Long-term effects of cropping system on red clover proportion and crude protein concentration in mixed leys

Långtidseffekter av odlingssystem på rödklöverandel och råproteinkoncentration i blandvallar

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Abstract

Red clover is an important crop in Sweden, being the most cultivated forage legume in the country. It has a high crude protein concentration and fast establishment, which makes it a welcome addition to a ley mixture. However, red clover is not persistent and generally little remains in the third harvest year. This is caused by a combination of several different factors such as management practices and diseases. To determine how these factors affect red clover persistence, literature on the subject was analysed and correlated with the results of an analysis of data from the long-term field experiment R8-71. R8-71 includes six-year crop rotations with different proportions of timothy-red clover ley in each rotation.

Diseases and pests are the most common causes of red clover decline, and without their presence, red clover cultivars can persist for more than eight years. The analysis of R8-71 revealed typical signs of both clover rot and root rot. There was a sharp decrease in red clover proportion in the third harvest year, which is typical when plants are affected by root rot. Over time there was a decrease of clover proportion in the first harvest year in the crop rotation with the most frequent use of timothy-red clover ley, a typical sign of clover rot.

It is known that the clover proportion of a ley and crude protein of the forage increases between the first and second harvest, and this was confirmed in this thesis. Having a three-year or five-year ley did not influence the persistence of clover within a crop rotation cycle.

The clover proportion in the first and second harvest of the first production year declined with each crop rotation cycle. The crop rotation with a five-harvest year ley was the only rotation where the decline of clover proportion in the first production year was significant, but it was only significantly different from the change in clover proportion of the two-harvest year cropping system.

In conclusion, red clover and crude protein concentration in the produced forage increased between the first and second harvest. Red clover persistence was not significantly affected by the proportion of ley within a rotation, but the proportion of red clover decreased over crop rotation cycles when the gap between leys with clover was less than two years.

Keywords: Red clover, Trifolium pratense *L., persistence, ley age, Sweden, crop rotation, harvest*

Sammanfattning

Rödklöver spelar en stor roll för svenskt jordbruk då den är den mest odlade foderbaljväxten i landet. Den har en hög råproteinkoncentration och en snabb etablering vilket gör den till ett välkommet tillskott i vallfröblandningen. Rödklöver är dock inte uthållig i vallen och det är vanligt att en tredjeårsvall innehåller näst intill ingen rödklöver. Detta orsakas av en kombination av olika faktorer så som odlingsåtgärder och sjukdomar. För att utröna hur dessa faktorer påverkar rödklöverplantans uthållighet, har litteratur inom ämnet analyserats och korrelerats till resultatet av en analys av det långliggande fältexperimentet R8-71. R8-71 inkluderar sexåriga växtföljder med olika andel rödklöver-timotejvall i växtföljden.

Sjukdomar och skadegörare visade sig vara de vanligaste orsakerna till minskningen av rödklöver. När dessa inte är närvarande kan rödklövern uppnå en ålder av över åtta år. Analysen av data från R8-71 antyder att både klöverröta och rotröta påverkat rödklövern i försöket. Det var en skarp nedgång av klöverandel under vallens tredje skördeår, vilket kan hänföras till angrepp av rotröta då detta är typiska symptom. Över tid noterades en nedgång av klöverandelen redan i förstaårsvallen i växtföljden med störst andel timotej-rödklövervall, ett typiskt tecken på klöverröta.

Det är sedan tidigare känt att rödklöver- och råproteininnehåll i en vall stiger mellan första och andra skörd, vilket bekräftades i denna studie. Det fanns ingen skillnad mellan påverkan på klöverns uthållighet mellan en växtföljd med en treårig vall jämfört med fem inom den sexåriga växtföljden.

Klöverandelen i första och andra skörd det första produktionsåret minskade för varje växtföljdsomlopp. Växtföljden med en femårig produktionsvall var den enda där minskningen för det första produktionsåret var signifikant men minskningen var endast signifikant olik från förändringen av klöverinnehåll i växtföljden med en tvåårig produktionsvall.

Sammanfattningsvis ökade rödklöver och råproteinkoncentrationen i det producerade fodret mellan första och andra skörd. Rödklöverns uthållighet påverkades inte signifikant av andelen vall inom en växtföljdsrotation, men rödklöverhalten minskade över växtföljdscyklerna om avbrottet mellan vall med klöver var mindre än två år.

Nyckelord: Rödklöver, Trifolium pratense *L., uthållighet, vallålder, Sverige, växtföljd, skörd*

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Abbreviations

BCA	Biological control agent
CP	Crude protein
DM	Dry matter
DS	Developmental stage
Ley 1/H1	Harvest year 1
LS mean	Least Squares mean
RCP	Red clover proportion

1. Introduction

Grass-clover ley has been common in Swedish crop rotations for a long time. It made its debut in the middle of the 19th century when the previously meadow focused system changed to make way for the new cropping system, more focused on nutrient cycling using cereals and grass/legumes in rotation (Rösiö, 1904; Fogelfors, 2015). In the end of the 19th and beginning of the 20th century, farmers in northern Sweden started transitioning from using naturally-sown leys, to sowing grass and clover mixtures (Hellström, 1917). Seeds for naturally-sown leys were often collected from what had fallen down on the floor of lofts or haystacks (Hellström, 1917; Kårhe 1996).

The mixture of grasses and legumes in a ley has many uses, such as feed for animals, green manure, cover crop and reduction of certain weed populations. Grass and legumes have different growth rhythms, which causes the first harvest to have a large proportion of grass in comparison to legumes. However, in the regrowth, the proportion of legume biomass increases (Steen, 1970 see Nilsdotter-Linde, 2001). Legumes in the mixture contribute to increasing the yield and the digestible crude protein (CP) concentration by fixing their own nitrogen (Frankow-Lindberg, 1983; Fogelfors, 2015).

1.1. Red clover in rotational leys

Forage legumes incorporated into the crop rotation can improve sustainability by increasing the soil fertility, reducing cereal diseases and increasing soil porosity (Kahnt, 1999). Red clover (*Trifolium pratense* L.) is a legume that is frequently used in leys, it is second only to lucerne (*Medicago sativa* L.) as a forage legume world-wide and is the most commonly cultivated forage legume in Sweden (Boller *et al.*, 2010; Halling & Larsson, 2017). In northern Sweden it was preceded by alsike clover (*Trifolium hybridum* L.) that had a better fit for the climate conditions in the past, before new sufficiently winter hardy cultivars of red clover were produced (Hellström, 1917). In Europe it is frequently used in grass-clover mixtures during a 2-4 year-period (Boller *et al.*, 2010), but in Sweden 2-3 years for a clover-grass ley is standard practice (Fogelfors, 2015). Red clover is an important forage crop also further south, as it is the second most important legume used for fodder production in the UK, where it is a common source of nitrogen in organic silage swards (Laidlaw & Frame, 2013). In general, red clover's low persistence makes it a poor fit for the UK, as forage production is trending towards long-duration leys.

Red clover has many advantages in relation to other forage legumes. It is easy and fast to establish, making it a good choice for renewing already established leys (Casler & Undersander, 2019). Red clover can grow in many kinds of soils but prefers to grow in well drained clay soils (Hellström, 1917). Ideally, the soil should have a clay content of between 15 and 25% and be rich in potassium (Osvald, 1959). Red clover can withstand pH down to 5.5 though it prefers higher as there will be more nodulation with a higher pH (Mulder & Van Veen, 1960; Casler & Undersander, 2019). It is possible to produce favourable growing conditions even in more unfavourable soils with the help of modern interventions such as liming, drain pipes and the addition of fertiliser (Friberg, 1991).

A common grass-clover mixture used in the north of Sweden is red clover and timothy (*Phleum pratense* L.), due to their winter hardiness and similar heading dates (Fogelfors, 2015). The species available for sown leys in northern Sweden in the beginning were few. Easily acquired seeds were available for timothy and different species of clover (Hellström, 1917). A mixture of red clover and timothy has been used in a long-term field experiment located in Västernorrland, Jämtland and Västerbotten at the different sites Offer, Ås and Röbäcksdalen, respectively, since the 1960s up until 1986 when the experiment was reduced to one site, Offer (Hagsand *et al.*, 1961; Hagsand *et al.*, 1987). Since the beginning the experiment has included four different crop rotations representing six-year rotations of animal production focused farming, farming focused on producing cash crops and farming including both practices. The rotations include ley of different durations: a five-year ley, a three-year ley, a two-year ley, and one using ley as green manure (Hagsand *et al.*, 1961). The inclusion of the different frequencies and the duration of the experiment makes it suitable for studies on red clover persistence.

1.2. Aim and hypothesis

The purpose of this thesis was to determine how the botanical composition and forage quality of a timothy-red clover ley is affected by management and disease, with a focus on red clover persistence, through a literature study and experimental data. The experimental data will specifically be used to observe the effects of ley age on the percentage of red clover and quality of the ley at the different harvest times. The first hypothesis is that there is an increase in crude protein concentration and red clover proportion with each harvest within a season and a decrease between seasons. The second is that a larger share of ley in the crop rotation will cause a faster decrease of the red clover proportion and crude protein concentration within a crop rotation cycle as well as between crop rotation cycles.

2. Materials and methods

2.1. Literature study

Search engines used: Google, Google scholar, SLU library. Some articles and books were ordered in physical copies from the SLU library when they were unavailable online. Literature was also provided by the supervisor of this project as well as purchased from second-hand bookshops.
Search terms:

- Latin: *Trifolium pratense*, *Fusarium*, *Phoma medicaginis*, *Sclerotinia trifoliorum*, *Cylindrocarpon destructans*
- English: Clover rot, Root rot, Red clover, Persistence, Ley, Ley age, Frequency, Sward, Variety, Cultivar, Early, Middle, Late, Ploidy, Fertiliser effect, Host range, Crop rotation
- Swedish: Vall, Sverige, Rödklöver, Rotröta, Klöverröta, Baljväxter

The articles found were reviewed for relevance and references of interest were checked for availability and reviewed.

2.2. Offer, Ås and Röbäcksdalen field trials 1963-1986

Below is the description of the long-term field trial as it was designed in 1963-1986 as well as how the statistical analysis of the dataset was performed.

2.2.1. Field trial experimental design, management and samplings

The ley data that were used in this thesis originates from SLU's long-term field experiment R8-71 including the sites Offer, Ås and Röbäcksdalen between the years 1963 and 1986. The different sites started their first cycle at different times, the first cycle at Ås began in 1960, at Offer it began in 1961 and at Röbäcksdalen it began two years later, in 1963 (Hagsand *et al.,* 1961). In 1963, all sites carried the scheduled crops, which is why this year was chosen as the starting point of this thesis. The experiment consisted of two replicates of each crop rotation year (12 plots per crop rotation A, B, C and D), yielding 48 plots at each of the sites. Table 1 contains brief information on the different sites location and the soils physical and chemical properties.

Table 1. Brief overview of the three sites location and soil physical and chemical properties

Site	Clay content (%)	Soil texture	pH	Latitude	Longitude
Offer	20-25[1]	Silty clay loam[1]	6[2]	63.14°N[2]	17.75°E[2]
Ås	20[1]	Gravelly loam[1]	6.4[2]	63.25°N[2]	14.56°E[2]
Röbäcksdalen	10-15[1]	Clayey silt loam[1]	6[2]	63.81°N[2]	20.24°E[2]

[1]*(Ericson, 1992),* [2]*(Zhou* et al.*, 2019).*

The different crop rotations included in field experiment R8-71 represented different stages between an animal production farm and a pure crop production agriculture (Hagsand *et al.*, 1961). In Table 2 the different crop rotations are presented: rotation D represented pure crop production and therefore only used the under-sown ley as green manure. The ley was ploughed in early summer and then followed by a fallow. Crop rotation B and C included a mixture of oat and peas which was used as green fodder. All leys were harvested two times in a season, with the exception of rotation C, where the second harvest year was only harvested once before it was ploughed (Hagsand *et al.*, 1961). The ley in each crop rotation was sown at a seed rate of 15 kg/ha germinating seeds of timothy and 10 kg/ha germinating seeds of red clover.

Table 2. Six-year crop rotation at Ås, Röbäcksdalen and Offer in the years 1963-1986 for treatments A, B, C and D including timing for 1st and 2nd harvest for leys[3]

A	**Harvest (1st/2nd)**	**B**	**Harvest (1st/2nd)**	**C**	**Harvest (1st/2nd)**	**D**
Barley with under sown ley		Barley with under sown ley		Barley with under sown ley		Barley with under sown ley
Ley 1	Silage stage/First half of August	Ley 1	Silage stage/First half of August	Ley 1	Silage stage/First half of August	Fallow
Ley 2	Silage stage/First half of August	Ley 2	Hay stage/Second half of August	Ley 2	Hay stage/-	Winter rye
Ley 3	Hay stage/Second half of August	Ley 3	Hay stage/Second half of August	Winter rye		Peas
Ley 4	Hay stage/Second half of August	Oat + Peas		Potato		Potato
Ley 5	Hay stage/Second half of August	Rape-seed		Oat + Peas		Carrots

[3]*(Hagsand* et al.*, 1961).*

The first-year ley was harvested on the same date, regardless of crop rotation. The first harvest was taken in the silage harvest stage a few days before the heading of timothy (Hagsand *et al.*, 1961), which corresponds to developmental stage (DS) 45 (Gustavsson, 2011). The second harvest was performed in the first half of August (Hagsand *et al.*, 1961). After the first harvest year the harvest dates differ between crop rotations, with the first harvest the second year of rotation B and C being done in the hay harvest stage, and the second harvest being postponed until the second half of August, as shown in Table 2. In crop rotation A the first harvest is not performed in the hay harvest stage until year three. When the ley is harvested in the hay stage it corresponds to timothy DS 50-61, the period from the beginning of heading to when the spike starts to flower (Gustavsson, 2011). After a revision of the experiment in 1967, implemented during the start of each second cycle between 1967 and 1969, all the second harvests were performed simultaneously within the period 10th–25th of August (Hagsand *et al.*, 1967).

