Effects of Shade, Water and Species Mix on Perennial Ryegrass and Red Clover Competition

A Simulated Silvopasture System

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Abstract

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A silvopasture system is an agroforestry system specifically designed for the production of trees, tree products, forage for grazing and livestock. Research has shown that silvopasture systems can be an environmentally and economically feasible alternative to traditional land uses.

Two potential silvopasture forage species, perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pretense* L.), were exposed to shade, low water levels and varying species mixes. They were grown in a greenhouse for 14 weeks and were harvested at two occasions. The light treatments were full light, 50% shade or 80% shade. The water levels were either a well watered treatment or a water level where the plants were exposed to water stress. There were three different species mixes; 75% grass / 25% clover, 50% grass / 50% clover or 25% grass / 75% clover. After harvest the biomass was measured, development stage determined, and protein content and neutral-detergent fiber (NDF) content analyzed. The objectives of the study were to investigate how a grass species and a legume species in different mixes are affected by different levels of shade and water.

The biomass increased with increased light and water. The grass dominated mix gave a higher biomass than the clover dominated mix for both species. Only the clover showed differences in development stages between the treatments. The maturity of the plants was increased by increased exposure to water and light. In the second harvest both the grass and the clover increased their protein concentration when the shade levels increased. The grass had a higher protein concentration when grown in a low water treatment while the clover had a higher protein concentration when grown in a high water treatment. The grass dominated mix increased the protein concentration for the grass while the clover dominated mix increased the protein concentration for the clover. The actual amount of protein in the plants followed the variations of the biomass; a larger plant had a higher protein content and vice versa. The NDF levels varied between treatments, crops and harvests.

The second harvest generally showed larger effects of the treatments than the first harvest. The reason was that the plants started to grow in an untreated condition until they were well established for the first harvest, while they had to start regrowing in the treated environment for the second harvest.

Agrovoc: Perennial ryegrass, red clover, silvopasture, shade, water, species mix, biomass, development stage, protein, NDF

Sammanfattning

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Silvopasture system är agroforestry system särskilt utvecklade för produktion av virke, trädprodukter, bete och animalieproduktion. Forskningsresultat har visat att silvopasture system kan vara miljömässigt och ekonomiskt lönsamma jämfört med traditionell markanvändning.

Två potentionellt möjliga betesväxter, engelskt rajgräs (*Lolium perenne* L.) och röd klöver (*Trifolium pretense* L.), utsattes för skugga, låg vattennivå och varierande artblandningar. De odlades i växthus i 14 veckor och skördades vid två tillfällen. Ljusbehandlingarna var fullt ljus, 50 % skugga och 80 % skugga. Vattennivåerna var antingen en välvattnad behandling eller en behandling där växterna utsattes för vattenstress. Tre olika artblandningar användes; 75 % gräs / 25 % klöver, 50 % gräs / 50 % klöver och 25 % gräs / 75 % klöver. Efter skörd mättes biomassa, utvecklingsstadierna bestämdes och proteininnehåll och fiber (NDF) innehåll mättes. Målsättningen med studien var att undersöka hur en gräsart och en baljväxtart i varierande artsammansättningar påverkas av olika nivåer av skugga och vatten.

Biomassan ökade med ökat ljus och vatten. Den gräsdominerade artblandningen resulterade i en högre biomassa för både gräset och klövern än den klöverdominerade artblandningen. Endast klövern visade variation i utvecklingsstadier mellan de olika behandlingarna. Utvecklingen av klöverplantorna påskyndades av ökat ljus och god vattentillgång. Både gräset och klövern ökade sin proteinkoncentration när skuggningsnivåerna ökade i andra skörden. Gräset hade en högre proteinkoncentration när det växte under vattenstress medan klövern hade en högre proteinkoncentration när den växte under god vattentillgång. Gräset ökade sin proteinkoncentration då det växte i den gräsdominerade artblandningen, medan klövern hade högst proteinkoncentration i den klöverdominerade artblandningen. Mängden protein per planta följde variationerna i biomassa; ju större växt, desto större mängd protein innehöll den. NDF koncentrationen varierade med behandling, art och skörd.

Den andra skörden visade generellt större effekt av behandlingarna än den första skörden. Anledningen till detta var att växterna inför den första skörden började växa i en obehandlad miljö tills de var väl etablerade, medan de inför den andra skörden började sin återväxt i full behandling.

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1. Introduction

A silvopasture system is an agroforestry system specifically designed for the production of trees, tree products, and forage for grazing livestock (USDA, 1997). USDA (1997) states that "Overall, silvopastures can provide economic returns while creating a sustainable system with many environmental benefits." (p.1). This has been demonstrated by studies in southern US that have shown that silvopasture is an environmentally and economically feasible alternative to traditional land uses (Husak and Grado, 2002). Also Grado, Hovermale and Louis (2001) showed in a study that silvopasture might be favorable to some grazing treatments even before the final harvest of trees is concluded.

An agroforestry system is a complex ecosystem. As in any natural ecosystem the different species included in the agroforestry system will affect each other in various ways. In a silvopasture system, the main species will be one or several types of trees with a ground vegetation of grasses and legumes in either pure or mixed stands. The forage species will compete with each other for water and light, but can also benefit from each other with for example nitrogen fixation from the legumes. The trees will affect the forage species by the shade they are causing, which might limit the growth of the forages. Trees will also use a lot of water, which can create competition for this important medium. On the other hand, these effects might also benefit the forages by limiting strong sunlight and thereby drought, and protect the pasture from harsh temperatures and winds.

1.1 Shade

"Light is used by plants not only as a source of energy for photosynthesis, but also as a signal for growth and morphogenesis. Indeed, there is hardly any process in plant life, from seed germination to flowering, that is not affected by light." (Srivastava, 2002, p.663).

Research studies for how grasses perform under shaded conditions show that the yield is likely to decrease (Kephart, Buxton and Taylor, 1992; Vartha, 1973; Lin *et al*., 1999) but forage quality may improve under such stress (Kephart and Buxton, 1993). However, most species possess some level of shade tolerance. Vartha (1973) showed that perennial ryegrass decreases in dryweight when exposed to shade but even at 5% of full light the grass had some biomass production. A study by Lin *et al*. (1999) showed that several cool season grass species (i.e. ryegrass) possessed some shade tolerance to 50% shade, and did not show significant differences in dryweight compared to 0% shade. For most of the grasses the dryweight at 80% shade was significantly different from the dryweight at full light. A somewhat contradicting response was presented by Garrett and Kurtz (1983), who showed that tall fescue can produce higher yield in shaded conditions, than when grown in the open. The forage quality response to shade can be exemplified by a study by Kephart and Buxton (1993) that showed increased protein concentration and decreased NDF levels when exposing tall fescue (*Festuca arundinacea* L.), reed canarygrass (*Phalaris arundinacea* L.) and deertongue grass (*Panicum clandestinum* L.) to shade.

Different studies report different results in how legumes respond to shade. Lin *et al.* (1999) showed in their study with several different legumes that most of the legumes decreased in yield when exposed to shade. Redfearn, Buxton and Devine (1999) showed that soybeans intercropped with sorghum developed a higher forage quality but lower yield than monocropped soybean. For the intercropped soybean the NDF content was lower, the protein concentration in the stems where higher, but the protein content in the leaves were lower than the monocrop. Johnson *et al.* (2002) showed that the nutrient values for shaded peanut (*Arachis glabrata* Benth*)* were lower than when the peanut was grown in full sun. NDF levels increased and protein concentrations decreased in the shaded peanuts compared to the full light plants.

1.2 Water

When a plant is exposed to water stress, one of the earliest effects will be a reduction in vegetative growth. This has been believed to be caused by a loss in turgor pressure which reduces cell enlargement of the plant. This is probably not the only explanation to the effects of water stress. It is also believed that a lack of water transportation between the xylem and the leaf occurs with water stress, which would reduce leaf expansion. Also, desiccation of the plant can lead to membrane damages and therefore metabolic disruptions in the cell. In addition to these factors, photosynthesis can be affected by water deficit due to closure of the stomata and direct effects of low water levels on the photosynthetic system (Hopkins and Hüner, 2004).

Sheaffer *et al*. (1992) showed in a study with reed canarygrass, smooth bromegrass (*Bromus inermis* L.), orchardgrass (*Dactylis glomerata* L.) and timothy (*Phleum pretense* L.) that grass forage yields were reduced by drought and that droughted forages had a higher forage quality than the controls. The protein levels of the grasses increased and the fiber content decreased when the plants were exposed to drought.

Also legumes seem to respond to drought by getting a higher forage quality. Peterson, Sheaffer and Hall (1992) showed in a study with alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.), cicer milkvetch (*Astragalus cicer* L.) and red clover (*Trifolium pretense* L.) a consistently higher forage quality for droughted plants then their controls. The NDF levels decreased at all harvests. The protein concentrations sometimes increased but this response was varying. Also Halim *et al*. (1989b) showed a lower cell wall concentration in stem bases in water stressed alfalfa plants compared to the controls. The cell wall concentration in the leaves was generally not affected by drought. The same study showed a higher protein concentration in stems, but lower protein concentration in leaves in draught exposed alfalfa compared to the controls. The authors suggest a translocation of nitrogen from rapidly senescing leaves to stems to be the cause of this response. The dryweight of legumes seems to decrease with water stress. In a study by Grimes, Wiley and Sheesley (1992) alfalfa experienced a linear yield loss when the soil water potentials dropped to below -1MPa (10 bar).

