

**DYNAMICS IN VEGETATION COMPOSITION**  
**PLANT – PATHOGEN INTERACTIONS**

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## Summary

Plant community composition is known to be dynamic due to abiotic and biotic factors. However little is known about the interactive effect of above- and belowground herbivores on plant community composition and dynamics, but recently it has become apparent that the interaction of both spatially separated herbivores may be an important factor in determining plant growth and consequently also dynamics in plant community composition.

Aboveground herbivores such as cattle can create regeneration niches by trampling and via their dung. Smaller herbivores, such as rabbits and large rodents, commonly burrow, creating other kinds of recruitment opportunities. However belowground herbivores can play an important role in community dynamics as well.

Soil-borne organisms can have both positive and negative effects on plant growth. A positive effect occurs when the presence of soil biota increases plant growth or survival. A well-known example of a positive effect of soil biota on plant growth is the arbuscular mycorrhizal fungi growing on roots of plants. A negative effect occurs when the presence of soil-borne pathogens decrease plant growth or plant survival. The most well known example of negative effects comes from agricultural systems. The growth of the same crop on the same field year after year is known to decrease productivity.

In natural systems plant growth can also be regulated by the negative feedback of plant specific pathogens. Van der Putten et al. (1997) have shown the importance of soil-borne pathogens during succession in coastal sand dunes. They found that species that were grown in soils from previous and later successional species were reduced most in soils from the later successional species, indicating that species disappear during succession from sites where the soil has become colonised with specific growth-reducing pathogens on the one hand. But, on the other hand, indicating that later successional species are able to grow in sites from which previous successional plants have disappeared.

Bever (1994) and Bever et al. (1997) found evidence that the soil community can contribute to the maintenance of diversity within plant communities. Soil biota can be involved in the creation of micro sites that are favoured by different plant species and thus creating certain patchiness in the vegetation, often observed in grasslands.

In a long-term exclosure experiment in the Junner Koeland in the Netherlands temporal dynamics and spatial heterogeneity in the plant community were observed as well. In this area temporal plant community dynamics and spatial plant community heterogeneity seem to be increasing at sites primarily grazed by voles, while the community composition in the areas grazed by large herbivores is more constant. These dynamics in the vole grazed parts do not appear to be solely dependent on changes in the abiotic conditions, for when plant species are sensitive to such changes, it is expected that all species show a similar pattern over time and space, which is not the case.

In this study I will try to answer the following questions regarding plant population dynamics in grazed and ungrazed grasslands. (1) Can the presence of soil-borne pathogens contribute to temporal and spatial variation in plant species composition in a grassland? (2) Does the presence of cattle inhibit this effect, i.e. are plant community dynamics controlled by soil organisms in the absence of cattle only? Sub questions will be: (1a) is the growth of specific plant species negatively related to the cover of that same plant species in the field and (2a) is the variance in response of the plant species less variable in cattle grazed soils compared to vole grazed soils? (3a) Are abiotic circumstances more important for fluctuations in plant growth and differences in plant growth between treatments than soil borne pathogens?

To answer these questions I will set up a greenhouse experiment, in which I will study the growth responses of four different dominant plant species (*H. lanatus*, *F. rubra*, *A. capillaris* and *R. acetosa*). The plants will be grown on "cattle grazed" and "voles grazed" soils from a long-term experiment in the Junner Koeland (the Netherlands).

Total biomass was not significantly different between cattle and vole-grazed soils, nor were shoot and root biomass. However in general total biomass and root biomass tend to be higher on cattle-grazed soils and shoot biomass tends to be higher on vole-grazed soils. When total biomass derived in the experiment was compared to the abundance of that same species in the field, it showed a positive effect for *H. lanatus*, *F. rubra* and *A. capillaris*, but a negative effect for *R. acetosa* independent of grazing treatment.

For the abiotics, pH was significantly more alkaline in cattle-grazed areas and regression analysis with total biomass mostly showed positive trends (more total biomass in more alkaline soils). The Soil Organic Matter (SOM) content was also higher in cattle-grazed areas although not significantly. Regression analysis showed a negative trend for all species (less total biomass with higher SOM content), except for *F. rubra* which showed no trend at all. Potential mineralization and nutrient content of nitrate was higher in vole-grazed sites, while ammonium was higher in cattle-grazed sites. Only *F. rubra* showed a positive trend in total biomass versus potential mineralization, all other species were constant. Finally, soil texture was found to have a large impact on some analysis. Blocks A and B were found to be more sandy, while blocks C, D and E had higher clay content. The block effects were consistent with the partition in texture.

Growth of a specific plant species is negatively related to the cover of that same plant species in the field. And also the variance in growth with which the plants respond is less variable in cattle-grazed soils compared to vole-grazed soils. This response in plant growth appears not to be consistent with the variance of nitrate content and potential mineralization, but the nutrient contents and pH are probably very important factors for the dispersal and living conditions for soil-borne bacteria. Finally, the coefficient of variation points in the direction of presence of soil-borne pathogens determining plant growth. Therefore they can contribute to temporal and spatial variation in plant species composition. And since the cattle-grazed soils contain more nutrients and also show a higher biomass without actual grazing during the experiment, it is possible that the presence of cattle inhibits the effect of soil-borne pathogens.

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## Introduction

Plant community composition is known to be dynamic due to abiotic and biotic factors. Examples of abiotic factors affecting community dynamics are: weather, nutrient availability, light availability etc. Biotic factors influencing dynamics in community composition can for instance be the presence of herbivores in the system. The effect of aboveground herbivores on plant communities has been studied very well. The magnitude and direction of the impact on plant species richness in grasslands vary strongly between habitat and herbivore type. Herbivores have been found to reduce species richness in low productive systems, whereas they can increase species richness in more productive systems<sup>21, 23, 25, 20</sup>. Size differences between herbivores determine their foraging selectivity and food quality requirement<sup>14</sup>. On the other hand large herbivores can create regeneration niches by trampling and via their dung. Smaller herbivores, such as rabbits and large rodents, commonly burrow, creating other kinds of recruitment opportunities<sup>19</sup>. However belowground herbivores can play an important role in community dynamics as well. Especially little is known about the interactive effect of above- and belowground herbivores on plant community composition and dynamics, but recently it has become apparent that the interaction of both spatially separated herbivores may be an important factor in determining plant growth and consequently also dynamics in plant community composition<sup>12, 22, 24</sup>.

Soil-borne organisms can have both positive and negative effects on plant growth. A positive effect occurs when the presence of soil biota increases plant growth or survival. A well-known example of a positive effect of soil biota on plant growth are the arbuscular mycorrhizal fungi growing on roots of plants. The fungi obtain carbon from the plant and in return the fungi transport nitrogen to the plant via their hyphal network<sup>1, 26</sup>. This nitrogen would not be available to the plant otherwise, since it originates from mineral nitrogen and is too complex for the plant to take-up. On the other hand, Bardgett et al. (1998) state that amounts of available C are often important in regulating the biomass of soil micro flora and the fauna that feed upon them. The effects of above-ground herbivory on root C flow manifest themselves as short-term changes in plant C allocation and root exudation. The shifts in C allocation can therefore influence the biomass and density of the soil biota.

A negative effect of the presence of soil-borne pathogens can be decreased plant growth or plant survival. The most well known example of negative effects comes from agricultural systems. The growth of the same crop on the same field year after year is known to decrease productivity. This is explained by the negative feedback of plant specific pathogens which have accumulated in the root zones of these crops and are limiting plant growth<sup>27, 11</sup>. Therefore, a system of crop rotation made its way into agriculture<sup>8</sup>, reducing the accumulation of species specific pathogens and thus minimizing reduced crop growth.

In natural systems plant growth can also be regulated by the negative feedback of plant specific pathogens. For example, Van der Putten et al. (1997) have shown the importance of soil-borne pathogens during succession in coastal sand dunes. They found that species that were grown in soils from previous and later successional species were reduced most in soils from the later successional species, indicating that species disappear during succession from sites where the soil has become colonised with specific growth-reducing pathogens on the one hand. But, on the other hand, indicating that later successional species are able to grow in sites from which previous successional plants have disappeared.

Other studies also showed that pathogens were able to speed up succession<sup>10, 28, 33</sup>. Dobson et al. (1994) summarizes some examples of epidemics caused by a number of pathogens in dominant species that have led to major changes in the structure of forests and woodlands. Examples of this are: Chestnut

blight, caused by *Cryphonectria parasitica* an aboveground fungus, in the Eastern United States<sup>28, 33</sup> and Dutch elm disease in Britain and Western Europe, caused by *Ceratocystis ulmi* which is another fungus, this time living in the vascular system of the tree and transmitted via beetles or via root grafts<sup>10</sup>. The trees that died from these diseases left large open spaces in the forests, giving small seedlings a chance to grow and maintaining a certain patchiness or mosaic in the forest. These open spaces differ from open spaces created by cutting down trees, since the dying process is fairly slow and the surrounding vegetation can adapt to the changed circumstances.

The previous example is mostly based on distribution in time, but spatial distribution of soil organisms can also determine the spatial distribution of the plant species and therefore also plant community composition. For example, Bever (1994) and Bever et al. (1997) found evidence that the soil community can contribute to the maintenance of diversity within plant communities<sup>7, 8</sup>. Soil biota can be involved in the creation of micro sites that are favoured by different plant species and thus creating certain patchiness in the vegetation, often observed in grasslands<sup>9</sup>.

In a long-term exclosure experiment in the Junner Koeland in the Netherlands temporal dynamics and spatial heterogeneity in the plant community were observed as well<sup>2</sup>. In this area temporal plant community dynamics and spatial plant community heterogeneity seem to be increasing at sites primarily grazed by voles, while the community composition in the areas grazed by large herbivores is more constant, with *Agrostis capillaris* and *Festuca rubra* as the dominant species.

These dynamics in the vole-grazed parts do not appear to be solely dependent on changes in the abiotic conditions, for when plant species are sensitive to such changes, it is expected that all species show a similar pattern over time and space. The observed patterns in the field do not seem to be dependent only on such changes, since not all species show the same pattern (fig. 1).

While the community composition is more or less constant in the areas grazed by large herbivores, the vole-grazed areas show much more dynamics in community composition. These dynamics could be explained by different factors, for example, dynamics in the abiotic soil properties, a "litter" feedback or a negative feedback of soil pathogens. Abiotic soil properties will probably contribute to community composition differences between cattle-grazed and vole-grazed areas, but I expect that they are not so dynamic from year to year and especially between different plots, which lay close together, that they are the only factors determining oscillations in plant community composition. Although figure 1 shows that differences in cover percentage in fertilized and unfertilized areas fluctuate, the pattern with which this occurs is similar only the amplitude is smaller. This indicates that not only abiotic conditions are responsible for the observed dynamics in the field, but on top of that another mechanism is probably present.

