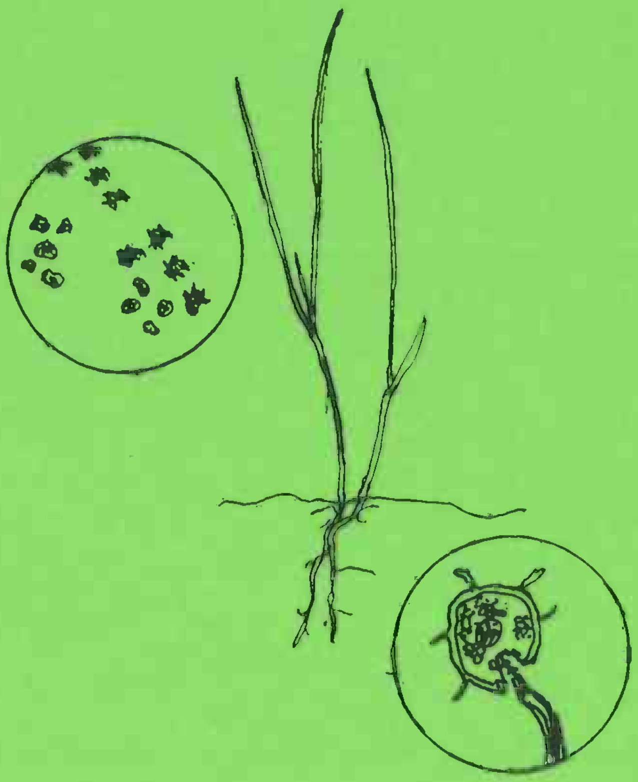
IMPACTS OF ABOVE - AND BELOW-GROUND HERBIVORY ALONG A SUCCESSIONAL GRADIENT



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Summary

Above- and below-ground herbivory can have differnent effects on structuring vegetation. The aim of this research was to investigate their importance along a successional gradient in coastal sand dunes at the island of Schiermonnikoog. Since in this system nutrients may play an important role, nutrient limitation was also investigated. A herbivore excluding experiment was done at three stages of the chronosequence, consisting of four blocks with a different grazing treatment: excluding above-ground herbivory by rabbits and hares, excluding below-ground herbivory by nematodes, excluding both above- and below-ground herbivory and a control. In each exclosure a fertilization experiment was carried out with adding N, P, N and P control with no fertilization.

Nutrients were limiting in the system: N in all stages, P in stage 1 and 2. Consequently, they may play a role in succession. Excluding above-ground herbivory caused biomass as a whole to increase, whereas the impact was different among species: mostly decreasing on low-stature or early successional species (like *Sedum acre* or Mosses, and increasing on high-stature or late successional species (like *Festuca rubra* and other grasses). Reducing below-ground herbivory by nematodes did have no impact on biomass and on plant species composition. Interactions between above-ground and below-ground herbivory were not found.

INTRODUCTION

Nutrient limitation is one of the important features of early stages of vegetation succession; mostly nitrate (N) and/or phosphate (P) are limiting. Limitation will decrease with succession, for organic matter and thus nutrients will begin to accumulate. By this an increase of biomass production is made possible (Grime 1977, Tilman 1985, Van Andel et al. 1993).

Since nutrients are scarce in earlier stages, this will cause the plants to have special adaptions: tolerance to low nutrient levels (Grime 1977), or an ability to compete for nutrients (Tilman 1985, 1990). During succession a shift takes place from nutrient limitation to light limitation (due to nutrient and biomass increase), and therefore light competition will become important (Tilman 1985, 1990). Thus an early successional species should invest more in below-ground parts (higher biomass production and/or allocation), whereas a later successional species should invest more in above-ground parts.

A factor that can have a great impact on vegetation succession is herbivory. It can affect rate and direction in many various ways: indirectly via e.g. accelerating nutrient turnover and thus nutrient availability, or directly by taking away biomass. Competitive relations between plant species will be modified by herbivores: when a dominant plant is eaten by a herbivore, the subdominant species will have a relative advantage. Thus plants can be benefitted and disadvantaged, depending on the herbivore's preferences and the plants' ability to withstand grazing. This is called apparent competition (Louda *et al.* 1990). In a situation with grazing, species that are adapted to nutrient-poor soils can replace species that are characteristic for relatively nutrient-rich soils. Two mechanisms are known to explain this: Firstly, the plant species characteristic for nutrient-poor circumstances has features that enable it to increase biomass allocation to structures that enhance nutrient absorption (Tilman 1988), or it has features that enable it to restrict their nutrient losses due to grazing (Grime 1979, Berendse and Elberse 1990). In a pot experiment differences in nitrogen loss resulting from clipping had an important impact upon the competitive balance between plant species under nutrient-poor conditions: *Festuca rubra*, a species from relatively poor soils, was able to out-compete *Arrhenaterium elatius*, a species from relatively nutrient rich habitats when clipped, but not without clipping (Berendse *et al.* 1992).

Herbivory can roughly be divided into two types: above- and below-ground herbivory, of which different effects are known. Above-ground herbivory reduces standing crop, and can change competitive interactions between plants. Since early successional species have invested relatively more in below-ground and later species more in above-ground parts, taking away above-ground biomass will have a greater impact on plants that have invested more in above-ground parts. Therefore above-ground herbivory will favour low-stature or early successional plants, or in "Tilman terms", will favour good nutrient competitors. Because of this, above-ground herbivory is said to have a slowing down or retrogressive effect on succession (Oksanen *et al.* 1981, Bakker 1985, Brown 1990, Van Andel *et al.* 1993).

According to Grime (1977) species diversity is low in early, nutrient-poor successional stages, then increases in mid-succession an finally decreasing again in late, nutrient-rich stages. A few stress tolerators (Grime) or nutrient competitors Tilman) will dominate in early stages, whereas in late and final stages this will be a few highly competitive (Grime) or light-competitive (Tilman) species. As we have seen, above-ground herbivory will retard succession, so it is to be expected that grazing will cause an increase of species number in nutrient-rich (late successional) habitats, while it reduces species number in nutrient-poor (early successional) habitats. Some field observations did show this, indeed (Bakker 1985, Louda *et al.* 1990).

For below-ground herbivory the situation is the other way around; it reduces root biomass, and since earlier plants will presumably have invested more in below-ground parts, below-ground herbivory will have a greater impact on these than on later successional plants. So contrary to above-ground herbivory, it can favour high-stature or late successional plant species, or in "Tilman terms" good light competitors. Hence, it is said to have an accelerating effect on succession (Brown 1990, Van der Putten *et al.* 1993). Experiments with sterilised dune soil showed that below-ground herbivory indeed favours later successional plants: these "soil-borne diseases" (nematodes) seem to be plant-specific, and do not infect invading species (Van der Putten *et al.* 1993, Van der Putten & Peters 1997/unpublished). This is similar to the prediction that along gradients (spatial or temporal) there will be a change in above-ground herbivores, since food resources of the herbivores change along that same gradient (Louda *et al.* 1990). Furthermore, in primary succession of drift sand landscapes changes were found in nematode community structure (De Goede *et al.* 1993).

Interactive effects of above- and below-ground herbivory have not been described very often so far, and it will be interesting to find out in what respect these types of herbivory influence vegetation processes.

The coastal sand dune system is a habitat in which primary vegetation succession can be observed very well. Along the European Wadden Sea islands tend to grow in the east and be destructed in the west by the sea, including the dunes on the islands. Hence, a chronosequence of dune vegetation is to be expected (Grootjans 1995). In available dunes above-ground herbivores are mostly rabbits and hares, whereas important below-ground herbivores are nematodes and insect larvae.

AIM

The aim of this research is to investigate the importance of above- and below-ground herbivory on structuring vegetation along a successional gradient in coastal sand dunes. Furthermore, nutrient limitation will be investigated.

