The relationship between nutritional quality of plants and seed intake by large herbivores

RuG

student: Sander IJ. Jonker
supervisors: A.M. Mouissie
J.P. Bakker

December 2003

Community and Conservation Ecology Group
Rijks*universiteit* Groningen

D 766

Abstract

Many former agricultural fields become available for nature development. These fields have to be colonised by seeds of target species as often the topsoil together with the seed bank will be removed. Colonisation by target species can be stimulated by fencing the nature development site together with so called source sites. Then herbivores may disperse the seeds by epizoochory and endozoochory. This study focuses on endozoochory.

We studied the relationship between seed survival in cattle and of seed uptake by fallow deer and nutritional quality of the foliage. Three hypotheses have been tested: (1) seeds of plants with relatively high nitrogen content are overrepresented in the dung of Scottish Highland cattle compared with the seed supply in the same area; (2) intake of seeds by fallow deer is positively correlated with nitrogen content of foliage within plant species grown on different nitrogen supply; (3) intake of seeds by fallow deer is positively correlated with nitrogen content of the foliage between three plant species (*Agrostis capillaris*, *Juncus effusus* and *Erica tetralix*).

To test the first hypothesis the correlation between nitrogen content of various plants at Dellebuursterheide and seed composition in the dung of cattle was tested. In this survey it cannot be concluded that there was a significant correlation. But there was a positive correlation between Ellenberg nitrogen indicator value and nitrogen content. To test the second hypothesis *Plantago major* and *Stellaria media* were grown upon soils with different nitrogen supply and used in a food choice experiment. In neither of the species nitrogen content in foliage and seed intake did significantly correlate. In *Plantago major* there was a significant increase in seed uptake with increasing length of the fruiting stems. In *Stellaria media* there was a significant correlation between nitrogen content of the fruiting stems and the percentage foraging time.

To test the third hypothesis the three species were used in a food choice experiment. There was no significant correlation between nitrogen content of the plants and uptake of seeds. Nitrogen content of plants on itself seems to be no good indicator for the potential seed dispersal. But together with other researches it is shown that heathland species are dispersed less than species from areas that are richer in nutrients. Therefore we recommend to exclude grazing from heathlands, especially in summer.

Contents

page

1 **Introduction** 1

2 **Materials and methods** 4

2.1 Relationship between endozoochory and plant nitrogen content: a field study 4
2.1.1 Study site
2.1.2 Nitrogen content of plant species
2.1.3 Data analysis

2.2 Food choice experiment fallow deer 6
2.2.1 Cultivation of plants offered to fallow deer
2.2.2 Experimental design
2.2.3 Data analysis

3 **Results** 9

3.1 Relationship between endozoochory and plant nitrogen content: a field study 9
3.1.1 Relationship between nitrogen content of plant species and seed dispersal
3.1.2 Nitrogen contents compared with Ellenberg nitrogen indicator values

3.2 Food choice experiment fallow deer 14
3.2.1 Selectivity of fallow deer for quality and quantity of *Plantago major*
3.2.2 Selectivity of fallow deer for quality and quantity of *Stellaria media*
3.2.3 Choice between three species

4 **Discussion** 21

4.1 Relationship between N-content of plants and seed density in cattle dung
4.2 The role of N-contents of plants in diet selection of fallow deer
4.3 Limitations of our study

Acknowledgements 24

References 25

Appendices

1 Introduction

Nowadays many agricultural fields become available for ecological restoration. In many of these places the topsoil is removed in order to get the right abiotic conditions (i.e. nutrient poor) for the restoration and development of heathlands or species-rich grasslands. But with the removal of the soil the seed bank is also removed. In order to recover, these areas have to be colonised by target plant species. As plants are sessile their colonisation depends on successful dispersal of their seeds to new sites. The larger the area that is covered through dispersal the higher the chance of having a successful colonisation as the possibility of finding a convenient site becomes larger. Plant seeds can be dispersed by wind, water, animals and machines (Bakker *et al.* 1996). The results of wind dispersal would probably be small as wind tunnel experiments with 22 species of pasture and heath showed that wind dispersal occurs only on a scale of a few meters (Zijlstra 1992, Klooker *et al.* 1999). Dispersal by animals is considered to have a larger range, therefore it has been thought that colonisation by target species can be stimulated by fencing the nature development site together with so called source sites and graze them by different herbivore species (Klooker *et al.* 1999).

Seeds can be dispersed by small animals (i.e. ants) and larger animals (birds, mammals, etc.) in their fur (epizoochory), in their gut (endozoochory) or by scatter hoarding (dysochory). Specific feeding habits, home range and migration movement complicate the possible seed dispersion patterns by zoochory (Harper 1977, Bonn & Poschlod 1998). The number of seeds dispersed by animals is first determined by the seed output of a plant species (Nathan & Muller-Landau 2000). In endozoochory the size and range of dispersal depends further on the amount of seeds that is taken in, the percentage of seeds that is still viable after the passage through the alimentary tract, the retention time inside the animal and the home range of the animal (figure 1.1) (Bonn & Poschlod 1998). Viability of seeds after gut passage is determined by the resistance of particular species to the process of chewing and gut passage. Seed survival through animals is related to the degree of hardness of seeds. Small and hard seeds have less chance of being crushed by teeth and are more resistant for the enzymes and acids than larger and softer seeds (Gardener *et al.* 1993, Frankton & Mulligan 1987). The retention time, which differs between herbivore species, also plays a key role in the survival of seeds: the longer the retention time the lower the number of viable seeds (Bonn & Poschlod 1998).

Seeds that are still viable in the herbivore dung have a competition advantage above seeds that are dispersed by wind or epizoochory as large faecal droppings have at least four effects on plants, beneath and around it: (1) smothering and exclusion of light from the adjacent plants; (2) local disturbance of the nutrient relations; (3) changes in the pattern of grazing around the pat which animals tend to avoid; (4) creation of an island for colonisation (Harper 1977).

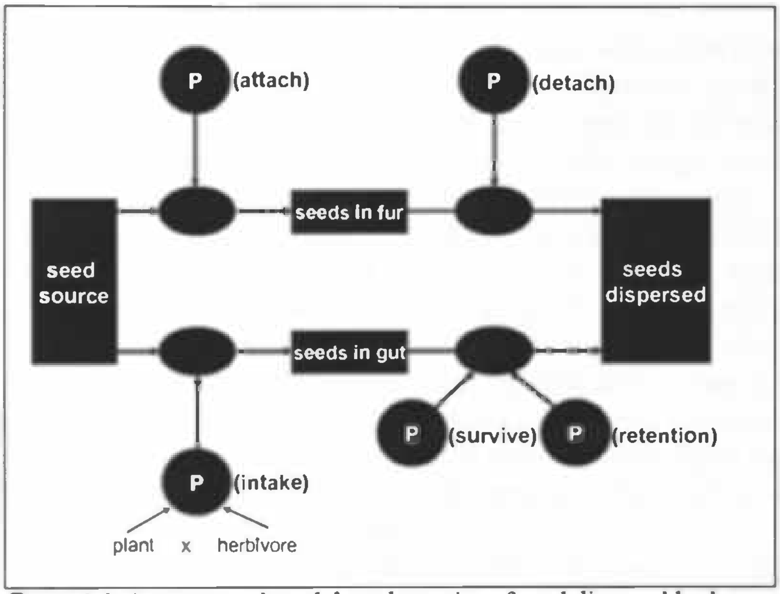

Figure1.1: A conceptual model on dynamics of seed dispersal by large herbivores. Epizoochory is shown by the upper part and endozoochory by the lower part (from Mouissie (unpublished)).

The present study focuses on the endozoochory by large herbivores. We studied the first step in the process of endozoochory; the intake of seeds by large herbivores. And we compared this with the nutritional quality of the seed source (lower left part of figure 1.1). Wallis de Vries (1994) distinguishes four levels of factors which determine foraging behaviour of animals: the spatial scale, currencies, information and the individual context. The spatial factor will be determined by landscape, plant community, patches and plants. Currencies are the availability of energy, proteins and minerals in the environment. Information about seasonal change, spatial variation, sampling and visual and tactile cues can influence herbivore behaviour. The implementation of this information depends on the individual context which is formed by body condition, individual experience, social experience and other priorities like predation pressure.

Seed intake can happen accidentally or intentionally. Seed intake happens intentionally when an animal deliberately takes in a seed as food source. When intake happens accidentally, the animal does not want the seeds but the foliage or a fruit. The 'foliage is the fruit'-hypothesis of Janzen (1984) states that some plant species with small seeds are evolved to be consumed by large herbivores. It says foliage to herbivores is like berries to birds. When these herbivores eat the foliage of the plants they automatically take in the seeds. These seeds survive inside the animal and are dispersed when they leave the animal. The plant is tasty when the seeds are ready: the plant concentrates on the selectivity of the herbivore. This would be formed co-evolutionary (mutualism). On the other hand it is possible that the plant tends to avoid being eaten and that the seeds survive by coincidence. The seeds have become more resistant to survive the route through the animal ('making the best of a bad job') (Janzen 1984).