In a number of studies, summarised in Bardgett et al. (1998), higher numbers of bacteria, bacterial biomass and bacterial activity have been found in grazed areas<sup>3, 4, 5, 17</sup>. This could include soil-borne pathogens that exert negative effects on plant growth, although it is not certain whether the species of bacteria found in these studies are harmful to plants.

Another factor that might contribute to dynamics in the plant community is the "litter" feedback. Tillman et al. (1991) described that oscillations in the dynamics of the perennial grass *Agrostis scabra* resulted from time delayed inhibitory effects of plant litter on subsequent growth. It was argued that in productive areas litter inhibits growth of plants in the subsequent growing season by intercepting light. Consequently, plant growth will be decreased until the litter is decayed. Plant species that are more capable of growing in these circumstances may become more dominant in that area.

A third factor that might explain the dynamics in the plant community is the negative feedback by soil pathogens to the plant communities. Bever et al. (1997)

stated that negative feedback is the process in which the presence of a plant species changes the soil community in a manner that decreases the growth of that particular plant species relative to other species. This is due to a build-up of plant specific pathogens in the rhizosphere. The different factors mentioned above have the biggest effect on oscillations in systems where for instance large herbivores can not interrupt these processes. In the field experiment it has been observed that the oscillations are less strong in cattle-grazed sites, which may be explained by the dampening effects that large grazers such as cattle have on the oscillations. For example, by removing aboveground biomass as forage, there will be less litter accumulation and thus less "litter" feedback. On the other hand, the negative effect of soil organisms may also be less strong in areas grazed by large aboveground herbivores (fig. 2), since the abundance of plant species that are susceptible to grazing may increase when herbivores are absent. Consequently, their species specific pathogens increase as well and when the pathogen load becomes too high, performance and abundance of the specific plant species will diminish and will finally be replaced by a species that is less sensitive to these specific pathogens. However, when herbivores are present in this system, they may exert a stronger effect on plant growth than these pathogens, leading to a more stable community composition over time. This is because the palatable species decline, since they are grazed upon, whereby grazing tolerant species can maintain and become dominant regardless of the presence of their plant specific soil organisms. Thus, aboveground grazing has a stronger effect on community dynamics than belowground plant pathogens, however in the absence of aboveground grazers soil organisms may take over control of plant community dynamics.

In this study I will try to answer the following questions regarding plant population dynamics in grazed and ungrazed grasslands. (1) Can the presence of soil-borne pathogens contribute to temporal and spatial variation in plant species composition in a grassland? (2) Does the presence of cattle inhibit this effect, i.e. are plant community dynamics controlled by soil organisms only in the absence of cattle? Sub questions will be: (1a) is the growth of specific plant species negatively related to the cover of that same plant species in the field and (2a) is the response of the plant species less variable in cattle-grazed soils compared to vole-grazed soils? (3a) Are abiotic circumstances more important to the response of plant growth than soil borne pathogens?

With presence of large above ground herbivores the composition of the plant community is more or less constant, whereas in the absence of large herbivores the belowground community will have an important impact on plant species composition or its dynamics. The abundance of different plant species will be determined by the presence of plant species specific pathogens and the specific plant will have a disadvantage compared to the other plant species in the community.

Therefore the hypothesis is that cattle have a top-down control on the vegetation by altering the competitive relationship between plant species. Consequently, the effects that pathogens may have on plant species abundance are suppressed.

The presence of a plant at a certain location will negatively affect the growth of the same species at that same location in the following year (negative feedback). Therefore the plant abundance in the field will be negatively correlated to the biomass from the experiment.

To answer these questions I will set up a greenhouse experiment, in which I will study the growth responses of four different dominant plant species. The plants will be grown on "cattle-grazed" and "vole-grazed" soils from a long-term experiment in the Junner Koeland (the Netherlands).

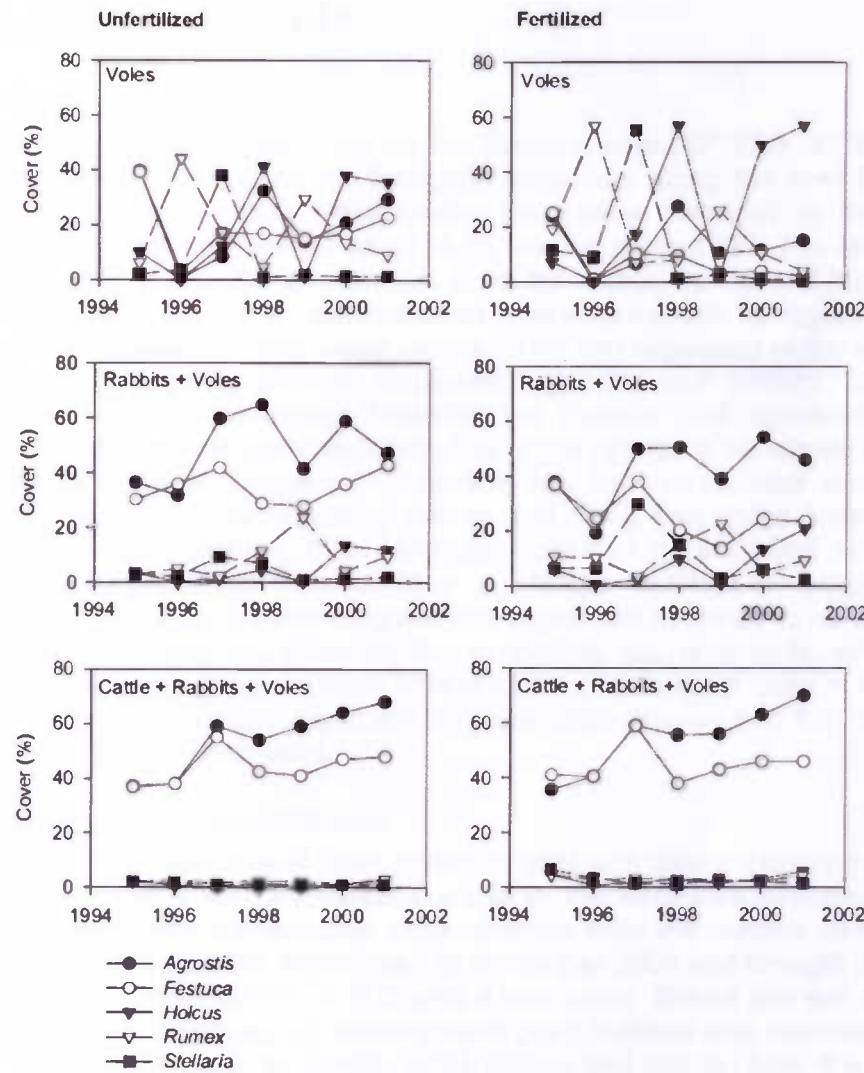


Figure 1. Cover percentage of 5 different species in the Junner Koeland area in the different grazing regimes<sup>2</sup>.

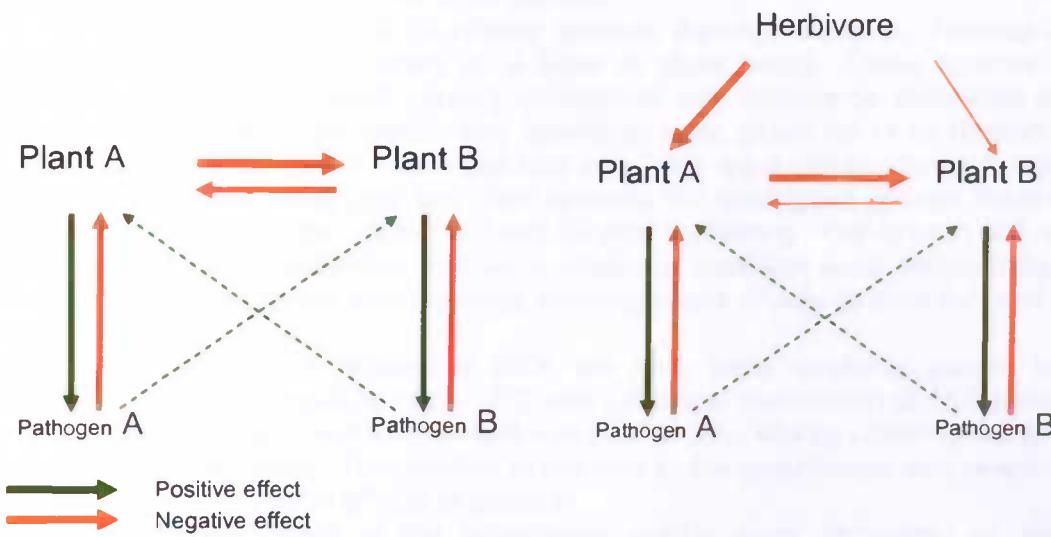


Figure 2. Schematic of the plant-pathogen interactions in the soil. In the right figure herbivores are added and mediate as inhibitors of the pathogen effect on the plants<sup>32</sup>.

## Materials and methods

### Experimental set-up field

The study is performed in the Junner Koeland area ( $52^{\circ} 32'N$ ,  $6^{\circ} 36'E$ ), a 100 ha nature reserve including 50 ha floodplain grassland along the river Overijsselse Vecht in the Northeast of the Netherlands. The site is managed by the National Forest Service (Staatsbosbeheer) as a nature reserve since 1967. The area used to be common grazing land for the farmers from the village Junne and has probably been grazed by livestock since medieval times. The area used to be regularly flooded, but since the canalisation of the Vecht (about 1910) and regulation of the water level, the frequency of flooding strongly decreased over the last century. In 1994 an exclosure experiment was set-up following the Russian doll approach to study herbivore impact under natural conditions. Using this approach vertebrate herbivores were excluded stepwise from large to small in five exclosures, later reverred to as blocks. Outside the exclosure cattle, rabbits and voles can graze (referred to as cattle-grazed or C in graphs), then cattle are excluded by a barbed wire, leaving access to rabbits and voles (referred to as R). Finally rabbits were excluded using chicken mesh, leaving only voles to graze the vegetation (referred to as vole-grazed or V in graphs)<sup>2</sup>. Every experimental block consists out of 6 plots with different treatments. Two of the six plots were fertilized and therefore not used in the current experiment. For the current experiment only the cattle-grazed and the vole-grazed plots in the exclosures were used.