METHODS

Study area

The study was carried out in the dunes of the Dutch Waddensea island of Schiermonnikoog. At three stages, situated in a chronosequence of primary dune succession (fig. 1), a herbivory manipulating and fertilization experiment was carried out.

When starting the experiment in 1994, the youngest stage was approximately 5 years old, with a vegetation dominated by *Sedum acre* and *Festuca rubra.*

The second stage was approximately 20 years old, with a vegetation dominated by *Festuca rubra*, other abundant species were *Agrostis stolonifera, Trifolium repens* and *Odontitis verna.*

The third stage was approximately 100 years old, with a vegetation dominated by *Holcus lanatus*, *Carex arenaria* and mosses *(Bryopsida)*. Other abundant species were *Festuca ovina* and *Luzula campestris.*

The organic litter layer increases with stage (Van der Veen and Caldeway 1997).

Above-ground herbivores were Lagomorphs: Rabbit (*Oryctolagus coniculus*) and Common Hare (*Lepus europaeus*). Herbivory intensity was measured in a former study (Van der Veen and Caldeway 199&) by counting hare and rabbit droppings (fig. 2). Stage 3 had highest dropping numbers, stage 1 and 2 did not differ significantly.

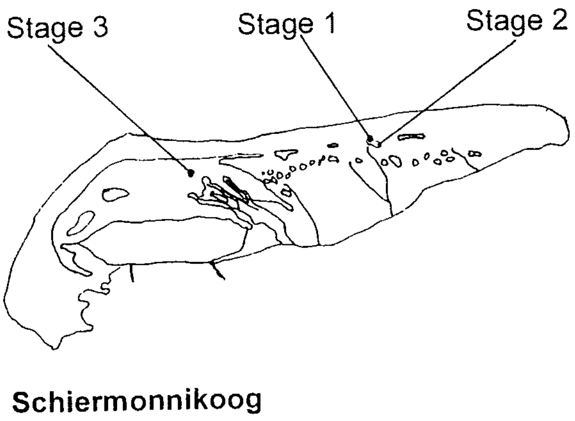

Fig. 1: The island of Schiermonnikoog. Along the north side of the island a chronosequence of dunes can be found. Stages are indicated in the figure; stage 1= 5 years old, stage 2= 20 years old and stage 3= 100 years.

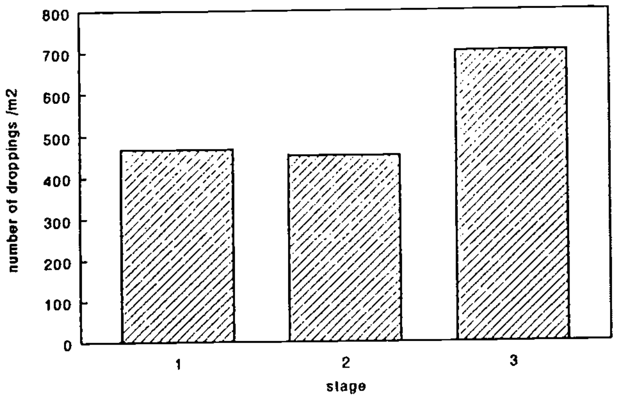

Fig. 2: Hare and rabbit grazing intensity. The herbivory intensity is measured by counting rabbit droppings during the year 1994.

Experimental design

An overview of the total experiment in given in figure 3.

1. Herbivory manipulation

To investigate the influence of above- and below-ground herbivory, a herbivore excluding experiment was done, consisting of four blocks with a different grazing treatment. Each block had two plots of 0.50 x 0.50 m. The treatments were:

- excluding above-ground herbivory by rabbits and hares
- excluding below-ground herbivory by nematodes
- excluding both above- and below-ground herbivory
- control, in which herbivory was not excluded

Above-ground herbivory was excluded by installing an exclosure. The fence was installed into the soil up to 0.40 m below ground level, to prevent digging by rabbits. The height of the fence was 0.50 m above ground level, the mesh width approximately 2.5 cm. The fence was installed in the second week of May 1994.

Below-ground herbivory was excluded using the nematicide Nemacure®. The nematicide was supplied 7 cm below ground level with a density of 10 g/m². This was done by stabbing a shovel at short distances (10 cm) up to 7 cm into the ground, pulling the shovel slightly backwards and dispersing the nematicide pallets into this small gap. From 1994-1996, the nematicide was supplied each year in April.

This was done with three replicates at every stage. In each plot was measured light availability at soil surface, total below-ground biomass, and above-ground biomass of individual species.

2. Fertilization

To test whether nitrogen (N) and phosphorous (P) are limiting factors at the three stages, in each exclosure a fertilization experiment was carried out with two replicates. The treatments were:

Adding
- nitrogen (N)
- phosphate (P)
- N and P
- control with no fertilization

From 1994-1996, in each plot the nutrients were supplied in April with concentrations of 16 g·m². Above-ground biomass of individual species was measured in each subplot.

Description of measurings

1. Light availability at soil surface

At September 4 and 9 1996, light availability was measured by using a stick containing 10 light-sensible cells (PAR). The stick was stuck at soil level into every plot twice, in both opposite diagonal directions. To be able to express light availability at soil surface as a percentage of incoming sunlight, the stick was also held above the vegetation (ca. 1 m above soil surface). To be sure that light availability was measured only in the vegetation, only the three middle cells of the stick were used for analysis.

2. Below-ground biomass per plot

Root biomass was measured by taking soil samples with a soil core sampler (⌀ 7.0 cm, depth 20 cm). Roots were extracted by washing away the sand on sieves, then dried at 70°C for at least 24 h and weighed.

3. Above-ground biomass per plot

At September 23 and 24, above-ground biomass was harvested per half plot (0.25 x 0.5 m). These vegetation samples were sorted out per species, dried at 70°C for at least 24 h and weighed.

Measuring nematode numbers

From every herbivory treatment soil samples were taken using an auger (⌀ 0.79 cm, depth 10 cm). From every treatment 5 samples were taken from each replicate, and these were put together as one bulk sample.

Extraction of the nematodes from the soil samples was done with the method of Oostenbrink (1960). From the roots and organic material, nematodes were extracted with the Baermann funnel method (Hooper 1986). Before the extractions, the soil samples were weighed to express the number of nematodes per gram soil.

The extracted nematodes of each sample (from both methods) were diluted in 100 ml of water. From this solution 2 x 5 ml was taken to count the nematodes under a stereo-microscope.

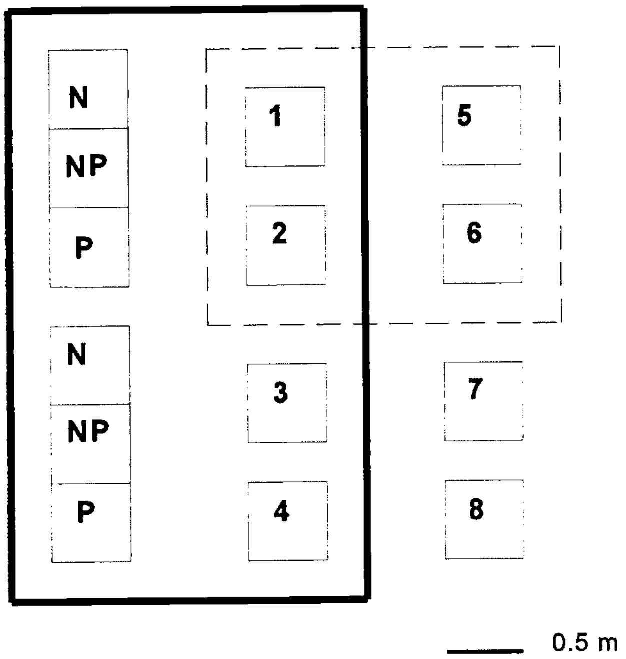

Fig. 3: **Overview of the experiment.** N = nitrogen fertilization, P= phosphate fertilization, NP= fertilization with both nitrogen and phosphate.
— Exclosure — — Nematicide

Species identification was done after the nematodes were fixated with formaldehyde to prevent their disintegration of. Plant feeding nematodes have been identified and counted.

