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Weed invasion in different species compositions with and without perennial ryegrass

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#### Abstract

Though perennial ryegrass (*Lolium perenne*) is a desired species in roughage production because of its nutritive qualities, it is not very persistent and — when used in species mixtures — often disappears after the first year. This may give weeds the chance to invade the space now left open. In this study, different species compositions — both including and excluding perennial ryegrass — were compared with each other as to their resilience to such an invasion, using data from both the first and the second year of ley. Additional factors of comparison were the inclusion of species with contrasting functional traits in the mixtures (fast-establishing vs. slowly establising and N<sub>2</sub>-fixing vs. nonfixing), the level of nitrogen fertilisation (moderate vs. low) and location (northern Norway (Holt) vs. the middle of the country (Kvithamar)). Yield levels were also analysed for all these factors.

Perennial ryegrass was found to have a weed suppressive effect, strongest in the first year, but still present in the second year, when the abundance of ryegrass was far lower. The effect of the presence of this species on yield levels, however, differed between the years: mixtures including perennial ryegrass had higher yields in the first year, but lower yields in the second year.

The presence of fast-establishing species in the mixture was found to have the main weed suppressive effect. Mixtures containing both fast-establing species and at least one  $N_2$ -fixing species gave the highest yields; the mixture without any fast-establishing species gave the lowest yields.

The level of nitrogen fertilisation did not show any weed suppressive effect, though individual weed species may have been affected. The lower level of nitrogen fertilistation gave consistently lower yields.

Weed abundance was much higher at Holt than at Kvithamar. Yields were higher at Kvithamar than at Holt.

The point frequency method, a non-destructive way of estimating the biomass of different species in a plot usually used in natural vegetation, was evaluated for use in cultivated grassland. It provided results mostly agreeing with those obtained by harvesting and separating, though there were some limitations in the practicality of its use.

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## Chapter 1

# Introduction

## 1.1 Leys in Norway

Of the ca. 1 million ha of cultivated land in Norway, about 48% is used as temporary grassland (ley). When permanent grassland and cereals and oilseed crops are added, ca. 80% of Norwegian agricultural land is devoted to the production of ruminant feed. Norwegian leys are mostly used to produce silage, the importance of which has increased over the past years (there was a 17% rise in silage intake per cow per year between 2003 and 2013).

Leys in Norway are cut between one and four times per season, depending on the local climate. They are fertilised with both animal manure and mineral fertiliser for an average total of 177 kg N, 20 kg P and 107 kg K per ha per year. The species that are used most are the grass species timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), perennial ryegrass (*Lolium perenne*) and smooth meadow-grass (*Poa pratensis*) and the clover species red clover (*Trifolium pratense*) and white clover (*Trifolium repens*). The most popular seed mixtures contain timothy, meadow fescue and red clover, while perennial ryegrass is mostly used in pure stands.

Because of the environmental conditions in Norway (low temperatures and great variation in daily photoperiod — especially in the north), varieties from further south in Europe are often insufficiently adapted, resulting in low winter survival. Therefore, Norwegian varieties are preferred: for timothy and meadow fescue, not much else is used; for perennial ryegrass, Norwegian varieties have recently become available and their share is now increasing.

Between 2003 and 2013, the per area yields of Norwegian grasslands did not increase, remaining far below estimated potential yields. Several reasons for this have been suggested: poor soil structure and drainage because of heavy machinery use, low incentives for structural improvement (because of a high percentage of rented farmland and a subsidy system based on cultivated area rather than yield) and the low price of concentrate feed as compared to silage production. The present study is part of a bigger project studying affordable methods for increasing grassland yields, in order to make roughage production more cost efficient. (Steinshamn et al., 2016)

#### 1.1.1 Sown species

A short overview follows of the principal characteristics of the grass and legume species used in this study.

#### 1.1.1.1 Perennial ryegrass (Lolium perenne)

L. perenne is a desired species in roughage production because of its excellent nutritive qualities: high digestibility and high contents of proteins, soluble carbohydrates and Na. Its yields are highest at high levels of nitrogen fertilisation. It is a fast-establishing and very competitive species, but is not very persistent when cut only (as opposed to grazing). It is sensitive to cold winters. (Peeters et al., 2004)

In Norway, *L. perenne* has become more popular, mostly along the coast in the south of the country, where winters are milder. It is often used in pure stands, though usually in mixtures of different varieties. (Steinshamn et al., 2016)

#### 1.1.1.2 Timothy (Phleum pratense)

*P. pratense* is a very productive species which has good nutritive qualities: its digestibility is comparable to that of *L. perenne*, though its content of soluble carbohydrates is lower and its Na content is very low. It responds well to high nitrogen fertilisation levels, but produces good yields also at moderate levels. It is competitive, but less so than *L. perenne*. *P. pratense* is very tolerant of cold winters and therefore exceptionally suited to northern climates. (Peeters et al., 2004)

*P. pratense* is the most used grass species in Norway — more than 70% of Norwegian produced grass seeds sold belong to this species —, and its share is still increasing. Especially popular is the variety 'Grindstad', a landrace that has been in use since 1915. (Steinshamn et al., 2016)

#### 1.1.1.3 Meadow fescue (Festuca pratensis)

F. pratensis is a productive species which has good nutritive qualities that can be compared to those of L. perenne. It responds well to high nitrogen fertilisation levels, but produces good yields also at moderate levels. It is competitive, but less so than L. perenne; it is not persistent under cutting-only regimes. F. pratensis is very tolerant of cold winters and therefore especially suited to northern climates. (Peeters et al., 2004)

In Norway, its popularity has declined somewhat in recent years, though it is still a part of many mixtures. Of Norwegian produced grass seeds sold, *F. pratensis* has a share of about 15%. (Steinshamn et al., 2016)

#### 1.1.1.4 Smooth meadow-grass (Poa pratensis)

The nutritive qualities of *P. pratensis* compare unfavorably with those of *L. perenne*, especially when it comes to digestibility. It is not very productive in the first 2-3 years after sowing, but becomes more prevalent with the spread of its rhizome system and reaches a good level of productivity. It grows slowly in spring; yields in the summer are higher. *P. pratensis* is very tolerant of cold winters and therefore especially suited to northern climates. (Peeters et al., 2004)

In Norway, *P. pratensis* is often used in mixtures, though mainly in leys with a combined regime of grazing and cutting. (Steinshamn et al., 2016)

#### 1.1.1.5 Common bent (Agrostis capillaris)

A. capillaris has a lower digestibility than L. perenne or P. pratense. It does not need a high nitrogen fertilisation level, though productivity is not very high. It is not very competitive, though it is persistent and spreads with rhizomes and stolons. It grows slowly in spring; yields in the summer are higher. A. capillaris is very tolerant of cold winters and therefore exceptionally suited to northern climates. (Peeters et al., 2004)

In Norway, A. capillaris is not much used in leys, though it is often part of mixtures used for lawns and golf courses. (Steinshamn et al., 2016)

#### 1.1.1.6 Red clover (*Trifolium pratense*)

T. pratens is a highly productive species for the first 2-3 years, though not after that. It is included in mixtures because of its  $N_2$ -fixing abilities and has good nutritive qualities, though it is not as digestible as L. perenne. It may require some nitrogen fertilisation for establishment, but after that its productivity is lowered by the application of nitrogen. Cultivars that are tolerant of cold winters are available. (Frame et al., 1998)

In Norway, *T. pratense* is one of the main species included in mixtures for roughage production. (Steinshamn et al., 2016)

#### 1.1.1.7 White clover (*Trifolium repens*)

T. repens is included in mixtures beause of its  $N_2$ -fixing abilities. It has a higher digestibility than other legumes and has also good nutritive qualities otherwise. It may require some nitrogen fertilisation for establishment, but after that its productivity is lowered by the application of nitrogen. A good development of stolons is crucial for the persistence of T. repens. Cultivars that are tolerant of cold winters are available. (Frame et al., 1998)

In Norway, T. repens is often used in mixtures, though mainly in leys with a combined regime of grazing and cutting. (Steinshamn et al., 2016)

## 1.2 Mixtures

In recent years, the benefits of species mixtures in grasslands as opposed to monocultures have been the subject of several studies. In general, mixtures have been observed to produce higher yields than the average of pure stands of the component species (overyielding) and can often exceed the yields of pure stands of the most productive species (transgressive overyielding) (Cardinale et al., 2007, Finn et al., 2013). Reasons for this diversity effect include better utilisation of resources in different niche spaces (niche differentiation), positive interactions between species (facilitation) and selection of the species that are most productive under certain environmental conditions (sampling effect) (Hooper et al., 2005).

