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1 **Title: Does atmospheric nitrogen deposition lead to greater nitrogen and**
2 **carbon accumulation in coastal sand dunes?**

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21 acidification, plant diversity
22

Abstract

Atmospheric nitrogen (N) deposition is thought to accelerate ecological succession, causing a loss of diversity in species-rich dune grasslands and hampering restoration goals. We tested whether elevated atmospheric N deposition results in faster accumulation of soil C and soil N, using three high-resolution chronosequences of up to 162 years in coastal sand dunes with contrasting N deposition and soil base status (high N deposition calcareous and acidic dunes in Luchterduinen, the Netherlands (LD) and low N deposition calcareous dunes in Newborough, UK (NB)). We also used the process model CENTURY to evaluate the relative contribution of N deposition, climate, and soil pH. In contrast to our hypothesis we found that accumulation of soil C and N was greatest at the low N deposition site NB. Model simulations indicated a negative interaction between high N deposition and symbiotic N_2 fixation. From this we conclude that high N deposition suppresses and replaces N_2 fixation as a key N source. High N deposition led to lower soil C:N only in the early stages of succession (<20 years). The data also revealed accelerated acidification at high N deposition, which is a major concern for restoration of dune grasslands. More data are needed from acidic dunes from low N deposition areas to assess pH effects on soil C and N pools. Therefore, while N accumulation in soils may not be an issue, both acidification and plant community change due to elevated availability of mineral N remain major conservation problems. Restoration in degraded dune grasslands should focus on maintaining habitat suitability, rather than N removal from soil pools.

1   Introduction

In coastal dune ecosystems, the accumulation of soil organic matter (SOM) during primary and secondary succession is a fundamental driver in the development of fixed dune grasslands (Olff et al. 1993; Ranwell 1972; Van der Meulen and Jungerius 1989). In the early stages of succession, sandy soils are low in SOM and have a small nitrogen (N) pool, so the availability of mineral N and water can limit plant productivity (Bartholomeus et al. 2012; Bohnert and Jensen 1996; Johnsen et al. 2014; Tilman et al. 1996). Therefore, it has been suggested that a higher input of mineral N from atmospheric N deposition for several decades will accelerate succession of dune ecosystems by enhancing biomass production and litter input (Jones et al. 2004; Remke et al. 2009a, 2009b; Veer and Kooijman 1997), leading to increased soil carbon and N stocks (Jones et al. 2008, 2013). The enhanced accumulation of soil C and N may hamper conservation and restoration of low productive dune grasslands with a high biodiversity, even after atmospheric N deposition has reduced to low levels.

The mechanisms by which atmospheric N deposition may alter soil processes are both direct and indirect. Evidence from some experiments and from gradient studies suggests that extra N boosts plant productivity and plant tissue N content (Jones et al. 2004; Plassmann et al. 2009; Remke et al. 2009a, 2009b; Van den Berg et al. 2005), although these effects are not always observed in the field (Ford et al. 2016; Ten Harkel and Van der Meulen 1996). The increased plant productivity enhances litter input, which accelerates accumulation of soil C and N. Accumulation of N in the soil may also be affected by changes in C:N ratio of the humic layer. C:N ratio is important because it controls many soil processes, with faster mineralisation as C:N falls below certain thresholds (Rowe et al. 2006). High N deposition may lead to increased plant tissue-N content and therefore a decreased C:N ratio in soil (Mulder et al. 2013; Remke et al. 2009b; Sardans and Penuelas 2012). However, a gradient study in fixed dune grasslands suggests that a contrasting outcome for soil C:N ratios is also possible, where increased biomass production due to N deposition can actually increase C:N ratios by priming the system with carbon-rich biomass (Jones et al. 2004). The direct and indirect effects of N deposition may lead to higher N mineralisation, creating even more available N to fuel faster plant growth (Berendse 1998; Sparrius et al. 2012). At the same time, high N deposition, high N mineralisation, and a low soil C:N ratio may also increase leaching losses (Phoenix et al. 2003; Rowe et al. 2006).

Accumulation rates of C and N in the soil are also influenced by natural factors. Soil pH may exert a major influence on organic matter accumulation since it controls decomposition rates. In addition, nitrification rates are also pH sensitive (Kemmitt et al. 2006), causing interactive effects of pH on N dynamics in the soil. Soil pH also governs phosphorus availability which together with N is usually the key limiting nutrient in dunes (Kooijman and Besse 2002; Kooijman et al. 2016). Moreover, soil pH declines during succession because of soil organic matter accumulation, and decalcification. A high atmospheric N deposition also enhances acidification, due to elevated input of reduced N (NH_x), which produces protons when it is nitrified in the soil (Van Breemen et al. 1984). Thus, effects of elevated N deposition may differ between calcareous and acidic dunes and between different successional stages.

A main natural source of N is biological N_2 fixation by symbiotic and by free-living non-symbiotic bacteria. In dunes, rates of N_2 fixation from these natural sources can be high when *Hippophae rhamnoides* is present; it can fix 0.05-0.45 kg N ha^{-2} per day (Hassouna and Wareing 1964; Kumler 1997; Stewart and Pearson 1967; Stuyfzand 1993), which is equivalent to 9.1-82.1 kg N ha^{-2} yr^{-1} if N_2 fixation takes place during 50% of the year. In

95   temperate grasslands symbiotic N_2 fixation ranges 0.1-10 kg N ha^{-2} yr^{-1}, and non-symbiotic N_2
96   fixation 0.1-21 kg N ha^{-2} yr^{-1} (Reed et al. 2011). Non-symbiotic N fixation may be hampered
97   by high amount of available N relative to available P (Eisele et al. 1989), indicating negative
98   effects of N deposition on non-symbiotic N fixation. Furthermore, symbiotic N fixing plants
99   are more abundant in calcareous dunes than in acidic dunes (Weeda et al. 1987). This means
100 that contribution of N fixation on soil N accumulation may differ between calcareous and
101 acidic dunes and under high and low N deposition.

103 A major challenge in studying these multiple effects of N deposition on soil C and N
104 accumulation is that soil development is a slow process, resulting from minor shifts in the
105 balance between production and decomposition of SOM. Most fertilization experiments in the
106 field do not run for long enough to detect enhanced N or C pools in the soil (Ford et al. 2016;
107 Remke 2010). Even long-running experiments struggle to detect small changes in large soil
108 pools, while gradient studies can be confounded to a greater or lesser extent by other co-
109 occurring gradients. However, chronosequence studies provide a technique to infer changes in
110 soil processes over longer time-scales (Knops and Tilman 2000; Stevens and Walker 1970),
111 provided certain assumptions are met (Johnson and Miyanishi 2008). In addition, process-
112 based soil development models can be used to test the influence of driving factors over long
113 time scales by varying climate, N deposition and soil conditions as inputs.

115 In this study, we used data from three robustly constructed chronosequences in two sites with
116 different levels of N deposition. The site with low N deposition is located in Wales, and
117 consists of calcareous dunes. The site with high N deposition is located in the Netherlands,
118 and consists of both calcareous and acidic dunes. We tested differences between the three
119 chronosequences in accumulation rates of soil C and soil N, soil C:N ratios, and soil pH,
120 patterns of vegetation structure and plant species richness. We further simulated the soil
121 development over a 75 year period using the CENTURY model (Metherell et al. 1993), to
122 evaluate the potential effects of N deposition, climate, and biological N_2 fixation. The
123 research questions were:
124   (1) Do dunes under high N deposition have greater accumulation of soil C and soil N, and
125 lower soil C:N ratios than those under low N deposition; and is this reflected in the pattern of
126 vegetation succession?
127   (2) What are the relative contributions of atmospheric N deposition, climate and N_2
128 fixation to C and N accumulation in calcareous and acidic dunes?

130 2   **Materials and methods**

132 2.1   *Site description*

134 This study uses three chronosequences constructed at two temperate coastal dune ecosystems
135 in Europe: Newborough Warren (hereafter called NB) in North Wales, United Kingdom
136 (53:08N 4:21W), and Luchterduinen (hereafter called LD) in the Netherlands (52:18N,
137 4:30E). NB only consists of calcareous dunes with a $CaCO_3$ content of approximately 1.7 %
138 in the young stages and 0.5-0.7 % in old dune grasslands in the top 15 cm. LD has
139 chronosequences in both calcareous and acidic dunes. In the calcareous dunes succession
140 starts at a $CaCO_3$ content of 1.2 to 2.2 %, and ends up in old stages with a content of 0-1.7 %
141 in the top 15 cm. Here, decalcification depth of old grasslands has a range of 0-22 cm. In the
142 acidic dunes succession starts at a $CaCO_3$ content of 0.3-1.2 %. The top soil layer is
143 decalcified ca. 10-20 years after the start of succession. Decalcification depth of old
144 grasslands is typically deeper than 30 cm. NB and LD have broadly similar climatic

conditions (annual precipitation: 850 mm in NB and 805 mm in LD, annual average temperature: 10.2 °C in NB and 9.7 °C in LD, for the period of 1931 - 2014), but there are some difference in the seasonal patterns (Appendix A). LD has been exposed to a high level of atmospheric N deposition in the last decades with a peak of ca. 30-37 kgN ha^{-1} yr^{-1} during 1970-1990, whereas atmospheric N deposition level in NB has not been as strongly elevated and remained within the range of ca. 5 – 10 kgN ha^{-1} yr^{-1} (Figure A.6 in Appendix A).