Statistical analysis

Light availability was tested with a univariate analysis of variance, regression analysis of light availability and above-ground biomass was done with a multivariate, simple linear regression.

All other variables (except per species biomass) were tested using a univariate analysis of variance. According to Shen (1995), variables should be nested if it is not clear whether the variable is bound to a site. Herbivory can vary with stage, and does as was shown for above-ground herbivory (fig 2). So the level of herbivory treatment is not identical, and the treatments are nested within site and stage. Since the fertilization treatment is the same at every stage, it is not nested within stage, but only within site. Nesting within site was done, because by this variables are weighted parallel. Per species biomass was tested using a multivariate analysis of variance. Testing was done per stage, variables were nested within site.

If necessary, data were transformed to improve homogeneity. Data in figures are showed untransformed. The statistical analysis was done using the program SPSS/PC+.

RESULTS

Results of all statistical analysis are given in the appendix.

Fertilization

Overall effects

Above-ground biomass (fig. 4) increases in all stages when fertilizing with nitrate (N). Fertilization with phosphate (P) had a significant effect on biomass, too, but its effect seems to be variable: in stage 1 and 2 it is increasing, in stage 3 decreasing. There was no interaction effect of N and P.

The percentage dead biomass of total biomass (fig. 5) was significantly influenced by stage and fertilization with N and with P. Percentage dead increased when fertilized. Percentage dead biomass also increases with stage.

Species number (fig 6) was effected significantly by stage and N-fertilization. Species number increased with stage, decreased due to fertilizing.

Effects on species, per stage

In table 1 in the appendix is given an overview of the species found at every stage.

In stage 1 (fig 7) N-fertilization has influenced plant species. Dead biomass and *Festuca rubra* increase, the dicots (*Sedum acre* and *Leontodon saxatilis*) and *Agrostis stolonifera* decrease.

Stage 2 (fig. 8) was also influenced by the N-fertilization treatment. Dead biomass increases (or decomposition rate decreases), and two plant species, *Festuca rubra* and *Potentilla anserina* increase. Other species showed no significant response to fertilization.

In stage 3 (fig. 9) no significant effects of fertilization were found at species level.

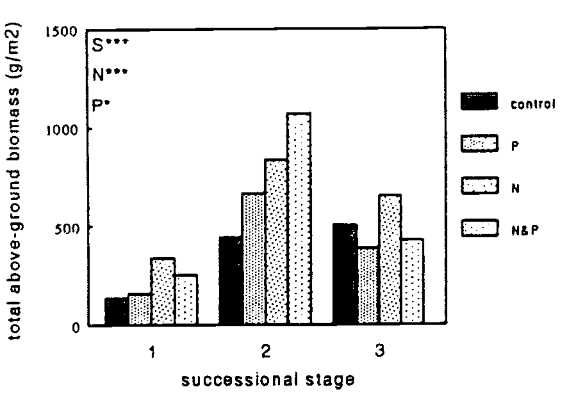

Fig. 4: **Effects of fertilization and successional stage on above-ground biomass.** Statistical significances are indicated as follows: S= stage, N= fertilization with nitrogen, P= fertilization with phosphate; * p< 0.05, ** p<0.01, *** p<0.001.

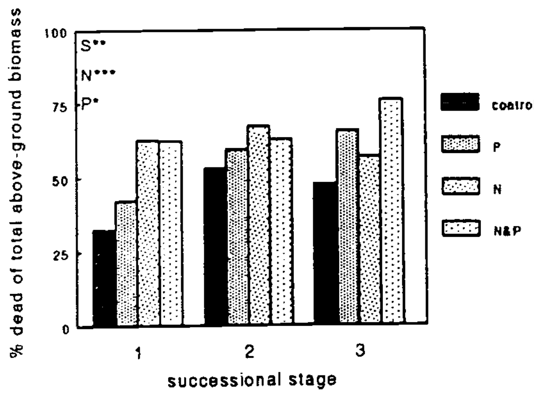

Fig. 5: **Effects of fertilization and successional stage on percentage dead biomass.** Statistical significances are indicated as follows: S= stage, N= fertilization with nitrogen, P= fertilization with phosphate; * p< 0.05, ** p<0.01, *** p<0.001.

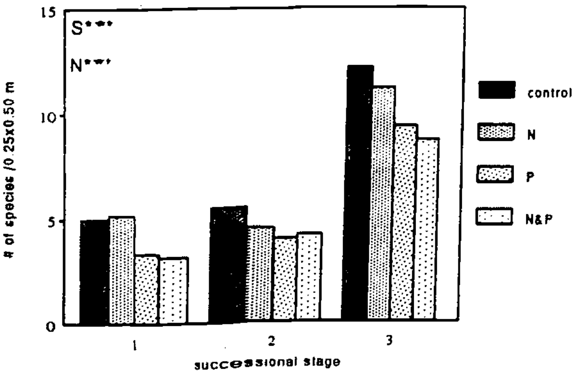

Fig.7: **Stage 1:The effect of fertilization and successional stage on species biomass.** Statistical significances are indicated as follows: N= fertilization with nitrogen;
* p< 0.05, ** p<0.01, *** p<0.001.

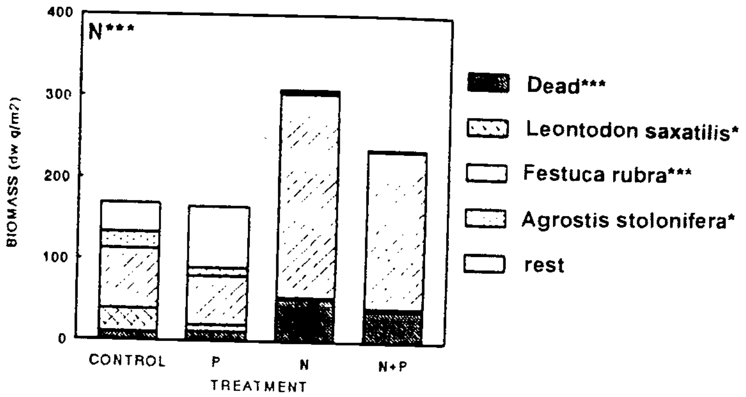

Fig. 6: **Effects of fertilization and successional stage on number of species.** Statistical significances are indicated as follows: S= stage, N= fertilization with nitrogen, P= fertilization with phosphate; * p< 0.05, ** p<0.01, *** p<0.001.

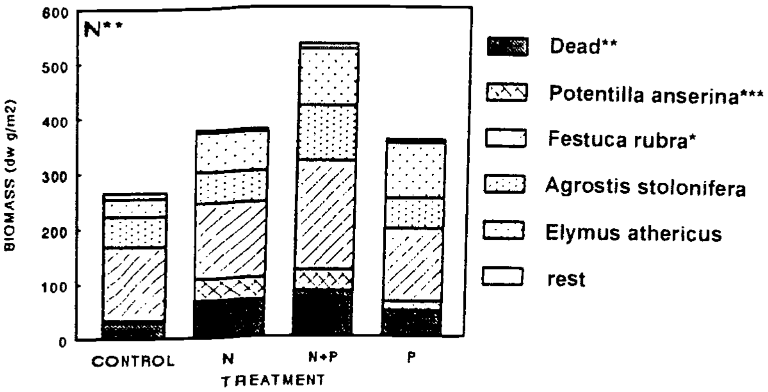

Fig.8: **Stage 2:The effect of fertilization and successional stage on species biomass.** Statistical significances are indicated as follows: N= fertilization with nitrogen;
* p< 0.05, ** p<0.01, *** p<0.001.