### Experimental set-up greenhouse

To study the response of plant growth to soils with different grazing history we collected soil in cattle and vole-grazed areas in the exclosure experiment in the Junner Koeland. These soil samples were collected from the surface 20 cm directly adjacent to the plots from the experiment in November 2006 and brought back to the laboratory where it was stored at  $4^{\circ}C$  until it was used. Before the soil was put in pots, it was homogenised and all aboveground plant material was removed from the soil, just as all insects (worms, larvae, ants), stones and woody roots, the remaining roots were cut in one cm pieces. Per plot four pots of 15,5 cm width were filled and weighed. Each pot was covered with tin foil to prevent desiccation and growth of algae and put in the greenhouse to be planted.

Preceding this, seeds of *Holcus lanatus*, *Agrostis capillaris*, *Festuca rubra* and *Rumex acetosa* were sown on a layer of glass pearls. These species were selected, since they showed (cyclic) oscillations and dominance outbreaks in the Junner Koeland exclosure experiment. Seedlings were grown for 14 to 19 days after which they were planted. In each pot four seedlings were planted through holes in the tin foil, resulting in 40 pots per plant species. For each plant species there were 20 pots containing 'cattle'-grazed soil and 20 pots containing 'vole'-grazed soil. In the following two weeks seedlings that were unable to establish were replaced by new seedlings. In some cases also replaced seedlings were unable to establish and were again replanted.

On the 24th of November 2006 the pots were randomly placed in the greenhouse at a temperature of  $21/15^{\circ}C$  with additional illumination of 15/9 h rhythm. During the experiment moisture content was kept at 30% w/w by watering 2-3 times a week with distilled water. The position of the pots in the greenhouse was re-arranged weekly to avoid structural effects of position.

In the sixth week of the experiment, aphids were discovered on several leaves of *Rumex acetosa*. All plants of this species were deloused with a mixture of soft soap and alcohol. The presence of the aphids and the delousing methods did not have an effect on the growth of the plants ( $P>0,05$ ).

After 12 weeks of growing, the experiment was harvested. All aboveground biomass was removed and dried 70°C for 24 hours, after which they were weighed. After removal of the shoots the soil was removed from the pots and divided in two, with each part containing half of the remaining stubble. One of these parts was stored at 4°C for further experimental use. From the other half the roots were washed and dead roots, little sticks and stones were removed, after which the samples were dried at 70°C for 24 hours and biomass was weighed. Since the soil from the pots was cut in half, the root biomass, found after washing of roots, was multiplied by two to have a weight for the entire pot. The total biomass is the biomass of the shoots and roots added up.

During the experiment all plants were measured every two weeks from the basis to the tip of the longest leaf of every plant from *H. lanatus*, *F. rubra* and *A. capillaris*. For *R. acetosa* the three largest leaves of every plant were measured from the base of the leave to the tip in a straight line alongside the main nerve. In addition the number of leaves per plant was counted.

### **Abiotics**

At the start of the experiment abiotic soil characteristics were measured. Sub samples of the collected soil were used which were homogenised and all fresh plant material was removed form the soil samples.

Moisture content was determined in the soil samples, after drying small samples of soil at 105°C over night. The average moisture content of the field soil has also been set as average moisture content for the greenhouse experiment.

Also pH and pH KCl were determined in the soil samples. For the pH protocol 15 g of soil was used and dissolved in 20 ml of demineralised water. To determine pH KCl, 2,5 ml KCl 1N was added to the samples after determining pH, after which it was mixed over night.

Soil Organic Matter (SOM) was determined after removal of all above ground material and large roots from the soil. Small amounts of this soil were put in porcelain cups and put in an oven where the samples were burned at 550°C over night. At this temperature only the present organic material is burnt and no additional chemical analyses are necessary using the loss-on-ignition method<sup>9</sup>.

To determine the mineral nitrogen content of the field soil, small bottles were filled with 25 g of soil after which 60 ml 1N KCl was added and the samples were mixed over night.

In half of these incubation tubes the initial amounts of inorganic nitrogen ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) present at the time when samples were collected, were measured. Part of this soil was used to determine potential mineralization rates in the soil in November 2006, soil cores were collected in PVC tubes of 16 cm length with a diameter of 4,3 cm. Two of these tubes were collected at every plot. The last part of these samples was also used to measure the other initial abiotic parameters described above. The other half of these tubes was incubated for five weeks at 20°C after which the inorganic nitrogen content was measured again. The data used in this study for potential mineralization are based on the difference in  $\text{NO}_3^-$  content between November and December.

### **Data analysis**

To test whether root-, shoot-, total biomass, shoot length or abiotic soil properties were different between the treatments (cattle-grazed vs. vole-grazed), I used general linear models (GLMs). The models were set up using root-, shoot, total biomass, shoot length and abiotics as response variables. Grazing treatment was entered as a fixed factor, and block was entered as a random factor. In each pot four plants were grown, all measurements within one pot were averaged. All data were tested for normality and homogeneity of variances. When necessary, data were log-

transformed to improve normality and homogeneity of variances. To test for relationships between plant growth in the experiment and field factors such as abiotic soil properties and abundance of plant species I used regression analysis entering total biomass as dependent factors and abundance of plant species or abiotics as independent factors. All statistical analyses were performed in Statistica 7.0. (StatSoft. Inc, 1984-2004)

## Results

### Plant growth

#### Biomass

Total biomass was not significantly different between cattle and vole-grazed soils, nor were shoot and root biomass. However *F. rubra* tended to have higher total biomass on cattle-grazed soils than on vole-grazed soils ( $p=0.063$ ;  $F_{1,30}=6.556$ ), which was probably due to higher root biomass of *F. rubra* in cattle-grazed soils ( $p=0.03$ ;  $F_{1,30}=10.210$ ). Although the other three species also have higher total biomass in cattle-grazed soils, these differences are not significant (fig. 3). On the contrary shoot biomass was generally higher in the vole-grazed soils.

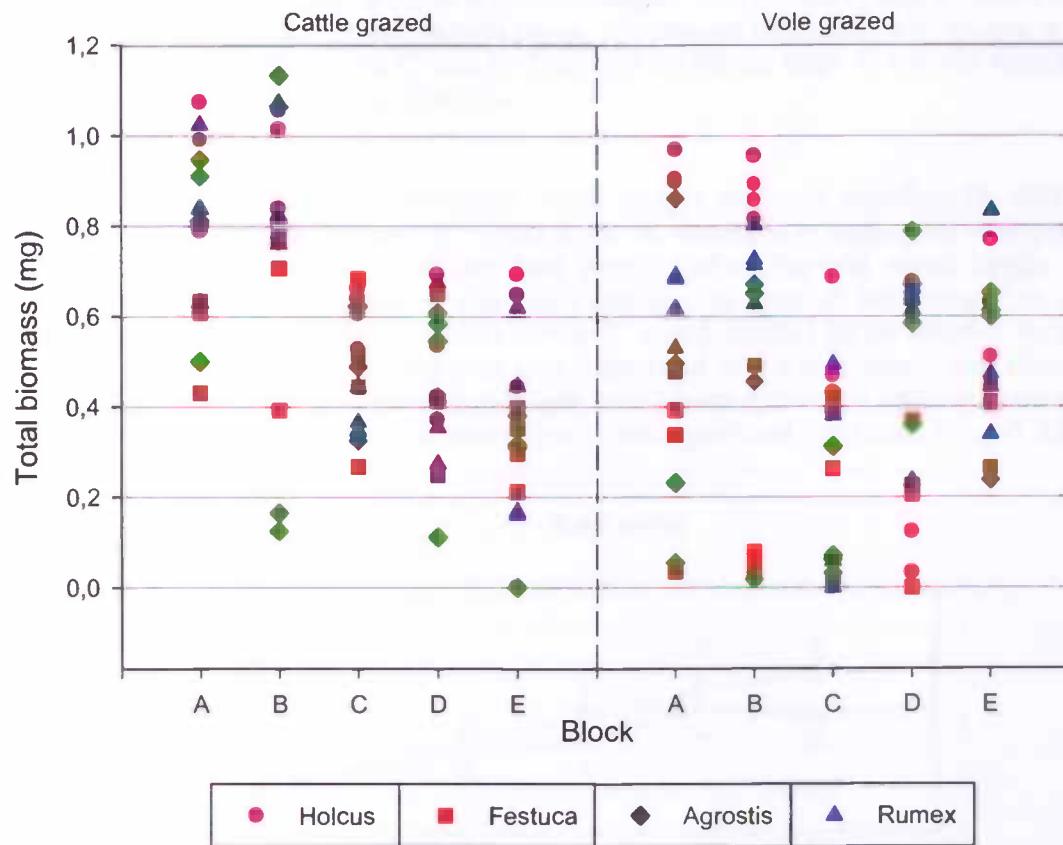


Figure 3. Total biomass of the four experimental species. Per block four pots were present.

### Block effect (biomass)

Since the experimental area lies in a former river arm there may be differences in abiotic soil properties such as pH, nutrient content, mineralization and soil texture. As a result of this growth responses in the five different blocks may be very different. For *H. lanatus* the block effect (fig 4) was significant for total biomass ( $p=0.020$ ;  $F_{4, 30}=10.836$ ), shoot biomass ( $p=0.020$ ;  $F_{4, 30}=10.752$ ) and root biomass ( $p=0.018$ ;  $F_{4, 30}=11.461$ ). Although not significant ( $P=0.057$ ;  $F_{4, 30}=5.918$ ), *R. acetosa* shows a similar pattern to *H. lanatus*. This indicates that the plants react to very local differences, which can point in the direction of the presence of small-scale abiotic differences or maybe of small-scale spatial variation in the presence of soil-borne organisms.

For all experimental species the block effect shows a clustering pattern. On the one hand blocks A and B show a similar pattern, with blocks D and E on the other hand, while block C is an intermediate block, sometimes clustering with blocks A and B and sometimes with blocks D and E. This can be traced back to the soil texture of these blocks mentioned under abiotics.

### Shoot length

When the experiment was harvested shoot length was not significantly different between the 3 grasses *H. lanatus*, *F. rubra* and *A. capillaris* in cattle and vole-grazed soils for all plant species. However, four weeks before harvest shoot length was significantly different for some of the plant species. *H. lanatus* was longer in vole-grazed areas ( $p=0.044$ ;  $F_{1, 30}=8.435$ ), while *F. rubra* tended to be shorter in vole-grazed sites. For *R. acetosa* leaf size was measured and it was found that plants in vole-grazed soils have a slightly bigger leaf than plants grown on cattle-grazed soils (fig. 5), however the difference in leave size is not significant ( $p=0.300$ ;  $F_{1, 30}=1.430$ ).