The chronosequences were established using high resolution aerial photographs available at least since 1940s in NB (1947, 1951, 1966, 1971, 1982, 1990, 2006; Jones et al. 2008), and 1930s in LD (1938, 1958, 1968, 1979, 1990, 2001, 2006, 2011; Aggenbach et al. 2013). When a bare spot in an aerial photo becomes vegetated in the aerial photo of the subsequent year, we assumed that succession started on that spot at the average year between the two sequential aerial photos. The succession age of the spot was calculated as the period between the year of succession started and the year of soil sampling (i.e. 2012). The age of the spots which were already vegetated in the oldest aerial photos were estimated with aid of additional historical records. In NB age of oldest stage (162 year) was estimated from historical maps (Jones et al. 2008), and second oldest stage (61 year) estimated from reconstructed aeolian history (Jones et al. 2010). In LD the age of the oldest stage was set at 97 year based on general records of aeolian history. In total, we have selected 48 plots in NB all with calcareous topsoil (ranging from 0 to 162 years old at year 2012) and 110 plots in LD (ranging from 0 to 97 years old at year 2012). LD plots were split in calcareous (N=48; referred as LD calcareous) and quickly decalcifying dunes (N=62; referred as LD acidic). An overview of the plots is given in Table B.1 in Appendix B. All plots ranging from bare sand to dry dune grasslands are independent from the phreatic aquifer.

2.2 *Soil sampling*

For each plot, volumetric soil samples were taken from 0 cm to 15 cm depth in 2012 or 2013 for NB and in 2012 for LD. The soil samples were weighed to calculate bulk density and, after removing large roots, dried at 65 °C and machine-ground. Soil organic C and N were measured by combustion on a Carlo Erba CSN analyser, after acidification to remove carbonates. pH in topsoil (0-5 cm depth for LD, 0-6 cm depth for NB) was measured by extracting fresh soils with demineralised water with a ratio 1:2.5 (w/v).

2.3 *Vegetation recording*

In all plots of LD, bare sand cover and species composition of vascular plants, mosses and lichens were recorded in 1 m x 1 m plots during summer 2012. For NB, species composition and bare sand cover were recorded in 2 m x 2 m plots (n = 21) as part of an earlier chronosequence study at the same site (Jones et al. 2008, 2010), which shared many locations with the plots used for soil sampling in 2012. Succession age of the vegetation plots ranged from 5 to 150 years. We used bare sand cover as a vegetation structure parameter, and we calculated total species number for each plot as a measure for biodiversity.

2.4 *Statistical analysis*

Changes in soil C pool, soil N pool, soil C:N ratio, and soil pH over successional age were fitted for each chronosequence separately. Following relationships in Jones et al. (2008) a 3-parameter logistic curve ($a1$/(1+exp(($a2$-age)/$a3$))) was fitted to the increase in C pools and N pools over time. An asymptotic curve ($a1$+($a2$-$a1$)*exp(-exp($a3$)*age)) was fitted to both C:N

ratio and pH as in both cases the data approach an asymptote. Parameter *a1* is referred as 'asymptotic' value in both curves. Parameter *a3* of the logistic curve is the age value at the inflection point of the curve (with a low value indicating a strong increase), and parameter *a2* is the scale parameter on the input axis. For the asymptotic curve parameter *a2* is the intercept and *a3* the log rate. Testing of differences between pairs of curves and the curve parameters was then conducted in R. The R functions SSasymp and SSlogis were used to fit the curves, within the function nlsList. Significant differences of N deposition were evaluated by comparing NB and LD calcareous, and differences due to initial calcium carbonate content by comparing LD calcareous and LD acidic. Differences between curves for individual parameters were assessed as significant when these were higher than twice the standard error of the difference.

Changes in bare sand cover and total species number along successional age were also fitted for each chronosequence separately. For bare sand cover we used a logistic curve (*a1*+*a2**exp(-*age*/*a3*)). For total species number different regression models were selected: a logistic curve (*a1*/(1+exp((*a2*-*age*)/*a3*))) for NB and LD calcareous, and a 2-order polynomial curve for LD acidic. Testing of differences between curve pairs for bare sand cover was conducted in the same way described above. This testing was not conducted for total species number, because the vegetation plots differed in size for LD and NB, and for LD calcareous and LD acidic, the best fit was acquired with different regression models.

2.5   *CENTURY Model*

To simulate development of soil and vegetation, we used the CENTURY model (Metherell et al. 1993). The CENTURY model is internationally used and well validated with empirical data for both agricultural and natural ecosystems across biomes (e.g. Kelly et al. 2000; Schimel 1994). CENTURY dynamically simulates decomposition of soil organic matter and associated dynamics of soil C and N, growth of vegetation, and hydrology. See Appendix C for more details about the model specification and optimization procedure. We optimized three model parameter values, which control maximum plant production, nutrient uptake by plant, and decomposition rate, based on observation data of soil C, soil N, and plant biomass. All three optimized parameter are global (i.e. not site-specific) parameters, not facilitating responses to any local factors such as N deposition level or soil pH.

2.6   *Site-specific model input data*

We used historical records of N deposition level (wet plus dry, NH_x plus NO_y) of NB and LD (Figure A.6 in Appendix A). For NB, the back-calculated national profile for UK was calibrated to Newborough (Jones et al. 2008). For LD, the national average of N deposition level in the Netherlands (CBS et al. 2015) was corrected for the local N deposition level of LD for the period after 1946, by using the proportion of the local over the national average in 2014 (Velders et al. 2015), i.e. 84%.

Monthly average values of precipitation, minimum and maximum temperature were taken from weather station RAF Valley for NB (<8 km from the plots) and weather station De Bilt for LD. The weather data of De Bilt were corrected for LD by using the proportion of difference between the De Bilt and the closest weather station to LD, Valkenburg (<15 km from the plots) in the recent year records. For computation of potential evapotranspiration, we used a simplified Penman equation (Linacre 1977), multiplied with 0.75 to convert from lake surface to vegetated surface.

245

246 We assumed that the asymbiotic N_2 fixation (i.e. N_2 fixation by free-living microorganisms)
247 occurred at a constant rate both in NB and in LD. We used the median value of temperate
248 unfertilized grasslands (Reed et al. 2011), 5.7 kgN ha^{-2} yr^{-1}. The fixed N was added to the
249 ammonium pool of mineral N in the topsoil. In the CENTURY model symbiotic N_2 fixation is
250 assumed to occur when soil mineral N is not sufficient to satisfy the plant N demand, having
251 taken into account all the other factors which limit plant growth (e.g. temperature, moisture).
252 The N fixers fix N in newly assimilated biomass with a C:N ratio of 26.7 gC gN^{-1}. We set an
253 extra parameter value to define the proportion of symbiotic N fixers, and parameterized it
254 based on vegetation records of each area. This parameter controls how much fraction of the
255 plant N demand, at maximum, can be fulfilled by symbiotic N_2 fixation. Since there was no
256 clear temporal trend in the observed proportion of symbiotic N fixers during succession, we
257 used average abundance (i.e. cover in percentage) of symbiotic N fixers from vegetation
258 record values all through the succession period: 6% for NB (N=21), 5.4% for calcareous
259 dunes in LD (N=48), and 0.14% for acidic dunes in LD (N=62). Note that effects of soil
260 acidity on process rates are not included in the CENTURY model. Therefore, difference in
261 model input values between calcareous and acidic sites in LD is merely the proportion of
262 symbiotic N fixers.

263

264 2.7 *Model simulation and analysis of model outputs*

265

266 Succession of soil and vegetation was simulated for the three chronosequences (NB
267 calcareous, LD calcareous, and LD acidic) for 75 times each (i.e. 1-year-old dune to 75-year-
268 old dune). Each simulation starts from bare soil (see Appendix C for model initial values). For
269 model simulation of each age, we used different data series of N deposition and meteorology
270 of the corresponding years (e.g. 1938 – 2012 for 75-year-old simulation, 1939-2012 for 74-
271 year-old simulation, etc.). Plausibility of the model was tested by comparing the model output
272 of soil C and N accumulation, soil C:N ratio, and above-ground plant productivity for the
273 three chronosequences with their field observation data of multiple ages (see Appendix B for
274 more details).

275

276 The scenarios with actual conditions as described above are hereafter referred to as 'actual
277 scenario'. In order to disentangle the contribution of different factors on soil and vegetation
278 succession, we ran extra scenarios for 75 years (from 1938 to 2012). First, for each of the
279 three sites, the model was run with a low N deposition (hereafter referred to as 'lowN
280 scenario'). For this, we used the N deposition level of 1900 in the Netherlands, i.e. 0.5 kgN
281 ha^{-1} yr^{-1}. Second, the model was run with absence of symbiotic N_2 fixation (hereafter referred
282 to as 'noNfix scenario'). Third, we ran the model with the low N deposition level and no
283 symbiotic N fixers (hereafter referred as 'lowN+noNfix scenario'). Since the difference
284 between sites in lowN+noNfix scenario is merely due to the meteorological conditions, this
285 scenario can be considered as baseline scenario reflecting only the effect of climate. We
286 consider the difference between the lowN+noNfix scenario and lowN scenario as the effect of
287 symbiotic N_2 fixation; the difference between lowN+noNfix scenario and noNfix scenario as
288 the effect of atmospheric N deposition; the difference between lowN+noNfix scenario and
289 actual scenario as the combined effect of symbiotic N_2 fixation and atmospheric N deposition.
290 When the combined effect of symbiotic N_2 fixation and atmospheric N deposition was larger
291 or smaller than the sum of effects of symbiotic N fixation and atmospheric N deposition, we
292 consider the interactive effects of these two factors as positive or negative, respectively.

293

294 **3   Results**

3.1   Observed soil C and N accumulation under low and high N deposition levels

For all chronosequences, regression models for C and N accumulation had a high r^2 and significant p-values for the regression parameters (p<0.001) (Table 1). Soil C pool rose with increasing age and levelled off after around 60-80 years (Figure 1) to a level of approximately 2.3 kg C m^{-2} in LD and 2.8 kg C m^{-2} in NB. Soil N also increased with age and levelled off to a level of 0.18 kg N m^{-2} in LD and 0.24 kg N m^{-2} in NB (Figure 1). Difference in initial calcium carbonate content between acidic and calcareous dunes, only tested for LD, had no effect on the final C and N pool (curve asymptotes), and other regression parameters (Table 1). Unexpectedly, the final pools of C and N were significantly higher at the low N site in NB than at the high N calcareous site in LD (p=0.039 for C pool, p=0.015 for N pool). In NB, accumulation of soil N was much higher than the cumulative inputs from atmospheric N deposition (Figure B.1.b in Appendix B). For example, N deposition accounted for only 26 % of the N pool at the succession age 65 years. In LD cumulative N deposition was equivalent to 112 and 92 % of the N pool at age 75 years respectively for the calcareous and acidic chronosequences.