1.3 Mix

Legumes have the ability to fixate nitrogen, the nutrient most limiting to productivity. The nitrogen fixation can be high enough to provide self-sufficiency of nitrogen for legume crops (Heichel and Henjum, 1991). Fertilization requirements can be reduced when forage legumes are grown in mixes with grasses, since nitrogen can be transferred from the legume to the grass (Heichel and Henjum, 1991). The choice of species, and in which combinations they are used, can markedly affect their productivity and forage quality (Sollenberger, Templeton and Hill, 1984). Heichel and Henjum (1991) found in their study that red clover grown with reed canary grass fixed in average 63 kg N per ha during a four year period. Grasses have an advantage in areas with high soil N fertility while legumes are more competitive in low N fertility soils due to their ability to fixate nitrogen (Schwinning and Parsons, 1996). Sollenberger *et al*., (1984) found that as legume seed rates were increased, both the legume and grass component of mixed swards produced more biomass and nitrogen. Also Turkington (1996) found that generally, perennial ryegrass and white clover (*Trifolium repens* L.) mixes used resources more effectively and were more productive than their corresponding monoculture. The same conclusion was made by Annicchiarico and Piano (1994), who did a study on different white clover genotypes grown together with different grass species.

The results of competition between plants are determined by the density, horizontal distribution and relative time of emergence of the plants (Håkansson, 1997). Interactions among plants in a mixture begin during seedling growth and establishment. Both red clover and perennial ryegrass can be described as very aggressive (Sollenberger *et al.*, 1984). A lot of studies have been done on perennial ryegrass and white clover and Turkington (1996) states that interaction between the two are complex, and change dramatically with pasture age, harvest date, and density.

The canopy structure will be highly affected by the mix of species in the forage system. Grasses have narrow, vertical leaves while legumes have wide, horizontal leaves. A stand with grasses with mainly erect leaves will facilitate light penetration compared to a stand of clover with mainly horizontal leaves (Joggi, Hofer, and Nösberger, 1983). When white clover was grown under perennial ryegrass the responses of the legume was a lower biomass, fewer new branches produced, and increased petiole length (Markuvitz and Turkington, 2000). When comparing the different parts of the above ground plant parts as a percentage of the total biomass, it could be seen that the proportion of leaves and stolons decreased while the proportion of petioles increased in this study.

Competition between plant species can be studied in many ways. Two contrasting experimental designs, additive and replacement series, have been used to study interactions in mixed plant stands (Jolliffe, Minjas and Runeckles, 1984). Replacement series have been used widely (Jolliffe *et al.,* 1984) but can easily be misinterpreted since it is difficult to determine comparable densities for different plant species (Håkansson, 1997). Both interspecific and intraspecific competition will occur in a mixed stand but it might be difficult to distinguish between the two. Jolliffe *et al.* (1984) suggest in a study an alternative approach to study competition so that the effects of the two forms of interactions can be separated. Jolliffe *et al.* (1984) also shows that species often respond more strongly to increased densities of their own species than increased densities of other species. Still, an increased density of any plant in a mixed stand will increase competition if there is not a very strong niche differentiation between the plants (Håkansson, 1997).

1.4 Development

Forage quality almost always declines as forages mature. The trend is that the protein concentration declines while the fiber concentration increases with maturity. When a plant matures it will go from a mainly leafy stage to developing more and more stems until it finally flowers and sets seeds. Leaves are usually of a much higher forage quality than stems (Barnes *et al*., 2003). Comparing the amount of leaves with the amount of stems of a plant gives a measure which is commonly referred to as the leaf:stem ratio. Studies with legumes, including red clover, have shown that maturity gets delayed with increasing water stress (Peterson *et al.*, 1992; Halim *et al*., 1989a). These two studies also showed that the leaf:stem ratio of the studied legumes increased with increasing water stress. In another report Halim *et al.* (1989b) mention that water stress at the vegetative stage of alfalfa had no effect on the leaf:stem ratio, but when the plants reached bud stage an effect from the previous water stress was visible. The authors believe this effect resulted from retardation in stem growth. Halim *et al.* (1989a) suggests that the slowing of plant maturation and growth during water stress highly contributes to the change in forage quality, but should not be seen as the only explanation to the response.

1.5 Objective

The model for this research project is a silvopasture system in central Indiana composed of black walnut trees and forage species. The system is being grazed by sheep. In this study the forage part of the silvopasture system has been investigated by simulating an agroforestry system in a greenhouse. The objectives of this study were to investigate how a grass species and a legume species in different proportions to each other, are affected by various levels of shade and water. Some of the potential effects a silvopasture system in central Indiana can have on the forage species have thereby been included in the study. The goal was to analyze the possible responses of two specific forage species to varying levels of shade and water.

2. Material and methods

One grass species (perennial ryegrass, *Lolium perenne* L.) and one legume species (red clover, *Trifolium pratense* L.) were mixed in three different relationships (75%/25%, 50%/50%, and 25%/75%, where the percentage refers to the number of plants) in pots. The pots were treated with three different light-levels (full light, 50% shade and 80% shade) and two different water-levels (field capacity and a lower water-level) in a total of 18 treatment combinations. The project was done as a split plot experiment, with four replicates, which sums up to 72 pots. The pots were placed close to each other with a boarder of pots around each block. These boarder pots contained grass and legumes in the relationship 50%/50%. There were 168 boarder pots and all together the experiment contained 240 pots. See Appendix 1.

2.1 Species

One grass species and one legume species were used in this experiment; perennial ryegrass (PR) and red clover (RC). The growth period was limited for this project and therefore it was important that the species were fast growing, especially in the beginning of their life cycles. A certain shade tolerance was necessary since the species were used as potential agroforestry species. Since the agroforestry system that was simulated is being grazed, the species also needed to be resistant to disturbances like grazing. Perennial ryegrass and red clover fulfill all of these requirements (Barnes *et al.*, 2003, Fogelfors, 2001).

2.2 Material

2.2.1 Pots

The pots that were used were 35 cm high, 16x16 cm at the top and narrowing towards the bottom, giving a capacity of containing 8 liters. These deep pots where chosen to give the plants as much space as possible to not hamper the root development. Squared pots were used so that they could be placed close together to get a similar environment for all the pots in terms of competition from the neighboring pots.

2.2.2 Soil

The soil that was used was a 366-P ScottsCoir Growing Medium containing a mix of coconut coir pith, vermiculite, composted pine bark, perlite and starter nutrients. This growing medium is known to have good water holding capacity and little shrinkage when drying (Scotts, 1998).

2.3 Sowing

The seeds were pre-germinated on moist filter paper in Petri-dishes for 4 to 6 days. The sealed Petri-dishes were placed in a closed paper box in the green house (about 23°C) during the germination period.

When the roots of the germinated seeds had reached 5 to 20 mm they were transplanted to the pots. Each pot was planted with 12 seedlings in the combinations:

Mix 9:3 = 9 perennial ryegrass, 3 red clover

Mix 6:6 = 6 perennial ryegrass, 6 red clover
Mix 3:9 = 3 perennial ryegrass, 9 red clover

The border pots were planted like combination 6:6.
The pots were checked after emerging and new seedlings were transplanted where emergence failed to ensure the specific combinations between the two species.

2.4 Green house climate

The plants were grown in a green house with a requested day temperature of 75F (23.9°C) and a night temperature of 64F (17.8°C). The switches between day and night temperatures were done at sunrise and sunset. The humidity was set to 60% during day time and 50% during the night. At indoor temperatures above 80F (26.6°C) curtains covered the roof of the green house. Extra lights were turned on from 7 in the morning to 7 at night. The lamps used were 1000W, high-pressure sodium discharge lamps.

2.5 Shade

The sub-blocks where shaded to receive three different light levels; full light, 50% shade, and 80% shade. These shading levels were chosen from the results of a study by Lin et al. (1999) that showed significant differences in above ground weight for ryegrass when grown in 80% shade but not 50% shade compared to 0% shade while red clover biomass seemed to decline more when shaded at these levels.

The shading was done with a 53% shading light black UV resistant, high density polyethylene shade cloth, a 79% shading heavy black shade cloth (same material), and no shade cloth at all on the sub-blocks with full light. The shade cloth was placed on a frame of wooden poles, about 50 cm above the top of the pots. The cloth was cut in the corners to let it hang down on the sides about 25 cm to protect from the sun to come in from the sides. Still, the cloth was not allowed to reach all the way down to the pots but instead leave about 25 cm gap to facilitate the air-flow and as far as possible avoid differences in for example air temperature and humidity between the different light treatments. The sub-blocks were placed as far apart as the benches permitted, and the roof of the cloth was placed as low as possible to avoid shading effects on the bordering sub-blocks.

2.6 Water

When the seedlings were established and the treatments started the pots were checked for the need of water three times a week. The well-watered pots were usually given water at all three occasions while the pots with a lower water level were only watered once or twice a week. The need for watering varied greatly with the different light-treatments, where the full-light treatments dried up easily while the 80% shade treatments hardly needed water even once a week. The watering needs also varied with the weather.

All pots were watered on Mondays in connection to fertilization. Before this occasion the moisture content of the pots was determined with a Delta-T Devices ThetaMeter. Three samples were taken from each pot which gave a moisture content in m^3 water per m^3 soil. These numbers were used as a guideline of how much water each pot should have. The need of watering was also determined by the visible moisture content of the plants as well as the weight of the pots. The water potentials were held at approximately 5-15 bar for the low water level and 1-1/3 bar for the high water level. The wilting point is the soil water content at a soil water potential of 15 bar. The field capacity is considered to be the moister staying in the topsoil after being thoroughly wetted and allowed to drain. The field capacity can be estimated to 1/3 bar (Kutilek and Nielsen, 1994).

A water retention curve was made for the soil to translate the moisture content of the soil to water potentials. Samples of the soil were put in pressure plates, soaked, and left in 0.1, 1, 5 and 15 bar pressure. After a few days when the moisture content of the soil samples had stabilized, they were weighed, dried and weighed again. The relationship between these numbers gives a water retention curve (Hillel, 1982), which is shown in Appendix 2.

To calibrate the outputs from the ThetaMeter, small pots were filled with soil and watered thoroughly. The small pots were used to give a value of the moisture content of the whole pot and not only the surface, since the rods of the ThetaMeter only reached about 8cm. The ThetaMeter output was registered, as well as the weight of the pot. The ThetaMeter measures and the weighing were repeated until the pots were very dry. The results give a correlation between the moisture content shown by the ThetaMeter and the actual moisture content. See Appendix 3.

