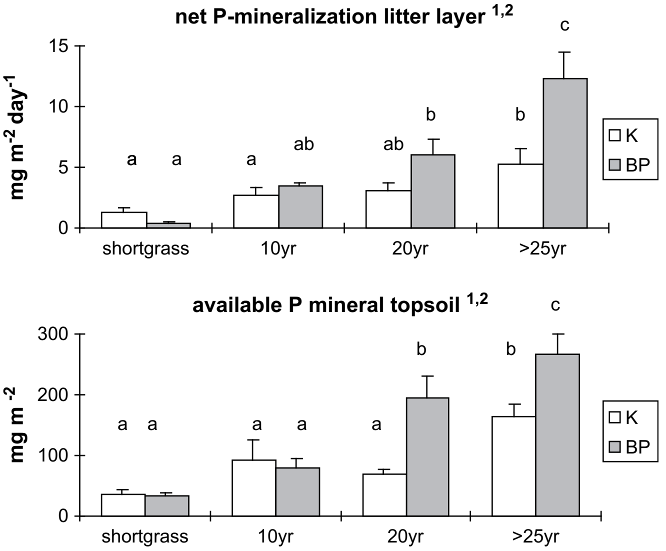

Fig. 2. Net P-mineralization in the litter layer and plant-available P in the mineral topsoil (0–10 cm) of coastal dune grasslands in Kopjesduinen (K) and former beach plain (BP) in different stages of grass-encroachment. 10 yr = tallgrass vegetation for 5–15 yr; 20 yr = tallgrass vegetation for 15–25 yr; >25 yr = tallgrass vegetation for >25 yr. Mean values ($n = 5$–10) and standard errors. ^{1} = significant differences in two-way ANOVA between the two sites; ^{2} = significant differences in two-way ANOVA between stages of grass-encroachment ($p < 0.05$). Different letters indicate significant differences between particular stages or sites for a particular parameter.

shifted with soil organic matter content. In Kopjesduinen, SOM was generally below 4%, and mineral Fe increased to 80% of the total amorphous fraction. In Beach Plain, however, with SOM of 3–10%, mineral Fe was generally below 40%, as most Fe was converted to organic forms, as part of organic matter complexes.

The shift from mineral to organic Fe with soil organic matter content also affected different P-fractions, such as mineral P (P_{min}) and oxalate-extractable P (P_{ox}). In the acid soils of this study, P_{min} mainly included P bound to amorphous iron (hydr)oxides, and some iron phosphates. P_{ox} consisted of P bound to amorphous iron (hydr)oxides as well, but in addition of P bound to Fe-organic matter complexes. If P_{min} was higher than P_{ox} , which happened in Kopjesduinen when mineral Fe was higher than approximately 40% and SOM below 3%, the surplus probably consisted of iron phosphates (Fig. 3B), which are insoluble when iron concentrations are high. When mineral Fe was higher than 40%, plant-available P ($P_{available}$) was indeed very low (Fig. 3C). If P_{ox} was higher than P_{min}, which happened in Beach Plain, when mineral Fe dropped below approximately 40%, and SOM increased above 3%, the surplus probably consisted of P bound to Fe-organic matter complexes, which constitutes a more or less reversible binding. This was supported by the increase in $P_{available}$ when mineral Fe dropped below 40%.

4. Discussion

In this study, we tested whether two dune zones with acid, iron-rich soils, but low or high grass-encroachment, differed in nutrient availability, and whether this changed with duration of grass-encroachment. Higher grass-encroachment in the former Beach Plain seemed clearly associated with an increase in SOM. In Kopjesduinen, the zone with low grass-encroachment, SOM was significantly lower than in Beach Plain. However, in contrast to expectations (Gerlach et al., 1994; Kowalchuk et al., 1997; Booth et al., 2005), higher SOM in Beach Plain did not lead to higher net N-mineralization. In the litter layer, N-mineralization increased in

Table 2
Microbial characteristics of coastal dune grasslands in Kopjesduinen (K) and former beach plain (BP) in different stages of grass-encroachment (0 = shortgrass; 10 = tallgrass vegetation for 5–15 yr; 20 = tallgrass vegetation for 15–25 yr; >25 = tallgrass vegetation for >25 yr). NM-efficiency = efficiency of net N-mineralization per unit microbial C. Mean values ($n = 5$–10) and standard errors.

