UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Resilience in coastal dune grasslands: pH and soil organic mattereffects on P nutriti on, plant strategies, and soil communities

Kooijman, A.M.; Morriën, E.; Jagers op Akkerhuis, G.; Missong, A.; Bol, R.; Klumpp, E.; van Hall, R.; van Til, M.; Kalbitz, K.; Bloem, J.

Ecosphere

Resilience in coastal dune grasslands: pH and soil organic matter effects on P nutrition, plant strategies, and soil communities

Annemieke Kooijman,[1],† Elly Morriën,[1,2] Gerard Jagers op Akkerhuis,[3] Anna Missong,[4] Roland Bol,[1,3] Erwin Klumpp,[3] Rutger van Hall,[1] Mark van Til,[5] Karsten Kalbitz,[1,6] and Jaap Bloem[3]

[1]*Department of Ecosystem and Landscape Dynamics, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park, P.O. Box 94062, Amsterdam 1090 GB The Netherlands*
[2]*Terrestrial Ecology, NIOO-KNAW, P.O. Box 50, Wageningen 6700 AB The Netherlands*
[3]*Wageningen University and Research Centre, P.O. Box 47, Wageningen 6700 AA The Netherlands*
[4]*Institute of Bio- and Geosciences (IBG-3) Agrosphere, Forschungszentrum Jülich, Wilhelm-Johnen-Straße, Julich 52425 Germany*
[5]*Waternet, P.O. Box 94370, Amsterdam 1090 GJ The Netherlands*
[6]*Soil Resources and Land Use, Technische Universität Dresden, Pienner Straße 19, Tharandt 01737 Germany*

Abstract. Soil organic matter (SOM) and pH are key ecosystem drivers, influencing resilience to environmental change. We tested the separate effects of pH and SOM on nutrient availability, plant strategies, and soil community composition in calcareous and acidic Grey dunes (H2130) with low, intermediate, and/or high SOM, which differ in sensitivity to high atmospheric N deposition. Soil organic matter was mainly important for biomass parameters of plants, microbes, and soil animals, and for microarthropod diversity and network complexity. However, differences in pH led to fundamental differences in P availability and plant strategies, which overruled the normal soil community patterns, and influenced resilience to N deposition. In calcareous dunes with low grass-encroachment, P availability was low despite high amounts of inorganic P, due to low solubility of calcium phosphates and strong P sorption to Fe oxides at high pH. Calcareous dunes were dominated by low-competitive arbuscular mycorrhizal (AM) plants, which profit from mycorrhiza especially at low P. In acidic dunes with high grass-encroachment, P availability increased as calcium phosphates dissolved and P sorption weakened with the shift from Fe oxides to Fe-OM complexes. Weakly sorbed and colloidal P increased, and at least part of the sorbed P was organic. Acidic dunes were dominated by nonmycorrhizal (NM) plants, which increase P uptake through exudation of carboxylates and phosphatase enzymes, which release weakly sorbed P, and disintegrate labile organic P. The shifts in P availability and plant strategies also changed the soil community. Contrary to expectations, the bacterial pathway was more important in acidic than in calcareous dunes, possibly due to exudation of carboxylates and phosphatases by NM plants, which serve as bacterial food resource. Also, the fungal AM pathway was enhanced in calcareous dunes, and fungal feeders more abundant, due to the presence of AM fungi. The changes in soil communities in turn reduced expected differences in N cycling between calcareous and acidic dunes. Our results show that SOM and pH are important, but separate ecosystem drivers in Grey dunes. Differences in resilience to N deposition are mainly due to pH effects on P availability and plant strategies, which in turn overruled soil community patterns.

Key words: arbuscular mycorrhizal (AM) plants; atmospheric N deposition; bacteria; fungi; Grey dunes H2130; iron; nonmycorrhizal (NM) plants; soil community network.

† **E-mail:** a.m.kooijman@uva.nl

INTRODUCTION

Soil organic matter (SOM) and pH are major ecosystem drivers that influence soil communities, plant-available nutrients, and vegetation in many ways (Ellenberg et al. 1974, Swift et al. 1979). In coastal dune grasslands, which belong to the priority habitat Grey dunes (H2130) of the EU habitat directive (European Union 1992), pH and SOM may be especially important. In many ecosystems, pH and SOM change together during succession, but in Grey dune areas, these factors may be clearly separated. Older dunes with acidic topsoils alternate with younger calcareous dunes, both consisting of mosaics of low and high SOM, due to differences in eolian activity (Aggenbach et al. 2017). Also, in the dunes all soils are sandy, which precludes differences in P sorption (Shen et al. 2011) and microbial community composition (Hassink 1994) due to texture. In industrialized countries, pH and SOM are also important to Grey dunes because different dune zones respond in a different way to high atmospheric N deposition (Bobbink et al. 2010, Kooijman et al. 2017). Grass-encroachment, that is, the dominance of tall graminoid species and associated loss of plant diversity, is generally higher in acidic than in calcareous Grey dunes, and more pronounced on soils with high than with low SOM (Fig. 1; Remke et al. 2009a, b, Kooijman et al. 2017).

Soil organic matter and pH may influence resilience to high N deposition in Grey dunes through differences in soil community composition, which in turn affect microbial respiration and N cycling (Swift et al. 1979). Soil organic matter is a food resource for bacteria and fungi, which are in turn eaten by protists and microarthropods, and soil communities usually increase during succession in microbial biomass, microarthropod diversity, and network interaction strength (Kaufmann 2001, Frouz et al. 2008, Morriën et al. 2017). Soil pH is probably more important in shaping microbial communities (Fierer and Jackson 2006, Lauber et al. 2009), as calcareous soils are usually dominated by bacteria and acidic soils by fungi (Blagodatskaya and Anderson 1998, Bååth and Anderson 2003, Mulder et al. 2005, Kooijman et al. 2008, Rousk et al. 2009, 2010). Calcareous, bacteria-dominated soils are generally associated with higher respiration

and biological activity than acidic, fungi-dominated soils (Aerts and Chapin 2000, Schimel and Bennett 2004, De Vries et al. 2011, Kooijman et al. 2016). However, because N immobilization is higher for bacteria than for fungi, net N mineralization, which is a proxy for N availability to the vegetation, may actually show the opposite pattern (Zöttle 1960, Davy and Taylor 1974, Verhoeven et al. 1990, Kooijman et al. 2008, 2016). Bacteria have higher N demand and lower C:N ratios than fungi (Moore et al. 2005), due to their faster life cycles and use of amino acids for osmoregulation rather than carbohydrates (Measures 1975, Kuehn et al. 1998). This may imply that availability of N to the vegetation is lower in calcareous than in acidic Grey dunes, which may explain why grass-encroachment is higher in the latter.

Soil pH and SOM may also influence resilience to high N deposition through their effects on P nutrition (Kooijman et al. 1998, 2017). Phosphorus is an essential nutrient for plant growth, which may reduce effects of high N deposition when this is a limiting factor. However, different forms of P, such as inorganic P in primary and secondary minerals, sorbed P, organic P, and colloidal P systematically change during succession and soil development (Walker and Syers 1976, Hinsinger 2001, Shen et al. 2011). This means that P availability to the vegetation may change with pH and/or SOM. In calcareous dunes, primary and secondary calcium phosphates may be abundant (Walker and Syers 1976, Kooijman et al. 1998), but P availability to the vegetation may still be low due to their low solubility at high pH (Lindsay and Moreno 1966). During bedrock weathering and pedogenetic development, P is released from primary and secondary minerals (Walker and Syers 1976). In calcareous dunes, this process is especially important around pH 6.5, when calcium phosphates dissolve together with calcium carbonates (Lindsay and Moreno 1966) and become available to the vegetation (Kooijman and Besse 2002, Kooijman et al. 2017). In acidic dunes, iron phosphates may occur, but P availability to the vegetation may be restricted by their low solubility around pH 4 (Lindsay and Moreno 1966). Besides being part of primary and secondary minerals, orthophosphate can also be sorbed to amorphous Fe and Al (hydr)oxides or complexes of Fe and Al with SOM, with Fe and

Fig. 1. Characteristic vegetation in different coastal dune grasslands (Grey dunes; H2130) in the Amsterdamse Waterleidingduinen, the Netherlands. (A) Calcareous dunes with low soil organic matter content (SOM). (B) Calcareous dunes with intermediate/high SOM. (C) Acidic dunes with low SOM. (D) Acidic dunes with intermediate/high SOM. Photographs (A–C) were taken by Mark van Til and photograph (D) by Leo van Breukelen, Waternet Amsterdam.

Al acting as bridging agents (Walker and Syers 1976, Hamad et al. 1992, Gerke 2010). The distinction between these forms of Fe (and Al) in the soil is important, as sorption of P to Fe oxides is stronger than to Fe-OM complexes (Kooijman et al. 2009, Gerke 2010), but it is unknown how they are influenced by pH and SOM. In acidic dunes, soils with low SOM were dominated by Fe oxides and soils with high SOM by Fe-OM complexes (Kooijman et al. 2009), but this has not yet been studied in calcareous dunes. Phosphorus can also be organic, due to uptake by plant roots and microorganisms (Turner et al.

2007). Organic P can again be mineralized to orthophosphate, but also be sorbed or precipitated (Celi et al. 2003, Prietzel et al. 2016). It is unknown to which extent organic P, or its mineralization by microbial communities, is influenced by pH and SOM, although it is likely that the importance of organic P increases with higher SOM. A relatively unknown P fraction is formed by colloidal P, that is, nanoparticles and fine colloids smaller than 0.5 μm which move through the soil solution (Bol et al. 2016, Missong et al. 2017). Influences of pH and SOM on colloidal P in dune soils are also still unknown.

Differences in P fractions and availability over pH and SOM gradients may in turn lead to differences in plant strategies (Lambers et al. 2008). While nitrate and ammonium are usually taken up by plant roots through mass flow, P uptake is restricted by low mobility of the phosphate ion in the soil (Walker and Syers 1976, Hinsinger 2001). Cooperation with mycorrhizal fungi may improve P uptake, but different groups of mycorrhizal plants exploit different P sources (Cairney and Meharg 2003, Read and Perez-Moreno 2003, Smith and Read 2008, Smith et al. 2011). In coastal dune grasslands, arbuscular mycorrhizal (AM), ericoid mycorrhizal (ErM), and nonmycorrhizal (NM) plants may all occur (Ernst et al. 1984, Van Til and Mourik 1999), but distribution patterns probably change with pH and SOM. The AM and NM plants mainly use inorganic P and can be found at both high pH and low pH (Emmerton et al. 2001, Read and Perez-Moreno 2003, Smith and Smith 2011, Cardoso et al. 2017). For AM plants, mycorrhizal benefits are especially high when P is a limiting factor (Hoeksema et al. 2010). However, predominance of AM plants does not necessarily lead to high biomass production, as high rates of mycorrhizal infection are usually associated with low relative growth rates (Koziol and Bever 2015), due to the high costs to support the fungal network. Ericoid mycorrhizal plants primarily use organic nutrients and occur especially at low pH, due to their tolerance to heavy metals (Cairney and Meharg 2003).

The general aim of this study was to disentangle the separate effects of SOM and pH on different ecosystem compartments in Grey dunes such as vegetation, soil nutrients, and soil communities. The more specific aim was to better explain differences in resilience to high N deposition between dune zones and unravel whether they were due to differences in P availability and plant strategies, differences in N availability and soil communities, or both. The study consisted of two separate, but complementary parts: a P nutrition and a soil community study. For each study, we selected plots in calcareous and acidic dunes with low, intermediate, and/or high SOM, albeit in different locations. Both studies analyzed basic soil properties, vegetation patterns, mycorrhizal plant strategies, and microbial cycling of C and N. The P nutrition study further

concentrated on different fractions of P, including in nanoparticles and fine colloids, and distribution of amorphous Fe (and Al) over Fe oxides and Fe-OM complexes. The soil community study further concentrated on soil community composition, microarthropod diversity, and plant–animal networks. The research questions were as follows: (1) What are the separate effects of pH and SOM on different ecosystem compartments? (2) How can these effects help explain differences in resilience to high N deposition between dune zones?

MATERIALS AND METHODS

Site selection

The study was conducted in the Amsterdam Water Supply Dunes (AWD), which belong to the lime- and iron-rich dunes of the Netherlands (Eisma 1968). Initial lime content is 4–8%, and sand grains are coated with amorphous Fe oxides, with values of 6 mmol Fe/kg (Kooijman et al. 1998). The study area consists of calcareous and acidic dune zones, with SOM gradients in both, due to differences in eolian activity (Aggenbach et al. 2017). Calcareous sites were mainly located in the outer dunes close to the sea, which formed in the 17th century, and still have calcareous topsoils (Van Til and Mourik 1999). Acidic sites were located in the inner dunes, which formed in the 11th century, and have become decalcified to more than a meter depth. In all sites, groundwater tables are several meters below the soil surface.

Atmospheric N deposition along the Dutch coast currently ranges around 15 kg N·ha^{-1}·yr^{-1}, but values amounted to 30 kg N·ha^{-1}·yr^{-1} in the 1990s (Kooijman et al. 2017). Critical N loads, above which negative effects occur, are 15 kg N·ha^{-1}·yr^{-1} for calcareous Grey dunes and 10 kg N·ha^{-1}·yr^{-1} for acidic Grey dunes (van Dobben and van Hinsberg 2008). In the last decade, approximately 10% of the grasslands was covered with tallgrass vegetation in calcareous dunes, but approximately 80% in acidic dunes (Kooijman et al. 2017).

Plot selection and field survey

In the P nutrition study, five replicate plots of approximately 3 × 3 m were selected according to stratified random sampling procedures

throughout calcareous and acidic dunes zones with low, intermediate, and high SOM. The location of the 30 sampling points was determined with GPS (Appendix S1: Table S1). Samples for P nutrition (2009), chemical and physical fractionation (2010), and nanoparticle and fine colloid analysis (2017) were collected in different years, but within the same plots, and the same period of April–May. Also, pH, SOM, and/or bulk density were measured in different campaigns, to test that samples of the same plot were indeed similar. Interannual variations in temperature and soil moisture may have influenced the results to some extent, but we assumed that differences between sites were more important.

In the soil community study, five replicate plots of approximately 2 × 2 m were selected according to stratified random sampling procedures in particular calcareous and acidic dune areas with low and high SOM. The location of the 20 sampling points was determined with GPS (Appendix S1: Table S1). All parts of the study were conducted within the same year (2011), but aboveground vascular plant biomass was sampled in 2012 as well, to reduce variation in biomass production between dry and wet years.

Vegetation sampling

In both the P nutrition and the soil community study, with plot sizes of 3 × 3 and 2 × 2 m, respectively, plant species composition was recorded in each plot in May. Cover of bare sand, vascular plants, and cryptogam layer were estimated in percentage. Nomenclature followed van der Meijden (2005) for vascular plants, van Tooren and Sparrius (2007) for bryophytes, and Aptroot et al. (2011) for lichens. Vascular plants were listed as AM, ErM, ectomycorrhizal (EcM) plants, or NM species according to various sources (Read et al. 1976, Ernst et al. 1984, Wang and Qiu 2006, Brundett 2009). However, ErM and EcM plants did generally not occur, except for some *Salix repens* in some of the calcareous plots of the P nutrition study with intermediate or high SOM.

Aboveground vascular plant biomass was sampled for the analysis of its nutrient content in both studies, and for estimates of standing crop in the soil community study. In the P nutrition study, aboveground vascular plant biomass was collected in each of the 30 plots in April. In the soil community study, aboveground vascular plant biomass was collected in each of the 20 plots in July, in 25 × 25 or 50 × 50 cm plots, depending on the amount of vegetation. In addition, healthy leaf samples were collected for *Carex arenaria*, a NM plant present in all 20 plots, although with high cover values only in acidic dunes. In the soil community study, collection of aboveground vascular plants was repeated one year later, but only for estimates of the standing crop.

Soil sampling

In the P nutrition study, topsoil samples (0–10 cm) were collected in each of the 30 plots in April, in metal rings for the analysis of basic soil characteristics, fractionation of C, P, Fe, and Al, and laboratory incubation experiments. Organic layers were absent, even in the most acidic sites. Topsoil samples (0–10 cm) were also collected for the analysis of P, Fe, and Al in nanoparticles and fine colloids <0.5 μm, which technically belong to the soil solution (Missong et al. 2017). As this is a relatively new and elaborate method, a subset of 18 of the 30 plots was used, consisting of three of the five replicate plots per situation.

In the soil community study, topsoil samples (0–5 cm) were collected in each of the 20 plots, in May and October. Sampling depth was lower than in the P nutrition study, because the topsoil included most of the SOM, microorganisms, and microfauna, which would otherwise become too diluted. For soil mesofauna, however, a standard depth of 7.5 cm was used (Jagers op Akkerhuis et al. 2008). For bulk density, additional samples were collected in metal rings of 5 cm depth and 100 cm^3 volume. Organic layers were absent, even in the most acidic sites.

In the soil community study, both May and October samples were used for the analysis of basic soil parameters, phospholipid-derived fatty acid (PLFA) analysis of the microbial community, and laboratory incubation experiments. In May, samples were further used for microscopic counting of bacteria, fungi, amoeba, and flagellates, measurements of bacterial activity, and determination of microarthropods such as Collembola and Acari. In October, samples were further used for determination of microbial C and N.

Analysis of soil samples and aboveground biomass

For both the P nutrition and the soil community study, vegetation and soil samples were stored at 4°C until further analyses, except for the subsamples for PLFA analysis (Frostegård et al. 1991), which were frozen the same day. Vegetation samples were dried (48 h at 70°C), weighed, and ground. For aboveground vascular plant biomass, data of the two sampling years in the soil community study were combined to one mean value for each of the 20 plots. The soil samples for bulk density were dried (48 h at 105°C), and gravimetric moisture content and bulk density were determined. After homogenization by hand, pH values were determined in demineralized water, using a 1:2.5 weight:volume ratio. Lime content of the soil was measured with a Shimadzu TOC-VCPH analyzer (Shimadzu, Kyoto, Japan). Vascular plant C and N contents were determined with a Vario EL cube Elementar CNS analyzer (Vario EL cube, Elementar GmbH, Langenselbold, Germany), and P content, after microwave destruction with HNO_3, with Optima-8000 ICP-OES (Perkin Elmer, Waltham, MA, USA). Analyses of C, N, and P were conducted for bulk aboveground plant material and *C. arenaria* leaves. For plant N:P ratios, values around 15 g/g are characteristic for balanced N and P uptake, values around 10 g/g for N limitation, and values around 20 g/g for P limitation (Olde Venterink et al. 2003).

Soil C content was measured with loss on ignition (LOI) at temperatures of 375°C, at which organic matter disappears, but calcium carbonate not yet, and/or with an Elementar CNS analyzer. If not measured simultaneously, SOM was assumed to consist of 50% of organic C. For the 30 plots of the P nutrition study, both LOI and CNS methods were applied. For the 20 plots of the soil community study, the May samples were analyzed with LOI and the October samples with a CNS analyzer. Carbon contents per kg soil were converted to values per m^2 in the upper 5 (soil community study) or 10 cm (P nutrition study) of the topsoil with help of bulk density, which showed average values of 1.34 ($\pm$0.06) g/cm^3 for soils with low organic matter content, 1.17 ($\pm$0.09) g/cm^3 for soils with intermediate organic matter content, and 0.97 ($\pm$0.06) g/cm^3 for soils with high organic matter content. Soil N content was measured with an CNS analyzer in the P nutrition study and the October samples of the

soil community study, and with $H_2SO_4/H_2O_2/Se$ destruction in the May samples of the soil community study. In the soil community study, soil C content slightly differed between May and October ($P = 0.038$), possibly due to differences in method and/or exact sampling location within the plot. However, pH, N content, and C:N ratio did not differ between sampling periods, and response patterns with respect to pH and SOM were the same. For the data analysis of the soil community study, samples of May and October were combined to one mean value per plot.

Fractionation of soil organic matter

Physical fractionation of SOM was conducted according to van Mourik and Blok (2008) in the P nutrition study and Cerli et al. (2012) in the soil community study. On average, the light fraction accounted for 54% of total organic C in the first and 63% in the latter study, probably due to the sandy soils, which restrict formation of heavy clay–humus complexes. However, as values did not consistently differ between pH and SOM classes, the data are not further treated.

Analysis of humin, humic acids, and fulvic acids was applied in the P nutrition study, as humic acids may increase P availability (Du et al. 2013). Humic acids are polymeric complexes with ions such as Fe and Al, and are important to P sorption. Insoluble humin was separated from humic and fulvic acids in 0.1 mol/L NaOH solution (Stevenson 1994). Separation of humic and fulvic acids was done by precipitation with 2 mol/L H_2SO_4. Humin and humic acids were frozen, dried, weighed, and ground, and C contents were determined with an Elementar CNS analyzer. For fulvic acids, which remained in liquid, organic C was measured with an Skalar SA 1074 autoanalyzer (Skalar Analytical B.V., Breda, the Netherlands). For calcareous soil, inorganic C was measured with a Shimadzu TOC-VCPH analyzer.

Selective extraction of P

Selective extractions of P were applied to dried and ground soil samples of the 30 plots in the P nutrition study. The data should be treated as potential rather than actual amounts, because selective extractions give only a rough indication of different forms, and reactive surfaces are increased due to grinding of the laboratory samples.

Total P was determined after heating 1 g of ground soil sample at 500°C in order to digest organic matter (Westerman 1990, Kooijman et al. 1998, 2009). Samples were subsequently extracted with 50 mL 0.5 mol/L H_2SO_4. Total P was measured spectrophotometrically with a Cecil CE1010 spectrometer (Akribis Scientific Limited, Pickmere, UK) with a sulfuric acid/ammonium molybdate/ascorbic acid/potassium antimonyl tartrate solution at 880 nm. Inorganic P was extracted in the same way as total P, but with non-heated samples. Organic P was calculated as the difference between total P and inorganic P. The fraction of P sorbed to amorphous, poorly crystalline Fe and Al was determined according to Schwertmann (1964), by extraction in the dark with 0.073 mol/L ammonium oxalate and 0.05 mol/L oxalic acid at pH 3. This method yields P sorbed to Fe and Al oxides, but also to organic Fe-OM and Al-OM complexes, which differ in binding strength (Kooijman et al. 2009, Gerke 2010).