2.7 Fertilization

Once a week, on Mondays, all the pots were fertilized with one cup (0.237 liter) of fertilizer solution. This fertilizer was prepared by the green house and contained in mg/liter: 200 N, 29 P, 167 K, 67 Ca, 30 Mg, and micronutrients. The amount given to the pots were calculated after the need of nitrogen. When given one cup per week during the growth period, the plants received nitrogen equivalent to 210 kg/hectare. This high fertilization rate was chosen to ensure rapid plant growth since the time for the study was limited. The red clover seeds where not inoculated, and therefore no nitrogen fixation should have occurred.

2.8 Rotation

To minimize errors in the project, the pots were rotated once a week. This was done by moving each pot within a sub block one step in clockwise direction. The pots were always kept in the same sub block.

2.9 Harvest

The pots were harvested at two occasions; 8 weeks after planting and then after an additional 6 weeks.

At the harvest occasion the height of the plants were measured. The value used was an average maximum height of all the plants of the same species in one pot.
The development stage was determined by a SLU scale used for scoring the maturity stages in forage grasses and legumes. See Appendix 4.

2.10 Analysis

The plants were put in paper bags and dried for at least three days in approximately 60°C. When dried, the samples were taken out of their bags and weighed. Parts of the samples from harvest one was by mistake stored in room temperature over night before weighed which mean they absorbed some moisture before they were weighed. After redrying and reweighing a few samples it seemed like the weight increases of the samples were up to 4%. The losses in material due to handling of some samples implied a bigger error than this weight increase and therefore the data from the slightly moist samples were used. The samples from the second harvest were taken from the oven and weighed immediately.
After the samples were weighed they were grinded and stored in airtight plastic bottles until further analysis.

The forage quality was measured with near infrared reflectance spectroscopy (NIRS). An equation for perennial ryegrass was used for the perennial ryegrass and an equation for alfalfa was used for the red clover when the NIRS was calibrating the results from the spectroscopy. Data for neutral detergent fiber (NDF) and crude protein (CP) were collected.

2.11 Statistical methods

The differences in dry weight, protein content and NDF-levels between the different crops, harvests and treatments were measured with the least significant difference (LSD-values) for the means, using SAS GLM procedure (SAS Institute Inc, 1999). The values for protein per plant were transformed to log-values before analysis following the model for transformation by Box, Hunter and Hunter (1978). All other values were used in their original form.

3. Results

The outcome of the study was evaluated through the biomass, development stage, protein content and NDF content of the plants. The results are shown in tables after each section. All the significant interactions are based on LSD-values (least significant difference) for p-values of 0.05. Individual factors are shown even when these factors were included in significant interactions with other factors. This was done to give an overall picture of the response of the plants as well as complement the data where the interactions were not significant for both crops and both harvests.

3.1 Biomass

The effect on the biomass was expressed as dry weight per plant and as the ratio between the dry weights for the grass versus the dry weight of the legume.

3.*1.1 Dry weight per plant*

The dry weights of the plants were affected by all the different treatment factors (shade, mix and water). In Table 1, there is a significant interaction between shade and mix for differences in dry weight for the second harvest. The pattern is more defined for the clover than for the grass. There were significant differences in performance of both the grass and the legume between at least mix 9:3 and mix 3:9 within the full light treatment. The dry weights are significantly higher in mix 9:3 than in mix 6:6 and mix 3:9 for the grass in the 50% shaded treatment, while there was no significant difference for this treatment in the clover. Also the 80% shade treatment is the same for all mixes in both the grass and the legume. When looking at shade as an individual factor it can be seen that the biomass decreased significantly when the shade levels changed from full light to 50% shade to 80% shade (Table 2). An exception was the ryegrass in the first harvest where 50% shade was not significantly different from either the full light treatment or the 80% shade level. The ryegrass still follows the same trend as it does in the second harvest.

Mix as an individual factor shows that the biomass per plant was higher in mix 9:3 than mix 6:6 and mix 3:9 for the legume (Table 3). For the grass (second harvest) also mix 6:6 was significantly different from mix 9:3 and 3:9, which gave a clear relationship showing that the biomass per plant increased when the grass was in majority (Table 3).

The significant interaction between shade and water (Table 4) shows that the grass gets a significantly higher dry weight when growing in a high water level compared to the low water level when grown in full light. For the other light treatments there is no such difference. The legume on the other hand also shows a significant difference in dry weight between the water levels when grown in 50% shade. The dry weights decrease with increased shade regardless of the water level.

Red clover showed a significant interaction between mix and water for both harvests (Table 5). In mix 9:3 and mix 3:9 in the first harvest, and mix 9:3 in the second harvest, biomass production decreased significant with a lower water level. In the high watered treatment there is a significant difference in dry weight between mix 9:3 and the other mixes while all the mixes in the low water treatment have the same biomass production.

Perennial ryegrass showed a higher biomass production for the high water treatment than the low water treatment for both harvests (Table 6).

Table 1. Influence of shade and mix on dryweight/plant (g) in perennial ryegrass (PR) red clover (RC)

	PR, H2			RC, H2		
	Mix			Mix		
Shade	9:3	6:6	3:9	9:3	6:6	3:9
0%	0.86 *Aa*	0.71 A*a*	0.33 *Ba*	1.58 *Aa*	0.98 *Ba*	0.87 *Ba*
50%	0.48 *Ab*	0.28 *Bb*	0.26 *Ba*	0.71 *Ab*	0.54 *Ab*	0.41 *Ab*
80%	0.21 *Ac*	0.13 *Ab*	0.13 *Aa*	0.16 *Ac*	0.1 *Ac*	0.11 *Ac*
$LSD_{0.05}$↔ (A-C)			0.2			0.42
$LSD_{0.05}$↕ (a-c)			0.23			0.27
p-value shade*mix			0.0122			0.0276

Mix=relation of grass:legume plants
H2=second harvest

Table 2. Influence of shade on dryweight/plant (g) in perennial ryegrass (PR) and red clover (RC)

Shade	PR, H1	PR, H2	RC, H1	RC, H2
0%	0.53 *a*	0.63 *a*	0.54 *a*	1.14 *a*
50%	0.37 *ab*	0.34 *b*	0.37 *b*	0.56 *b*
80%	0.23 *b*	0.16 c	0.14 *c*	0.12 *c*
$LSD_{0.05}$	0.17	0.18	0.08	0.15
p-value shade	0.0130	0.0021	0.0001	0.0000

H1=first harvest, H2=second harvest

Table 3. Influence of mix on dryweight/plant (g) in perennial ryegrass (PR) and red clover (RC)

Mix	PR, H2	RC, H1	RC, H2
9:3	0.52 *a*	0.40 *a*	0.82 *a*
6:6	0.37 *b*	0.33 *b*	0.54 *b*
3:9	0.24 *c*	0.31 *b*	0.46 *b*
$LSD_{0.05}$	0.11	0.07	0.17
p-value mix	0.0001	0.0280	0.0003

Mix=relation of grass:legume plants
H1=first harvest, H2=second harvest

Table 4. Influence of shade and water on dryweight/plant (g) in perennial ryegrass (PR) and red clover (RC)

	PR, H1		PR, H2		RC, H1		RC, H2	
	Water							
Shade	HW	LW	HW	LW	HW	LW	HW	LW
0%	0.63 *Aa*	0.44 *Ba*	0.84 *Aa*	0.43 *Ba*	0.66 *Aa*	0.41 *Ba*	1.54 *Aa*	0.74 *Ba*
50%	0.39 *Ab*	0.35 *Aab*	0.36 *Ab*	0.32 *Aab*	0.42 *Ab*	0.32 *Ba*	0.73 *Ab*	0.38 *Bb*
80%	0.25 *Ab*	0.21 *Ab*	0.16 *Ab*	0.15 *Ab*	0.14 *Ac*	0.14 *Ab*	0.14 *Ac*	0.11 *Ac*
$LSD_{0.05}$↔ (A-C)		0.09		0.16		0.1		0.24
$LSD_{0.05}$↕ (a-c)		0.18		0.26		0.1		0.22
p-value shade*water		0.0252		0.0013		0.0015		0.0002

HW=high water level, LW=low water level
H1=first harvest, H2=second harvest

Table 5. Influence of mix and water on dryweight/plant (g) in red clover(RC)

	RC, H1		RC, H2	
Mix	HW	LW	HW	LW
9:3	0.51 *Aa*	0.29 *Ba*	1.2 *Aa*	0.44 *Ba*
6:6	0.35 *Ab*	0.3 *Aa*	0.64 *Ab*	0.44 *Aa*
3:9	0.37 *Ab*	0.26 *Ba*	0.57 *Ab*	0.35 *Aa*
$LSD_{0.05}$↔ (A-C)	0.1		0.24	
$LSD_{0.05}$↕ (a-c)	0.1		0.24	
p-value mix*water	0.0363		0.0029	

Mix=relation of grass:legume plants
HW=high water level, LW=low water level
H1=first harvest, H2=second harvest

Table 6. Influence of water on dryweight/plant (g) in perennial ryegrass (PR) and red clover (RC)

Water	PR, H1	PR, H2	RC, H1	RC, H2
HW	0.42 *a*	0.45 *a*	0.41 *a*	0.80 *a*
LW	0.33 *b*	0.30 *b*	0.28 *b*	0.41 *b*
$LSD_{0.05}$	0.05	0.09	0.05	0.14
p-value water	0.0013	0.0017	0.0000	0.0000

HW=high water level, LW=low water level
H1=first harvest, H2=second harvest

3.1.2 Grass:legume ratio for dry weight

In the second harvest, the ratio between the grass and the legume increased with shade, which shows that the grass has a better growth in a shaded environment than the legume (Table 7).