	Microbial C litter^{1,2} (g m^{-2})	Microbial C topsoil^{1,2} (g m^{-2})	Respiration litter^{1,2} (g m^{-2} day^{-1})	Respiration topsoil (g m^{-2} day^{-1})	NM-efficiency litter^{1,2} (mg g^{-1} day^{-1})	NM-efficiency topsoil^{1} (mg g^{-1} day^{-1})
K0	7.1 (1.8) b	18 (3) a	2.4 (0.2) b	3.9 (0.5) a	0.5 (0.4) a	4.3 (1.2) ab
K10	4.8 (0.6) a	21 (3) a	1.0 (0.1) ab	4.3 (0.5) a	2.4 (0.3) b	4.8 (1.1) b
K20	4.7 (0.4) a	23 (2) a	1.9 (0.2) b	2.9 (0.3) a	3.5 (0.5) c	3.0 (0.5) ab
K > 25	6.4 (1.5) a	20 (1) a	1.4 (0.6) ab	3.7 (0.3) a	4.3 (1.0) c	4.2 (0.5) ab
BP0	2.4 (0.5) a	18 (2) a	0.5 (0.0) a	3.9 (0.5) a	1.8 (0.3) ab	4.3 (0.3) ab
BP10	4.9 (0.5) a	28 (4) b	1.3 (0.3) ab	3.8 (0.7) a	4.2 (0.4) c	3.1 (0.5) ab
BP20	8.6 (1.2) b	31 (2) b	2.8 (0.4) bc	3.3 (0.3) a	3.4 (0.2) bc	2.5 (0.5) a
BP > 25	9.2 (1.5) b	33 (2) b	3.8 (0.7) c	3.9 (0.3) a	5.0 (0.5) c	2.7 (0.2) a

Different letters indicate significant differences between particular stages or sites for a particular parameter.
[1] Significant differences in two-way ANOVA between the two sites.
[2] Significant differences in two-way ANOVA between stages of grass-encroachment ($p < 0.05$).