Close to the harvest, two samples á 50 × 50 cm were cut from each replicate and put together in a pooled sample (Hagsand *et al.*, 1961). The botanical analysis was analysed for both harvests and the samples were separated into different fractions. The first harvest was separated at species level even for the weed fraction, while the second harvest was divided into the three fractions; legumes, grass and herbal weeds, before they were dried and weighed (Hagsand *et al.,* 1967).

For the crude protein analysis, two samples weighing at least 3 kg in fresh weight from each replicate were collected, after which they were cut into smaller fractions and dried (Hagsand *et al.*, 1961). Before the samples were sent for conventional feed analysis, the samples from the two corresponding replicates at each site were mixed and ground. For this thesis only results from the crude protein analysis were used, and this analysis was performed using the Kjeldahl method (Nordic Committee on Food Analysis, 1976).

When this long term field trial was designed it was determined that the cultivars should be replaced if a superior cultivar was introduced on the market (Hagsand *et al.*, 1961). There have been several different cultivars of red clover used during the length of the experiment, as summarised in Table 3.

Disa and Bjursele are diploid red clover cultivars adapted to the climate of northern Sweden (Friberg, 1991). Bjursele has a similar yield to the diploid cultivar Reko (Andersson, 1983; Hagsand & Landström, 1984), and it has also shown to have a better tolerance for low pH than an average red clover species (Friberg, 1991). Both Bjursele and Reko are late flowering cultivars (Rufelt, 1979).

The diploid cultivar Björn was introduced into the field experiment between 1979 and 1980 (Ericson, 2021) Björn has resistance towards stem and bulb nematodes (*Ditylenchus dipsaci* (Kühn) Filipjev) (Svalöf Weibull AB, 1996), and has been documented to yield more than its predecessors (Andersson, 1983). In addition, it has in field trials proven to be well adapted to the climate of central northern Sweden (Friberg, 1991).

Table 3. Cultivars of red clover used 1963-1986, starting year in parenthesis[4]

Site	Cultivars				
Offer	Offer (1963)	Disa (1967)	Reko (1977)	Björn (1979)	
Ås	Offer (1963)	Disa (1966)	Reko (1977)	Björn (1979)	
Röbäcksdalen	Offer (1963)	Disa (1968)	Bjursele (1969)	Reko (1977)	Björn (1980)

[4]*(Ericson, 2021).*

During the crop rotation both potassium and phosphorus were applied in the amounts presented in Tables 4 and 5, not including the potassium and phosphorus added by fertilisation with manure. Table 4 includes changes in the potassium fertilisation plan after the experiment was revised in 1967 (Hagsand *et al.*, 1967). The application took place in the spring except for the application to winter rye which was applied before sowing in late summer (Hagsand *et al.*, 1961).

Table 4. The amount of potassium applied each full rotation in kg K/ha, numbers within parenthesis is potassium fertilisation as it was changed to in 1967-1969[3]

A		**B**		**C**		**D**	
Barley with under sown ley	60	Barley with under sown ley	80	Barley with under sown ley	120	Barley with under sown ley	60
Ley 1	-	Ley 1	-	Ley 1	-	Fallow	-
Ley 2	60	Ley 2	60	Ley 2	40	Winter rye	80
Ley 3	60	Ley 3	-	Winter rye	80	Peas	60 (80)[5]
Ley 4	-	Oat + Peas	40	Potato	100	Potato	200
Ley 5	40	Rapeseed	40	Oat + Peas	60	Carrots	160 (140)[5]
Total	220		220		400		560

[3]*(Hagsand* et al.*, 1961),* [5]*(Hagsand* et al.*, 1967).*

Table 5. The amount of phosphorus applied each full rotation in kg P/ha[3]

A		**B**		**C**		**D**	
Barley with under sown ley	35	Barley with under sown ley	35	Barley with under sown ley	35	Barley with under sown ley	25
Ley 1	-	Ley 1	-	Ley 1	-	Fallow	-
Ley 2	15	Ley 2	15	Ley 2	15	Winter rye	25
Ley 3	15	Ley 3	15	Winter rye	15	Peas	25
Ley 4	15	Oat + Peas	15	Potato	35	Potato	35
Ley 5	15	Rapeseed	20	Oat + Peas	15	Carrots	35
Total	95		100		115		145

[3]*(Hagsand* et al.*, 1961).*

Manure was applied in the autumn to benefit the following year's crop as shown in Table 6 (Hagsand *et al.*, 1961). The manure was either applied as solid or liquid manure, if it was liquid and had a dry matter content of around 10%, twice the specified amount was applied (Hagsand *et al.*, 1967). Each ton of manure was estimated to contain 1 kilogram of phosphorus and 4.5 kilograms of potassium.

Table 6. Amount of applied manure in ton/ha during a full crop rotation[3]

A		B		C		D	
Barley with under sown ley	30	Barley with under sown ley	-	Barley with under sown ley	-	Barley with under sown ley	-
Ley 1	-	Ley 1	-	Ley 1	-	Fallow	-
Ley 2	-	Ley 2	-	Ley 2	-	Winter rye	-
Ley 3	-	Ley 3	30	Winter rye	-	Peas	-
Ley 4	30	Oat + Peas	-	Potato	40	Potato	-
Ley 5	-	Rapeseed	30	Oat + Peas	-	Carrots	-
Total	60		60		40		-

[3]*(Hagsand* et al.*, 1961).*

Table 7 describes the N fertilising strategy for the whole crop rotation during the experiments first cycle. The amount of nitrogen fertiliser used during the first- and second-year ley for crop rotations A, B and C was adapted to the clover proportion. For the leys, all the mineral fertiliser was applied in the spring, and none was applied after the first or second harvest (Hagsand *et al.*, 1961).

Table 7. The amount of N fertiliser in kg N/ha was determined and applied using the following scheme, the application in leys was adjusted to red clover proportion (RCP) the first and second harvest year[3]

A			**B**			**C**			**D**	
Crop	RCP	N	Crop	RCP	N	Crop	RCP	N	Crop	N
Barley with under sown ley		-	Barley with under sown ley		15	Barley with under sown ley		15	Barley with under sown ley	30
Ley 1	High	-	Ley 1	High	-	Ley 1	High	-	Fallow	-
	Low	30		Low	30		Low	30		
	None	60		None	60		None	60		
Ley 2	High	30	Ley 2	High	30	Ley 2	High	30	Winter rye	15
	Low	60		Low	60		Low	60		
Ley 3		60	Ley 3		45	Winter rye		15	Peas	15
Ley 4		45	Oat & Peas		15	Potato		60	Potato	100
Ley 5		60	Rape-seed		30 + 45	Oat & Peas		15	Carrots	40 + 30
Total	High	195		High	180		High	135		230
	Low	255		Low	240		Low	195		
	None	285		None	270		None	225		

[3]*(Hagsand* et al.*, 1961).*

The nitrogen fertiliser plan was revised in 1967 as shown in Table 8 and was implemented during the experiments second cycle at all sites (Hagsand *et al.*, 1967).

With this new plan if the clover content was below 50% in the first two ley harvest years, the ley was fertilised with 90 kg of N per hectare split into one spring application and one application shortly after the first harvest. The replicates were always fertilised according to the replicate with the smaller proportion of clover. The red clover proportion of the leys was assessed both in spring and autumn.

Table 8. The amount of N fertiliser in kg N/ha as revised in 1967 was determined and applied using the following scheme, the application in leys was adjusted to red clover proportion (RCP) the first and second harvest year[5]

A			**B**			**C**			**D**		
Crop	RCP	N	Crop	RCP	N	Crop	RCP	N	Crop	RCP	N
Barley with under sown ley		10	Barley with under sown ley		15	Barley with under sown ley		15	Barley with under sown ley		30
Ley 1	>50%	30	Ley 1	>50%	30	Ley 1	>50%	30	Ley 1	>50%	30
	<50%	60 + 30		<50%	60 + 30		<50%	60 + 30	Fallow	<50%	60
Ley 2	>50%	30	Ley 2	>50%	30	Ley 2	>50%	30	Winter rye		15
	<50%	60 + 30		<50%	60 + 30		<50%	60 + 30			
Ley 3		60 + 30	Ley 3		60 + 30	Winter rye		15	Peas		15
Ley 4		60 + 30	Oat & Peas		15	Potato		75	Potato		100
Ley 5		60 + 30	Rape-seed		60 + 60	Oat & Peas		15	Carrots or Swede		45 + 30
Total	>50%	340		>50%	300		>50%	180			265
	<50%	460		<50%	420		<50%	300			

[5]*(Hagsand* et al.*, 1967).*

In 1971 in a separate project, an inspection and soil sampling were performed on long-term field trials at different sites, among them was Offer, Ås and Röbäcksdalen (Olvång & Björkman, 1972). Stem and bulb nematodes were found at the sites Offer and Ås, but not Röbäcksdalen.

2.2.2. Statistical analysis of R8-71 dataset

For the statistical analysis, the two statistical software's JMP® (SAS Institute Inc., 2021) and SAS 9.4® (SAS Institute Inc., 2013) were used. The models produced were Mixed-effects models where year was set as a random variable and the remaining as fixed.

The ley dataset contained several zeros as the ley aged, and in time, the clover percentage reached zero. To solve this problem, if the clover percentage was zero the set variable a (clover proportion) was set as 0.005 and harvest years 4 and 5 were not analysed since the red clover percentage at this point was at or close to zero. Crop rotation D was not included in this analysis since the ley was only used as green manure and was never harvested. The analyses of crude protein concentration and botanical composition were made treatment-wise, which meant that the replicates within each site could not be used as a factor in the statistical analyses.

Variables:

$x = year - 63$ Year 0 (x = 0) is set as 1963.

$a = red\ clover\ proportion$ If red clover proportion is 0, then a is 0.005.

The variable a facilitated the transformation of the clover percentage values in the dataset and improved variance distribution when necessary, by converting the function so that it becomes linear.

Since the ley proportion in the crop rotations are dissimilar, two models with different purposes were used when comparing differences between the treatments. The different levels of significance were classed as follows $*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$.

- Model 1: Harvest year 1 (H1) was compared between the three treatments A, B and C. This made it possible to analyse the difference between harvest 1 and 2 and the long-term effect of the treatments on red clover proportion and crude protein concentration in H1 using the continuous time variable x.
- Model 2: Harvest year 1 (H1), harvest year 2 (H2) and harvest year 3 (H3) were used to compare treatments A and B. This meant that it was possible to determine if there was a significant decrease in red clover proportion and crude protein concentration as the ley aged and if there was any significance behind the choice of treatment. C was not included since it did not have a third harvest year.

Model 1

A separate dataset was created containing only harvest year 1 (H1) with treatments A, B and C and the different sites. Both crude protein concentration and red clover percentage were analysed in JMP using the models in Table 9 and 10.

Table 9. Mixed-effects model used in JMP, all effects including year are random

Fixed effects	Random effects
Site	Year
Treatment	Site*Year
Cut	Year*Treatment
Site*Treatment	Site*Year*Treatment
Site*Cut	Year*Cut
Treatment*Cut	Site*Year*Cut
Site*Treatment*Cut	Year*Treatment*Cut

The model analysing trend over time included both the continuous time variable x as a fixed-effect variable and the factor year as a random-effects factor (Table 10). This produced a trend displaying the long-term change over time in clover proportion and crude protein concentration while allowing yearly random deviations from that trend.

Table 10. Mixed-effects model used in JMP, adding the time variable x, the age of the experiment starting from 1963, to determine if there is a significance in trend over time. All effects including year were random

Fixed effects	Random effects
x	Year
Site*x	Site*Year
Treatment*x	Year*Treatment
Cut*x	Site*Year*Treatment
Site*Treatment*x	Year*Cut
Site*Cut*x	Site*Year*Cut
Treatment*Cut*x	Year*Treatment*Cut
Site*Treatment*Cut*x	
Site	
Treatment	
Cut	
Site*Treatment	
Site*Cut	
Treatment*Cut	
Site*Treatment*Cut	

When analysing red clover percentage, the variable was transformed using logit to improve the variance distribution.

$$logit = \log(\frac{a}{1-a})$$

Model 2

As in Model 1, a separate dataset was created but instead included H1, H2 and H3 for treatments A and B. Crude protein and red clover percentage were analysed using the models in Table 11.