Table 7. Influence of shade on dryweight/plant (g) perennial ryegrass:red clover ratio

Shade	H2
0%	0.65 *b*
50%	0.78 *b*
80%	1.58 *a*
$LSD_{0.05}$	0.71
p-value shade	0.0401

H2=second harvest

3.2 Development stage

The development stages of the plants differed depending on species, harvest and treatment according to Figure 1. The perennial ryegrass stayed in a vegetative stage throughout the experiment, regardless of harvest and treatment. The plants were therefore evaluated as development stage 1 (data not shown). The red clover varied in development stage depending on harvest and treatment. In the first harvest, all samples but two were evaluated to development stage 1. The remaining two were development stage 2 and were both high water treatments, with one in 0% shade, mix 9:3 and the other 50% shade, mix 6:6 (data not shown). A larger variation was found in the second harvest. A couple of samples had reached development stage 3 and several samples were evaluated as development stage 2. These were found in the 0% and 50% shaded treatments with the major part in 0% shade. The plants in the 80% shaded treatment all belonged to development stage 1. From the data can also be seen that the high watered samples tended to reach a higher development stage than the low watered treatments. Which mix the plants belonged to did not seem to make any difference. These data were not statistically evaluated and are shown in Figure 1.

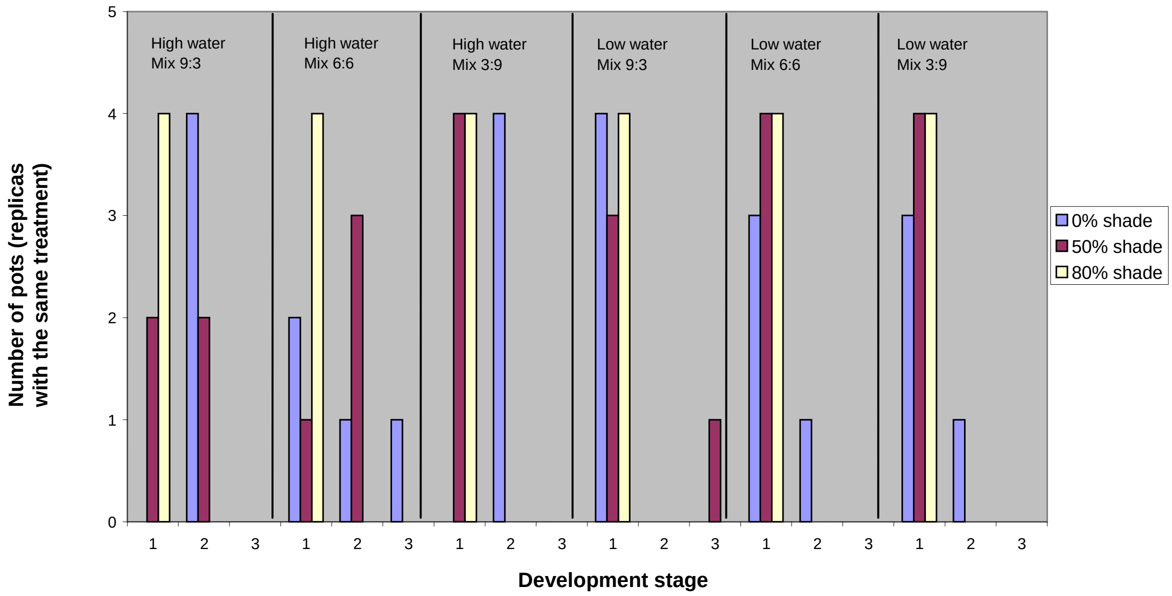

Figure 1. Development stages for red clover in different mixes and water levels, harvest 2. Development stage based on SLU-scale, mix=relation between grass and legume, high water=high water level, low water=low water level.

3.3 Protein content

The protein content of the plants was expressed in four different ways. First the protein concentration (%) was measured. This data was also used to create a ratio between the perennial ryegrass and the red clover. In addition to this, a measure of actual protein content (g/plant) was calculated. Also for this measure a ratio between the two species was created, but no significant interactions were found for this measure.

3.3.1 Protein concentration

A significant interaction was found between shade and water for red clover in the second harvest (Table 8).The protein concentration of the clover increases significantly when the level of shade increases for both the high and low water treatment. In the 0% shade treatment, there was a significant difference between the protein concentrations of the plants in the different water levels, where the low water level gave a higher protein concentration. In the 50% and 80% shade treatments there was no significant difference in protein concentration. When looking at shade as an individual treatment (Table 9) it can be seen that in the second harvest there was a clear response to shade where 0% shade had a much lower protein concentration than 50% and 80% shade. The first harvest also had significant differences between the light treatments, but there were no clear relationships.

The water treatment had significant differences for red clover, (first harvest only) and perennial ryegrass, (second harvest only) (Table 10). The red clover showed a significantly higher protein concentration for the high water treatment while the perennial ryegrass had a significantly higher protein concentration when grown under low water conditions.

The protein concentration increased significantly for the grass when occurring in mix 9:3 compared to mix 3:9, with mix 6:6 intermediate (Table 11). The protein concentration increased significantly for the legume when occurring in mix 3:9 compared to mix 9:3 (Table 11). Mix 6:6 was intermediate here as well, but not significantly different from the other mixes. Thus, the protein concentration of both the grass and the legume increased when the specific crop was dominating the stand.

Table 8. Influence of shade and water on protein concentration (%) of red clover (RC)

	RC, H2	
Shade	HW	LW
0%	16.57 *Bb*	19.02 *Ab*
50%	20.16 *Aa*	19.86 *Aab*
80%	20.37 *Aa*	20.31 *Aa*
$LSD_{0.05}$↔ (A-C)		1.18
$LSD_{0.05}$↕ (a-c)		1.26
p-value shade*water		0.0030

HW=high water level, LW=low water level
H2=second harvest

Table 9. Influence of shade on protein concentration (%) of perennial ryegrass (PR) and red clover (RC)

Shade	PR, H1	PR, H2	RC, H1	RC, H2
0%	26.21 *a*	20.81 *b*	22.09 *b*	17.79 *b*
50%	25.54 *b*	21.51 *a*	22.82 *a*	20.01 *a*
80%	26.25 *a*	21.7 *a*	22.36 *ab*	20.34 *a*
$LSD_{0.05}$	0.37	0.67	0.56	1.07
p-value shade	0.0061	0.0393	0.0482	0.0027

H1=first harvest, H2=second harvest

Table 10. Influence of water on protein concentration (%) of perennial ryegrass (PR) and red clover (RC)

Water	PR, H2	RC, H1
HW	21.05 *b*	22.87 *a*
LW	21.63 *a*	21.98 *b*
$LSD_{0.05}$	0.39	0.37
p-value water	0.0042	0.0000

HW=high water level, LW=low water level
H1=first harvest, H2=second harvest

Table 11. Influence of mix on protein concentration (%) of perennial ryegrass (PR) and red clover (RC)

Mix	PR, H1	RC, H1
9:3	26.40 *a*	22.2 *b*
6:6	26.10 *b*	22.32 *ab*
3:9	25.51 *c*	22.75 *a*
$LSD_{0.05}$	0.25	0.45
p-value mix	0.0000	0.0468

Mix=relation of grass:legume plants
H1=first harvest

3.3.2 Grass:legume ratio for protein concentration

There was a significant interaction between shade and water for the ratio between the protein concentration of the grass and the legume in the second harvest (Table 12). The table shows that the grass got an advantage of the legume when they occurred in full light and high water treatment.

When looking at shade individually (Table 13) for the first harvest, there seemed to be an advantage for the grass in 0% shade and 80% shade, while the ratio was lower for 50% shade. In the second harvest, there was a more uniform relationship when the grass occurred in a larger proportion in 0% shade than in 50% and 80% shade.

Table 14 shows the individual effects of the water treatments. The ratio between the grass and the legume shows that the difference in protein concentration between the two species was significantly larger in the low water treatment than the high water treatment. That is, the grass increased its protein level more than the legume when occurring in a low water environment, or reversed; the legume decreased its protein concentration more than the grass when grown in full light.

The different mixes showed significant differences between the grass:legume ratios (Table 15). The ratio between the grass and the legume was significantly higher in mix 9:3 compared to mix 3:9 in both harvests. Mix 6:6 was intermediate in the first harvest but the same as mix 9:3 in the second harvest. These results mean that either the grass increased in protein when occurring in mix 9:3, or the legume increased in protein when occurring in mix 3:9.

Table 12. Influence of shade and water on protein concentration (%) perennial ryegrass:red clover ratio

	H2			
Shade	HW		LW	
0%	1.25	*Aa*	1.12	*Ba*
50%	1.06	*Ab*	1.1	*Aa*
80%	1.07	*Ab*	1.07	*Aa*
$LSD_{0.05}$↔ (A-C)				0.07
$LSD_{0.05}$↕ (a-c)				0.09
p-value shade*water				0.0057

HW=high water level, LW=low water level
H2=second harvest

Table 13. Influence of shade on protein concentration (%) perennial ryegrass:red clover ratio

Shade	H1		H2	
0%	1.19	*a*	1.19	*a*
50%	1.12	*b*	1.08	*b*
80%	1.18	*a*	1.07	*b*
$LSD_{0.05}$		0.02		0.09
p-value shade		0.0010		0.0322

H1=first harvest, H2=second harvest

Table 14. Influence of water on protein concentration (%) perennial ryegrass:red clover ratio

Water	H1	
HW	1.15	*b*
LW	1.18	*a*
$LSD_{0.05}$		0.02
p-value water		0.0010

HW=high water level, LW=low water level
H1=first harvest

Table 15. Influence of mix on protein concentration (%) perennial ryegrass:red clover ratio

Mix	H1		H2	
9:3	1.19	*a*	1.14	*a*
6:6	1.17	*b*	1.13	*a*
3:9	1.13	*c*	1.07	*b*
$LSD_{0.05}$		0.02		0.05
p-value mix		0.0000		0.0161

Mix=relation of grass:legume plants
H1=first harvest, H2=second harvest

3.3.3 Protein per plant

A significant interaction was found between shade and mix for perennial ryegrass in the second harvest (Table 16). In Table 16, it can be seen that the amount of protein in the grass increased with light in all 3 mixes. The amount of protein was significantly higher in mix 9:3 than mix 3:9 for the full light treatment and the 50% shade treatment.