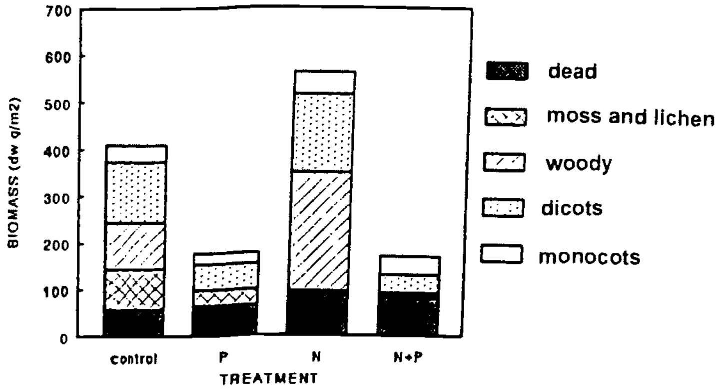

Fig.9: **Stage 3:The effect of fertilization and successional stage on species biomass.** Statistical significances are indicated as follows: N= fertilization with nitrogen;
* p< 0.05, ** p<0.01, *** p<0.001.

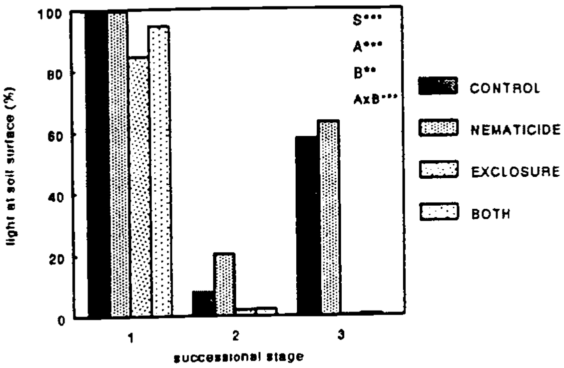

Fig. 10: Light availability at soil surface, expressed as percentage of incoming light above the vegetation. Statistical significances are indicated as follows: : S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

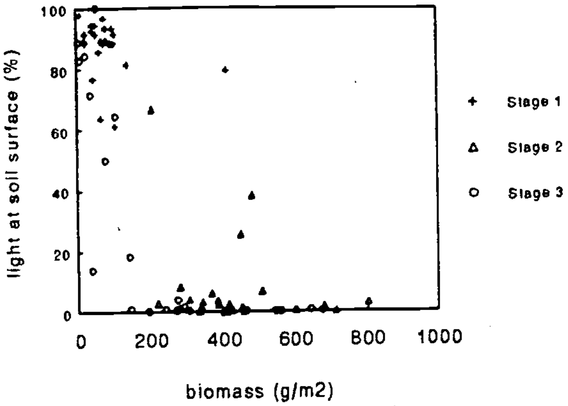

Fig. 11: Light availability at soil surface in relation to above-ground biomass, $r^2=0.599$.

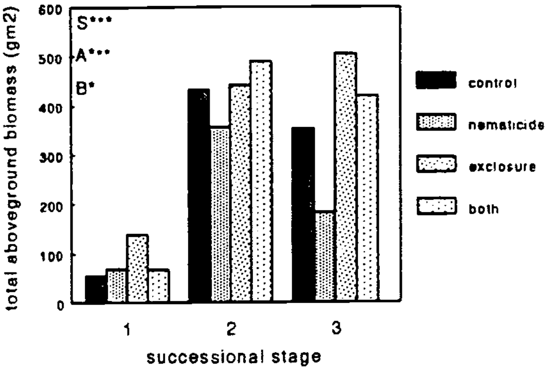

Fig 12: Effects of successional stage, excluding above-ground and below-ground herbivory on above-ground biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

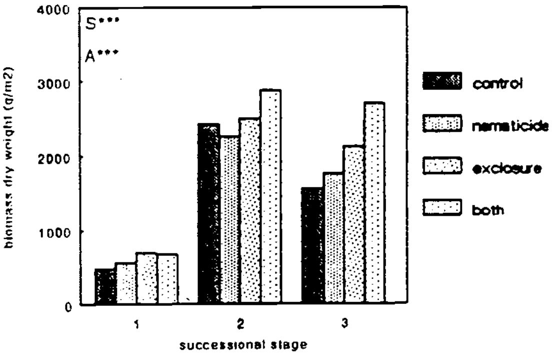

Fig 13: Effects of successional stage, excluding above-ground and below-ground herbivory on below-ground biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

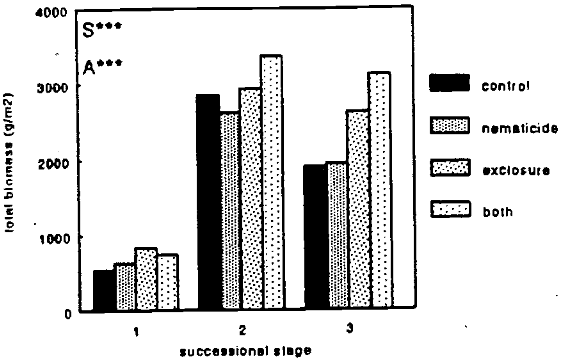

Fig14: Effects of successional stage, excluding above-ground and below-ground herbivory on total biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

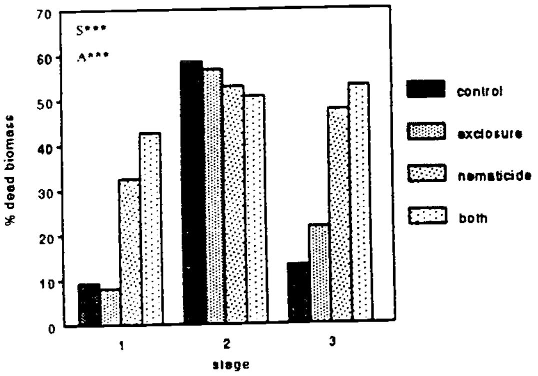

Fig 15: Effects of successional stage, excluding above-ground and below-ground herbivory percentage dead biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

Herbivory

Overall effects

Light availability (i.e. light at soil surface) is plotted with successional stage and treatment (fig. 10), and relation to above-ground biomass (fig. 11). Stage, excluding above-ground herbivory, excluding below-ground herbivory had significant effects on light availability. Furthermore, there was an interaction effect between excluding above-ground and below-ground herbivory. Light availability is highest in stage 1, lowest in stage 2 and intermediate in stage 3. In all stages excluding above-ground herbivory decreases light availability.

The relation between light availability and above-ground biomass was moderate. With increasing biomass light availability decreases. Since biomass increases with stage (see fig. 12 and 13), light availability will decrease. Except for some outliers, this is the case: stage 1 has lowest biomass, but highest light availability, the latter is decreasing from stage 1. Moss biomass was skipped from analysis, since it was not possible to get the stick under the moss layer in stage 3.

Above-ground, below-ground and total biomass (fig. 12, 13 and 14) increase with stage and due to excluding above-ground herbivory. Excluding below-ground herbivory has no significant effect on below-ground and total biomass. There was no interaction between excluding above- and below-ground herbivory. The impact of excluding above-ground herbivory on biomass seems to be lowest in stage 2, where biomass is of a comparable order as stage 3, but herbivory intensity comparable to stage 1. The impact is greatest in stage 1 (2.5 x). The percentage dead biomass is effected by stage and excluding above-ground herbivory (fig. 15).