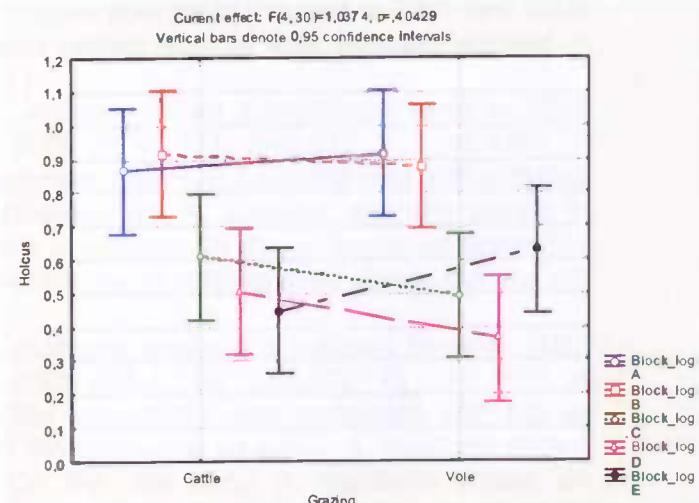


Figure 4. Block effect for total biomass of *H. lanatus* per grazing treatment.

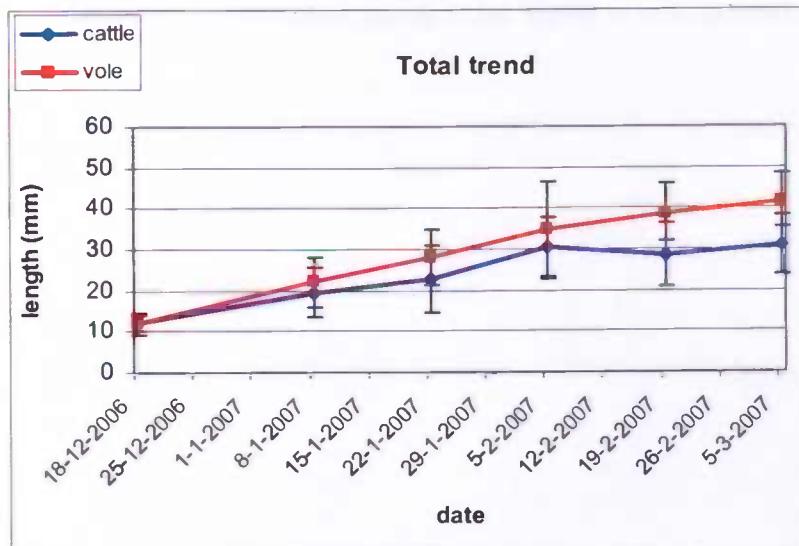


Figure 5. Leaf length of *R. acetosa* during the experiment for both grazing treatments.

## Regression

The abundance of the four experimental species in the field in 2005 and 2006 (previous growing seasons) has also been plotted against total biomass derived in the greenhouse experiment (fig. 6; table 1).

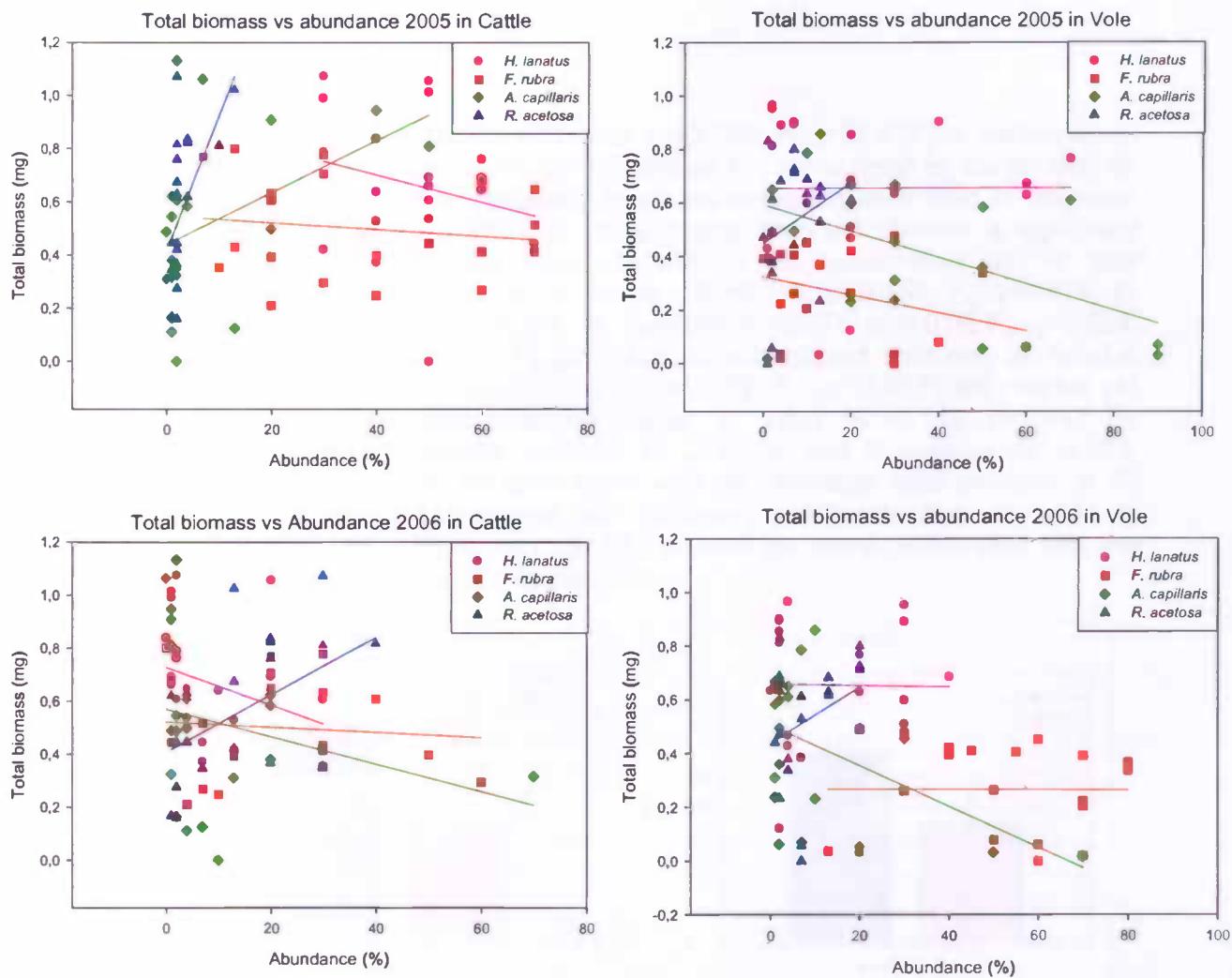
In the cattle-grazed soils only *R. acetosa* shows a significant trend in 2005 ( $R^2=0.321$ ;  $p=0.009$ ;  $F_{1, 18}=8.510$ ) and 2006 ( $R^2=0.233$ ;  $p=0.031$ ;  $F_{1, 18}=5.480$ ). A higher biomass of *R. acetosa* in the experiment seems to be correlated with a higher abundance of *R. acetosa* in the field. However for *R. acetosa*, only the trends in cattle-grazed areas in 2005 and 2006 are significant. In the vole-grazed areas the trends for *R. acetosa* show the same direction as in cattle-grazed areas, but are not significant.

In the vole-grazed soils only *A. capillaris* shows a significant trend in 2005 ( $R^2=0.266$ ;  $p=0.034$ ;  $F_{1, 18}=5.257$ ) and 2006 ( $R^2=0.258$ ;  $p=0.022$ ;  $F_{1, 18}=6.250$ ). A lower total biomass for *A. capillaris* in the experiment was correlated with higher abundance of *A. capillaris* in the field. In the cattle-grazed areas *A. capillaris* shows two opposite trends over the years. On the one hand *A. capillaris* shows an increasing total biomass in the experiment, with an increase in abundance of *A. capillaris* in 2005, but on the other total biomass of *A. capillaris* decreases with increasing abundance of *A. capillaris* in the field in 2006.

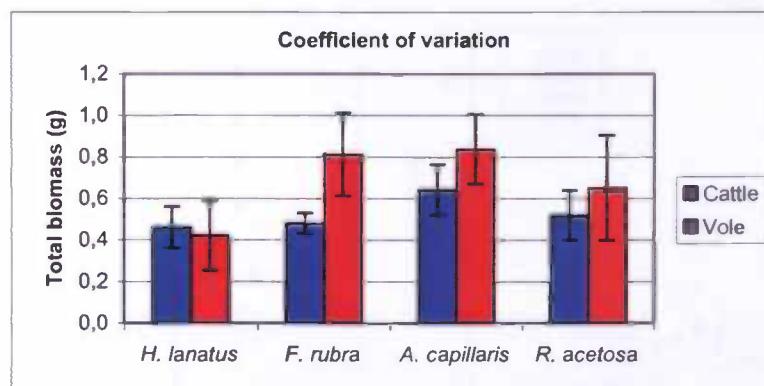
For *H. lanatus* and *F. rubra* also some trends were observed, although they were not significant. Total biomass of *H. lanatus* in the experiment was negatively correlated with the abundance of *H. lanatus* in the field within cattle-grazed areas for both years, while there was no relationship for *H. lanatus* in vole-grazed areas for both years. *F. rubra*, although not very strong, also shows a negative relationship for its total biomass in the experiment versus its abundance in the field, irrespective of the treatment and in both years.

## Coefficient of Variation: Total biomass

In figure 7 the coefficient of variation (CV) for total biomass is shown, which points out heterogeneity of data. It seems that in the vole-grazed soils the coefficient of variation is highest for all plant species except for *H. lanatus*, although this is not significant. *F. rubra* is the only species that is almost significant ( $p=0.068$ ;  $F_{1, 0}=6.195$ ) for total biomass. This means that total biomass within vole-grazed blocks seems to be more variable compared to cattle-grazed blocks. When looked at the coefficient of variation for root biomass, *F. rubra* does show a significant difference ( $p=0.04$ ;  $F_{1, 0}=8.627$ ) with higher variation in vole grazed soils. Variation in shoot biomass showed no significant differences, but variation tends to be higher in vole-grazed areas.