Table 11. Mixed-effects model used in JMP, all effects including year are random

Fixed effects	**Random effects**
Site	Year
Treatment	Site*Year
Harvest year	Year*Treatment
Cut	Year*Harvest year
Site*Treatment	Year*Cut
Site*Harvest year	Site*Year*Treatment
Site*Cut	Site*Year*Harvest year
Treatment*Harvest year	Site*Year*Cut
Treatment*Cut	Year*Treatment*Harvest year
Harvest year*Cut	Year*Treatment*Cut
Site*Treatment*Harvest year	Year*Harvest year*Cut
Site*Treatment*Cut	Site*Year*Treatment*Harvest year
Site*Harvest year*Cut	Site*Year*Treatment*Cut
Treatment*Harvest year*Cut	Site*Year*Harvest year*Cut
Site*Treatment*Harvest year*Cut	Year*Treatment*Harvest year*Cut

Similar as in Model 1, the clover percentage dependant variable *a* was transformed using logit.

3. Results

Red clover persistence is connected to many different factors. Included below are the results of the literature study, determining possible causes of red clover's low persistence and methods to increase it, together with the results from long-term experiments at Ås, Offer and Röbäcksdalen.

3.1. Literature study

Red clover is a commonly used legume in Swedish leys with its fast establishment but slower summer growth (Fogelfors, 2015). Its persistence is much more limited than that of some other commonly used legumes like white clover (*Trifolium repens* L.), that also has a faster summer growth. There are many known reasons for the low persistence of red clover. Because red clover plants do not spread vegetatively, the same taproot must deal with diseases, damage to the growing point by harvesting and/or grazing and cold winter conditions year after year. This often leads to a third-year ley consisting to a small extent of red clover and a decline in the ley crude protein concentration if there is no addition of extra N fertiliser (Frankow-Lindberg, 1983; Nilsdotter-Linde *et al.*, 2002).

3.1.1. The effect of ley management on red clover persistence

When choosing management for a ley, there are a lot of components to consider; cultivar choice, fertiliser application strategy, stubble height, grazing or no grazing, the number of harvests and harvest dates. All these components in turn, depend on where the ley is located.

There are many different cultivars of red clover that mature at varying rates, causing their heading times and regrowth to be different from one another, the options are early, intermediate and late flowering (Halling & Larsson, 2017). Late flowering cultivars are considered to be the most persistent compared to early and intermediate (Black *et al.*, 2009; Halling & Larsson, 2017). Early flowering cultivars are the most commonly cultivated red clovers in the US (Frame *et al.*, 1998). Early cultivars of red clover have been determined to have the least persistence in both the US and Sweden (Taylor *et al.*, 1990; Frame *et al.*, 1998; Halling & Larsson, 2017). In exchange for better persistence they instead produce most of their yield early in the season and have the best regrowth of the three options (Frame *et al.*, 1998; Halling & Larsson, 2017). The differences between early and late cultivars have become less distinct over time as new cultivars have been bred and introduced on the market (Frame *et al.*, 1998).

A study, performed in the Netherlands that compared early maturing Swiss landraces (also called Mattenklee) with early and intermediate traditional European cultivars, stated that the persistence of the Swiss landraces was greater than that of the traditional cultivars (Hoekstra *et al.*, 2018). The Swiss landraces have shown similar potential in Sweden, where in a master's thesis study, a five year old ley of Mattenklee cultivars exhibited the same disease index as a three year old red clover ley including the Swedish intermediate cultivar SW Ares (Älmefur, 2020). Mattenklee was bred to withstand a greater number of harvests and have better persistence than other red clover cultivars (Boller, 2000). It has been shown to outperform other cultivars in a second-year ley and it is regularly grown together with high competition grasses, such as Italian ryegrass (Boller, 2000).

Beyond the early, intermediate and late cultivars, there is the choice between diploid or tetraploid red clover. The tetraploid cultivars produce more dry matter (DM) yield on average (Sheldrick *et al.*, 1986; Fogelfors, 2015; Amdahl *et al.*, 2016; Hoekstra *et al.*, 2018), and are more persistent than their diploid counterpart due to a greater resistance to diseases and pests, like clover rot and stem nematodes (Åkerberg *et al.*, 1963 see Yamada & Hasegawa, 1990; Frame *et al.*, 1998; Vleugels *et al.*, 2013). However, tetraploid red clover is not always guaranteed to be superior to the diploid cultivars as specific breeding efforts have resulted in the introduction of new and improved cultivars (Frame, 1976 see Frame *et al.*, 1998).

Diploid red clover has been improved through the years in several aspects such as stress resistance, persistence and seed production (Frame *et al.*, 1998). An experiment performed at the research station Hurley in England concluded that while the tetraploid cultivars in most cases yielded more than the diploid, there was no significant difference in plant density when the experiment was finished (Sheldrick *et al.*, 1986). The experience of tetraploid red clover having superior persistence differs between different locations (Taylor & Quesenberry, 1996), in Europe and Japan better persistence of tetraploids has been recorded (Yamada & Hasegawa, 1990; Tomaszewski, 1989 see Taylor & Quesenberry, 1996), while in the US the diploid cultivars have performed better (Taylor & Wiseman, 1985 see Taylor & Quesenberry, 1996). A Norwegian study summarising five different experimental series, stated that the comparable success of tetraploid clover in relation to diploid was dependent on the location (Marum, 2006). The tetraploid cultivar performed the best in more northern areas in relation to diploid cultivars in the same area, the differences sometimes being up to 100%. Similarly, a new tetraploid cultivar that was introduced in Japan in the 1990s named Taisetsu demonstrated a larger yield and better persistence than its diploid parent cultivar Sapporo, particularly in snowy areas (Yamada & Hasegawa, 1990). Sapporo was used in crosses with other cultivars a few years earlier in a field trial that compared non-flowering (cultivars that do not produce flowers in the year of seeding) to flowering cultivars (Sawai *et al.*, 1986). This trial indicated that non-flowering cultivars are more persistent due to producing more fibrous roots from the crown at the base of the stem during the seedling stage.

Fertilisation strategy is an important part of providing red clover in a ley, no matter the cultivar, with the prerequisites to persist over the growing seasons. Grass-clover leys have a lower response to increased nitrogen (N) fertiliser than pure grass leys since grass is more responsive to fertilisation than clover and larger amounts of fertiliser risks the clover being outcompeted by the grass (Frankow-Lindberg, 2017). In Northern Ireland a N fertiliser application trial was performed on six-year-old grass and red clover leys where the N application in the seventh-year ley did not result in a decreasing effect on the clover content (McBratney, 1987). However, in the eight-ley year, there was a significant decrease in the clover yield for the fertilised ley on average, which was attributed to the fertilisation. There was no decrease in clover content within the treatments due to disease but rather to the application of nitrogen (McBratney, 1987).

An increase of N fertilisation can cause a decrease in the concentration of CP where at the same time there will be an increase in metabolisable energy in the sward due to an increase of the grass proportion (Frankow-Lindberg, 1983). The decrease in red clover content with increased fertilisation was demonstrated in a field experiment in southern and central Sweden where two different N fertiliser rates were used, 0 and 100 kg N/ha (Nilsdotter-Linde *et al.*, 2002). The treatment receiving 100 kg of nitrogen had a lower CP concentration, mainly due to the decreased clover content. In 1980 to 1986 in northern Sweden, 29 field trials were established to analyse the effect of different nitrogen fertilisation levels in a grass-clover ley in comparison to a pure grass ley over three harvest years (Gustavsson, 1989). It was determined that the grass-clover ley needed 100 kg/ha less nitrogen fertiliser per year to reach the same amount of DM as the grass ley and even less to reach the same crude protein concentration. At the lower fertilisation levels, in the first harvest of each season, the clover proportion increased as the ley aged and for the higher levels it decreased. A field trial performed in the climate of northern Sweden with a crop rotation of 12 years, determined the effect of ley age and fertiliser application on the leys botanical composition (Hagsand & Landström, 1984). The results confirmed that the red clover persisted within 20-40 percent in the three first harvest years if N fertiliser application was low and that it decreased with an increase in N fertiliser application and ley age. In addition, it established the fertiliser effect on crude protein concentration where a pure grass ley proved to respond with a faster increase as the fertiliser N application increased than the grass-clover ley. Red clover contains a higher concentration of crude protein than grass (Frankow-Lindberg, 1983), and a mixed ley will yield more crude protein with less fertiliser applied than a pure grass ley (Hagsand & Landström, 1984).

Depending on the purpose of the ley and its percentage of red clover, the recommended fertilisation strategy will look different. A general concept is, the higher the clover percentage, the lower the need for fertiliser (Frankow-Lindberg, 2017). However, fertiliser application with the majority being applied in the spring is said to be beneficial for red clover persistence (Frankow-Lindberg, 2017). A single application of 50-75 kg/ha is suggested, as there is evidence that it is stimulating for red clover development (Crowley, 1975 see Frame *et al.*, 1998), this is similar to the Swedish recommendations for an economically optimal fertiliser strategy for a ley with less than or equal to 20% clover (Frankow-Lindberg, 2017). If the clover proportion is higher, it is not considered economically justifiable to apply N before the first harvest. Above 30% clover it is not recommended to fertilise, unless there is a need to increase maximum DM yield (Frankow-Lindberg, 2017). With a harsher climate in the north of Sweden compared to the south it can be advantageous to withhold fertilising the first year to avoid the red clover plants being overshadowed before the first winter (Friberg, 1991).

Like cultivar choice and fertiliser amount, the number of harvests taken within a season and the interval in between each harvest, can influence red clover persistence. Red clover needs time to recover before winter after the last harvest, a time period of 45 days before temperatures reach below freezing has been recommended (Lang, 1985 see Taylor & Quesenberry, 1996). On average, two harvests per season in Sweden is common practice when harvesting a red clover ley (Halling *et al.*, 2004). However, it has become more and more common to take three harvests in a season even in northern parts of Sweden. At Luttugården in Norrbotten, in a four-year crop rotation used for organic animal production, three harvests were taken each season (Wirsén, 2019). The ley was kept for three harvest years before it was resown. The farm had a decline of red clover proportion in the third year, but to make up for the decrease the ley mixture sometimes contained white clover.

Field trials conducted in central and southern Sweden that compared a two and three harvest system over the duration of three consecutive years, stated that the DM production decreased faster between seasons in the three-harvest system (Nilsdotter-Linde *et al.*, 2002). However, the digestibility of the forage was higher with three harvests compared to two. A similar experiment was performed in Kvinnersta and Rådde in 2005-2007 using two or three harvests (Wallenhammar *et al.*, 2014). A decrease in red clover proportion between harvest year two and three was found for both harvest systems, and there was no significant difference between them. At Röbäcksdalen, Umeå, a study on ley yield and quality was established in 2006 with two harvest frequencies, two or three harvests per season (Nilsson *et al.*, 2011). The experiment was observed for three harvest years and the results suggested that there was a decrease in ley persistence in the three-harvest system. In Quebec, Canada, a three-year field trial with the same two harvest frequencies, resulted in no significant difference in red clover persistence between the two systems over two production years (Coulman & Kielly, 1988). Though it was found that the two-harvest system yielded more DM in the first harvest year.

In the period between 1978 and 1982, field experiments were conducted at 37 locations distributed across Sweden with the purpose to determine the effect of harvest frequency and date of harvest on botanical composition (Tuvesson, 1986). Two or three harvests were applied, but at the end of the trial, no significant difference could be seen on clover content in the second-year ley between the two treatments. Treatments with three, up to six harvests were tested in England and the assessment concluded that a harvest frequency of more than three harvests in a season was unjustified as the amount of yield decreased greatly and the quality increase was small (Sheldrick *et al.*, 1986).

The climate conditions and length of the growing season in some of the studies mentioned differ from the ones present in northern Sweden. Recommendations available for harvest frequencies in northern Sweden end up being approximately two harvests per season to give the individual red clover plants enough time to recover before winter, due to the short growing season (Friberg, 1991).

As for the best time to harvest, a study performed in Wisconsin, Madison, implementing different harvest dates and frequencies, concluded that the best time to harvest the ley is when the red clover is at 20% bloom as it has less of an effect on its persistence than a harvest at 40% bloom (Wiersma *et al.*, 1998).

In relation to harvest date and frequency, there is evidence that suggests that stubble height when harvesting plays a role in red clover persistence and crude protein concentration. A study performed at sites around Uppsala between the years 1972 and 1974, observed the different effects between the stubble heights 4, 8 and 12 cm, respectively (Fagerberg, 1979). The 4 cm stubble yielded the largest harvest in total, seen over the two harvest years, but the largest harvest in the second-year ley corresponded with the 12 cm stubble in the first-year harvest. Unlike yield, the crude protein concentration continuously increased with an increase in stubble height (Fagerberg, 1979). A similar study was made in Flahult at an experimental farm using different harvest dates and stubble heights. The long stubble of 8-10 cm proved to be beneficial for percent clover the following seasons first harvest compared to the short stubble of 3-4 cm, especially if the harvest was performed during an unfavourable harvest date in September (Lustig, 1965). A second trial at the same location using 3-4 cm short stubble and 6-8 cm long stubble did not yield an equally distinct effect but still showed the benefit of a longer stubble in the month of September.