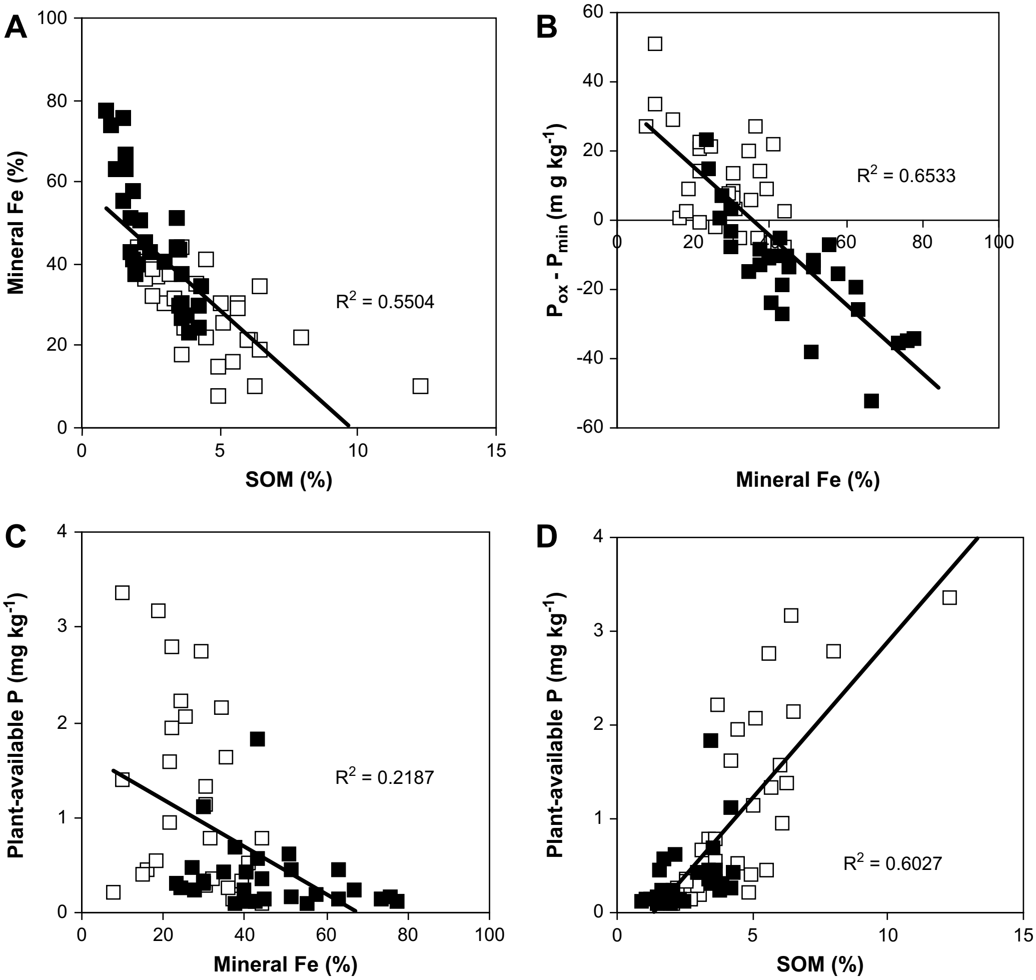

Fig. 3. Relationships between soil organic matter, mineral Fe and P-availability in coastal dune grasslands ($n = 58$). ■ = Kopjesduinen; □ = former Beach Plain. (A) Soil organic matter (SOM) and mineral amorphous Fe; (B) mineral amorphous Fe and the difference between oxalate-extractable P and mineral P, as indicator of P-fixation in iron phosphates (higher P_{min}) versus light P-sorption to iron-organic matter complexes (higher P_{ox}); (C) mineral amorphous Fe and plant-available P; (D) SOM and plant-available P. All correlations were significant ($p < 0.05$).

later stages of grass-encroachment, but did not differ between dune zones. The mineral topsoil was not affected by dune zone or stage of grass-encroachment at all. Possibly, increase in net N-mineralization with higher SOM in the mineral topsoil mainly occurs in very early stages of succession, when the N-cycle is still building up (Gerlach et al., 1994). In acid soils, bioturbation is low, which may reduce input of fresher litter from the litter layer even in older stages of grass-encroachment (Nierop and Verstraten, 2004). In older soils, SOM may thus increasingly consist of older and more stable organic matter, with lower rates of decomposition and N-mineralization, which counteracts a higher mass. This was supported by the increase in SOM and microbial mass in Beach Plain, but clear decrease in microbial activity, so that the net result in respiration and N-mineralization was the same as for Kopjesduinen with lower SOM.

4.1. *Grass-encroachment as a self-reinforcing process*

Even though N-mineralization in the mineral soil was not affected by grass-encroachment, the clear increase in net mineralization of N and P in the litter layer suggests that grass-encroachment is at least partly a self-reinforcing process. This is in accord with Veer and Kooijman (1997) and Kooijman and Besse (2002), who found consistently higher net N-mineralization in tallgrass than shortgrass vegetation in the upper 20 cm of the soil. In both Kopjesduinen and Beach Plain, net N-mineralization increased 1.4 times from initial to the oldest stages of grass-encroachment, which is (almost) enough to account for the increase in aboveground biomass with time. The self-reinforcing aspect of grass-encroachment is likely stimulated by the clonal lifestyle of species such as *C. arenaria*, which facilitates exploitation of soil resources (D'hertefeldt and Jónsdóttir, 2003).

The increase in net N-mineralization with duration of grass-encroachment was partly due to increased mass of the litter layer, but also to higher efficiency per unit microbe. The latter could have been due to lower microbial N-demand, as N-content generally increases in older litter (Parton et al., 2007). However, litter C:N ratios only differed between dune zones, and not between stages of grass-encroachment. Nevertheless, with both increased mass and more efficient N-mineralization, litter layers became more and more important, and account for a substantial part of net N-mineralization and aboveground biomass in older tallgrass stages.

The self-reinforcing effect of grass-encroachment seemed stronger in Beach Plain than in Kopjesduinen, which may be explained by higher P-availability in older Beach Plain stages. In contrast to N, availability of P increased in the mineral topsoil with age of grass-encroachment. This may reflect a cause of grass-encroachment rather than a result, because P is generally less affected by litter input than N, but mainly regulated by soil chemistry (Kooijman et al., 1998). Grass-encroachment may have started earlier in soils with high P-availability, because high atmospheric N-deposition can be used more efficiently if P is not a limiting factor (Kooijman and Besse, 2002).