In the present study we tested if there is a relationship between the nutritional quality of plants and seed intake by large herbivores. In general the research focused on the question: are seeds of palatable plants dispersed better than seeds of plants that are less palatable and which habitat type contains plants that are more palatable. Within this main question three hypotheses are tested.

First hypothesis (H1): seeds of plants with relatively high nitrogen content are overrepresented in the dung of Scottish Highland cattle compared with the seed supply in the same area. To test this hypothesis the nitrogen contents of plants were compared with the seed composition in the dung. These seed compositions are coming from a previous study by M. Mouissie to find out the seed survival and viability after passing the digestion tract of cattle (Scottish Highland cattle) as well as to predict the nutrient and seed dispersal into the area of Dellebuurster Heide. Mouissie *et al.* (unpublished) found positive correlations between the Ellenberg nitrogen indicator values of plants and the numbers of plant seeds that were found in the dung of cattle. Ellenberg nitrogen indicator values of the specific plants were determined in order to establish the relationship between the nutritional quality of plants and the area they are expected to grow upon. Plant samples were taken from the Dellebuurster Heide at the same moments in the year as the samples of cattle dung were collected by M. Mouissie.

Second hypothesis (H2a): intake of seeds by fallow deer (*Cervus dama*) (figure 1.2) is positively correlated with nitrogen content of foliage within plant species grown on different nitrogen supply. It is thought that plants have higher nutritional quality and are more attractive for herbivores when having relatively much protein. To test this hypothesis *Plantago major* and *Stellaria media* were grown upon soils with different nitrogen supply and used in a food choice experiment with fallow deer. These intermediate feeders are thought to selectively minimise the fibre content in their diet as well as exploiting any better source of nutrients to optimise their intake (Geist 1982).

Third hypothesis (H2b): intake of seeds by fallow deer is positively correlated with nitrogen content of the foliage between three plant species. *Agrostis capillaris*, *Juncus effusus* and *Erica tetralix* were used for this experiment as these plants play important roles on heathlands. In this experiment fallow deer (*Cervus dama*) were fed plants that contained varying amounts of nitrogen to get insight in the possible preference of deer for nitrogen rich plants. Fallow deer are appropriate study herbivores qualified as intermediate feeders (Hofmann 1985). This means they have a preference for certain high quality food but are not too fussy in their food choice as concentrate selectors. Hence we expect our results to apply to a wide range of herbivores.

Figure 1.2: A couple of male fallow deer (*Cervus dama*).[1]

2 Materials & Methods

2.1 Relationship between endozoochory and plant nitrogen contents: a field study

Hypothesis 1: seeds of plants with relatively high nitrogen content are overrepresented in the dung of Scottish highland cattle compared with the seed supply.

2.1.1 Study site

The Dellebuursterheide (200 ha) is situated in east-Friesland, and is a remnant of extensive heathlands along the river Tjonger. The study site consists of the Delleboeren (heathland, bog and forest), the Hoorn (river dunes) and former arable fields of which the topsoil is removed (25 ha) (Klooker *et al.* 1999). Almost the whole site (180 ha) is grazed year-round since 1979 by livestock (cattle, horses and sheep) of which the number fluctuates (Jager 1999). Plants were sampled from the heathland (nutrient-poor), the mesotrophic grassland (moderate nutrient level) and the oligotrophic grassland (nutrient-rich) (Table 2.1).

Table 2.1: Species sampled for nitrogen analysis. Per species the family, habitat type and sampling month are indicated.

species	family	habitat type	December	March	June
Erica tetralix	Ericaceae	heathland	x	x	x
Calluna vulgaris	Ericaceae	heathland	x	x	x
Holcus lanatus	Poaceae	mesotrophic	x		x
Molinia caerulea	Poaceae	heathland	x	x	x
Agrostis capillaris	Poaceae	oligo/meso		x	x(2)
Festuca ovina	Poaceae	oligotrophic			x
Galium saxatile	Rubiaceae	oligotrophic	x		x
Juncus effusus	Juncaceae	heathland	x	x	x
Cerastium fontanum	Caryophyllaceae	mesotrophic	x		x
Plantago lanceolata	Plantaginaceae	mesotrophic			x
Rumex acetosella	Polygonaceae	mesotrophic			x
A. capillaris + G. saxatile	mixture	oligotrophic	x		
F. ovina + A. capillaris	mixture	oligotrophic		x	
H. lanatus + P. trivialis	mixture	mesotrophic		x	
bulk grasses	mixture	oligotrophic	x		
short vegetation	mixture	mesotrophic		x	

2.1.2 Nitrogen contents of plant species

In order to get a complete image of the food opportunities of the animals, samples are taken of plants that have seeds as well as plants that do not, but that can serve as an alternative food source. Plant samples were taken from different parts of the study site on December 18th 2002, March 25th 2003 and June 17th 2003 (Table 2.1). A manual sampling method was used in order to get representative samples. The survival rates that came out of the earlier research of Mouissie *et al.* (unpublished) were compared with the N-content of the plant samples to test the correlation between dispersal of seeds of a plant and quality of its leaves. N-content is often used as quality indicator (Lesage *et al.* 2000; Semiadi *et al.* 1995). With the amount of nitrogen the amount of proteins is counted indirectly, especially when you leave the NO_3-form out of account. Food quality can also be estimated by determining %NDF (neutral detergent fibre), %ADF (acid detergent fibre) and amount of hard tissues such as lignin (Lesage *et al.* 2000; Wilmshurst *et al.* 1994). But the analyses for these indicators would have taken too long for the research period, so the choice was made to do N-analyses only. The N-analysis is done with the Kjeldall-method (appendix 1), adapted to leave the nitrogen in nitrate form out of account. So only the nitrogen in proteins is analysed.

2.1.3 Data analysis

The results for every plant species were compared with the amount of seeds in cattle dung and the Ellenberg nitrogen indicator value. For every sampling date the species were analysed with a One-way ANOVA and post hoc Tukey HSD (P=0.05) in order to test which species differed in N-contents. The nitrogen contents of the plant samples that contained seeds were compared with the ratio of seeds in cattle dung and supply using a One-way ANOVA. Also nitrogen contents and Ellenberg nitrogen indicator values of every species were compared.

2.2 Food choice experiment fallow deer

Hypothesis 2a: intake of seeds by fallow deer (*Cervus dama*) is positively correlated with nitrogen content of foliage of *Plantago major* and *Stellaria media* grown on different nitrogen supply.
Hypothesis 2b: intake of seeds by fallow deer is positively correlated with nitrogen content of the foliage between three plant species.

2.2.1 Cultivation of plants offered to fallow deer

The plant species that were used for this feeding experiment were *Plantago major* (Plantaginaceae) and *Stellaria media* (Caryophyllaceae), respectively a species with the seeds placed outside the foliage and a species with the seeds between the foliage (Figure 2.1). Both species have small and hard seeds, what is supposed to be an advantage in surviving the alimentary tract of herbivores. Small seeds will not be crushed very easily when the herbivore chews the plant material and when the seeds are hard the digestive acids and enzymes can do less harm. Both species are found flowering on cattle dung pats (Mouissie *et al.* unpublished). Both species normally germinate in spring. *Stellaria media* develops seeds year round and *Plantago major* in July, but because they are grown in the greenhouse they were fructificated earlier. Both species set seed within the year of germination. The plants were cultivated in 10-liter trays with a sand/potting compost-mixture (sand: potting compost 3:1) and different N-levels (Table 2.2).

Table 2.2: Scheme of the amount of seeds and the NH_4NO_3-additions for *Plantago major* and *Stellaria media* in the greenhouse as a preparation for the feeding experiment with fallow deer. *P. major*: 1st addition is February 19th and 2nd addition is March 19th 2003. *S.media*: 1st addition is May 20th and 2nd addition is June 12th 2003.

P. major	sown	1st addition	2nd addition
poor	0.37 g	x	x
medium	0.37 g	250 ml 31.98 mM	250 ml 31.98 mM
rich	0.37 g	250 ml 608.0 mM	250 ml 608.0 mM

S.media	sown	1st addition	2nd addition
poor	0.5 g	x	x
poor +	0.5 g	x	100 ml 31.98 mM
medium	0.5 g	250 ml 31.98 mM	x
medium +	0.5 g	250 ml 31.98 mM	250 ml 31.98 mM
rich	0.5 g	250 ml 152.0 mM	x
rich +	0.5 g	250 ml 152.0 mM	250 ml 31.98 mM

For both species the levels of nitrogen were chosen in order to get no overdose resulting in dead plants or doses that are too low resulting in a lack of differentiation between the different levels (Van Andel 1974, De Graaf 2000). Per tray 0.37 gram seeds of *Plantago major* and 0.5 gram seeds of *Stellaria media* were sown to get equally and fully covered trays. The N-levels in the soils are approximately 1.6 mM, 2.4 mM and 16.8 mM for the trays with *Plantago major*. In order to realize these levels different amounts of nitrogen were added (Table 2.2). The poor soil consists only out of sand and potting soil. Because of the potting soil the nitrogen concentration is initially circa 1.6 mM. The other two soils, medium and rich, have nitrogen added. An NH_4NO_3-solution was chosen to keep the levels of other elements equal for every treatment. To influence quality rather than quantity, only nitrogen was differentiated by adding NH_4NO_3 to the soil. Nevertheless for control the sizes of the plants are also taken into account. Before the start of the feeding four different qualities were determined for each tray. Some material was taken from the plants (circa 7 grams ~ approximately 1 gram dry weight) for analysing the N-content. From each tray the height of the leaves and the height of the inflorescences were measured as well as the number of inflorescences.