3.1.2. Red clover diseases

Root rot (a complex of many different fungi and bacteria such as *Fusarium* spp., *Phoma* spp. and *Cylindrocarpon destructans* (Zinss) Scholten) and clover rot (caused by the ascomycete *Sclerotinia trifoliorum* Erikss.) are two common diseases plaguing red clover, causing premature reduction in the number of individual plants.

Root rot

In Finland during the 1960s in a country wide investigation, it was determined that root rot was a more common occurrence and cause of decline in yield of red clover, than clover rot (Ylimäki, 1967). The first survey in Sweden with the purpose to determine the incidence of root rot in the country was performed in the 1970s and yielded similar results as the survey in Finland (Rufelt, 1986a). Root rot proved to be vastly prevalent and affected plants already in the seeding year. The disease causes a discoloration in the taproot as shown in Figure 1 and usually originates in old stubble or damaged sections of the root and continues to spread through the root tissue (Rufelt, 1994).

Figure 1. Root rot symptoms in a red clover taproot. Photo: Sanna Bergqvist

A greenhouse study performed in Canada investigated the host range of several *Fusarium* species extracted from pea cultivars (Safarieskandari *et al.*, 2021). Among the species were *Fusarium avenaceum* (Fr.) Sacc. and *F. oxysporum* Schlect. that are known to cause root rot in red clover (Taylor & Quesenberry, 1996), and the subspecies *F. solani* f. sp. *pisi* (Fsp). The study indicated that both *F. avenaceum* and *F. solani* f. sp. *pisi*. were, in addition to peas, pathogenic on cultivars of faba bean (*Vicia faba* L.), dry bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) but it did not document any infection or significant difference in emergence for wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) or rye (*Secale cereale* L.) (Safarieskandari *et al.*, 2021). *F. avenaceum* was a few years before isolated from spring/winter wheat and barley originating from Finland and Canada (Lysøe *et al.*, 2014). It is one of the most generalist *Fusarium* species where isolates have shown potential to infect cultivars distantly related to the host it was isolated from (Nalim *et al.*, 2009). Two strains of *F. avenaceum* isolated in Sweden and tested in a pot experiment produced severe symptoms on pea and bean as well as on lucerne, white and red clover (Lager & Gerhardson, 2002). *F. avenaceum* and *F. acuminatum* Ell. & Ev. were two of the most commonly isolated *Fusarium* species in Sweden in the previously mentioned 1970s survey together with findings of *Phoma medicaginis* var. *pinodella* Malbr. & Roum. and *Cylindrocarpon destructans* (Rufelt, 1986a). In Finland similar findings were made by Ylimäki (1967) where *Cylindrocarpon destructans*, *F. acuminatum* and *F. avenaceum* were isolated but there were no mentions of *P. medicaginis* var. *pinodella* outside of an infection experiment.

Phoma medicaginis var. *pinodella*, a fungus which is pathogenic on red clover and pea (French, 2004; Watson *et al.*, 2017), was found to be frequent in the south of Sweden (Rufelt, 1986a). Tunisian isolates of the same species have in a growth chamber experiment proven pathogenic on dry bean, lucerne, barrel medic (*Medicago truncatula* Gaertn.), pea as well as showing mild symptoms on chickpea (Djebali, 2013). In Sweden, *P. medicaginis* var. *pinodella* obtained from red clover showed pathogenicity on pea and lucerne but the symptoms were less severe than the ones caused by *Fusarium* (Lager & Gerhardson, 2002).

A study monitoring the different pathogens of root rot deduced that *C. destructans* was the cause behind the most severe symptoms (Almquist, 2016). In 2015 an organic forage experiment was set up in Sweden where different species of forage legumes were evaluated in regards to their root rot resistance (Wallenhammar *et al.*, 2021). The forage legumes, red clover, birdsfoot trefoil (*Lotus corniculatus* L.), lucerne and white clover all housed the *C. destructans* pathogen in their roots.

Root rot is in general a slow plant disease (Ylimäki, 1967; Rufelt, 1979), and its effects are noticed to the greatest extent in the second harvest year onwards, the number of affected plants increase each year starting as early as the year of seeding (Ylimäki, 1967; Rufelt, 1986a). The disease progresses quickly during summer with a slower development during winter (Rufelt, 1986a). Two causes of increased severity of root rot have been observed in Sweden. The first being red clover age, where an older red clover plant had a greater disease index (Rufelt, 1979; Stoltz & Wallenhammar, 2010), the second was a positive correlation found between decreasing root nutrients and increased severity (Stoltz & Wallenhammar, 2010). Plant losses because of root rot is most common in spring when the plants are weaker, due to sparse access to stored nutrients or injuries sustained during winter (Ylimäki, 1967; Öhberg, 2008). If the disease-causing organism is already present in the soil and infect the plant in the early stages before emergence it can cause pre-emergence killing or damping off, greatly reducing the stand of red clover (Ylimäki, 1967; Kilpatrick *et.al.*, 1954 see Rufelt, 1986a). Root rot is in a way an opportunistic disease, it benefits from all elements that can weaken the red clover plant such as harvest frequency, date of last harvest, presence of other pathogens etc. (Rufelt, 1979). Root rot infection itself does not always kill the plant, but weakens it, making it more likely to succumb to additional stresses (Rufelt, 1979).

Clover rot

Clover rot is not the most important disease on red clover in Sweden, but it is the most serious and it is present throughout the whole country (Öhberg, 2004; Öhberg, 2008). Similarly to root rot, clover rot can cause a large reduction of the red clover population in a ley which leads to a decline in yield and ley crude protein concentration (Ylimäki, 1967; Scott, 1984). Clover rot is largely dependent on favourable temperature and moisture (Loveless, 1951), and it is the most serious in northern regions that experience winters with thick snow cover (Ylimäki, 1967). *Sclerotinia trifoliorum* produces resting structures in the form of sclerotia as shown in Figure 2. These sclerotia can survive in the soil for an extended amount of time, causing disease in host plants more than ten years after the last susceptible crop was grown at the location (Dillon Weston *et.al.*, 1946 see Öhberg, 2008).

The sclerotia of clover rot normally lie dormant during summer (Serikstad *et al.*, 2013), with infection developing during late autumn or early spring, causing the largest decline in red clover population the following winter and spring (Hanson & Kreitlow, 1953; Öhberg, 2008). Newly established plants are the most sensitive towards clover rot and the greatest loss takes place in spring after the first winter (Öhberg, 2004). If the conditions are not favourable for infection, the fungus can be latent in the plant until the conditions are suitable (Serikstad *et al.*, 2013). It has been indicated quite recently that clover rot damage was only present in the northern and eastern parts of Finland even though it was common in the whole country a few decades earlier (Yli-Mattila *et al.*, 2009).

Figure 2. Sclerotia from Clover rot. Photo: Sanna Bergqvist

Grazing or frequent harvesting, risks weakening the red clover plants that have low resistance against grazing and trampling, leaving them vulnerable for severe disease or poor overwintering (Yli-Mattila *et al.*, 2009). However, autumn grazing or harvesting is suggested as a way to mitigate clover rot by removing ascospores in places like eastern England (Loveless, 1951; Hanson & Kreitlow, 1953; Scott, 1984), as long it is performed with care (Loveless, 1951; Scott, 1984). If the harvest or grazing is performed at a late stage there is risk of jeopardising the plant's winter hardiness which in itself can cause the plant to be less tolerant (Öhberg, 2004). White clover was for a time recommended as an option in England instead of red clover in regions where clover rot was prevalent (Scott, 1984). This continued until white clover started to experience severe infections of clover rot and the practice was abandoned. A survey performed in Denmark on white clover suggested that there could be a correlation between the presence of nematodes and the infection of clover rot (Hansen & Søegaard, 2009).

S. trifolium is known to primarily infect forage legumes that includes around 100 species that have been identified as hosts for the fungus (Saharan & Mehta, 2008). Swedish investigations of the fungus *S. trifolium* host range included nine different forage species and all of them proved susceptible to clover rot (Öhberg, 2008). The nine species assessed were white clover, red clover, alsike clover, lucerne, both birdsfoot trefoil and big birdsfoot trefoil (*Lotus pedunculatus* Cav.), sainfoin (*Onobrychis viciifolia* Scop.), yellow sweetclover (*Melilotus officinalis* (L.) Lam.) and goat's-rue (*Galega orientalis* Lam.).

Prerequisites for disease

An outbreak of disease is dependent on the right conditions being present, but different diseases have different prerequisites (Figure 3). Root rot is considered a more detrimental disease on red clover since it is more consistently a problem year after year when the soil is infected, unlike clover rot that needs specific abiotic factors to reach its full potential (Ylimäki, 1967). Root rot has previously often been misidentified as clover rot on damaged plants in the spring, when they are difficult to visually differentiate from each other (Ylimäki, 1967).

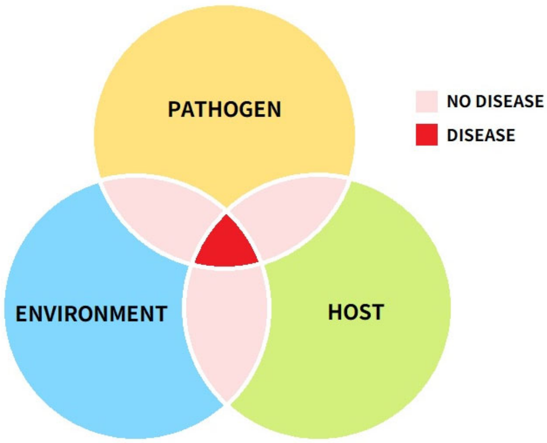

Figure 3. Disease triangle, prerequisites for an epidemic.

Foliar necrosis caused by clover rot develops the fastest at 15-18 °C (Raynal, 1989 see Frame *et al.*, 1998), but *S. trifolium* can grow and cause infection in the range of temperatures from below zero up to around 24 °C (Hanson & Kreitlow, 1953). Moisture is essential for the fungus to grow and for its sclerotia to survive for a long time (Hanson & Kreitlow, 1953), while dry conditions or flooding can cause a decrease in clover rot infection and severity (Dillon Weston *et.al.*, 1946 see Öhberg, 2008). Root rot is not as dependent on a specific climate to be problematic, but it still affects the rate of development. Root rot progresses faster in southern Sweden compared to northern Sweden, as in the south it can continue to develop and spread even during winter due to the milder climate (Rufelt, 1979; Rufelt, 1986a).

Methods of control

Root rot is to a large extent a result of different management practices. Harvest frequency plays a large role in root rot infection where an increase in number of harvests per season contributes to an increase in disease index together with a late last harvest, not giving the plant time to recover before winter (Rufelt, 1979). A greenhouse experiment performed with the purpose to determine the frequency of possible harvests before the root rot index is affected, concluded that two harvests per season were appropriate for leys in Sweden (Rufelt, 1986b). Root rot is not a disease caused by poor crop rotation since the fungi responsible are polyphagous, providing the organism with the ability to persist until the next time red clover is grown (Rufelt, 1979). In spite of that, crop rotation has been recommended as a control measure since the disease causing organisms tend to accumulate if red clover is grown often (Taylor & Quesenberry, 1996). Grazing of red clover produces a similar effect to an increase in harvest frequency since grazing often means frequent defoliation and a low stubble, in addition to damage from trampling which can become an access point for root rot or other fungal diseases (Rufelt, 1979; Lager, 2002).

Choice of cultivar can aid in decreasing root rot infection – late flowering cultivars tend to store more of their carbohydrates in their roots during autumn which gives them a greater inherent survivability and a better tolerance towards root rot (Rufelt, 1979). Studies in Canada have also revealed the possibility to breed for resistance towards root rot but the procedure demands parental cultivars with a large genetic variation (Coulman & Lambert, 1995). A Swedish field trial evaluating 18 common cultivars for resistant properties towards root rot found that none of the ones selected for the evaluation had any resistance towards the disease (Almquist, 2016). Results have been found indicating benomyl as an effective substance against root rot (Leath *et al.*, 1973), but the same results have not been obtained in Sweden (Rufelt, 1980). Currently, the substance benomyl is not permitted for use in the EU (European Commision, 2021).

Brassicol is a fungicide that has been tested as a control for both root rot and clover rot. It has not proven effective towards root rot but has potential for control of clover rot (Bengtsson, 1961; Rufelt, 1980). However, like benomyl, the use of brassicol is currently not permitted in the EU (European Commision, 2021).

Biological control can be used as an option instead of chemical agents, *Coniothyrium minitans* Campbell is a fungus that can be used for this purpose to control fungi of the *Sclerotinia* species (Öhberg & Bång, 2010). Its pathogenic effect on *S. trifoliorum* was established already in 1957 with samples collected throughout southern England (Tribe, 1957). The commercially available biological control agent (BCA) Contans®WG based on *C. minitans,* was used in a field experiment performed at Ås in the county of Jämtland in Sweden in an already established ley, consisting of red clover, timothy and meadow fescue, as well as a pure stand of red clover (Öhberg & Bång, 2010). In the already established ley (sown the year before the Contans®WG application) the BCA was able to reduce the number of apothecia by autumn the following year but not the number of dead plants. In the pure red clover stands the BCA application was either applied before sowing or on the emerged seedlings. Of the two cultivars used in the pure stands, the diploid Jesper and the autotetraploid SW Torun, the treatment only had a significant effect on SW Torun where an increase in plant winter survival was documented. The reason for the difference between the cultivars remained undetermined.

