"This is a post-peer-review, pre-copyedit version of an article published in Grass and Forage Science. The final authenticated version is available online at: http://dx.doi.org/<u>10.1111/gfs.12427</u>

Variation in rate of phenological development and morphology between red clover varieties: Implications for clover proportion and feed quality in mixed swards

Shahid Nadeem^{1-*}, Håvard Steinshamn¹, Elin Halvorsen Sikkeland¹⁺, Anne-Maj Gustavsson² and Anne Kjersti Bakken¹

¹Norwegian Institute of Bioeconomy Research, P.O. Box 115, 1431 Ås, Norway

²Swedish University of Agricultural Sciences (SLU), SE-901 83, Umeå, Sweden

present address: Norwegian University of life Sciences (NMBU), 1430 Ås, Norway

⁺present address: Norwegian Agricultural Extension Service (NLR) Trøndelag, Namsengata 4, 7863 Overhalla, Norway

* Corresponding author: Shahid Nadeem, E-mail: <u>shahid.nadeem@nmbu.no</u>

Abstract

Red clover (*Trifolium pratense*) grown in mixtures with grasses often constitutes a lower proportion of total yield in spring than in summer growth. A more even red clover proportion between the harvests would benefit forage quality and management at feeding. We investigated whether inclusion of early versus late maturing red clover varieties could reduce this disproportionality. In a two-year field trial harvested three times per season, each of six red clover varieties were grown in two grass mixtures. Rate of phenological development did not differ during spring growth, but did so in regrowth after first and second cuts. Here the earliest varieties constituted the highest proportion. At all harvests, the early varieties had lower crude protein concentrations and a higher content of neutral detergent fibre (NDF) and indigestible NDF, than the late varieties. Clover proportion was higher in swards with a mixture of timothy and meadow fescue than in swards with perennial ryegrass during the first year and lower in the second year. It is concluded that developmental rate should be explored further as a key character for red clover competiveness in spring growth of rapidly elongating grasses.

Keywords: Trifolium pratense, phenology, grass swards, clover proportion, forage quality.

1. Introduction

In Scandinavia, red clover is often grown together with temperate grasses in mixed swards under 2-3-cut systems (Halling et al., 2004). Inclusion of this species in grasslands increases the dry matter (DM) yield and forage quality (Halling et al., 2002; Sturludóttir et al., 2014), by exploiting its high forage quality, biological nitrogen fixation (BNF) and adaption to different environmental conditions (Abberton and Marshall, 2005; Nyfeler et al., 2011; Rasmussen et al., 2012). The most used complementary grass species are timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.) and perennial ryegrass (*Lolium perenne* L.) (Steinshamn et al., 2016).

Cropping red clover in mixed swards entails certain challenges, which may hinder the farmers to exploit its full potential. During spring, it is an inferior competitor for light in erect grass stands and thus constitutes a low proportion of total biomass, whereas later in summer it may dominate the yield of moderately fertilized swards (Pineiro and Harris, 1978; Riesinger and Herzon, 2008; Eriksen et al., 2014). This disproportional content of red clover in first and later harvests may lead to differences in feed quality of the mixture that may imply challenges to the farmers with difficulties in feeding with more than one type of silage at a time.

The architectural characteristics of red clover varies between genotypes and is important for the species' persistence and regrowth capacity in mixed swards with grasses (Van Minnebruggen et al., 2014). Ross et al. (2001) also demonstrated that red clover plants competing with weeds adapted their architecture according to environmental conditions.

The competitive ability of red clover in mixed swards may also depend on companion grass species (Hay and Hunt, 1989). Timothy has a very synchronous generative development in spring growth and slow regrowth rates and low tiller density after cutting, while perennial ryegrass and meadow fescue tiller populations will be more heterogeneous and be able to regenerate from vegetative tillers sooner after cutting (Davies 1988; Höglind et al., 2005; Seppanen et al., 2010). The homogenous, tall and erect timothy stand in spring may supress the vegetative clover, while the long lag period of timothy for regrowth after cutting may facilitate clover dominance. However, it is not obvious which principal type of grass sward that will supress red clover most and when.

The feed quality of red clover changes over the growing season according to morphological and phenological development and does also depend on frequency of harvest (Hetta et al., 2004; Kuoppala et al., 2008; Kleen et al., 2010). In harvesting regimes applied at high latitudes, the crude protein, energy content and the dry matter digestibility are usually higher in first harvest than in second and third harvest (Bakken et al., 2009). This may partly be due to higher temperature during regrowth than in the first growth and to high stem to leaf ratio in red clover regrowth.