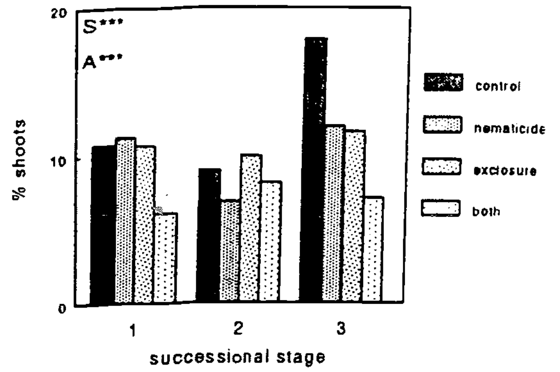

Fig. 16: **Effects of successional stage, excluding above-ground and below-ground herbivory on percentage shoots.** Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

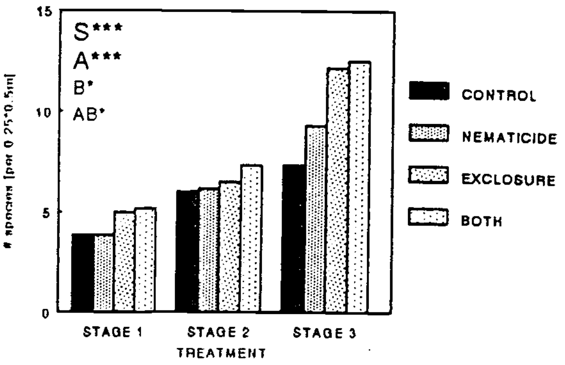

Fig. 17: **The effect of successional stage, excluding above-ground and below-ground herbivory on number of species.** Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

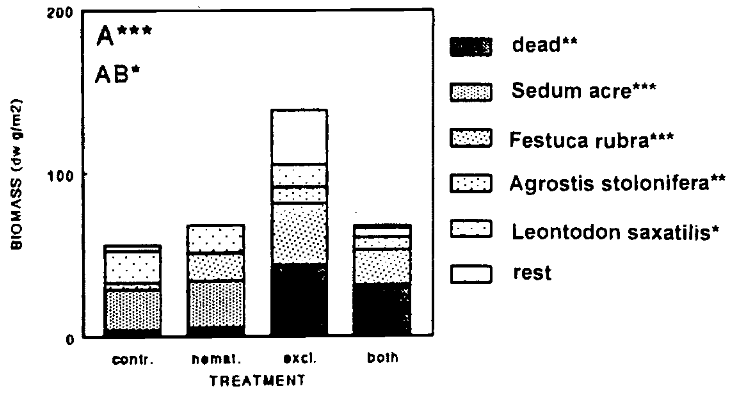

Fig. 18: Stage 1: The effect of successional stage, excluding above-ground and below-ground herbivory on species biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

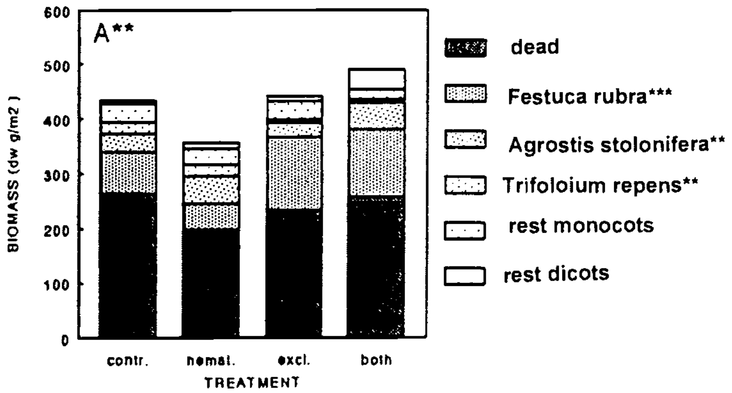

Fig. 19: Stage 2: The effect of successional stage, excluding above-ground and below-ground herbivory on species biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

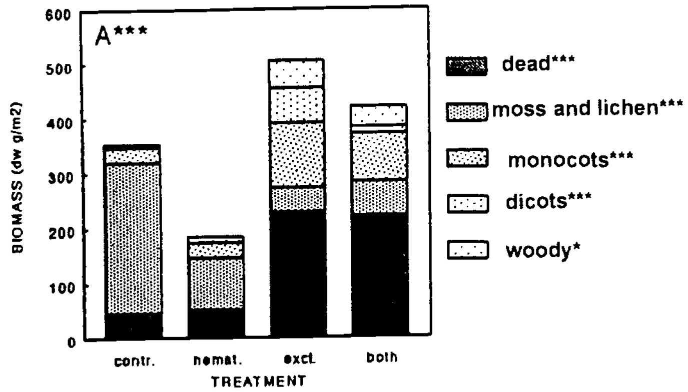

Fig. 20: Stage 3: The effect of successional stage, excluding above-ground and below-ground herbivory on species biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

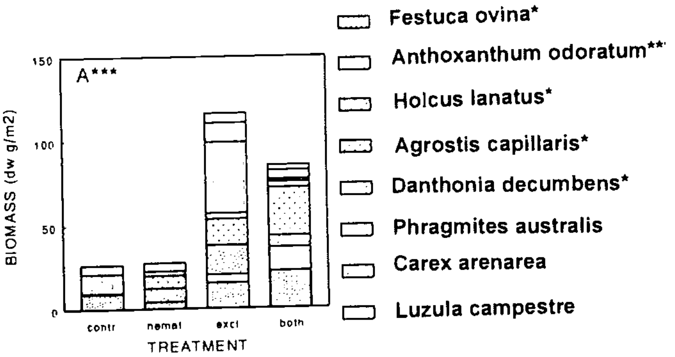

Fig. 21: Stage 3: The effect of successional stage, excluding above-ground and below-ground herbivory on monocot biomass. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * p< 0.05, ** p<0.01, *** p<0.001.

The analysis of percentage shoots (fig. 16) was done without moss and lichen biomass, since they are anatomically different and are usually not taken in an shoot-root ratio analysis. The percentage shoots of total living biomass is a measure of shoot-root ratio (s/r). Significant effects were found of stage and excluding above-ground herbivory: decrease of percentage shoots with stage, and increase due to excluding above-ground herbivory.

Effects on species

In the statistical per stage analysis sometimes species were grouped, by this effects were found which were not significant at species level. In table 3.1 is given an overview of the species found at every stage.

The number of species increases with stage, excluding above-ground herbivory and excluding below-ground herbivory (fig. 17). The increase of vascular plant species is highest in stage 3.

In all stages the exclosures (above-ground herbivory exclusion) had effects at species level. In stage 1 (fig. 18) grasses (*Festuca rubra* and *Agrostis stolonifera*) and dead biomass increase, whereas the dicots (*Sedum acre* and *Leontodon saxatilis*) decrease. Other species were not effected significantly. In stage 2 (fig. 19) *Trifolium repens* decreased, and *Festuca rubra*, *Agrostis stolonifera* and *Potentilla anserina* increased. In stage 3 (fig. 20 and 21) excluding above-ground herbivory caused an increase of all vascular plants as one group, and a decrease of bryophytes and lichens. As a group, herbaceous dicots were increased, but considering the species separately, no effect was found. Increasing effects where found separately on 5 grasses (*Festuca ovina*, *Holcus lanatus*, *Agrostis capillaris*, *Anthoxanthum odoratum* and *Danthonia decumbens*) and the woody plants (*Betula pubescens*, *Salix repens* and *Rubus fruticosus*) as a group.

The biomass of bryophytes is almost negligible in stages 1 and 2, lichens were not found there, but in stage 3 they are very abundant, and dominant outside the exclosure. The percentage of moss and lichens of total living biomass (fig. 22) decreases strongly due to excluding above-ground herbivory in stage 3.

Nematode numbers

In fig. 23 and 24 are shown numbers of all nematodes and plant feeding nematodes per stage and treatment. Significant effects were stage and excluding below-ground herbivory. Nematode numbers are increasing with stage, and decrease strongly due to the nematicide.

Furthermore, the species composition was differing per stage (data not shown in this report).

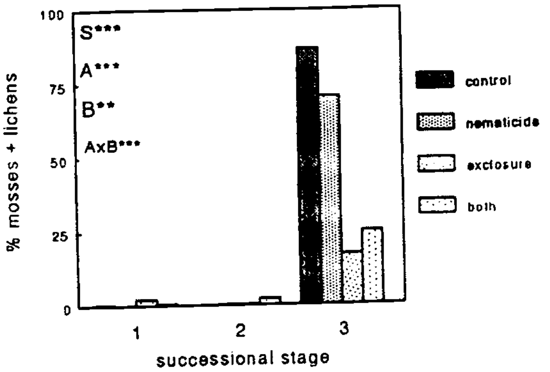

Fig. 22: The effect of successional stage, excluding above-ground and below-ground herbivory on percentage moss and lichen (of total above-ground biomass). Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * $p < 0.05$, ** $p<0.01$, *** $p<0.001$.