4.2. *Grass-encroachment stimulated by high P*

In principle, iron-rich soils have low P-availability (Jensen et al., 1992; Lamers et al., 1998), and iron is capable of P-sorption even in calcareous soils (Hamad et al., 1992; Golterman, 1997). In Dutch coastal dunes, P-availability was indeed higher in iron-poor than iron-rich soils (Kooijman et al., 1998). In the present study, molar Fe:P ratios, which ranged from 2.8 in Kopjesduinen to 2.3 in Beach Plain, seemed high enough to expect reduction of P-availability (Jensen et al., 1992). Also, P-saturation index (van der Zee and Riemsdijk, 1988) ranged from 0.08 to 0.13, which is clearly below the saturation value of 0.30, at which all P-binding sites are supposed to be occupied.

Nevertheless, even if total iron (and aluminium) was high in both zones, P-availability clearly differed between Kopjesduinen and Beach Plain. In wetlands, P-availability may increase when Fe(III) becomes unavailable due to reduction of sulphate and iron (Lamers et al., 1998). In terrestrial soils, P-availability may increase when Fe(III) is incorporated in organic matter complexes (Kooijman et al., 1998). Extraction methods usually yield more than one P-form, which make it impossible to separate P-fractions exactly. However, as in Borggaard et al. (2006), a comparison between different methods may allow at least for some differentiation. In acid soils, where calcium phosphates play no role (Lindsay and Moreno, 1966), both P_{min} and P_{ox} contain P adsorbed to iron oxides. However, P_{min} may in addition contain iron phosphates, while P_{ox} also contains P sorbed to Fe-organic matter complexes. Higher P_{min} than P_{ox} may thus suggest a surplus of iron phosphates. Higher P_{min} than P_{ox} was mainly found in Kopjesduinen, where mineral Fe was higher than approximately 40% and SOM below 3%, and plant-available P low as well. In contrast, in Beach Plain, P_{ox} was generally higher than P_{min}. The surplus thus probably consisted of P bound to Fe-organic matter complexes, which constitutes a more or less reversible binding (Sinha, 1971; Koenin and Hooper, 1976), and may lead to higher amounts of plant-available P.

Higher P-availability in Beach Plain than in Kopjesduinen thus suggests that accumulation of organic matter is important, even if P is mainly sorbed to Fe in organic matter complexes, rather than to organic matter directly (Borggaard et al., 2006). Higher organic matter content may be due to higher age of Beach Plain soils, but probably also to the slightly lower position in the landscape. Kopjesduinen have always been above the water level. However, Beach Plain, where groundwater levels have been (far) below the soil surface for the past 150 years, due to drinking water mining and low water levels in adjacent polders (van Til and Mourik, 1999), may have been temporarily flooded in winter before. Higher water levels may have stimulated accumulation of organic matter due to retarded decomposition, but also led to reduction, dissolution and reoxidation of iron (Koenin and Hooper, 1976), which may explain the shift from mineral to organic forms. This shift may also be a more contemporary process. In Kopjesduinen, which have never been flooded, mineral iron decreased when soil organic matter content became higher as well. Nevertheless, large parts of the dunes consist of former beach plains (dune slacks), alternated with dune ridges. The iron-rich hinterdunes, which were supposed to be P-poor due to P-fixation in iron phosphate, may thus actually contain large areas with high P-availability, due to high organic matter content and a shift from mineral to organic iron.