To the best of our knowledge, no one has investigated the relationship between red clover phenological development and competitiveness with different grass species during spring growth of perennial leys harvested for silage production. In the present study, we tested if there were any relationship between the rate of phenological development in red clover and its competitive ability, performance and feed quality in different grass swards. The overall aim was to find strategies to overcome the disproportional content of clover between cuts and thus to better exploit its potential in silage production. The specific hypotheses were as follows:

1. In spring growth, the proportion of red clover in mixed swards will be higher with early than with late maturing red clover varieties.

- 2. The proportion of red clover will be higher in swards with a mixture of timothy and meadow fescue as companion grasses than with a mixture of perennial ryegrass varieties.
- 3. At the same harvesting date, the nutritive value will differ between early and late red clover varieties.

In order to test the above hypotheses, we conducted a field experiment with mixed swards including six red clover varieties (early and late maturing) from different latitudes of Europe and two different grass mixtures.

2. Material and methods

2.1 Experimental site

The field experiment was established in 2013 at Kvithamar research station of Norwegian Institute of Bioeconomy Research (NIBIO) in Stjørdal, Central Norway (63°30N, 10°54 E; 30 m altitude). At Kvithamar the long-term normal value (1961-1990) for annual precipitation is 896 mm, of which 416 mm occurs during the growing season (May to September), and the average growing season temperature is 11.7 °C. The soil was a silty loam (21% clay and 63% silt) overlying silty clay loam, classified as typic Cryaquoll (Soil Survey Staff, 1998), Mollic Gleysol (WRB 1998) and an Orthic Humic Gleysol (CSSC, 1998). The plough layer contained 5-6% organic matter (by weight). Weather conditions at the experimental site in the study period are shown in Figure S1.

2.2 Treatment description and grass-clover establishment

Based on advice from respective breeders, six red clover varieties were selected to cover a wide range in rate of phenological development. The red clover varieties were Lea (Graminor, Norway), Betty (Swaløf Weibull AB, Sweden), Calisto (DLF, Denmark), Atlantis (Norddeutsche Pflanzenzucht Hans-Georg Lembke (NPZ), Germany), Dalfia (Agroscope, Switzerland), and Ilte (Joegeva, Estonia). They were each sown together with two different grass mixtures according to a randomized block design with three replicates. One grass mixture was composed of a mixture of perennial ryegrass varieties (Barpasto, Calibra, Dunluce, Prana, Storm, Figgjo, Birger, Fia and Trygve), while the other grass mixture consisted of timothy (var. Grindstad) and meadow fescue (var. Fure).

The field experiment was established on 3^{rd} July 2013. The seed rates for the two mixtures were 35 kg ha⁻¹ (5 kg red clover + 30 kg ryegrass) and 25 kg ha⁻¹ (5 kg red clover + 12.5 kg timothy + 7.5 kg meadow fescue). Before sowing, farmyard manure (containing 1.7 kg NH₄ - N Mg⁻¹) was spread at the rate of 20 Mg ha⁻¹, and mineral fertilizer as NPK (23-3-10) at the rate of 60 kg N ha⁻¹. All plots were harvested once on 26 August in 2013.

2.3 Field management during the production years

During the production years in 2014 and 2015, 160 kg N ha⁻¹ yr⁻¹ of mineral N was supplied in a compound fertilizer (25-2-6 % NPK), distributed as 0.5, 0.25, 0.25 in spring, after first cut and after second cut respectively. All plots were harvested three times per year at a stubble height of 7 cm using a Haldrup plot harvester (Haldrup, Løgstør, Denmark). Spring harvest was taken at early heading stage of timothy, defined as when the tip of the inflorescence was visible above the flag leaf on 10% of the tillers (5 and 22 June in 2014 and 2015, respectively). Second harvest was taken on 21 and 28 July and the third on 1 and 15 September in 2014 and 2015, respectively. A subsample of approximately 1 kg was manually harvested by scissors (just before the main harvest) from two randomly selected areas in each plot and sorted for estimation of red clover and grass proportion in the total biomass and later analyses of forage quality of the sorted components. Total biomass fresh-weight from each plot was measured by balance on the harvester, while a subsample of

approximately 1 kg was taken for drying and calculation of the dry matter content of the mixed sward. All samples were dried at 60°C for 48h.