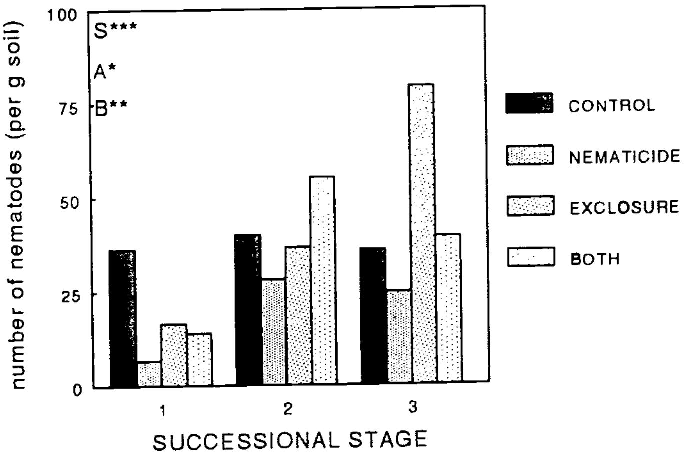

Fig. 23: The effect of successional stage, excluding above-ground and below-ground herbivory on the total number of nematodes. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * $p < 0.05$, ** $p<0.01$, *** $p<0.001$.

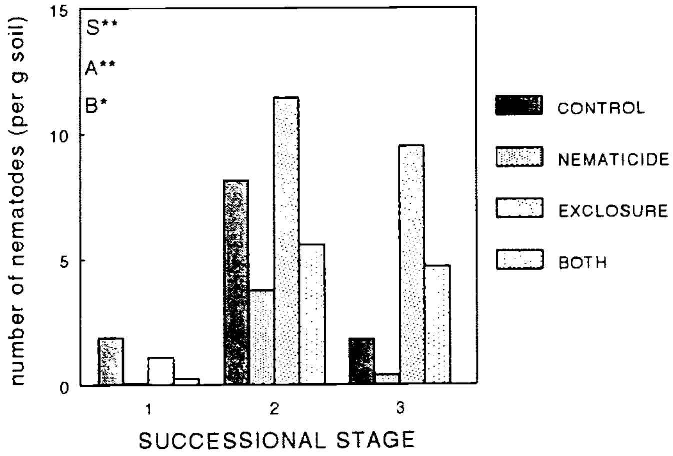

Fig. 24: The effect of successional stage, excluding above-ground and below-ground herbivory on number of plant feeding nematodes. Statistical significances are indicated as follows: S= stage, A= excluding above-ground herbivory, B= excluding below-ground herbivory; * $p < 0.05$, ** $p<0.01$, *** $p<0.001$.

Discussion

Nutrients

Inside the exclosures and without any other treatment, above-, below-ground and total biomass were lowest in the stage 1, intermediate in stage 2 and highest in stage 3 (fig 12, 13 and 14). This is consistent with the idea that biomass should increase with succession (Grime 1977, Tilman 1985, Van Andel 1993). In all stages fertilization increased above-ground biomass, and both N and P had significant effects. However, excluding above-ground herbivory increased biomass, too, so the fertilization effect is superimposed on the herbivory excluding effect. The vegetation is "recovering" from herbivory, and this vegetation regrowth is accelerated by nutrient addition. So at least part of the biomass increase due to fertilization is actually an exclosure effect. But still biomass increase is higher when fertilized, thus it can be concluded that nutrients are limiting. Increase of biomass is relatively higher at the 20 year old stage (stage 2) compared to that of 5 year old (stage 1), but is lower again in the 100 year old stage (stage 3). This is consistent with the idea that dunes form a nutrient poor system, and that limitation decreases with succession.

That at stage 3 the amount of biomass in the P-fertilized treatments is decreased, is due to the fact that these plots did not contain any woody plants (*Betula pubescens*, see fig. 9). Woody plants can form a big part of the total above-ground biomass, as they did in several other plots in stage 3. Why they were not growing there is difficult to say. An explanation could be that *B. pubescens* seedlings, or their associated mycorrhiza, cannot stand high phosphate concentrations, probably due to changing the N/P-ratio when fertilizing with P.

Herbivory and biomass

Above-ground biomass increased when above-ground herbivory was excluded. The impact of excluding herbivory on biomass was greatest in stage 1 and lowest in stage 2. This seems strange, for herbivory intensity is highest in stage 3. However, the impact of herbivory can higher in earlier stages, because plants have also to cope with low nutrient levels, which makes it more difficult to withstand biomass loss due to grazing (Grime 1979, Oksanen *et al.* 1981, Van Andel *et al.* 1993). When above-ground biomass is increasing, light availability at soil surface should decrease, which was the case in this experiment (see fig. 11). Then it is expectable that light competition or light stress is becoming an important factor with biomass increase. However, competition was measured within the same experiment during the first year, and there appeared to be no light competition (Caldeway 1995). Furthermore, Wilson and Tilman (1991) found that light competition begins to play a role when above-ground biomass reaches levels of 1200 $g \cdot m^2$, and this is not the case in this experiment. Alternatively, it can be that not light plays a role, but features that enable plants to tolerate herbivory. For instance, features that enable plants to restrict nutrient losses due to grazing (Grime 1979, Berendse and Elberse 1990). Moreover, it is imaginable that nutrient competition is not decreasing with succession, but increasing, because nutrient stress is decreasing. Another factor indicating that at least in stage 1 light does not play an important role, is that the vegetation cover in stage 1 was very sparse, also inside the exclosure and that thus light availability was still high enough there. Thus it could be that not light competition, but nutrient competition is the factor that plays an important role in all stages. This looks like a combination of the theories of Grime and Tilman: tolerance to low nutrient levels as well as competition ability. For the fertilization treatment now three factors can be distinguished: Firstly, there is no more removal of biomass by herbivores, secondly, nutrient availability increases with stage and finally, fertilization causes an increase of biomass and probably, nutrient competition. But still, competition is not measured in this experiment, and to investigate its influence, another experiment should be done.

With above-ground biomass, also below-ground biomass increased. Since the formation of root biomass is depending for a big deal on assimilation products, and thus on the amount of shoot biomass, it is logical that when shoot biomass is increased, root biomass increases, too.

In spite of the number of plant feeding nematodes being reduced (fig. 24), no effects were found on plant species composition and on below-ground biomass (fig 13). Only above-ground biomass was decreased. The possibility that plants suffered slightly from the nematicide can be excluded: the manufacturer guaranteed no suffering of crops; simple pot experiments showed no damage to plants, and also transplanted plants of a competition measurement within the experiment in the first year did not show signs of damage due to nematicide application. The number of nematodes increased with stage. This is expectable, since root biomass (the nematodes' food resource) is also increasing. There can be several reasons why the reduction of nematodes did not affect root biomass. First of all, the damage nematodes cause to the roots may be small, so that the plants are almost not hindered by nematode root herbivory. Secondly, the reduction of nematodes might not have been enough; there are still some nematodes left after the treatment. However, the measuring of nematode numbers was done in September when the effect of nematicide application started to decrease. A quick recovery of the nematode population due to invasion from surrounding parts or from cyst stadia could explain the number of nematodes still found (De Goede 1993). An effective way of dealing with below-ground herbivores can be sterilizing the soil, but this is almost impossible. Van der Putten *et al.* (1993) did this in a pot experiment. The disadvantage then is that all organisms in the soil will be killed, also those that benefit the plants (like symbiontic bacteria and mycorrhiza). And moreover, in this way the herbivores are not isolated. The situation below the soil is very complex, and there are lots of interactions that are not fully or not at all understood. So by eliminating one group of organisms, consequences for the rest of the soil system cannot be foreseen.