For shade treatment individually, there was a clear relationship for both species and both harvests that the amount of protein increased significantly when light increased (Table 17). Also mix as an individual treatment factor showed clearly that the amount of protein was significantly higher in mix 9:3 than in mix 3:9 (Table 18). This was shown for perennial ryegrass in both harvests and for red clover in the second harvest.

The significant interaction between shade and water is showed by Table 19. In both crops and both harvests the amount of protein increased significantly with light for both the high and low water treatment. In the 0% shade the amounts of protein increased significantly with the higher water level for both the grass and the clover. For clover there was also a significant difference in the 50% shade while the 80% shade showed no difference between the two water levels for either crop. Water as an individual factor, showed a significantly higher amount of protein in the high water treatment compared to the low water treatment for both crops at both harvests (Table 20).

The interaction between mix and water was significant for red clover in the second harvest (Table 21). In mix 9:3, the high water level gave a significantly higher amount of protein than the low water level. In the other mixes the differences were not significant. In the low water level all the mixes gave the same amount of protein per plant.

Table 16. Influence of shade and mix on protein/plant (g) of perennial ryegrass (PR)

	PR, H2		
	Mix		
Shade	9:3	6:6	3:9
0%	0.069 *Aa*	0.056 *Ba*	0.028 *Ca*
50%	0.042 *Ab*	0.026 *Bb*	0.023 *Bab*
80%	0.02 *Ac*	0.012 *Ac*	0.013 *Ab*
$LSD_{0.05}$↔ (A-C)			0.011
$LSD_{0.05}$↕ (a-c)			0.013
p-value shade*mix			0.0056
Mix=relation of grass:legume plants			
H2=second harvest			

Table 17. Influence of shade on protein/plant (g) of perennial ryegrass (PR) and red clover (RC)

Shade	PR, H1	PR, H2	RC, H1	RC, H2
0%	0.056 *a*	0.051 *a*	0.048 *a*	0.076 *a*
50%	0.039 *b*	0.03 *b*	0.035 *b*	0.045 *b*
80%	0.024 *c*	0.015 *c*	0.013 *c*	0.012 *c*
$LSD_{0.05}$	0.015	0.014	0.007	0.012
p-value shade	0.0050	0.0021	0.0000	0.0000

H1=first harvest, H2=second harvest

Table 18. Influence of mix on protein/plant (g) of perennial ryegrass (PR) and red clover (RC)

Mix	PR, H1	PR, H2	RC, H2
9:3	0.045 *a*	0.044 *a*	0.057 *a*
6:6	0.039 *b*	0.031 *b*	0.04 *b*
3:9	0.035 *b*	0.022 *c*	0.036 *b*
$LSD_{0.05}$	0.006	0.008	0.01
p-value mix	0.0101	0.0000	0.0043

Mix=relation of grass:legume plants

H1=first harvest, H2=second harvest

Table 19. Influence of shade and water on protein/plant (g) of perennial ryegrass (PR) and red clover (RC)

	PR, H2		PR, H2		RC, H1		RC, H2	
	Water							
Shade	HW	LW	HW	LW	HW	LW	HW	LW
0%	0.066*Aa*	0.046 *Ba*	0.065 *Aa*	0.038 *Ba*	0.06 *Aa*	0.037 *Ba*	0.095 *Aa*	0.057 *Ba*
50%	0.041*Ab*	0.037 *Aab*	0.032 *Ab*	0.029 *Aab*	0.041 *Ab*	0.029 *Ba*	0.058 *Ab*	0.031 *Bb*
80%	0.024*Ac*	0.023 *Ab*	0.015 *Ac*	0.015 *Ab*	0.014 *Ac*	0.013 *Ab*	0.013 *Ac*	0.01 *Ac*
$LSD_{0.05}$↔ (A-C)	0.008		0.011		0.009		0.017	
$LSD_{0.05}$↕ (a-c)	0.015		0.015		0.009		0.015	
p-value shade*water	0.0079		0.0014		0.0073		0.0267	

HW=high water level, LW=low water level

H1=first harvest, H2=second harvest

Table 20. Influence of water on protein/plant (g) of perennial ryegrass (PR) and red clover (RC)

Water	PR, H1	PR, H2	RC, H1	RC, H2
HW	0.207 *a*	0.037 *a*	0.038 *a*	0.055 *a*
LW	0.176 *b*	0.027 *b*	0.026 *b*	0.033 *b*
$LSD_{0.05}$	0.005	0.006	0.005	0.01
p-value water	0.0016	0.0030	0.0000	0.0000

HW=high water level, LW=low water level

H1=first harvest, H2=second harvest

Table 21. Influence of mix and water on protein/plant (g) of red clover (RC)

	RC, H2	
Mix	HW	LW
9:3	0.078 *Aa*	0.035 *Ba*
6:6	0.044 *Ab*	0.035 *Aa*
3:9	0.044 *Ab*	0.028 *Aa*
$LSD_{0.05}$↔ (A-C)		0.017
$LSD_{0.05}$↕ (a-c)		0.017
p-value mix*water		0.0180

HW=high water level, LW=low water level
Mix=relation of grass:legume plants
H2=second harvest

3.4 Fiber content

Fiber content was measured as NDF, neutral detergent fiber. The concentration (%) of NDF was analyzed as well as the ratio between the NDF concentration of the grass and the NDF concentration of the legume.

3.4.1 NDF concentration

A significant interaction between shade and mix was found for perennial ryegrass in the first harvest (Table 22). The NDF content of the grass increased significantly with increased shade level in all different mixes except for 80% shade where mix 9:3 and 6:6 gave the same NDF concentration. In all shade levels the NDF content increased significantly from mix 9:3 to mix 3:9. In the full light treatment also mix 6:6 was significantly different from 9:3 and 3:9. Thus, NDF increased when the grass occurred in a lower quantity compared to the legume. Shade as an individual factor showed that in the first harvest, both the grass and the legume had a significantly increased NDF content when the shade increased from full light to 80% shade (Table 23). This relationship was stronger for the ryegrass than for the clover. In the second harvest the relationship for the legume was the reversed and the NDF content decreased significantly when shade increased.

Within the mix treatment the NDF content of the grass increased significantly when the grass occurred in a smaller amount compared to the legume (mix 3:9)(Table 24). In the second harvest, the legume had a significantly higher NDF content when occurring in mix 6:6 than in mix 9:3 and 3:9 (Table 24).

For NDF concentration, there was also a significant interaction between mix and water for red clover in the second harvest (Table 25). The high water treatment had a significantly higher NDF content than the low water treatment in mix 6:6 and 3:9. Mix 9:3 showed the same trend but the difference was not significant. The clover showed no difference in NDF content between the three mixes within the low water level. Within the high water level, clover in mix 6:6 seemed to have a higher NDF content than clover in the other mixes. With water as an individual factor, it can be seen that the NDF levels increased significantly with water in both the grass and the legume for the second harvest (Table 26).

Table 22. Influence of shade and mix on NDF concentration (%) of perennial ryegrass (PR)

	PR, H1		
	Mix		
Shade	9:3	6:6	3:9
0%	38.20 *A a*	39.71 *B a*	42.73 *C a*
50%	40.61 *A b*	42.06 *B b*	43.06 *C b*
80%	42.02 *A c*	42.36 *A b*	44.02 *B b*
$LSD_{0.05}$↔ (A-C)			1.45
$LSD_{0.05}$↕ (a-c)			1.24
p-value shade*mix			0.0396

Mix=relation of grass:legume plants
H1=first harvest

Table 23. Influence of shade on NDF concentration (%) of perennial ryegrass (PR) and red clover (RC)

Shade	PR, H1	RC, H1	RC, H2
0%	40.21 *c*	32.81 *b*	40.26 *a*
50%	41.91 *b*	33.07 *b*	38.7 *b*
80%	42.80 *a*	34.2 *a*	36.36 *c*
$LSD_{0.05}$	0.47	0.68	1.22
p-value shade	0.0000	0.0065	0.0010

H1=first harvest, H2=second harvest

Table 24. Influence of mix on NDF concentration (%) of perennial ryegrass (PR) and red clover (RC)

Mix	PR, H1	PR, H2	RC, H2
9:3	40.27 *c*	48.94 *c*	38.06 *b*
6:6	41.37 *b*	49.88 *b*	39.04 *a*
3:9	43.27 *a*	51.22 *a*	38.22 *b*
$LSD_{0.05}$	0.53	0.89	0.73
p-value mix	0.0000	0.0000	0.0367

Mix=relation of grass:legume plants
H1=first harvest, H2=second harvest

Table 25. Influence of mix and water on NDF concentration (%) of red clover (RC)

	RC, H2	
Mix	HW	LW
9:3	38.24 *Ab*	37.87 *Aa*
6:6	40.2 *Aa*	37.88 *Ba*
3:9	38.96 *Ab*	37.49 *Ba*
$LSD_{0.05}$↔ (A-C)		1.11
$LSD_{0.05}$↕ (a-c)		1.11
p-value mix*water		0.0478

Mix=relation of grass:legume plants
HW=high water level, LW=low water level
H2=second harvest

Table 26. Influence of water on NDF concentration (%) of perennial ryegrass (PR) and red clover (RC)

Water	PR, H2	RC, H2
HW	50.54 *a*	39.13 *a*
LW	49.49 *b*	37.74 *b*
$LSD_{0.05}$	0.73	0.6
p-value water	0.0064	0.0000

HW=high water level, LW=low water level
H1=first harvest, H2=second harvest

3.4.2 Grass:legume ratio for NDF

Significant differences were found for the grass:legume ratio within the shade treatment in both harvests (Table 27). In the second harvest, the ratio was significantly higher in 80% shade than in 0% and 50% shade. Thus the NDF content of the grass increased more with shade then the NDF content of the legume. In the first harvest, the relationship was not as clear. The 50% shade treatment had a higher ratio than the 0% shade treatment while the 80% shade was not significantly different from either of them.

Also the mix treatment showed significant differences for the NDF grass:legume ratio in both harvests (Table 28). The ratios increased when the legume occurred in a higher quantity. In other words, the NDF levels of the grass increased more than the NDF levels of the legume when the stand was grown in mix 3:9 compared to mix 9:3.