3.2   Observed soil C:N ratio

For LD calcareous and LD acidic the asymptotic regression models of C:N ratio with age had a moderate r^2, and low p-values for the model parameters (p<0.001) ,while for NB r^2 was very low (Table 1). In NB, C:N ratio did not change over time. In both LD chronosequences, C:N ratio rose in the early stage (0-20 year) and then levelled off to approximately 13.5 (Figure 1). Differences in N deposition had a significant effect (p=0.008), leading to a lower intercept in LD calcareous compared to NB. The asymptotic values did not differ, which means that N deposition only affects the C:N ratio in the early stages (< 20 years). For LD there was no clear effect of calcium carbonate content on C:N ratio (p=0.052).

3.3   Observed topsoil pH

All chronosequence regression curves of pH on age had a high r^2 and significant p-values (p<0.001) for all regression parameters (Table 1; Figure 1). N deposition had no effect on intercept and asymptote, indicating that initial and final pH values were the same for NB and LD calcareous. However, N deposition strongly affected the log rate (*a3*), indicating faster acidification in LD calcareous than in NB (p<0.001). Acidification was also strongly affected by initial calcium carbonate content (p<0.001), with the asymptotic values being much higher in LD calcareous than in LD acidic (5.8 and 4.0, respectively).

3.4   Bare sand cover and total plant species number

In all chronosequences, bare sand cover declined quickly when succession starts, and became low after 30 years (Figure 2). The regression curves only differed for the asymptotic parameter between NB and LD calcareous. In LD calcareous, plots in the older stages still had some bare sand (0-30 %), while in NB bare sand cover was very low (0-3 %). For total species number (vascular plants, mosses, and lichens), both calcareous chronosequences follow the same pattern: a strong increase between 0 and 20 years, and at older age species richness stays constant (Figure 2). The species number was higher in NB compared to LD calcareous, probably due to the larger plot size. The trend in LD acidic differed strongly from

both calcareous chronosequences. Species richness increased in early succession stages, but declined again between 39-49 and 97 years.

3.5   Model plausibility for predicting soil and vegetation succession

Model performance against observed data is shown in Figure B.1 in Appendix B for C pool, N pool, C:N ratio, and above-ground vascular plant biomass. The model predicted soil C accumulation reasonably well. Soil N accumulation was underestimated, especially for NB and LD acidic. Soil C:N ratio was overestimated by the model, but the increase in soil C:N ratio in the beginning of succession, which was observed clearly in LD, was well reproduced by the model. The predicted above-ground plant production was in the same range for old stages of NB, lower for young stages of NB, and generally higher for LD.

3.6   Contribution of different N sources to soil N accumulation

The CENTURY model outputs reveal the contribution of different factors to N accumulation and their relative importance on soil development. After 75 years of succession, the effect of climate on N accumulation was similar between LD and NB (Figure 3). In the two calcareous areas, there was a negative interactive effect of symbiotic N fixers and atmospheric N deposition, i.e. elevated N deposition switched off a part of the symbiotic N_2 fixation. In the calcareous dunes of NB, with low N deposition, the potential contribution of atmospheric N deposition to total N pools in the soil was relatively low, which suggests that most N came from symbiotic N fixers. In LD calcareous, where atmospheric N deposition level was high, the contribution of N deposition was higher than in NB. However, in LD calcareous, symbiotic N fixers also contributed strongly to N accumulation, but much of the excess N was lost from the system through leaching. In LD acidic, the contribution of atmospheric N deposition to soil N accumulation was very large in comparison to that of symbiotic N_2 fixation, because symbiotic N fixers were hardly present.

4.   Discussion

4.1   Effects of elevated N deposition on accumulation of soil C and soil N

Our hypothesis was that a long period of N deposition speeds up the accumulation of soil N and C. However, the empirical data of accumulation of soil N and C in the calcareous chronosequences indicated the opposite, despite the 2.6 times higher cumulative atmospheric N deposition in LD than in NB over the past 75 years. The model outputs suggest that microbiological N_2 fixation was an important N source for ecosystem development at both sites, but with considerable excess N leached at the high N deposition site, consistent with observations in other studies (Stuyfzand 1993; Ten Harkel et al. 1998). However, the model outputs suggested that N_2 fixation was considerably lower at the high N deposition site, suggesting that N deposition switched off much of the symbiotic N_2 fixation. N_2 fixation can be switched off when mineral N in the soil is sufficient for demand (Tang et al. 1999), as shown by experiments with N-fixing species present such as *Hippophae rhamnoides* (Kato et al. 2007) and *Trifolium repens* (Macduff et al. 1996), as well as asymbiotic N_2 fixation in temperate grasslands (Keuter et al. 2014).

Higher N accumulation in NB might also be explained by the higher plant productivity, due to slightly lower drought stress in NB than LD. The model results suggested that differences in climate had little effect on the outcomes for soil C and N pools, because drought stress in LD

simultaneously reduced both plant growth and decomposition of soil organic matter (Appendix A). However, the lower Ellenberg moisture values coupled with greater bare sand cover in LD, although only significant in older successional stages, suggested that drought stress may exert a stronger negative effect on productivity in LD than NB. To access the effect of drought stress on N and C accumulation, more extensive analyses of soil moisture conditions are needed based on high frequency meteorological data and local soil moisture properties. Higher N accumulation in NB may also be associated with higher abundance of eutrophic species (as indicated by higher Ellenberg N values for NB than LD; results not shown), and might be linked to differences in geochemical properties of the dune sand between NB and LD.

A gradient study showed that high N deposition led to three times higher soil organic matter content in old acidic dunes, while there was no effect on calcareous dunes (Remke et al. 2009b). Therefore, acidic dunes may be more sensitive to enhanced N accumulation due to N deposition. However, we could not make the equivalent comparison for acidic dunes since we are not aware of any similar high resolution chronosequences spanning this age range at low N deposition sites.

4.2   Effect of elevated N deposition on soil C:N ratio

Our empirical data show an effect of atmospheric N deposition on the quality of organic matter (i.e. lower C:N ratios in high N deposition sites) in early successional stages. A direct effect of high N deposition would be an increased uptake of N by plants, and therefore a decrease of the C:N ratio of plant biomass and litter (Berendse 1998; Remke et al. 2009b; Van den Berg et al. 2005). Also, high N deposition may, especially in young stages, stimulate growth of microorganisms that generally have lower C:N ratios than soil and plant litter (Cleveland and Liptzin 2007). However, the lack of difference in C:N ratio in older stages remains unexplained, despite contrasting evidence for both lower (Remke et al. 2009b) and higher (Jones et al. 2004) C:N ratios observed in the field.

4.3   Model uncertainty

While our model was able to reproduce the overall patterns of long-term soil succession in dune grasslands, there were some mismatches between the model prediction and field observations. Firstly, symbiotic and asymbiotic N_2 fixation is one of the largest unknowns in the N inflow of ecosystems (Reed et al. 2011), yet their contribution to N and C accumulation could be significant (Pluis and De Winder 1989). Sensitivity analysis showed that the modelled N accumulation was strongly affected by the parameters that control symbiotic and asymbiotic N_2 fixation (Appendix B). Thus, improved parameterization for N_2 fixation is a step to improve the model. Secondly, our model underestimated soil C and N accumulation for acidic sites. The only mechanism of pH effects included in our model is the higher amount of symbiotic N fixers in calcareous sites than acidic sites. However, pH potentially influences other processes too, such as plant productivity, SOM decomposition and N transformations, which would require additional site-specific measurements to adequately parameterise the model. Thirdly, we did not include grazing effects in the model due to lack of quantitative information about grazing intensity in the past, whereas our plots have been under different grazing regimes of cattle, sheep, and wild rabbits. Since grazing can have considerable effects on SOM accumulation in dune grasslands (Kooijman and Smit 2001), improvement of the model performance relies on exact records of the grazing history for each plot.

4.4   Effects of N deposition on soil acidification and plant species diversity

Acidic dune grasslands are sensitive to accelerated decalcification and acidification of the topsoil due to atmospheric pollution because of the relatively low acid buffer capacity (Kooijman and Besse 2002; Remke et al. 2009b; Stuyfzand 1993). The acidic chronosequence in the high deposition area showed a fast drop of topsoil pH, which was much stronger than the trend of pH in chronosequences at Spiekeroog (Germany) and South Haven Peninsula (UK) with a comparable calcium carbonate content in the pioneer stage and with a relative low deposition (Gerlach et al. 1994; Wilson 1960). Acidification affected the plant communities, with a drop in species richness in acidic grasslands when topsoil pH falls below 4.5-5.0, while in the calcareous grasslands species richness stayed constant, and topsoil pH remained high (5.5-7.0). This was reinforced by a decline in basiphilous species like *Viola curtisii, Cerastium semidecandrum* and an increase in acidophilous species like *Cladonia portentosa* and *Teesdalia nudicaulis.* The rapid acidification in the acidic dune grasslands due to N deposition therefore creates a legacy effect that will hamper restoration.