The percentage shoots decreased with stage and increased when above-ground herbivory was excluded. The expected stage effect would be a relative increase, because with ongoing succession plants would invest more in above-ground parts. It is possible that this is still a herbivory effect; in stage 3 the pressure of herbivory was highest, and as was seen before, it might be that above-ground parts still have to "recover". Just like standing crop, the percentage shoots is still increasing. The effect of excluding above-ground herbivory on percentage shoots was increasing, and thus consistent with the expectation that the impact on plants that have invested more in above-ground parts should be greater than that on plants that have invested more in below-ground parts. This supports the presumption that decrease of percentage shoots with stage is still a herbivory effect. Reduction of below-ground herbivory did not have a significant effect on the percentage shoots. This is surprising, when regarding the results in fig. 16: it is obviously decreasing. The below-ground biomass showed a slight increase (not significant, though), so it might be that there was an effect of nematode reduction, and that this effect might become more visible when the experiment would be carried out for a longer period of time.

Species composition

It was predicted that with ongoing succession, species will become taller, and this will be enhanced when fertilized. Considering the situation without herbivory (i.e. within the exclosures), stage 1 is dominated by *Festuca rubra*, *Agrostis stolonifera* and slightly *Leontodon saxatilis*; in stage 2 *F. rubra* is dominating. *F. rubra* is a grass of ea. primary dunes, and settles right after the dune colonizer *Ammophila arenaria* (Weeda *et al.* 1994). Thus in dunes it is an early successional species, together with *Leontodon saxatilis*, but the latter is occurring more in slightly opener primary dune stages (Weeda *et al.* 1994). *L. saxatilis* was also out-competed when fertilized, indicating that it will disappear with ongoing succession, which was the case: In stage 2 it is still present, but only with very low biomass. *Potentilla anserina* has come in then, a species growing on almost every soil type, except on very poor soils. When fertilized its biomass increased, and it did not occur in stage 1, which is only a few hundred metres away. *Agrostis stolonifera* is said to be able to grow in all kinds of habitats, and is favoured by fertilization (Weeda *et al.* 1994). However, in stage 1 fertilization was decreasing biomass of *A. stolonifera*, and in stage 2 no effect was measured (fig. 18 and 19). It could be that *A. stolonifera* is not able to compete with *F. rubra* in this stage (see further on).

Stage 3 then has a totally different species composition compared to stage 1 and 2; it is dominated by a variety of monocots, some dicots in higher numbers, and also woody plants have come in. Although there was no significant effect of fertilization at species level, it seems as if all vascular plants are slightly increased, and mosses and lichens decreased. Some species, like *Agrostis capillaris*, *Holcus lanatus* and *Anthoxanthum odoratum* can grow in a variety of habitats, but mostly on soils with enough organic material and nutrients. Other species, like *Luzula campestris*, *Festuca ovina*, *Danthonia decumbens* and *Carex arenaria* are more characteristic of poorer soils, mostly they prefer low P-concentrations. Also *Betula pubescens* is characteristic for poorer soils, though a certain amount of humus is necessary (Weeda *et al.* 1997). So in stage 3 some mid-successional species are starting to occur, as well as species that already have features that are characteristic for many climax stadia, i.e. mostly forest or woody plants.

As was already stated, above-ground herbivory puts advantage to good N-competitors and disadvantage to L-competitors. Consequently, species that benefit from fertilization and out-compete others should be good light competitors (though light competition was not found here) and can be expected to be limited when grazed. Or the other way around, nutrient competitors or low stature plants will be limited when herbivory is excluded. In stage 1 can be seen (fig. 18) that *Sedum acre* and *Leontodon saxatilis*, low stature species of early stages, decrease when herbivory is excluded. In stage 2 *Trifolium repens* decreased, a species of diverse, mostly short vegetation (Weeda *et al.* 1994). Stage 3 was more intensively grazed than the other stages, and this was visible in the vegetation: it was dominated by mosses and lichens, thus very low stature species, and all other vascular plants were grazed to the same height as the mosses. As a consequence of excluding above-ground herbivory, all vascular plant species increased in biomass, and as said before, even woody plants came in. In fact they do not have to come from far away, because in stage 3 the grazed fields are surrounded by thickets of *Betula*, *Salix*, *Rosa*, *Rubus* and *Sambucus*. This indicates that succession is indeed delayed or perhaps stopped by herbivores.

Other factors and items

An item not mentioned yet is dead biomass. More dead biomass means accumulation of nutrients, a decrease of light availability at soil surface, possibly higher nutrient availability, and consequently acceleration of succession. In this experiment, the percentage dead biomass was increased by excluding above-ground herbivory (fig. 15) and by fertilizing (fig 5). In stage 1 and 2 Festuca produced the greatest amount of dead biomass, and it is possible that by mainly this other species were inhibited. This could explain why *Agrostis* did not increase there. Thus grazing decreases the amount of dead biomass, and consequently this may be another factor why succession is delayed.

Bryophytes and lichens form the bulk biomass outside the exclosure in stage 3 (fig. 20 and 22), this is also observed in other studies in dunes with rabbit grazing (Ten Harkel & Van der Meulen 1995, Van der Hagen 1995). The first explanation is that mosses are not very much preferred by the rabbits, whereas many monocots and to some extent several dicots form the major diet of rabbits (Bhadresa 1987, Dress 1988). Hence, it is a question of apparent competition. Since mosses and lichens are very low-stature, they were out-competed because of light depletion when the exclosure was installed. Light competition was not measured for this very low-stature organisms, so it cannot be excluded. Another feature of mosses and lichens is that they are able to stand severe droughts, and can use very small amounts of water supply (Keizer *et al.* 1985). In stage 3 could be observed that the soil was very dry outside the exclosure, and that it even contained uncovered spots because of digging by the rabbits. It is expectable that the water capacity of the soil will be very low there. As a consequence, water competition caused by herbivory is a second factor playing a role. In this study it was not possible to measure light availability under the sometimes very dense moss and lichen cover. Keizer *et al.* (1985) found out that in a very dense bryophyte layer light availability can be less than 5%. From that study it also appeared that seedling emergence can be very hard within such a dry moss layer, especially combined with grazing. These factors then favour the bryophytes and lichens.

The number of species was effected negatively by grazing (all stages) and fertilization (stage 1 and 2). The effect of grazing is consistent with the prediction of decrease of species under nutrient poor conditions. Under richer circumstances (stage 3) the situation is more complex. Firstly, even stage 3 is still not really rich in nutrients (indicated by the kind of occurring species and the fertilization experiment). Secondly, the moss and lichen species have not been identified. From other studies appeared that these can form a very diverse community (Van der Hagen 1995). So it might be that species number of all plants and lichens together is reduced. On the other hand, it is known from dunes that some mosses can become very dominant, especially the exotic *Campylopus introflexus* (Keizer *et al.* 1985, Weeda *et al.* 1995). Thus a conclusion about total species richness cannot be drawn from the results of stage 3.

Whether herbivores have either delayed or stopped succession is difficult to say. Rabbits have been on Schiermonnikoog for several centuries, and it can be expected then that they had influence on vegetation since the very beginning of formation of the studied dunes. Another factor is that rabbit (and hare) populations are characterized by fluctuations of numbers due to climate, diseases etc (Watt 1981). Thus when the rabbit population has crashed, vegetation will make a rapid progress in succession. But when the rabbit population is recovering again (and this is possible within short time), their influence may become retrogressive again. So it seems that there is an interaction of the processes. Besides, another question is whether succession would have taken a totally different direction if herbivores had not been there. Since herbivores are able to modify vegetation composition, some species never might occur, even if herbivores would disappear from a system.

Conclusions

Nutrients were limiting in the system: N in all stages, P in stage 1 and 2. Consequently, they may play a role in succession. Excluding above-ground herbivory caused biomass as a whole to increase, whereas the impact was different among species: mostly decreasing on low-stature or early successional species, and increasing on late successional species. Reducing below-ground herbivory by nematodes did have no impact on biomass and on plant species composition, although if the experiment would last longer, effects might become visible. Interactions between above-ground and below-ground herbivory were not found.