Recent studies of species mixtures containing both grass and legume species have all confirmed their positive effects on yield. Finn et al. (2013) found overyielding for more than 97% of mixtures and transgressive overyielding for ca. 60% throughout the three years of their experiment. They divided the studied species into categories according to two pairs of functional traits: N<sub>2</sub>-fixing vs. nonfixing and fast-establishing vs. temporally persistent. Interactions between species with complementary traits were found to be positive across all sites, indicating the importance of these traits for the diversity effect. Sturludóttir et al. (2014) found similar results in a study focusing on the more demanding climatic conditions of Northern Europe and Canada, using mixtures of timothy, smooth meadow-grass, red clover and white clover.

An experiment conducted in Norway by Ergon et al. (2016) — studying mixtures of perennial ryegrass, tall fescue (*Festuca arundinacea*), red clover and white clover under a low nitrogen fertilisation regime — likewise found overyielding and transgressive overyielding of mixtures as compared to pure stands. Overyielding was higher in a five-cut system as compared to a three-cut system and strongest in the second year (of three). In addition to being higher, mixture yields were also more stable through the years than those of pure stands. Positive species interactions were found between the grass species, sometimes between the grass and legume species, but not between the legume species.

Brophy et al. (2017), studying mixtures including species from the same categories as used by Finn et al., found a positive correlation between the strength of the diversity effect and the abundance of legumes in the previous year. Change in relative abundance between sown species was most strongly correlated to their relative growth rate: those with the highest relative growth rate (usually the temporally persistent grass species) became more dominant through the years. Legumes became less abundant in subsequent years, though less so for temporally persistent than for fast-establishing legumes. The positive effects of legumes were still present even when contributing less than 20% to total dry matter yield.

## 1.3 Weeds

Negative effects of weeds in cultivated grasslands include the use of resources otherwise available to the sown species — resulting in lower yields —, a decline in feed quality and the necessity of using herbicides that can be damaging to the environment; all of these entail significant costs. If seed mixtures could be composed such that weed invasion is minimised, this would have beneficial implications for the cost efficiency of roughage production.

In addition to their positive effects on yield, species mixtures have been observed to reduce weed biomass in comparison with monocultures. Some recent studies have investigated the mechanisms behind this effect in order to be able to optimise seed mixture composition. Tracy et al. (2004), though they found no correlation between species richness and weed abundance in pastures, concluded that a more even distribution of species seemed to have a suppressive effect. By contrast, Picasso et al. (2008) found an exponential decrease in weed biomass with species richness for all combinations of location and cutting regime, though there was a large effect of the inclusion of weed-suppressive species in the mixture (i.e. species that performed well in pure stands). Combinations of  $C_3$ -grasses and legumes had a lower weed biomass than plots with only  $C_3$ -grasses or only legumes; inclusion of  $C_4$ -grasses did not suppress weeds further.

Finn et al. (2013) found — in the experiment mentioned earlier — a median weed biomass lower than 4% of total yield in mixtures; for pure stands, the median percentage of weeds increased over the three years of the experiment: 15%, 20% and 32% respectively. Sturludóttir et al. (2014) found similar results, concluding that selection effects alone (i.e. the greater likelihood of the presence of a weed suppressive species in a mixture) could not be responsible for weed suppression, but that the more efficient resource use by mixtures resulted in a lower availability of these resources for weeds.

Suter et al. (2017) compared pure stands and mixtures of grass and legume species with contrasting functional traits: N<sub>2</sub>-fixing vs. nonfixing and shallow-rooted vs. deep-rooted. They found that combining species with contrasting traits increased weed suppression, and describe three mechanisms involved in this suppression: (1) higher N-availability through N<sub>2</sub>-fixing resulting in higher biomass yield and resource capture by the sown species, specifically: (2) higher interception of light by the sown species and (3) higher uptake of nitrogen by the grass species from the surface soil layer and below; this confirms similar findings by Frankow-Lindberg (2012).

In a large study, Connolly et al. (2018) found a strong weed-suppressive effect of mixtures as opposed to monocultures, mostly independent of the sown proportion of the component species (i.e. no strong effect of evenness was found). Transgressive suppression (i.e. a lower weed biomass than in the best performing monoculture) was found for all mixtures and in all of the three years of the experiment. High light interception was ensured by combining fast-establishing and temporally persistent species.

## 1.4 Questions and hypotheses

The present study wil try to answer the following questions:

- 1. What is the effect of the presence of perennial ryegrass in species mixtures on the amount of weeds present?
  - Perennial ryegrass is expected to reduce the amount of weeds in the first year, but increase the amount of weeds in the second year (because of the open spaces left due to its low winter survival).
- 2. What is the effect of a lower level of nitrogen fertilisation on the amount of weeds present?
  - As sown species are better able to make use of extra nitrogen and thereby gain a competetive edge, a lower nitrogen level is expected to favour weeds. However, differences between individual weed species may occur.
- 3. What is the effect of the (combinations of) functional traits ( $N_2$ -fixing vs. nonfixing; plant height and speed of establishment) on the amount of weeds present?
  - The amount of weeds is expected to be lower in mixtures containing a mix of the different functional traits.
- 4. How do all these factors influence yield?
  - Perennial ryegrass was found by Leraand (2018)— who studied the same experimental plots as the ones used in this study to increase yields in the first year of ley; this is not expected to be the case in the second year. The lower level of nitrogen fertilisation is expected to lower yields. Mixtures combining species with contrasting functional traits are expected to give the highest yields.

An additional objective of this study is the evaluation of the point frequency method (see "Materials and methods") for use in cultivated grasslands.

## Chapter 2

## Materials and methods

## 2.1 Experimental outline

At different locations throughout Norway, experimental plots sown with mixtures composed of different grass and clover species were established in spring/early summer 2016. The species and cultivars used and their functional traits are listed in Table 2.1. The locations considered in this study were NIBIO Kvithamar in the middle of the country  $(63^{\circ}49'N, 10^{\circ}88'E; 28 \text{ m.a.s.l.})$  and NIBIO Holt in the far north  $(49^{\circ}65'N, 18^{\circ}91'E; 14 \text{ m.a.s.l.})$ . Following a simplex design (see e.g. Cornell, 2002), at both of these locations 30 different mixtures were sown on two plots each: one would receive a normal level of nitrogen fertilisation and to the other one a lower level would be applied. In this study, only a subset of these mixtures was included, as detailed in Table 2.2. As can be seen, the mixtures were selected in such a way that — apart from the pure stands — contrasting pairs of mixtures differing only in the prensence or absence of perennial ryegrass were included. The plots — measuring 7 m by 1.5 m, of which 5.5 m by 1.5 m were harvested — were ordered randomly at each location. At Kvithamar, plots were harvested three times a year; at Holt, plots were harvested two times.

Species		Cultivar	N <sub>2</sub> -fixing?	Plant height and speed of establishment
Perennial ryegrass	Lolium perenne	Figgjo	No	High/fast
Timothy	Phleum pratense	Grindstad	No	High/fast
Meadow fescue	Festuca pratensis	Vestar	No	High/fast
Smooth meadow-grass	Poa pratensis	Knut	No	Low/slow
Common bent	Agrostis capillaris	Leikvin	No	Low/slow
Red clover	Trifolium pratense	Gandalf	Yes	High/fast
White clover	Trifolium repens	Litago	Yes	Low/slow

Table 2.1: Species and cultivars used; functional traits

In the year of establishment all plots were fertilised equally (at a moderate level), with one or two clearing cuts as necessary. Starting from the first ley year two different fertiliser treatments were applied, as detailed in Table 2.3 (Holt) and Table 2.4 (Kvithamar).