4.5   Implications for nature management

Our results indicate that high N deposition does not accelerate C and N accumulation in calcareous dunes due partly to suppression of N_2 fixation at high mineral N inputs, and also due to leaching of excess N as a result of the low retention capacity for N in dune soils. However, we cannot draw conclusions about accelerated accumulation rates in acidic dune grasslands due to lack of low N sites. Since soil N pools in calcareous Grey dunes are not increased by legacy effects of elevated N accumulation, then topsoil removal for this reason is not necessary. However, while N pools are not elevated, there is a wide literature documenting eutrophication effects in calcareous dunes such as declines in plant species richness (Field et al. 2014; Kooijman et al. 2016), possibly due to increased availability of soil mineral N (Jones et al. 2004). In addition, increased decalcification and acidification due to N deposition also reduces plant species biodiversity of dune grasslands. Restoration in dune grassland exposed to a high N (and legacy S) load should therefore focus on measures that maintain habitat suitability and restore a high base status of the topsoil (e.g. Jones et al. 2016). Soil restoration can be achieved by stimulating small-scale aeolian processes that create base-rich soils in deflation zones with renewed soil succession, and by deposition of calcareous sand in existing dune grasslands (Brunbjerg et al. 2014; Van Boxtel et al. 1997). Another measure is sod-cutting of superficially decalcified soils, which increases topsoil pH and favours basiphilous dune grassland species on a short time scale (Van Til and Kooijman 2007). Remke et al. (2009b) found that the vegetation of old stage acidic dune grasslands is very sensitive to acidifying N deposition in a relatively low range (wet deposition 5-8 kgN ha^{-1} yr^{-1}). Therefore, for old acidic dune grasslands, these measures are sustainable only when N load drops below this range.

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654

Figure 1. Changes in topsoil C pool, N pool, C:N ratio and pH with succession age for Newborough (NB calcareous), acidic Luchterduinen (LD acidic), and calcareous Luchterduinen (LD calcareous). Lines are the regression models regressed by age (NB calcareous: grey; LD calcareous: black; LD acidic: dashed black).

<1.5 column>
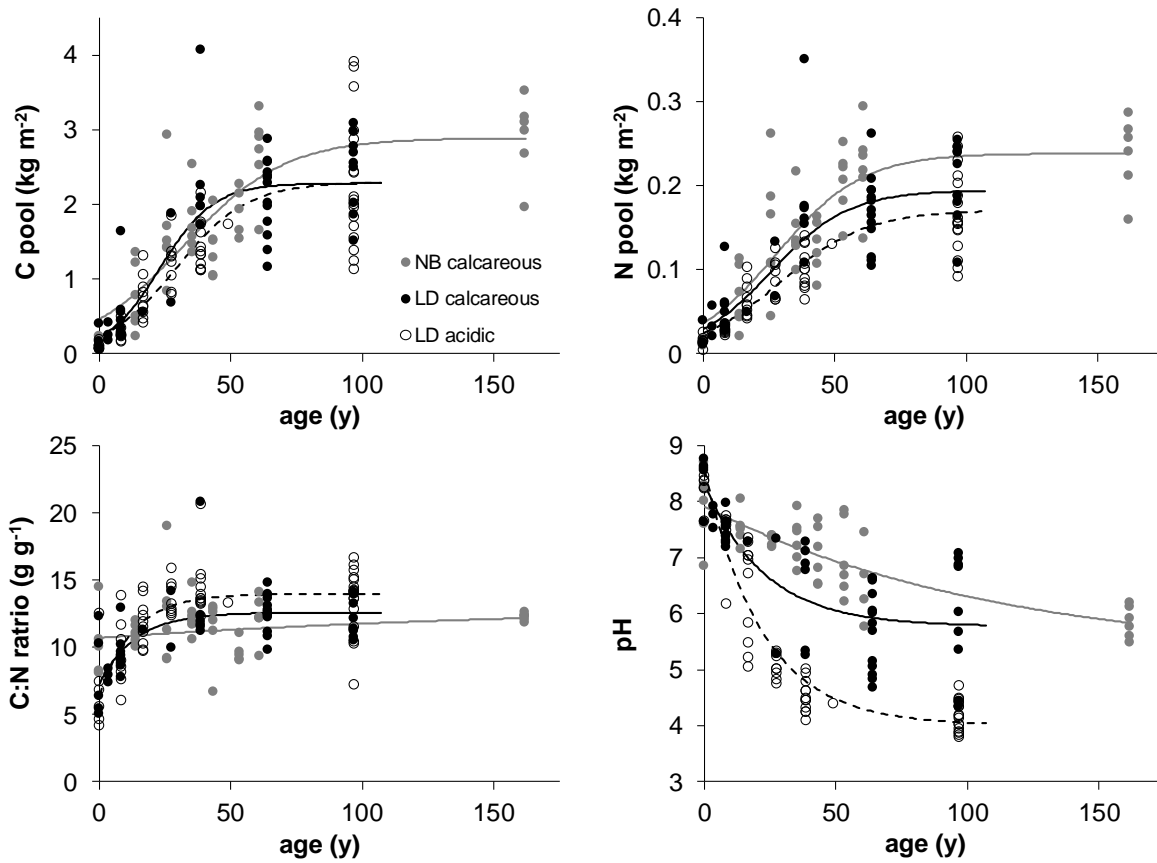
Figure 2. Changes in bare sand cover and total species number (vascular plants, mosses, and lichens) with succession age for Newborough (NB calcareous), acidic Luchterduinen (LD acidic), and calcareous Luchterduinen (LD calcareous). Lines are the regression models regressed by age (NB calcareous: grey; LD calcareous: black; LD acidic: dashed black). Note the different sampling area for species richness: NB is species per 4 m^2, LD is species per 1 m^2.
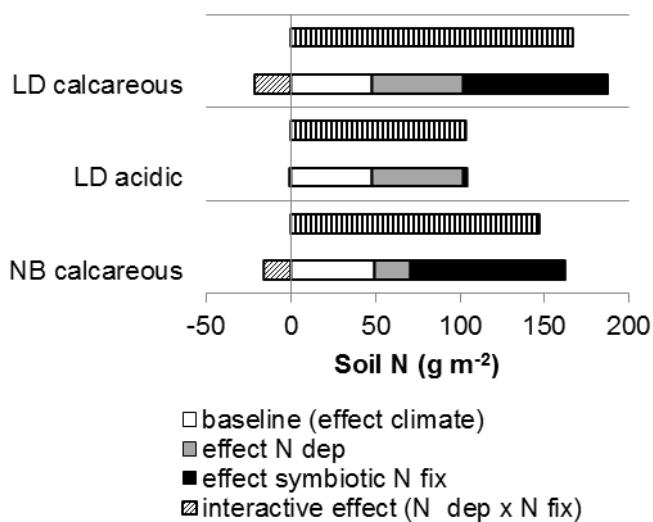
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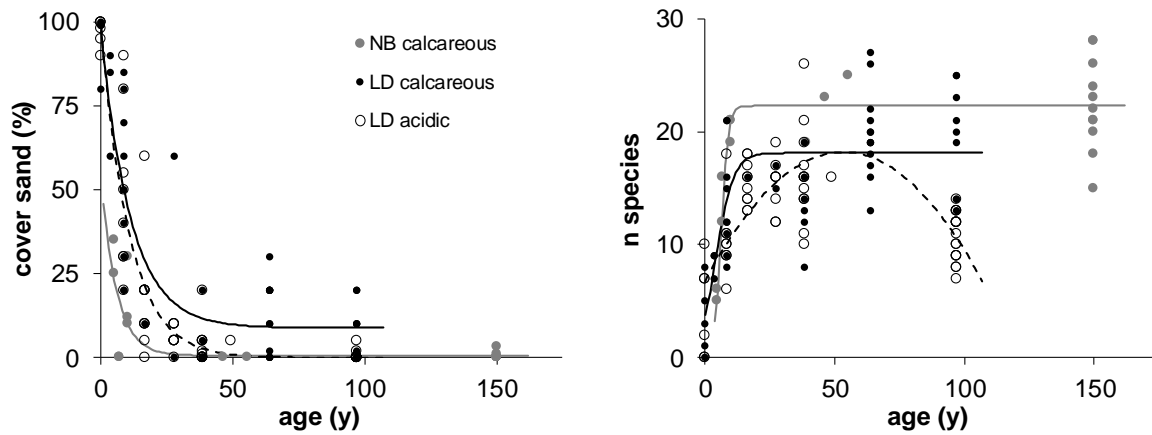
Figure 3. CENTURY outputs for N pool in actual scenario and contributions of atmospheric
N deposition, symbiotic N fixers, and climate to soil N pool after 75 years of simulation (from
1938 to 2012) for Newborough (NB calcareous), acidic Luchterduinen (LD acidic), and
calcareous Luchterduinen (LD calcareous). See section 2.7 for how contributions were
calculated from a series of simulation scenarios.

<1 column>

□baseline (effect climate)
▨effect N dep
■effect symbiotic N fix
▨interactive effect (N dep x N fix)

18

Table 1. Statistics of the regression models of C pool, N pool, C:N ratio and pH of the topsoil on succession age for the chronosequences in Newborough (NB calcareous), Luchterduinen calcareous (LD calcareous) and Luchterduinen acidic (LD acidic). C pool and N pool were fitted with a logistic model: $(a1/(1+\exp((a2-age)/a3))$; C:N ratio and pH were fitted with an asymptotic model: $a1+(a2-a1)*\exp(-\exp(a3)*age)$. P-values model parameters: * P<0.001. Where significant, differences between chronosequences for each model parameter are indicated with letters.