Due to the previously mentioned more limited host range of clover rot than root rot, it is possible to utilise crop rotations to reduce the clover rot pathogen (Leath, 1985). But for the crop rotation to be effective, perennial legumes should not be grown at the same location for a period of over five years (Öhberg, 2004), though a period of four to five years without a host crop has also been suggested (Leath, 1985). If the clover rot infections are serious, red clover should not be grown at the location for a minimum of eight years (Dillon Weston *et.al.*, 1946 see Öhberg, 2008). However, a well thought out crop rotation will not eradicate the disease as it can survive on some species of weed (Leath, 1985). In addition to a good crop rotation, the sclerotia of clover rot can be prevented from forming apothecia by ploughing deeper than 5 cm (Williams & Western, 1965), though buried sclerotia can survive for more than seven and a half years (Pape, 1937 see Öhberg, 2008).

Breeding efforts through the years have produced several clover rot resistant cultivars with resistance of different degrees. In Belgium a field experiment was initiated where 117 accessions of red clover were screened for resistance against *S. trifoliorum* (Vleugels *et al.*, 2013). The experiment determined that tetraploid cultivars had a significantly higher resistance on average than diploid cultivars but that there was no significant difference in susceptibility between cultivars, landraces and wild populations. This was previously confirmed in the Netherlands in an inoculation trial screening for resistance in different diploid and tetraploid cultivars and families (Dijkstra, 1964). An infection trial in Norway, using different isolates of *S. trifoliorum* assessed nine red clover families, each consisting of one diploid and one autotetraploid derived from the same parent (Vestad, 1960). It was deduced that the autotetraploid of each family was significantly more resistant to clover rot than the diploid. Similar effects have been seen in northern Sweden where the diploid and autotetraploid pair Bjursele and Betty achieved the same result in a field trial (Öhberg *et al.*, 2008). However, the same cultivars in a controlled environment displayed the opposite relationship where the diploid Bjursele proved more resistant (Öhberg *et al.*, 2005). In that controlled environment experiment, it was simultaneously determined that late flowering cultivars had a higher resistance towards clover rot on average than the intermediate cultivars. This has also proven to be true in a field environment in the south of Sweden (Öhberg, 2008). The previously mentioned diploid cultivar Jesper and its autotetraploid equivalent SW Torun have, unlike Bjursele and Betty, not outperformed one another in clover rot resistance (Öhberg & Bång, 2010). It should be mentioned that outside of bred resistance, well-adapted cultivars can have an inherent resistance to the conditions where they are grown (both biotic and abiotic), making them more persistent than they would have been at other locations (Taylor & Smith, 1978).

3.2. Red clover persistence at Offer, Ås and Röbäcksdalen 1963-1986

Below are the results from Model 1 and 2, analysing the red clover data from the three different sites. Cut 1 and 2 are referring to the harvest number and treatment A, B and C to the different crop rotations. Focus was put on red clover proportion (RCP) and CP concentration. All error bars in the figures are constructed from the confidence interval.

3.2.1. Model 1

Clover proportion

The results from model 1, comparing the first harvest year in each crop rotation of the three treatments A, B and C, for red clover proportion are summarised in Table 12. To be able to determine change over time, model 1 included the continuous time variable x. For clover proportion the factors site, treatment and cut were significant, together with the two interactions Site*Treatment and Treatment*x.

Table 12. Results from the Mixed-effects model, modelling the effects of site, treatment and cut on clover proportion.[a] DFnum and DFden are the numerator and denominator degrees of freedom, respectively.

Source	DFnum	DFden	F Value	Prob > F
Site	2	45.7	6.65	0.003**
Treatment	2	44.4	27.22	<0.001***
Cut	1	23.2	169.75	<0.001***
Site*Treatment	4	89.3	4.16	0.004**
Site*Cut	2	44.9	1.66	0.201
Treatment*Cut	2	45.7	2.09	0.135
Site*Treatment*Cut	4	87.7	0.63	0.645
Treatment*x	2	41.8	3.89	0.028*

[a] *Level of significance:* * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

As a mean across sites, treatments and year, cut two had the highest clover proportion with a LS mean of approximately 0.69 (Figure 4, Table 18 in appendix) and was statistically different from cut one with a LS mean of 0.34.

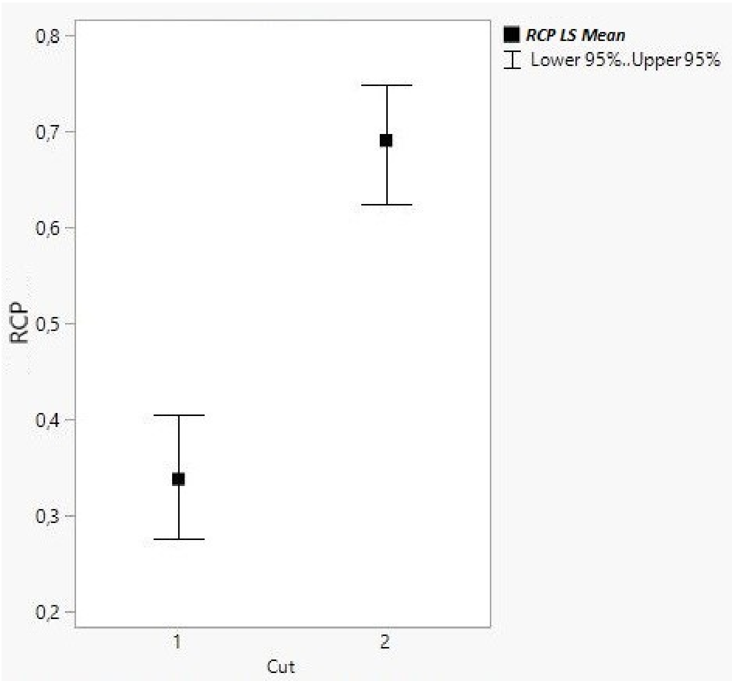

Figure 4. Red clover proportion as affected by cuts (1 and 2) the first harvest year, as a mean across sites (Offer, Ås and Röbäcksdalen), treatments (A, B and C) and year.

Within sites Offer and Ås the first harvest year, treatment C had a significantly higher clover proportion than A and B as a mean across cut and year (Figure 5, Table 19 in appendix). At Röbäcksdalen, treatment C was however not significantly different from neither A nor B. The RCP LS mean of Offer was the highest between the different sites on average across treatment, cut and year.

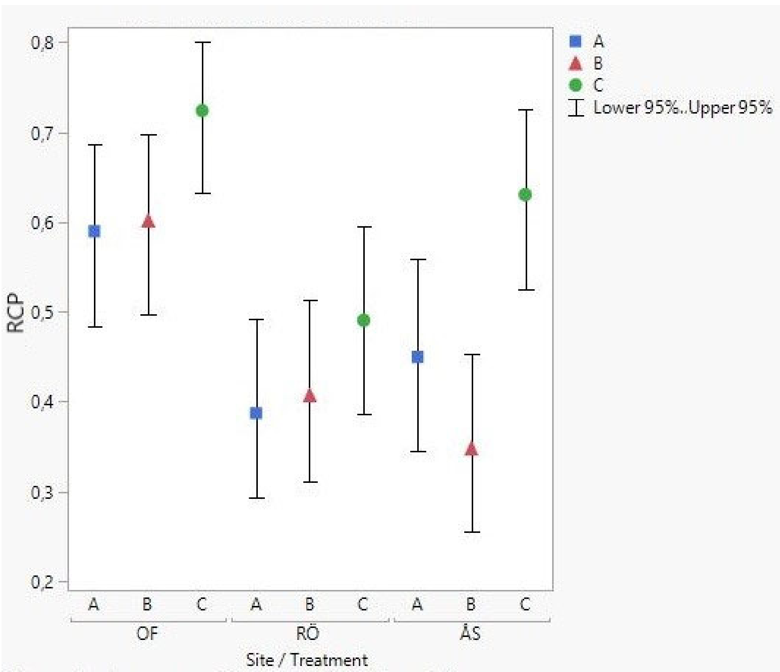

Figure 5. Red clover proportion as affected by treatments (A, B and C) and sites (Offer, Röbäcksdalen and Ås) the first harvest year, as a mean across cuts (1 and 2) and year.

The only treatment that had a significant negative effect on clover proportion the first harvest year over time was treatment A (Figure 6, Table 13). B and C did affect clover proportion significantly over time, though slope B is close to being significant at the 0.05 level. Both the trends for A and for B were significantly different from trend C while A and B were not significantly different from each other.

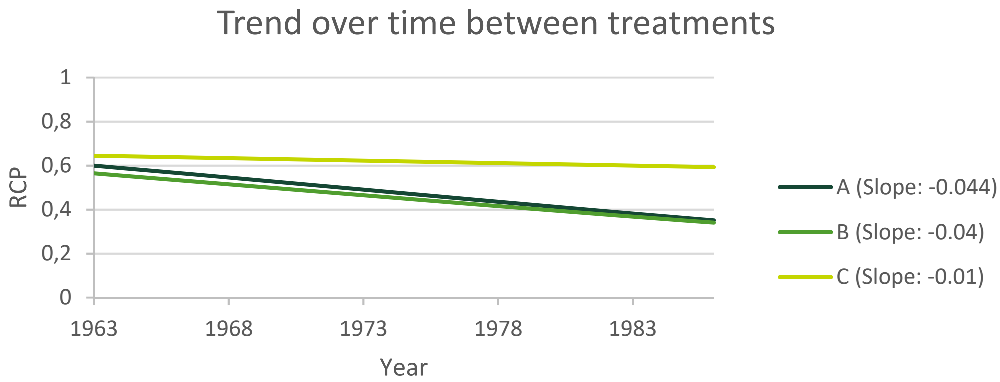

Figure 6. Effect of treatments (A, B and C) over time on change in clover proportion the first harvest year.

Table 13. Results from analysing the effect of treatment (A, B and C) over time on clover proportion the first harvest year across a mean of cuts (1 and 2) and sites (Offer, Röbäcksdalen and Ås)[a]

	DF	t Value	Pr > |t|
Trend A-B	41.7	- 0.31	0.754
Trend A-C	41.8	- 2.56	0.014*
Trend B-C	41.9	- 2.24	0.030*
Slope A	30.8	- 2.25	0.032*
Slope B	30.9	- 2.03	0.051
Slope C	30.7	- 0.49	0.629

[a] *Level of significance:* * $P \leq 0.05$.

Crude protein concentration

Similarly to clover proportion, crude protein concentration the first harvest year was significantly affected by the factors site, cut and treatment, together with the time variable x. The interactions between treatment*cut, treatment*x and treatment*cut*x were also significant (Table 14).

Table 14. Results from the Mixed-effects model, modelling the effects of site, treatment and cut on crude protein concentration, level of significance[a]

Source	DFnum	DFden	F Value	Prob > F
Site	2	46.1	6.36	0.004**
Treatment	2	46.1	18.63	<0.001***
Cut	1	22.9	9.65	0.005**
Site*Treatment	4	91.1	1.78	0.139
Site*Cut	2	45.4	2.12	0.131
Treatment*Cut	2	45.7	8.15	<0.001***
Site*Treatment*Cut	4	91.0	1.06	0.379
x	1	22.1	14.60	<0.001***
Treatment*x	2	44.3	4.46	0.017*
Treatment*Cut*x	2	44.1	5.71	0.006**

[a] *Level of significance:* * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Site had a significant effect on crude protein concentration where there was a significantly smaller amount of crude protein in the harvested biomass at Röbäcksdalen compared to Ås and Offer (Figure 7, Table 20 in appendix).

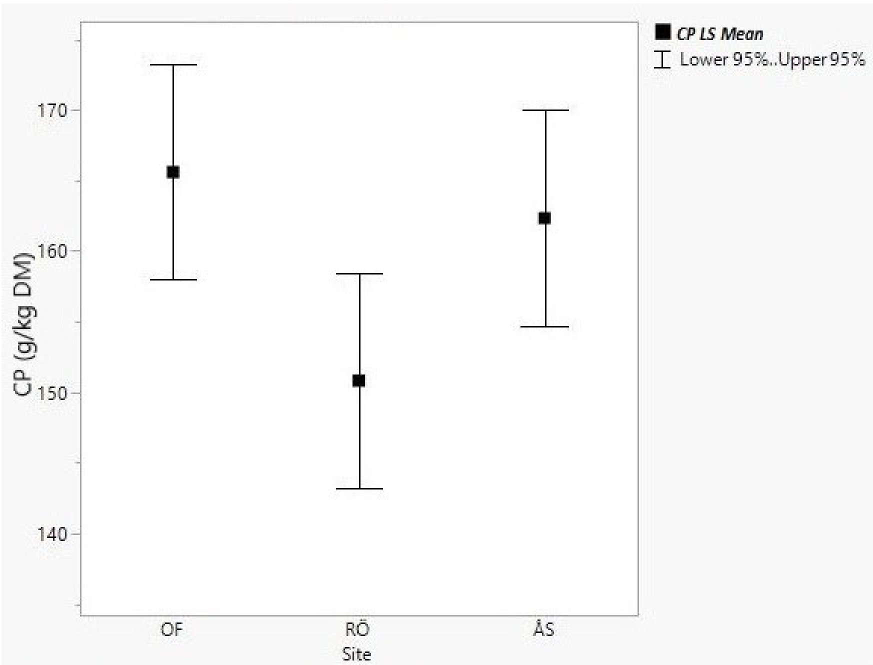

Figure 7. Site effect on crude protein concentration the first harvest year, as a mean across cuts (1 and 2), treatments (A, B and C) and year.