**Figure 6. Total biomass in the experiment versus abundance of the same species in the field, for all experimental plants and both grazing treatments in 2005 and 2006.**



**Figure 7. Coefficient of variation of the four experimental plants.**  
**Showing the heterogeneity of the data.**

## Abiotics

### pH

First of all pH is significantly higher ( $p=0.006$ ;  $F_{1,0}=28.421$ ) in cattle-grazed soils compared to vole-grazed soils. pH KCl shows the same trend as the pH (fig. 8). Overall the pH KCl is higher (alkaline) in the cattle-grazed soils than in the vole-grazed soils ( $p=0.072$ ;  $F_{1,0}=5.88$ ). In cattle-grazed soils pH showed a significant (positive) relationship with the total biomass in the experiment for all four experimental plant species (fig. 9): *H. lanatus*  $R^2=0.318$ ;  $p=0.010$ ;  $F_{1,18}=8.374$ , *F. rubra*  $R^2=0.258$ ;  $p=0.022$ ;  $F_{1,18}=6.274$ , *A. capillaris*  $R^2=0.275$ ;  $p=0.018$ ;  $F_{1,18}=6.824$ , *R. acetosa*  $R^2=0.532$ ;  $p=0.0003$ ;  $F_{1,18}=20.456$ . In vole-grazed soils only *H. lanatus* shows the same significant trend ( $R^2=0.431$ ,  $p=0.002$ ;  $F_{1,18}=13.657$ ), with higher pH (alkaline), total biomass also becomes higher. *F. rubra* ( $R^2=0.113$ ;  $p=0.147$   $F_{1,18}=2.292$ ), *A. capillaris* ( $R^2=0.071$ ;  $p=0.256$ ;  $F_{1,18}=1.375$ ) and *R. acetosa* ( $R^2=0.053$ ;  $p=0.327$ ;  $F_{1,18}=1.016$ ) show no clear trend with pH. Although total biomass of *R. acetosa* has a tendency to increase with increasing pH (more alkaline), while *A. capillaris* has the opposite tendency. pH KCl showed the same relationship with the biomass of all plant species in the experiment.

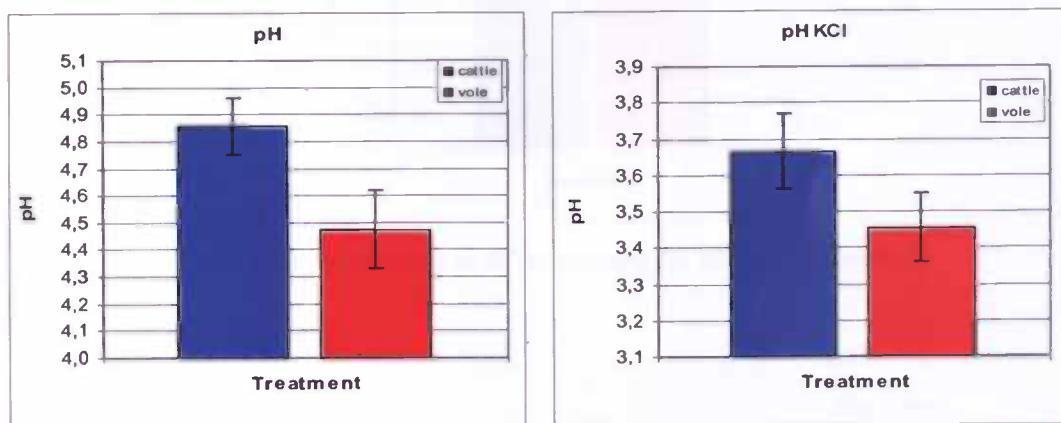


Figure 8. Differences between grazing treatments for pH and pHKCl.

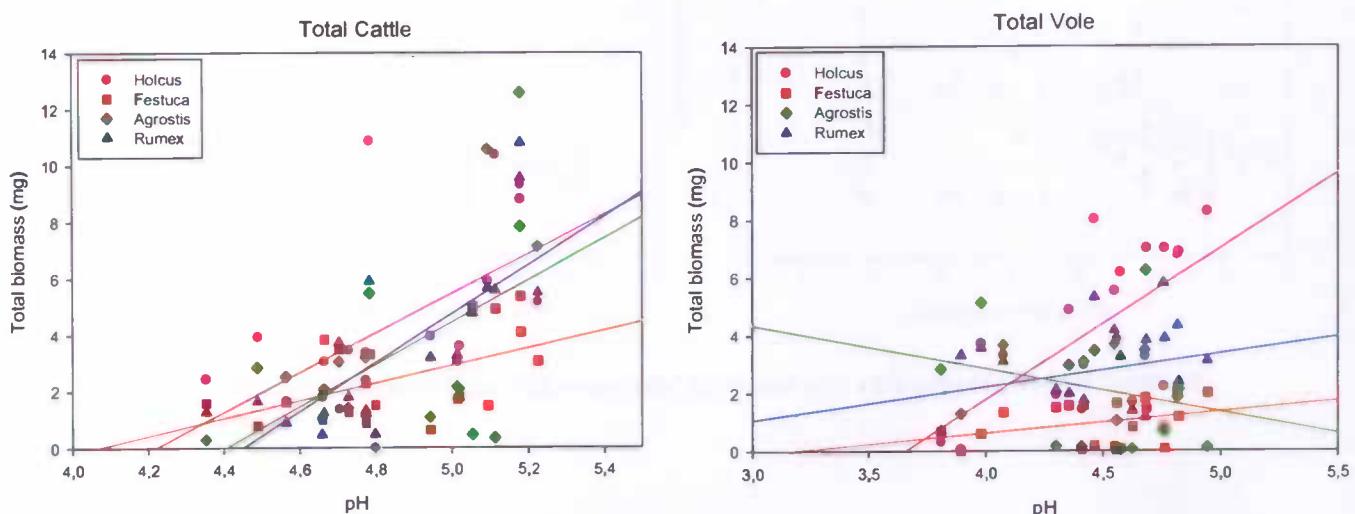


Figure 9. Relationships between total biomass and pH for both grazing treatments.

### Soil Organic Matter

The Soil Organic Matter (SOM) content (%) tends to be higher in cattle-grazed soils (fig. 10;  $p=0.081$ ;  $F_{1,0}=5.377$ ), however this pattern is only present in block A, while all other blocks show the opposite (vole higher than cattle).

In general, total biomass is negatively related to SOM (fig. 11), so total biomass decreases with increasing SOM content. *R. acetosa* and *H. lanatus* produce less biomass with lower SOM on both cattle ( $R^2=0.399$ ;  $p=0.003$ ;  $F_{1,18}=11.947$  and  $R^2=0.478$ ;  $p=0.0007$ ;  $F_{1,18}=16.504$ ) and vole-grazed soils ( $R^2=0.211$ ;  $p=0.042$ ;  $F_{1,18}=4.814$  and  $R^2=0.594$ ;  $p=0.00001$ ;  $F_{1,18}=26.323$ ). *A. capillaris* only showed a significant ( $R^2=0.219$ ;  $p=0.037$ ;  $F_{1,18}=5.060$ ) negative relationship in cattle-grazed soils, but there also was a trend towards lower biomass with lower SOM in vole-grazed soil. In contrast, *F. rubra* did not show any pattern with a change in SOM on both cattle and vole-grazed soils.

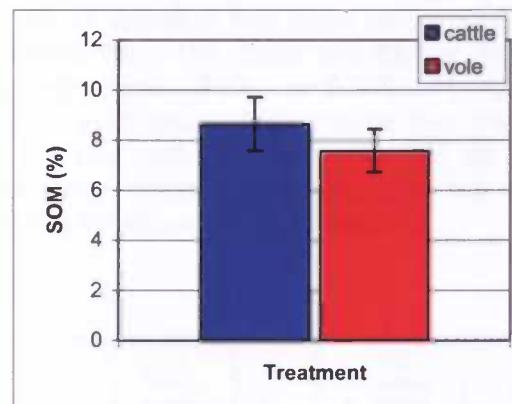


Figure 10. Difference in SOM content for both treatments.

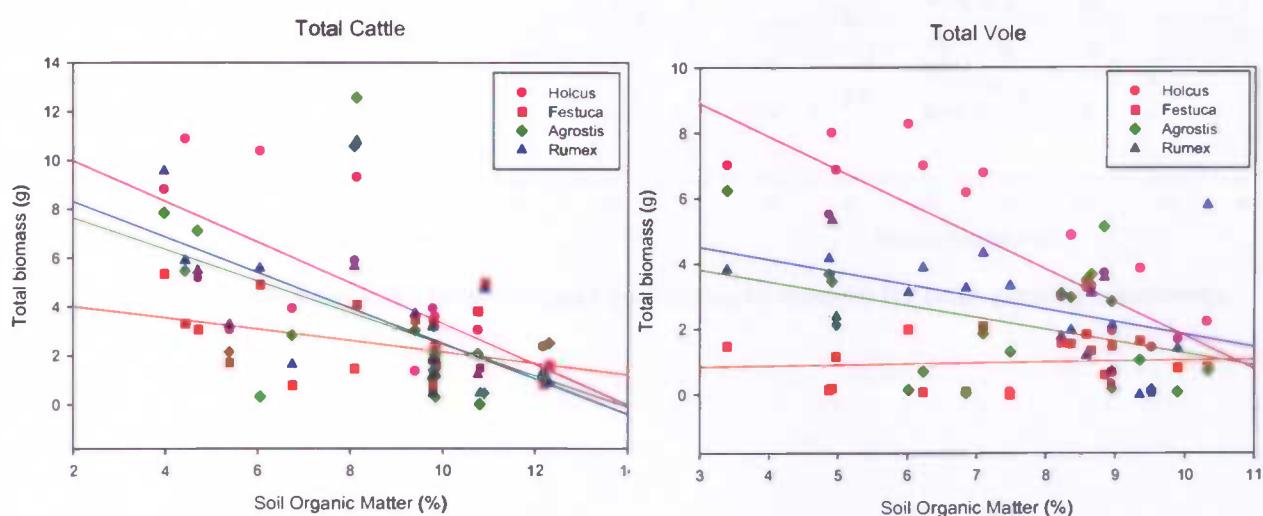


Figure 11. Relationships between total biomass and soil organic matter content.