<1.5 column>

Variabels	chronosequence	r^2	a1 ave±SD	a2 ave±SD	a3 ave±SD
C pool	NB calcareous	0.71	$2.89 \pm 0.21^{*a}$	$31.9 \pm 4.4^{*}$	$19.3 \pm 3.9^{*a}$
	LD calcareous	0.81	$2.28 \pm 0.11^{*b}$	$22.9 \pm 3.3^{*}$	$10.3 \pm 2.2^{*b}$
	LD acidic	0.70	$2.29 \pm 0.13^{*b}$	$29.1 \pm 3.0^{*}$	$13.5 \pm 2.9^{*b}$
N pool	NB calcareous	0.68	$0.24 \pm 0.02^{*a}$	$27.9 \pm 4.2^{*}$	$16.4 \pm 3.7^{*}$
	LD calcareous	0.78	$0.19 \pm 0.01^{*b}$	$25.6 \pm 4.4^{*}$	$14.8 \pm 3.3^{*}$
	LD acidic	0.76	$0.17 \pm 0.01^{*b}$	$29.3 \pm 3.0^{*}$	$16.4 \pm 3.1^{*}$
C:N ratio	NB calcareous	0.06	13.16 ± 6.83	$10.7 \pm 0.6^{*a}$	-5.2 ± 4.3
	LD calcareous	0.46	$12.59 \pm 0.45^{*}$	$7.4 \pm 0.8^{*b}$	$-2.6 \pm 0.5^{*}$
	LD acidic	0.52	$13.96 \pm 0.46^{*}$	$6.5 \pm 0.9^{*b}$	$-2.6 \pm 0.3^{*}$
pH	NB calcareous	0.62	$5.19 \pm 0.79^{*ab}$	$7.9 \pm 0.2^{*a}$	$-4.7 \pm 0.5^{*a}$
	LD calcareous	0.63	$5.77 \pm 0.24^{*a}$	$8.3 \pm 0.3^{*ab}$	$-3.1 \pm 0.4^{*b}$
	LD acidic	0.89	$4.01 \pm 0.14^{*b}$	$8.6 \pm 0.2^{*b}$	$-3.1 \pm 0.1^{*b}$

694

695

Appendix A. Climatic conditions and atmospheric N-deposition level in Newborough and Luchterduinen

<online>

**Climatic conditions in Newborough and Luchterduinen and its effects on soil and vegetation in CENTURY model**

Difference in meteorological data between Newborough (NB) and Luchterduinen (LD)

NB has warmer and wetter winter than LD, whereas LD has higher precipitation and higher potential evapotranspiration in summer than NB (Figure A.1). The ratio of precipitation to potential evapotranspiration in summer, which indicates the aridity, is slightly lower in LD than NB (i.e. LD experiences more drought stress in summer than NB) (Figure A.2).

Higher drought stress in LD compared to NB was also reflected in plant species composition. Average Ellenberg values for moisture were in general slightly higher for NB than LD (Figure A.3), indicating that LD has more species adapted for dry conditions.
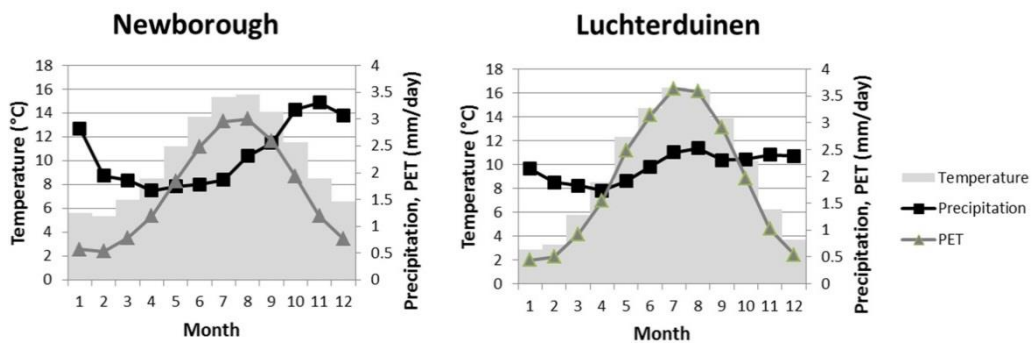
Figure A.1. Monthly average of temperature, precipitation, and potential evapotranspiration between 1931 and 2014.
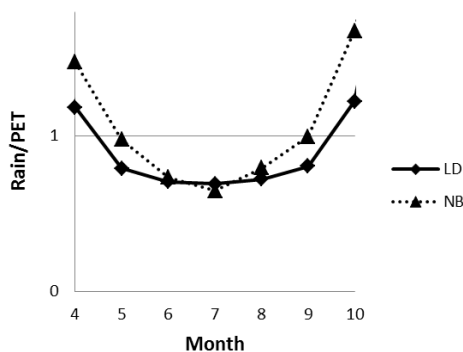
Figure A.2. Monthly average ratios of precipitation to potential evapotranspiration from April to October. Average values were calculated using the data of 1931 to 2014.
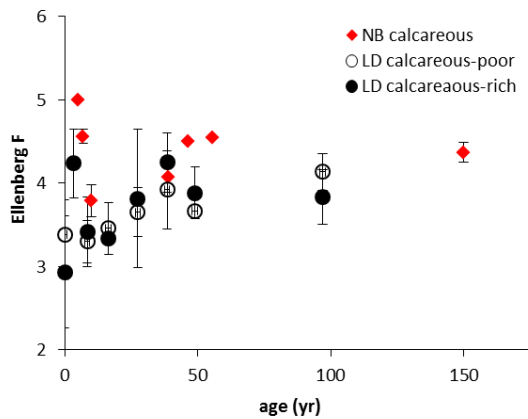
Figure A.3. Average Ellenberg value for moisture of the plant species recorded in the plots. For each vegetation record average values were calculated based on presence/absence data. Points indicate averages of vegetation records and bars standard deviation.

Effects of climate on soil and vegetation in CENTURY model

In the CENTURY model, soil moisture influences SOM decomposition, plant growth, shoot death, and root death, and soil temperature influences SOM decomposition and plant growth. On one hand, SOM decomposition and plant growth were reduced by low soil moisture content slightly more strongly in LD than in NB (Figure A.4 a&b) and shoot and root death rate were higher in LD than NB. On the other hand, SOM decomposition and plant growth were reduced by low soil temperature more strongly in NB than in LD (Figure A.4 c&d). Altogether, the influence of climatic factors (i.e. soil moisture and soil temperature) on SOM decomposition and plant growth was almost indifferent between LD and NB, and so was that on soil C and N accumulation.
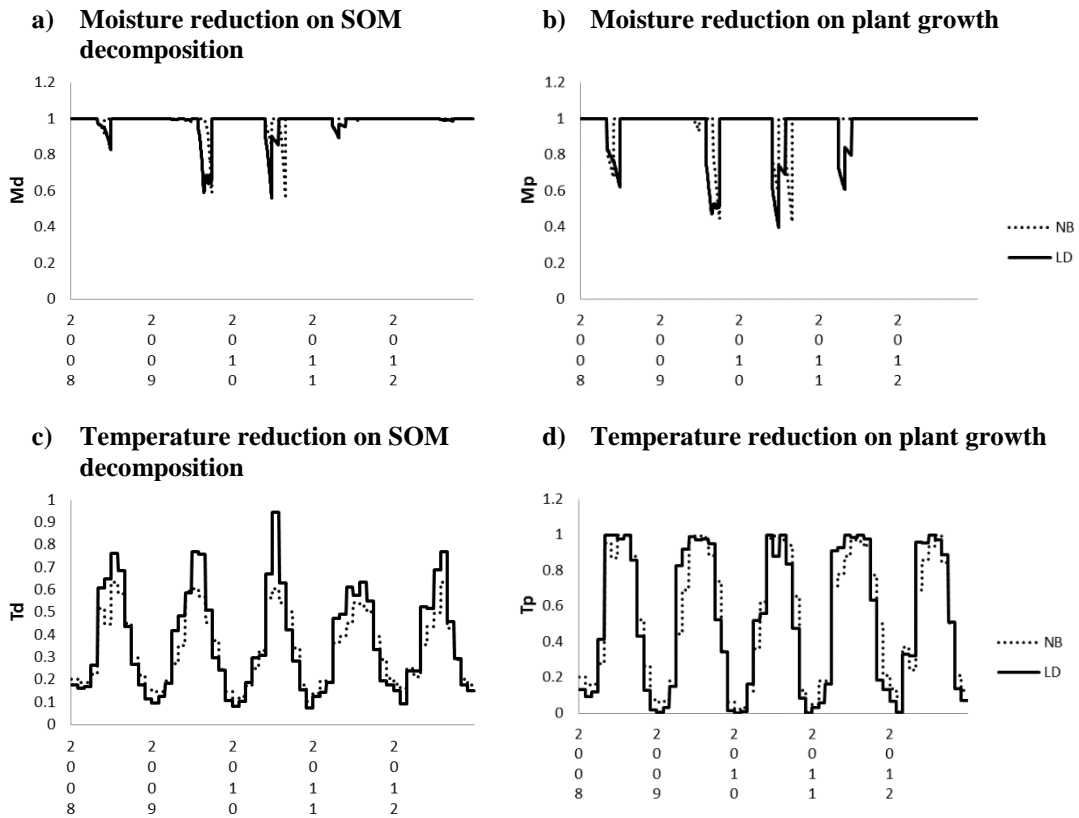
Temporal changes in climate over years

In last decades annual precipitation was slightly increasing in LD (Figure A.5a) and therefore the difference in annual precipitation between LD and NB became smaller. Annual average temperature and potential evapotranspiration were increasing in last decades for both LD and NB (Figure A.5b, A.5c).

a) Moisture reduction on SOM decomposition

b) Moisture reduction on plant growth

c) Temperature reduction on SOM decomposition

d) Temperature reduction on plant growth

Figure A.4. Soil moisture reduction term on soil organic matter decomposition (a) and on plant growth (b) and soil temperature reduction term on soil organic matter decomposition (c) and on plant growth (d) in the CENTURY model. The reduction terms were computed from a 5-year simulation (from 2008 to 2012) with low atmospheric N deposition level and no symbiotic N fixation (thus the difference between NB and LD is caused merely due to climate). Value 1 means there is no reduction due to soil moisture or temperature, whereas value 0.5 means that soil moisture or temperature reduce SOM decomposition or plant growth to 50 %.