Total P consisted of inorganic and organic P, which each consisted of solid and sorbed P fractions. These fractions could not be measured directly, but minimum and maximum estimates could be calculated, based on the measured amounts of inorganic, organic, and sorbed P (Kooijman et al. 2009, 2019). In calcareous dunes, inorganic P was generally larger than sorbed P, which means that at least part of the inorganic P belonged to the soil solid phase, probably as calcium phosphates (Hinsinger 2001). In this case, the minimum estimate of solid inorganic P was the surplus of inorganic P. When inorganic P was smaller than sorbed P, the minimum estimate of solid inorganic P was zero. The maximum estimate for solid inorganic P was the amount of inorganic P itself. In acidic dunes, sorbed P was generally larger than inorganic P, which means that at least part of the sorbed P was organic. In this case, the surplus of sorbed P represented the minimum estimate for sorbed organic P. When sorbed P was smaller than inorganic P, the minimum estimate for sorbed organic P was zero. The maximum estimate for sorbed organic P was sorbed P itself.

Weakly sorbed P was determined according to Mehlich (1984), using 0.015 mol/L ammonium fluoride, 0.20 mol/L glacial acetic acid, 0.25 mol/L ammonium nitrate, and 0.013 mol/L nitric acid. This method usually yields lower amounts of sorbed P than the oxalate extraction by Schwertmann (1964) used above. Mehlich extraction was applied on 26 of the 30 soil samples, because four samples, from calcareous and acidic dunes with low or high SOM, were used for a pioneer study on colloidal P (Missong et al. 2017). Two calcareous samples with low and intermediate SOM showed much higher values than the others and were discarded. One of them also showed unrealistic values for many microbial parameters, possibly due to fertilization with animal excrements.

Selective extraction of amorphous Fe and Al

Total amorphous Fe and Al were measured for the 30 plots of the P nutrition study with the oxalate extractions for sorbed P described above (Schwertmann 1964). One calcareous sample with low SOM, which also showed extreme values for Mehlich-extractable P, showed unrealistically low values for oxalate-extractable Fe, and all variables related to this were discarded. Organic Fe and Al, which belongs to complexes of Fe and Al with SOM, were measured according to McKeague et al. (1971) by alkaline Na pyrophosphate/NaOH extraction at pH 9.8. Inorganic amorphous Fe and Al, which belongs to Fe and Al oxides, was calculated as the difference between total amorphous and organic Fe and Al. As already mentioned, selective extractions should be used with some care. Part of the organic Fe and Al may consist of small inorganic Fe and Al particles, especially in podzol soils with mobilization and precipitation of Fe (Jeanroy and Guillet 1981). However, in the acidic dunes of this study, podzolization did not yet occur.

Water-dispersible nanoparticles and fine colloids

The chemical composition of nanoparticles and fine colloids, which technically belong to the water-soluble fraction in the soil solution, was only determined in the P nutrition study. For each of the 18 plots, gravimetric moisture content of the topsoil was determined, and water-dispersible colloids extracted according to Missong et al. (2017). Field-fresh soil was suspended in deionized water in a ratio of 1:2 (w:v) and shaken. The suspension was diluted fourfold and left to sediment for 10 min and the supernatant soil extract decanted. Part of the supernatant was centrifuged for 5 min at 4000 g to obtain water-dispersible colloids smaller than 500 nm.

Another part was centrifuged at 10,000 g, after which the remaining colloid-free electrolyte phase was decanted, and the residue freeze-dried and weighed to calculate the average colloid weight of the extracts.

The nanoparticles and fine colloids were size separated with Postnova Analytics asymmetric field-flow fractionation, which was coupled online to an Agilent 750 ICP-MS (Agilent, Santa Clara, CA, USA), to analyze P content of the different colloidal fractions, as well as the main P-binding constituents Fe and Al (Missong et al. 2017). Particle size resolution of the applied FFF separation was checked by analyzing latex standards of the sizes 21, 64, 100, and 600 nm under the same conditions. The particles were focused in the flow chamber for 12 min at a cross flow of 2.5 mL/min. After focusing, the cross flow decreased for 30 min, which allowed the particles to be separated according to size, with the smallest nanoparticles coming first. The largest particles were released when the cross flow was zero, which lasted for 30 min. However, nanoparticles were rare and most particles belonged to fine colloids of 200–300 nm, so different size fractions were combined to one colloidal fraction. Total P, Fe, and Al in the bulk sample were also analyzed with ICP-MS, and soluble fractions were calculated by subtraction of colloidal from total values.

Microbial cycling of C, N, and P

Potential net mineralization of N was measured in laboratory incubation experiments in both the P nutrition and the soil community study, but net mineralization of P only in the first. Fresh, homogenized samples were put into large petri dishes, brought to optimal moisture levels, and stored at 20°C in the dark for six weeks (P nutrition study) or four weeks (soil community study). Ammonium, nitrate, and phosphate concentrations of fresh and incubated samples were extracted with 50 mL 0.05 mol/L K_2SO_4 solution and measured with a Skalar segmented flow autoanalyzer. Net N and P mineralization were calculated from differences between incubated and fresh samples. For the soil community study, net N mineralization per g C did not differ between the May and October samples, and values were combined in one mean value for each of the 20 plots.

Microbial C, N, and P were measured in the 30 plots of the P nutrition study with the chloroform fumigation extraction procedure (Brooks et al. 1985). In the 20 plots for the soil community study, microbial C and N were measured with the same procedure, but only for the October samples. Fumigated samples were flushed for 24 h with chloroform and extracted with 0.05 mol/L K_2SO_4 immediately afterward, to prevent microbial regrowth. Dissolved organic matter, ammonium, nitrate, total N, and phosphate were measured in fumigated and non-fumigated samples at the start and end of the incubation period, using a Skalar segmented flow autoanalyzer. Microbial C, N, and P concentrations were calculated from the differences between fumigated and non-fumigated samples.

In the P nutrition study, the net increase in microbial C, N, and P during the incubation experiment was used as a proxy for microbial uptake. Also, total (net) microbial C, N, and P release was calculated as the sum of the net mineralization of C, N, and P and their microbial uptake. Microbial C:N, C:P, and N:P ratios were calculated as well. For microbial N:P ratios, values around 3.1 g/g indicate balanced availability of N and P, while clearly higher values point to P limitation (Cleveland and Liptzin 2007). One calcareous sample with low SOM, which also showed extreme values for Mehlich-extractable P and oxalate-extractable Fe, had unrealistic microbial values in fresh soil, and was discarded for all microbial parameters. For three calcareous samples, microbial P and/or N was very low after incubation, and the associated nutrient ratios were discarded.

Microbial respiration was determined in both the P nutrition and the soil community study, albeit with different methods. In the P nutrition study, respiration was measured at the start and end of the incubation experiment by measuring CO_2 concentrations in the headspace with a Carlo Erba Varian gas chromatograph according to Tietema (1992). In the soil community study, in the May samples, respiration was based on the amount of oxygen used during the incubation experiment (Bloem et al. 2006). In the October samples, respiration was measured with a respicond (Nordgren 1988), in which the cumulative amount of CO_2 over the incubation period was measured via the decrease in electrical conductivity in the KOH solution due to the formation

of K_2CO_3. In the soil community study, respiration rates did not differ between May and October, and values were combined to one mean value for each of the 20 plots.

With microbial nutrient contents, net mineralization, and respiration, proxies for microbial efficiency could be calculated. Respiration quotient was calculated as respiration per unit microbial C. Net N mineralization per unit C respired was calculated as a proxy for microbial N use efficiency; high values correspond with low microbial immobilization and vice versa (Kooijman et al. 2008, 2016).

Microbial community composition

Microbial community composition was only determined in the soil community study. In May, bacterial biomass was determined by confocal laser scanning microscopy (Bloem et al. 1995) and fungal biomass by microscopic counting after staining with fluorescent dyes (Bloem and Vos 2004). In addition to hyphal length, percentages of active hyphae and unstained hyphae were determined using epifluorescence alternated with transmitted light illumination. Part of the unstained fungi were melanized fungi. Melanized fungi are light yellow to dark brown and contain the pigment melanin, which is considered as adaptation to stress (Butler and Day 1998).

In addition to direct counting, bacterial activity was measured by incorporation rates of [^{14}C]leucine, an amino acid used for protein synthesis, and [^{3}H]thymidine, a precursor of thymine which is one of the four base components of DNA (Bloem and Bolhuis 2006). Leucine incorporation is thus reflecting bacterial protein synthesis and thymidine incorporation bacterial growth rate.

In both May and October, microbial community composition was determined by analysis of PLFAs, which are essential membrane components of all living cells, but absent in storage products or dead cells (Frostegård et al. 1991). We used PLFA analysis rather than quantitative PCR (Rousk et al. 2010), because we focused on living microbial biomass. Lipids were extracted with a solution of chloroform, methanol, and citric acid buffer, and fractionated into neutral lipids, glycolipids, and phospholipids on silica gel columns. The phospholipids were hydrolyzed to fatty acids and collected by liquid extraction with hexane, dried, redissolved, and stored at −20°C prior to analysis.

In total, 17 different PLFAs were used, with one characteristic for fungi (18:2n6c) and eleven for bacteria (Gram-positive bacteria: i-15:0, i-16:0, a-15:0, i-17:0, and 17:0; Gram-negative bacteria: 16:1n7, cy17:0, and cy19:0; other bacteria: 14:0, 16:0, and 18:0). Unfortunately, we did not measure 16:1x5c, which is characteristic for AM fungi. The total amounts of Gram-positive and Gram-negative bacteria were calculated, as well as their relative proportion. The latter may be influenced by protist grazing, as Gram-positive bacteria are slightly larger than Gram-negative bacteria (Griffiths et al. 1999, Rønn et al. 2002), but also by pH, as Gram-positive phyla such as *Actinobacter* and *Fimicutes* are more tolerant to acidic conditions (Chodak et al. 2015). The relative proportion of Gram-positive bacteria was calculated as Gram-positive/(Gram-positive + Gram-negative). Fungal:bacterial (F:B) ratios were calculated, based on the amount of fungal and bacterial PLFAs. Potential differences in microbial community composition between May and October were tested with principal component analysis (PCA; Ter Braak 1988), but were very small, and for each PLFA, the May and October values were combined to one mean value for each of the 20 plots.

Microfauna and mesofauna

The numerical abundance of soil animals was only measured in the soil community study. The main bacterivores are often protists (amoeba and flagellates), which are small in size, but large in numbers, and can grow as rapidly as their prey. Protists were enumerated by the most probable number method after Rønn et al. (1995) with the use of fourfold dilution series in 1/10 Tryptic Soy Broth medium. The same soil suspension as prepared for bacterial and fungal counts was used, diluted in microtiter plates; after 2 and 4 weeks of incubation at 18°C, these were checked microscopically for the presence of amoebae and flagellates. Nematodes were not sampled, because they were thought less important in coastal dune soils, which was supported by the general absence of nematode predators.

Microarthropods (Acari and Collembola) constitute an important part of the soil fauna and prefer particular food resources (Siepel and Maaskamp 1994, Coleman and Hendrix 2000). Many Collembola feed on fungal hyphae, although they may change diet when necessary

(Sauer and Ponge 1988, Endlweber et al. 2011). Microarthropods were extracted during one week with the Tullgren method and determined with gel-based subsamples (Jagers op Akkerhuis et al. 2008). Taxa consisted of species, genera, or families, depending on which extent determination was possible. In total, 65 different taxa were distinguished: 55 mites and 10 Collembola. Microarthropod taxa were grouped into ten different feeding guilds (Siepel and de Ruiter-Dijkman 1993, Jagers op Akkerhuis et al. 2008): herbivores (grazers and browsers), fungivores (grazers and browsers), predators (of arthropods, nematodes, or in general), and opportunists (omnivores, herbofungivorous grazers, and opportunists). Browsers digest cell content only, while grazers digest both cell content and cell walls. Nematode predators were only found in small numbers in one plot and combined with the general predators. Soil macrofauna was not sampled, because earthworms are rare in dry sandy dune soils (Nijssen et al. 2014).

Statistical analysis

For variables measured in both the P nutrition and the soil community study, three-way general linear models were applied, with study type (P nutrition or soil community), pH class (calcareous and acidic), and SOM class (low, intermediate, and/or high) as independent parameters (Cody and Smith 1987). The model was as follows: response variable = study, pH, SOM, study × pH, study × SOM, pH × SOM, and study × pH × SOM. Even though the studies were conducted in different locations, and differed in depth of the soil samples, plot size for vegetation relevés, and sampling period of vascular plant biomass, this was done to test potential responses to pH and SOM in the most vigourous way. For this analysis, the high SOM plots of the soil community study were assigned to the intermediate SOM class, because the amount of soil organic C was more comparable with the intermediate SOM plots of the P nutrition study than with the high SOM plots. Differences were significant for P values < 0.05, and differences between individual groups were post hoc-tested with LS means tests.

For variables only measured in one of the studies, two-way general linear models were applied, with pH (calcareous and acidic) and SOM class (low, intermediate, and/or high) as independent parameters. The model was as follows: response variable = pH, SOM, and pH × SOM. Differences were significant for P values < 0.05, and differences between individual groups were post hoc-tested with LS means tests.

In the P nutrition study, relationships between different fractions of P and Fe and/or pH were tested with Spearman correlation tests, and correlations were significant when $P < 0.05$ (Cody and Smith 1987). To test how much of the variance in the percentage of organic Fe would be explained by pH and SOM, a stepwise multiple linear regression was performed, with the actual pH and SOM content as independent variables (Cody and Smith 1987). The model was as follows: organic Fe = pH SOM/forward. To further test relationships between pH, SOM, P fractions, and AM and NM plants, a correlation-based network analysis was conducted, based on Spearman correlation tests for individual sets of parameters. This was done with actual pH and SOM values, eleven variables related to P nutrition (total P, inorganic P, minimum estimates of solid inorganic P, inorganic Fe, organic P, organic Fe, minimum estimates of sorbed organic P, weakly sorbed P, colloidal P, and P released by microorganisms), and AM and NM plant species number or relative cover. To test which of the P-related variables were most important for AM and NM plants, stepwise multiple linear regressions were applied with the eleven P-related variables as independent variables. The model was as follows: AM species, NM species, or AM (or NM) relative cover = total P, inorganic P, minimum estimates of solid inorganic P, inorganic Fe, organic P, organic Fe, minimum estimates of sorbed organic P, weakly sorbed P, colloidal P, and P released by microorganisms/forward.

For the soil community study, a correlation-based network analysis was applied to test relationships between the actual pH and SOM values, AM and NM plant cover, seven different functional groups in the soil community (bacteria, fungi, bacterial feeders, fungal feeders, herbivores, omnivores, and predators), soil respiration, and net N mineralization. The relative importance of different factors was tested with stepwise multiple linear regression, with pH, SOM content, and AM and NM plant cover as independent variables. The model was as

follows: bacteria, fungi, bacterial feeders, fungal feeders, herbivores, omnivores, predators, soil respiration, or net N mineralization = pH, SOM content, AM plant cover, and NM plant cover/forward.

In order to identify key players in the hypothetical causal relationships between soil communities and pH or SOM, *d*-separation tests were performed (Shipley 2000, 2003). The causal parents and children were chosen from our hypothetical directed acyclic graph (DAG), in which no feedback loops are allowed. Model A tested whether pH could be a causal factor to the relative abundance of AM plants (a) and bacterial growth rates (b), measured as incorporation of thymidine (pmol·g^{-1}·h^{-1}), which in turn could affect numbers of amoeba in the soil (c). Model B tested whether SOM could be a causal factor for bacterial growth rates (a) and the relative abundance of active fungi (b), which both in turn could affect net N mineralization (c and d). The causal parents and children were then transformed into a basis set of *d*-separation claims, stating only those components that were d-separated by another component in the DAG. The *d*-separation claims were then transformed into statistical claims. For the hypothetical causal relationships, we assumed linear relations, and the correlations between our *d*-separation claims were tested. The residual errors of these correlations were then added and tested with a Fisher's *C* test, with two times the number of claims as degrees of freedom. If the *P* value was lower than 0.05, there was too much unexplained variation in the causal model to hold, and the null model was rejected. However, when the *P* value was above 0.05, the null model was accepted. When the model was accepted, we could calculate the individual parameters that represent the slope of the relation between the components in the DAG. Library ggm and piecewiseSEM were used in R3.5.1 (R Core Team 2018).

For individual plant species and microarthropod taxa, interactions between them were tested in a correlation-based network analysis according to Morriën et al. (2017). This was done for the 26 plant species and 38 microarthropod taxa present in at least 3 of the 20 plots. The Spearman rank correlation matrix was calculated on the whole dataset, using pairwise comparisons, but excluding zeros. The obtained matrix was filtered

and loaded in Cytoscape 3.6.1 to visualize interactions with *R*-values > 0.6. *K*-means clustering on the correlation matrix was used to divide the species into 8 corresponding clusters, with specific behavior regarding the habitat conditions. Differences in total network structure between the four dune sites with different pH and/or SOM were made visible by only using species present in those habitats.

Principal component analysis was applied within the program Canoco (Ter Braak 1988), to analyze mutual relationships between plant and soil characteristics. This analysis was applied for the P nutrition and the soil community study separately. For the P nutrition study, 63 plant and soil variables as described in the previous sections were used to construct the PCA axes, and for the soil community study 48. Missing values, which are not accepted by the program, were replaced by mean values for the other replicates in the group. For each variable, values were standardized because they differed in size and magnitude, but otherwise PCA was used with standard options. Variables related to pH and SOM were used as environmental factors, to test correlations with the PCA axes after they had been extracted, as independent test of the relative importance of pH and/or SOM. Because analysis of soluble and colloidal fractions in the P nutrition study was based on three instead of five replicates, and the two missing values in each group replaced by their means, PCA was also applied without them. However, this did not change the main patterns, and they were included in the final analysis.

Results

Basic soil characteristics

The P nutrition and soil community studies did not differ with respect to lime content or pH in the soil (Table 1; Appendix S1: Table S2). As expected, in both studies, calcium carbonate was only present in calcareous dunes and pH of the topsoil was significantly higher in calcareous than in acidic dunes. Soil C content did also not differ between studies when expressed as a percentage, although values in kg/m^2 were higher in the P nutrition study, due to differences in soil depth. In both studies, soil C and N showed a significant increase from low to high SOM, in

Table 1. Topsoil characteristics in different coastal dune grasslands in the Netherlands.

Site	Lime content (%)	pH	Soil C (%)	Soil C (kg m^{-2})	Soil N (g/m^2)	C:N ratio (g/g)	Humin (% soil C)	Humic acids (% soil C)
P nutrition study (0–10 cm)								
C1	1.4 (0.7)[A]	7.2 (0.7)[C]	0.9 (0.3)[B]	0.9 (0.3)[A]	98 (33)[B]	9.6 (2.7)[A]	66 (12)[B]	16 (7)[C]
C2	1.7 (0.7)[A]	7.5 (0.2)[C]	1.4 (0.2)[C]	1.6 (0.3)[B]	168 (34)[C]	9.7 (0.3)[A]	76 (4)[C]	8 (4)[B]
C3	4.1 (3.9)[A]	7.5 (0.7)[C]	3.3 (0.5)[E]	2.7 (0.2)[D]	261 (27)[D]	10.3 (0.9)[A]	87 (10)[D]	1 (1)[A]
A1	…	5.8 (0.7)[B]	0.4 (0.0)[A]	0.5 (0.1)[A]	40 (7)[A]	13.2 (1.9)[B]	45 (4)[A]	30 (5)[D]
A2	…	4.1 (0.2)[A]	1.3 (0.3)[B]	1.3 (0.6)[B]	109 (49)[B]	12.3 (0.5)[B]	46 (4)[A]	35 (2)[DE]
A3	…	4.0 (0.1)[A]	2.5 (0.4)[D]	2.2 (0.2)[C]	173 (6)[C]	12.8 (1.3)[B]	45 (6)[A]	36 (4)[E]
Soil community study (0–5 cm)								
C1	2.9 (1.2)[A]	8.2 (0.2)[D]	0.9 (0.2)[A]	0.6 (0.2)[A]	46 (9)[A]	12.6 (0.6)[A]	…	…
C2	1.8 (1.1)[A]	7.1 (0.7)[C]	2.0 (0.5)[B]	1.2 (0.2)[B]	97 (17)[C]	13.2 (1.8)[A]	…	…
A1	…	5.2 (0.6)[B]	1.1 (0.4)[A]	0.7 (0.3)[A]	44 (17)[A]	16.9 (2.8)[B]	…	…
A2	…	3.8 (0.1)[A]	2.0 (0.4)[B]	1.2 (0.2)[B]	75 (14)[B]	16.2 (1.6)[B]	…	…

Notes: C1, calcareous soil with low soil organic matter (SOM); C2, calcareous soil with intermediate SOM; C3, calcareous soil with high SOM; A1, acidic soil with low SOM; A2, acidic soil with intermediate SOM; A3, acidic soil with high SOM. Values given are means (n = 5) and standard deviations. Different letters indicate significant differences for a particular parameter in a particular study between the soil types ($P < 0.05$).

both calcareous and acidic dunes. Soil C:N ratios were higher in the soil community than in the P nutrition study, possibly because the shallower samples contained more fresh organic material, which generally has a higher C:N ratio than more decomposed forms. However, in both studies, C:N ratios were also significantly influenced by pH and showed significantly lower values in calcareous than in acidic dunes. Chemical fractionation of SOM was only applied in the P nutrition study and was especially influenced by pH. Humin contents were higher in calcareous dunes, but humic acids in acidic dunes. Humic acids, aromatic complexes with metal ions such as Fe and Al, accounted for only a few percent of soil organic C in calcareous dunes, but increased to 30–36% in acidic dunes.