### Potential mineralization

The inorganic nitrogen parameters  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were also measured. These two abiotic parameters show opposite patterns from each other, which is logical considering the following:  $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^+$  or in reverse: nitrification.  $\text{NO}_3^-$  is highest in vole-grazed soils ( $p=0.005$ ;  $F_{1,0}=29.926$ ), while  $\text{NH}_4^+$  is highest in cattle-grazed soils ( $p=0.029$ ;  $F_{1,0}=11.146$ ). Potential mineralization ( $\text{NO}_3^-$ ) was significantly higher ( $p=0.002$ ;  $F_{1,0}=55.490$ ) in vole-grazed soils compared to cattle-grazed soils. And as expected for  $\text{NH}_4^+$  it is the other way around, the coefficient of variation is higher in cattle-grazed soils ( $p=0.049$ ;  $F_{1,0}=7.859$ ). While the previous abiotic parameters show different trends in the regression analysis with total biomass, this is not the case for the potential mineralization of inorganic nitrogen ( $\text{NO}_3^-$ ), with the exception of *F. rubra* in the vole-grazed soils (fig.12). Total biomass of *F. rubra* increases slightly with increasing nitrogen content ( $R^2=0.231$ ;  $p=0.032$ ;  $F_{1,18}=5.409$ ), while biomass of the other plant species does not change with nitrogen content.

Also the coefficient of variation has been determined for both nitrate content and potential mineralization (fig. 13). Both are higher in the cattle-grazed plots compared to vole-grazed plots ( $\text{NO}_3^-$   $p=0.005$ ;  $F_{1,0}=29.926$  and potential mineralization  $p=0.002$ ;  $F_{1,0}=55.490$ ). This means that there is more variation for these two parameters in the cattle-grazed plots. Of all the abiotic parameters described in this section only nitrate content and potential mineralization showed a significant difference for the coefficient of variation.

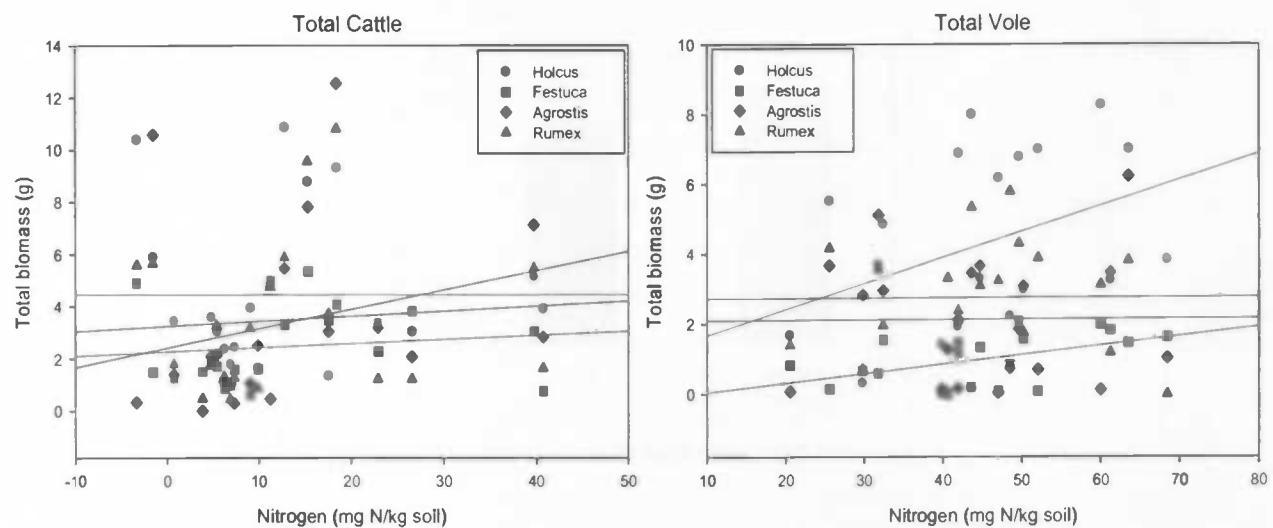
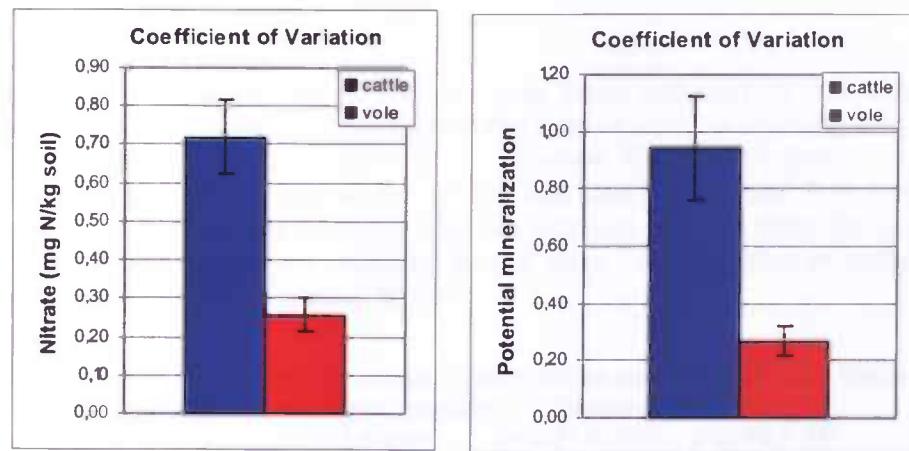


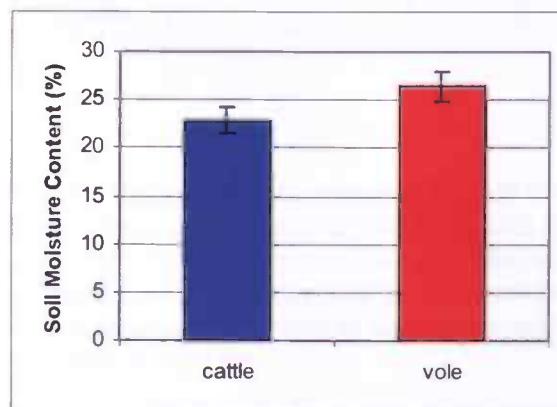
Figure 12. Relationship between total biomass and nitrogen content for both grazing treatments.



**Figure 13. Coefficient of Variation for nitrate content and potential mineralization of nitrate.**

### Soil moisture

Soil moisture content is highest in vole-grazed soils (fig.14;  $p=0.026$ ;  $F_{1,0}=11.875$ ), except block D which shows the opposite of the general pattern with a higher moisture content in cattle-grazed soils.



**Figure 14. Difference in soil moisture content**

### Total biomass dependency

After the separate analyses of all factors, they were included in one analysis to determine whether abundance of species in the field in 2006 is a strong predictor for the reaction observed in total biomass or whether the abiotic factors are more important. In table 1 the significances of the test are shown and it is visible that especially pH is a very strong predictor for total biomass. On the other hand it shows that also abundance is a good predictor in the case of *H. lanatus* in cattle-grazed soils and *A. capillaris* in vole-grazed soils.

**Table 1. Determinants for total biomass of all species and both grazing treatments.**

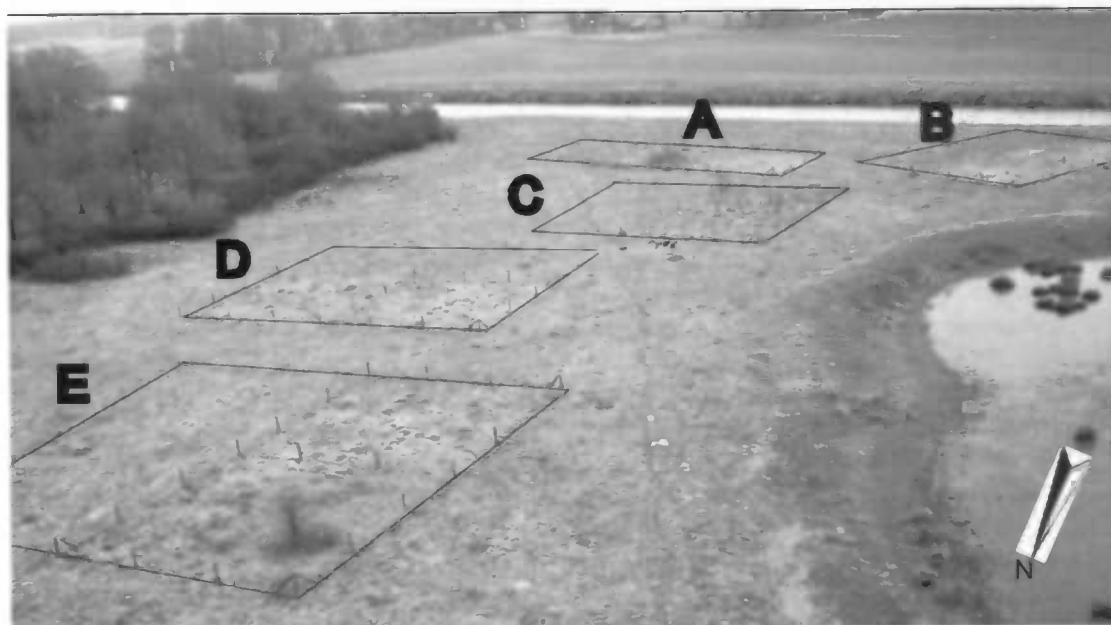
Relationships: negative = - /positive = +

Significance:  $p < 0.001 = +++/- -$ ,  $p < 0.01 = ++/-$ ,  $p < 0.05 = +/-$

Species	Treat.	pH	Mineral.	Moist	SOM	Abund.
<i>H. lanatus</i>	Cattle				-	-
	Vole	+++		-	-	
<i>F. rubra</i>	Cattle					
	Vole					
<i>A. capillaris</i>	Cattle	0.056			--	-
	Vole					
<i>R. acetosa</i>	Cattle	+				
	Vole					

### Soil texture

The exclosures are located next to a former branch of the river Vecht (fig. 9). After the soil texture analysis it was found that blocks A and B are sandier, this is logical since they lie closest to the river as it runs now. Blocks C, D and E were found to have higher clay content than the others and these three blocks are further away from the present riverbed and are lying next to each other with C closest to the present riverbed followed by D and finally E.