a)

b)

c)
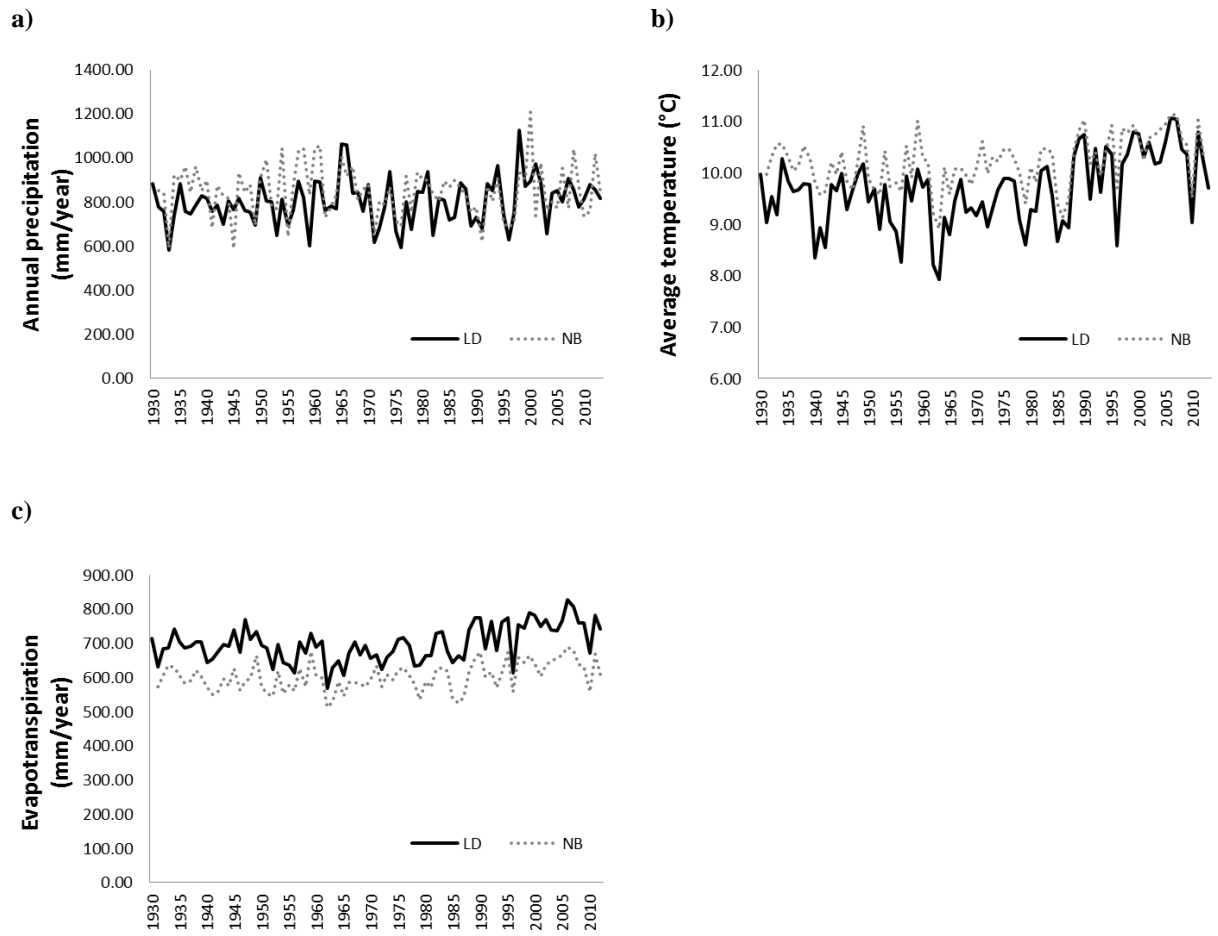
Figure A.5. Annual average values of precipitation (a), daily temperature (b), and potential evapotranspiration (c) of LD and NB from 1931 to 2012.

761   **Atmospheric N deposition**

762

763   Historical atmospheric N deposition levels in NB and LD are shown in Figure A.6.

764   See Section 2.6 for the source of the data. Atmospheric N deposition level was similar

765   between LD and NB till ca. 1920's. After that LD had higher levels (except during the

766   World War II period), with a peak around 1970-1990 reaching almost 40 kgN ha^{-1} yr^{-1}

767   ^{1}. NB had only slightly elevated levels of atmospheric N deposition in the last

768   decades, ranging between 5-10 kgN ha^{-1} yr^{-1}.

769
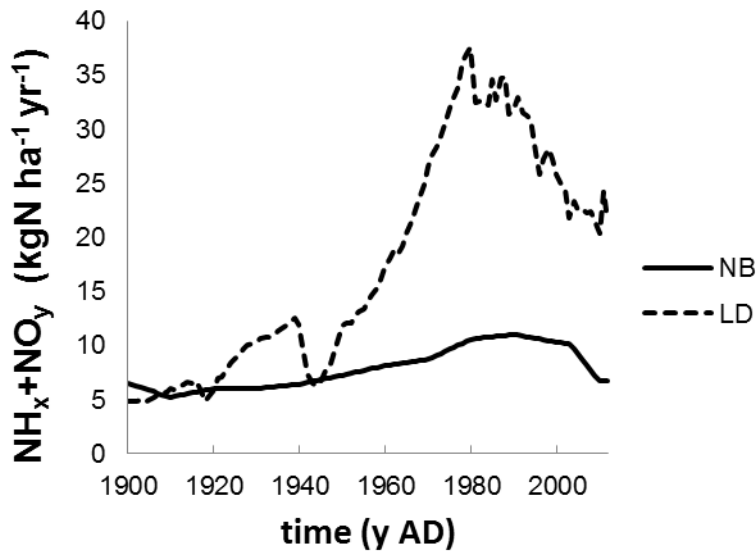
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771   **Figure A.6.** Atmospheric N deposition (wet plus dry, NH_x plus NO_y) of NB and LD from 1900 to
772   2012.

773

Appendix B. Model plausibility analysis and model sensitivity analysis

<online>

Model plausibility analysis

Observation dataset

To test the plausibility of the model, we used soil C, soil N, and soil C:N ratio of three chronosequences which were used for the statistical analysis in the main text. In addition, we also used above-ground biomass of vascular plants. Above-ground biomass of vascular plants (g m^{-2}) was measured in 2012 as standing crop in summer. The biomass data is available only for a part of the plots for NB. See Table B.1 for overview of the observation dataset for each variable. Since we have simulated for 75 years only, the observed data of oldest successional stages (i.e. 162 years old for NB, 97 years old for LD) were compared to the modelled values of 75 years simulation.

Comparison between model prediction and observation

The model predicted soil C-accumulation reasonably well, although the difference between calcareous and acidic sites in LD was overestimated by the models (Figure B.1a). Soil N accumulation was underestimated by the model, especially for NB and acidic LD dunes (Figure B.1b). For calcareous dunes, the model predicted lower N accumulation in NB than in LD, while the measured values of N-accumulation were higher for NB than LD. Soil C:N ratio was constantly overestimated by the model (Figure B.1c). The increase in soil C:N ratio in the beginning of succession, which was observed clearly in LD, was reproduced by the model. Predicted above-ground plant production was in the same range for old stages of NB, lower for young stages of NB, and generally higher for LD (Figure B.1d).
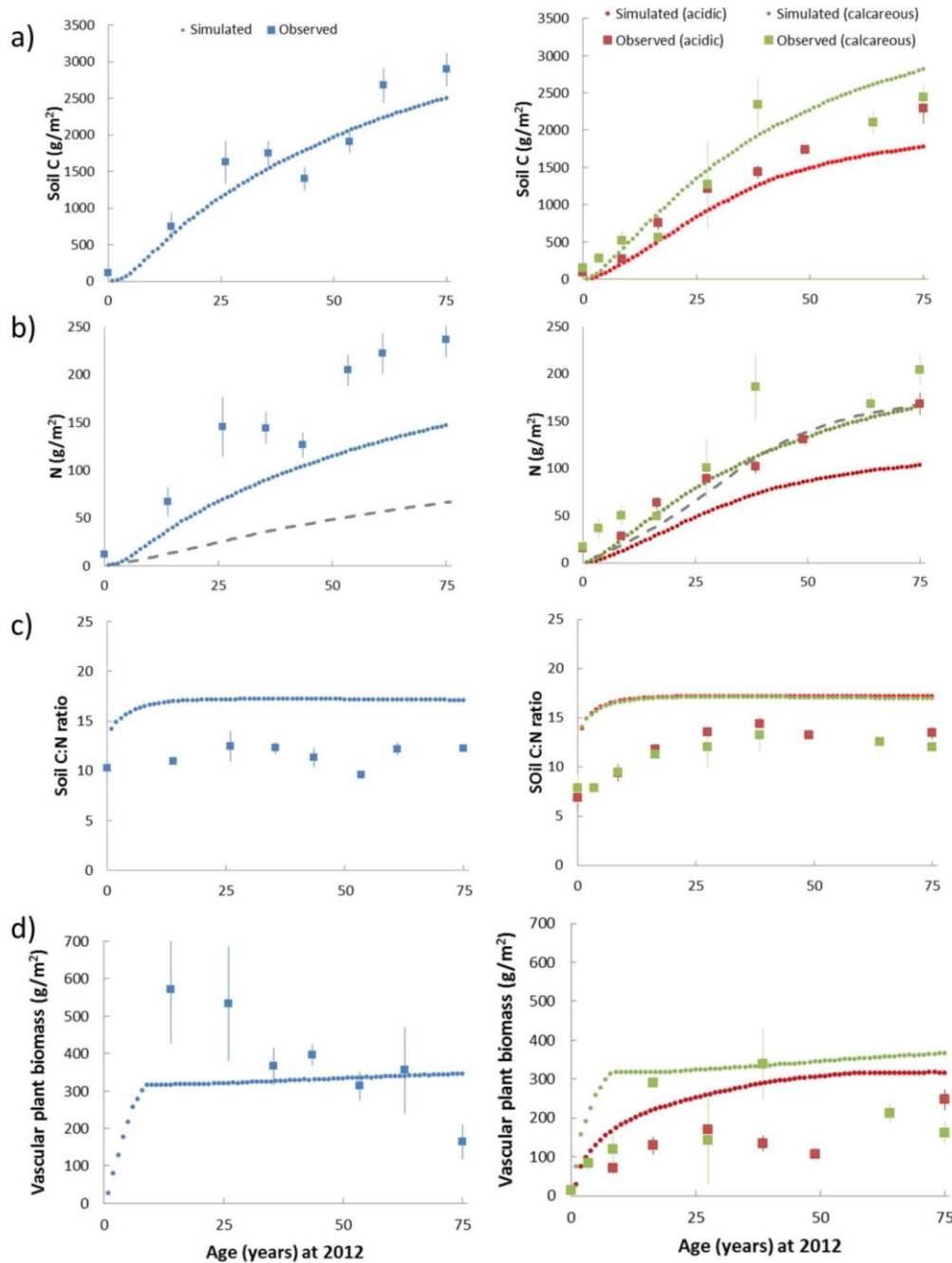
804
805 **Figure B.1.** Simulation results of soil C pool (a), soil N pool (b), soil C:N ratio (c) and vascular plant
806 biomass (d) for calcareous-rich Newborough (NB; left) and calcareous and acidic Luchterduinen (LD;
807 right). Model was run for 75 times for 1-year-old dune (i.e. started as bare sand from January 1st 2012,
808 simulated till December 31th 2012) up to 75-year-old at year 2012 (i.e. started as bare sand from
809 January 1938, simulated till December 31th 2012) with an interval of 1 year. Thus, each point
810 corresponds to a simulation exercise (i.e. the output value at the end of the simulation period).
811 Observed values (average ± SE) in chronosequence are shown with squares. 49-year –old acidic dune
812 of LD and 16.5-year-old calcareous dune of LD have only one observation record and therefore have
813 no SE bars. For the presentation purpose, the observation values of the oldest plots (i.e. 162-year-old in
814 NB and 97-year-old in LD) were plotted as 75-year-old in these figures. Dotted lines in Figure b) are
815 cumulative amount of atmospheric N deposition during each simulation period.
816