Vascular plant strategies

In both the P nutrition and the soil community study, plant species composition and strategies clearly differed between calcareous and acidic dunes (Table 2). Arbuscular mycorrhizal species such as *Viola curtisii* and *Galium verum* were present especially in calcareous dunes. Nonmycorrhizal species such as *Rumex acetosella* and *Luzula campestris* were, however, more common in acidic dunes. The NM species *C. arenaria* was found in almost all 50 plots, but with low cover values in calcareous dunes, while it dominated the vegetation in acidic dunes.

Plant species numbers were generally higher in the P nutrition than in the soil community study, probably due to the larger plot size (Fig. 2; Appendix S1: Table S3). However, in both studies, the total number of vascular plant species was significantly higher in calcareous than acidic dunes. This was also the case for AM plants. The relative number of AM plant species accounted for approximately 75% of the vascular plant species in calcareous dunes, but only around 30% in acidic dunes. The number of NM species did not change with pH, but the relative number increased from calcareous to acidic dunes. Nonmycorrhizal species accounted for only approximately 25% of the

Table 2. Plant species composition in different coastal dune grasslands in the Netherlands.

| Plant species	Type	P nutrition study						Soil community study			
		C1	C2	C3	A1	A2	A3	C1	C2	A1	A2
Phleum arenarium	AM	3	…	…	…	…	…	4	…	…	…
Erodium lebelii	AM	5	5	…	1	…	…	5	5	…	…
Festuca rubra	AM	5	4	…	…	…	…	4	…	…	…
Myosotis ramossissima	AM	5	3	…	…	…	…	…	…	…	…
Sedum acre	AM	3	1	…	…	…	…	4	2	…	…
Viola curtisii	AM	3	1	…	…	…	…	2	4	2	…
Syntrichia ruralis	Moss	5	4	…	2	…	…	4	3	…	…
Cladonia ciliata	Lichen	2	3	…				…	…	…	…
Saxifraga tridactylitis	AM	2	2	…	…	…	…	…	…	…	…
Ononis repens	AM	…	…	…	…	…	…	3	3	…	…
Koeleria macrantha	AM	…	…	…	…	…	…	1	2	…	…
Rubus caesius	AM	…	…	…	…	…	…	1	5	…	…
Cynoglossum officinale	AM	5	5	1	…	…	…	1	4	1	…
Jacobea vulgare	AM	5	5	4	…	…	…	1	5	…	1
Veronica arvense	AM	2	3	1	…	…	…	…	5	…	…
Galium verum	AM	1	5	4	…	…	…	1	3	…	1
Veronica officinalis	AM	1	1	2	…	…	…	…	…	…	…
Glechoma hederacea	AM	…	3	3	…	…	…	…	…	…	…
Salix repens	AM	…	3	2	…	…	…	…	…	…	…
Taraxacum officinale	AM	…	3	3	…	…	…	…	…	…	…
Cerastium fontanum	NM	…	2	3	…	…	…	…	…	…	…
Carex flacca	NM	…	…	5	…	…	…	…	…	…	…
Potentilla reptans	AM	…	…	5	…	…	…	…	…	…	…
Thymus pulegioides	AM	…	…	5	-	…	…	…	…	…	…
Viola canina	AM	…	…	4	-	…	…	…	…	…	…
Fissidens adianthoides	Moss	…	…	4	-	…	…	…	…	…	…
Pseudoscleropodium purum	Moss	…	2	4	2	2	1	…	…	…	…
Hypnum cupressiforme	Moss	3	5	3	5	5	2	1	5	1	4
Cladonia foliacea	Lichen	4	3	…	3	1	…	4	4	5	1
Cerastium semidecandrum	NM	5	4	…	3	…	…	4	5	4	…
Carex arenaria	NM	5	5	4	5	5	5	5	5	5	5
Luzula campestris	NM	1	4	3	…	2	3	…	3	…	3
Calamagrostis epigejos	AM	1	5	4	1	5	4	…	…	1	3
Festuca ovina	AM	…	1	5	3	4	4	…	…	…	…
Rumex acetosella	NM	…	3	1	3	3	3	…	…	5	1
Corynephorus canescens	NM	…	…	…	5	…	…	…	…	5	1
Teesdalia nudicaulis	NM	…	…	…	3	…	…	…	…	…	…
Hypochaeris radicata	AM	…	…	…	…	…	…	…	…	2	3
Aira praecox	NM	…	…	…	…	…	…	…	…	3	…
Cladonia portentosa	Lichen	…	…	…	1	3	1	…	…	5	5
Dicranum scoparium	Moss	…	1	1	…	3	5	…	…	1	3
Campylopus introflexus	Moss	…	…	…	…	…	…	…	…	5	…
Hypnum jutlandicum	Moss	…	…	…	…	2	5	…	…	…	…

Notes: C1, calcareous soil with low soil organic matter (SOM); C2, calcareous soil with intermediate SOM; C3, calcareous soil with high SOM; A1, acidic soil with low SOM; A2, acidic soil with intermediate SOM; A3, acidic soil with high SOM; AM, arbuscular mycorrhizal plant; NM, nonmycorrhizal plant. Values indicate the numbers of times a particular species was found within a particular group (n = 5).

vascular plant species in calcareous dunes, but around 70% in acidic dunes.

In the P nutrition study, vascular plant cover was slightly higher than in the soil community study, and cryptogam cover slightly lower, probably due to differences in sampling locations (Fig. 3). In both studies, vascular plant cover significantly increased from low to higher SOM. Aboveground vascular plant biomass was only measured in the soil community study, but also

P-nutrition study
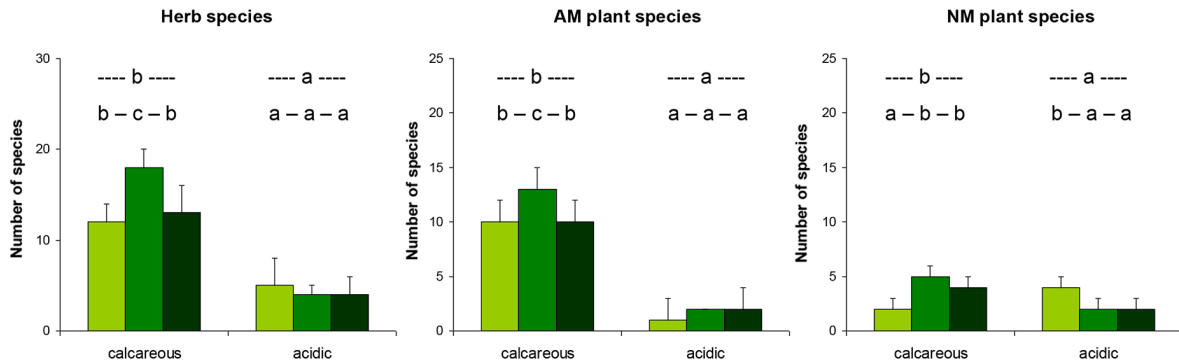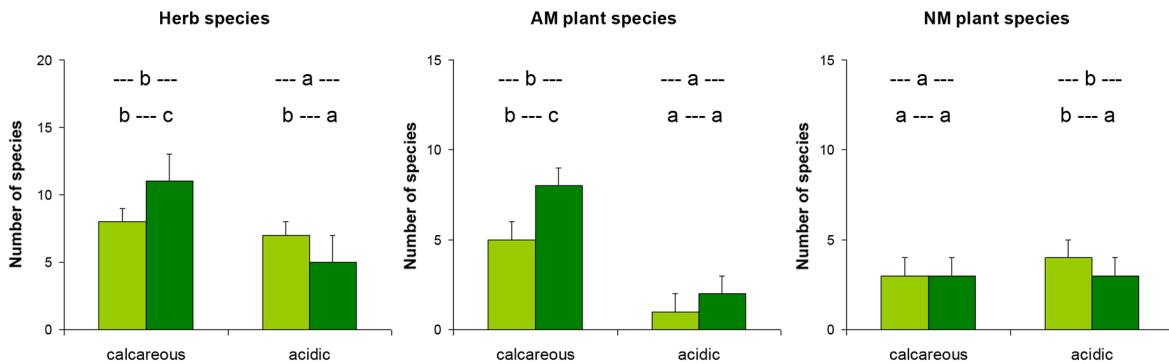

Soil community study

Fig. 2. Number of plant species in coastal dune grasslands in calcareous and acidic dunes with different soil organic matter (SOM) in the Netherlands. Herb species are vascular plant species; arbuscular mycorrhizal (AM) species, AM plant species; and nonmycorrhizal (NM) species, NM plant species. Light green bars are low SOM; green bars, intermediate SOM; and dark green bars, high SOM. Values given are means (n = 5) and standard deviations. Different letters in the upper row indicate significant differences for a particular parameter between calcareous and acidic dunes (P < 0.05). Different letters in the lower row indicate significant differences for a particular parameter between all four-six soil types.

increased with SOM. In calcareous dunes, aboveground biomass increased from 52 ($\pm$34) g/m^2 at low SOM to 159 ($\pm$118) g/m^2 at higher SOM. In acidic dunes, biomass was significantly higher than in calcareous dunes, and increased from 93 ($\pm$31) g/m^2 at low SOM to 248 ($\pm$98) g/m^2 at higher SOM.

The cover of AM plants increased from low to higher SOM, but especially changed with pH, and was in both studies significantly higher in calcareous than in acidic dunes. The relative AM plant cover was approximately 80% of the vascular plant cover in calcareous dunes, but approximately 20% in acidic dunes. Cover of NM plants

also significantly increased with SOM but was especially high in acidic dunes. The relative NM plant cover increased from approximately 20% of the vascular plant cover in calcareous dunes to approximately 80% in acidic dunes.

Vascular plant nutrient contents

Vascular plant N and P contents were slightly higher in the P nutrition than in the soil community study, probably because vegetation samples were collected earlier in the growing season. However, in both studies, plant N and P contents were significantly higher in calcareous than in acidic dunes, probably

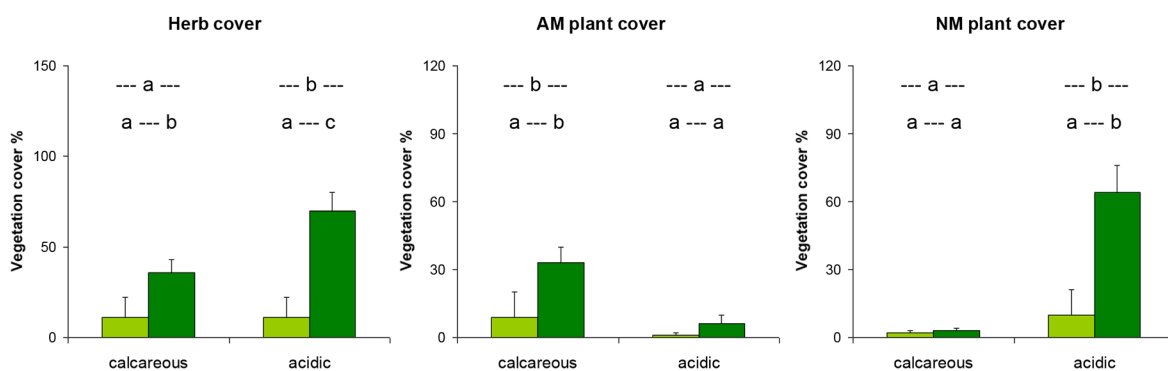**P-nutrition study**
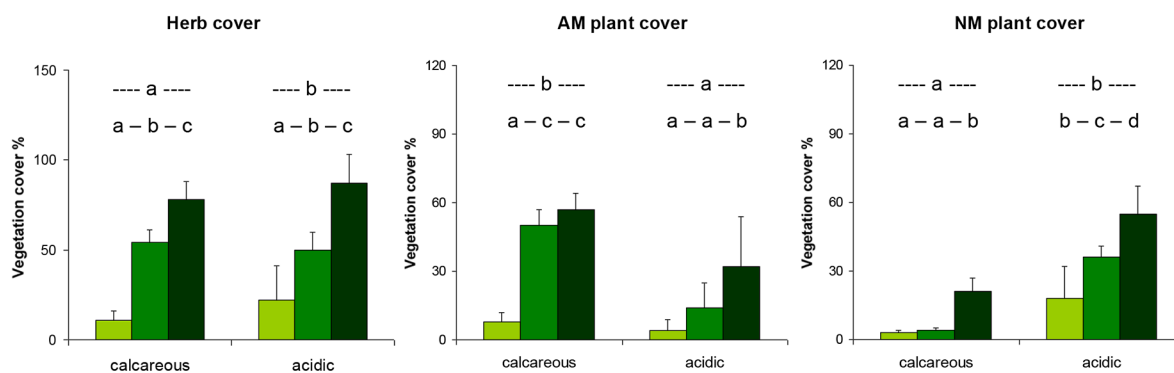
Soil community study

Fig. 3. Absolute cover values of the vegetation in coastal dune grasslands in calcareous and acidic dunes with different soil organic matter (SOM) in the Netherlands. Herb species are vascular plant species; arbuscular mycorrhizal (AM) species, AM plant species; and nonmycorrhizal (NM) species, NM plant species. Light green bars are low SOM; green bars, intermediate SOM; and dark green bars, high SOM. Values given are means ($n = 5$) and standard deviations. Different letters in the upper row indicate significant differences for a particular parameter between calcareous and acidic dunes ($P < 0.05$). Different letters in the lower row indicate significant differences for a particular parameter between all four-six soil types.

associated with the predominance of AM plants (Fig. 4). Nevertheless, in both calcareous and acidic dunes, foliar N:P ratios pointed to balanced availability of N and P to the AM- or NM-dominated vegetation, with values of 13–14 g/g in the P nutrition study and 12–13 g/g in the soil community study. In contrast, for the NM plant *C. arenaria*, which was sampled in all plots of the soil community study, foliar N:P ratios largely differed between dune zones. This NM species showed low plant P contents in calcareous dunes, and N:P ratios of 22–25 g/g, which clearly point to P limitation. In acidic dunes, however, plant P contents were significantly higher than in calcareous dunes, and N:

P ratios decreased to 15–16 g/g, which points to balanced supply of N and P.

Different fractions of P

Different forms of P clearly differed between dune zones (Appendix S1: Table S4, Fig. S1). Total P did not differ with SOM, but this fraction was significantly higher in calcareous than acidic dunes, with average values of 20.4 and 15.2 g/ m², respectively. In both calcareous and acidic dunes, inorganic P significantly decreased from low to high SOM, due to conversion of inorganic to organic P. However, inorganic P was more strongly influenced by pH and especially decreased from calcareous to acidic dunes, due
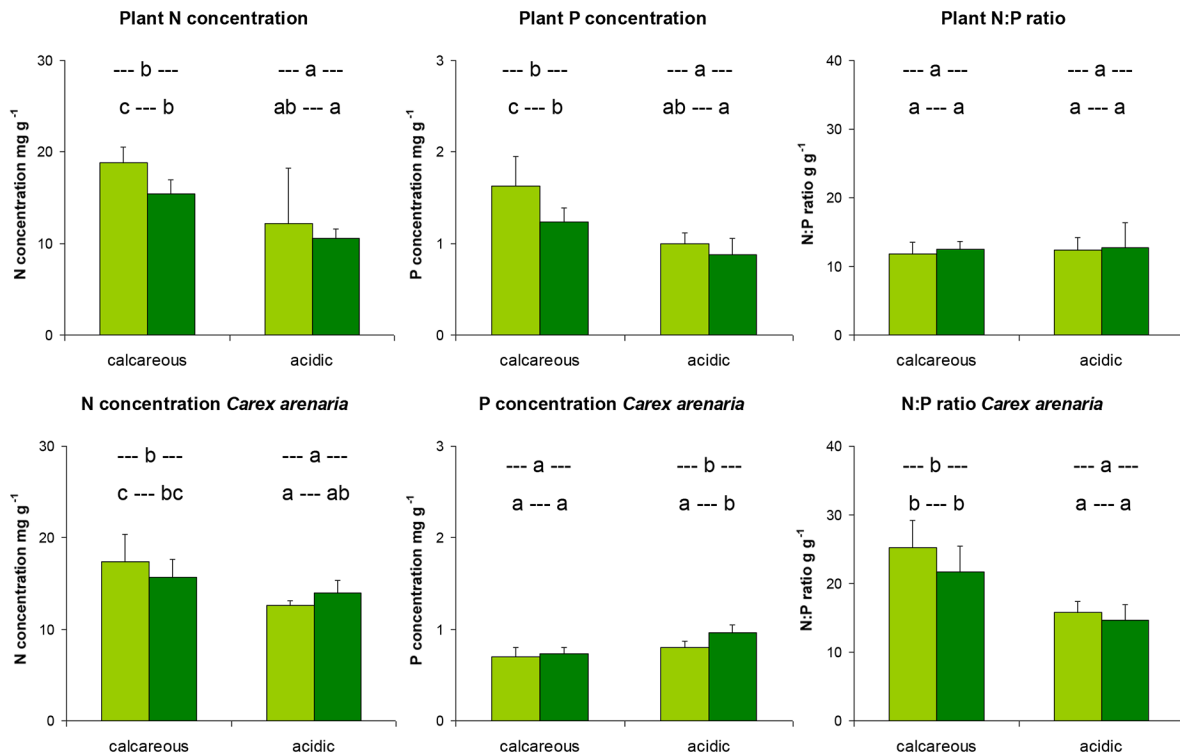
Fig. 4. Vascular plant nutrient contents and ratios in coastal dune grasslands in calcareous and acidic dunes with different soil organic matter (SOM) in the Netherlands. Plant N and P contents and N:P ratios are based on arbuscular mycorrhizal plant-dominated vegetation in calcareous dunes and nonmycorrhizal plant-dominated vegetation in acidic dunes. The NM plant *Carex arenaria* occurred in both dune zones. Light green bars are low SOM, and dark green bars are high SOM. Values given are means ($n = 5$) and standard deviations. Different letters in the upper row indicate significant differences for a particular parameter between calcareous and acidic dunes ($P < 0.05$). Different letters in the lower row indicate significant differences for a particular parameter between all four-six soil types.

to dissolution of calcium phosphates. Inorganic P thus gradually decreased from 11.6 g/m^2 in calcareous dunes with low SOM to 4.1 g/m^2 in acidic dunes with high SOM. In calcareous dunes, inorganic P was higher than sorbed P, which means that at least part of the inorganic P belonged to the soil solid phase, probably as calcium phosphates (Fig. 5). With the measured values of inorganic and sorbed P, minimum and maximum amounts of solid inorganic P could be estimated. Both estimates for solid inorganic P clearly decreased from calcareous to acidic dunes.

Organic P did not differ between calcareous and acidic dunes, but significantly increased from low to high SOM, due to conversion of inorganic to organic P. On average, organic P accounted for 36% of total P at low SOM, 56% at intermediate SOM, and 67% at high SOM. Sorbed P did also not differ between dune zones, but its relative importance increased from 29% of total P in calcareous dunes to 44% in acidic dunes. Also, in acidic dunes, sorbed P was higher than inorganic P, especially at intermediate and high SOM, which means that at least part of the sorbed P consisted of organic P. With the measured values of inorganic and sorbed P, minimum and maximum estimates could be calculated for the amount of sorbed organic P (Fig. 5). The maximum estimates for sorbed organic P did not differ between dune zones, and only increased with SOM. However, the minimum estimates showed that at least some of the sorbed P was organic in acidic dunes with
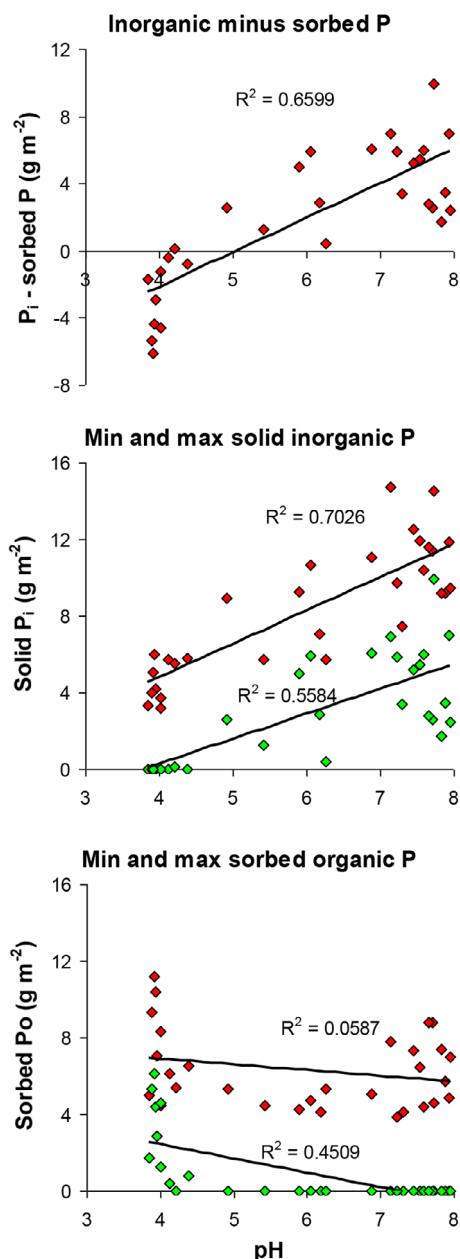
Fig. 5. Relationships between pH and inorganic P minus sorbed P, minimum (green) and maximum (red) estimates for solid inorganic P, and minimum (green) and maximum (red) estimates for sorbed organic P. All correlations were significant ($P < 0.05$), except for the maximum estimates for sorbed organic P.

intermediate and high SOM. In these places, sorbed organic P amounted to at least 24–26% of total organic P, or at least 16–19% of total P.

Mehlich-extractable P, a fraction of weakly sorbed, plant-available P, was significantly higher in acidic than calcareous dunes, with average values of 1.0 and 0.6 g/m^2, respectively. Colloidal P, which technically belongs to the soil solution, was also significantly higher in acidic than in calcareous dunes, with average values of 0.10 and 0.05 g/m^2, respectively. Colloidal P accounted for approximately half of total dissolved P in all dune zones, which means that total dissolved P also increased from high to low pH.