Table 27. Influence of shade on NDF concentration (%) perennial ryegrass:red clover ratio

Shade	H1		H2	
0%	1.23	*b*	1.27	*b*
50%	1.27	*a*	1.29	*b*
80%	1.25	*ab*	1.36	*a*
$LSD_{0.05}$	0.03		0.04	
p-value shade	0.0350		0.0039	

H1=first harvest, H2=second harvest

Table 28. Influence of mix on NDF concentration (%) perennial ryegrass:red clover ratio

Mix	H1		H2	
9:3	1.21	*c*	1.28	*b*
6:6	1.24	*b*	1.28	*b*
3:9	1.29	*a*	1.35	*a*
$LSD_{0.05}$	0.03		0.03	
p-value mix	0.0000		0.0001	

Mix=relation of grass:legume plants
H1=first harvest, H2=second harvest

4. Discussion

When the red clover samples were analyzed with NIRS, an equation for alfalfa was used for the estimations of the forage quality values. It can be questioned if red clover and alfalfa are so alike that the equations are interchangeable. However, for this study it is assumed that these two plants are alike and the error this assumption might have caused is ignored.

Generally it can be believed that the perennial ryegrass was benefited by the high nitrogen fertilization (Schwinning and Parsons, 1996). This might for example have caused higher biomass for the grass than the legume and affected the protein content of the plants. The protein content of the ryegrass (around 21 to 26% depending on treatment) agrees with results from a study by Hoffman *et al.,* 1993, where perennial ryegrass at an early development stage (second node) contained 23.3% protein. The protein content of the red clover (around 17 to 23% depending on treatment) are somewhat lower than the protein content found in red clover in the study by Hoffman *et al.* (1993). In this study, red clover at a late vegetative state had 24.5% protein.

4.1 Shade

Shade clearly affected biomass negatively for both the grass and the legume. This result was expected since it agrees with previous studies (Kephart *et al.*, 1992; Vartha, 1973; Lin *et al.*, 1999; Redfearn *et al.*, 1999). Still there can be seen some shade tolerance of the grass since the 50% shade level in the first harvest was not different from the full light treatment, or the 80% shade treatment. Also this result agrees with Lin *et al.* (1999). The differences were larger in the second harvest, possibly because the plants started to grow in an untreated condition until they were well established in the first harvest, while they had to start regrowing in the shaded environment for the second harvest. Also there seemed to be a larger effect on the clover than the ryegrass, which indicates that perennial ryegrass had a stronger shade tolerance than red clover. This is confirmed by the grass:legume ratio for the dryweight that shows that the grass had a better growth than the legume in a shaded environment.

For perennial ryegrass, there was no difference in dryweight per plant between the shade levels when occurring as a minority, while the dry weight showed differences when the number of grass plants was equal to, or exceeding the number of clover plants. Possibly this had to do with the shade level the red clover potentially already provided by its horizontal leaf arrangement (Joggi *et al.*, 1983).

Shade clearly had an effect on the development stage of red clover. Since the ryegrass remained in a vegetative stage for all the shade treatment it is difficult to draw any conclusion of the effect of shade on the grass. To do this, the project would have needed to continue for a longer time period.

The effect on the protein concentration of the plants when exposed to shade differed with species and harvest. The higher protein concentrations in both crops in the 50% and 80% shade in the second harvest indicates a higher forage quality when a plant is exposed to shade. The varying results in the first harvest indicate that the relationship might not be that clear. The results from the second harvest agree with the results of Kephart and Buxton (1993), Redfearn *et al.* (1999) as well as Johnson *et al.* (2002). The ratios between the grass and the legume followed the same uneven patterns between the harvests, but in the second harvest the grass kept the protein concentrations better than the legume when growing in full light. This was expected since the clover had a higher development stage in the second harvest than the grass, and this probably affected the protein concentration. An explanation for the differences between the harvests can be, as for the dry weight, that for the first harvest, the plants started to grow in an unshaded condition. Therefore the shade treatments might not have been in full effect. For the second harvest the plants were exposed to shade during the whole growth period and the shade effect was more pronounced. The interaction between shade and water for red clover in the second harvest showed a larger difference between the shade levels in the high water treatment than the low water treatment, which probably had to do with the differences in development stages between the water treatments.

The clear patterns of protein content per plant between the shade levels can be easily understood when comparing the protein content to the biomass. A smaller plant should naturally contain a smaller amount of protein. The differences in protein concentration were evidently not high enough to make up for the loss of protein due to a lower biomass. The relationship between protein content and biomass can also, at least partly, explain why the mix with a majority of grass had a higher amount of protein per plant in the 0% and 50% shade treatments than the mix with a majority of clover, since the biomass followed a similar trend. Also, for the interaction between shade and water, the differences in protein per plant exactly followed the differences in dry weight per plant.

Most literature agree that the fiber content of plants is likely to decrease when shade increases (Kephart and Buxton, 1993; Redfearn *et al*., 1999). There are also studies showing the opposite response to shade (Johnson *et al*., 2002). In this study red clover followed the pattern of decreased NDF concentration in the second harvest when exposed to shade, while both crops had the reversed pattern in the first harvest. The differences in NDF concentration of the red clover between the two harvests can be explained with the difference in development stages of the plants between the first and the second harvest. When ryegrass occurred as a minority in the pots, the highest NDF levels were received. This could be an effect of a possibly increased shade level provided by the horizontal leaf arrangement of the red clover (Joggi *et al*., 1983). The grass:legume ratio for the second harvest makes sense, since the NDF concentration of the legume decreased with shade and the grass had no significant differences. For the first harvest, both the legume and the grass responded with increased NDF levels when exposed to shade and therefore the ratio between them will not show a distinct pattern.

4.2 Water

As Hopkins and Hüner (2004) stated, reduction in vegetative growth will be one of the first responses to water stress. That is the result received in this study for both crops, in both harvests. In the results, it can be seen that there was only a difference between the high and the low water treatment in 0% shade for perennial ryegrass, and in 0% and 50%

shade for the red clover. This probably occurred because the pots in the 0% shade treatment dried out much faster than the other shade treatments and therefore it was easier to keep a sufficient difference in water content of the soil. The 80% shade treatment was the treatment that held moisture the longest, which also was reflected in the response of the plants. The high watered red clover occurring in the mix with grass as the majority, were distinguished in both harvests for having a higher biomass than the low watered treatment. In the first harvest this was also true for the mix with clover as a majority. Since the mix dominated by grass should have received more light, (Joggi *et al.*, 1983) the plants in these pots should not only have grown better but also dried out faster. Therefore, it is natural to see a difference in biomass between the water levels in this mix. This argument is contradicted by the result of the clover dominated mix in the first harvest, which for the biomass experienced the same response as the grass dominated mix. The response of the mix dominated by grass might be more accurate than the mix dominated by clover, since the differences between the water levels might have been more sufficient in the grass dominated pots due to quicker drying of the soil. The lack of significant differences in ratio between the biomass of the grass and the legume when exposed to water stress show that the two species have a similar response in growth to water availability.
The response in development stage for the red clover strongly agrees with studies by Peterson *et al.* (1992) and Halim *et al.* (1989a) who suggest that maturity gets delayed with an increasing water stress. Not enough growing time was given for the perennial ryegrass to show its response.

The response of the protein concentrations of the plants to different water levels varied with crop. The grass increased its protein concentration when exposed to water stress while the legume had the reversed response. The response of the grass agrees with the results of a study by Sheaffer *et al.* (1992). A study by Peterson *et al.* (1992) showed varying results in protein concentration when red clover was exposed to water stress. Halim *et al.* (1989a) showed higher protein concentrations in stems but lower protein levels in leaves when alfalfa was exposed to drought. These studies indicate that the response of protein concentration in legumes to shade is somewhat unclear. The interaction between shade and water for red clover in the second harvest shows that it was only in the 0% shade level that there was a difference in protein concentration between the water levels. This result was probably received since the 0% shade treatment dried out more than the other shade levels did. Only red clover in the second harvest showed this significant interaction. This can be understood since the red clover treated with 0% shade reached a higher development stage than in the other treatments in the second harvest. These plants also grew larger and received a higher biomass than the other clover plants. The larger grass:legume ratio for the protein concentrations in the draughted condition compared to the well watered condition can be explained by the decrease in protein concentration in the legume when exposed to draught. There was no difference in protein concentration for the grass between the water treatments in the first harvest, and therefore the difference in ratio has to be caused by the legume.

The actual protein content per plant was higher in the high water treatment then the low water treatment for both harvests and both crops. This relationship is easily explained with the direct relationship to the dry weight of the plants. For perennial ryegrass in the second harvest, this trend should be somewhat counteracted by the reversed trend in protein concentration. In the interaction between shade and water the same relationships can be seen as for the biomass. In red clover, second harvest, the water treatments only had an effect in the mix dominated by grass. The protein levels in the other mixes were not significantly different between the water levels. Also these effects on protein content agree with the effect on the biomass, which should be a likely explanation to the interaction. The biomass of the red clover was also affected in the first harvest, but the differences in biomass were not as great as in the second harvest. This could be the reason why the protein content was not significantly affected in the first harvest even if the biomass was.

The NDF levels were higher in the high water treatment than the low water treatment in the second harvest for both crops. This result agrees with studies by Sheaffer *et al*. (1992), Peterson *et al.* (1992) and Halim *et al*. (1989b). The interaction between mix and water for red clover, second harvest, showed that the NDF concentration of the clover was only affected in the mix dominated by clover, or in the mix with an equal amount of grass and clover plants. In the grass dominated mix, red clover had the same NDF concentration both in the high water level and the low water level. Why these results were received is not easily explained and further studies would be needed to answer this question.