The LS means of crude protein for the first cut in each treatment was lower than the LS means for the second cut per treatment, however the difference between first and second cut was only significant for treatment B (Figure 8, Table 21 in appendix). The low CP concentration of the first cut for treatment B was statistically different from all other cuts.

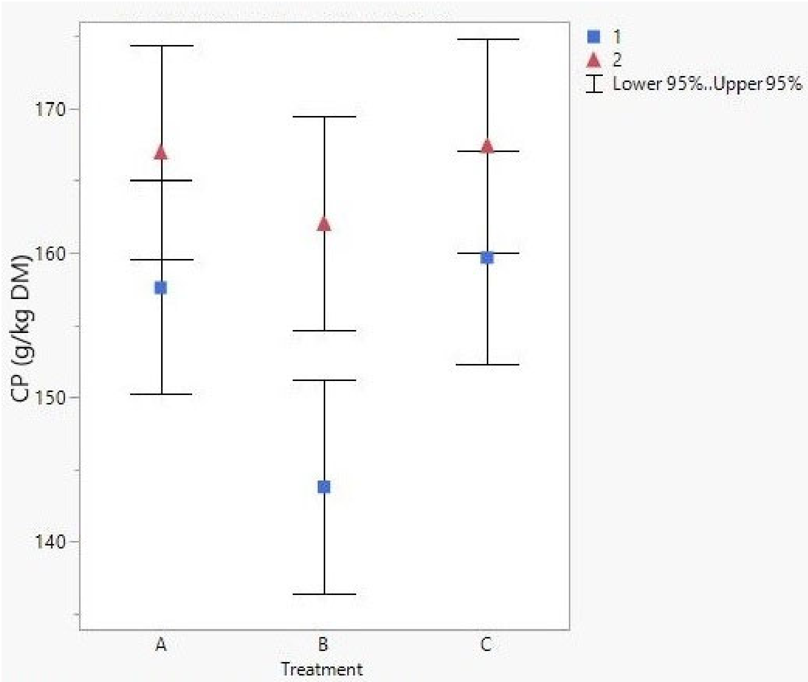

Figure 8. Effect of the interaction between treatments (A, B and C) and cuts (1 and 2) on crude protein concentration in the first harvest year, as a mean across sites (Offer, Röbäcksdalen and Ås and year).

The effect of the interaction treatment*cut on crude protein concentration the first harvest year was significant over time (Figure 9, Table 15). Treatment A had a significant decrease for both cut one and two over time, treatment B and C only had a significant change over time for cut two. The only trends that were significantly different from each other were the first cut for treatment A compared with the first cut of treatment C and B.

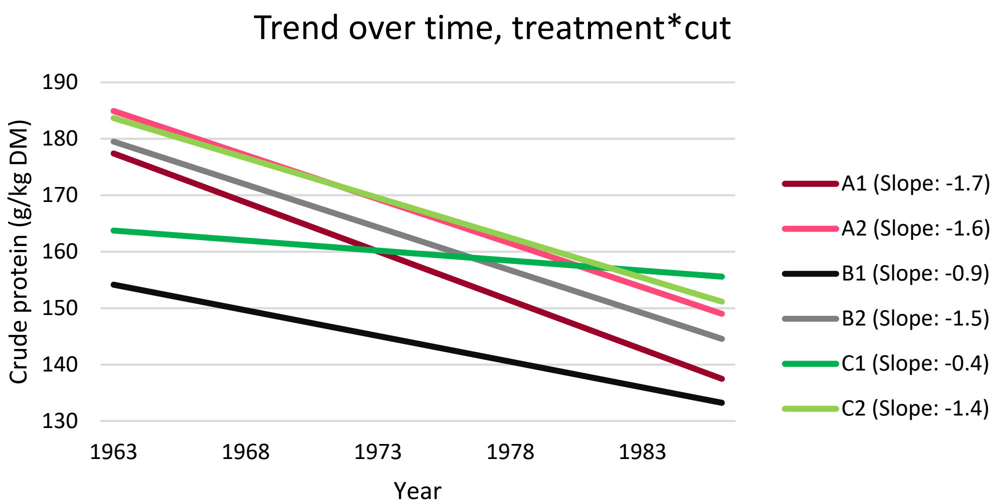

Figure 9. Effect of the interaction between cuts (1 and 2) and treatments (A, B and C) on the change in crude protein concentration over time the first harvest year.

Table 15. Results from analysing the effect of the interaction between treatments (A, B and C) and cuts (1 and 2) over time on crude protein concentration the first harvest year, as a mean across sites (Offer, Röbäcksdalen and Ås) and year[a]

Treatment/Cut	DF	t Value	Pr > |t|
A1-A2	28.7	- 0.29	0.770
B1-B2	28.7	1.03	0.310
C1-C2	28.7	1.80	0.083
A1-B1	79.5	- 2.62	0.011*
A1-C1	79.4	- 4.38	<0.001***
B1-C1	79.4	- 1.76	0.082
A2-B2	79.5	- 0.14	0.891
A2-C2	81.6	- 0.47	0.643
B2-C2	81.6	- 0.33	0.743
Slope A1	58.7	- 3.73	<0.001***
Slope A2	58.7	- 3.36	0.001**
Slope B1	58.7	- 1.96	0.055
Slope B2	58.7	- 3.27	0.002**
Slope C1	58.2	- 0.77	0.447
Slope C2	59.0	- 3.04	0.004**

*[a] Level of significance: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001.*

3.2.2. Model 2

Clover proportion

For Model 2, comparing the three harvest years of treatment B to the three first harvest years of treatment A, the factors site and treatment are no longer significant whereas the factors harvest year and cut are (Table 16). There are significant two-way interactions for Site*Harvest year, Site*Cut and Harvest year*Cut, and significant three-way interactions for Site*Treatment*Harvest year and Treatment*Harvest year*Cut. Treatment C was excluded since it only has a two-harvest year ley.

Table 16. Results from the Mixed-effects model, modelling the effects of sites (Offer, Röbäcksdalen and Ås), treatments (A and B), harvest years (H1, H2 and H3) and cuts (1 and 2) on clover proportion[a]

Source	DFnum	DFden	F Value	Prob > F
Site	2	45.8	2.74	0.075
Treatment	1	23.6	0.24	0.627
Harvest year	2	46.6	119.82	<0.001***
Cut	1	23.0	120.16	<0.001***
Site*Treatment	2	44.5	27.58	<0.001***
Site*Harvest year	4	91.3	3.11	0.019*
Site*Cut	2	44.1	4.35	0.019*
Treatment*Harvest year	2	44.9	0.53	0.592
Treatment*Cut	1	24.2	0.01	0.937
Harvest year*Cut	2	44.9	50.02	<0.001***
Site*Treatment*Harvest year	4	92.2	1.43	0.230
Site*Treatment*Cut	2	46.3	3.73	0.032*
Site*Harvest year*Cut	4	87.2	0.92	0.459
Treatment*Harvest year*Cut	2	47.6	8.62	<0.001***
Site*Treatment*Harvest year*Cut	4	91.6	0.93	0.451

[a] *Level of significance:* * $P \leq 0.05$, *** $P \leq 0.001$.

For the first and second cut for every treatment at each site, the first cut had a clover proportion that was significantly lower than the clover proportion of the second cut (Figure 10, Table 22 in appendix). None of the first cuts differed significantly between each other over the different sites and treatments. On the other hand, while none of the second cuts for treatment A significantly differed, the second cut for treatment B at Offer had a significantly higher clover proportion than the second cut for the same treatment at Ås. The second cut at Offer for treatment A and B were not significantly different from each other while at Röbäcksdalen the second cut of A contained significantly less clover than the same cut for B, opposite to the relationship at Ås.

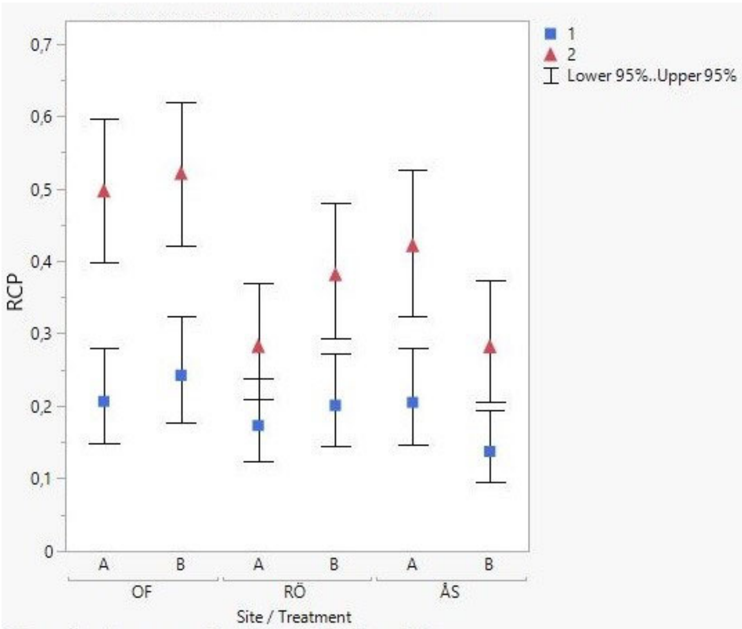

Figure 10. Effect of the interaction between sites (Offer, Röbäcksdalen and Ås), treatments (A and B) and cuts (1 and 2) on clover proportion as a mean across harvest years (H1, H2 and H3) and year.

There was one first and second cut in the three-way interaction Treatment*Harvest year*Cut that was not significantly different from each other for harvest year 3 (H3), treatment A (Figure 11, Table 23 in appendix). When comparing the second cut of H1, H2 and H3 for both treatments, the difference between the two second cuts within the same ley age was not statistically significant. Between the different ley ages, the second cuts were statistically different from each other. There was no statistically significant difference between the first cuts of H1 and H2 independent of treatment.

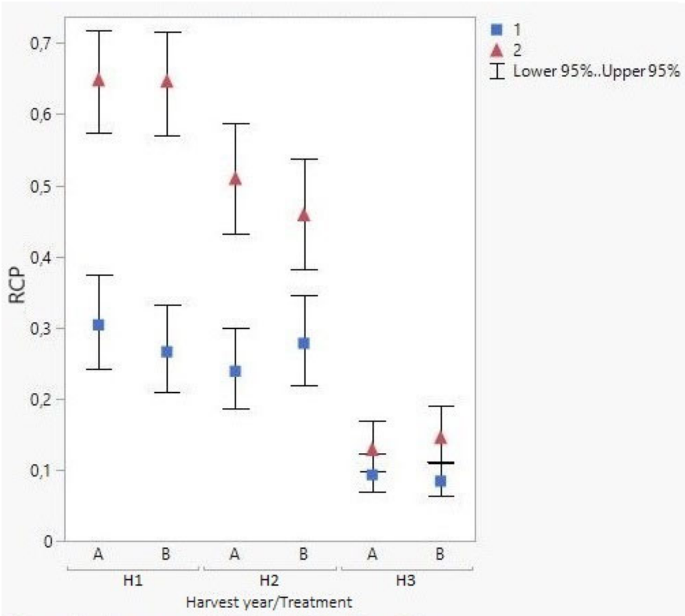

Figure 11. Effect of the interaction between treatments (A and B), harvest years (H1, H2, and H3) and cuts (1 and 2) on clover proportion as a mean across sites (Offer, Röbäcksdalen and Ås) and year.

Crude protein concentration

Unlike clover percentage, crude protein concentration was significant for all included factors, place, treatment, harvest year and cut. There were several significant two-way interactions, site*treatment, site*harvest year, treatment*harvest year, treatment*cut and harvest year*cut. There were also three significant three-way interactions, site*treatment*cut, site*harvest year*cut and treatment*harvest year*cut (Table 17).

Table 17. Results from the Mixed-effects model, modelling the effects of sites (Offer, Röbäcksdalen and Ås), treatments (A and B), harvest years (H1, H2 and H3) and cuts (1 and 2) on crude protein concentration[a]

Source	DFnum	DFden	F Value	Prob > F
Site	2	46.2	4.65	0.014*
Treatment	1	22.5	61.36	<0.001***
Harvest year	2	46.4	54.35	<0.001***
Cut	1	23.0	81.77	<0.001***
Site*Treatment	2	46.0	5.67	0.006**
Site*Harvest year	4	89.1	2.69	0.036*
Site*Cut	2	45.6	0.66	0.520
Treatment*Harvest year	2	46.7	22.53	<0.001***
Treatment*Cut	1	23.1	95.58	<0.001***
Harvest year*Cut	2	43.8	41.82	<0.001***
Site*Treatment*Harvest year	4	92.5	0.15	0.964
Site*Treatment*Cut	2	46.2	5.17	0.009**
Site*Harvest year*Cut	4	91.3	4.06	0.005**
Treatment*Harvest year*Cut	2	46.1	107.67	<0.001***
Site*Treatment*Harvest year*Cut	4	90.3	2.18	0.077

[a] *Level of significance:* * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Over the different ley ages and treatments, two of the first and second cuts were not statistically different from each other in terms of crude protein, as seen for treatment A, H1 and H2 (Figure 12, see Table 24 in appendix). The difference between the remaining first and second cuts was statistically significant. The second cut for treatment B H2 was statistically different from the second cuts of H3 and second cut of treatment A H2. The difference between the first cuts of H3 and the first cut of treatment B H2 was not statistically significant. As an overall mean across site, cut, harvest year and year, treatment B produced less crude protein than treatment A over the three harvest years (Figure not shown).