Different fractions of Fe and Al

For total amorphous Fe, values in g/m^2 were on average four times higher than for Al (Appendix S1: Table S5, Fig. S2). For both Fe and Al, inorganic and organic fractions showed different distribution patterns over dune zones. Inorganic Fe, which consists of poorly crystalline, amorphous Fe oxides, was not influenced by SOM, but significantly higher in calcareous than in acidic dunes. On average, inorganic Fe showed values of 60.1 g/m^2 in calcareous dunes and 16.6 g/m^2 in acidic dunes. Inorganic Al did not differ with pH, but was significantly influenced by SOM, albeit in an unsystematic way. The two fractions combined mainly differed between dune zones, with significantly higher values for inorganic Fe + Al in calcareous than in acidic dunes.

Organic Fe, which is part of organic Fe-OM complexes, significantly increased from low to high SOM, but especially from high to low pH, in accord with the higher amount of humic acids. On average, organic Fe showed values of 18.6 g/m^2 in calcareous dunes and 53.0 g/m^2 in acidic dunes. Organic Al and organic Fe + Al showed similar patterns and were significantly higher in acidic than in calcareous dunes, especially at intermediate and high SOM. In the colloidal and truly dissolved fraction, Fe and Al also increased from high to low pH. Colloidal Fe and Al accounted for 52–54% of the total dissolved Fe and Al in all soil types. For Al, the total amount and the soluble and colloidal fractions were significantly higher in acidic than in calcareous dunes. For Fe, these patterns were less clear, but soluble Fe was also higher in acidic than calcareous dunes.

For P sorption to the soil solid phase, Fe is generally more important than Al. For P sorption, it

is also important whether amorphous Fe consists of Fe oxides or Fe-OM complexes, as sorption to the first is stronger than to the latter. In contrast to expectations, the percentage of organic Fe differed with pH rather than with SOM. In a stepwise multiple linear regression, pH explained 73% of the variance in the percentage of organic Fe, but SOM only 5%. Organic Fe accounted for approximately 10% of the amorphous Fe at pH 8, but increased to approximately 90% at pH 4 (Fig. 6). This means that sorbed P is mainly strongly sorbed to Fe oxides in calcareous dunes, but relatively weakly to Fe-OM complexes in acidic dunes. These patterns were supported by Mehlich-extractable P, which consists of weakly sorbed P, and indeed showed a positive relationship with the percentage of organic Fe.

Microbial cycling of C, N, and P

The P nutrition and soil community study differed in microbial C and N in fresh soil, as well as respiration during the incubation experiments, probably due to differences in soil depth (Appendix S1: Table S6). Also, microbial C showed a clear increase during the incubation period in the P nutrition study, while the soil community study showed a slight decrease. Nevertheless, microbial C and N were significantly higher in calcareous than in acidic dunes and increased from low to higher SOM (Fig. 7). Respiration and net N mineralization were not generally influenced by pH and SOM, although they showed significantly higher values for acidic than calcareous dunes in the soil community study. Nitrification amounted to 90–100% of net N mineralization, irrespective of pH or SOM (data not shown). Respiration quotient was significantly higher at low than at higher SOM, probably due to the larger amount of fresh organic matter. However, net N mineralization per unit C respired, which is a proxy for microbial N use efficiency and immobilization, did not differ between any of the studies or soil types.

Changes in microbial N and P during incubation, measured in the P nutrition study, differed between calcareous and acidic dunes (Fig. 8; Appendix S1: Table S7). In fresh samples, microbial C:N ratios were similar in the two dune zones, even though microbial C and N were both higher in calcareous dunes. However, microbial
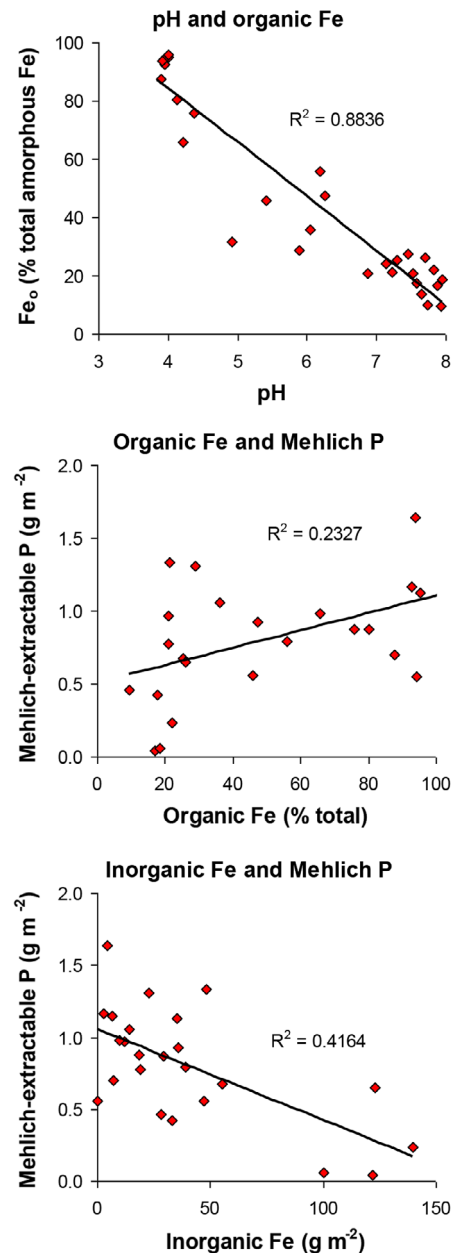

Fig. 6. Relationships between different forms of Fe and P: pH and organic Fe, as percentage of total amorphous Fe, the percentage of organic Fe and Mehlich-extractable P, and the amount of inorganic Fe and Mehlich-extractable P. All correlations were significant ($P < 0.05$).

C:N strongly increased during incubation in calcareous dunes, but remained the same in acidic dunes. In calcareous dunes, even though microbial C clearly increased during incubation,
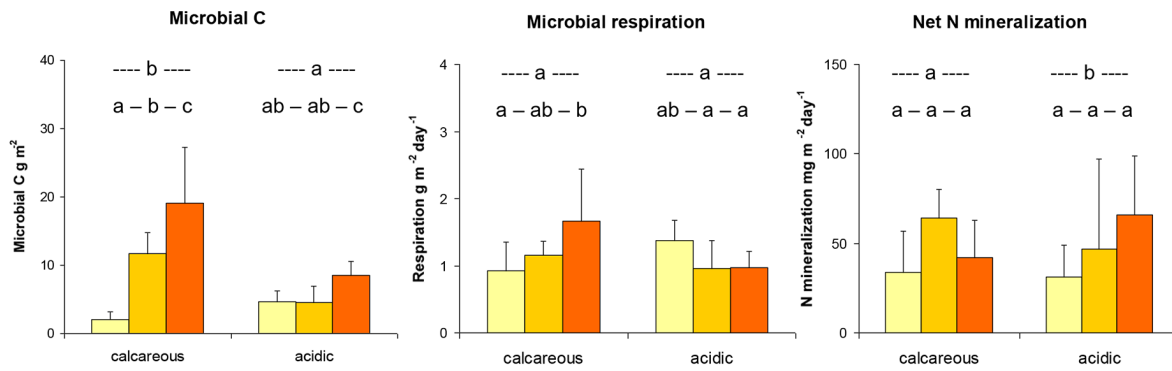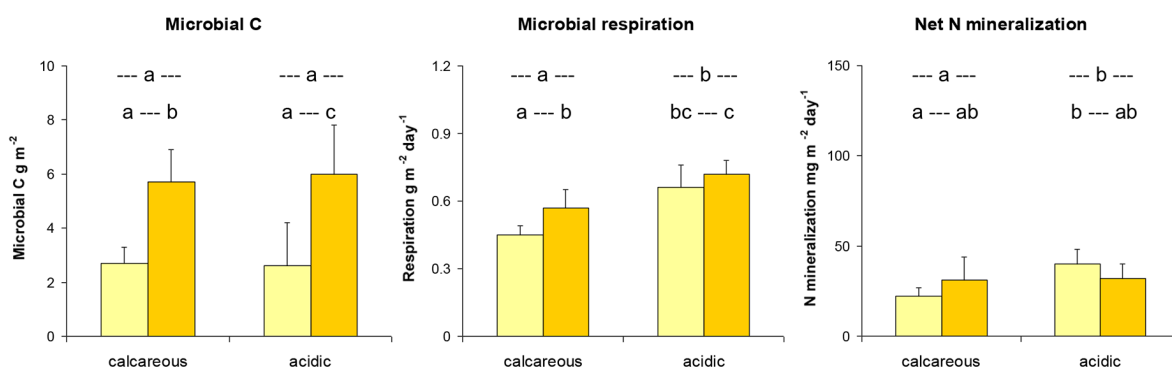
P-nutrition study (0-10 cm)

Soil community study (0-5 cm)

Fig. 7. Microbial characteristics in coastal dune grasslands in calcareous and acidic dunes with different soil organic matter (SOM) in the Netherlands. Light yellow bars are low SOM; yellow bars, intermediate SOM; and orange bars, high SOM. Values given are means ($n = 5$) and standard deviations. Different letters in the upper row indicate significant differences for a particular parameter between calcareous and acidic dunes ($P < 0.05$). Different letters in the lower row indicate significant differences for a particular parameter between all four-six soil types.

microbial N more or less decreased, perhaps because AM fungi were no longer supported by plant roots. In acidic dunes, microbial N increased together with microbial C. Net N mineralization and total microbial N release did not differ between calcareous and acidic dunes. However, in calcareous dunes, almost all of total N release was used for net N mineralization, while in acidic dunes, a large part went into microbial growth.

Cycling of microbial P also differed between calcareous and acidic dunes. In fresh soil, microbial P was higher, and C:P ratios lower, in calcareous than in acidic dunes. In calcareous dunes, microbial C:P ratios showed relatively low values around 35 g/g in fresh soil, probably

due to the presence of AM fungi. In acidic dunes, microbial C:P ratios amounted to 77 g/g in fresh soil, which points to P limitation for the microorganisms in the presence of plant roots. However, these patterns were converted during the incubation period. In calcareous dunes, microbial P only slightly increased during incubation, perhaps because AM fungi were no longer supported by plant roots. As a result, average microbial C:P ratios in calcareous dunes increased from 35 to 65 g/g. In acidic dunes, microbial P uptake during incubation was significantly higher than in calcareous dunes, and average microbial C:P ratios decreased from 77 to 31 g/g. Total microbial P release over the incubation period was also higher in acidic than in
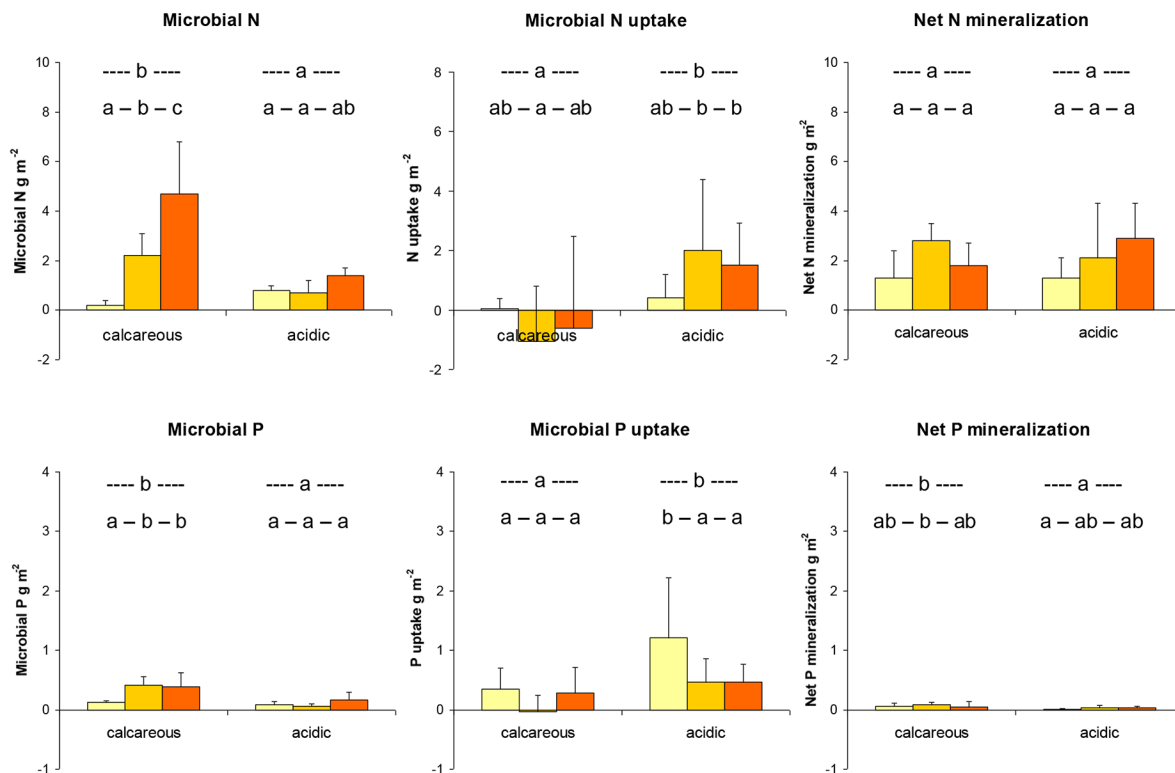
Fig. 8. Characteristics of microbial cycling of N and P in coastal dune grasslands in calcareous and acidic dunes with different soil organic matter (SOM) in the Netherlands. Light yellow bars are low SOM; yellow bars, intermediate SOM; and orange bars, high SOM. Values given are means (n = 5) and standard deviations. Different letters in the upper row indicate significant differences for a particular parameter between calcareous and acidic dunes ($P < 0.05$). Different letters in the lower row indicate significant differences for a particular parameter between all six soil types.

calcareous dunes, which points to higher decomposition of organic P. Most of the P was used for microbial uptake rather than net P mineralization, to relieve the P stress inherited in the presence of plant roots.

P nutrition and plant strategies

In the correlation-based network analysis, the actual amount of SOM content showed positive correlations with total P, organic P, sorbed P, and organic Fe, and a negative one with inorganic Fe (Fig. 9). However, pH had much stronger effects on P nutrition and showed significant positive and negative correlations with two groups of variables. Positive correlations with pH were found for the high-pH group, consisting of total P, inorganic P, minimum estimates of solid inorganic P, and inorganic Fe. These parameters also mostly positively correlated which each other

(data not shown). Negative correlations with pH were found for the low-pH group, consisting of organic Fe, minimum estimates of sorbed organic P, Mehlich-extractable P, and colloidal P. These parameters also mostly positively correlated with each other.

The variables in the high-pH group in turn positively correlated with the number of AM plant species and the relative cover of AM plants. However, AM plants showed negative correlations with the low-pH group, as well as with total microbial P release during the incubation experiment. In a stepwise multiple linear regression, 82% of the variance in the number of AM plant species was explained by inorganic P, with positive effects on AM plants, and by total microbial P release, with negative effects (Table 3). For the relative cover of AM plants, colloidal P and inorganic P explained
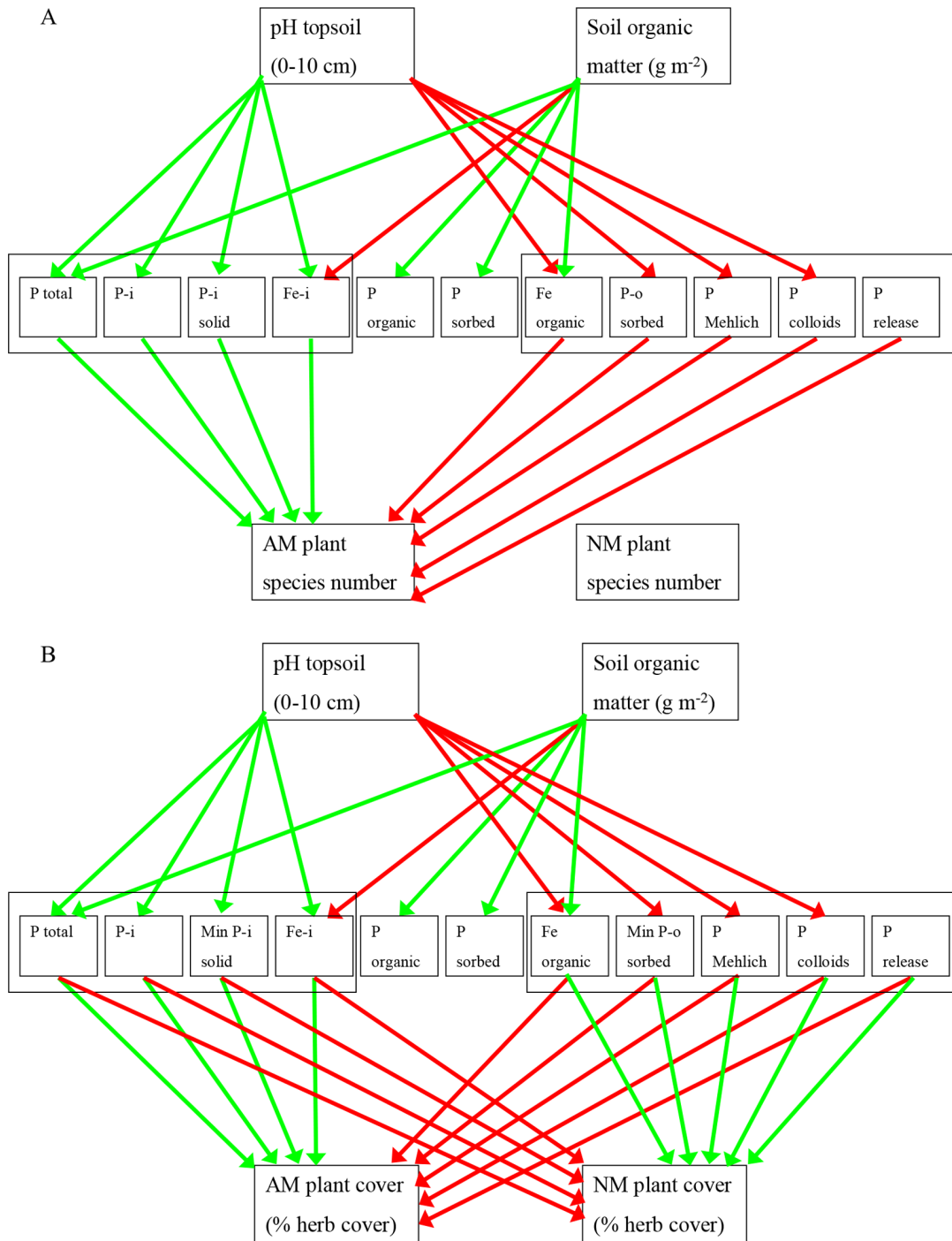
Fig. 9. Correlation-based network analysis of different variables related to P nutrition and arbuscular mycor-rhizal (AM) and nonmycorrhizal (NM) plant strategies. Only significant correlations are indicated ($P < 0.05$). (A) Positive (green) and negative (red) correlations with pH, soil organic matter content (SOM), variables related to P nutrition, and number of AM and NM plant species. (B) Positive (green) and negative (red) correlations with pH, SOM SOM, variables related to P nutrition, and the relative cover of AM and NM plant species, as percentage of total vascular plant cover.

70% of the variance together, with negative effects on AM plants for the first and positive effects for the latter.

The number of NM species was not significantly correlated with any variable related to P nutrition. In a stepwise multiple linear regression, Mehlich-extractable P, with positive effects on NM plant species, only explained 24% of the variance. However, the relative cover of NM plants showed significant positive correlations with the variables of the low-pH group and with total microbial P release and negative ones with the high-pH group. As for the relative cover of AM plants, colloidal P and inorganic P explained 70% of the variance together, but with positive effects on NM plants for the first and negative effects for the latter.

Soil community composition

Soil organic matter and pH were both important to soil communities (Appendix S1: Table S8). In accord with expectations, bacteria and fungi significantly increased from low to higher SOM (Fig. 10). Fungi were also more abundant in acidic than in calcareous dunes, in both PLFA analyses and counts. In addition, melanized hyphae, which generally develop under stressed conditions, were more abundant in calcareous than in acidic dunes, and showed values of 31–51% of total fungal biomass in the first, but only 6–12% in the latter.

Bacterial community composition differed between dune zones, with more Gram-negative bacteria in calcareous dunes and more Gram-positive bacteria in acidic dunes. However, contrary to expectations, total bacterial biomass was not higher in calcareous than in acidic dunes, but

did not differ between dune zones. Bacterial growth rates, measured as incorporation of labeled thymidine, were even higher in acidic than in calcareous dunes. As a result, differences in F:B ratios were lower than expected. Fungal: bacterial ratios were significantly higher in acidic than calcareous dunes, but actually only in acidic dunes with low SOM. In acidic dunes with higher SOM, F:B ratios did not differ from those in calcareous dunes.

Many groups of soil animals significantly increased in abundance from low to higher SOM, such as herbivores, fungivores, omnivores, and predators (Fig. 11). However, bacterivores, which consisted of protists such as amoeba and especially flagellates, were not influenced by SOM. Bacterial feeders were significantly more abundant in acidic than in calcareous dunes, in accord with the higher bacterial growth rates. In contrast, fungivores were more abundant in calcareous than in acidic dunes, despite the lower amount of fungi. This was especially true for fungivorous browsers, which were the dominant fungivores in all soil types.

Soil community composition and plant strategies

In the correlation-based network analysis, the actual amount of SOM had positive effects on almost all functional groups of soil organisms, except bacterivores (Fig. 12). Bacteria, fungi, fungivores, herbivores, omnivores, and predators showed significant positive correlations with this factor. Correlations with actual pH values were only negative and significant for the amount of fungi, bacteria, and omnivores. The cover of AM plants was positively correlated with SOM content, and in turn with

Table 3. Stepwise multiple linear regression of the relationships between P nutrition and AM and NM plants, with eleven variables related to P nutrition as independent variables.