4.3 Mix

The biomass of the plants was affected by in which species mix they were grown. In the grass dominated mix, where the proportions were 75% grass versus 25% legume, both the grass and the legume had a higher biomass per plant than in the other mixes. These results are contradicting to the results from the study by Sollenberg *et al.* (1984) that showed that the biomass increased for both the legume component and grass component of mixed stands of grass and legumes with increased legume seedling rates. The differing results are probably due to the high fertilization rate that was used in this study, which caused a situation where the plants did not benefit from a higher rate of legumes as they would do with a lower fertilization rate. A possible explanation to the increased biomass in the grass dominated mix can also be that the canopy structure of the grass will be more open than the canopy of the legume and therefore more light will penetrate it (Joggi *et al.*, 1983). If more light reaches the plants in the grass dominated mix, the biomass should also be higher since that effect of light has been shown in this study. This theory is also supported by the article by Sollenberg *et al.* (1984) where the authors mention that shading by the companion legume may have undesirable effects on ryegrass when grown in legume-ryegrass mixtures. Also Annicchiarico and Piano (1994) found in their study with white clover and several different grasses that the inter-specific competition mainly occurred for light, since their plants were grown in a relatively favorably environment with respect to water and nutrients. This theory is based on the assumption that the grass dominated mix in fact was more open, and more light penetrated the canopy. Even though it seems like a reasonable explanation it must be seen as speculative, since the light interception was not measured in this study.

In the ryegrass, second harvest, there is a difference in the effects of shade on biomass between the different mixes. The differences between the shade levels are larger in the grass dominated mix than the clover dominated mix. This indicates that the canopy structure influences the shade effects. A denser canopy should hamper the shade effects, since the plants are already providing shade themselves. When comparing the mixes with the water levels, it was found that the mix dominated by grass had a higher biomass than the other mixes in the high water treatment, while all the mixes produced the same biomass in the low water treatment. A suggestion why the grass dominated mix is not producing more biomass than the other mixes when growing in the low water treatment, is that since this mix lets through more light than the other mixes it will also be drier than the others. Therefore the growth will be hampered despite the better light conditions. When considering the theory of the higher light penetration in the grass dominated mix, combined with the affect of light on development stage, it can be believed that there would be a tendency towards higher maturity in the mix dominated by grass. There are no such clear results in this study. The only tendency in this direction was that the high watered, grass dominated mix, had a few more samples in the higher development stages than the other mixes.

The protein concentration in the plants increased when the specific species occurred as a majority in the pots. This response has no easy explanation and further investigations would be needed to give an answer to why this result was achieved. Interactions between plants are complex and change over time (Turkington, 1996). The response in protein concentration to mix occurred in the first harvest, when the plants were recently established, which can be a reason for this specific effect.

The grass:legume ratio for the protein concentration was higher in the mix where grass occurred as a majority compared to the mix where clover was the dominating species. This is the same pattern as can be seen within protein concentration, for the effects of mix on the grass, which had a higher protein concentration in the grass dominated mix than the mix dominated by clover. The clover had the opposite relationship but the differences were not as large as for the grass.

The amount of protein per plant was higher in the grass dominated mix than the clover dominated mix, which is the same pattern as for the dry weight. This result therefore makes sense since a larger plant is more likely to contain more protein than a smaller plant, as long as the concentrations remain on a fairly constant level. In all mixes there are differences in protein content between the different light levels. The grass dominated mix, and the mix with equal amount of grass and clover plants, show this relationship stronger than the clover dominated mix. Also these trends closely follow the trend of the biomass. In red clover, in the second harvest, the grass dominated mix is distinguished for giving higher protein content in the high watered treatment than the other mixes. In the low watered treatment there is no difference between the mixes. Once again, the same relationship can be found for the biomass, which probably is the major cause for the trends in protein content.

Perennial ryegrass clearly showed a higher fiber content when growing as a minority compared to when growing in the grass dominated mix. This result has a positive relationship to the result for shade and should be an effect of the increased shade level the red clover possibly provided (Joggi *et al.*, 1983). Red clover had a higher NDF content in the mix with the same amount of grass and clover, compared to the mixes where one of the species grew as a majority. The reason why red clover showed that response is not clear and further investigations would be needed to explain this reaction. For perennial ryegrass, in the first harvest, an interaction was found between shade and mix. All the different shade levels show the same trend; a higher NDF content in the clover dominated mix than in the grass dominated mix. For the interaction between mix and water (red clover in the second harvest), the mix with equal amount of grass and clover was distinguished for giving a higher NDF concentration than the other mixes in the high water treatment. In the low water treatment, all mixes had the same NDF concentration. The same response of the red clover was shown in mix as an individual factor. The reason why this occurred is unclear and also here further studies are needed to confirm and explain the phenomenon. The grass:legume ratio for the NDF content is higher in the clover dominated mix than in the grass dominated mix. Theoretically, either the grass increases its NDF content, or the legume decreases the same when occurring in a mix with mostly clover compared to a mix with mostly grass. When comparing this result to the effect of mix on the NDF content, it can be seen that the NDF content for the grass increases when it occurs as a minority. There is no clear relationship for the legume. The differences in the grass:legume ratio therefore have to be due to the changes in NDF content of the grass.

5. Summary and conclusions

5.1 Overall summary and conclusions

5.1.1 Shade

Shade affected biomass negatively for both the grass and the legume. The differences between the shade levels were larger in the second harvest than in the first harvest. The reason probably was because the plants started to grow in an untreated condition until they were well established in the first harvest, while they had to start regrowing in the shaded environment for the second harvest.

Shade only had an effect on the development stage of the red clover.

The effect on the protein concentration of the plants when exposed to shade differed with species and harvest. The higher protein concentrations in both crops in the 50% and 80% shade in the second harvest, indicates a higher forage quality when a plant is exposed to shade. The varying results in the first harvest indicate that the relationship might not be that clear.

The protein content of the plants followed a positive relationship to the biomass.

Red clover had a decreased NDF concentration in the second harvest when exposed to shade, while both crops had the reversed pattern in the first harvest.

5.1.2 Water

A higher water level gave, in general, a higher biomass. This trend was more pronounced in the treatments where the plants were exposed to more light.

The maturity of the red clover was delayed with increased water stress.

The response of the protein concentrations of the plants to different water levels varied with crop. The grass increased its protein concentration when exposed to water stress while the legume had the reversed response.

The actual protein content per plant was higher in the high water treatment than the low water treatment for both harvests and both crops. This response followed the dry weight of the plants.

The NDF levels were higher in the high water treatment than the low water treatment in the second harvest for both crops.

5.1.3 Mix

The mix in which the grass and the legume grew, affected the biomass of the plants. In the grass dominated mix, both the grass and the legume had a higher biomass per plant than in the other mixes.

The maturity of the plants was not affected by the mix of the species.

The protein concentration of a certain species increases when this species occurred as a majority in the pots. Why the plants responded this way could not be easily explained. The amount of protein per plant was higher in the grass dominated mix than the clover dominated mix. This was the same pattern as for the dryweight.

Perennial ryegrass had a higher fiber content when growing in the clover dominated mix than the grass dominated mix. This might have been an effect of the increased shade level caused by the red clover in the clover dominated mix. Red clover had a higher NDF content in the mix with equal amount of grass and clover than the other mixes. The reason why red clover responded this way could not be explained and requires further studies.

Generally it can be suspected that the perennial ryegrass was benefited by the high nitrogen fertilization, which might have affected the biomass and protein concentration of the grass. The results of the red clover might have been affected by the use of an equation for alfalfa when analyzed with NIRS.

5.2 Limitations of this research

One major restriction for this study was the limited amount of time available for the experiment. The perennial ryegrass and a large part of the red clover stayed in the vegetative stage throughout the study, which can be suspected to highly affect the outcome of the forage quality. Also the timing of the project, which was carried out from February through May, might have affected the results. Plants might respond differently to artificial light compared to natural sunlight, and the distribution of the light intensities was different than it would be during a natural growing season. The difficulties of keeping a consistent difference in water level for the 80% shade level highly affected the outcome of this environmental factor. The high nitrogen levels might have affected the ryegrass and the red clover differently, and probably the ryegrass has been benefited by the fertilization. The alfalfa equation used for the NIRS analysis of red clover is also a possible source of error.

The objectives of this study was to combine shade, water and species mixes into one study to see how these factors affect two selected forage species. When analyzing the results of this study, and applying them to real silvopastrial systems, one must keep in mind that there are several other factors influencing the ground vegetation as well. This study was performed in a green house environment, under beneficial nutrient availability and with no other competition than from the opposite forage species. In a natural silvopastrial system, there will be tree roots and weeds, possibly limited availability of nutrients, varying climatic conditions, possibly soil compaction and wear from grazing animals, stresses caused by insects and diseases, etc. All these factors will play a part in the performance of the ground vegetation. Consequently, only a few of the possible factors influencing a silvopastrial system have been included in this study.

5.3 Contribution of this research

When studying the influences of environmental and biological factors on forage species, most literature discuss one factor at a time. The objectives in this study were to look at three possible factors combined and investigate their effects on two forage species. I believe that this study can have contributed with a deeper understanding of the reactions of the forage species to a complex growth situation.

5.4 Future research

Several studies have been done on shade and water and their influence on forage species. Not as many have been done considering species in a mix, and certainly not on how species mixes are affected by light and water stresses. This study gave one set of results, but repeated studies should be done to confirm and give surety to the results received. Clarifications are also needed regarding specific parts of this study, for example regarding how the development stage of perennial ryegrass is influenced by shade, water and mix, and the influences of mix and shade on NDF concentrations.