Figure 2.1: *Stellaria media* (left) and *Plantago major* were the species used in this experiment.[2,3]

2.2.2 Experimental design

The experiment was carried out in a deer enclosure in Lieren hosting five fallow deer. The first plant species that was used for the experiments was *Plantago major*. The trays were placed randomly (by drawing lots) in a matrix with circa 2 meters space in between (Figure 2.2a). At a time interval of 15 seconds notations were made for each tray whether a deer was eating from it or not. With the interval times the endurance of the visits was determined. The inflorescences were counted after 5, 20, 60 and 120 minutes to obtain an overview of the foraging behaviour during the course of time.

The second time the *Stellaria media*-trays were offered to the fallow deer. The number of inflorescences was determined as well as the height of the plants and the N-content of the leaves and the inflorescences. Fourteen trays were put randomly in a matrix (Figure 2.2b). Again at a time interval of 15 seconds notations were made for each tray whether a deer was eating from it or not. The number of inflorescences left was counted for each tray after 20, 60 and 80 minutes.

The last feeding experiment was done with three different species that roughly represent the plant quality composition of heathland-grassland mosaics in the Netherlands, namely, *Erica tetralix*, *Juncus effusus* and *Agrostis capillaris*. Three trays of each species were placed randomly in a matrix after the number of inflorescences was counted (Figure 2.2c). Just like the other experiments the number of visits was counted by making notations at intervals of 15 seconds. At two points in time (after respectively 20 and 40 minutes) during the experiment the fruiting stems were counted to see if there were differences between the species.

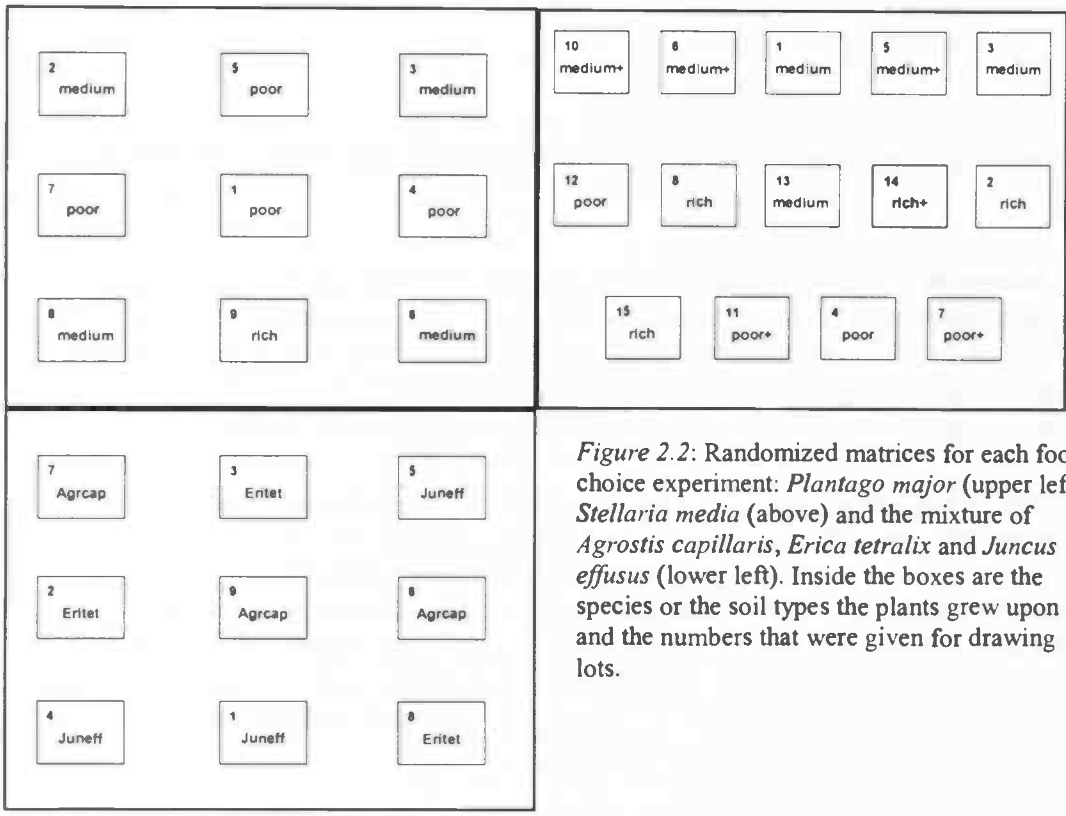

Figure 2.2: Randomized matrices for each food choice experiment: *Plantago major* (upper left), *Stellaria media* (above) and the mixture of *Agrostis capillaris*, *Erica tetralix* and *Juncus effusus* (lower left). Inside the boxes are the species or the soil types the plants grew upon and the numbers that were given for drawing lots.

2.2.3 Data analysis

After the experiment the relationships between the eating time of the animals and the various parameters of the different trays were tested using Pearson's correlation. This is also done for the percentage of eaten inflorescences and the different parameters. The nine trays were classified into three groups of different nitrogen contents: 0-6, 6-10 and >10 mg N/g plant material. The differences between percentages eaten fruiting stems through time between the three different nitrogen content groups in *Plantago major* were compared using a One-way ANOVA and Tukey HSD-test. Differences between the three different species are determined using a Univariate Analysis of Variance (ANOVA) and Tukey HSD.

3 Results

3.1 Relationship between endozoochory and plant nitrogen contents: a field study

3.1.1 Relationship between nitrogen content of plant species and seed dispersal

In the samples of December 18th the heathland species have the lowest nitrogen amounts followed by species from the oligotrophic and mesotrophic grasslands. Non seeding *Juncus effusus* individuals have significantly higher nitrogen contents than the individuals with seeds. Poaceae and Rubiaceae have the highest nitrogen contents (Table 3.1A).

In the samples of March 25th differences in N-contents of the plants from the different habitat units is clearer: heathland species are lowest, followed by oligotrophic and mesotrophic grassland species (Table 3.1B).

In June the species of the different habitat units are more or less mixed up: the clear division is no longer there. *Juncus effusus* and *Molinia caerulea* belong no longer to the species that are lowest in nitrogen. There is even no significant difference between species (Table 3.1C).

Table 3.1: Nitrogen contents of plant samples (mg N/g plant) taken at different habitat units (n = numbers of samples). Samples were taken at three dates: December 18th 2002 (A), March 25th 2003 (B) and June 17th (C). The results have been obtained with a Tukey HSD (α=0.05). Different letters indicate significant differences between the species.

A) December 18th 2003

sample	habitat	n	nitrogen content
Molinia caerulea	heathland	3	6.5 ± 1.3 a
Erica tetralix	heathland	3	10.3 ± 0.29 b
Calluna vulgaris	heathland	4	10.7 ± 0.51 bc
Juncus effusus	heathland	3	13.1 ± 0.58 bc
A. capillaris + G. saxatile	oligotrophic	2	15.5 ± 0.74 bcd
bulk grasses	oligotrophic	2	15.9 ± 0.25 d
J. effusus seedless	heathland	2	18.6 ± 0.11 de
Holcus lanatus	oligotrophic	3	22.8 ± 1.7 ef
Cerastium fontanum	mesotrophic	2	22.9 ± 5.4 ef
Galium saxatile	oligotrophic	3	23.2 ± 2.0 ef
Holcus lanatus	mesotrophic	2	24.9 ± 0.92 f

B) March 25th 2003

sample	habitat	n	nitrogen content
Molinia caerulea	heathland	4	7.4 ± 1.7 a
Erica tetralix	heathland	4	10.5 ± 0.33 b
Juncus effusus	heathland	5	11.7 ± 1.9 bc
Calluna vulgaris	heathland	4	14.1 ± 0.56 bcd
Agrostis capillaris (dry)	oligotrophic	4	15.6 ± 1.3 cd
Festuca ovina + A. capillaris	oligotrophic	3	17.1 ± 2.3 d
H. lanatus + P. trivialis	mesotrophic	3	22.5 ± 1.2 e
short vegetation	mesotrophic	3	29.2 ± 2.7 f

C) June 17th 2003

sample	habitat	n	nitrogen content
Erica tetralix	heathland	3	12.5 ± 0.95 a
Calluna vulgaris	heathland	3	12.8 ± 0.82 a
Cerastium fontanum	mesotrophic	2	15.6 ± 2.3 a
Festuca ovina	oligotrophic	3	15.8 ± 0.58 a
Rumex acetosella	oligotrophic	3	15.9 ± 1.5 a
Molinia caerulea	heathland	3	16.4 ± 2.8 a
Agrostis capillaris	oligotrophic	3	17.7 ± 2.4 a
Juncus effusus	heathland	3	18.7 ± 2.7 a
Holcus lanatus	mesotrophic	3	20.1 ± 2.2 a
Plantago lanceolata	mesotrophic	3	20.6 ± 4.8 a
Galium saxatile	oligotrophic	3	20.8 ± 3.4 a
Agrostis capilaris	mesotrophic	3	23.6 ± 10.7 a

The nitrogen content of plants at Dellebuursterheide that carried seeds were compared with the data from Mouissie *et al.* (unpublished) on seed density in cattle dung. These seed amounts were divided by the relative abundance in the area to get the cattle/supply ratio. In December the plants that carried seeds were *Calluna vulgaris*, *Erica tetralix*, *Juncus effusus* and *Molinia caerulea* (Table 3.2A). The correlation between nitrogen content and cattle/supply ratio turned out to be insignificant (P = 0.21). In March the same species carried seeds as in December (Table 3.2B). Again there was no significant correlation (P = 0.40). In June the species carrying seeds were *Juncus effusus*, *Rumex acetosella*, *Cerastium fontanum* and *Holcus lanatus* (Table 3.2C). There was no significant correlation (P = 0.67).