Response factor	Independent factor	Partial R^2	F	Total R^2
AM plant species	Inorganic P	0.56	13.76**	
	Total microbial P release	0.26	13.77**	0.82
NM plant species	Mehlich-extractable P	0.24	3.40	0.24
AM or NM cover†	Colloidal P	0.50	11.19**	
	Inorganic P	0.20	6.66*	
	Minimum estimate sorbed organic P	0.07	2.85*	0.77

Notes: AM, arbuscular mycorrhizal; NM, nonmycorrhizal. Only significant contributions are given.
*$P < 0.05$; **$P < 0.01$.
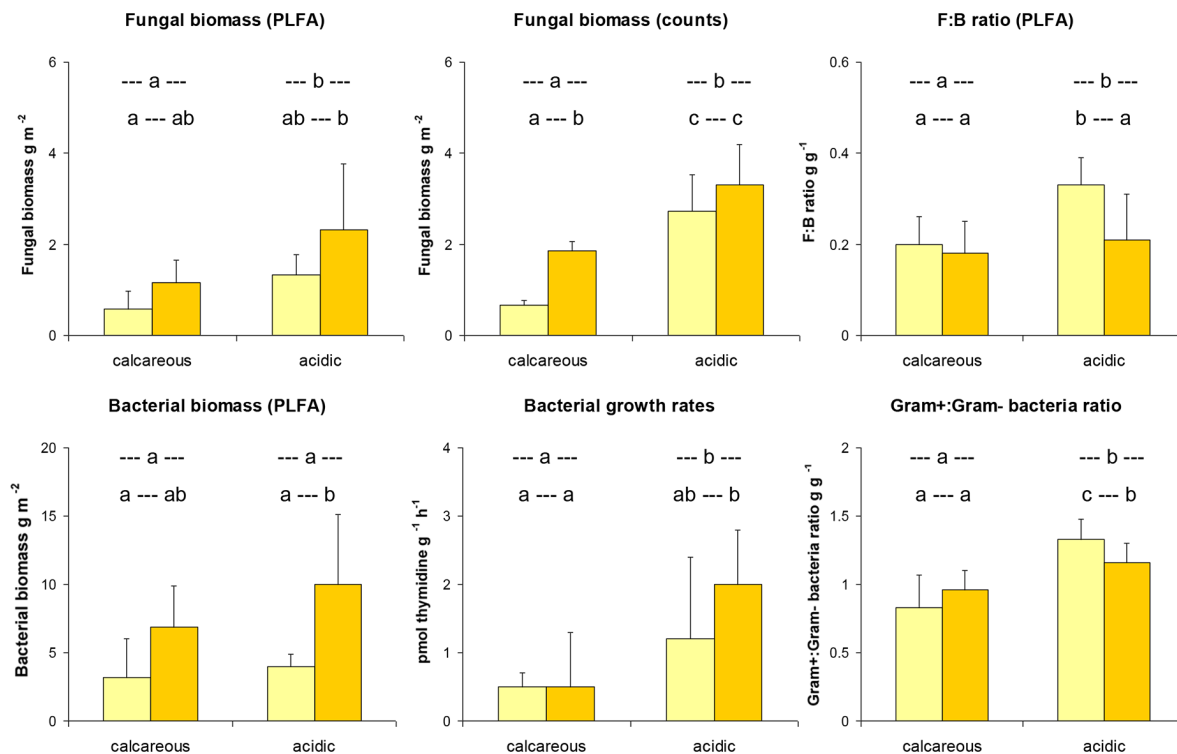† Percentage of herb layer.

Fig. 10. Characteristics of bacteria and fungi in coastal dune grasslands in calcareous and acidic dunes with different soil organic matter (SOM) in the Netherlands. Light yellow bars are low SOM; yellow bars, higher SOM. Values given are means ($n = 5$) and standard deviations. Different letters in the upper row indicate significant differences for a particular parameter between calcareous and acidic dunes ($P < 0.05$). Different letters in the lower row indicate significant differences for a particular parameter between all four soil types.

fungivores and predators. The cover of NM plants was negatively influenced by pH, but NM plants in turn positively correlated with bacteria, fungi, and omnivores. Most functional groups except bacterivores correlated with other groups, and only in a positive way. Bacteria correlated with fungi, and both with omnivores, which included some herbofungivores. Fungivores and herbivores correlated with omnivores and predators. Omnivores showed correlations with all other groups except bacterivores, and predators with fungivores, herbivores, and omnivores.

The soil community thus correlated with SOM content and pH directly, but also indirectly via the cover of AM and NM plants. In the stepwise multiple linear regression, for most functional groups, the four factors together explained 61–66% of the variance (Table 4). However, for fungi, bacterivores, and herbivores, only 29–40% of the variance was explained. Soil pH was the most important factor for fungi and bacterivores, which both showed higher values at low pH. Soil organic matter content was most important for bacteria, herbivores, and predators, and on a secondary level for omnivores, which all increased with higher SOM. The cover of AM plants was most important for fungivores, which increased with AM plants, and on a secondary level for bacteria, which decreased with AM plants. The cover of NM plants was most important for omnivores, and on a secondary level for bacteria, predators, and fungivores. Bacteria, omnivores, and predators increased with NM plants, but fungivores decreased with NM plants.

Relationships between functional soil groups and cycling of C and N were less consistent. Respiration positively correlated with fungi and bacterivores, which together explained 41% of the
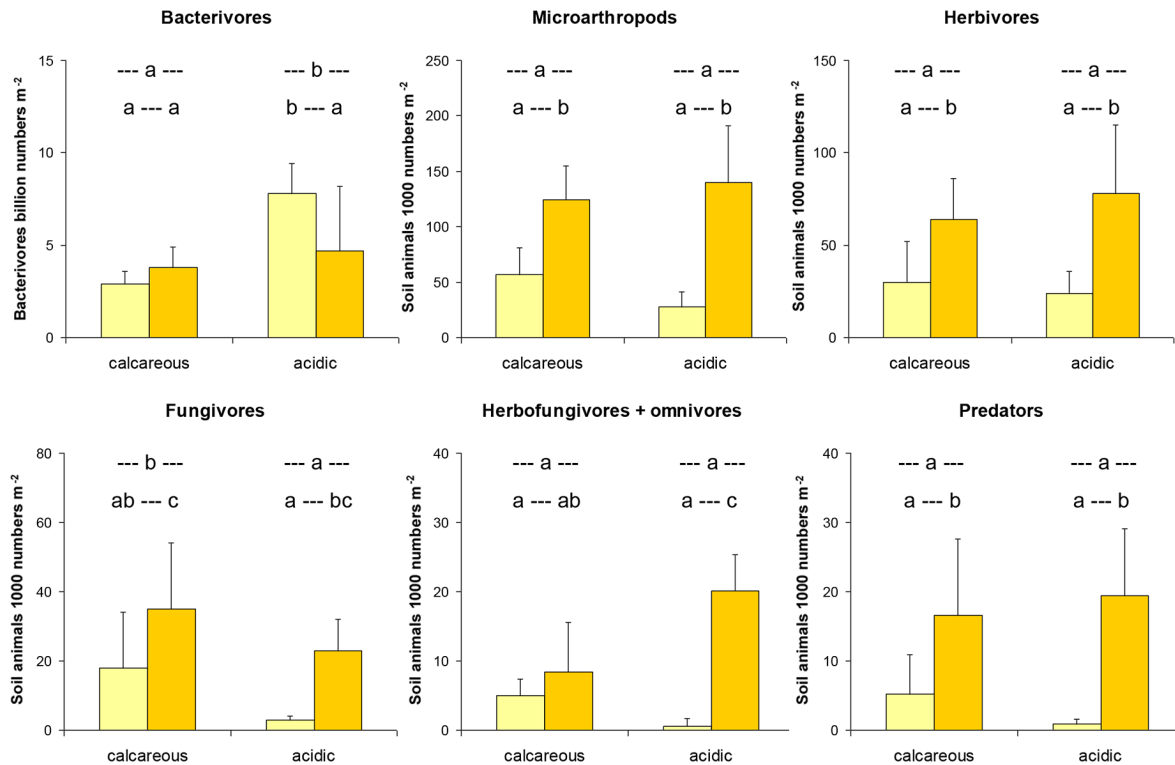
Fig. 11. Functional groups in the soil community in coastal dune grasslands in calcareous and acidic dunes with different soil organic matter (SOM) in the Netherlands. Light yellow bars are low SOM; yellow bars, higher SOM. Values given are means ($n = 5$) and standard deviations. Different letters in the upper row indicate significant differences for a particular parameter between calcareous and acidic dunes ($P < 0.05$). Different letters in the lower row indicate significant differences for a particular parameter between all four soil types.

variance. Net N mineralization only showed a positive relationship with bacterivores, which explained 25% of the variance.

In the analysis of causal relationships between soil communities and pH, significant cause and effect relationships were found for pH with the relative abundance of AM plants, and with bacterial growth rates, which in turn influenced abundance of amoeba (Fig. 13, Table 5). *D*-separation tests revealed that high pH stimulated the relative abundance of AM plants and fungi and thus increased the relative importance of the fungal channel, but reduced bacterial growth rates, and importance of the bacterial channel. Low pH did the opposite and reduced abundance of AM plants and fungi, but increased importance of the bacterial channel. Higher SOM mainly fed the bacterial channel through higher bacterial growth rates, which in turn led to higher rates of

net N mineralization. The relative abundance of active fungi was, however, negatively influenced by high SOM.

Microarthropod diversity

Microarthropod diversity was influenced by SOM rather than pH (Table 6). The number of taxa increased especially from low to higher SOM, and many taxa were only found at higher SOM, more than half of them being predators. Most taxa were found in both calcareous and acidic dunes. However, taxa such as the herbofungivore *Scutovertex minutus*, the fungivore *Paratullbergia callipygos,* and predators such as *Anystidae* and *Hypoaspis* were only found in calcareous dunes.

Distribution patterns of microarthropods and their relation to vascular and cryptogam plant species were further studied in a co-occurrence
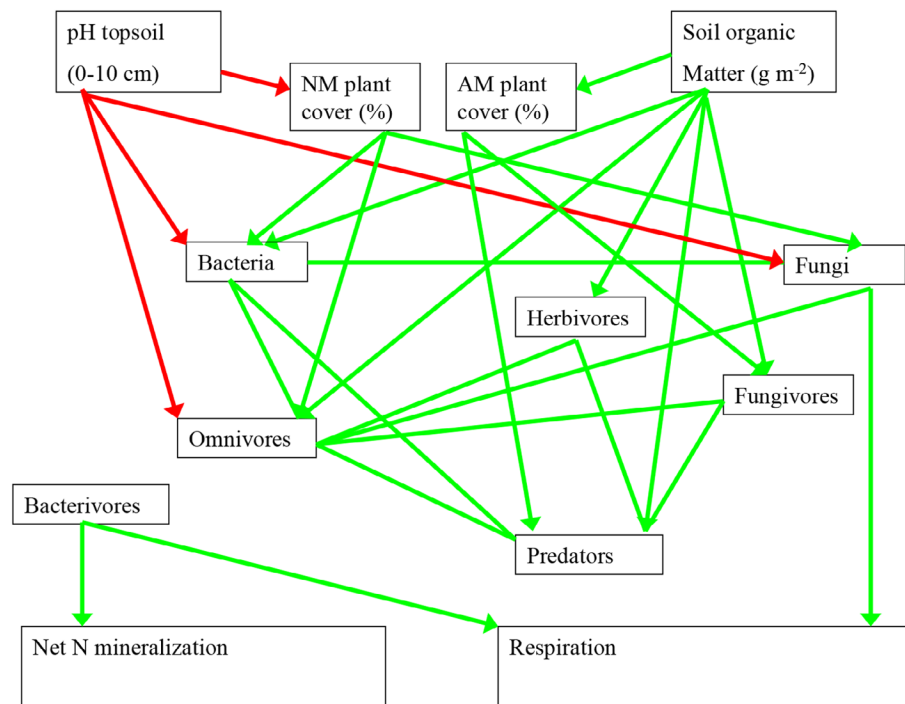
Fig. 12. Correlation-based network analysis of soil community characteristics and arbuscular mycorrhizal (AM) and nonmycorrhizal (NM) plant cover. Only significant correlations are indicated ($P < 0.05$). Positive correlations are given in green and negative correlations in red.

network analysis. In the network analysis, plant species and microarthropod taxa were divided into eight network clusters (Fig. 14). Clusters 1, 2, and 3 contained plants and animals of calcareous dunes, and clusters 6 and 7 animals and/or plants of acidic dunes. Clusters 4 and 5 contained only animal taxa, including herbivores, fungivores, and predators. Cluster 8 had no connection to particular dune areas and consisted of a few common animal taxa.

The four dune areas clearly differed in network density and importance of these clusters. In both calcareous and acidic dunes, dense and complex networks were mainly found at higher SOM. Connectance ranged around 8% of all possible connections in most dune types, but was 5% in acidic dunes with low SOM.

Calcareous dunes with low SOM had a relatively small network, consisting of 39 nodes and 55 connections. They mainly belonged to cluster 1 with pioneer plants, cluster 2 with AM plants + fungivorous browsers, and fragments of

the animal clusters 4 and 5. Calcareous dunes with higher SOM had denser and more complex networks, with 50 nodes and 95 connections. The network consisted of four main clusters, such as cluster 2 with AM plants + fungivorous browsers, cluster 3 with older dune plants + predators, and the animal clusters 4 and 5.

In acidic dunes, the number of nodes was slightly higher than in calcareous dunes, but the number of connections was lower. Acidic dunes with low SOM had very small and partly isolated networks, with only 29 nodes and 18 connections. Only cluster 7 with acidic pioneer plants was important, and a small network existed of fragments of the animal clusters 4 and 5, and cluster 6 with acidic plants + fungivorous browsers. Acidic dunes with high SOM, however, had a dense and complex network with 42 nodes and 71 connections in three main clusters, consisting of the animal clusters 4 and 5, and cluster 6 with acidic plants and animals.

Table 4. Stepwise multiple linear regression of the relationships between different characteristics of the soil community, with pH, soil organic matter content, and AM and NM plant cover as independent variables.

Response factor	Independent factor	Partial R^2	F	Total R^2
Fungi based on PLFA (g/m^2)	pH value	0.40	11.89**	0.40
Fungivores (no./m^2)	AM plant cover	0.56	23.34***	
	NM plant cover	0.09	4.65*	0.66
Bacteria based on PLFA (g/m^2)	Soil OM content	0.33	11.62**	
	NM plant cover	0.14	7.68*	
	AM plant cover	0.13	4.39*	0.61
Bacterivores (no./m^2)	pH value	0.17	3.59	
	NM plant cover	0.17	4.40	0.34
Herbivores (no./m^2)	Soil OM content	0.29	7.21*	0.29
Omnivores (no./m^2)	NM plant cover	0.48	16.71***	
	Soil OM content	0.18	9.22**	0.66
Predators (no./m^2)	Soil OM content	0.50	18.12***	
	NM plant cover	0.11	5.16*	0.64
Respiration (g/m^2)	Fungi	0.22	4.08*	
	Bacterivores	0.19	5.66*	0.41
Net N mineralization (g/m^2)	Bacterivores	0.25	6.01*	0.25

Notes: AM, arbuscular mycorrhizal; NM, nonmycorrhizal; PLFA, phospholipid-derived fatty acid. Only significant contributions are given.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Principal component analysis

Mutual relationships between plant strategies, P nutrition, microbial nutrient cycling, and soil communities were further tested in principal component analyses (PCAs). As the sampling sites and measured parameters differed between the P nutrition and soil community studies, PCA was conducted for each study separately. For both PCAs, variables directly related to pH and SOM were included as "environmental" variables, to independently test the importance of each factor. In both analyses, pH and SOM strongly correlated with the first or second PCA axis, but not with the other. In the P nutrition study, the first axis was explained by pH, with R-values of 0.90–0.95 for pH class and the actual pH values, and the second axis by SOM, with R-values of 0.83–0.94 for SOM class and the actual amounts of SOM. In the soil community study, the first axis was explained by SOM, with R-values of 0.75–0.86, and the second by pH, with R-values of 0.70–0.91. Together, the two axes explained 83% of the cumulative variance of the species–environment relationship in the P nutrition study and 90% in the soil community study.

Many of the variables related to vegetation, P nutrition, and soil community correlated with either SOM or pH, in both positive and negative ways (Fig. 15). High SOM was mainly related to biomass of plants, microorganisms, and soil animals. High correlations with high SOM were thus found for vascular plant cover, aboveground vascular plant biomass, bacteria, and most groups of microarthropods. Total N, organic P, and organic Fe in the soil were also associated with high SOM. In contrast, only few variables were characteristic for low SOM, such as the cover of bare sand, and the relative amount of inorganic P.

High and low pH mainly correlated with plant and animal strategies and different forms of P and Fe. The number of vascular plant species, AM plant species, relative AM plant cover, microbial P, and fungivorous browsers all increased with high pH. High values for total P, inorganic P, inorganic Fe, and the N:P ratio of the NM plant *C. arenaria* were characteristic for high pH as well. In contrast, low pH was characterized by high relative number and cover of NM plants. This was associated with variables related to high P availability, such as high relative amount of sorbed P, organic Fe and humic acids, high minimum estimates of sorbed organic P and colloidal P. Microbial C:N and C:P ratio were also higher at low pH, as was the decrease in microbial C:P ratio during incubation. In the soil community, both fungi (based on counts) and flagellates were characteristic for low pH.
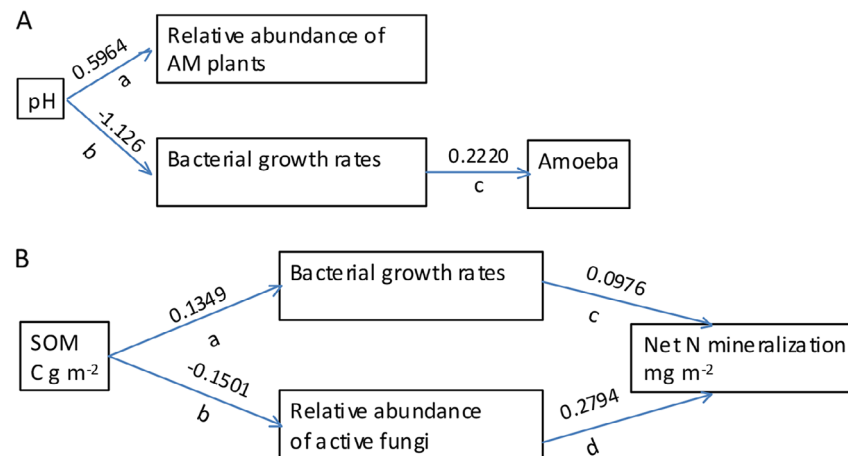
Fig. 13. *D*-separation tests conducted as a form of piecewise structural equation modeling. Model A tested whether pH could be a causal factor to the relative abundance of arbuscular mycorrhizal (AM) plants (a) and bacterial growth rates (b), measured as incorporation of thymidine (pmol·g^{-1}·h^{-1}), which in turn could affect numbers of amoeba in the soil (c). Model B tested whether soil organic matter (SOM) could be a causal factor for bacterial growth rates (a) and the relative abundance of active fungi (b), which both in turn could affect net N mineralization (c and d). Both models were valid as cause and effect model (model A: Fisher's *C* test: 4.490854, df: 4, *P*: 0.34633; model B: Fisher's *C* test: 2.938, df: 4, *P*: 0.568).

Table 5. Characteristics of the models used in the *d*-separation tests described in Fig. 14.

Model	Estimate	Adjusted R^2	F	df	P
Model A					
a	0.5964	0.1008	3.13	18	0.0938
b	−1.126	0.3326	10.47	18	0.0045
c	0.2220	−0.04195	0.235	18	0.6337
Model B					
a	0.1349	−0.02316	0.57	18	0.46
b	−0.15011	0.06958	2.421	18	0.1371
c	0.09759	−0.08418	0.2624	17	0.7723
d	0.27949	−0.08418	0.2624	17	0.7723

Notes: Model A tested whether pH could be a causal factor to the relative abundance of AM plants (a) and bacterial growth rates (b), measured as incorporation of thymidine (pmol·g^{-1}·h^{-1}), which in turn could affect numbers of amoeba in the soil (c). Model B tested whether soil organic matter could be a causal factor for bacterial growth rates (a) and the relative abundance of active fungi (b). In turn, net N mineralization could be affected by bacterial growth rates (c) and active fungi (d). Both models A and B were significant as cause and effect model (model A: Fisher's *C* test: 4.490854, df's: 4, *P*: 0.34633; model B: Fisher's *C* test: 2.938, df's: 4, *P*: 0.568). In model A, individual regressions were significant for pH and bacterial growth rates ($P < 0.05$). In model B, the individual regressions were not significant.

Discussion

The aim of this study was to disentangle the effects of SOM and pH on vegetation, soil nutrients, and soil communities in Grey dunes, in order to better explain differences in resilience to high N deposition between calcareous and acidic dunes. The results were surprising and showed that SOM mainly influenced biomass parameters, but that pH led to fundamental differences in P nutrition and plant mycorrhizal strategies, which in turn overruled the normal patterns in soil community composition and N cycling.

SOM and plant biomass

For the vegetation, SOM was especially important to vascular plant cover and aboveground biomass. Although vascular plant biomass may increase SOM in the form of litter input, it may also respond to SOM due to availability of nutrients. Although net mineralization of N and P was not influenced by SOM directly, organic P and sorbed P clearly increased with SOM. High SOM may also lead to higher water-holding capacity, which is important in an area where temperatures at the soil surface on sunny days may increase to 60°C (Stoutjesdijk and Barkman 1992). In the study area, volumetric soil moisture content at field capacity was approximately 5% in dune soils with low SOM but increased to 25% in soils with high SOM (Y. Fuyita, *unpublished data*).

Table 6. Distribution of microarthropod taxa in soils of different dune areas.