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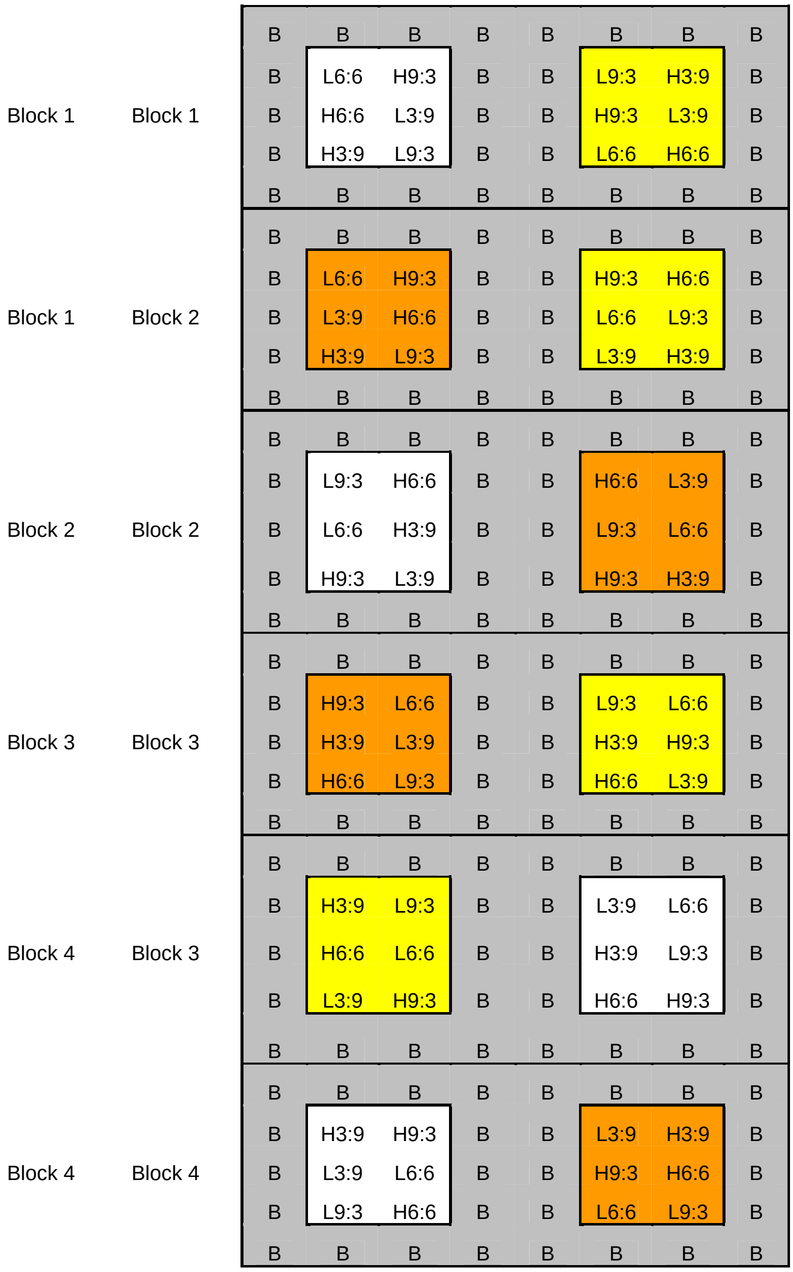
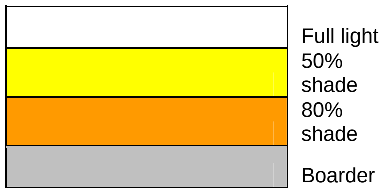
Appendix 1





R = replication

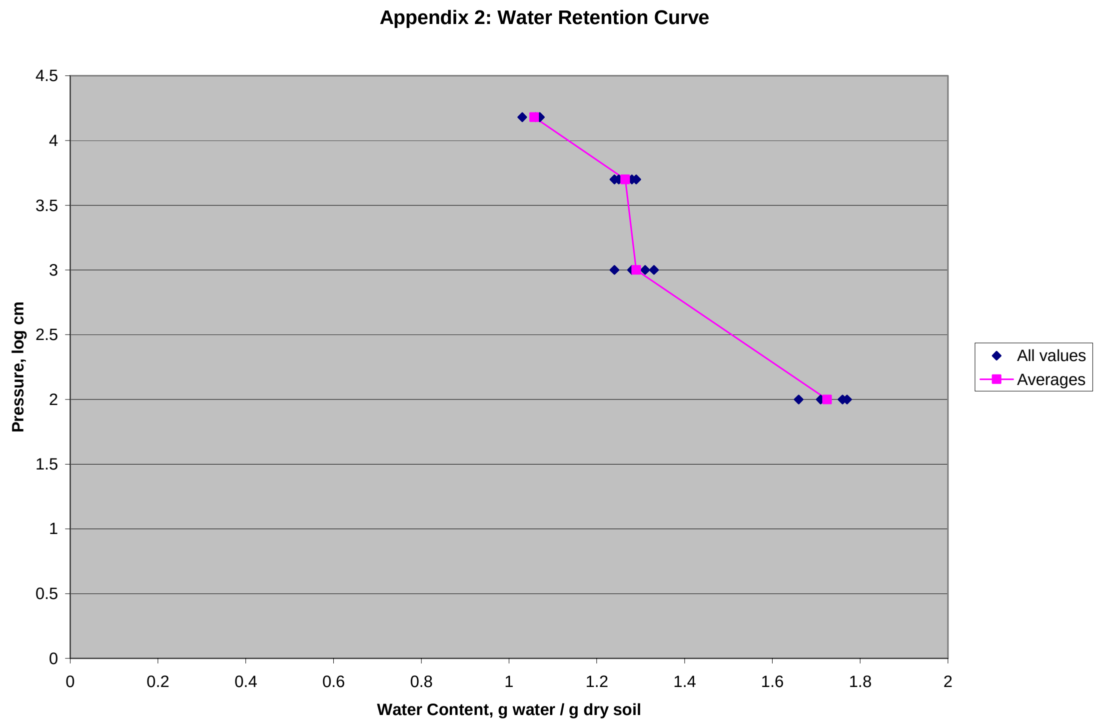
B = boarder pot

H = high water level

L = low water level

9:3 = 75% grass, 25% legume
6:6 = 50% grass, 50% legume
3:9 = 25% grass, 75% legume

Appendix 2



Appendix 2: Water Retention Curve
Pressure, log cm
Water Content, g water / g dry soil
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
All values
Averages

Appendix 3

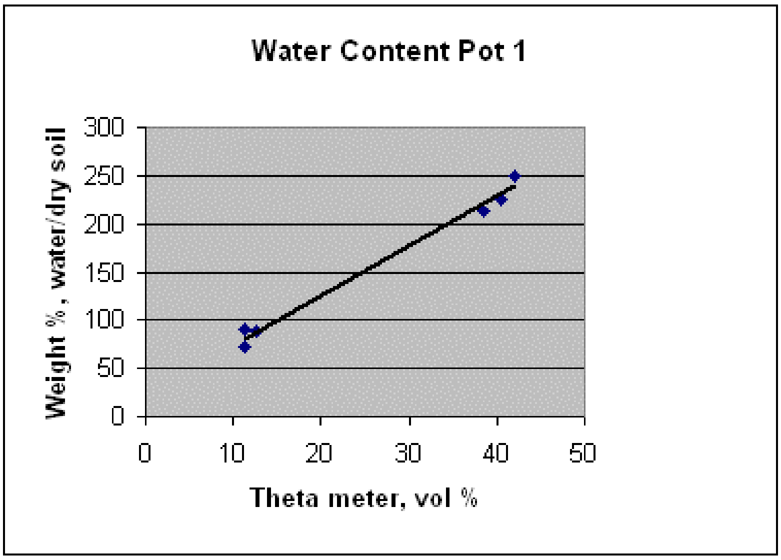


Figure 1. Water content in pot 1. Theta meter output versus actual water content

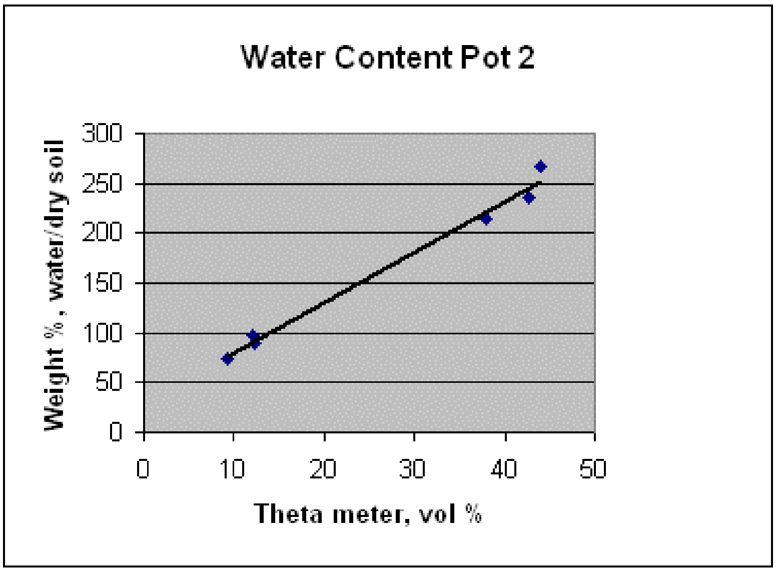


Figure 2. Water content in pot 2. Theta meter output versus actual water content

Appendix 4

Scales for determination of development stages for forage grasses and legumes

Code	Stage	Description
1	Leaf	Only leaves and extended leave sheets visible
2	Stem elongation	At least one internode visible on half of the shoots
3	Pre-booting	Part of panicle or head visible on some shoots
4	Early booting	At least half of the panicle or head is visible above the flag leaf on at least half of the shoots
5	Booting	When a part of the penduncle is visible below the panicle or head on at least half of the shoots
6	Anthesis	When the anthers are visible
7	Post anthesis	When the pollen distribution has ceased

Table 1. Description of scale used for scoring the maturity stages in forage grasses (SLU scale)

Code	Stage	Description
1	Leaf	Only leaves and petioles visible
2	Stem elongation	Most of the plants have visible internodes, i.e. at least 1 cm between the attachments of the petioles
3	Pre-bud	The shoot apex of the main stalk visible on at least some of the plants
4	Bud	Individual buds in the shoot apex visible on most of the plants
5	Pre-anthesis	Open flowers visible on the flower head of the main stalk on some plants
6	Anthesis	Open flowers visible on the flower heads of the side stalks on most plants
7	Post anthesis	The flowers on the main stalk are withered and the sepals are starting to darken on most plants

Table 2. Description of scale used for scoring the maturity stages in forage legumes (SLU scale). Own translation.