2.4 Determination of plant survival, phenological stage and morphological characteristics

In spring 2014 and 2015, we recorded the frequency of red clover plants in each plot along 0.5 m of a randomly selected seeding row. This was to discover differences between varieties in a composite character covering germination success and winter mortality.

The phenological stage of three permanently marked red clover plants in each plot was recorded in early spring, two weeks before harvest and one day before harvest. New plants were selected if the previous ones died in course of study. The stage of single plants was expressed as a mean stage by count (MSC), calculated from numeric indices assigned to all shoots individually. The indices ranged from 1.0 (vegetative, first leaf emerging) to 4.0 (seed formation stage) and was developed according to the system evolved for grasses by Moore et al. (1991). The indices have been listed in table S1.

The phenological stages of grass species were evaluated according to the scale suggested by Gustavsson et al. (2011). At first harvest in 2014, 57% of the perennial ryegrass tillers were in the booting stage (39-50) and 16% in the stem elongation stage (30-38). The corresponding figures for timothy were 32 and 39%. At first harvest in 2015, 33% and 37% of the perennial ryegrass tillers were in the booting and stem elongation stages, respectively, and for timothy the corresponding figures were 53 and 39%.

Just before each harvest, the height of the grasses and red clover in the undisturbed canopy was estimated by entering a thin measuring stick into the sward at three locations in each plot, and recording the height of the nearest plants from soil surface to the top leaf/head in vegetative and reproductive stages. The clover plants marked for phenological observations were then carefully cut by scissors at a stubble height of 7 cm, and dissected into leaves, stems and flowering organs. The different fractions were weighed after drying at 60 $^{\circ}$ C.

2.5 Nutritional quality analysis

The dried composite and sorted yield samples of grass and clover were chopped and milled in a Cyclotec 1093 sample mill (Foss companies, Hillerød, Denmark) with a 1-mm sieve, and scanned with a near infrared reflectance spectrophotometer (NIR systems 6500, Silver Spring MD, USA) (Fystro and Lunnan 2006). Based on multivariate statistics using ISI software (NIRS2, Ver. 4.00, Intrasoft International, Silver Spring, MD, USA) a set of samples (20 % of total) was selected for chemical analysis at the Dairy One Forage testing laboratory Ithaca, NY, USA. Crude protein (CP) concentration was determined by using AOAC Method 990.03 and ash contents by using AOAC Method 942.05 (AOAC,1990). Heat-stable α -amylase and sodium sulphite treated neutral detergent fibre (NDF) was determined using an ANKOM fibre analyser (ANKOM Technology Corporation,Fairport, NY) based on procedures described by Van Soest et al. (1991). Digestibility of NDF was determined *in vitro* after incubation for 48 h using the ANKOM Daisy II Filter Bag Technique, ANKOM Technology, Macedon, NY. Based on the chemical analyses at Dairy One Forage Testing Laboratory, a local calibration was developed and applied for prediction of quality of the whole populations of samples scanned by NIR.

2.6 Statistical Analysis

All data were analysed by analysis of variance (ANOVA) using linear mixed models, according to the procedure MIXED in statistical analysis system (SAS, release 9.3, 2002-2010, SAS institute Inc.,Cary, NC, USA). The parameters were modelled with grass mixture (1, 2) and clover variety (1-6) as fixed factors and replicate was treated as a random factor. The fixed factor year (2014,

2015) was included as a repeated statement when data for both years were modelled together. The interactions between red clover variety, grass mixture and year were included in the models. For the dependent variable clover proportion in yield, an additional mixed model ANOVA was conducted with clover plant density in spring as covariable. Tukey's test was used for pairwise comparisons of means within harvests, with a level of significance of ($p \le 0.05$). Bivariate Pearson correlation analyses were performed using the procedure CORR in SAS.