**Figure 15. The Junner Koeland with the five enclosures. The river Vecht is visible in the top of the picture and on the right side the former river arm is visible.**

## Discussion

Although not significant total biomass is higher in cattle-grazed areas, while shoot biomass is higher in vole-grazed areas. When total biomass is compared to the abundance in the field in 2006 two significant relationships are visible. *A. capillaris* shows a significant negative trend with abundance of the same species in the field, which is to be expected when it concerns the negative feedback from species specific pathogens in the soil. However, *R. acetosa* shows the opposite with a significant positive trend. This could be happening when the community is re-establishing itself and the soil-borne pathogens did not yet accumulate in the root zone of these plants. For the abiotic factors in this experiment pH is significantly more alkaline in cattle-grazed areas. Soil Organic Matter (SOM) is although not significant also higher in cattle-grazed areas and the ammonium content is significantly higher in cattle-grazed areas, with nitrate showing the opposite as was expected due to the pathways they go through when present in the soil. Potential mineralization showed the same pattern as ammonium and nitrate. Finally moisture content in the soil was significantly higher in the vole grazed areas.

At the beginning of this study several questions were posed about plant-pathogen interactions. Van der Putten et al. (1993)<sup>30</sup> already showed that these interactions are very important in succession in coastal fore dunes. Shipton (1977)<sup>27</sup>, Bruehl (1987)<sup>11</sup> and Bardgett et al (1993)<sup>3</sup> carried out several experiments to demonstrate the plant-pathogen interactions in agricultural systems. In this study we tried to prove that plant-pathogen interactions are also present in grasslands and that grazing by herbivores influences this interaction. Because negative feedback of pathogens may result in dynamics in vegetation composition, we asked whether a negative relationship between field abundance and experimental biomass occurs. It was expected that a negative relationship between field abundance and experimental biomass occurs when the numbers of pathogens of a certain species have accumulated in its root zone, which will lead to lower biomass of that plant species when grown on the same soil again, i.e. plants will experience a negative soil feedback. However, in the present study the negative relationship between abundance of a certain plant species in the field and total biomass in the experiment was not very apparent. However, we did find some relationships between total biomass in the experiment and abundance in the field.

For instance, total biomass of *A. capillaris* decreases with abundance of the same species in the field, in the vole-grazed soils. This could be due to a negative feedback effect of species specific pathogens in the soil, which inhibit growth of the specific plants, which in this case is *A. capillaris*. The same effect was found by Olff et al. (2000)<sup>24</sup>. The species used in their experiment grew better on unsterilized soil from the site where it had increased in cover during recent years and did worse on unsterilized soil from the site where it had decreased recently. Van der Putten et al. (1993)<sup>30</sup> found that succession in coastal fore dune vegetation was also determined by soil-borne bacteria. They stated that if soil-borne pathogens and parasites are involved in the degeneration of a certain species, then succeeding plant species must be tolerant of the soil-borne diseases of their preceding species. Biomass of their experimental species was reduced by soil-borne diseases of their own and of their successors. Moreover, Olff et al (2000)<sup>24</sup> found that negative soil feedback could lead to small-scale shifting mosaics of *A. capillaris* and *F. rubra* in a grassland. On the other hand *R. acetosa* shows a positive trend between field abundance and experimental biomass which could be due to a decreasing population of *R. acetosa* in the previous years and as *R. acetosa* is re-establishing the soil biota are not yet present in large numbers and therefore still harmless to plants. It might also be the case that growth of *R. acetosa* is more dependent on abiotic circumstances in the

soil than by biotic feedback. The places where it has been growing in the field had better abiotic circumstances for this plant species leading to a positive relationship.

On the other hand it could be the case that this species is more dependent on abiotic circumstances compared to soil-borne pathogens. We asked whether it could be the case that within grassland systems abiotic circumstances are more important to the response of plant growth than soil borne pathogens? We measured different abiotic variables in the field soil and related them to experimental biomass. For example, nitrate concentrations were higher in vole-grazed sites, which can be due to the larger amount of litter, just as the potential mineralization rate of nitrate. Nitrate is converted via nitrification or denitrification processes<sup>16</sup>, both of which are mediated by specialized bacteria. Thus maybe, bacteria concentrations are higher in vole-grazed sites. In contrast, ammonium concentrations are significantly higher in cattle-grazed areas. Bakker et al. (2003)<sup>2</sup> found that when herbivore densities in the grazing treatments were compared based on their daily energy expenditure at twice basal metabolic rate, the herbivore assemblages appeared to be very similar. However large herbivores like cattle deposit a large amount of urine at once. Compared to that, voles only lose a few drops at a time. The potential mineralization rates of ammonium are also higher in cattle-grazed sites and are not mediated by bacteria, since it is a process with oxygen as main reactor. For plants growing in most field situations, the two elements that most commonly determine plant performance are nitrogen and phosphorous<sup>16</sup>. It is therefore expected that plants in the vole-grazed areas show a higher total biomass. Only the opposite is true, since total biomass is highest in cattle-grazed areas.

However, as stated before, the pattern found in total biomass is mostly determined by root biomass, which is also higher in cattle-grazed soils. Shoot biomass on the other hand is higher in vole grazed soils. While preparing the soil samples for the experiment, it was also observed that more roots were present in the soil from the cattle-grazed sites. However no significant differences were found between cattle and vole-grazed soils. Fitter (1997)<sup>16</sup> states that the best characterized response to deficiency of soil-based resources is a change in the pattern of growth, favouring root growth over shoot growth. Since nitrate content and potential mineralization rates are lower in the cattle-grazed areas, it is expected that more roots are present in these soils. However, Guitian et al. (2000)<sup>18</sup> found the opposite: root-biomass was significantly affected by defoliation treatment, declining with increasing intensity of defoliation. There were no significant relationships between total biomass and nitrogen, except for a positive relationship for *F. rubra* on vole-grazed soils. This could also point in the direction of plant growth not being solely dependent on nutrient availability.

Soil Organic Matter (SOM) content was highest in cattle-grazed sites and therefore it would again be expected that less roots are present in these soils. However, higher SOM content in cattle-grazed soils is only found in block A, all of the other blocks show a higher SOM content in the vole-grazed soils. The shoot biomass is also higher in vole-grazed soils compared to cattle-grazed soils. Therefore it is possible that the plants have invested more in shoot growth in these soils, since enough nutrients are available in the soil, especially nitrate which is also higher in vole-grazed soils. This corresponds to what was stated by Fitter (1997)<sup>16</sup>. The relationship between total biomass and SOM content is negative. So, the higher the SOM content, the lower the total biomass in the experiment. SOM is the basic substance from which a number of nutrients originate. Since it is expected that plants grow better when enough nutrients are available it was expected that total biomass and SOM would have a positive relationship.

SOM affects a large number of soil properties it includes: aggregate stability (which affects soil structure); cation exchange capacity (affects nutrient availability), nutrient release rate by mineralization (microbial activity in releasing N, P and other elements) and water-holding capacity<sup>35</sup>. In the experiment a significant block effect

was found for almost all measured parameters and for all four experimental plant species. This clustering effect can be due to the different soil types which are present in the field. Sandy soils (block A and B), with low SOM content are more susceptible for acidification of the soil. However, the pH becomes more acidic further away from the sandy soils.

pH is also linked to nitrate and ammonium content. The nitrate content is higher in vole-grazed soils, which can lead to more acidic soils. Nitrate is converted via nitrification or denitrification processes<sup>16</sup>, both of which are mediated by specialized bacteria. So, high nitrate content points to high concentrations of these specialized bacteria and maybe bacteria concentrations are higher in vole-grazed sites. However, higher nitrate concentration lead to higher pH and consequently biological activity of soil bacteria<sup>33</sup> is expected to be lower. This in turn could lead to less dynamics in vegetation composition. However the experimental biomass shows a positive trend with more alkaline soils, in which biological activity would be higher and thus more dynamics would be visible. Another effect of more acidic soils is suppression of root growth<sup>33</sup> and since the pH is more acidic in vole-grazed sites, less roots can be expected compared to cattle-grazed soils. A smaller amount of roots leads to a reduced uptake of for instance nitrate<sup>33</sup> which was shown to be higher in vole-grazed areas. When more nitrate remains in the soil the pH becomes more acidic and probably reduces the numbers of bacteria; which in the end amplifies the dynamics in the vegetation composition. So, we expected that mineralization rates and nitrate concentrations would be very important determining plant growth; however, it seems that pH is a more important factor.

Until now all abiotic and biotic predictors for total biomass were shown separately. When an analysis was done using several abiotic factors and abundance in the field in 2006, especially pH showed a very strong significant effect on total biomass. Also moisture content and SOM showed significant relationships with total biomass. This shows that total biomass is very dependent on the abiotic circumstances in which the plant grows. However, for two species and in two different grazing treatments abundance also showed a significant relation with total biomass. Therefore, we can not exclude abundance as a predictor for total biomass and thus there may still be a role for biotic soil feedback in determining plant community composition and dynamics, but it seems that the abiotic factors are more important determinants in this grassland system.

However there is another approach to see if soil-borne pathogens have an effect on vegetation dynamics and if this effect is limited by grazing of herbivores. This can also help with the conclusion to what extent plant growth is dependent on abiotic factors compared to biotic factors. By using the Coefficient of Variation (CV) it is possible to determine if the response of the plant species is less variable in cattle-grazed soils compared to vole-grazed soils? The CV shows to what extent heterogeneity is present in the data. Based on total biomass, data from this study show that in vole-grazed areas plants react more variable in growth compared to plants grown on the cattle-grazed soils. This is consistent with what Bakker (2002)<sup>2</sup> found during the experiment. The reaction of the blocks within in the experiment reacted more variable in the vole-grazed areas. There was even a lot of variation between the different plots within the blocks. This could indicate that cattle-grazed soils contain less species specific bacteria that can inhibit plant growth. This is also expected since the species composition in space in the vole grazed areas is very dynamic, while in cattle-grazed areas the herbivores keep the composition more stable in space and over time by grazing<sup>2</sup>. On the other hand this variation can also be due to abiotic factors like nutrient content or pH. It is expected that due to the large variations of plant growth in the plots, also different nutrient patches could come into existence, when the plants and the soil give feedback to each other. The patchiness of abiotic factors is also expected when the ammonium concentrations are taken into account, since cattle produce large depositions of urine compared to

the very small amounts produced by the voles. In the CV for all abiotic parameters measured in this study, only potential mineralization and nitrate content have significant differences between the grazing treatments. However, they show the opposite of the CV for total biomass. It is expected that plants react to the availability of nutrients. Caldwell *et al.* (1996)<sup>13</sup> found that root density in their experiment was higher in nutrient enriched sites. Fitter (1997)<sup>16</sup> also shows graphs of for instance the nitrogen cycle with the major transformations that determine nutrient availability to the plant. This corresponds to the idea that when plants are dependent on, in this case, nitrogen they should behave according to the same variation patterns. So biomass is expected to show a similar pattern to nitrate content or potential mineralization if plant growth is dependent on nutrient availability. In this study it is found that nitrate content and potential mineralization are more constant in vole-grazed soils and thus biomass is expected to be more constant in these soils as well. However, the CV of total biomass shows the opposite. Total biomass is more variable in vole-grazed soils compared to cattle-grazed soils. This shows that biomass does not react directly to nitrate content or potential mineralization in the soils, but that another mechanism is present in the system. This effect may be caused by soil borne pathogens that influence plant growth.