Microarthropod taxon	Feeding guild	C1	C2	A1	A2
Microtydeus	HB	5	5	5	5
Nanorchestes	HG	5	5	3	5
Tydeidae	HB	4	5	5	5
Paratydeidae	FB	4	3	5	4
Stigmaeidae	PA	3	3	3	5
Eupodes	OO	2	2	1	4
Leioseius bicolor	PG	2	2	1	2
Xenylla	FB	1	5	5	4
Tectocepheus velatus	OHF	1	5	3	5
Bdella	PA	1	4	5	5
Pygmephorus	FB	1	…	1	3
Scutovertex minutus	OHF	4	…	…	…
Paratullbergia callipygos	FB	4	4	…	…
Anystidae	PA	2	1	…	…
Hypoaspis	PG	1	2	…	…
Tyrophagus	FB	5	5	3	…
Tetranychidae	HB	…	3	…	…
Trhypochthonius tectorum	HB	…	5	1	3
Peloptulus phaenotus	FG	…	3	1	3
Speleorchestes	FG	…	2	2	1
Scutacarus	FB	…	…	…	5
Cryptognathidae	PG	2	…	4	…
Pseudocheylidae	PG	2	…	3	…
Tarsonemus	HB	1	3	…	4
Cunaxidae	PA	1	3	…	3
Brachychthoniidae	FG	1	2	…	5
Liochthonius lapponicus	FG	1	2	…	2
Amblyseius	PA	…	4	…	1
Zercon triangularis	PG	…	3	…	1
Microppia minus	FG	…	3	…	3
Erythraeidae	PA	…	2	…	2
Entomobrya nivalis	OHF	…	1	…	4
Asca bicornis	PG	…	1	…	4
Isotoma	FB	…	1	…	3
Hypoaspis aculeifer	PG	…	1	…	3
Rhagidia	PA	…	1	…	3
Oppiella nova	FG	…	1	…	2
Pseudisotoma sensibilis	FB	…	1	…	2

Notes: C1, calcareous soils with low soil organic matter (SOM); C2, calcareous soils with high SOM; A1, acidic soils with low SOM; A2, acidic soils with high SOM; HB, herbivorous browser; HG, herbivorous grazer; FB, fungivorous browser; FG, fungivorous grazer; PA, predator on arthropods; PG, general predator; OHF, herbofungivorous omnivore; OO, opportunistic omnivore. Numbers indicate the amount of times that a taxon was present in plots of a particular site ($n = 5$). Only taxa found in at least three plots are included.

pH and plant strategies

For plant species composition, pH was more important than SOM. The number of plant species was higher in calcareous than in acidic dunes, irrespective of SOM, in accord with the generally higher diversity for calcicole than calcifuge plant species in Europe, which better survived the last ice age (Ewald 2003). Moreover, calcareous dunes were dominated by AM plants, and acidic dunes by NM plants, as confirmed by our causal relation tests.

We did not measure actual root colonization with AM fungi, but for plants capable of forming AM, the NM condition is nearly always unnatural (Smith et al. 2011). For some species, the AM or NM status is not entirely clear. *Sedum acre* may be considered as NM because of its NM family (Olsson and Tyler 2004) but was clearly mycorrhizal in the Dutch coastal dunes (Ernst et al. 1984). Also, Cyperacaeae may be more mycorrhizal than previously considered (Muthukumar et al. 2004), but *C. arenaria* is still seen as a NM species (Drigo et al. 2007). This was supported by its aboveground P content in calcareous dunes, which was much lower than in the AM-dominated vegetation. In any case, even if some plant species would shift in AM or NM status, calcareous dunes would still be dominated by AM plants and acidic dunes by NM plants. This is supported by tree species in calcareous dunes such as *Crataegus monogyna* and *Euonymus europeus*, which are also AM plants.

Predominance of AM plants in calcareous dunes

Arbuscular mycorrhizal symbiosis occurs in 70–80% of vascular plant families (Brundett 2009, Smith and Smith 2011). Many AM plants and fungi are found in calcareous habitats, such as chalk grasslands and deciduous forests on limestone (Kovacs and Szigetvari 2002, Bååth and Anderson 2003). In Kooijman (2010), approximately 80% of the shrub and tree species in limestone forests consisted of AM plants. Arbuscular mycorrhizal plants may predominate in calcareous habitats, because they take up inorganic P such as calcium phosphates (Read and Perez-Moreno 2003, Jansa et al. 2011, Smith and Smith 2011). Primary and secondary calcium phosphates are abundant in calcareous parent materials and can be dissolved by local acidification in the root zone (Walker and Syers 1976, Marschner 1995, Hinsinger 2001).

In our study, inorganic P was indeed higher in calcareous than in acidic dunes. Also, in calcareous dunes, at least part of the inorganic P belonged to the soil solid phase, such as calcium
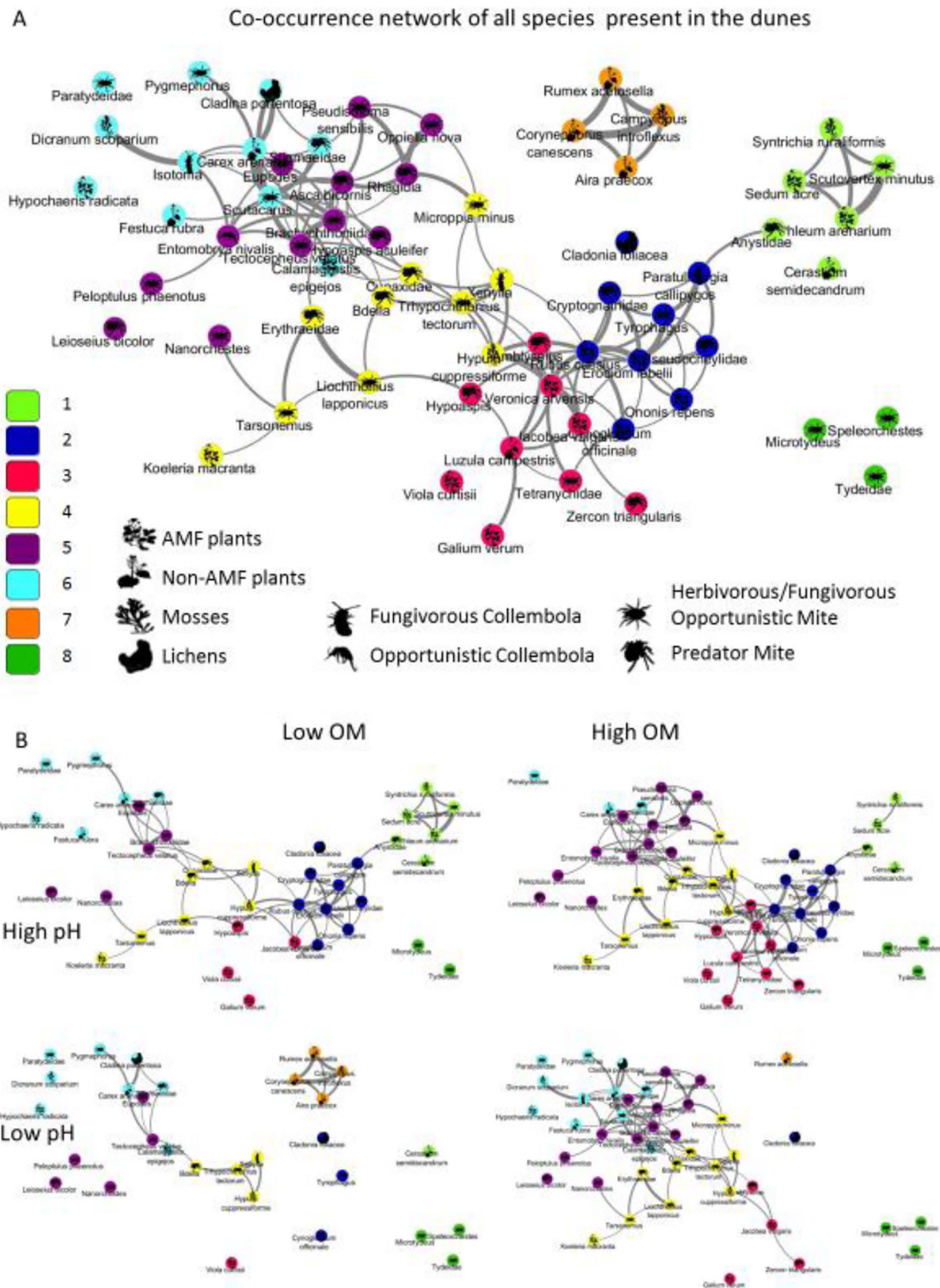
Fig. 14. Network analysis between plant species and microarthropod taxa in calcareous and acidic dunes with low and high soil organic matter (SOM). (A) Entire network based on all 20 plots. (B) Networks for particular dune areas, based on the taxa occurring there. The names and positions of the taxa in B are the same as in A.

Mainly correlated with high SOM	Mainly correlated with low SOM
Vascular plant cover AM plant cover Aboveground vascular plant biomass Total soil N Soil C:P ratio Organic P Relative amount organic P Organic Fe Microbial C Bacterial PLFA Number of microarthropod taxa Mites Herbivorous grazers Fungivorous grazers Herbofungivores General predators Predators on arthropods	Bare sand Relative amount inorganic P Colloidal Fe

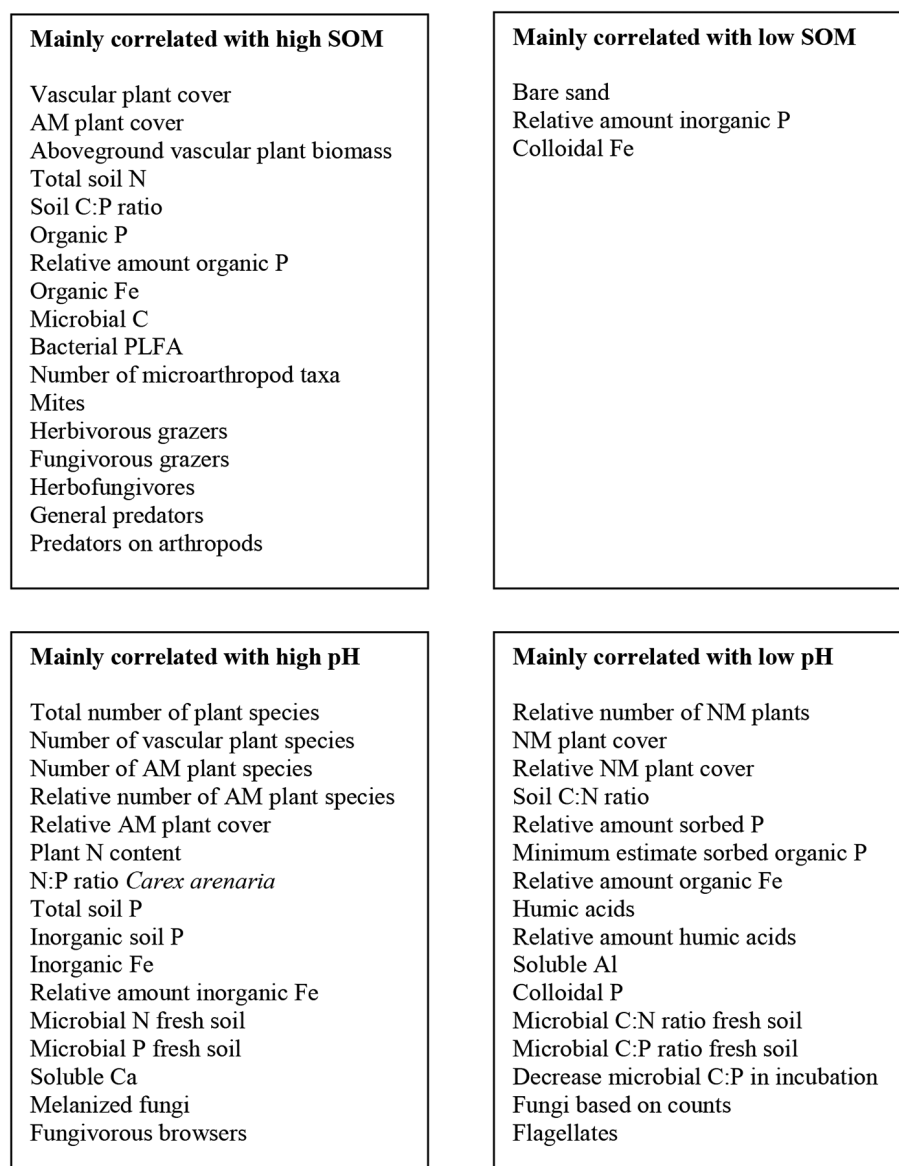| Mainly correlated with high pH	Mainly correlated with low pH
Total number of plant species Number of vascular plant species Number of AM plant species Relative number of AM plant species Relative AM plant cover Plant N content N:P ratio *Carex arenaria* Total soil P Inorganic soil P Inorganic Fe Relative amount inorganic Fe Microbial N fresh soil Microbial P fresh soil Soluble Ca Melanized fungi Fungivorous browsers	Relative number of NM plants NM plant cover Relative NM plant cover Soil C:N ratio Relative amount sorbed P Minimum estimate sorbed organic P Relative amount organic Fe Humic acids Relative amount humic acids Soluble Al Colloidal P Microbial C:N ratio fresh soil Microbial C:P ratio fresh soil Decrease microbial C:P in incubation Fungi based on counts Flagellates

Fig. 15. Principal component analyses (PCAs) of variables related to vegetation characteristics, P nutrition, and soil communities. Separate PCAs were conducted for the P nutrition and soil community study, but in both cases, pH and soil organic matter (SOM), tested as environmental variables, strongly correlated with the first or second axis. Variables with correlations (R) with the first or second axis higher than 0.6 or lower than −0.6 were grouped according to their preference for high or low SOM or pH.

phosphates. Arbuscular mycorrhizal fungi may have little direct access to calcium phosphates themselves, but the external mycelium may be associated with phosphate-solubilizing bacteria, which act as mycorrhizal helpers (Frey-Klett et al. 2007). The AM fungi have a special affinity for phosphate (Cardoso et al. 2017) and scavenge this from large volumes of soil (Smith et al. 2011). This was supported by the much higher microbial P content in calcareous than in acidic dunes, as well as the lower microbial C:P ratios. The P transfer by the symbiotic fungi can be

quite effective and accounts for as much as 70% of the P acquired by the plant (Wang et al. 2017). In our study, vascular plant P contents were indeed higher for the AM-dominated vegetation in calcareous dunes than for the NM-dominated vegetation in acidic dunes. Also, foliar N:P ratios indicated that P was not a limiting factor for AM plants (Olde Venterink et al. 2003).

However, AM benefits for the plant are often inversely related to P availability in the soil (Johnson et al. 1997, Smith and Read 2008, Hoeksema et al. 2010). The P uptake benefits for the host plant will outweigh the costs of carbon supply to the fungus mainly when P availability limits its plant growth. In calcareous dunes, P availability to the vegetation is low, due to low solubility of calcium phosphates at high pH (Lindsay and Moreno 1966, Hinsinger 2001). Calcium phosphates can be dissolved by local acidification in the root zone, but often reprecipitate again. Also, in our calcareous dunes, P sorption was probably relatively strong. Sorbed P was primarily sorbed to Fe oxides, which constitutes a relatively strong form of binding (Gerke 2010, Yan et al. 2016). In calcareous dunes, the amount of total and inorganic Fe even increased from low to high SOM, which points to actual formation of Fe oxides. Also, the dominant form of phosphate in calcareous soils is HPO_4^{2-} (Hinsinger 2001, Shen et al. 2011), which is more strongly bound than $H_2PO_4^-$. At high pH, the phosphate ion may even be bound to Fe oxides in bidentate and binuclear fashion (Kim et al. 2011, Fink et al. 2016). In contrast, more plant-available P fractions such as Mehlich-extractable P, colloidal P, and total microbial P release during incubation were lower than in acidic dunes.

Low P availability in calcareous dunes was supported by the P concentrations in the NM plant *C. arenaria*, which were significantly lower than in acidic dunes. Also, N:P ratios of *C. arenaria* were 22–25 g/g in calcareous dunes, which clearly point to P limitation (Olde Venterink et al. 2003). Predominance of AM plants in calcareous dunes may thus be explained by high amounts of inorganic P, their main P source, but especially by the low availability of P due to low solubility of calcium phosphates at high pH, strong P sorption to Fe oxides, and low amounts of plant-available P in general, which increases the mycorrhizal benefits.

Predominance of NM plants in acidic dunes

In our study, acidic dunes were dominated by NM plants. A shift from AM to NM plants in mineral soils with high and low pH was also noticed by Olssen and Tyler (2004). These authors found mainly AM plants on limestone rocks in southern Sweden, but predominance of NM plants on acidic rock types, which they attributed to differences in exchangeable soil phosphate.

Despite these findings, predominance of NM plants in acidic dunes was rather surprising. On a global scale, NM plants in dry habitats are especially known from highly weathered, acidic, Fe-rich, and P-impoverished soils, which use adaptations such as proteoid and cluster roots to increase P uptake (Lambers et al. 2008). Also, in the temperate climates of NW Europe, acidic soils in open habitats are usually occupied by ErM plants, such as *Calluna vulgaris* and *Empetrum nigrum* (Ellenberg et al. 1974). However, ErM fungi mainly use organic nutrient sources (Emmerton et al. 2001, Cairney and Meharg 2003) and may not establish when organic layers on top of the mineral soil have not yet developed. In the dunes of our study, organic layers were still absent, and the ErM plant *C. vulgaris* is only found in much older dunes, which formed several thousands instead of hundred years ago (Van Til and Mourik 1999).

Arbuscular mycorrhizal plants may be less common in acidic dunes, because arbuscular fungi are sensitive to low pH (Van Aarle et al. 2002). However, AM plants and fungi do occur in acidic soils (Kluber et al. 2012), especially in tropical climates (Cardoso et al. 2017). In tropical soils, inorganic P may be present in Fe or Al phosphates, but their availability is restricted due to low solubility at low pH (Lindsay and Moreno 1966, Hinsinger 2001). Also, in acidic tropical soils, phosphate ions may be strongly sorbed to Fe oxides and even become occluded (Walker and Syers 1976, Fink et al. 2016). Like in calcareous soils, inorganic P is present in acidic tropical soils, but its low availability may increase mycorrhizal benefits for AM plants (Hoeksema et al. 2010).

Less inorganic P in acidic dunes

Predominance of NM plants in acidic dunes may thus be due to pH-induced changes in P

availability rather than sensitivity of the AM fungi to acidic conditions. Calcium phosphates generally dissolve below pH 6.5 (Lindsay and Moreno 1966, Hinsinger 2001), which would reduce the amount of inorganic P in acidic soils, but also the mycorrhizal benefits for AM plants (Hoeksema et al. 2010). In acidic dunes, the amount of inorganic P was indeed lower than in calcareous dunes. Some AM plants may persist in acidic dunes, such as *Calamagrostis epigejos*, but this is a fast-growing species with high relative growth rates, which usually have low rates of mycorrhizal infection (Koziol and Bever 2015).

More weakly sorbed P in acidic dunes

While inorganic P decreased from calcareous to acidic dunes, sorbed P became more important. This favors NM plants, which have different strategies for P uptake than AM plants (Lambers et al. 2008, Richardson et al. 2009, Raven et al. 2018). Nonmycorrhizal plants exudate large amounts of carboxylates, small organic molecules, such as citrate and oxalate, which release sorbed P by ligand exchange. This is a highly effective mechanism (Gerke et al. 2000, Johnson and Loeppert 2006, Gerke 2015), which can also release sorbed P in acidic dunes.

In our study, the proportion of sorbed P increased from 29% of total P in calcareous dunes to 44% in acidic dunes. Also, the amount of weakly sorbed Mehlich-extractable P increased from calcareous to acidic dunes, which means that sorbed P became more weakly bound. This was partly due to the shift in the dominant form of phosphate from HPO_4^{2-} to $H_2PO_4^-$ (Hinsinger 2001, Shen et al. 2011). However, the shift from strong P sorption to Fe oxides in calcareous dunes to weak sorption to Fe-OM complexes in acidic dunes played a role as well (Kooijman et al. 2009, Gerke 2010, Yan et al. 2016), especially because Fe is generally more important to P sorption than Al or calcite (Hamad et al. 1992, Shang et al. 1992). Organic acids may block or eliminate part of the P sorption sites (Daly et al. 2001), and with $H_2PO_4^-$ and Fe-OM complexes, phosphate ions can only be bound in monodentate fashion (Kim et al. 2011, Fink et al. 2016).

Shift from Fe oxides to Fe-OM complexes with pH

Although Fe-bound P is usually measured in P fractionation methods, differences in the form of

amorphous Fe and the implications for P binding are often ignored (Gerke 2010). The shift from Fe oxides to Fe-OM complexes with pH becomes especially clear when other Fe-rich dunes in the Netherlands are included (Kooijman et al. 1998, 2009). The compiled dataset consisted of more than 120 dune plots and showed an R^2 value of 0.76 for the relationship between pH and the proportion of organic Fe (Fig. 16). The proportion of organic Fe increased from close to zero at pH 8, to approximately 100% at pH 4, irrespective of SOM. In Kooijman et al. (2009), plant-available P increased when the proportion of organic Fe was higher than 50% of total amorphous Fe, which is below pH 5.5 according to this figure.

The shift from Fe oxides to Fe-OM complexes is probably caused by higher sorption of organic molecules to Fe oxides at low pH. In Gu et al. (1994), sorption of organic molecules to Fe oxides was much higher at pH 4 than at pH 7. At low pH, the hydroxyl groups of the Fe oxides become protonated, which attracts organic anions, which then replace the protonated hydroxyl groups, and transform Fe oxides into Fe-OM complexes (Fink et al. 2016). The shift from Fe oxides to Fe-
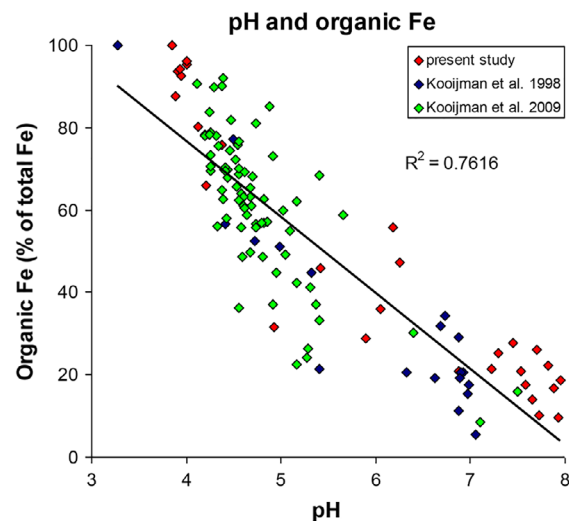

Fig. 16. Relationship between pH and organic Fe in calcareous and acidic coastal dunes, given as percentage of total amorphous Fe. Red dots represent values from the present study, and blue and green dots from earlier studies in Fe-rich coastal dunes (Kooijman et al. 1998, 2009). The correlation between pH and the percentage of organic Fe was significant ($P < 0.05$).