Table 3.2: Nitrogen content of plant species having seeds, sampled on December 18th 2002 (A), March 25th 2003 (B) and June 17th 2003 (C). The seeds in cattle dung and seed supply ratio (c/s-ratio) of the same species is also shown. There was no significant correlation in any of the sampling sets.

A) 18th December 2002

species	N-content	c/s-ratio
Calluna vulgaris	0.00223	10.74
Erica tetralix	0.00334	10.26
Juncus effusus	0	13.06
Molinia caerulea	0.00393	6.53

B) 25th March 2003

species	N-content	c/s-ratio
Calluna vulgaris	0.00006	14.08
Erica tetralix	0	10.48
Juncus effusus	0.0771	11.10
Molinia caerulea	0	7.47

C) 17th June 2003

species	N-content	c/s-ratio
Juncus effusus	0.0121	18.66
Rumex acetosella	0	15.93
Cerastium fontanum	0.0133	15.56
Holcus lanatus	0.0147	20.06

3.1.2 Nitrogen contents compared with Ellenberg nitrogen indicator value

Nitrogen contents of all plants sampled at Dellebuurster Heide were compared with their Ellenberg nitrogen indicator values (Figures 3.1, 3.2 and 3.3). The samples taken in December (Figure 3.1) and March (Figure 3.2) show a significant positive correlation between the nitrogen content of the species and their Ellenberg nitrogen indicator value (respectively $P = 0.026$ and $P = 0.038$). In the samples of June this correlation is no more significant ($P = 0.066$). Each species has nitrogen contents between 12 and 20 mg N/g plant material (dry weight) (Figure 3.3).

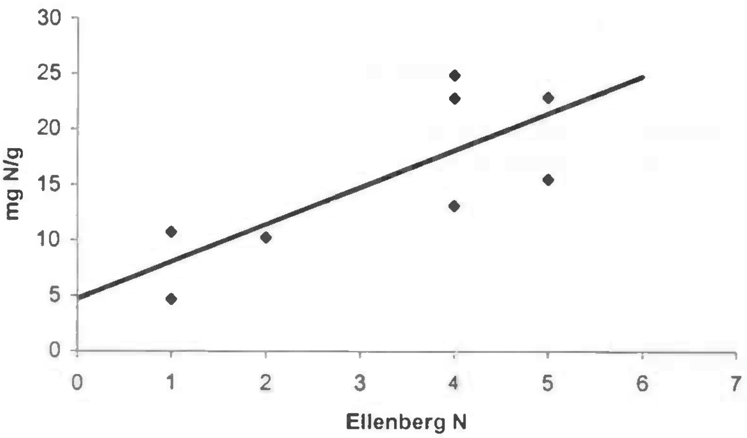

Figure 3.1: Nitrogen content versus Ellenberg N of the plant samples taken at Delleburen on December 18th 2002. Linear regression: $y = 3.3x + 4.7$, $R^2 = 0.59$. $P = 0.026$.

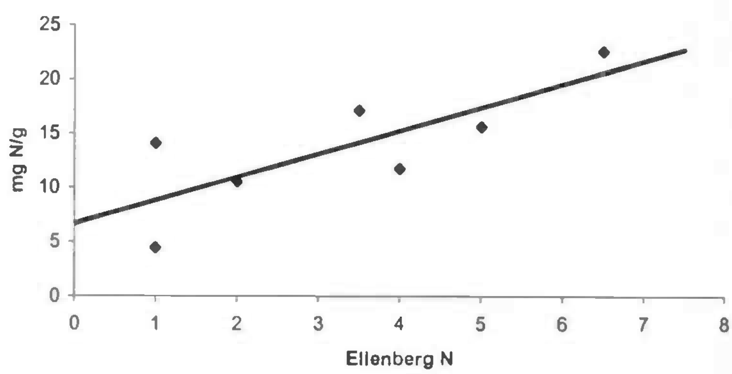

Figure 3.2: Nitrogen content versus Ellenberg N of the plant samples taken at Delleburen on March 25th 2003. Linear regression: $y = 2.1x + 6.7$, $R^2 = 0.61$. $P = 0.038$.

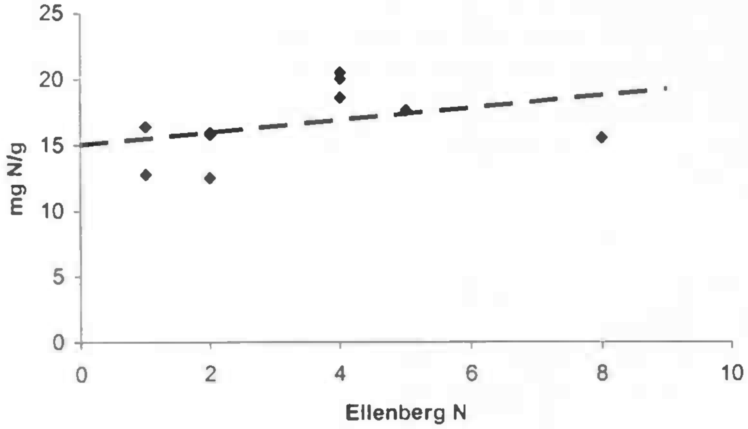

Figure 3.3: Nitrogen content versus Ellenberg N of the plant samples taken at Delleburen on June 17th 2003. Linear regression: 0.47x + 15.0, $R^2 = 0.14$. $P = 0.066$.

3.2 Food choice experiment fallow deer

3.2.1 Selectivity of fallow deer for quality and quantity of Plantago major

The rich group apparently has got the most eaten seeds followed by the medium group, although this was still the other way round after 300 seconds. From the start the poor group was eaten the least. The differences are at any time insignificant according to the One-way ANOVA (Figure 3.7).

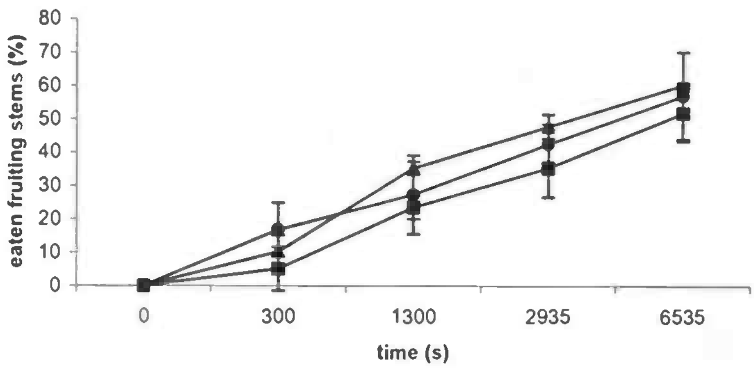

Figure 3.7: Eaten fruiting stems through time: percentages of seeds eaten per group are put against time (▲= >10 mg N/g, ● = 6-10 mg N/g, ■ = 0-6 mg N/g). No significant differences: (300s) $P = 0.18$, (1300s) $P = 0.39$, (2935s) $P = 0.30$, (6535s) $P = 0.68$. Error bars indicate standard error of the mean.

When the percentage of eaten fruiting stems is compared with nitrogen content the various points are scattered and there is no significant correlation between the two parameters: Pearson's Rho = 0.151, ($P = 0.70$) (Figure 3.8).

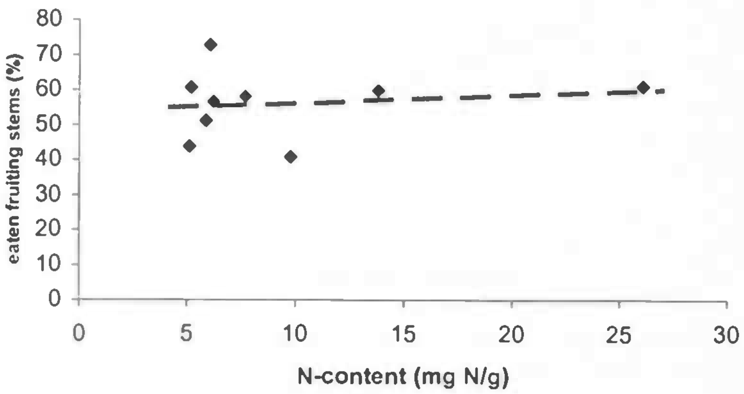

Figure 3.8: Eaten fruiting stems of *Plantago major* by fallow deer versus nitrogen content. Linear regression: $y = 0.21x + 54.0$, $R^2 = 0.023$. No significant correlation: $P = 0.70$.