No.	L. perenne	Р.	<i>F</i> .	Р.	Α.	Т.	T. repens
		pratense	pratensis	pratens is	capillar is	pratense	
1	1	0	0	0	0	0	0
2	0	1	0	0	0	0	0
8	0.14	0.14	0.14	0.14	0.14	0.14	0.14
9	0.2	0.2	0.2	0.2	0.2	0	0
10	0	0.25	0.25	0.25	0.25	0	0
12	0	0.17	0.17	0.17	0.17	0.17	0.17
27	0	0.33	0.33	0	0	0.33	0
28	0	0	0	0.33	0.33	0	0.33
29	0.25	0.25	0.25	0	0	0.25	0
30	0.25	0	0	0.25	0.25	0	0.25

Table 2.2: Species mixtures studied (proportion of seed weight recommended for pure stands)

Table 2.3: Fertiliser treatments (A=normal, B=low level of N) at Holt from first ley year (in kg/daa; A.f.h. = After first harvest, Fullgj=Fullgjødsel (complete fertiliser))

Treat- ment	Total N	Time	Fullgj 8-5-19	Opti KAS	Fullgj 18-3-	Ν	Р	K	Ca	S
					15					
А	17	Spring	62.5	18.5		10	3.1	11.9	0.7	7.3
		A.f.h.		13	20	7	0.5	2.9	0.3	0.8
В	8.5	Spring	62.5	0		5	3.1	11.9	0.7	7.3
		A.f.h		0	20	3.5	0.5	2.9	0.3	0.8

Table 2.4: Fertiliser treatments (A=normal, B= low level of N) at Kvithamar from first ley year (in kg/daa; A.f.h. = After first harvest, A.s.h. = After second harvest, Fullgj=Fullgjødsel (complete fertiliser))

Treat-	Total	Time	Fullgj	Opti	Fullgj	Ν	Р	Κ	Ca	$\mathbf{S}$
ment	Ν		8-5-19	$\mathbf{KAS}$	18-3-					
					15					
A	24	Spring	60	18		9.6	3.0	11.4	0.7	7.0
		A.f.h.		13	20.5	7.2	0.5	3.0	0.3	0.8
		A.s.h.		13	20.5	7.2	0.5	3.0	0.3	0.8
В	12	Spring	60	0		4.8	3.0	11.4	0.7	7.0
		A.f.h		0	20.5	3.6	0.5	3.0	0.3	0.8
		A.s.h		0	20.5	3.6	0.5	3.0	0.3	0.8

## 2.2 Methods

#### 2.2.1 Botanical composition

#### 2.2.1.1 Point frequency method

In the second year of ley, approximately three weeks before the first and second harvest, the botanical composition of each plot was recorded using the point frequency method. This method, which is normally used to estimate the biomass of different species in natural vegetation, is non-destructive and has a low observer bias (Bråthen & Hagberg, 2004), and was therefore considered to be a promising way to estimate biological composition in cultivated grassland also, as was done in the present study.

A steel triangle with pins at each angle was placed at 16 positions spread more or less evenly throughout each plot, resulting in 48 pin positions per plot. For each pin, the number of contact points between the pin and a particular species (intercept frequency) was noted, with a maximum of 6 hits per species per pin (for practical considerations; see discussion). During the registrations before the first harvest, it was also noted whether the pin hit any plant litter.

## 2.2.1.2 Visual estimation

Immediately before each harvest, the botanical composition of each plot was estimated visually. The proportion of each sown species was estimated as a percentage of the total biomass; the same was done for the categories of dicotyledonous and monocotyledonous weeds, though not further divided into individual species.

#### 2.2.1.3 Separating after harvesting

Before harvesting, a representative sample of ca. 300 g was taken from each plot, leaving stubbles of ca. 6-7 cm. The samples were taken at random from multiple locations in the plots, then stored in plastic bags in a refrigerator (or, if stored for longer than a week, in a freezer). Samples were separated into individual sown species and dicotyledonous and monocotyledonous weeds (not further divided into species, except for the first harvest of the second ley year). Dry matter weight was then determined after drying the samples for 48 hours at  $60^{\circ}$ C.

## 2.2.2 Yield

Yields for each plot were weighed with the Haldrup harvester; dry matter yields were then determined on the basis of the dried samples described above.

## 2.2.3 Phenology

Samples of at least 30 shoots were taken from edge plots (or outer edges of experimental plots) containing pure stands of timothy fertilised at the moderate level. This was done every time when point frequency method registrations took place. On the basis of these samples, the mean stage count was determined (a measurement of phenological stage; see Bakken et al., 2005). The results are shown in Table 2.5.

Table 2.5: Mean stage count of timothy at the time of the point frequency method registrations

Harvest	Kvithamar	Holt
1	1.49	1.60
2	1.51	1.41

## 2.3 Data analysis

Data were analysed using multi-way ANOVA, with the following factors:

- 1. Inclusion of perennial ryegrass in the mixture (True or False)
- 2. Level of nitrogen fertilisation (normal or low)
- 3. Location (Kvithamar or Holt)
- 4. Mixture (1-4, see Table 2.6)

Table 2.6: Species mixtures analysed

No.	L. perenne	P. pratense	F. pratensis	P. pratensis	A. capillaris	T. pratense	T. repens
1	X/-	Х	Х	Х	Х	Х	Х
2	X/-	Х	Х	Х	Х	-	-
3	X/-	Х	Х	-	-	Х	-
4	X/-	-	-	Х	Х	-	Х

All analyses were done in Python, using the packages pandas and statsmodels. Visualisations were made using the packages seaborn and matplotlib.

## Chapter 3

## Results

All figures should be read in the following way:

- R: Inclusion of perennial ryegrass (True or False)
- N: Level of nitrogen fertilisation (H=moderate, L=low)
- S: Location (K=Kvithamar, H=Holt)
- B: Species composition (R=pure stand of perennial ryegrass, T=pure stand of timothy, for 1-4 see Table 2.6)

Data from pure stands are only included in figures comparing species compositions. In bar plots visualising interactions, error bars indicate 95% confidence intervals. All ANOVA tables can be found in the appendix.

## 3.1 First year of ley

### 3.1.1 Yield

An analysis of dry matter yield in the first year of ley showed that all factors had a significant effect (nitrogen fertilisation level: p < 0.001, presence or absence of perennial ryegrass: p < 0.001, species composition: p = 0.002 and location: p < 0.001). There was a significant interaction between presence of ryegrass (p = 0.006) and species composition and a tendency towards interaction between presence of ryegrass and location (p = 0.06).

The effects of all factors are shown in Figure 3.1. It can be seen that presence of perennial ryegrass increased yield and that a lower level of N fertilisation decreased yield; yields were generally higher at Kvithamar than at Holt. Compositions 1 and 3 performed better than compositions 2 and 4.

Interactions are shown in Figure 3.2. The effect of perennial ryegrass on yield was stronger in composition 4 than in the others; the effect also seems to have been somewhat larger at Holt than at Kvithamar.



Figure 3.1: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on yield (in kg DM per daa) in the first year of ley.



Figure 3.2: Interaction between presence or absence of perennial ryegrass and (a) species composition and (b) location; effect on yield (in kg DM per daa) in the first year of ley.

#### 3.1.2 Total amount of weeds

#### 3.1.2.1 First harvest

An analysis of the total amount of weeds (as DM yield) as determined by separating after harvesting showed significant effects for presence or absence of perennial ryegrass (p = 0.001), species composition (p = 0.002) and location (p < 0.001). Interactions between presence of ryegrass and location (p = 0.006) and between species composition and location (p = 0.02) were also significant.

Figure 3.3 shows the effects of the different factors. Presence of ryegrass reduced the amount of weeds; more weeds were present at Holt than at Kvithamar; plots with composition 4 had most weeds, while those with composition 3 had least. Interactions are shown in Figure 3.4. The effects of ryegrass and species composition were more pronounced at Holt than at Kvithamar.



Figure 3.3: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on total amount of weeds (in kg DM per daa) in the first year of ley (first harvest).



Figure 3.4: Interaction between (a) location and presence or absence of perennial ryegrass and (b) species composition and location; effect on total amount of weeds (in kg DM per daa) in the first year of ley (first harvest).