OM complexes from high to low pH may even explain why the total amount of sorbed P did not differ between dune zones, even though sorption became weaker. P sorption capacity may be higher for Fe oxides than for Fe-OM complexes, but also increases from high to low pH (Yan et al. 2016). In the latter study, the total amount of P sorbed to goethite at pH 7 did not differ from P sorbed to complexes of goethite and humic acids at pH 3.

Predominance of Fe-OM complexes over Fe oxides at low pH may especially occur in temperate climates, where decomposition of organic matter and weathering of primary minerals is retarded due to the relatively low temperatures (Blume et al. 2016).

Fe-OM complexes may also be common in organic soils such as peatlands (Kooijman et al. 2019). However, formation of Fe-OM complexes may be restricted in tropical soils with high Fe and low organic matter content. In the tropical soils of Thaymuang et al. (2013), poorly crystalline iron was approximately six times higher than in our temperate zone dune soils, which are considered Fe-rich (Eisma 1968, Kooijman et al. 1998). However, organic C contents in the tropical soils were approximately seven times lower. This may restrict formation of humic Fe-OM complexes and stimulate strong P binding to Fe oxides, which may explain why many acidic tropical soils are dominated by AM rather than NM plants (Cardoso et al. 2017).

Part of the weakly sorbed P in acidic dunes is organic

In acidic dunes, at least part of the sorbed P consisted of organic P, which accounted for at least 24–26% of total organic P or 16–19% of total P. This is important for NM plants, which do not use organic P directly, but can release the phosphate ion from labile organic P by exudation of phosphatase enzymes (Richardson et al. 2009, Güsewell 2017, Raven et al. 2018). In calcareous dunes, part of the sorbed P may also have been organic, but this could not be determined. In any case, this fraction would probably be more strongly sorbed than in acidic dunes, due to the high amount of Fe oxides.

We did not measure different forms of organic P, but most of it probably consists of inositol

hexakiphosphate, or IHP (Turner et al. 2007). This is a principal storage form of P in vegetation and seeds, and consists of cyclic acids, saturated with up to six phosphate groups. With so many phosphate groups, IHP is generally strongly bound to the soil solid phase (McKercher and Anderson 1989, Celi et al. 2003) and may be incorporated in high molecular humic substances or in complexes with Ca, Fe, and Al (Shang et al. 1992, Celi et al. 2003, Prietzel et al. 2016). The stronger affinity of IHP for Al than for Fe (Shang et al. 1992) may even explain why most Al was organic, even in calcareous dunes.

However, IHP is not a suitable P source for NM plants (Pérez Corona et al. 1996, Güsewell 2017). In Güsewell (2017), *Carex* spp. showed high exudation of phosphatases for labile organic P and grew well on this P source, but not at all on the more complex IHP. Labile P is probably present in the sorbed fraction, as this is less strongly bound than IHP (McKercher and Anderson 1989). Also, labile organic P is bound preferentially to Fe rather than Al (Shang et al. 1992), which means that labile organic P is weakly bound in acidic dunes, due to predominance of Fe-OM complexes (Gerke 2010, Fink et al. 2016). As already indicated, this is especially useful for NM plants, which not only exudate carboxylates to release weakly sorbed P (Lambers et al. 2008, Raven et al. 2018), but also produce enzymes to disintegrate labile organic P (Güsewell 2017).

In our study, NM plants indeed showed efficient P uptake in acidic dunes. The NM plant *C. arenaria* was clearly P-limited in calcareous dunes, but its N:P ratios decreased from 22–25 to 15–16 g/g in acidic dunes, which indicates that P was not a limiting factor any longer (Olde Venterink et al. 2003). The NM-dominated vascular plant vegetation even showed N:P ratios of 12–13 g/g, which point to N limitation and excess P. Efficient P uptake by NM plants in acidic dunes was supported by the microbial community, which was P-limited in the presence of plant roots. The microorganisms showed high total P release during the incubation experiment, probably due to high availability of weakly sorbed labile organic P. However, in fresh soil, in the presence of plant roots, microbial N:P ratios were much higher than the critical value of 3.1 g/g (Cleveland and Liptzin

2007). Microbial C:P ratios were also high, and the average values of 77 g/g of the present study were comparable to the 69 g/g for fresh acidic dune soil found in earlier research (Kooijman et al. 2009).

More colloidal P in acidic dunes

Colloidal P has mainly been studied in stream waters, because it largely contributes to P transport (Bol et al. 2016), and soils have received less attention (Missong et al. 2017). However, in contrast to less mobile forms of P, colloidal P with size below 0.5 μm can travel with the water flow. Fine colloids consist for a large part of organic matter (Missong et al. 2018) and can be seen as small Fe-Al-OM complexes, to which P is weakly sorbed. It is thus not surprising that colloidal P, as well as the total amount of colloids, was significantly higher in acidic than in calcareous dunes. Also, like weakly sorbed P, colloidal P consisted partly of organic P (A. Missong, *unpublished data*), which can be disintegrated by the phosphatase enzymes exudated by NM plants. The increase in colloidal P thus further contributes to high P availability in acidic dunes and was the major explanatory factor for the relative cover of NM plants.

Changes in soil communities with SOM

The soil community was influenced by SOM more than by pH. Most microbial and soil animal groups increased with SOM, in accord with general changes in soil communities during succession (Kaufmann 2001, Frouz et al. 2008, Morriën et al. 2017). Soil organic matter may be important to the soil community because it increases water-holding capacity, and many soil organisms are sensitive to drought stress (Verhoef and van Selm 1983, Xu et al. 2012). Drought stress was supported by the high proportion of drought-tolerant Gram-positive bacteria, which was much higher than in alpine grasslands with a wetter climate (Fuchslueger et al. 2014).

However, SOM may be especially important for soil communities because it drives the food chain. For bacteria and fungi, SOM is a major food resource, which in turn stimulates bacterial and fungal feeders, and then omnivores and predators. The *d*-separation tests even suggest that SOM feeds the bacterial channel more than the fungal channel. Herbivores probably mainly

profit from SOM through the increase in plant biomass. Network diversity and complexity also increased with SOM, in accordance with Morriën et al. (2017). Also, animal–animal interactions became more important at higher SOM and the network clusters with only animals more obvious. These findings suggest that animal diversity in the soil is mainly driven by food supply.

Changes in soil communities with pH

Although SOM was more important, the soil community was also influenced by pH, especially through the changes in P availability and plant strategies from calcareous to acidic dunes. The influence of AM and NM plants probably even overruled the normal patterns of bacteria-dominated communities in calcareous soils and fungi-dominated communities in acidic soils. Such patterns have been firmly established for many soil types (Blagodatskaya and Anderson 1998, Bååth and Anderson 2003, Mulder et al. 2005, Kooijman et al. 2008, Rousk et al. 2009, 2010), but appeared not valid for coastal dunes in the Netherlands.

According to expectations, fungi were indeed less common in calcareous than acidic dunes, and more stressed, as shown by the higher percentage of melanized fungi (Butler and Day 1998). Melanized hyphae are unknown from AM fungi (Treseder and Lennon 2015) but may protect saprotrophic and some EcM fungi against, for example, drought stress. However, fungal feeders were more abundant in calcareous than in acidic dunes, probably due to the presence of AM fungi. This was especially the case for fungivorous browsers, which only digest the cell content, such as the mite *Tyrophagus* and the collembole *P. callipygos*. The latter species was characterized as fungivorous browser by Jagers op Akkerhuis et al. (2008), even though it may use other food than mycorrhizal fungi (Sauer and Ponge 1988). Also, *P. callipygos* was clearly incorporated in the network cluster with AM plants. Mycorrhizal fungi are probably more nutritious than saprophytic fungi, which was supported by the higher microbial nutrient contents in calcareous than in acidic dunes.

Bacterial pathway more important in acidic dunes

In many ecosystems, bacteria are more abundant in calcareous than in acidic soils

(Blagodatskaya and Anderson 1998, Bååth and Anderson 2003, Mulder et al. 2005, Kooijman et al. 2008, Rousk et al. 2009, 2010). However, in our study, bacterial biomass did not differ between dune zones, and bacterial growth rates and the amount of bacterial grazers were even higher in acidic dunes. As a result, bacterial biomass and bacterial grazers negatively correlated with pH instead of positively, and *d*-separation tests suggested that low pH actually increased the bacterial pathway. The deviating results of our study may be due to differences in land use. De Vries et al. (2012) showed that plants have the potential to influence soil communities through their belowground inputs, and Drigo et al. (2007) that AM and NM plants may have different effects. Our study was conducted in natural grasslands, with AM plants in calcareous dunes and NM plants in acidic dunes. In contrast, Rousk et al. (2009, 2010) used agricultural fields without natural vegetation, and in the Beech forests of Kooijman et al. (2008, 2016), undergrowth vegetation was also very sparse. In Mulder et al. (2005), although natural ecosystems such as woodlands and heathlands were included, most plots belonged to agro-ecosystems.

In calcareous dunes, colonization of roots by AM fungi may lead to lower total sugar output to the rhizosphere (Jansa et al. 2011). This may reduce the amount of bacteria, as sugar is an important bacterial food resource. In contrast, in acidic dunes, NM plants may have positive effects on bacteria. Nonmycorrhizal plants generally exudate small carboxylates such as citrate and oxalate to mobilize weakly sorbed P (Gerke 2015). However, these organic molecules may also be decomposed by bacteria (Bruce 1934, Hajna 1934) and thus form a bacterial carbon source (Morris and Allen 1994). Nonmycorrhizal plants also release phosphatase enzymes (Güsewell 2017), which may serve as bacterial carbon source as well (Beaman et al. 1988). These hypotheses need to be further tested, but predominance of NM plants and associated strategies for P uptake in acidic dunes may thus strengthen the bacterial pathway. Although pH effects were partly confounded by SOM, positive effects of NM plants on bacteria in acidic dunes were also noted by Drigo et al. (2007).

Bacterial community composition also changed over the pH gradient, and the proportion of Gram-positive bacteria was higher in acidic than in calcareous dunes, in accord with Bååth and Anderson (2003). Gram-positive bacteria are generally considered to be more drought-tolerant (Fuchslueger et al. 2014), but drought stress probably occurs in both calcareous and acidic dunes. The increase in Gram-positive bacteria may also reflect an increase of acid-tolerant phyla such as *Actinobacter* and *Fimicutes* (Chodak et al. 2015). However, grazing pressure by protists, which prefer the smaller Gram-negative bacteria and were more common in acidic than in calcareous dunes, may have played a role as well (Griffiths et al. 1999, Rønn et al. 2002).

Minor differences in net N mineralization

Although net N mineralization in calcareous soils is often lower than in acidic soils (Zöttle 1960, Davy and Taylor 1974, Verhoeven et al. 1990), due to higher microbial N demand and immobilization (Measures 1975, Kuehn et al. 1998, Moore et al. 2005), differences in N cycling in our study were remarkably low. Laboratory incubation experiments should be seen as potential rather than actual nutrient availability in the field (Schimel and Bennett 2004). Also, microbial C both increased and decreased in incubation experiments, which may have influenced net N mineralization. In addition, in calcareous dune samples, net N mineralization was probably influenced by AM fungi, which were unsupported by plant roots during the incubation period. Nevertheless, none of the incubation experiments showed higher microbial N uptake for calcareous than for acidic dunes. Also, net N mineralization per unit C respired, a proxy for microbial N use efficiency and immobilization (Kooijman et al. 2008, 2016), did not differ between dune zones in any of the incubation experiments. This may be due to the shift in soil communities in response to predominance of AM and NM plants. In our study, differences in bacterial and fungal pathways between calcareous and acidic dunes were reduced by AM and NM plants, which in turn may have reduced differences in N cycling between the two dune zones.

Resilience to high N deposition

In calcareous dunes, resilience to high N deposition is higher than in acidic dunes, as indicated

by lower rates of grass-encroachment (Remke et al. 2009a, Kooijman et al. 2017) and higher critical N loads (Van Dobben and van Hinsberg 2008). Our findings suggest that these differences in resilience are due to differences in P availability and plant strategies, rather than differences in soil communities and N availability. Calcareous dunes were not dominated by bacteria, and net N mineralization was not consistently lower than in acidic dunes. In fact, N availability to the vegetation may even be higher in calcareous than acidic dunes, due to uptake of N by AM fungi (Johansen et al. 1994, Wang et al. 2017).

High resilience to N deposition in calcareous dunes can be explained by low P availability to the vegetation, due to low solubility of calcium phosphates in the soil and strong P sorption to Fe oxides. Both N and P are essential nutrients (Olde Venterink et al. 2003), and if P is a limiting factor, extra N may not lead to increased biomass production. In calcareous dunes, AM plants increase P uptake with help of AM fungi (Hoeksema et al. 2010, Smith and Smith 2011), but plant biomass probably remains low as long as P is a limiting factor. Mycorrhizal symbiosis costs a lot, especially when photosynthesis is limited by other factors such as soil moisture (Fitter 1991, Raven et al. 2018). To support the mutualistic interaction, up to 20% of the photoassimilates in host plants may flow to mycorrhizal root systems (Wang et al. 2017). As a result, high mycorrhizal infection leads to low relative growth rates (Koziol and Bever 2015). Mycorrhizal costs for the host plant may be so high that the relationship becomes parasitic and limits plant biomass production (Johnson et al. 1997, Wilson et al. 2001, Koziol and Bever 2015), or may restrict formation of flowers (Dixon 2000). In calcareous dunes, AM plants may also increase resilience to grass-encroachment with high nutrient contents in aboveground biomass. The highly nutritious plants may attract more rabbits, which counteract grass-encroachment by grazing.

In acidic dunes, high N deposition may lead to increased plant biomass production, because P availability is also high, especially with respect to weakly sorbed and colloidal P. High N deposition may even further increase P availability through increased exudation of phosphatase enzymes for labile organic P under N-rich conditions (Güsewell 2017). For NM plants, increased root exudates, which help with P uptake, would also cost a lot of carbohydrates (Raven et al. 2018). Exudation of organic acids may account for 2–3% of dry root weight (Kirk et al. 1999), although it is unknown whether such costs are higher than mycorrhizal costs for AM plants. However, the dominant NM species *C. arenaria* is a clonal plant with high carbohydrate reserves in its rhizomes (Noble et al. 1979). In areas with low N deposition along the Baltic Sea, *C. arenaria* showed N:P ratios of 7–8 g/g, which points to high P supply, but N-limited growth conditions (Remke et al. 2009a, b). In such areas, *C. arenaria* was present in small amounts, and the vegetation dominated by lichens. However, in acidic dunes with high N deposition, *C. arenaria* has become a dominant plant species, which decreased plant diversity in many NW-European countries (Remke et al. 2009a, b, Kooijman et al. 2017).

Calcareous dunes are thus in better shape than acidic dunes in terms of plant diversity and conservation of natural values, at least as long as pH values remain high. However, in a temperate climate, calcareous dunes are sensitive to acidification, especially when atmospheric deposition is also high (Aggenbach et al. 2017). Dissolution of calcium carbonates may also increase with climate change, when Dutch dunes receive more rainfall (IPCC 2014). Acidification is a problem for AM plants, not only because of dissolution of calcium phosphates below pH 6.5 (Lindsay and Moreno 1966, Hinsinger 2001), but also because of the shift from Fe oxides to Fe-OM complexes, and the associated increase in plant-available P around pH 5.5.

In coastal dunes, stimulation of natural dynamics and blowout activity may be a way to increase the pH through replenishment of the soil with fresh sand. In calcareous dunes, it should be possible to keep the pH of the topsoil high enough to prevent dissolution of calcium phosphates, which will safeguard the presence of AM plants and maintain high plant diversity. In acidic dunes, such high pH values can probably only be reached inside the blowouts (Aggenbach et al. 2017). However, in acidic dunes, most calcium phosphates have probably already dissolved long ago, and it may be sufficient to increase pH to values above 5.5. At such values, the vegetation probably already mainly consists of NM plants, but relatively small ones such as the red-list species

Corynephorus canescens and *Teesdalia nudicaulis* (Van der Meijden 2005). Below pH 5.5, availability of weakly sorbed (organic) P will increase, and the vegetation becomes dominated by larger and fast-growing NM plants such as *C. arenaria*. In that case, only grazing of the vegetation by cattle or sheep may help improve plant diversity, and only if they eat it.

Concluding Remarks

The aim of this study was to separate the effects of pH and SOM on plant diversity, nutrient availability, and soil communities in the EU priority habitat Grey dunes. The results demonstrate that differences in pH in sandy coastal dunes of the temperate zone lead to fundamental changes in plant strategies, from AM plants in calcareous to NM plants in acidic dunes. This shift may not be due to pH directly, as AM plants and fungi occur in acidic soils as well, but to fundamental shifts in P nutrition. In calcareous dunes, P availability was low despite high amounts of inorganic P, due to low solubility of calcium phosphates and strong P sorption to Fe oxides at high pH, which increases mycorrhizal benefits for AM plants. In acidic dunes, sorbed P became more important, consisting of both inorganic and organic P, which were mainly weakly sorbed to Fe-OM complexes. This is more favorable for NM plants, which in turn stimulated the bacterial pathway. For the vegetation, SOM was mainly important for plant cover and biomass. For the soil community, SOM was also a main driver for microbial biomass and numerical abundance of soil animals, but also determined diversity and complexity of the food web. Nevertheless, the shift from AM to NM plant strategies with pH clearly influenced microbial community composition, and overruled the normal bacterial and fungal pathways in calcareous and acidic soil. It should be noted that more research is needed, but the findings do suggest that P nutrition and associated plant strategies, as well as their influence on soil communities and N cycling, are crucial to understand differences between dune zones in resilience to high N deposition. Phosphorus is usually treated on a secondary level, but for coastal Grey dunes may be even more important than C and N.

Literature Cited

Aerts, M. A. P. A., and F. S. Chapin. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of process and patterns. Advances in Ecological Research 30:1–67.

Aggenbach, C. J. S., A. M. Kooijman, Y. Fujita, H. van der Hagen, M. van Til, D. Cooper, and L. Jones. 2017. Does atmospheric nitrogen deposition lead to greater nitrogen and carbon accumulation in coastal sand dunes? Biological Conservation 212:416–422.

Aptroot, A., K. van Herk, and L. Sparrius. 2011. Veldgids Korstmossen van duin, heide en stuifzand. Bryologische en Lichenologische werkgroep van de KNNV, Zeist, the Netherlands.

Bååth, E., and T. H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry 35:955–963.

Beaman, L., M. Paliescheskey, and B. L. Beaman. 1988. Acid phosphatase stimulation of the growth of nocardia asteroids and its possible relationship to the modification of lysosomal enzymes in macrophages. Infection and Immunity 56:1652–1654.

Blagodatskaya, E. V., and T. H. Anderson. 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO_2 of microbial communities in forest soils. Soil Biology and Biochemistry 30:1269–1274.

Bloem, J., and P. R. Bolhuis. 2006. Thymidine and leucine incorporation to assess bacterial growth rate.

Pages 142–149 *in* J. Bloem, D. W. Hopkins, and A. Benedetti, editors. Microbiological methods for assessing soil quality. CABI, Wallingford, UK.

Bloem, J., D. W. Hopkins, and A. Benedetti. 2006. Microbiological methods for assessing soil quality. CABI, Wallingford, UK.

Bloem, J., M. Veninga, and J. Shepherd. 1995. Fully automatic determination of soil bacterium numbers, cell volumes and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Applied and Environmental Microbiology 61:926–936.

Bloem, J., and A. Vos. 2004. Fluorescent staining of microbes for total direct counts. Pages 861–874 *in* G. A. Kowalchuk, F. J. De Bruijn, I. M. Head, A. D. L. Akkermans, and J. D. Van Elsas, editors. Molecular microbial ecology manual. Second edition. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Blume, H. P., G. W. Brümmer, H. Fleige, R. Horn, E. Kandeler, I. Kögel-Knaper, K. Stahr, and B. M. Wilke. 2016. Scheffer/Schachtschabel soil science. Springer, Berlin, Heidelberg, Germany.

Bobbink, R., et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20:30–59.

Bol, R., et al. 2016. Dissolved and colloidal phosphorus fluxes in forest ecosystems—an almost blind spot in ecosystem research. Journal of Plant Nutrition and Soil Science 79:425–438.

Brooks, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17:837–842.

Bruce, W. F. 1934. The decomposition of citric acid by *Bacillus aertrycke*. Journal of Biological Chemistry 107:119–129.

Brundett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320:37–77.

Butler, M. J., and A. W. Day. 1998. Fungal melanins: a review. Canadian Journal of Microbiology 44:1115–1136.

Cairney, J. W. G., and A. A. Meharg. 2003. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. European Journal of Soil Science 54:735–740.

Cardoso, E. J. B. N., M. A. Nogueira, and W. Zangaro. 2017. Importance of mycorrhizae in tropical soils. Pages 245–268 *in* J. L. de Azevedo and M. C. Quecine, editors. Diversity and benefits of microorganisms from the tropics. Springer International Publishing, New York, New York, USA.

Celi, L., G. De Luca, and E. Barberis. 2003. Effects of interaction of organic and inorganic P with ferrihydrite and kaolinite-iron oxide systems of iron release. Soil Science 168:479–488.

Cerli, C., L. Celi, K. Kalbitz, G. Guggenberger, and K. Kaiser. 2012. Separation of light and heavy organic matter fractions in soil – Testing for proper density cut-off and dispersion level. Geoderma 170:403–416.

Chodak, M., M. Gołębiewski, J. Morawska-Płoskonka, K. Kuduk, and M. Niklińska. 2015. Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. Annals of Microbiology 65:1627–1637.

Cleveland, C. C., and D. Liptzin. 2007. C:N:P stoichiometry in soil: Is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85:235–252.

Cody, R. P., and J. K. Smith. 1987. Applied statistics and the SAS programming language. Elsevier Science, Amsterdam, The Netherlands.