Furthermore none of the parameters that were analysed showed a significant correlation with the amounts of fruiting stems that were eaten during the experiment. The correlation between foliage height and eaten fruiting stems was not significant (*P*

= 0.36). Fruiting stem height and the height the fruiting stems pointed out of the foliage did show a positive correlation with the percentage of eaten fruiting stems but was still not significant (respectively $P = 0.067$ and $P = 0.087$).

The fallow deer did not eat longer at trays that had high nitrogen contents than at those with low ($P = 0.57$). This was the case for every period within the experiment (data not shown). The initial number of fruiting stems did have a significant correlation with foraging time ($P = 0.017$) (Figure 3.9).

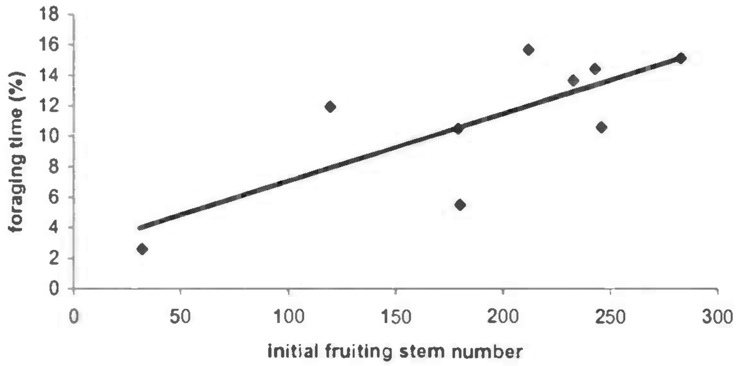

Figure 3.9: Initial fruiting stem number in *Plantago major* versus foraging time by fallow deer in percentages. Linear regression: $y = 0.048x + 1.9$, $R^2 = 0.54$. $P = 0.017$.

Fruiting stem height did also have a positive, significant ($P = 0.029$) correlation with foraging time (Figure 3.10).

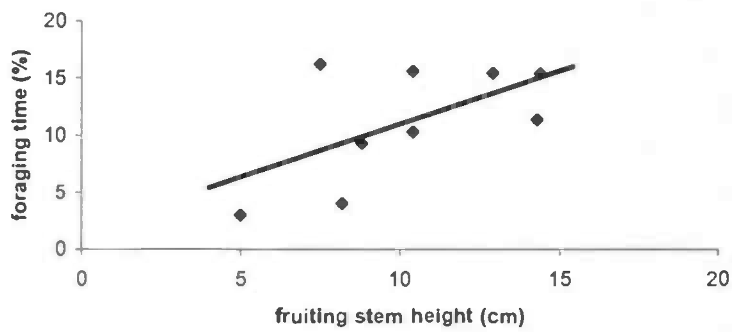

Figure 3.10: Fruiting stem height of *Plantago major* versus foraging time by fallow deer in percentages have a positive correlation. Linear regression: $y = 0.92x + 1.7$, $R^2 = 0.35$. $P = 0.029$.

The fallow deer seem to be more attracted by the size of the plants than by the N-content: N-content is not significantly correlated with the amount of seeds eaten during the experiment neither with the time spent at the different trays. During the first 20 minutes of the experiment the fallow deer spent significantly more time at trays with higher foliage but the foliage height was not significantly correlated with the number of eaten fruiting stems.

3.2.2 Selectivity of fallow deer for quality and quantity of Stellaria media

In *Stellaria media* there was no significant correlation between nitrogen content of the foliage and percentage of eaten inflorescences in this experiment ($P = 0.94$) (Figure 3.11). Also the initial number of fruiting stems did not significantly influence the percentage of eaten fruiting stems ($P = 0.64$) (data not shown).

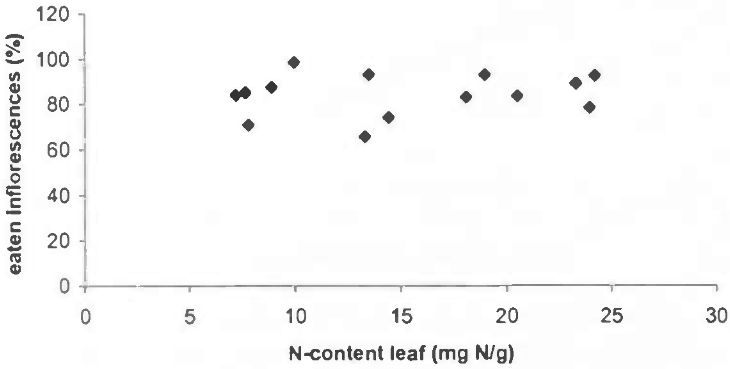

Figure 3.11: Nitrogen content of the leaves of *Stellaria media* versus percentage of eaten fruiting stems by fallow deer. $P = 0.94$.

Time spent per tray seemed to increase with nitrogen content of the leaves, but this is not significant ($R^2 = 0.45$, $P = 0.30$, Figure 3.12).

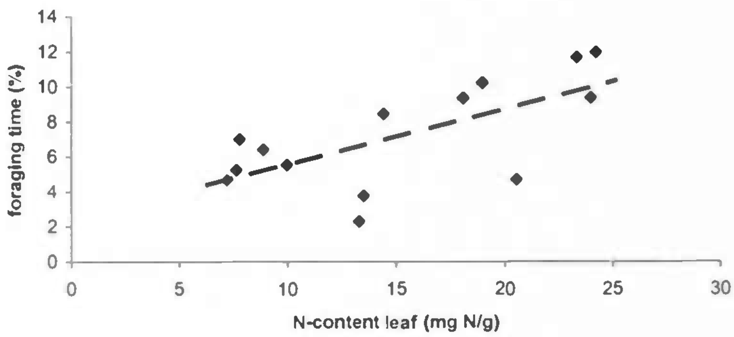

Figure 3.12: Nitrogen content of the leaves of *Stellaria media* versus foraging time in percentages by fallow deer. Linear regression: $y = 0.31x + 2.4$, $R^2 = 0.45$. $P = 0.30$.

Percentage eaten fruiting stems did not have significant correlation with the nitrogen content of fruiting stems ($P = 0.79$) (data not shown). Nitrogen content of the fruiting stems and foraging time showed a significant correlation ($P < 0.001$) and explained 84% of the variation ($R^2 = 0.84$) (Figure 3.13).

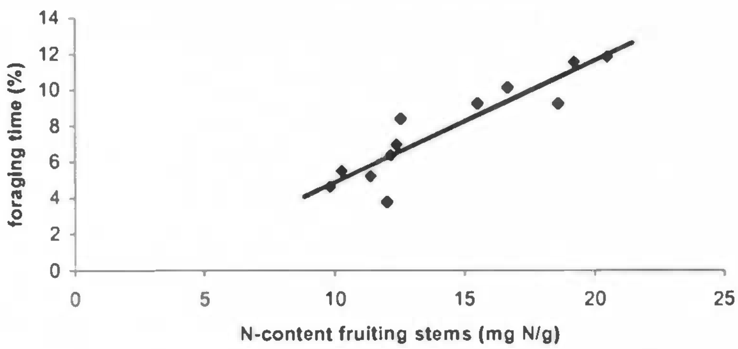

Figure 3.13: Nitrogen content in fruiting stems of *Stellaria media* versus foraging time in percentages by fallow deer. Significant correlation. Linear regression: $y = 0.68x - 1.9$, $R^2 = 0.84$, $P < 0.001$.

The height of the leaves of *Stellaria media* was not correlated with the percentage of eaten fruiting stems by fallow deer (data not shown), but leaf height was positively correlated with foraging time of fallow deer ($R^2 = 0.79$, $P < 0.001$, Figure 3.14).

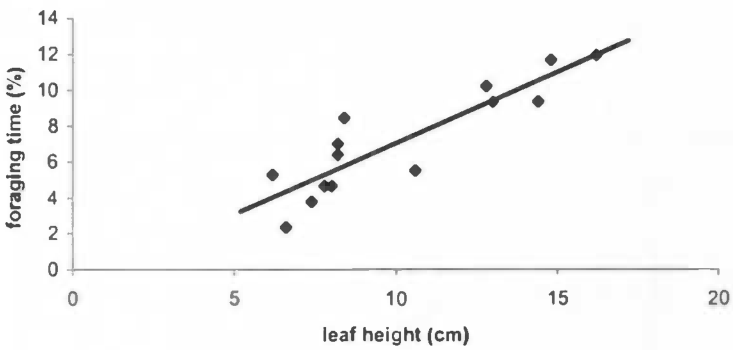

Figure 3.14: Leaf height of *Stellaria media* versus foraging time by fallow deer. These two parameters are significantly correlated. Linear regression: $y = 0.79x + 0.87$, $R^2 = 0.79$, $P < 0.001$.