#### 3.1.2.2 Second harvest (Holt)

An analysis of the total amount of weeds (as DM yield) as determined by separating after harvesting showed significant effects for presence or absence of perennial ryegrass (p = 0.02) and a tendency towards an effect for species composition (p = 0.08). There was a tendency towards interaction between presence of ryegrass and species composition (p = 0.07).

Figure 3.5 shows the effect of all factors. There were less weeds where perennial ryegrass was present, and more weeds in composition 4 than in the others. Figure 3.6 shows the interaction between presence of ryegrass and species composition; it can be seen that there only was a higher amount of weeds in composition 4 if no perennial ryegrass was present.



Figure 3.5: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation and (c) species composition on total amount of weeds (in kg DM per daa) in the first year of ley (second harvest at Holt).



Figure 3.6: Interaction between presence or absence of perennial ryegrass and species composition; effect on total amount of weeds (in kg DM per daa) in the first year of ley (second harvest at Holt).

#### 3.1.2.3 Third harvest (Kvithamar)

An analysis of the total amount of weeds (in DM yield) as determined by separating after harvesting showed significant effects for level of nitrogen fertilisation (p = 0.01) and species composition (p = 0.002) and a tendency towards an effect for presence or absence of perennial ryegrass (p = 0.08). Interactions betweeen level of nitrogen fertilisation and species composition (p = 0.006) and presence of ryegrass and species composition (p = 0.009) were also significant.

Figure 3.7 shows the effects of all factors. Higher amounts of weeds occured at the higher level of nitrogen fertilisation and in compositions 4 (especially) and 2; the effects of ryegrass were somewhat unclear. Figure 3.8 shows how the amount of weeds in composition 4 was higher when ryegrass was absent and when the level of nitrogen fertilisation was higher.



Figure 3.7: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation and (c) species composition on total amount of weeds (in kg DM per daa) in the first year of ley (third harvest at Kvithamar).



Figure 3.8: Interaction between (a) presence or absence of perennial ryegrass and species composition and (b) nitrogen fertilisation level and species composition; effect on total amount of weeds (in kg DM per daa) in the first year of ley (third harvest at Kvithamar).

#### 3.1.2.4 Visual estimation

Total DM yield of weeds for the first ley year were determined on the basis of visual estimations. An analysis showed that the effects of presence or absence of perennial ryegrass (p < 0.001), species composition (p < 0.001) and location (p < 0.001) were significant and that there was a tendency towards an effect of the level of nitrogen fertilisation (p = 0.08). Interaction between presence of ryegrass and species composition (p = 0.01), presence of ryegrass and location (p = 0.007) and species composition and location (p = 0.008) were also significant.

Figure 3.9 shows the effects of all factors. Presence of perennial ryegrass reduced the amount of weeds; less weeds were present at Kvithamar than at Holt; composition 3 had the least amount of weeds, composition 4 the most. Figure 3.10 shows how the effect of ryegrass and species composition were more pronounced at Holt than at Kvithamar and how the effect of ryegrass was especially pronounced in composition 4.



Figure 3.9: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on total amount of weeds (in kg DM per daa) in the first year of ley (sum of all harvests; visual estimation).



Figure 3.10: Interaction between (a) location and presence or absence of perennial ryegrass, (b) species composition and location and (c) species composition and presence or absence of perennial ryegrass; effect on total amount of weeds (in kg DM per daa) in the first year of ley (sum of all harvests; visual estimation).

## 3.2 Second year of ley

#### 3.2.1 Yield

An analysis of dry matter yield in the second year of ley showed that all factors were significant (p < 0.001 for all factors) and that there was a significant interaction between species composition and location (p = 0.003).

In Figure 3.11, the effects of all factors are visualised. While the presence of perennial ryegrass produced higher yields in the first year of ley, in the second year the effect was reversed. The effects of level of nitrogen fertilisation and location were comparable to those in the first year (higher yields with a higher level of nitrogen fertilisation; higher yields at Kvithamar). The effect of species composition was also similar to the first year (compositions 1 and 3 performed better), but was stronger now. The yields of the pure stands of perennial ryegrass, however, had dropped in the second year.

Figure 3.12 shows the interaction between species composition and location; the differences between the compositions were larger at Kvithamar than at Holt.



Figure 3.11: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on yield (in kg DM per daa) in the second year of ley.



Figure 3.12: Interaction between species composition and location; effect on yield (in kg DM per daa) in the second year of ley.

#### 3.2.2 Total amount of weeds

#### 3.2.2.1 First harvest

An analysis of the total amount of weeds (as DM yield) as determined by separating after harvesting showed significant effects for species composition (p < 0.001) and location (p < 0.004) and a significant interaction between these two factors (p < 0.01).

Figure 3.13 shows the effects of all factors: there were more weeds at Holt than at Kvithamar and more weeds in composition 4 than in the other ones. Figure 3.14 shows how the effect of species composition was far stronger at Holt than at Kvithamar.



Figure 3.13: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on total amount of weeds (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.14: Interaction between species composition and location; effect on total amount of weeds (in kg DM per daa) in the second year of ley (first harvest; separated).

Another analysis of the total amount of weeds was made on the basis of the data obtained using the point frequency method. In addition to species composistion (p = 0.005) and location (p = 0.03), also presence or absence of perennial ryegrass was found to be significant here (p = 0.05). There was also a significant interaction between presence of ryegrass and species composition (p = 0.02).

Figure 3.15 shows the effects of all factors. Presence of ryegrass resulted in less weeds; there were more weeds at Holt than at Kvithamar; composition 4 had more weeds than the other compositions. Pure stands of ryegrass and (especially) timothy also had a large amount of weeds.

Figure 3.16 shows how the effect of species composition was only present when no ryegrass was included in the mixture.



Figure 3.15: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on total amount of weeds (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.16: Interaction between species composition and presence or absence of perennial ryegrass; effect on total amount of weeds (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).

#### 3.2.2.2 Second harvest

The total amount of weeds for the second harvest was only analysed on the basis of the data obtained using the point frequency method. The analysis showed significant effects for species composition (p = 0.03) and location (p < 0.001) and a significant interaction between these two factors (p = 0.03). There was also a tendency towards interaction between presence of ryegrass and species composition (p = 0.09).

Figure 3.17 shows the effects of all factors. There were more weeds at Holt than at Kvithamar and composition 4 had more weeds than the other compositions. Pure stands of ryegrass and timothy had a larger amount of weeds than any mixture.

Figure 3.18 shows how the effect of species composition was only present at Holt and how composition 4 only had more weeds when no ryegrass was included in the mixture.



Figure 3.17: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on total amount of weeds (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).



Figure 3.18: Interaction between (a) species composition and location and (b) species composition and presence or absence of perennial ryegrass; effect on total amount of weeds (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### 3.2.2.3 Visual estimation

Total DM yield of weeds for the second ley year were determined on the basis of visual estimations. An analysis showed that the effects of species composition (p = 0.05) and location (p < 0.001) were significant. There were no significant interactions.

Figure 3.19 shows the effects of all factors. Less weeds were present at Kvithamar than at Holt and composition 4 had the largest amount of weeds.



Figure 3.19: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on total amount of weeds (in kg DM per daa) in the second year of ley (sum of all harvests; visual estimation).

#### 3.2.3 Individual weed species

In this section the results for selected individual weed species are shown. Since most weed species only occurred at one of the locations, the number of observations was limited and a meaningful statistical analysis was not possible. Only for *T. officinale* ANOVAs were performed, for the other species the results are only presented visually. Species with a negligable number of observations are not included.

#### 3.2.3.1 Northern dock (Rumex longifolius)

*R. longifolius* was only found at Holt. The effects of the different factors on the amount of *R. longifolius* at the first harvest are shown in Figure 3.20 (in kg DM per data as measured after harvesting and separating) and Figure 3.21 (in average number of hits per pin as measured using the point frequency method). Figure 3.22 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

The data obtained from separating suggested a higher amount of R. longifolius at higher levels of nitrogen fertilisation; while this was not supported by the point frequency method data from the first harvest, the point frequency method data from the second harvest showed a similar effect. The data obtained from separating showed a higher amount of R. longifolius in composition 4 and — in one case — in composition 1. The point frequency method data from both harvests showed higher amounts of R. longifolius in composition 1, 3 and 4, with composition 2 having least. None of the data sets showed a clear effect of the presence or absence of perennial ryegrass.