817 **Table B.1.** Age classes per chronosequence with number of plots and values (average and SD) for C
818 pool, N pool, C: N ratio and pH of the topsoil, and standing crop of vascular plants.
819

| age class	interval	N	C pool	N pool	C:N ratio	pH	standing crop
y	yr yr^{-1}		kg m^{-2}	kg m^{-2}	g g^{-1}		g m^{-2}
Newborough (NB)							
0	0	6	0.12 ± 0.06	0.012 ± 0.003	10.2 ± 2.6	8.4 ± 0.2	
14	6-22	6	0.75 ± 0.45	0.067 ± 0.037	11.0 ± 0.7	8.1 ± 0.2	570 ± 351
26	22-30	6	1.63 ± 0.70	0.145 ± 0.076	12.5 ± 3.7	7.9 ± 0.3	533 ± 370
35.5	30-41	6	1.75 ± 0.43	0.144 ± 0.040	12.3 ± 1.4	7.9 ± 0.5	366 ± 121
43.5	41-46	6	1.40 ± 0.38	0.126 ± 0.031	11.4 ± 2.4	7.8 ± 0.3	396 ± 68
53.5	46-61	5	1.90 ± 0.31	0.204 ± 0.040	9.6 ± 0.8	7.7 ± 0.3	314 ± 94
61	61-65	6	2.68 ± 0.57	0.222 ± 0.052	12.2 ± 1.6	7.5 ± 0.3	355 ± 282
162	>65	6	2.90 ± 0.54	0.237 ± 0.046	12.3 ± 0.3	6.5 ± 0.5	164 ± 115
Luchterduinen (LD) calcareous							
0	0	5	0.15 ± 0.15	0.017 ± 0.012	7.8 ± 3.2	8.4 ± 0.4	14 ± 20
3.5	1-6	3	0.28 ± 0.12	0.036 ± 0.019	7.9 ± 0.5	7.7 ± 0.2	84 ± 36
8.5	6-11	9	0.51 ± 0.44	0.050 ± 0.031	9.5 ± 1.4	7.5 ± 0.2	119 ± 138
16.5	11-22	1	0.55	0.049	11.3	7.3	290
27.5	22-33	2	1.27 ± 0.84	0.100 ± 0.045	12.0 ± 3.0	6.3 ± 1.5	143 ± 162
38.5	33-44	6	2.34 ± 0.86	0.186 ± 0.084	13.2 ± 3.7	6.4 ± 0.9	339 ± 224
64	54-74	13	2.10 ± 0.51	0.168 ± 0.044	12.6 ± 1.4	5.7 ± 0.7	213 ± 89
97	>74	9	2.44 ± 0.53	0.205 ± 0.048	12.0 ± 1.4	6.0 ± 1.1	162 ± 89
Luchterduinen (LD) acidic							
0	0	6	0.10 ± 0.05	0.015 ± 0.007	6.9 ± 3.0	8.2 ± 0.3	13 ± 14
8.5	1-6	8	0.27 ± 0.11	0.028 ± 0.005	9.4 ± 2.5	7.4 ± 0.5	71 ± 36
16.5	11-22	10	0.75 ± 0.28	0.063 ± 0.021	11.8 ± 1.7	6.4 ± 0.9	129 ± 71
27.5	22-33	8	1.21 ± 0.34	0.089 ± 0.023	13.6 ± 1.3	5.0 ± 0.2	171 ± 106
38.5	33-44	12	1.44 ± 0.32	0.102 ± 0.024	14.4 ± 2.1	4.5 ± 0.3	135 ± 70
49	44-54	1	1.73	0.131	13.3	4.4	107
97	>74	17	2.29 ± 0.87	0.169 ± 0.049	13.5 ± 2.4	4.2 ± 0.3	247 ± 107

820
821

822 **Model sensitivity analysis of key parameters**

823

824 We tested how parameters controlling symbiotic and asymbiotic N fixation influence
825 soil N accumulation after 75 years of simulation (1938-2012). The proportion of
826 symbiotic N fixers has very strong effect on soil N accumulation around the range of
827 dune ecosystems (less than ca. 20%, i.e. *nfxrat* < 0.2) (Fig B.2a). Soil N accumulation
828 does not increase rapidly at higher proportion because the system becomes limited by
829 other factors (e.g. plant production becomes limited by water availability).

830

831 Asymbiotic N fixation does have linear effect on soil N accumulation (Fig B.2b). The
832 rate of increase is faster for NB than LD because NB receives less atmospheric N
833 deposition than LD and therefore overflow of N via leaching is less in NB.

834
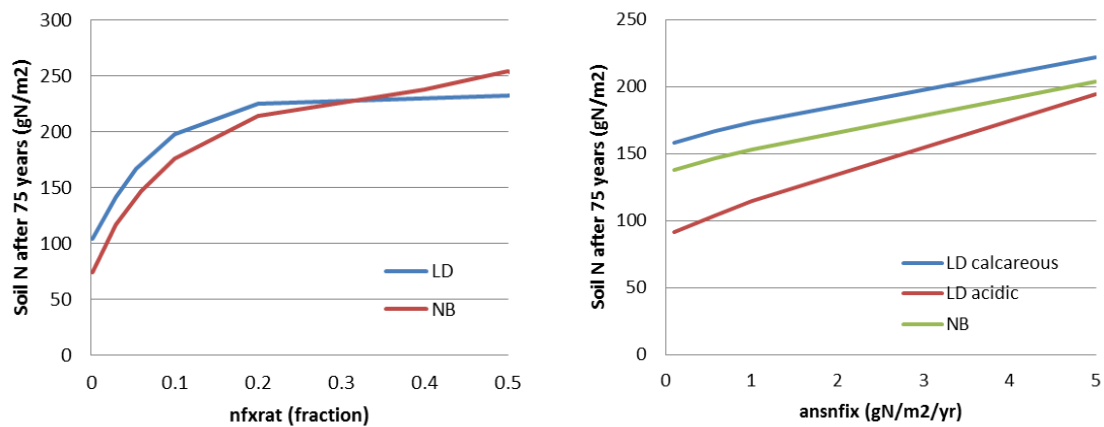
    a)   Proportion symbiotic N fixers'*nfxrat*'   b)   asymbiotic N fixation '*ansnfix*'

835 **Figure B.2.** Changes in model output values of soil N accumulation after 75 years simulation against
836 different levels of proportion of symbiotic N fixers '*nfxrat*' (Fig B.2a) and asymbiotic N fixation
837 '*ansnfix*' (Fig B.2b. The default values for the parameters are *nfxrat*=0.06 for NB, *nfxrat*=0.054 for LD
838 calcareous, *nfxrat*=0.0014 for LD acidic; *ansnfix*=0.57 gN m^{-2} yr^{-1} for all chronosequences.

839

Appendix C. Model description

<online>

CENTURY model

General settings

We reconstructed the CENTURY model based on the equations published in literature (Parton et al. 1998; Parton et al. 1987; Parton et al. 1993; Parton et al. 1988) and source code (Metherell et al. 1993). The model consists of different processes, e.g. plant production, SOM dynamics, hydrology. We used the equations and parameter values of CENTURY version 4 (Metherell et al. 1993) unless specified. When a parameter value is specific for different vegetation types, we took those for temperate grasslands ('TG'). The original CENTURY computes SOM dynamics weekly and plant production monthly, whereas we simulate them on a daily basis. Accordingly, parameter values with weekly and monthly rates were converted to daily rates (with a factor 1/7 and 1/30, respectively).

CENTURY uses multiple soil layers with constant depths. We set three soil layers of 20 cm (topsoil), 50 cm, and 50 cm depth. We assumed that all soil organic matter exist in the topsoil layer. Water and dissolved N moves between soil layers. We assumed that all roots are distributed in the topsoil layer. This means that plant took up nutrients only from the topsoil layer, and drought stress on plant production was controlled by moisture content of the topsoil. Mineral N pool was split into ammonium and nitrate pools, as in the daily version of CENTURY, DAYCENT (Del Grosso et al. 2002). With these pools, we also simulated nitrification and denitrification processes following the DAYCENT model.

Below is the brief explanation of each sub-module of CENTURY model, as well as modifications of equations and parameter values made for this study. For more detailed description of CENTURY model, see original literature (Parton et al. 1998; Parton et al. 1987; Parton et al. 1993; Parton et al. 1988).