3.2.3 Choice between three species: Erica tetralix, Juncus effusus and *Agrostis capillaris*

Erica tetralix, Juncus effusus and *Agrostis capillaris* had significantly different nitrogen contents ($P = 0.043$). Comparing these nitrogen-values with the percentages of eaten fruiting stems during the experiment resulted in a point scatter: this means that there is no significant correlation ($P = 0.79$) (Figure 3.15).

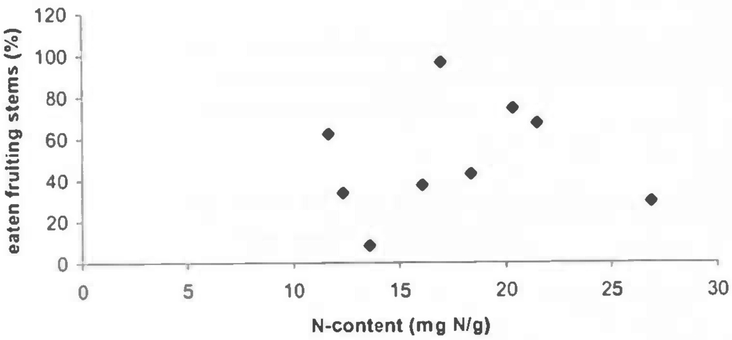

Figure 3.15: Nitrogen contents of three different species versus percentage eaten fruiting stems by fallow deer. $P = 0.79$. Three samples for every species (9 in total).

There seem to be differences in percentage eaten fruiting stems between the species but the standard errors of the results are too large, so the differences are not significant (Figures 3.16 and 3.17).

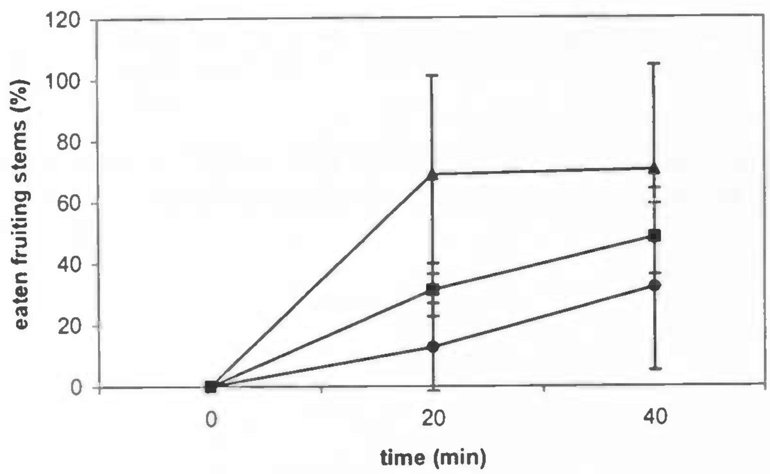

Figure 3.16: Percentage eaten fruiting stems per species through time during the experiment by fallow deer (● *Erica tetralix*, ■ *Juncus effusus*, ▲ *Agrostis capillaris*). Differences are not significant. Error bars indicate standard error of the mean.

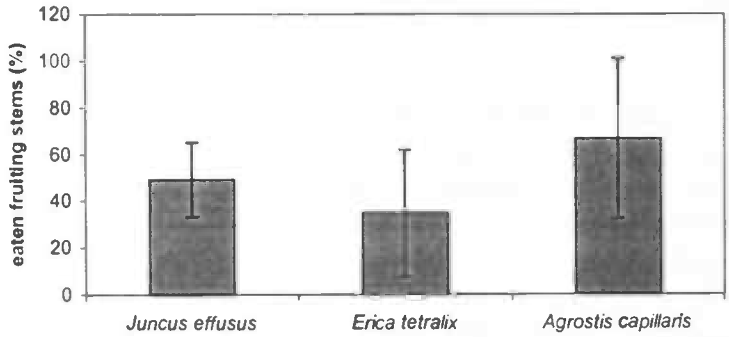

Figure 3.17: Percentage eaten fruiting stems per species at the end of the experiment (after 40 minutes). Differences are not significant: $P = 0.40$. Error bars indicate standard error of the mean.

Also the foraging time of the fallow deer does not differ significantly between the three species that were used (Figure 3.18).

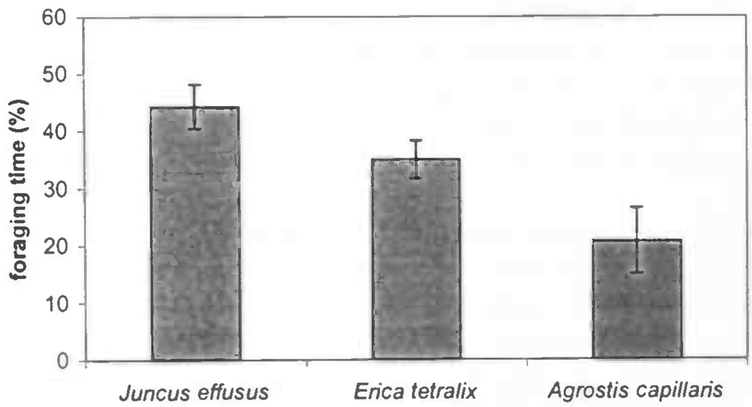

Figure 3.18: Foraging time by fallow deer in percentages per species. The differences are not significant: $P = 0.17$. Error bars indicate standard errors of the mean.

We noticed that fallow deer tried to eat the seeds of *Juncus effusus*, but grabbing also the stems of the plant made them eat the stems as well. Fallow deer were attracted by the seeds.

4 Discussion

4.1 Relationship between N-contents of plants and seed density in cattle dung

The first hypothesis, seeds of plants with relatively high nitrogen content are overrepresented in the dung of Scottish Highland cattle compared with the seed supply in the same area, cannot be tested with the results that came out of the survey done. There were not enough samples to obtain reliable correlation results. Malo and Suárez (1995) found that seed content of dung is correlated with seed supply in the field. But the study of Vos (2001) did not show correlation between seed content in the dung and seed supply in the field. So he concluded seed supply not to be the only important factor determining seed content in the dung.

When foraging on heathlands, the highest intake by cattle (up to 20% of total intake) of *Calluna vulgaris* and *Erica tetralix* takes place in the winter (Wallis de Vries 1994). In these months the nitrogen content of these species is lower than the rest of the year, so the intake must also depend on the quality of other available species or certain elements in the plants. Vos (2001) found that *Erica tetralix* was most abundant in Delleburen, but was not found in cattle dung samples. Cattle spent much time in heathland, probably using it as a resting site. Cattle graze little on resting sites, while dung deposition is probably not smaller than at foraging sites. In summer mesotrophic grassland may function as a seed source, while the heathland functions as a sink for seeds. During winter the heath may become an important seed source. *Calluna vulgaris* is added to their grass diet between November and March, and it can reach a maximum of 50% of all bites in January (Welch 1984, Bokdam & Gleichman 2000).

Vos (2001) found that the species dispersed most by endozoochory were *Poa trivialis*, *P. annua*, *P. pratensis*, *Cerastium fontanum* and Juncaceae. These species prefer soil which is rich in nutrients, contrary to heathland species. Being dispersed in cattle dung thus gives these species an advantage above the heath species because of release of nutrients that comes together with dung deposition (Dai 2000). Plants such as *Calluna vulgaris* in heather moorland are killed by more dung pats than they can colonize (Welch 1985). Vos (2001) concludes that endozoochorous dispersal by cattle is of little importance for the dispersal of target species. The number of seeds of target species is very small compared to the number of dispersed seeds of non-target species.

Ellenberg nitrogen indicator values correlate with the ratio of seed supply and amount of seeds in cattle dung, according to results of Mouissie *et al.* (unpublished). This means that plants from nutrient-rich fields will be dispersed better by cattle than plants from poorer fields. So from the positive correlations between Ellenberg nitrogen indicator values and nitrogen content of the plants we obtained one can indirectly conclude there is a relation between nitrogen content and seed dispersal by cattle. But it is plausible that for every species successful dispersal of seeds depends on different factors: the quality and abundance of species in the environment as well as the nutrient contents of the plants and the nutrients that are wanted by the animals at that very moment.

Taking the above mentioned results into account one can foresee that endozoochorous dispersal of seeds of target species does not always have the wanted effect. Non-target species are dispersed better and towards the heathlands, especially in summer, while cattle also take nutrients from the rich grasslands into the heathlands. It is recommendable to stop the grazing during summer time when most grasses have seeds, and restart the grazing in winter when the heath species are eaten and their seeds dispersed. This can be done for example by fencing the heathland out from the grassland. Very sensitive and threatened conservation sites should be grazed without contact with nutrient-rich grassland.