Coleman, C., and P. F. Hendrix. 2000. Invertebrates as webmaster in ecosystems. CABI, Cambridge, Massachusetts, USA.

Daly, K., D. Jeffrey, and H. Tunnet. 2001. The effect of soil type on phosphorus sorption capacity and desorption dynamics in Irish grassland soils. Soil Use and Management 17:12–20.

Davy, A. J., and K. Taylor. 1974. Seasonal patterns of nitrogen availability in contrasting soils in the Chiltern Hills. Journal of Ecology 62:793–807.

De Vries, F. T., M. E. Liiri, L. Bjørnlund, H. M. Setälä, S. Christensen, and R. Bardgett. 2012. Legacy effects of drought on plant growth and the soil food web. Oecologia 170:821–833.

De Vries, F. T., J. W. van Groenigen, E. Hoffland, and J. Bloem. 2011. Nitrogen losses from two grassland soils with different fungal biomass. Soil Biology and Biochemistry 43:997–1005.

Dixon, J. M. 2000. *Koeleria macrantha* (Ledeb.) Schultes (*K. alpigena* Domin, *K. cristata* (L.) Pers. pro parte, *K. gracilis* Pers., *K. albescens* auct. non DC.). Journal of Ecology 88:709–726.

Drigo, B., G. A. Kowalchuck, E. Yergeau, T. M. Bezemer, H. S. Doscher, and J. A. van Veen. 2007. Impact of elevated carbon dioxide on the rhizosphere communities of *Carex arenaria* and *Festuca rubra*. Global Change Biology 13:2396–2410.

Du, Z. Y., Q. H. Wang, F. C. Liu, H. L. Ma, B. Y. Ma, and S. S. Malhi. 2013. Movement of phosphorus in a calcareous soil as affected by Humic Acid. Pedosphere 23:229–235.

Eisma, D. 1968. Composition, origin and distribution of Dutch coastal sands between Hoek van Holland

and the island of Vlieland. Dissertation. Rijksuniversiteit Groningen, Groningen, The Netherlands.

Ellenberg, H., H. E. Weeber, R. Düll, V. Wirth, and W. Werner. 1974. Zeigerwerte von Pflanzen in Mitteleuropa. Verlag Erich Goltze GmbH & Co, Gottingen, Germany.

Emmerton, K. S., T. V. Callaghan, H. E. Jones, J. R. Leake, A. Michelsen, and D. J. Read. 2001. Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. New Phytologist 151:513–524.

Endlweber, K., L. Ruess, and S. Scheu. 2011. Collembola switch diet in presence of plant roots thereby functioning as herbivores. Soil Biology and Biochemistry 41:1151–1154.

Ernst, W. H. O., W. E. van Duin, and G. T. Oolbekking. 1984. Vesicular-arbuscular mycorrhiza in dune vegetation. Acta Botanica Neerlandica 33:151–160.

European Union. 1992. Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora. European Commission, Brussel, Belgium.

Ewald, J. 2003. The calcareous riddle. Folia Geobotanica 38:357–366.

Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences USA 103:626–631.

Fink, J. R., A. V. Inda, T. Tiecher, and V. Barrón. 2016. Iron oxides and organic matter on soil phosphorus availability: a review. Ciência e Agrotecnologia 40:369–379.

Fitter, A. H. 1991. Costs and benefits of mycorrhizas: implications for functioning under natural conditions. Experientia 47:350–355.

Frey-Klett, P., J. Garbaye, and M. Tarkka. 2007. Tansley review: the mycorrhiza helper bacteria revisited. New Phytologist 176:22–36.

Frostegård, Å., A. Tunlid, and E. Bååth. 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14:151–163.

Frouz, J., K. Prach, V. Pizl, L. Hanel, J. Stary, K. Tajovsky, J. Materna, V. Balık, J. Kalc, and K. Rehounkova. 2008. Interactions between soil development, vegetation and soil fauna during spontaneous succession in post mining sites. European Journal of Soil Biology 44:109–121.

Fuchslueger, L., M. Bahn, K. Fritz, R. Hasibeder, and A. Richter. 2014. Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytologist 201:916–927.

Gerke, J. 2010. Humic (organic matter)-Al(Fe)-phosphate complexes: an underestimated phosphate form in soils and source of plant-available phosphate. Soil Science 175:417–425.

Gerke, J. 2015. The acquisition of phosphate by higher plants: effect of carboxylate release by the roots. A critical review. Journal of Plant Nutrition and Soil Science 178:351–364.

Gerke, J., W. Römer, and L. Beissner. 2000. The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. II. The importance of soil and plant parameters for uptake of mobilized P. Journal of Plant Nutrition and Soil Science 162:213–219.

Griffiths, B. S., M. Bonkowski, G. Dobson, and S. Caul. 1999. Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. Pedobiologia 43:297–304.

Gu, B., J. Schmitt, Z. Chen, L. Llang, and J. F. McCarthy. 1994. Adsorption and desorption of natural organic matter on iron oxide: mechanisms and models. Environmental Science Technology 28:38–48.

Güsewell, S. 2017. Regulation of dauciform root formation and root phosphatase activities of sedges (Carex) by nitrogen and phosphorus. Plant and Soil 416:57–72.

Hajna, A. A. 1934. Decomposition of salts of organic acids by bacteria of the genus Salmonella. Journal of Bacteriology 29:253–258.

Hamad, M. E., D. L. Rimmer, and J. K. Syers. 1992. Effect of iron oxide on phosphate sorption by calcite and calcareous soils. Journal of Soil Science 43:273–281.

Hassink, J. 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biology and Biochemistry 26:1221–1231.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil 237:173–195.

Hoeksema, J. D., et al. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13:394–407.

IPCC. 2014. Climate change 2014: synthesis report. In R. K. Pauchari, editor. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, 151pp.

Jagers op Akkerhuis, G. A. J. M., W. J. Dimmers, P. C. J. van Vliet, P. W. Goedhart, G. F. P. Martakis, and R. G. M. de Goede. 2008. Evaluating the use of gel-based sub-sampling for assessing responses of terrestrial microarthropods (Collembola and Acari) to

different slurry applications and organic matter contents. Applied Soil Ecology 38:239–248.

Jansa, J., R. Finlay, H. Wallander, F. A. Smith, and S. E. Smith. 2011. Role of mycorrhizal symbioses in phosphorus cycling. Pages 137–168 *in* E. K. Bünemann, A. Oberson, and E. Frossard, editor. Phosphorus in action, soil biology. Volme 26. Springer-Verlag, Berlin, Heidelberg, Germany.

Jeanroy, E., and B. Guillet. 1981. The occurrence of suspended ferruginous particles in pyrophosphate extracts of some soil horizons. Geoderma 26:95–105.

Johansen, A., I. Jakobsen, and E. S. Jensen. 1994. Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen. Plant and Soil 160:1–9.

Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist 135:575–585.

Johnson, S. E., and R. H. Loeppert. 2006. Role of organic acids in phosphate mobilization from iron oxide. Soil Science Society America Journal 70:222–234.

Kaufmann, R. 2001. Invertebrate succession on an alpine glacier foreland. Ecology 82:2261–2278.

Kim, J., W. Li, B. L. Philips, and C. P. Grey. 2011. Phosphate adsorption on the iron oxyhydroxides goethite (a-FeOOH), akaganeite (b-FeOOH), and lepidocrocite (g-FeOOH): a ^{31}P NMR study. Energy & Environmental Science. https://doi.org/10.1039/c1ee02093e

Kirk, G. J. D., E. E. Santos, and M. B. Santos. 1999. Phosphate solubilization by organic anion excretion from rice growing in aerobic soil: rates of excretion and decomposition, effects on rhizosphere pH and effects on phosphate solubility and uptake. New Phytologist 142:185–200.

Kluber, L. A., S. R. Carrino-Kyker, K. P. Coyle, J. D. DeForest, C. R. Hweins, A. N. Shaw, K. A. Smemo, and D. J. Burke. 2012. Mycorrhizal response to experimental pH and P manipulation in acidic hardwood forests. PLOS ONE. https://doi.org/10.1371/journal.pone.0048946

Kooijman, A. M. 2010. Litter quality effects on undergrowth species diversity by mass of the organic layer, pH, soil moisture and N-dynamics in Luxembourg beech and hornbeam forests. Journal of Vegetation Science 21:248–261.

Kooijman, A. M., and M. Besse. 2002. On the higher availability of N and P in lime-poor than in lime-rich coastal dunes in the Netherlands. Journal of Ecology 90:394–403.

Kooijman, A. M., J. Bloem, B. R. van Dalen, and K. Kalbitz. 2016. Differences in activity and N demand between bacteria and fungi in a microcosm incubation experiment with selective inhibition. Applied Soil Ecology 99:26–39.

Kooijman, A. M., C. Cusell, L. Hedenäs, L. P. M. Lamers, I. S. Mettrop, and T. Neijmeijer. 2019. Reassessment of phosphorus availability in fens with varying contents of iron and calcium. Plant and Soil. https://doi.org/10.1007/s11104-019-04241-4

Kooijman, A. M., J. C. R. Dopheide, J. Sevink, I. Takken, and J. M. Verstraten. 1998. Nutrient limitations and their implications on the effects of atmospheric deposition in coastal dunes; lime-poor and lime-rich sites in the Netherlands. Journal of Ecology 86:511–526.

Kooijman, A. M., M. M. Kooijman-Schouten, and G. B. Martinez-Hernandez. 2008. Alternative strategies to sustain N-fertility in acid and calcaric Beech forests: low microbial N-demand versus high biological activity. Basic and Applied Ecology 9:410–421.

Kooijman, A. M., I. Lubbers, and M. van Til. 2009. Iron-rich dune grasslands: relations between soil organic matter and sorption of Fe and P. Environmental Pollution 157:3158–3165.

Kooijman, A. M., M. van Til, E. Noordijk, E. Remke, and K. Kalbitz. 2017. N-deposition and grass-encroachment in calcareous and acidic Grey Dunes (H2130) in NW-Europe. Biological Conservation 212:406–415.

Kovacs, G. M., and C. Szigetvari. 2002. Mycorrhizae and other root-associated fungal structures of the plants of a sandy grassland on the Great Hungarian Plain. Phyton 42:211–223.

Koziol, L., and J. D. Bever. 2015. Mycorrhizal response trades off with plant growth rate and increases with plant successional status. Ecology 96:1768–1774.

Kuehn, K. A., P. F. Churchill, and K. Suberkropp. 1998. Osmoregulatory responses of fungi inhabiting standing litter of the freshwater emergent macrophyte *Juncus effusus*. Applied Environmental Microbiology 64:607–612.

Lambers, H., J. A. Raven, G. R. Shaver, and S. E. Smith. 2008. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution 23:95–103.

Lauber, C. L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111–5120.

Lindsay, W. L., and E. C. Moreno. 1966. Phosphate phase equilibria in soils. Soil Science Society of America Proceedings 24:177–182.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London, UK.

McKeague, J. A., J. E. Brydon, and N. M. Miles. 1971. Differentiation of forms of extractable iron and aluminium in soils. Soil Science Society of America Journal 35:33–38.

McKercher, R. B., and G. Anderson. 1989. Organic phosphate sorption by neutral and basic soils. Communications in Soil Science and Plant Analysis 20:723–732.

Measures, J. C. 1975. Role of amino acids in osmoregulation of non-halophilic bacteria. Nature 257:398–400.

Mehlich, A. 1984. Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis 12:1409–1416.

Missong, A., R. Bol, V. Nischwitz, J. Krüger, F. Lang, J. Siemens, and E. Klumpp. 2017. Phosphorus in water dispersible-colloids of forest soil profiles. Plant and Soil 427:71–86.

Missong, A., S. Holzmann, R. Bol, V. Nischwitz, H. Puhlmann, K. von Wilpert, J. Siemens, and E. Klumpp. 2018. Leaching of natural colloids from forest topsoils and their relevance for phosphorus mobility. Science of the Total Environment 634:305–315.

Moore, J. C., K. McCann, and P. C. de Ruiter. 2005. Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. Pedobiologia 49:499–510.

Morriën, E. M., et al. 2017. Soil networks become more connected and take up more carbon as nature restoration progresses. Nature Communications. https://doi.org/10.1038/ncomms14349

Morris, S. J., and M. F. Allen. 1994. Oxalate-metabolizing microorganisms in sagebrush steppe soil. Biology and Fertility of Soils 18:255–259.

Mulder, C., H. J. van Wijnen, and A. P. van Wezel. 2005. Numerical abundance and biodiversity of below-ground taxocenes along a pH gradient across the Netherlands. Journal of Biogeography 32:1775–1790.

Muthukumar, T., K. Udaiyan, and P. Shanmughavel. 2004. Mycorrhiza in sedges – an overview. Mycorrhiza 14:65–77.

Nijssen, M., B. Wouters, J. Vogels, A. M. Kooijman, H. van Oosten, C. van Turnhout, M. Wallis de Vries, J. Dekker, and I. Janssen. 2014. Begrazingsbeheer in relatie tot herstel van faunagemeenschappen in droge duingraslanden. Eindrapportage 2009–2013. Rapport nr. 2014/OBN 190-DK. Directie Agrokennis, Ministerie van Economische Zaken, Driebergen, the Netherlands.

Noble, J. C., A. D. Bell, and J. H. Harper. 1979. Population biology of plants with clonal growth I. The morphology and structural demography of *Carex arenaria*. Journal of Ecology 67:983–1008.

Nordgren, A. 1988. Apparatus for the continuous long-term monitoring of soil respiration rate in larger numbers of samples. Soil Biology and Biochemistry 20:955–958.

Olde Venterink, H., M. J. Wassen, A. W. M. Verkroost, and P. C. de Ruiter. 2003. Species richness-productivity patterns differ between N-, P-, ad K-limited wetlands. Ecology 84:2191–2199.

Olsson, P. A., and G. Tyler. 2004. Occurrence of non-mycorrhizal plant species in south Swedish rocky habitats is related to exchangeable soil phosphate. Journal of Ecology 92:808–815.

Pérez Corona, M. E., I. van der Klundert, and J. T. A. Verhoeven. 1996. Availability of organic and inorganic phosphorus compounds as phosphorus sources for *Carex* species. New Phytologist 133:225–231.

Prietzel, J., W. Klysubun, and F. Werner. 2016. Speciation of phosphorus in temperate zone forest soils as assessed by combined wet-chemical fractionation and XANES spectroscopy. Journal of Plant Nutrition and Soil Science 179:168–185.

R Core Team. 2018. R-version 3.5.1. 2018: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raven, J. A., H. Lambers, S. E. Smith, and M. Westoby. 2018. Costs of acquiring phosphorus by vascular land plants: patterns and implications for plant coexistence. New Phytologist 217:1420–1427.

Read, D. H., H. K. Koucheki, and J. Hodgson. 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. New Phytologist 77:641–653.

Read, D. J., and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytologist 157:475–492.

Remke, E., E. Brouwer, A. M. Kooijman, I. Blindow, H. Esselink, and J. G. M. Roelofs. 2009*a*. Even low to medium nitrogen deposition impacts vegetation of dry, coastal dunes around the Baltic Sea. Environmental Pollution 157:792–800.

Remke, E., E. Brouwer, A. M. Kooijman, I. Blindow, and J. G. M. Roelofs. 2009*b*. Low atmospheric nitrogen loads lead to grass encroachment in coastal dunes, but only on acid soil. Ecosystems 12:1173–1188.

Richardson, A. E., P. J. Hocking, R. J. Simpson, and T. S. George. 2009. Plant mechanisms to optimise access to soil phosphorus. Crop & Pasture Science 60:124–143.

Rønn, R., F. Ekelund, and S. Christensen. 1995. Optimizing soil extract and broth media for MPN-enumeration of naked amoebae and heterotrophic flagellates in soil. Pedobiologia 39:10–19.

Rønn, R., A. E. McCaig, B. S. Griffiths, and J. I. Prosser. 2002. Impact of protozoan grazing on bacterial community structure in soil microcosms. Applied and Environmental Microbiology 86:6094–6105.

Rousk, J., E. Bååth, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. International Society for Microbial Ecology Journal 4:1340–1351.

Rousk, J., P. C. Brookes, and E. Bååth. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75:1589–1596.

Sauer, E., and J. F. Ponge. 1988. Alimentary studies on the collembolan *Paratullbergia callipygos* using transmission electron microscopy. Pedobiologia 31:355–379.

Schimel, J. P., and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602.

Schwertmann, U. 1964. Differenzierung der eisenoxide des bodens durch extraktion mit ammoniumoxalat-lösung. Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde 105:194–202.

Shang, C., J. W. B. Stewart, and P. M. Huang. 1992. pH effect on kinetics of adsorption of organic and inorganic phosphates by short-range ordered aluminum and iron precipitates. Geoderma 53:1–14.

Shen, J., L. Yuan, J. Zhang, H. Li, Z. Bai, X. Chen, W. Zhang, and Z. Zhang. 2011. Phosphorus dynamics: from soil to plant. Plant Physiology 156:997–1005.

Shipley, B. 2000. Cause and correlation in biology: a user's guide to path analysis, structural equation and causal inference. Oxford University Press, Oxford, UK.

Shipley, B. 2003. Testing recursive path models with correlated errors using d-separation. Structural Equation Modeling 10:214–221.

Siepel, H., and E. M. de Ruiter-Dijkman. 1993. Feeding guilds of oribatid mites based on carbohydrase enzyme activities. Soil Biology and Biochemistry 25:149l–1497.

Siepel, H., and F. Maaskamp. 1994. Mites of different feeding guilds affect decomposition of organic matter. Soil Biolology and Biochemistry 26:1389–1394.

Smith, S. E., I. Jakobsen, M. Grønlund, and F. A. Smith. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology 156:1050–1057.

Smith, S. E., and D. Read. 2008. Mycorrhizal symbiosis. Third edition. Academic Press, Cambridge, Massachusetts, USA.

Smith, S. E., and F. A. Smith. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Reviews in Plant Biology 62:227–250.

Stevenson, F. J. 1994. Humus chemistry: genesis, composition, reactions. Wiley, New York, New York, USA.

Stoutjesdijk, P. H., and J. J. Barkman. 1992. Microclimate, vegetation and fauna. Opulus Press, Uppsala, Sweden.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in terrestrial ecosystem. Blackwell Scientific Publications, Oxford, UK.

Ter Braak, C. J. F. 1988. CANOCO – a FORTRAN program for canonical community ordination by partial detrended canonical correspondence analysis, principal component analysis and redundancy analysis. Technical report: LWA 88–02. Wageningen University, Wageningen, The Netherlands.

Thaymuang, W., I. Kheoruenromne, A. Suddhiprakarn, and D. L. Sparks. 2013. Organic matter stabilization by iron oxides in upland tropical red soils under tropical monsoonal and tropical savanna climates. Thai Journal of Agricultural Science 46:119–130.

Tietema, A. 1992. Abiotic factors regulating nitrogen transformations in the organic layer of acid forest soils: moisture and pH. Plant and Soil 147:69–78.

Treseder, K. K., and J. T. Lennon. 2015. Fungal traits that drive ecosystem dynamics on land. Microbiology and Molecular Biology Reviews 79:243–262.

Turner, B. L., L. M. Condron, S. J. Richardson, D. A. Peltzer, and V. J. Allison. 2007. Soil organic phosphorus transformations during pedogenesis. Ecosystems 10:1166–1181.

Van Aarle, I. M., P. A. Olsson, and B. Söderström. 2002. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. New Phytologist 155:173–182.

Van der Meijden, R. 2005. Heukels flora van Nederland, drieëntwintigste druk. Wolters-Noordhoff, Groningen, Germany.

Van Mourik, J., and S. Blok. 2008. Physical fractionation and cryo-coupe analysis of mormoder humus. Pages 199–210 *in* S. Kapur, A. Mermut, and G. Stoops, editors. New trends in soil micromorphology. Springer, Berlin, Heidelberg, Germany.

Van Til, M., and J. Mourik. 1999. Hieroglyfen in het zand: Vegetatie en landschap van de Amsterdamse Waterleidingduinen. Gemeentewaterleidingen, Amsterdam, The Netherlands.

Van Tooren, B. F., and L. B. Sparrius. 2007. Voorlopige verspreidingsatlas van de Nederlandse mossen. Bryologische en Lichenologische werkgroep van de KNNV, Zeist, The Netherlands.

Van Dobben, H. F., and A. van Hinsberg. 2008. Overzicht van kritische depositiewaarden voor stikstof, toegepast op habitattypen en Natura 2000-gebieden. Alterra-rapport 1654. Alterra, Wageningen, Germany.

Verhoef, H. A., and A. J. van Selm. 1983. Distribution and population dynamics of Collembola in relation to soil moisture. Holarctic Ecology 6:387–394.

Verhoeven, J. T. A., E. Maltby, and M. B. Schmitz. 1990. Nitrogen and phosphorus mineralization in fens and bogs. Journal of Ecology 78:713–726.

Walker, T. W., and J. K. Syers. 1976. The fate of phosphorus during pedogenesis. Geoderma 15:1–19.

Wang, B., and Y. L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363.

Wang, W., J. Shi, Q. Xie, Y. Jiang, N. Yu, and E. Wang. 2017. Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. Molecular Plant 10:1147–1158.

Westerman, R. L. 1990. Soil testing and plant analysis. Third edition. Soil Science Society of America, Madison, Wisconsin, USA.

Wilson, G. W. T., D. C. Hartnett, M. D. Smith, and K. Kobbemans. 2001. Effects of mycorrhiza on growth and decmography in tallgrass prairie forbs. American Journal of Botany 88:1452–1457.

Xu, G. L., T. M. Kuster, M. S. Günthardt-Goerg, M. Dobbertin, and M. H. Li. 2012. Seasonal exposure to drought and air warming affects soil Collembola and mites. PLOS ONE 7:e43102.

Yan, J., T. Jiang, Y. Yao, S. Lu, Q. Wang, and S. Wie. 2016. Preliminary investigation of phosphorus adsorption onto two types of iron oxide-organic matter complexes. Journal of Environmental Sciences 42:152–162.

Zöttle, H. 1960. Dynamik der Stickstoffmineralisation im Waldbodenmaterial. Plant and Soil 8:207–223.

Supporting Information