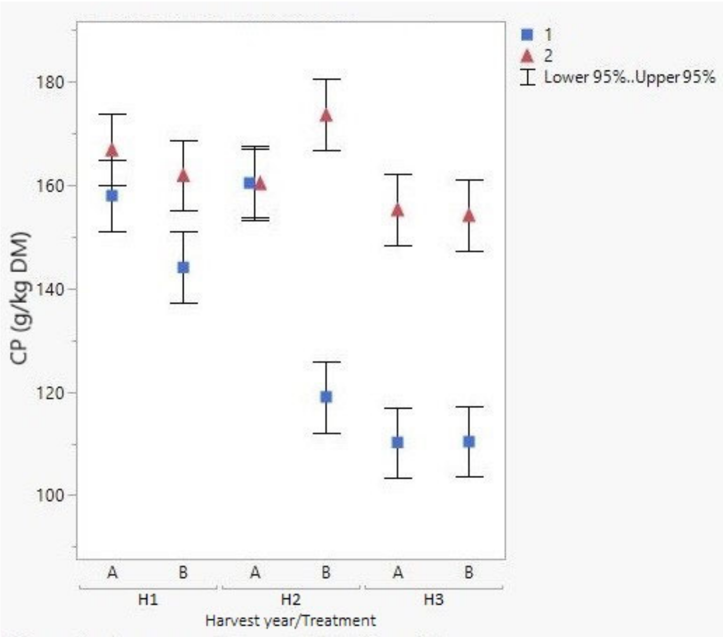

Figure 12. Effect of the interaction between treatments (A and B), cuts (1 and 2) and harvest year (H1, H2 and H3) on crude protein concentration as a mean across sites (Offer, Röbäcksdalen and Ås) and year.

4. Discussion

The hypothesis stating that there would be an increase of crude protein concentration and red clover proportion from the first to the second harvest was supported by the results from the long-term field experiments at Offer, Ås and Röbäcksdalen. The cause behind this is mentioned by Steen (1970) as read in Nilsdotter-Linde (2001) where it is stated that grass has a faster growth rate earlier in the season than legumes have, which causes the first harvest to contain a large proportion of grass compared to legumes. Sometime after the first harvest, the legumes have their fastest rate of growth and the second harvest will end up including a larger proportion of legumes than the first.

The second hypothesis stating that a larger share of ley in the crop rotation will cause a faster decrease of the red clover proportion and crude protein concentration is more complex and demands a more complex answer. In the study by McBratney (1987) it is evident that red clover can persist more than eight years without the presence of pests and diseases. Many of the studies found in the literature were not performed for more than three years as red clover is assumed not to persist in any significant capacity after that. This is also made apparent by the results from the analysis of the long-term experiment R8-71, where there is a significant decrease in red clover proportion between year two and three (Figure 11). From this information it is possible to deduce that the most common cause of red clover expiry is pests and diseases.

No specific investigation for fungal diseases in the leys was made between 1963 and 1986 at the R8-71 locations. In 2008, Öhberg (2008) mentions incidences of clover root rot at the field trial site Ås, same as Öhberg *et al.* (2008), with the addition of documentation of infections at Röbäcksdalen. This suggests that at least clover rot infection at these sites is highly likely, assuming the pathogen was also present in 1963-1986. The survey of long-term field trials performed by Olvång & Björkman (1972) noted the presence of the stem and bulb nematode at the sites Ås and Offer in 1971, which could possibly aid in the infection of clover rot as suggested by Hansen & Søegaard (2009). There was however no visible effect from the introduction of the stem and bulb nematode resistant red clover variety Björn. Further research is needed to elucidate the relationship between the two pathogens.

Since the ley data used in this thesis is quite old, an investigation into the presence of the pathogens at the three sites performed today would not give information about the presence of the pathogens in 1963-1986. The following analysis however, will be based on the assumption that both clover rot and root rot were present at the sites when the data was collected.

To combat pests and diseases it is important to have a well thought out crop rotation. Different pathogens are disadvantaged by different crop rotations, which makes it important to know what pathogens are present where the crops are to be sown. For clover rot a crop rotation with a gap of four to five years minimum where no host crop is grown is recommended to reduce clover rot incidence (Leath, 1985). The recommendations differ based on location and previous degree of disease incidence, at a location where clover rot incidence has been severe, a longer time period between hosts is recommended (Dillon Weston *et.al.*, 1946 see Öhberg, 2004; Öhberg, 2008).

Of the three crop rotations analysed, C has a three-year period where no host for clover rot is grown, though more research is necessary to with certainty exclude pea as a potential host. If the cause of the significantly lower clover proportion of crop rotation A and B compared to C is due to clover rot, this could indicate that it is enough to have a period of three years in between host crops since C does not seem to be as affected (Figure 5). It could also mean that it is not caused by clover rot and that something else is causing the difference, but this is difficult to confirm when such a long time has passed. Olvång & Björkman (1972) mentions a large presence of dandelions in crop rotation A and B, which could also be a cause of the lower clover proportion. In addition to this, ploughing was a more frequent practice in crop rotation C, which according to Williams & Western (1965) can be used as a method to combat clover rot. These three components (period of time between hosts, weeds and ploughing frequency) could possibly contribute to a slower decrease of clover proportion over time which would suggest that crop rotation B and C should have a lower decrease over time compared to A. This proved to be the case as A was the only crop rotation that had a significant decrease in the first harvest year over time. However, the difference between the trend for A and B was not statistically significant (Figure 6). This could indicate that if the period without a host crop is shorter than three years the pathogen population of clover rot will not decrease but rather increase, which would cause crop rotation A and B to not be significantly different from each other. In addition to this, harvest year four and five for crop rotation A, barely contained any clover, which would mean that clover rot would not have access to a red clover host during these two harvest years. Crop rotation A would then have a similar period without a host as crop rotation B. The perennial weed population could also have an effect on clover proportion over time as it competes with the sown cultivars for water and nutrients. In a similar manner it could have an effect on the average clover proportion across the different sites and harvests.

The trends of change in clover proportion over time were based on the first-year ley of each crop rotation which makes the decrease over time more likely to be caused by clover rot than root rot. According to Öhberg (2004), clover rot causes the greatest loss of red clover plants in spring after the first winter unlike root rot where Ylimäki (1967) mentions that the effect of the pathogen is mostly seen from the second harvest year onwards. The change of cultivars used through the years has not stopped the decrease of clover proportion over time. It is possible that the change has slowed the decrease, but the lack of visible effect of changing cultivar would suggest that the change of clover proportion over time is not strictly reliant on cultivar choice. No data about clover rot resistance was found on the cultivars Offer, Disa, Bjursele, Reko and Björn but diploid cultivars are said to be less persistent towards clover rot in general. Another possible explanation to the decrease over time, not discussed in detail in this thesis, is the change in soil properties as the trial progresses, which has been documented by Bolinder *et al.* (2010). Differences in soil quality is a plausible reason as to why clover proportion and crude protein concentration differ between sites (Figures 5 and 7). As there are dissimilar amounts of soil organic carbon and soil organic nitrogen at each site, it can be expected that the clover proportion will be affected differently depending on the location. The differences could also have been caused by differences in disease severity. Clover rot has been confirmed at Ås and Röbäcksdalen in later years, where at Ås, Öhberg *et al.* (2008) described clover rot killing 75-97% of red clover plants. Further research is required to confirm if the assumption that soil characteristics and disease severity can affect clover proportion to this extent is correct or should be disregarded.

There was a significant decrease in clover proportion between the second- and third-year ley for the first and second harvest for both crop rotation A and B (Figure 11). In addition to this, there was a significant decrease in the second harvests clover proportion of the second-year ley when compared to the second harvest of the first-year ley. Based on the previous statement by Ylimäki (1967) that root rot starts to be most noticeable the second year, root rot could be a plausible cause for the decrease as it is first visible the second harvest during the second production year of the ley. Rufelt (1986a) states that root rot progresses most rapidly during summer. Therefore, it is reasonable that the first harvest of the second-year ley is not as affected by root rot. The first and second harvests within the same ley year were not significantly different from each other when comparing the two crop rotations, A and B. In addition, they both started decreasing at the same time. This suggests that the crop rotation had no impact on the rate of decrease of the clover proportion, which corresponds with the observation made by Rufelt (1979) that it is not a disease caused by a poor crop rotation.

Because of its broad host range, root rot is more difficult to combat using crop rotation than clover rot, especially in areas like northern Sweden where the selection of available crops is more restricted. The fight against root rot should mainly focus on using management practices that keeps the plant as healthy as possible (Rufelt, 1994). Specific cultivars with root rot resistance seems to not yet be available, but are being developed and tested (Halling & Larsson, 2017).

The only deviating crude protein concentration was found for the first harvest of crop rotation B. It was significantly lower in CP concentration than the rest of the crop rotations (Figure 8). The reason for this could be the differences in management and clover proportion between the three crop rotations. Crop rotation A receives the most mineral N fertiliser over the six-year rotation (Table 6) and C the least. Crop rotation C had the highest clover proportion of the three on average while there was no significant difference between A and B. This indicates that the cause of the lower CP concentration for crop rotation B could be the lower clover proportion and the smaller total application of N mineral fertiliser. It is however difficult to draw this conclusion without knowing the exact application of fertiliser during the ley years and the nutritional status of the soil at sowing. Though Bolinder *et al.* (2010) has documented an accumulation of soil organic nitrogen for crop rotation A at the sites Offer and Röbäcksdalen which validates the statement to some extent.

When analysing trend over time for CP concentration in the first harvest year, it became clear that the interaction between crop rotation and harvest caused a decrease of CP over the years (Figure 9). However, the only crop rotation that had a decrease that was significant for both harvests was A. The decrease seen in crop rotation B and C were only significant for the second harvest. It is difficult to say why only the second harvest had a significant decrease of CP over time for B and C in the first harvest year. An explanation could be that the CP concentration of the first harvest is more dependent on the grass proportion of the ley than the second that is more dependent on clover. This would suggest that as the clover proportion decreases with time (Figure 6), so does the CP concentration of the second harvest. A flaw in this theory is that the decrease of clover proportion with time was only significant for crop rotation A, which makes this explanation unlikely. The second harvests crude protein concentration is largely dependent on the clover proportion of the ley if there is little or no addition of nitrogen fertiliser. Since there was no significant change in clover proportion for crop rotation B and C over time, that would suggest that the difference in crude protein concentration is due to fertilisation or other factors not observed in this thesis. A study mapping the specific amount of fertiliser applied over this period would need to be made to make a more accurate observation.

When observing the difference in CP concentration between the different ley ages, crop rotations and harvests, it was made evident that the CP concentration of the first harvest for crop rotation A in the second-year ley was significantly higher than that same harvest for crop rotation B (Figure 12). In contrast, the CP concentration of the second harvest remained approximately the same over the two crop rotations and increasing ley age. The differences in CP concentration can be explained by looking at the different harvest dates as they were in 1963 (Table 2). In crop rotation A the first harvest is taken at the silage harvest stage in both the first and second harvest year as well as a second harvest taken in the first half of August. The harvest dates for crop rotation B are the same as for A in the first harvest year, but after that it starts to differ. In the second year of crop rotation B the first harvest is taken at the hay harvest stage and the second is taken in the second half of August. CP concentration decreases as the plant develops and since the first harvest in this second year is taken at a later stage in plant development the resulting CP concentration will be lower. In the third-year ley the harvest dates for the two crop rotations are the same which is reflected in there being no significant difference between the two crop rotations. As mentioned, the second harvests had roughly the same CP concentration, despite the different harvest dates the second year and increasing ley age. This is probably because the time given for regrowth between the first and second harvest was about the same, meaning that the second harvest would take place in around the same developmental stage for both crop rotations. After the revision the time for regrowth differed about a week between the two rotations, with crop rotation B having the slightly shorter regrowth period. This explains the slightly higher CP concentration in the second harvest for B the second harvest year, but the difference was not significant compared to the other second harvests. As CP is an expression of concentration it is presumably not affected by ley age in the same way as clover proportion, which is why the CP concentration of the second harvests remain high even as the ley ages. The concentration can be the same, but the total yield of CP might decrease.