4.2 The role of N-contents of plants in diet selection of fallow deer

The second hypothesis, intake of seeds by fallow deer (*Cervus dama*) is positively correlated with nitrogen content of foliage within plant species grown on different nitrogen supply, was not verified. In each experiment, with *Plantago major* or *Stellaria media*, there was no significant correlation between nitrogen content of the foliage and percentage of eaten fruiting stems or time spent foraging at the relevant trays. In *Stellaria media* there was a significant correlation between nitrogen content of the fruiting stems and the percentage foraging time, but this can also be caused by the corresponding foliage height. Contrary to what was expected there is an increase in percentage eaten fruiting stems with increasing size of the fruiting stems. Therefore the fruiting stems themselves seem to be attractive for the deer. It was expected they would be avoiding the stems to eat the foliage only, and would be able to avoid the fruiting stems better as they were taller. But the fallow deer even bit off the stems deliberately instead of avoiding them. This refutes the possibility of the 'foliage is the fruit'-hypothesis (Janzen 1984) on *Plantago major*, because this hypothesis states that seeds are accidentally taken in while eating the foliage.

The last hypothesis, intake of seeds by fallow deer is positively correlated with nitrogen content of the foliage between three plant species, was also not verified. There was no significant correlation between nitrogen content of the plants and seed intake by fallow deer, although the three species had significantly different nitrogen contents. Also foraging time was not significantly different between the plant species.

Newman *et al.* (1995) predict with their 'optimal grazing model' that herbivores will not show a preference for high quality food, but a trade-off between intake rates, (internal) passage rate and absorption speed of nutrients. The preference depends on relative and absolute intake rates which can be modified by the relative abundance of the species. This may explain the insignificancy of the relations between nitrogen content and percentages foraging time and eaten fruiting stems in both *Plantago major* and *Stellaria media*. The model is also supported by the significant correlation between foliage height and percentage foraging time in *Stellaria media*.

Another intermediate selector (Hofmann 1985), red deer (*Cervus elaphus*) is found not to be always in the most optimal patch (Wilmshurst 1994) during an experiment with patches containing varying biomass. This can have several causes: individual variations between optima between the animals, errors in selecting the optimal patch or sampling plants to get information on the palatability. The last reason can also be the cause for the insignificant results in the food choice experiment with fallow deer. The trays were not large and the deer walked from tray to tray. Fallow deer are also supposed to be very opportunistic animals in their foraging behaviour (Geist 1982).

4.3 Limitations of our study

For the food choice experiments it certainly would have been better if there were more samples of each nitrogen level in both *Plantago major* and *Stellaria media*. Especially in *Plantago major* preparation problems resulted in a small number of usable trays. This small number resulted in data that could not give a reliable image of the relationship between nitrogen content and seed uptake.

Second, it is uncertain that the fallow deer in captivity are representative for the wild fallow deer in nature reserves. The deer in the enclosure are used to humans giving them all sorts of vegetables, so it is possible that they are not as selective about their forage as wild specimen. Maybe they are even more opportunistic as they are immediately attracted to what is brought in the enclosure.

The arrangement of the trays within the enclosure can also have influenced the foraging behaviour of the deer, as the trays at the corners of the matrix can be explored earlier by the deer than the others.

As nitrogen content of plants on itself is maybe not a good indicator for the potential seed dispersal one should focus on individual species. Diet selection studies have already been shifted from broad levels of forage types or plant parts towards the level of plant species. There are at least two reasons for this shift: (1) plant species within forage types differ greatly in physical properties, chemical composition and nutritional value; and (2) ecological and evolutionary interactions between herbivores and their food resources occur at the species level (and below), not at the broad level of forage type. However, diet selection at the plant species level is a very complex problem (Hanley 1997).

When having data about all important plant species of a nature reserve one can predict the processes that will occur when herbivores are used in the area for seed dispersal. One can estimate the influences a source site will have when fenced together with the nature development site. When the species of the source site are dispersed better by endozoochory it will have a positive influence on the development site. The other way round better dispersal of development site species will possibly lead to degradation of the source site.

Acknowledgements

Thanks go out to Maarten Mouissie for his assistance and encouragements, to Jacob Hoogendorf for his assistance and practical advice and to Nelly Eck for her advice and practical help in the laboratory. Special thanks go out to Ms. Hertjens for offering the deer camp for our food choice experiment.

References

Bakker, J.P., P. Poschlod, R.J. Strykstra, R.M. Bekker & K. Thompson (1996) Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica* 45: 461-490

Bokdam, J. & J.M. Gleichman (2000) Effects of grazing by free-ranging cattle on vegetation dynamics in a continental north-west European heathland. *Journal of Applied Ecology* 37: 415-431

Bonn, S. & P. Poschlod (1998) Zoochorie-Tiere und Ausbreiting. In: *Ausbreitingsbiologie der Pflanzen Mitteleuropas* p. 64-104 Quelle & Meyer Verlag, Wiesbaden

Dai, X. (2000) Impact of cattle dung on the distribution pattern of plant species in an alvar limestone grassland. *Journal of Vegetation Science* 11: 715-724

De Graaf, M.C.C. (2000) *Exploring the calcicole-calcifuge gradient in heathlands.* Thesis University of Nijmegen (KUN)

Frankton, C. & G.A. Mulligan (1987) *Weeds of Canada* New Canada Publications, NC Press Ltd., Toronto

Gardener, C.J., J.G. McIvor & A. Jansen (1993) Passage of legume and grass seeds through the digestive tract of cattle and their survival in faeces. *Journal of Applied Ecology* 30: 63-74

Geist, V. (1982) Adaptive behavioural strategies. In: *Elk of North America (Ecology and Management)* p. 219-277 Stackpole Books, Harrisburg

Hanley, T.A. (1997) A nutritional view of understanding and complexity in the problem of diet selection by deer (Cervidae). *Oikos* 79: 209-218

Harper, J.L. (1977) *Population Biology of Plants.* Academic Press, London

Hofmann, R.R. (1985) Digestive physiology of the deer – Their morphophysiological specialisation and adaptation. *Biology of Deer Production* 22: 393-407

Jager, H. (1999) *De vegetatie van de Delleboersterheide; Delleboersterheide, de Hoorn, Catspoele en Diakonievene.* It Fryske Gea, Olterterp

Janzen, D.H. (1984) Dispersal of small seeds by big herbivores: foliage is the fruit. *The American Naturalist* 123: 338-353

Klooker, J., R. van Diggelen & J.P. Bakker (1999) Natuurontwikkeling op minerale gronden; Ontgronden: nieuwe kansen voor bedreigde plantensoorten? Report Department of Plant Ecology, University of Groningen

Lesage, L., M. Crete, J. Huot & J.P. Ouellet (2000) Quality of plant species utilized by northern white-tailed deer in summer along a climatic gradient. *Ecoscience* 7: 439-451

Malo, J.E. & F. Suárez (1995) Herbivorous mammals as seed dispersers in a Mediterranean dehesa. *Oecologia* 104: 246-255

Nathan, R., & H.C. Muller-Landau (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* 15: 278-285

Newman, J.A., A.J. Parsons, J.H.M. Thornley, P.D. Penning & J.R. Krebs (1995) Optimal diet selection by generalist grazing herbivore. *Functional Ecology* 9: 255-268

Semiadi, G., T.N. Barry, P.D. Muir & J. Hodgson (1995) Dietary preferences of sambar (*Cervus unicolor*) and red deer (*Cervus elaphus*) offered browse, forage legume and grass species. *Journal of Agricultural Science* 125: 99-107

Van Andel, J. (1974) *An ecological study on Chamaenerion angustifolium (L.)* Thesis Amsterdam (VU)

Van Andel, J., J.P. Bakker & A.P. Grootjans (1993) Mechanisms of vegetation succession: a review of concepts and perspectives. *Acta Botanica Neerlandica* 42(4): 413-433

Vos, P.G. (2001) Seed dispersal by cattle. Student Report Department of Plant Ecology, University of Groningen

Wallis de Vries, M.F. (1994) *Foraging in a landscape mosaic. Diet selection and performance of free-ranging cattle in heathland and riverine grassland.* Thesis University of Wageningen

Welch, D. (1984) Studies in the grazing of heather moorland in North-East Scotland. I. Site descriptions and patterns of utilization. *Journal of Applied Ecology* 22: 179-195

Welch, D. (1985) Studies in the grazing of heather moorland in North-East Scotland. IV. Seed dispersal and plant establishment in dung. *Journal of Applied Ecology* 22: 461-472

Willson, M.F. (1992) The ecology of seed dispersal. In: Fenner, M. (ed.) *Seeds; the ecology of regeneration in plant communities.* p. 61-85 C.A.B. International, Wallingford

Weeda, E.J., R. Westra, C. Westra & T. Westra (1985-1994) *Nederlandse Oecologische Flora: wilde planten en hun relaties (part 1-5).* Publication of IVN i.c.w. VARA and VEWIN

Wilmshurst, J.F., J.M. Fryxell & R.J. Hudson (1994) Forage quality and patch choice by wapiti (*Cervus elaphus*). *Behaviour Ecology* 6: 209-217

Zijlstra, W. (1992) Winddispersie van zaden van 22 plantesoorten van hooilanden en vochtige heide: een windtunnelexperiment. Internal Report Department of Plant Ecology, University of Groningen

Pictures came from the following websites (15-5-2003):

1. www.natural-history-pictures.co.uk
2. www.wdr.de/studio/bielefeld/lokalzeitservice.vogelmiere/
3. www.yrttitarha.com/kanta/piharatamo/kuva.jpg