Figure 3.20: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of R. longifolius (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.21: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of R. longifolius (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.22: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of R. *longifolius* (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### 3.2.3.2 Creeping buttercup (Ranunculus repens)

*R. repens* was only found at Holt. The effects of the different factors on the amount of *R. repens* at the first harvest are shown in Figure 3.23 (in kg DM per data as measured after harvesting and separating) and Figure 3.24 (in average number of hits per pin as measured using the point frequency method).

Figure 3.25 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

The data obtained from separating suggested a higher amount of R. repens when perennial ryegrass was absent; composition 4 had more R. repens than the other mixtures. The same tendencies could be observed in the point frequency method data from both harvests.



Figure 3.23: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of R. repens (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.24: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of R. repens (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.25: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of R. repens (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### 3.2.3.3 Common dandelion (Taraxacum officinale)

T. officinale was found at both Holt and Kvithamar. An analysis of the dry matter yield of T. officinale at the first harvest only showed a tendency towards an effect for location. An analysis of the data obtained using the point frequency method showed significant effects for location (p < 0.001) and species composition (p = 0.03) and a tendency towards an effect for presence or absence of perennial ryegrass (p = 0.06). The interaction between species composition and location was also significant effect (p = 0.05). For the second harvest, the point frequency method data only showed a significant effect for location (p < 0.001).

The effects of all factors are shown in Figure 3.26 (first harvest; DM yield), Figure 3.27 (first harvest; point frequency method) and Figure 3.29 (second harvest; point frequency method). Common for all datasets was that T. officinale was more abundant at Kvithamar than at Holt. In addition, the point frequency method data from the first harvest shows that there was a higher presence of T. officinale in composition 4 — Figure 3.28 shows that this effect was only present at Kvithamar.



Figure 3.26: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. officinale (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.27: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. officinale (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.28: Interaction between species composition and location; effect on amount of T. officinale (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.29: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. officinale (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### 3.2.3.4 Thyme-leaved speedwell (Veronica serpyllifolia)

V. serpyllifolia was only present at Holt. The effects of the different factors on the amount of V. serpyllifollia at the first harvest are shown in Figure 3.30 (in kg DM per daa as measured after harvesting and separating) and Figure 3.32 (in average number of hits per pin as measured using the point frequency method). Figure 3.33 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

The data obtained from separating suggested a higher amount of V. serpyllifollia at the lower level of nitrogen fertilisation; composition 4 had more V. serpyllifollia than the other mixtures. Figure 3.31 shows that composition 4 only had more V. serpyllifollia at the lower nitrogen fertilisation level. The point frequency method data from both harvests also suggested an effect for the lower nitrogen fertilisation level, but the effect of species composition was absent.



Figure 3.30: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of V. serpyllifolia (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.31: Interaction between species composition and level of nitrogen fertilisation; effect on amount of V. *serpyllifolia* (in kg DM per daa) in the second year of ley (first harvest; sorted).



Figure 3.32: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of V. serpyllifolia (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.33: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of V. serpyllifolia (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### 3.2.4 Sown species

As with the weed species, the results for the sown species are only presented visually; since none of the species was included in all mixtures, the number of observations was limited, making statistical analysis more difficult. In addition, the seed ratios used differed between the mixtures; this would introduce an additional factor that would not have been taken account of in the analysis.

#### 3.2.4.1 Perennial ryegrass (Lolium perenne)

The effects of the different factors on the amount of L. perenne at the first harvest are shown in Figure 3.34 (in kg DM per data as measured after harvesting and separating) and Figure 3.35 (in average number of hits per pin as measured using the point frequency method). Figure 3.36 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

While the data obtained from separating suggested a higher amount of L. perenne at a higher level of nitrogen fertilisation, this effect was absent from the point frequency method data. For the first harvest, both methods showed a somewhat higher amount of L. perenne at Kvithamar than at Holt; this effect was not visible for the second harvest. The data obtaned from separating and the point frequency method data for the second harvest showed that composition 4 had more L. perenne than the other mixtures; this effect was not clear in the point frequency method data for the first harvest. The pure stands of L. perenne performed variably, though at the second harvest they had a higher yield of this species than any of the mixtures.



Figure 3.34: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of L. perenne (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.35: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of L. perenne (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.36: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of L. perenne (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### **3.2.4.2** Timothy (*Phleum pratense*)

The effects of the different factors on the amount of P. pratense at the first harvest are shown in Figure 3.37 (in kg DM per data as measured after harvesting and separating) and Figure 3.38 (in average number of hits per pin as measured using the point frequency method). Figure 3.39 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

Data for the first harvest from both methods showed a weak increase in the amount of P. pratense when perennial ryegrass was absent. This effect could not be observed for the second harvest. All datasets showed a higher amount of P. pratense at the higher level of nitrogen fertilisation. For the first harvest, both methods showed a somewhat higher amount of P. pratense at Kvithamar than at Holt; this effect was not clear for the second harvest. None of the datasets showed clear differences between the mixtures that contained P. pratense, though composition 2 seemed to have most P. pratense at the first harvest, while composition 1 seemed to have most at the second harvest. The pure stands of P. pratense had a much higher yield of this species than any of the mixtures.



Figure 3.37: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of P. pratense (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.38: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of P. pratense (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.39: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of P. pratense (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### **3.2.4.3** Meadow fescue (*Festuca pratensis*)

The effects of the different factors on the amount of F. pratensis at the first harvest are shown in Figure 3.40 (in kg DM per data as measured after harvesting and separating) and Figure 3.41 (in average number of hits per pin as measured using the point frequency method). Figure 3.42 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

For the first harvest, both methods showed a higher amount of F. pratensis when perennial ryegrass was absent; for the second harvest, this effect was not clear. A weak effect of the level of nitrogen fertilisation was visible at the second harvest (more F. pratensis at the higher level), but not at the first harvest. There was more F. pratensis at Holt than at Kvithamar, at least at the second harvest; the point frequency method data for the first harvest did not show a clear difference. No great differences were observed between the mixtures that contained F. pratensis, though perhaps composition 1 gave a somewhat lower yield of this species.



Figure 3.40: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of F. pratensis (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.41: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of F. pratensis (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.42: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of F. pratensis (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### 3.2.4.4 Smooth meadow-grass (Poa pratensis)

The effects of the different factors on the amount of P. pratensis at the first harvest are shown in Figure 3.43 (in kg DM per data as measured after harvesting and separating) and Figure 3.44 (in average number of hits per pin as measured using the point frequency method). Figure 3.45 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

For the first harvest, both methods showed a higher amount of P. pratensis when perennial ryegrass was absent; for the second harvest, this effect was still present, but less clear. No clear effects of the level of nitrogen fertilisation were visible. There was generally more P. pratensis at Holt than at Kvithamar, especially at the second harvest. Of the mixtures that contained P. pratensis, composition 4 consistently had the highest amount of this species, while composition 2 consistently had the lowest amount.



Figure 3.43: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of *P. pratensis* (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.44: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of P. pratensis (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.45: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of P. pratensis (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### **3.2.4.5** Common bent (Agrostis capillaris)

The effects of the different factors on the amount of *A. capillaris* at the first harvest are shown in Figure 3.46 (in kg DM per data as measured after harvesting and separating) and Figure 3.47 (in average number of hits per pin as measured using the point frequency method). Figure 3.48 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

The amount of A. capillaris observed was in general very low. No clear conclusions could be drawn regarding the amount of A. capillaris when perennial ryegrass was present or absent, though perhaps there was slightly more A. capillaris when ryegrass was absent. An effect of the level of nitrogen fertilisation was visible at the second harvest (more A. capillaris at the lower level), but not at the first harvest. There was somehat more A. capillaris at Kvithamar than at Holt, at least at the first harvest as measured with the point frequency method. At the first harvest, composition 4 contained the highest amount of A. capillaris and composition 1 contained the lowest amount; at the second harvest, A. capillaris was much more evenly distributed among the mixtures, though composition 2 contained somewhat more than the other two.