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APPENDIX

Table 1 A-C: Found species per stage. 0= controle, N= nitrogen fertilization, p= phosphate fertilization, N+P= nitrogen and phyosphate fertilization, A= exclusion of above-groeund herbivory, B= exclusion of below-ground herbivory, A+B= exclusion of above- and below-ground herbivory. Crosses indicate whether a species was present at a certain treatment. Nomenclature folows Van der Meijden 1990.

A. Stage 1	0	N	P	N+P	0	A	B	A+B
moss	x		x	x	x	x	x	x
Sedum acre	x	x	x	x	x	x	x	x
Erodium cicutarium dunense	x					x		
Linum cartharticum			x		x			
Oenothera erythrosepala	x		x	x		x		
Cirsium arvense	x	x				x		x
Leontodon saxatilis	x	x	x		x	x	x	x
Taraxacum officinale			x					
Hieracium pilosella	x		x		x	x		x
Festuca rubra	x	x	x	x	x	x	x	x
Agrostis stolonifera	x	x	x	x	x	x	x	x
Ammofila arenaria	x	x	x		x	x		x

B. Stage 2	0	N	P	N+P	0	A	B	A+B
moss	x		x			x	x	x
Cerastium fontanum	x	x	x	x	x	x	x	x
Potentilla anserina	x	x	x	x		x		x
Trifolium repens	x	x	x	x	x	x	x	x
Linum cartharticum	x		x		x	x	x	x
Odontitis vernus	x				x	x	x	x
Linaria vulgaris					x			
Sonchus arvensis		x	x	x			x	
Cirsium arvense	x	x		x	x	x		
Leontodon saxatilis	x				x	x	x	x
Festuca rubra	x	x	x	x	x	x	x	x
Poa pratensis	x	x		x		x		
Agrostis stolonifera	x	x	x	x	x	x	x	x
Elymus athericus	x	x	x	x	x	x	x	x
Ammofila arenaria					x		x	

C. Stage 3	0	N	P	N+P	0	A	B	A+B
lichen	x				x	x	x	x
moss	x	x	x	x	x	x	x	x
Salix repens		x					x	
Betula pubescens	x	x		x		x		x
Rumex acetosa	x	x	x	x	x	x	x	x
R. acetosella		x	x	x			x	
Stellaria graminea	x	x	x	x	x	x	x	x
Cerastium fontanum	x	x	x	x		x	x	x
Lychnis flos-cuculi				x				
Rubus fruticosus		x						
Potentilla anglica	x	x	x	x		x	x	
Vicia cracca							x	
Lotus comiculatus			x					x
Polygala vulgaris								x
Viola curtisii	x	x	x	x		x	x	x
Viola canina	x	x	x		x	x	x	x
Galium verum	x	x	x	x		x		x
Galium mollugo			x	x				x
Prunella vulgaris	x			x		x	x	
Veronica officinalis	x				x	x	x	x
Cirsium palustre	x	x				x		x
Cirsium arvense	x	x				x		
Luzula campestris	x	x	x	x	x	x	x	x
Festuca ovina	x	x	x	x	x	x	x	x
Poa pratensis	x	x	x	x		x	x	x
Anthoxanthum odoratum	x	x	x	x	x	x	x	x
Holcus lanatus	x	x	x	x	x	x	x	x
Agrostis capillaris	x	x	x	x	x	x	x	x
Phragmites australis	x	x	x	x		x		x
Danthonia decumbens	x	x	x	x		x		x
Carex arenaria	x	x	x	x	x	x	x	x

Table 2: **Fertilization, overall effects: results of univariate analysis of variance**. n.s.= not significant.

	Above-ground biomass		**Percentage dead**		**Number of species**	
	F	p	F	p	F	p
stage	61.27	0.00	7.83	0.001	139.22	0.00
site	3.90	0.004	4.34	0.002	6.75	0.00
N	5.54	0.00	4.91	0.00	6.22	0.00
P	5.54	0.045	2.54	0.023		n.s.

Table 3: **Fertilization, per species effects: results of multivariate analysis of variance.** The overall effects are in stage 1 N-fertilization (Hotellings p=0.00), and in stage 2 N-fertilization (Hotellings p=0.004) and (hotellings p=0.000). In stage 3 no significant effects of fertilization were found at species level, the table thus only gives p-values of stage 1 and 2. n.s.= not significant, empty cells indicate that a species was not occuring at a plot, and/or was not taken into the statistical analysis.

	Stage 1	**Stage 2**
	p	p
dead	0.00	0.002
Sedum acre	0.00	
Leontodon saxatilis	0.013	
Festuca rubra	0.00	0.034
Agrostis stolonifera	0.047	n.s.
Potentilla anserina		0.00
Trifolium repens		n.s.
Linum cartharticum		n.s.
Elymus athericus		n.s.
rest	n.s.	n.s.

Table 4: **Herbivory: Light availability.** The table gives F- and p-values for light availability tested as a univariate analysi of variance, and results of the regression analysis. The B-value is tested with a T-test.

	Light availability			
	F	p	**regression analysis**	
stage	3.221	0.00	r^2= 0.59949	B= -0.154640
site	4.63	0.001	F=104.77704	T= -10.236
A	39.66	0.00		p= 0.000
B	7.39	0.001		
A+B	5.81	0.00		

Table 5: Overall effects of excluding above- and below-ground herbivory: results of univarite analysis of variance.

5A.	Total biomass		Above-ground biomass		below-ground biomass		percentage shoots	
	F	p	F	p	F	p	F	p
stage	124.54	0.00	75.09	0.00	140.20	0.00	13.23	0.00
site	7.09	0.00		n.s	11.44	0.00	2.39	0.048
A	7.51	0.00	5.15	0.00	8.31	0.00	5.19	0.00
B		n.s.	5.15	0.011		n.s.		n.s.
A+B		n.s.		n.s.		n.s.		n.s.

5B.	% dead biomass		number of species		% moss and lichen	
	F	p	F	p	F	p
stage		0.00	117.35	0.00	348.93	0.00
site		0.013	6.75	0.00	2.74	0.027
A		0.00	8.88	0.00	30.25	0.00
B		n.s.	3.63	0.00	3.61	0.003
A+B		n.s.		n.s.	3.61	0.00

Table 6: Herbivory, per species effects: results of multivariate analysis of variance. The overall effects are in stage 1 excluding above-ground herbivory (Hotellings p=0.000), in stage 2 excluding above-ground herbivory (Hotellings p=0.006) and site (Hotellings p=0.000), and in stage 3 excluding above-ground herbivory (Hotellings p= 0.000). n.s.= not significant, empty cells indicate that a species was not occuring at a plot, and/or was not taken into the statistical analysis.

	stage 1	stage 2		stage 3
	p	p		p
dead	0.006	n.s	dead	0.00
Sedum acre	0.00		lichens	0.002
Leontodon saxatilis	0.015		moss	0.001
Festuca rubra	0.00	0.00	woody plants	0.011
Agrostis stolonifera	0.003	0.007	dicots	0.00
Potentilla anserina		0.00	*Luzula campestris*	n.s.
Trifolium repens		0.002	*Festuca ovina*	0.034
Linum cartharticum		n.s.	*Holcus lanatus*	0.032
Elymus athericus		n.s.	*Agrostis capillaris*	0.049
rest	n.s.	n.s.	*Anthoxanthum odoratum*	0.00
			Danthonia decumbens	0.032
			rest monocots	n.s.

Table 7: Nematode numbers: results of univariate analysis of variance. pf-nematodes= plant feeding nematodes, n.s.= not significant.

	total number of nematodes		number of pf-nematodes	
	F	p	F	p
stage	17.54	0.00	8.86	0.002
site				
A	4.65	0.041	9.77	0.005
B	9.24	0.006	5.38	0.031