Appendix 1

N-analysis after Kjelldal

Preparations
- Dry plant material in drying oven (48 hours by 70° C)
- Crush plant material
- Dry again (circa 1 hour by 70° C)

Analysis
- Weigh 0.2000 – 0.1500 grams of crushed and dried plant material and put it in a glass
- Add a selene-pill and 8 ml 96% H_2SO_4-solution
- Destruction (¾ hour)
- Destillation: add 20 ml 50% NaOH-solution and destillate to 10 ml borium acid
- Titrate with 0,05n H_2SO_4-solution until a sense of red appears: read number of ml
- Calculate nitrogen content with the following equation:

$$\frac{(\text{ml titrated sample} - \text{ml titrated blanco}) * 0.05}{\text{mg dry plant material}} = \text{meq N / mg}$$

(result times 14 to get mg N/mg)

Appendix 2

Timeschedules feeding experiment with fallow deer

Plantago major: 9 trays of plants, individual (1,2,3,4,5) notation at each time interval

time (s)	pos 1	pos 2	pos 3	pos 4	pos 5	pos 6	pos 7	pos 8	pos 9
0	2								
10	2						1		
20	2						1		
30	24						1		
40	24						1		
50	245						1		
60				2			1		
70					2			1	
80	45				2			1	
90					1	1			23
100									
110									
120									3
130						2		1	3
140						4		1	3
150									
160	1							4	
170		1						4	3
180		1						4	3
190			1						23
200									
210			1			23			
220			1			2345			
230			1			2345			
240			123			45			
250			1			45			
260					2	1			
270									
280								2	13
290									3
300									35
315			1						
330			1						
345			1						
360			14						
375		2				3			
390			1			3			
405	2			4		13			
420				2		3			
435					12	4			
450						14	5		
465								1	

480						3	45	1	
495					2	3	4	5	1
510					25			4	1
525					2				
540					2	4			
555	1								2
570									25
585								1	25
600					3			1	45
615					13				5
630				1	3				5
645					3				45
660					4				15
675				3				4	5
690				3				4	5
705				3					
720				3				5	
735				3				5	
750							3	4	
765							35	4	
780							345		
795							3	4	1
810							5		1
825					3		5		14
840						1			
855									
870									
885							5		
900			4			5			
915			4			5			
930			5						
945			45						
960			2						
975			35						
990	1		5			23			
1005	1		5		2				
1020	12		5	3					
1035	1			23					
1050			5	1			2		
1065			5		3				
1080	3		5						
1095	3					5		1	
1110	3							1	5
1125	34							1	5
1140	34							1	
1155	34							15	
1170	34						1	5	
1185	34						1	5	
1200	34						1	5	
1215	3			4				5	
1230									
1245	3				5				
1260	3				5	12		4	

1275	3		1	5	2				
1290			1	5	23				
1305	5		1						
1320	5	1	3						
1335									12
1350						1345			2
1365			1			4			2
1380			1		45				
1395					4				
1410						4			
1425									
1440									
1455									
1485									
1515									
1545									
1575									
1605									
1620			4	1					
1635			45						
1650			4						2
1665			45						2
1680	3		45			2			
1695	3		5		2	4			
1710	3				2				
1725	45								
1740	5						4		
1755							4		
1770									
1785									
1800									
1815									
1830									
1845									
1860									
1875									4
1890						3			
1905									45
1920									5
1935									
1950									
1965									
1980									
2010									
2025			1					4	
2040		1					24		
2055							2	5	
2070							4		
2085							4		
2100					5		4		
2115							4		
2130									
2145	4								

2160									
2175									
2190									
2205									
2220									
2235									
2250									
2265									
2280									
2295									
2310									
2325									
2340									
2355									
2370									
2400									
2430									
2460									
2475						45			
2490			4		5				
2505		4			5				
2520	4				5				
2535					5				
2550		5							
2565			4						
2580			45						
2595			4						
2610			4						
2625			4						
2640			4						
2655	3	4							
2670	235	4							
2685				2			5		
2700							5		
2715									
2730								4	
2745								4	
2760							3	4	
2775								35	
2790			1						5
2805						1			5
2820									
2835								3	5
2850								3	
2865									5
2880						5			
2895									
2910									
2925									
2940									
2955									
2970									

Stellaria media: 14 trays with plants; no individual deer countings

time (s)	pos1	pos2	pos3	pos4	pos5	pos6	pos7	pos8	pos9	pos10	pos11	pos12	pos13	pos14
0					3					2				
15				1	1				1	1				1
30				1	1				1	1				1
45				1	1				1	1				1
60					1			1	2	1				
75			1		1				2	1				
90			2	1					2					
105			2				1		2					
120			1						2			1	1	
135									1	1	1		1	
150		1	1	1			1						1	
165		2												
180		1					1		1				1	
195		1		1					1		1			
210	1				1		1		1	1				
225		1							1	1				
240								1	1		1		1	
255					1			1	1				1	
270			1		2			1	1					
285			1		1			1	1	1				
300		1		2			1		1					
315	1		1		1	1			1					
330			1											
345	1	1					1	1		1				
360		1				1		1		1	1			
375		1	1			1				1				
390		2			1	1								
405		2		1							1			
420		1		1		1	2							
435	1	1		1		1	1							
450	1		1			1	2							
465	1	1				1	2							
480	1					1		1			1			
495		1					2		1			1		
510		1					1	1						1
525		1	1				2			1				
540			1				1	1	1					
555			1	1			1		2					
570			1					1	1	1				
585			1	1					1					
600		1		1	1				2					
615		1		1						1				1
630				1			1		1					2
645				1			1			1			1	
660				1			1			2			1	
675								1		1			1	
690				1				1		1			2	
705				1			1			1				
720				1			1						2	
735			1				1	1					2	

750		1					2					1	1	
765							2				1	1		
780		1				1	1				1	1		
795		1				1					2	1		
810											1	1		
825		1									3	1		
840		3									2			
855		2	1				1				1			
870	1	2		1							2			
885	1							1			1			
900	1					1		1			2			
915	2							1			1			
930	1						1				1	1		
945	1						1							
960	1											2		
975												1		1
990										2				
1005				1	1				1	1				
1020				2	2					1				
1035			1	2						1				
1050		1	2	1				1						
1065		3	1					1						
1080	1	1				1		1						
1095		1				2								
1110		1				1	1				1		1	
1125		1									3		1	
1140										2				
1155									1	1				
1170									1	1				
1185									1					
1200									2					
1215														
1230														
1245								1	1					
1260				1				1						
1275												1		
1290											1			
1305											1			
1320											2			
1335														
1350														

3 species: 9 trays of plants (3 of each species)

time (s)	pos1	pos2	pos3	pos4	pos5	pos6	pos7	pos8	pos9
0									1
15						3		1	
30					1	2		1	1
45					1	1		1	
60					3	1			
75		1			2				
90		1					1	1	
105					1	1			
120						1	1		2
135							1	1	
150						1		1	
165			2		1				
180			2		1				
195			1			1			
210			2			1			
225									
240		1	1	1					
255						1			
270		1			1	1			
285					1	1		1	
300					1			1	1
315						2			
330						2		1	
345			1			1		1	
360					1	1		1	
375					1				
390						1		1	1
405			1			1	1		1
420							1		2
435						1			1
450						2			
465					2	1			
480		1			1	1			
495		2				1			1
510		1	1						
525		1	2		1				
540		1	1		1				
555		2	1		1		1		
570		1	1				1		
585			1	1					
600				2					
615				1				1	
630				1	1		1	1	
645					1				
660				1			1	1	
675				1			3		
690					1		2	1	
705							1	3	
720							1	2	2
735							1	1	2

750						2			2
765			1						3
780			1			1			2
795		1			1	1			1
810	1	2			1				
825				2					
840									
855									
870									
885				1					
900								1	
915								4	
930								1	1
945								1	1
960									1
975									
990									
1005									
1020							1		
1035							1		1
1050							1		
1065									
1080			1					1	
1095									
1110			1						
1125			1						
1140									
1155									
1170									
1185									
1200									
1215									
1230				1					
1245									
1260							1		
1275			1				1		
1290			1				1		1
1305			1				1		1
1320			1				1		1
1335			1				1		1
1350			1				1		
1365			1						
1380			1						
1395			2		1	1			
1410			2		1				
1425			2						
1440			2						
1455			2						
1470			1						
1485									1
1500			1						1
1515		1							1

1530					1				1
1545					1				
1560								1	1
1575								1	1
1590									1
1605								1	1
1620						1		1	
1635		1				1		1	
1650					1				
1665									
1680			1						
1695			1						
1710		1	1						
1725		2		1	1				
1740		2		1					
1755									
1770			1	2					
1785			1	1					
1800			1	1					
1815				1					
1830				1					
1845				1					
1860				1					
1875									
1890				1					
1905				1			1		
1920				1			1		
1935							2		
1950									1
1965									1
1980									1
1995									
2010									
2025		1							
2040		1							
2055									
2070		1							1
2085		1							
2100			1						2
2115			1						1
2130			1					2	
2145			1	1				2	
2160				1					
2175				1				1	
2190				1				3	
2205				1				1	
2220								2	
2235				1				2	
2250				1				2	
2265									
2280									