Figure 3.46: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of *A. capillaris* (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.47: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of *A. capillaris* (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.48: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of *A. capillaris* (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### **3.2.4.6** Red clover (*Trifolium pratense*)

The effects of the different factors on the amount of T. pratense at the first harvest are shown in Figure 3.49 (in kg DM per data as measured after harvesting and separating) and Figure 3.50 (in average number of hits per pin as measured using the point frequency method). Figure 3.51 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

For the first harvest, the point frequency method data showed a higher amount of T. pratense when perennial ryegrass was absent; the other datasets, however, did not show the same effect. The amount of T. pratense was higher at the lower level of nitrogen fertilisation in all datasets. There was somewhat more T. pratense at Kvithamar than at Holt, but this effect was not very clear at the second harvest. Composition 3 consistently contained more T. pratense than composition 1.



Figure 3.49: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. pratense (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.50: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. pratense (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.51: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. pratense (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### **3.2.4.7** White clover (*Trifolium repens*)

The effects of the different factors on the amount of T. repens at the first harvest are shown in Figure 3.52 (in kg DM per data as measured after harvesting and separating) and Figure 3.53 (in average number of hits per pin as measured using the point frequency method). Figure 3.54 shows the effects of all factors at the second harvest (only data obtained using the point frequency method were available here).

No clear effect of the presence or absence perennial ryegrass were present. There was more T. repens at the lower level of nitrogen fertilisation, but this effect was not very clear at the first harvest. There was consistently more T. repens at Kvithamar than at Holt. Composition 4 consistently contained more T. repens than composition 1.



Figure 3.52: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. repens (in kg DM per daa) in the second year of ley (first harvest; separated).



Figure 3.53: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. repens (in average number of hits per pin) in the second year of ley (first harvest; point frequency method).



Figure 3.54: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of T. repens (in average number of hits per pin) in the second year of ley (second harvest; point frequency method).

#### 3.2.5 Plant litter

The amount of plant litter before the first harvest was determined using the point frequency method. An analysis of the data showed significant effects for presence of absence of perennial ryegrass (p < 0.001) and species composition (p = 0.002) and a tendency towards an effect for location (p = 0.06). There was also a significant interaction between species composition and location (p = 0.008).

Figure 3.55 shows the effects of all factors. There was more plant litter where perennial ryegrass was present; there was more plant litter in composition 2 than in the other mixtures, but most in the pure stands of perennial ryegrass. There was somewhat more plant litter at Holt than at Kvithamar. Figure 3.56 shows how there was less plant litter in composition 1 at Kvithamar than at Holt, while the other mixtures performed similarly at both locations.



Figure 3.55: Effect of (a) presence or absence of perennial ryegrass, (b) level of nitrogen fertilisation, (c) location and (d) species composition on amount of plant litter (in fraction of pins hit) in the second year of ley (first harvest; point frequency method).



Figure 3.56: Interaction between species composition and location; effect on amount of plant litter (in fraction of pins hit) in the second year of ley (first harvest; point frequency method).

## Chapter 4

# Discussion

## 4.1 Yield

An analysis of the yield data for the first year of ley was done earlier by Leraand (2018), though this analysis also included data from NIBIO Særheim in the west of the country. When included, perennial ryegrass was dominant and contributed to higher yields than were obtained in the plots that did not contain this species. The effect of the other species in the mixtures was diminished when ryegrass was present, resulting in only small yield differences between the compositions. Without ryegrass, however, compositions 1 (all species) and 3 (only the fast-establishing species) outperformed the other two; composition 4, containing only the slowly establishing species, performed clearly worst.

In the second year of ley, the effect of perennial ryegrass on yield was reversed: yields were consistently lower when perennial ryegrass was included. This can be explained by the combination of the dominance of this species in the first year — hindering the establishment of the other species — and a low level of winter survival (Peeters et al., 2004). Ergon et al. (2016) also found this combination of dominance in the first year and decline in the second year. As in the first year, compositions 1 and 3 performed better than compositions 2 (all grasses) and 4, especially at Kvithamar — now also when ryegrass was included in the mixture, since the dominance of this species was no longer present. The high-performing mixtures were those that included fast-establishing species and at least one N<sub>2</sub>-fixing legume, confirming the findings of Finn et al. (2013) that the combination of N<sub>2</sub>-fixing and nonfixing species has a positive diversity effect, though not supporting their findings of a similar effect from the combination of fast-establishing and temporally persistent species. Perhaps a positive contribution of the slowly establishing species will be more visible in subsequent years.

In both years, the moderate level of nitrogen fertilisation gave consistently higher yields than the lower level; this was in line with earlier findings, e.g. Lunnan et al. (2017). A breakdown of the effects of the level of nitrogen fertilisation on the different sown species follows under "Individual species".

Though pure stands were not included in the statistical analysis, the highest yields in both years were from mixtures, providing another example of the transgressive<sup>1</sup> overyielding of mixtures that has been observed in earlier studies (e.g. Finn et al., 2013).

## 4.2 Total amount of weeds

The analysis of weed presence was made more difficult by the very low amount of weeds at Kvithamar in both years; this resulted in a heavy reliance on the data from only one location — Holt — for data that showed significant differences.

In the first year of ley, the presence of perennial ryegrass resulted in a lower amount of weeds (except at the third harvest at Kvithamar). The dominance of ryegrass in this year (Leraand, 2018) can very well explain this, since it is a highly competitive species with the potential to suppress other species (Peeters et al., 2004). These findings were also in line with Picasso et al.'s (2008) conclusion that a single species in a mixture can have a large suppressive effect. Since the dominance of perennial

<sup>&</sup>lt;sup>1</sup>Since only pure stands of two species were studied, the conclusion of transgressive overyielding cannot strictly be drawn; though, since *L. perenne* and *P. pratense* are among the most productive of the species included in the mixtures, the conclusion is likely.

ryegrass was no longer present in the second year of ley, it seems somewhat surprising that the inclusion of this species in the mixture still resulted in a lower amount of weeds — though this effect was only significant at the first harvest as measured with the point frequency method. An explanation might be that the dominance of perennial ryegrass in the previous year did not give the weeds a chance to establish themselves, resulting in a lower presence also in the second year. Another explanation might be that the low winter survival of perennial ryegrass resulted in a large amount of plant litter covering the soil, which then hindered the weeds from sprouting; it should be noted that the suppressive effect of ryegrass was strongest when measured with the point frequency method (i.e. three weeks before the first harvest, the earliest measuring date).

Comparing the different mixtures, composition 4 had consistently more weeds than the other three compositions, which performed similarly. The absence of fast-establishing species seems therefore to have been the deciding factor here, especially considering the fact that perennial ryegrass had the strongest relative suppressive effect in this composition (ryegrass being fast-establishing, it would have "taken over" the role of timothy and meadow fescue). The suppressive effect of mixtures as compared to pure stands (as found in earlier studies such as Connolly et al. (2017)) was only found in the data from the point frequency method, though there it was very clear. I must express my surprise that similar results were not obtained with the other methods, since the weed abundance in the pure stands seemed obvious to me when I observed the plots.

Somewhat surprisingly, the level of nitrogen fertilisation was not a factor in the total amount of weeds. This seems contrary to the findings by Suter et al. (2017) that the higher biomass of sown species resulting from higher nitrogen availability would have a suppressive effect. Though the biomass of the sown species was indeed lower at the lower level of nitrogen fertilisation (see under "Yield"), this did not seem to affect the amount of weeds present.

## 4.3 Individual species

#### 4.3.1 Sown species

At the first harvest of the second year of ley, the presence of perennial ryegrass resulted in lower amounts of meadow fescue and smooth meadow-grass and, to a certain extent, of timothy (and perhaps red clover). At the second harvest, these effects had disappeared or — in the case of smooth meadowgrass — weakened. This can be explained by the dominance of ryegrass in the previous year, which hindered the establishment of the other species. By the second harvest, due to the lower presence of ryegrass during the second year, the other species were able to claim the space previously occupied by ryegrass. It is unclear how the differences between the species (i.e. why the effect was strongest for meadow fescue and smooth meadow-grass) should be explained.