Plant production

Potential above-ground plant growth is calculated by multiplying the vegetation-specific maximum growth rate with three controlling factors; soil temperature, soil moisture, and self-shading. In our study, the maximum growth rate was obtained by the optimization procedure (see section 'Parameter optimization'). Allocation of biomass to shoot and root was set to be constant and calculated as a function of typical annual precipitation of the study sites, i.e. 80 cm yr^{-1}. To prevent unrealistically fast growth in the first years when the simulation starts from bare soils, we added an equation to restrict plant production relative to their current biomass, with the maximum relative growth rate (g g^{-1} d^{-1}). We used the mean value of maximum relative growth rate of 105 UK plants on a weekly basis, 1.2 g g^{-1} week^{-1} (Dawson et al. 2011), converted to a daily value with an assumption of exponential rather than arithmetic growth (i.e. 0.12 g g^{-1} d^{-1}).

Actual plant growth is controlled by the availability of N. C:N ratio of shoots and roots change dynamically within the minimum and maximum C:N ratios, depending

29

890 on available mineral N in soil and from symbiotic N_2 fixation. The minimum and
891 maximum C:N ratios of shoots were calculated as a function of shoot biomass. Since
892 the minimum and maximum C:N ratios of shoots in CENTURY ver.4 for temperate
893 grasslands (maximum C:N ratio 90-95, minimum C:N ratio 30-35) were much higher
894 than the range observed in our field study in Dutch dune ecosystems (Fujita et al.
895 2013b), we adapted these parameters to the empirical values in our dataset: maximum
896 C:N ratio 36 (50th percentile) – 59 (90th percentile); minimum C:N ratio 25 (10th
897 percentile) – 36 (50th percentile).
898
899 Available mineral N in soil for plant uptake, $Nava$, is restricted by root biomass as:
900
901 $Nava = mineralN \cdot (1 - riint \cdot \exp(-rictrl \cdot C_R \cdot ratbioC))$ (C.1)
902
903 where $mineralN$ is mineral N (nitrate plus ammonium) in topsoil (gN m^{-2}), C_R is the
904 amount of C in root (gC m^{-2}), $riint$ and $rictrl$ are the coefficient values to determine
905 the shape of root-size effect on N availability. The default values of $riint$ and $rictrl$ in
906 CENTURY ver.4 are 0.8 and 0.015, respectively. To restrict the nutrient uptake when
907 root biomass is very small in the beginning of succession, we changed the $riint$ value
908 from 0.8 to 0.99 (i.e. 1% of nutrient is available for roots when there is no root,
909 instead of 20 %). $rictrl$ value was obtained by the optimization procedure (see section
910 'Parameter optimization').
911
912 Symbiotic N fixation is assumed to occur when soil mineral N is not sufficient to
913 satisfy the plant N demand having taken into account all the other factors which limit
914 growth of plant (e.g. temperature, moisture). Symbiotic N fixation can occur up to a
915 maximum level of N fixed per C fixed, with a N:C ratio specific to each plant type
916 ('$snfxmx$'). Since CENTURY model assumes monoculture crop systems, the value of
917 $snfxmx$ is either 0 gN gC^{-1} (for non-N-fixers) or 0.0375 gN gC^{-1} (for legume crops
918 such as alfalfa). In natural ecosystems, however, symbiotic N-fixers (predominantly
919 legume species) are ubiquitous and they typically occupy a few percent of the total
920 cover. Thus, we introduced a new parameter, $nfxrat$ (fraction between 0 and 1), to
921 include the proportion of N fixers in plant production.
922
923 The maximum symbiotic N fixation ('$nfixmx$', gN m^{-2} d^{-1}) is computed as:
924
925 $nfixmx = P_P \cdot snfxmx \cdot nfxrat$ (C.2)
926
927 We used different values of $nfxrat$ for the three chronosequences based on measured
928 percentages of plant species associated with N fixers (see section 2.6). If available
929 mineral N plus maximum symbiotic N fixation is enough to support the potential
930 production with the maximum C:N ratio (i.e. N-poor biomass), actual production
931 equals to potential production. If not, actual production is reduced.
932
933 Death of shoots is calculated as a function of soil moisture and shading effects. In
934 addition, in the beginning of winter, 95% of living shoots die. Death of roots is
935 calculated as a function of soil moisture and soil temperature. Dead shoots flow into
936 standing dead pool, and then flow into surface litter pools (i.e. surface metabolic and
937 surface structural) with a function of lignin:N ratio. Dead roots flow into root litter
938 pools (i.e. belowground structural and belowground metabolic) with a function of
939 lignin:N ratio.

941 *Soil organic matter dynamics*

942

943 C in soils is divided into 8 pools (surface structural and metabolic, belowground
944 structural and metabolic, surface microbe, active, slow, passive). C in each pool is
945 decomposed with pool-specific maximum decomposition rates, multiplied with
946 reduction factors by soil moisture and soil temperature. We optimized the maximum
947 decomposition rate of the slow pool (see section 'Parameter optimization'). The
948 decomposed C flows into other pools, and some of the flows were influenced by
949 lignin content and soil texture.

950

951 *N dynamics*

952

953 Soil organic N flows are coupled with C flows. The outflow of N is proportional to
954 that of C, whereas the inflow of N into a pool is the product of the C inflow into the
955 pool and the N:C ratio of the pool. N:C ratios of soil pools varies, as N:C ratios of
956 inflows change dynamically depending on N concentration of the plant residues (for
957 surface microbe pool) or mineral N (for active, slow, and passive pools).

958

959 N is mineralised if in excess, and immobilized from mineral N pool if in shortage.
960 When potential N mineralisation is negative (i.e. net N immobilization) and if the
961 amount of ammonium concentration in soil is not enough to enable the
962 immobilization, decomposition is inhibited.

963

964 Nitrification and denitrification are modelled according to the daily version of
965 CENTURY, DAYCENT (Del Grosso et al. 2002), except for the pH-dependent terms
966 of nitrification and denitrification. Since mineral N pool in the top soil is not
967 separated into nitrate and ammonium (and therefore no preferential uptake of nitrate
968 or ammonium by plants, no difference in leaching rates between nitrate and
969 ammonium), the influence of the nitrification is restricted to the subsequent
970 denitrification in our model. Note that, in our study sites, denitrification hardly occurs
971 due to the prevailing dry conditions in the soil.

972

973 N leaching is computed as amount of mineral N (ammonium and nitrate) moving
974 from the 3rd soil layer to the layer beneath. We did not use the soil texture effect on N
975 leaching used in CENTURY ver.4, as it bases on empirical relationship which we
976 could not check if it is applicable to our target ecosystem. Instead, transfer of mineral
977 N in soil was simply calculated as the products of the water flows and the
978 concentration of mineral N in the originating layer.

979

980 N input via atmospheric deposition (dry plus wet, as ammonium or as nitrate) was
981 simply added to the ammonium pool and nitrate pool in the topsoil layer. The annual
982 rate of N deposition was divided by 365 to get the daily rate of N deposition.

983

984 Non-symbiotic N fixation (i.e. N fixation by free-living microorganisms) is
985 formulated either as a function of precipitation or as a function of N:P ratio in mineral
986 pool in CENTURY ver. 4. Since the coefficient values of these functions in
987 CENTURY were obtained by model tuning procedure (Parton et al. 1987) and
988 therefore not underpinned by theoretical or empirical evidence, we assumed simply a
989 constant rate of non-symbiotic N fixation all through the year. We used a the median

990 value of the study in temperate unfertilized grasslands (Reed et al. 2011), 0.57 gN m^{-2}
991 yr^{-1}. The fixed N is added to the ammonium pool of mineral N in the topsoil layer.
992
993 *Hydrology*
994
995 A simple hydrological model of CENTURY model (Parton et al. 1993) was used to
996 simulate water flows between soil layers. Snow and liquid snow were omitted. Plant-
997 related parameters (living biomass, standing dead materials, surface litter) were
998 updated every month from the plant production module of the model. Equations of the
999 hydrological module are fully described in Appendix B of Parton et al. (1993).
1000
1001 **Parameter optimization**
1002
1003 We optimized the following three model parameter values: the maximum above-
1004 ground plant growth per day under optimal condition (gC m^{-2} d^{-1}), the parameter
1005 controlling the impact of root biomass on nutrient availability for plants, and
1006 decomposition rate of the slow pool of soil organic matter (fraction/day). The
1007 parameter values were optimized using least-square object functions of regression
1008 methods using the software 'UCODE' (Hill and Tiedeman 2007). The purpose of this
1009 procedure is to optimize these global (i.e. not site-specific) parameters so that the
1010 model outputs are roughly tuned to typical ranges in dune ecosystems. Because
1011 incorporating pH as a controlling factor for decomposition in dune soils is still
1012 difficult (unpublished data), we calibrated one model for both calcareous and acidic
1013 soils. Therefore, we used observed data of LD, averaged over calcareous and acidic
1014 sites together, but not that of NB which has calcareous sites only. The used
1015 observation data was soil C, soil N, and above-ground plant biomass at the peak
1016 season of five succession ages.
1017
1018 **Model initial values**
1019
1020 For all sites, we assumed that dune succession starts from bare sand. Although our
1021 measurements indicated that bare sand contains a little amount of soil C and N, we
1022 assumed in the model that the initial bare sand does not contain any C and N. Ignoring
1023 those initial amounts of C and N hardly affected the model outputs. Grain analysis of
1024 similar soils to NB and LD suggested that there are only ignorable amount of clay and
1025 silt in the soils in our plots: no clay or silt in west coast dunes similar to NB (Allen et
1026 al., 2014), and only ~0.6% clay and ~1.7% silt in the same dune areas as LD (Fujita et
1027 al., 2013a). For simplicity, we used the same proportion of clay, silt, and sand content
1028 for both sites as model inputs: 0% for clay, 5% for silt, and 95 % for sand. Parameters
1029 to determine soil water retention characteristics (i.e. water content at wilting point,
1030 0.010, and at field capacity, 0.124) were calculated for pressure heads of -16000 cm
1031 and -100 cm, respectively, using soil physical parameters of typical Dutch sandy soils
1032 (Wösten et al. 2001).
1033
1034 Initial water content of three soil layers was obtained by running the hydrological
1035 model for 365 days prior to the start of the simulation date. Initial concentrations of
1036 mineral N (nitrate and ammonium) in the soil water were assumed to be zero.
1037
1038 **References**
1039

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