Surprisingly there is no significant effect of harvest date on the leys' clover proportion. This is possibly due to the difference in growth rate between grass and clover. Even if the first harvest is performed at a later date the clover will not yield more since it will be shaded by the grass and as a consequence grow slower until the ley is harvested, and sunlight can reach down into the sward. This biological mechanism could be interesting to investigate as an addition to a research study on how stubble height affects red clover persistence. Research is needed on the effects of stubble height on red clover persistence since the two trials previously mentioned performed by Fagerberg (1979) and Lustig (1965) were only performed for two harvest years and they were more focused on stubble height effect on yield, than red clover persistence.

The R8-71 experiment was in 1994 downsized to only include site Offer and one replicate in R8-71B. There are future possibilities for the use of this experiment, but changes can be made for it to include additional parameters that are interesting for future agriculture, in northern Sweden specifically. The following recommended changes will be made based on the current status of the experiment:

- After the experiment was revised in 1987 the chemical analysis of the CP concentration of the ley was discontinued (Hagsand *et al.*, 1987). To fully utilise the ley component of the crop rotation this should be brought back since it would make it possible to see how changes in the experimental setup affects CP concentration of the ley. For the same reason, the botanical analysis of the ley's regrowth should be reintroduced.

- An alternative that would demand larger changes to the experiment is to convert it and add additional harvest frequencies like three, or maybe even four harvests, to determine their effect on red clover persistence. There have been Swedish studies made on this subject, but often with more focus towards the yield quantity and quality, and not red clover persistence. Even if they were performed as persistence experiments, they were often not performed for a long enough time. With the changing climate, northern Sweden now has different prerequisites than previously, which adds to the importance of investigating the subject. If field trials are developed for variety testing of red clover, or for looking at the influence of different management strategies on red clover persistence, a third harvest year should always be included to be able to properly determine differences in persistence.
- The fertiliser strategy employed during 1963-1986 did not apply any nitrogen fertiliser for the regrowth of the ley. This was changed in 1987 to include an application for the regrowth in addition to the one applied in spring (Hagsand *et al.*, 1987). A future research opportunity could be to determine the effect of this change in fertiliser strategy on the red clover persistence. Frankow-Lindberg (2017) mentions that since grass-clover leys are the most common kind in Sweden, more fertiliser trial data is important to give more accurate recommendations. A new revision of the experiment's fertiliser strategy would also give an opportunity to compare the effects of modern-day recommendations with those in 1987, on red clover persistence.
- Introducing frequent pathogen and nematode monitoring into the experiment plan would make it possible to, in future years of the experiment, more accurately determine the cause behind changes in red clover proportion.

The inclusion of different harvest frequencies in a long-term experiment would make it possible to determine the effect climate change has on the possibility of increasing the harvest frequency without affecting red clover persistence. In addition to this, performing frequent pathogen monitoring by taking soil samples and doing DNA analysis would reveal any changes in the pathogen population in relation to management strategy and changing climate. With an increase in temperature, clover rot will probably become less common and root rot more prevalent, and this change will be possible to detect using long-term field experiments as they are performed at the same location for a long period of time.

Conclusion

In conclusion, red clover proportion and crude protein concentration increases between the first and second harvest due to differences in growth rate patterns between grass and clover. There is no difference in the rate of decrease of clover proportion within a crop rotation cycle between a ley frequency of three and five harvest years out of six. It is evident that continuous ley cropping, without a sufficient period of using non host crops or combating perennial weeds, leads to a decrease in clover proportion over time in the first harvest year. A lot of the literature on the subject of grass-clover leys is from the 80s and more current research is needed to better reflect the prerequisites we have today and the best management practices to go with them. Long-term field experiments can play an important role in the development of future agriculture if used to their full potential.

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Appendix

SAS Code

***Option 1 effect of treatment over time, clover proportion.**

```
data Logit ;
           set Alternativ_2_ledvist ;
           LogitA = log(a/(1 - a)) ;
run ;

PROC MIXED ASYCOV NOBOUND  DATA=Logit ALPHA=0.05;
CLASS Place Year Treatment Cut;
MODEL LogitA =  Place Treatment Place*Treatment Cut Place*Cut
Treatment*Cut Place*Treatment*Cut x x*Treatment / SOLUTION
DDFM=KENWARDROGER;
RANDOM Year Place*Year Year*Treatment Place*Year*Treatment
Year*Cut Place*Year*Cut Year*Treatment*Cut / SOLUTION ;
RUN;

PROC MIXED NOBOUND DATA=Logit ALPHA=0.05;
CLASS Place Year Treatment Cut;
MODEL LogitA =  Place Treatment Place*Treatment Cut Place*Cut
Treatment*Cut Place*Treatment*Cut x*Treatment / SOLUTION
DDFM=KENWARDROGER;
RANDOM Year Place*Year Year*Treatment Place*Year*Treatment
Year*Cut Place*Year*Cut Year*Treatment*Cut ;
estimate "Trend A - B" x*Treatment 1 -1  0 ;
estimate "Trend A - C" x*Treatment 1  0 -1 ;
estimate "Trend B - C" x*Treatment 0  1 -1 ;
lsmeans Treatment / at x = 0 ;
estimate "Slope A" x*Treatment 1 ;
estimate "Slobe B" x*Treatment 0  1 ;
estimate "Slope C" x*Treatment 0  0  1 ;
RUN;
```

***Option 1 effect of treatment*cut over time, crude protein.**

```
PROC MIXED ASYCOV NOBOUND  DATA=Alternativ_2_subset_Ledvisa_mede
ALPHA=0.05;
CLASS Place Year Treatment Cut;
MODEL  Crude_protein =  Place Treatment Place*Treatment Cut
Place*Cut Treatment*Cut Place*Treatment*Cut x Treatment*x
Treatment*Cut*x / SOLUTION DDFM=KENWARDROGER;
RANDOM Year Place*Year Year*Treatment Place*Year*Treatment
Year*Cut Place*Year*Cut Year*Treatment*Cut / SOLUTION ;
RUN;

PROC MIXED NOBOUND  DATA=Alternativ_2_subset_Ledvisa_mede
ALPHA=0.05;
CLASS Place Year Treatment Cut;
MODEL  Crude_protein =  Place Treatment Place*Treatment Cut
Place*Cut Treatment*Cut Place*Treatment*Cut Treatment*Cut*x /
SOLUTION DDFM=KENWARDROGER;
RANDOM Year Place*Year Year*Treatment Place*Year*Treatment
Year*Cut Place*Year*Cut Year*Treatment*Cut ;
```

```
estimate "Trend A1 - A2" Treatment*Cut*x  1 -1  0  0  0  0 ;
estimate "Trend B1 - B2" Treatment*Cut*x  0  0  1 -1  0  0 ;
estimate "Trend C1 - C2" Treatment*Cut*x  0  0  0  0  1 -1 ;
estimate "Trend A1 - B1" Treatment*Cut*x  1  0 -1  0  0  0 ;
estimate "Trend A1 - C1" Treatment*Cut*x  1  0  0  0 -1  0 ;
estimate "Trend B1 - C1" Treatment*Cut*x  0  0  1  0 -1  0 ;
estimate "Trend A2 - B2" Treatment*Cut*x  0  1  0 -1  0  0 ;
estimate "Trend A2 - C2" Treatment*Cut*x  0  1  0  0  0 -1 ;
estimate "Trend B2 - C2" Treatment*Cut*x  0  0  0  1  0 -1 ;

lsmeans Treatment*Cut / at x = 0 ;

estimate "Slope A1" Treatment*Cut*x  1  0  0  0  0  0 ;
estimate "Slobe A2" Treatment*Cut*x  0  1  0  0  0  0 ;
estimate "Slope B1" Treatment*Cut*x  0  0  1  0  0  0 ;
estimate "Slobe B2" Treatment*Cut*x  0  0  0  1  0  0 ;
estimate "Slope C1" Treatment*Cut*x  0  0  0  0  1  0 ;
estimate "Slobe C2" Treatment*Cut*x  0  0  0  0  0  1 ;

RUN;
```

Tables

Table 18. Red clover proportion LS means as affected by cuts (1 and 2) as a mean across treatments (A, B and C), sites (Offer, Röbäcksdalen and Ås) and year[a]

		Confidence interval	
Cut	**Red clover proportion LS mean**	**Lower 95%**	**Upper 95%**
1	0.34	0.28	0.41
2	0.69	0.62	0.75

[a] *The values are different at the significance level 0.05.*

Table 19. Tukey HSD test of clover proportion LS means as affected by the interaction between treatments (A, B and C) and sites (Offer, Röbäcksdalen and Ås) as a mean across cuts (1 and 2) and year[a]

			Confidence interval	
Site	**Treatment**	**Red clover proportion LS mean**	**Lower 95%**	**Upper 95%**
OF	**A**	0.59 bc	0.48	0.69
	B	0.60 bc	0.50	0.70
	C	0.72 a	0.63	0.80
ÅS	**A**	0.45 cd	0.34	0.56
	B	0.35 d	0.26	0.45
	C	0.63 ab	0.52	0.73
RÖ	**A**	0.39 cd	0.29	0.49
	B	0.41 bcd	0.31	0.51
	C	0.49 bcd	0.39	0.60

[a] *Values followed by different letters are significantly different from each other at the significance level 0.05.*

Table 20. Tukey HSD test of crude protein LS means as affected by sites (Offer, Röbäcksdalen and Ås) as a mean across cuts (1 and 2) and year[a]

		Confidence interval	
Site	**Crude protein LS Mean**	**Lower 95%**	**Upper 95%**
OF	165.60 a	157.98	173.21
ÅS	162.34 a	154.70	169.98
RÖ	150.82 b	143.20	158.44

[a] Values followed by different letters are significantly different from each other at the significance level 0.05.

Table 21. Tukey HSD test of crude protein LS means as affected by the interaction between treatments (A, B and C) and cuts (1 and 2) as a mean across sites (Offer, Röbäcksdalen and Ås) and year[a]

			Confidence interval	
Treatment	**Cut**	**Crude protein LS Mean**	**Lower 95%**	**Upper 95%**
A	**1**	157.59 a	150.18	165.00
	2	166.99 a	159.58	174.39
B	**1**	143.78 b	136.38	151.19
	2	162.05 a	154.64	169.45
C	**1**	159.67 a	152.27	167.06
	2	167.44 a	160.04	174.84

[a] Values followed by different letters are significantly different from each other at the significance level 0.05.

Table 22. Tukey HSD test of clover proportion LS means as affected by the interaction between sites (Offer, Röbäcksdalen and Ås), treatments (A and B) and cuts (1 and 2) as a mean across harvest years (H1, H2 and H3) and year[a]

				Confidence interval	
Site	**Treatment**	**Cut**	**Red clover proportion LS mean**	**Lower 95%**	**Upper 95%**
OF	**A**	**1**	0.21 ehijk	0.15	0.28
		2	0.50 abcf	0.40	0.60
	B	**1**	0.24 deghijk	0.18	0.32
		2	0.52 a	0.42	0.62
RÖ	**A**	**1**	0.17 jk	0.12	0.24
		2	0.28 bfghi	0.21	0.37
	B	**1**	0.20 hijk	0.14	0.27
		2	0.38 acde	0.29	0.48
ÅS	**A**	**1**	0.20 ehj	0.15	0.28
		2	0.42 abdg	0.32	0.53
	B	**1**	0.14 ik	0.10	0.19
		2	0.28 cefhj	0.21	0.37

[a] Values followed by different letters are significantly different from each other at the significance level 0.05.

Table 23. Tukey HSD test of clover proportion LS means as affected by the interaction between treatments (A and B), harvest years (H1, H2 and H3) and cuts (1 and 2) as a mean across sites (Offer, Röbäcksdalen and Ås) and year[a]

Treatment	Harvest year	Cut	Red clover proportion LS mean	Confidence interval Lower 95%	Upper 95%
A	H1	1	0.30 c	0.24	0.37
		2	0.65 a	0.57	0.72
	H2	1	0.24 cd	0.19	0.30
		2	0.51 b	0.43	0.59
	H3	1	0.09 f	0.07	0.12
		2	0.13 ef	0.10	0.17
B	H1	1	0.27 c	0.21	0.33
		2	0.65 a	0.57	0.72
	H2	1	0.28 c	0.22	0.35
		2	0.46 b	0.38	0.54
	H3	1	0.08 f	0.06	0.11
		2	0.15 de	0.11	0.19

[a] Values followed by different letters are significantly different from each other at the significance level 0.05.

Table 24. Tukey HSD test of crude protein concentration LS means as affected by the interaction between treatments (A and B), harvest years (H1, H2 and H3) and cuts (1 and 2) as a mean across sites (Offer, Röbäcksdalen and Ås) and year[a]

Treatment	Harvest year	Cut	Crude protein LS mean	Confidence interval Lower 95%	Upper 95%
A	H1	1	158.02 bc	151.16	164.88
		2	166.94 ab	160.08	173.80
	H2	1	160.70 abc	153.85	167.55
		2	160.19 bc	153.36	167.03
	H3	1	110.22 e	103.38	117.06
		2	155.36 bcd	148.50	162.22
B	H1	1	144.13 d	137.27	150.99
		2	161.98 abc	155.12	168.84
	H2	1	119.03 e	112.19	125.87
		2	173.67 a	166.82	180.52
	H3	1	110.36 e	103.52	117.20
		2	154.25 cd	147.38	161.11

[a] Values followed by different letters are significantly different from each other at the significance level 0.05.