4.3. *Different sensitivity to high N-deposition*

The results suggest that P-availability is important to the effect of high atmospheric N-deposition and grass-encroachment in coastal dunes, but acid soils are probably rather sensitive to high N-deposition already. In acid soils, net N-mineralization may be relatively high, because fungi, which may have lower N-requirements than bacteria (Hassink et al., 1993; Moore et al., 2005), are generally dominant groups of microorganisms (Bååth and Anderson, 2003; Kooijman et al., 2008). In acid soil, high atmospheric N-deposition may thus be used more efficiently (Kooijman and Besse, 2002). Naturally, microbial communities vary in time and space (Rose, 1988; de Boer et al., 1998, 2008), but in both Kopjesduinen and Beach Plain, except for older tallgrass stages in Beach Plain, net N-mineralization values per unit microbe were comparable to the high values found over a gradient of calcareous to acid beech forests (Kooijman et al., 2008). In addition, once started, grass-encroachment is a self-reinforcing process, and higher litter input leads to increased availability of N and P and further increases in biomass production (Veer and Kooijman, 1997). Also, high N-deposition may have stimulated grass-encroachment via nitrification. Nitrification is usually low under acid conditions (Prosser, 1989; Kowalchuk et al., 1997), but may increase in areas with high atmospheric N-deposition, where N is not a limiting factor (Kowalchuk et al., 1997; Kooijman and Besse, 2002). Nitrate-preference in species involved in grass-encroachment has been shown before (Nordin et al., 2005). Calcifuge species generally prefer ammonium to nitrate, because this is the dominant form of N under acid conditions (Falkengren-Grerup, 1995; Paulissen et al., 2004; Dijk and Eck, 2006). However, *C. arenaria* often already establishes in fresh sand with high pH, and perhaps prefers nitrate to ammonium. Similarly, *C. epigejos*, which can grow over a wide pH-gradient, may be nitrate-preferent as well.

Nevertheless, in spite of efficient use of N and high nitrification, sensitivity to N-deposition may mostly be affected by P-availability. Despite atmospheric N-deposition of 27–30 kg ha^{-1} yr^{-1} over the past decades (Dopheide and Verstraten, 1995), which is clearly above the critical load for dune grasslands of 10–20 kg N ha^{-1} yr^{-1} (Achermann and Bobbink, 2003; van Dobben and van Hinsberg, 2008), grass-encroachment was relatively low in Kopjesduinen, where P-availability is kept low by fixation in iron phosphate. Grass-encroachment may also be relatively low in calcareous soils close to the sea, with P-fixation in calcium phosphate (Kooijman and Besse, 2002). However, large parts of the dunes may actually have high P-availability, because P-fixing mechanisms are absent.

Nevertheless, according to the Dutch government (Natuurbalans, 2006), approximately 75% of the coastal dunes is not endangered by high N-deposition any more. Apart from high P-availability in large parts of the dunes, this view may be too optimistic for other reasons as well. Critical loads for Grey Dunes (Achermann and Bobbink, 2003; van Dobben and van Hinsberg, 2008) may be too high, because vegetation responses may occur at much lower levels of 5–8 kg ha^{-1} yr^{-1} already (Remke et al., 2009). Plassmann et al. (2009) also mention accumulative effects. Furthermore, for the coastal dunes, N-deposition, modelled with the OPS-model (van Jaarsveld, 2004), may be significantly underestimated, especially for ammonium, which showed two to four times higher values for measured than for modelled ammonium concentrations (Kooijman et al., in press). In addition, on a landscape scale, critical loads may be overestimated, due to aggregation of sensitive dune grasslands with less sensitive *Hippophae rhamnoides* L. scrub, which has (much) higher critical load because of N-fixing root symbionts (Lammers et al., 2005). Considering the responsibility to maintain and restore priority habitats in Natura 2000, it may be better to use critical loads of the most sensitive Grey Dunes, rather than aggregated 'actual' values. Also, given the two- to fourfold deviation between modelled and measured ammonium concentrations, actual N-deposition should be urgently measured, to reconsider to which extent N-deposition actually exceeds critical loads, and how much of the Dutch coastal dunes are still in danger.

5. Conclusions

The results of this study suggest that P-availability is a key factor with respect to sensitivity to high N-deposition in coastal dunes. Even iron-rich, decalcified dunes only seem to be relatively protected from grass-encroachment if soils are low in organic matter, in which P-availability is reduced by P-fixation in iron phosphate. However, when organic matter content rises above approximately 4%, and mineral forms of iron drop below approximately 40%, P-fixation apparently no longer occurs. Instead, P becomes relatively loosely bound to iron-organic matter complexes, which leads to higher P-availability and higher rates of grass-encroachment. The present estimation that 75% of the coastal dunes is not endangered by N-deposition any more may already be too optimistic, because actual N-deposition seems underestimated, and critical loads on a landscape scale overestimated. However, higher P-availability than expected in large parts of the dunes may even further increase the actual area where N-deposition still exceeds critical loads.

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