Of the grass species, the only one that showed a strong effect from the level of nitrogen fertilisation was timothy, which saw a clear reduction at the lower level. For the other grasses, effects were either absent or unclear (a lower amount of perennial ryegrass at the lower level was only seen from the separation data; an increase of common bent at the lower level was only seen at the second harvest). It seems, then, that timothy was solely responsible for the lower yields at the lower level of nitrogen fertilisation. This confirms the findings of Lunnan et al. (2017) that timothy yields were increased by a higher level of nitrogen fertilisation, while meadow fescue and smooth meadow-grass were not affected (perennial ryegrass and common bent were not studied). It does not confirm Peeters et al.'s (2004) observation that perennial ryegrass and meadow fescue perform best under high levels of nitrogen fertilisation, though it is in line with their statement that common bent does not need these.

The clover species both performed better at the lower level of nitrogen fertilisation (though this was somewhat less clear for white clover); this was as expected, since their  $N_2$ -fixing abilities give them a competitive edge at lower levels of nitrogen in the soil. Lunnan et al. (2017) found similar effects.

Perennial ryegrass, timothy, common bent, red clover and white clover were more abundant at Kvithamar than at Holt (to varying degrees), while there was more meadow fescue and smooth meadow-grass at Holt than at Kvithamar. It is difficult to pinpoint specific reasons why this would be so, and the varieties used may have been of some influence here.

Perennial ryegrass performed best in composition 4; since this mixture contained no other fastestablishing species, perennial ryegrass could take on this role without competition. Smooth meadowgrass performed worst in composition 2, which did not include any clover species; meadow fescue was not affected by the absence of clover (if anything, it performed better). This contradicts the findings of Lunnan et al. (2017), who saw a decrease of meadow fescue when clover was included in the mixture, while smooth meadow-grass was unaffected. Smooth meadow-grass performed best in composition 4, where it had the least amount of competition from other grass species (the amount of common bent was very low overall). Common bent also performed best in composition 4, at least at the first harvest. For timothy, no clear differences between the compositions were observed. Both clover species performed best in the mixture in which the other clover species was not present, though it cannot be concluded that this was the underlying reason.

### 4.3.2 Weed species

Because of the limited number of observations, it was very difficult to draw definite conclusions on individual weed species. Though some tendencies could be noted (less northern dock at the lower level of nitrogen fertilisation; more creeping buttercup when perennial ryegrass was absent; most creeping buttercup in composition 4), these should by no means be considered as clear results. For that, a larger experiment including more locations with am abundant and diverse weed flora would be needed.

## 4.4 Evaluation of the point frequency method

In order to find the relation between intercept frequency and biomass, a calibration has to be performed, such that conversion factors can be obtained for different (groups of) species (Bråthen & Hagberg, 2004). This, however, was not done in the present study. When assessing the relative prominence of individual species between plots, this was not a problem. For the measurement of the total amount of weeds, however, such a calibration should have been included; fortunately, since the weed populations were mostly dominated by single species (northern dock at Holt, common dandelion at Kvithamar) — which, on account of their size, would have similar conversion factors — this problem seems to have been mitigated.

In general, results from the point frequency method were mostly in agreement with the data obtained by separating after harvesting. Some of the differences that were observed (e.g. concerning the effect of nitrogen fertilisation on northern dock) could be accounted for by the fact that the point frequency method registrations were performed three weeks before the harvest — the effect only becoming measurable after that time —, while in the case of the suppressive effect of mixtures as opposed to pure stands, I suspect that the separation data did not reflect reality (see above).

While the data obtained with the point frequence method thus seem to be quite accurate in this study, the use of this method in cultivated grassland has some limitations. At the times when I performed my registrations, the stands had become quite dense, making it very difficult to make precise counts for all species. As a result, I was often forced to make estimations instead of registering the exact number. The sometimes quite windy conditions made this problem even worse. Also as a result from the density of the stands, a maximum of 6 hits per species per pin had to be implemented, as higher numbers were difficult to ascertain with any level of precision. These difficulties made the registration process quite time consuming, though I cannot compare the time required with that of the separation method, as I did not perform those registrations myself.

In conclusion, the point frequency method is a possible, non-destructive, alternative for estimating the botanical composition in cultivated grassland, though it has obvious limitations. Those limitations might be less problematic if registrations are performed at an earlier phonogical stage, when stands are less dense.

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# Appendix

All tables should be read in the following way:

- N: Level of nitrogen fertilisation
- R: Inclusion of perennial ryegrass
- B: Species composition
- S: Location

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	185440.50	185440.50	34.51	$5.46 \times 10^{-5}$
R	1.0	209952.00	209952.00	39.07	$2.97 \times 10^{-5}$
B	3.0	144185.25	48061.75	8.94	$1.78 \times 10^{-3}$
S	1.0	524800.13	524800.13	97.67	$2.06 \times 10^{-7}$
N:R	1.0	180.50	180.50	0.03	$8.57  imes 10^{-1}$
N:B	3.0	16025.25	5341.75	0.99	$4.26  imes 10^{-1}$
R:B	3.0	108906.75	36302.25	6.76	$5.50  imes 10^{-3}$
N:S	1.0	120.13	120.13	0.02	$8.83 \times 10^{-1}$
R:S	1.0	23005.12	23005.12	4.28	$5.90 \times 10^{-2}$
B:S	3.0	22942.13	7647.38	1.42	$2.81 \times 10^{-1}$
Residual	13.0	69851.75	5373.21		

Table 4.1: ANOVA of yield in the first year of ley

Table 4.2: ANOVA of the total amount of weeds (in kg DM per daa) in the first year of ley (first harvest)

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	26.4028	26.4028	0.0709	0.7943
R	1.0	6488.6269	6488.6269	17.4154	0.0011
B	3.0	9367.2533	3122.4178	8.3805	0.0023
S	1.0	9653.3776	9653.3776	25.9095	0.0002
N:R	1.0	185.7098	185.7098	0.4984	0.4926
N:B	3.0	1235.8956	411.9652	1.1057	0.3821
R:B	3.0	1821.3261	607.1087	1.6295	0.2308
N:S	1.0	7.5884	7.5884	0.0204	0.8887
R:S	1.0	4006.4452	4006.4452	10.7532	0.0060
B:S	3.0	4959.1520	1653.0507	4.4368	0.0235
Residual	13.0	4843.5490	372.5807		

Table 4.3: ANOVA of the total amount of weeds (in kg DM per daa) in the first year of ley (second harvest at Holt)

	df	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	6.003	6.003	1.467	0.313
R	1.0	93.123	93.123	22.759	0.017
В	3.0	77.845	25.948	6.342	0.082
N:R	1.0	4.840	4.840	1.183	0.356
N:B	3.0	9.113	3.038	0.742	0.594
R:B	3.0	86.122	28.707	7.016	0.072
Residual	3.0	12.275	4.092		

Table 4.4: ANOVA of the total amount of weeds (in kg DM per daa) in the first year of ley (third harvest at Kvithamar)

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	1.266	1.266	29.926	0.012
R	1.0	0.276	0.276	6.517	0.084
В	3.0	11.607	3.869	91.483	0.002
N:R	1.0	0.031	0.031	0.724	0.457
N:B	3.0	5.032	1.677	39.660	0.006
R:B	3.0	4.182	1.394	32.961	0.009
Residual	3.0	0.127	0.042		

Table 4.5: ANOVA of the total amount of weeds (in kg DM per daa) in the first year of ley (sum of all harvests; visual estimation)

	df	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	1583.156	1583.156	3.721	$7.58\times10^{-2}$
R	1.0	7612.546	7612.546	17.894	$9.83  imes 10^{-4}$
B	3.0	16461.179	5487.060	12.898	$3.41 \times 10^{-4}$
S	1.0	34952.358	34952.358	82.159	$5.56 \times 10^{-7}$
N:R	1.0	371.418	371.418	0.873	$3.67 \times 10^{-1}$
N:B	3.0	2319.452	773.151	1.817	$1.94 \times 10^{-1}$
R:B	3.0	6703.874	2234.625	5.253	$1.36 \times 10^{-2}$
N:S	1.0	612.150	612.150	1.439	$2.52  imes 10^{-1}$
R:S	1.0	4402.973	4402.973	10.350	$6.74 \times 10^{-3}$
B:S	3.0	7767.275	2589.092	6.086	$8.11 \times 10^{-3}$
Residual	13.0	5530.487	425.422		