This is another lead to the question if the presence of soil-borne pathogens contributes to temporal and spatial variation in plant species composition in a grassland? So combining first of all the analysis of total biomass versus several abiotic factors and abundance in the field in 2006, which shows that abiotics are very important determinants for total biomass, however the abundance in the field in 2006 can not be forgotten. Secondly, the coefficient of variance for abiotics and biotics which show the opposite patterns and therefore are a strong lead to abiotics being probably the stronger, but not the only determinants for total biomass.

But does the presence of cattle inhibit this effect, i.e. are plant community dynamics controlled by soil organisms only in the absence of cattle? We can not say with certainty that cattle inhibit the effect of soil-borne biota on plant growth. Although the CV of total biomass showed that less variation is present in the cattle-grazed soils. Guitian *et al.* (2000)<sup>18</sup> found that biomass of several plant species was lower in grazed situations. Total biomass and root biomass in the experiment are higher in cattle-grazed areas, while shoot biomass is higher in vole-grazed areas. Although grazing was not simulated during this experiment, the composition of soil-borne biota was formed in the previous situation where large herbivores were present; therefore it is possible that plants have a higher biomass in cattle-grazed soils compared to vole-grazed soils. On the other hand it is expected that soil borne pathogens have a larger inhibiting effect on plant growth in the vole-grazed sites, since their inhibiting effect in cattle-grazed sites is suppressed by the grazing of cattle. This would result in higher biomass in cattle-grazed soils. Bever (1994)<sup>7</sup> also found that plants grown in their own soil inoculum, or roots, were smaller compared to plants grown in soils from other plants. Indicating that, soil borne pathogens accumulate in the rhizosphere of a specific species and by doing so inhibit plant growth. But when the plants are moved to soils from other species and therefore without the specific soil borne pathogens, the plants grow larger. Blomquist *et al.* (2000)<sup>9</sup> found the same effect by sterilizing soil from a specific stand. They also found that biomass of plants reduced less when they grew on ant mound, which were found to contain fewer bacteria.

## **Conclusion**

The growth of some plant species is negatively related to the cover of that same plant species in the field. And also the variation in growth with which the plants respond is less variable in cattle-grazed soils compared to vole-grazed soils. This response in plant growth appears not to be consistent with the variance of nitrate content and potential mineralization, but the nutrient contents and pH are probably very important factors for the dispersal and living conditions for soil-borne bacteria. Thus, this variation points in the direction of the presence of soil-borne pathogens determining plant growth. Therefore they can contribute to temporal and spatial variation in plant species composition. And since the cattle-grazed soils contain more nutrients and also show a higher biomass without actual grazing during the experiment, it is possible that the presence of cattle inhibits the effect of soil-borne pathogens.

## Literature

1. Allen, E.B. and Allen, M.F., 1990, In: Grace, J.B., Tilman, D., Perspectives on plant competition. Academic Press, San Diego.
2. Bakker, E.S., 2003, Herbivores as mediators of their environment, the impact of large and small species on vegetation dynamics. PhD Thesis, University of Wageningen.
3. Bardgett, R.D., Frankland, J.C., and Whittaker, J.B., 1993, The effects of agricultural practices on the soil biota of some upland grasslands. *Agriculture, Ecosystems and Environment* 45:25-45
4. Bardgett, R.D. and Leemans, D.L., 1995, The effects of cessation of fertilizer application, liming and grazing on microbial biomass and activity in a reseeded upland pasture. *Biology and Fertility of Soils* 19:148-154
5. Bardgett, R.D., Leemans, D.K., Cook, R. and Hobbs, P.J., 1997, Seasonality in the soil biota of grazed and ungrazed hill grasslands. *Soil Biology & Biochemistry* 29:1285-1294
6. Bardgett, J.D., Wardle, D.A. and Yeates, G.W., 1998, Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry* 30(14):1867-1878
7. Bever, J.D., 1994 Feedback between plants and their soil communities in an old field community. *Ecology* 75(7):1965-1977
8. Bever, J.D., Westover, K.M. and Antonovics, J., 1997, Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* 85:561-573
9. Blomquist, M.M., Olff, H., Blaauw, M.B., Bongers, T. and Van der Putten, W.H., 2000, Interactions between above- and belowground biota: importance for small-scale vegetation mosaics in a grassland ecosystem. *OIKOS* 90:582-598
10. Brasier, C.M. ,1990, In: Ingram, D.S. and Williams, P.H., Advances in plant pathology (vol.5), Academic Press pp.55-118
11. Bruehl, G.W., 1987, Soilborne Plant Pathogens. Macmillan Publishing. CO., New York, New York.
12. Burdon, J.J., Jarosz, A.M., Kirby, G.C., 1989, Pattern and patchiness in plant-pathogen interactions – causes and consequences. *Annual review of Ecological Systems* 20:119-136
13. Caldwell, M.M. Manwaring, J.H., Durham, S.L., 1996, Species interactions at the level of fine roots in the field: influence of soil nutrient heterogeneity and plant size. *Oecologia* 106:440-447
14. Demment, M.W. & van Soest, P.J., 1985, A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *American Naturalist* 125:641-672
15. Dobson, A. and Crawley, M., 1994, Pathogens and the structure of plant communities. *TREE* 9(10):393- 397
16. Fitter, A.,1997, In: Crawley, M.J., Plant ecology, Blackwell science, Oxford. pp.51-72
17. Freckman, D.W., Duncan, D.A., and Larson, J.R. 1979, Nematode density and biomass in an annual grassland ecosystem, *Journal of Range Management* 32(6):318-422
18. Guitian, R. and Bardgett, R.D., 2000, Plant and soil microbial responses to defoliation in temperate semi-natural grassland. *Plant and Soil* 220:271-277
19. Huntly, N. & Reichman, O.J., 1994, Effects of subterranean mammalian herbivores on vegetation. *Journal of Mammalogy* 75:852-859
20. McNaughton, S.J., 1983, Compensatory plant growth as a response to herbivory. *OIKOS* 40:329-336

21. Milchunas, D.G. & Lauenroth, W.K., 1993, Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* 63:327-366
22. Mills, K.E., and Bever, J.D., 1998, Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology* 79(5):1595-1601
23. Olff, H. & Ritchie, M.E., 1998, Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution* 13:261-265
24. Olff, H., Hoorens, B., de Goede, R.G.M., van der Putten, W.H., Gleichman, J.M., (2000) Small-scale shifting mosaics of two dominant grassland species: the possible role of soil-borne pathogens. *Oecologia* 125:45-54
25. Proulx, M. & Mazumder, A., 1998, Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. *Ecology* 79:2581-2592
26. Read, D.J., 1983, The biology of mycorrhizae in Ericales. *Canadian Journal of Botany* 61:985-1004
27. Shipton, P.J., 1977, Monoculture and soilborne plant pathogens. *Annual Review of Phytopathology* 15:387-407
28. Stephenson, S.L., 1986, Changes in a former chestnut-dominated forest after a half century of succession. *American Midland Naturalist* 116(1):173-179
29. Tillman, D. and Wedin, D., 1991, Oscillations and chaos in the dynamics of a perennial grass. *Nature* 353:653-655
30. Van der Putten, W.H., van Dijk, C. & Peters, B.A.M., 1993, Plant-specific soil-borne diseases contribute to succession in foredune Vegetation. *Nature* 362:53-56
31. Van der Putten, W.H. and Peters, B.A.M., 1997, How soil-borne pathogens may affect plant competition. *Ecology* 78(6):1785-1795
32. Van der Putten, W.H. and Van der Stoel, C.D., 1998, Plant parasitic nematodes and spatio-temporal variation in natural vegetation. *Applied Soil Ecology* 10:253-262
33. Woods,F.W. and Shanks, R.E., 1959, Natural replacement of chestnut by other species in the great smoky mountains national park. *Ecology* 40(3):349-361
34. [www.dpi.vic.gov.au/web/root/domino/target10/T10manuals.nsf](http://www.dpi.vic.gov.au/web/root/domino/target10/T10manuals.nsf)  
Fertilising dairy pastures 4.5.2  
Department of Natural Resources and Environment.
35. [www.uvm.edu/vtvegandberry/factsheets/soilorganicmatter.html](http://www.uvm.edu/vtvegandberry/factsheets/soilorganicmatter.html)  
Grubinger, V., Soil organic matter: the living, the dead and the very dead, University of Vermont, Department of plant and soil science.

## Appendix 1. Significances of Relationship between Total biomass and Abundance in the field.

### Cattle-grazed soils

Species	2005			2006		
	p	R <sup>2</sup>	F <sub>1, 18</sub>	p	R <sup>2</sup>	F <sub>1, 18</sub>
<i>H. lanatus</i>	- 0.342	0.050	0.95	- 0.265	0.068	1.329
<i>F. rubra</i>	- 0.544	0.021	0.382	- 0.722	7.224e <sup>-5</sup>	0.131
<i>A. capillaris</i>	+ 0.061	0.181	3.981	- 0.280	0.065	1.242
<i>R. acetosa</i>	<b>+ 0.009</b>	0.321	8.510	<b>+ 0.031</b>	0.233	5.480

- negative relation

+ positive relation

### Vole-grazed soils

Species	2005			2006		
	p	R <sup>2</sup>	F <sub>1, 18</sub>	p	R <sup>2</sup>	F <sub>1, 18</sub>
<i>H. lanatus</i>	+ 0.995	1.926e <sup>-6</sup>	0.35e <sup>-4</sup>	- 0.958	1.592e <sup>-4</sup>	0.003
<i>F. rubra</i>	- 0.141	0.117	2.376	= 0.980	3.509e <sup>-5</sup>	0.001
<i>A. capillaris</i>	<b>- 0.034</b>	0.226	5.257	<b>- 0.022</b>	0.258	6.250
<i>R. acetosa</i>	+ 0.246	0.074	1.441	+ 0.166	0.104	2.081

- negative relation

+ positive relation

= constant relation