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	83834.89	83834.89	20.14	$6.10 \times 10^{-4}$
R	1.0	114373.49	114373.49	27.48	$1.59  imes 10^{-4}$
B	3.0	332239.34	110746.45	26.61	$8.04 \times 10^{-6}$
S	1.0	375952.88	375952.88	90.34	$3.23 \times 10^{-7}$
N:R	1.0	15.54	15.54	0.00	$9.52 \times 10^{-1}$
N:B	3.0	30652.20	10217.40	2.46	$1.09  imes 10^{-1}$
R:B	3.0	15216.75	5072.25	1.22	$3.42 \times 10^{-1}$
N:S	1.0	40.28	40.28	0.01	$9.23  imes 10^{-1}$
R:S	1.0	1580.63	1580.63	0.38	$5.48 \times 10^{-1}$
B:S	3.0	99782.38	33260.79	7.99	$2.84\times10^{-3}$
Residual	13.0	54101.47	4161.65		

Table 4.6: ANOVA of yield in the second year of ley

Table 4.7: ANOVA of the total amount of weeds (in kg DM per daa) in the second year of ley (first harvest; separated)

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	15.961	15.961	0.358	0.560
R	1.0	76.880	76.880	1.724	0.212
В	3.0	2276.876	758.959	17.024	$8.7  imes 10^{-5}$
S	1.0	526.501	526.501	11.810	0.004
N:R	1.0	10.580	10.580	0.237	0.634
N:B	3.0	21.986	7.329	0.164	0.918
R:B	3.0	34.647	11.549	0.259	0.854
N:S	1.0	72.601	72.601	1.628	0.224
R:S	1.0	141.120	141.120	3.165	0.099
B:S	3.0	770.531	256.844	5.761	0.010
Residual	13.0	579.574	44.583		

Table 4.8: ANOVA of the total amount of weeds (in average number of hits per pin) in the second year of ley (first harvest; point frequency method)

R(>F)
0.8526
0.0477
0.0048
0.0276
0.6348
0.4247
0.0173
0.3417
0.1546
0.3734

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	0.1276	0.1276	0.7390	0.4056
R	1.0	0.2931	0.2931	1.6971	0.2153
B	3.0	2.1850	0.7283	4.2175	0.0274
S	1.0	4.8991	4.8991	28.3680	0.0001
N:R	1.0	0.0007	0.0007	0.0038	0.9515
N:B	3.0	0.1120	0.0373	0.2162	0.8834
R:B	3.0	1.3801	0.4600	2.6639	0.0916
N:S	1.0	0.1027	0.1027	0.5945	0.4545
R:S	1.0	0.3011	0.3011	1.7436	0.2095
B:S	3.0	2.0228	0.6743	3.9044	0.0344
Residual	13.0	2.2451	0.1727		

Table 4.9: ANOVA of the total amount of weeds (in average number of hits per pin) in the second year of ley (second harvest; point frequency method)

Table 4.10: ANOVA of the total amount of weeds (in kg DM per daa) in the second year of ley (sum of all harvests; visual estimation)

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	722.5131	722.5131	0.3226	0.5797
R	1.0	5811.1746	5811.1746	2.5950	0.1312
B	3.0	22998.7703	7666.2568	3.4233	0.0495
S	1.0	50389.9496	50389.9496	22.5014	0.0004
N:R	1.0	7.4036	7.4036	0.0033	0.9550
N:B	3.0	10814.0906	3604.6969	1.6097	0.2352
R:B	3.0	12026.4173	4008.8058	1.7901	0.1986
N:S	1.0	147.7738	147.7738	0.0660	0.8013
R:S	1.0	5417.1559	5417.1559	2.4190	0.1439
B:S	3.0	11874.6286	3958.2095	1.7675	0.2028
Residual	13.0	29112.3833	2239.4141		

Table 4.11: ANOVA of the amount of T. officinale (in kg DM per daa) in the second year of ley (first harvest; separated)

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	84.417	84.417	0.409	0.534
R	1.0	47.680	47.680	0.231	0.639
В	3.0	634.386	211.462	1.024	0.414
S	1.0	863.237	863.237	4.179	0.062
N:R	1.0	125.687	125.687	0.608	0.449
N:B	3.0	105.371	35.124	0.170	0.915
R:B	3.0	185.312	61.771	0.299	0.826
N:S	1.0	0.081	0.081	0.000	0.984
R:S	1.0	0.412	0.412	0.002	0.965
B:S	3.0	372.866	124.289	0.602	0.625
Residual	13.0	2685.510	206.578		

	df	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	0.0018	0.0018	0.3316	$5.75\times10^{-1}$
R	1.0	0.0228	0.0228	4.2892	$5.88 \times 10^{-2}$
В	3.0	0.0628	0.0209	3.9375	$3.36 \times 10^{-2}$
S	1.0	0.4173	0.4173	78.4992	$7.19  imes 10^{-7}$
N:R	1.0	0.0036	0.0036	0.6696	$4.28\times10^{-1}$
N:B	3.0	0.0015	0.0005	0.0916	$9.63  imes 10^{-1}$
R:B	3.0	0.0437	0.0146	2.7373	$8.61\times 10^{-2}$
N:S	1.0	0.0062	0.0062	1.1685	$2.99\times10^{-1}$
R:S	1.0	0.0072	0.0072	1.3498	$2.66\times10^{-1}$
B:S	3.0	0.0561	0.0187	3.5184	$4.60 \times 10^{-2}$
Residual	13.0	0.0691	0.0053		

Table 4.12: ANOVA of the amount of T. officinale (in average number of hits per pin) in the second year of ley (first harvest; point frequency method)

Table 4.13: ANOVA of the amount of T. officinale (in average number of hits per pin) in the second year of ley (second harvest; point frequency method)

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	0.0014	0.0014	0.7353	$4.07\times 10^{-1}$
R	1.0	0.0005	0.0005	0.2647	$6.16  imes 10^{-1}$
В	3.0	0.0047	0.0016	0.8431	$4.94\times10^{-1}$
S	1.0	0.2658	0.2658	144.1176	$2.08\times10^{-8}$
N:R	1.0	0.0020	0.0020	1.0588	$3.22 \times 10^{-1}$
N:B	3.0	0.0029	0.0010	0.5196	$6.76 imes10^{-1}$
R:B	3.0	0.0074	0.0025	1.3431	$3.03  imes 10^{-1}$
N:S	1.0	0.0014	0.0014	0.7353	$4.07  imes 10^{-1}$
R:S	1.0	0.0005	0.0005	0.2647	$6.16  imes 10^{-1}$
B:S	3.0	0.0099	0.0033	1.7843	$2.00 \times 10^{-1}$
Residual	13.0	0.0240	0.0018		

Table 4.14: ANOVA of the amount of plant litter (in fraction of pins hit) in the second year of ley (first harvest; point frequency method)

	$d\!f$	$sum\_sq$	$mean\_sq$	F	PR(>F)
N	1.0	0.028	0.028	2.000	$1.81 \times 10^{-1}$
R	1.0	1.716	1.716	121.321	$5.80  imes 10^{-8}$
B	3.0	0.358	0.119	8.437	$2.27 \times 10^{-3}$
S	1.0	0.063	0.063	4.443	$5.50 \times 10^{-2}$
N:R	1.0	0.027	0.027	1.896	$1.92 \times 10^{-1}$
N:B	3.0	0.025	0.008	0.588	$6.33  imes 10^{-1}$
R:B	3.0	0.115	0.038	2.710	$8.81\times10^{-2}$
N:S	1.0	0.006	0.006	0.450	$5.14  imes 10^{-1}$
R:S	1.0	0.000	0.000	0.013	$9.11 \times 10^{-1}$
B:S	3.0	0.258	0.086	6.075	$8.16  imes 10^{-3}$
Residual	13.0	0.184	0.014		



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