3. Results

3.1 Phenological development, morphology and forage quality of red clover varieties

First harvest: There were a difference in MSC and leaf proportion between years, but not between variety and companion grass mixture (Tables 1). In the second year (Table 2), Atlantis and Dalfia were taller and developed less shoots per plant than Betty and Ilte. Red clover plants (except Betty and Lea) had higher dry weights in swards with perennial ryegrass than in swards with timothy/meadow fescue (significant year (Y) × grass mixture (GM) × variety (V) interaction, Table 1). Except for the variety Atlantis, clover plants developed more shoots when grown together with timothy/meadow fescue than with perennial ryegrass (GM x V interaction, Table 1). The red clover proportion of total DM yield differed between the years (5-14% in the first year and 38-54% in the second year) with Atlantis as the numerically highest and Calisto as the lowest (Table 2). Correlation analysis of pooled data (2014 and 2015) revealed a positive relationship between red clover MSC scores and clover proportion at first harvest (p<0.001, R²=0.54). A mixed model ANOVA (cfr. section 2.6) with number of living plants (plant density in 0.5 m seeding row) in early spring as covariate, revealed a significant (*p*=0.012) and positive relationship between number of plants and clover proportion. There were, however, no significant differences in plant

density between varieties, when this was analysed separately as response variable in an ANOVA. In the first year, red clover proportion was highest in the swards with timothy/meadow fescue, whereas it was the opposite in the second year (Table 2).

The NDFD and concentration of CP was lower in the second than the first year (Table 2). On average over two years, the two varieties Betty and Ilte had higher NDFD and concentration of CP, but lower NDF than the other varieties (Table 1 and S2). On average over two years, red clover plants had higher concentration of NDF in timothy/meadow fescue swards than in swards with perennial ryegrass but the differences were numerically small (1% of DM).

Second harvest: On average over years and companion grass, Atlantis and Dalfia were at more advanced phenological stages than Betty, Lea and Ilte in the second cut (Table 1 and S3). In all combinations of varieties and grass mixtures, red clover MSC was higher in second than the first year (Table 3), with exception for Atlantis and Lea grown together with timothy/meadow fescue and Ilte with perennial ryegrass (Y x G x V interaction, Table 1). On average over two years, Atlantis and Calisto were taller than Betty and Lea, while Dalfia had lower leaf proportion than Betty (Table S3). Red clover proportion of the total crop biomass was generally higher than in the first harvest, and was higher in swards with Atlantis than in swards with other varieties during the first year only (Table 3). During the second year, red clover proportion did not differ between the varieties. During the first year, Atlantis, Dalfia and Calisto had lower NDFD and concentration of CP, but Atlantis and Calisto had higher NDF concentration than Betty, Lea and Ilte (Table 3). During the second year, Dalfia had lower CP concentration than Betty and Lea, lower NDFD than Betty, Lea and Ilte and higher NDF concentration than Betty, Ilte and Atlantis. Overall, an opposite response of Dalfia in the two years caused the interaction between the variety and year (Table 1). In the second year, clover proportion of total biomass, NDFD and concentration of CP was higher, but NDF concentration was lower than in the first year (Table 3). Similar to first harvest on average over two years, red clover plants had higher concentration of NDF when grown together with timothy/meadow fescue than with perennial ryegrass (Table 1 and S3).

Third harvest: On average over years and companion grass Atlantis, Dalfia and Calisto were at more advanced phenological stages, taller and had lower leaf proportion than Betty, Lea and Ilte (Table 1 and S4). The ranking was the same in both years, but the differences in height was not significant in the second year (Table 4). For all varieties, red clover constituted more than 48% of total DM yield (Table 4). Clover proportion was higher in timothy/meadow fescue than in perennial ryegrass mixture in both years (Table 4), except for Betty and Calisto in the second year when clover proportion was higher in the ryegrass sward (Y x G x V interaction Table 1). The tallest and more generative red clover plants had lowest NDFD and concentration of CP but highest concentration of NDF in the first year (Table 4). On average over years and companion grass, Dalfia had lower NDFD, and CP but higher NDF than all varieties except Calisto (Table 1 and S4). On average over years, red clover plants developed more shoots, but had lower NDFD, concentration of CP and NDF in perennial ryegrass swards than in swards with timothy/meadow fescue (Table 1 and S4).

3.2 Yield and forage quality of grass

First harvest: Total DM yield was higher in swards with perennial ryegrass than in swards with timothy/meadow fescue in the first year, but the opposite was observed in the second year (Table 2). Total DM yield was not affected by red clover variety (Table 2). Timothy/meadow fescue plant canopies were taller than perennial ryegrass canopies both years (Table 2). On average over years, perennial ryegrass had higher NDFD and lower concentration of NDF than timothy/meadow fescue

(Table 1 and S2). Grass plant height and feed quality did not differ between swards according to red clover variety.

Second harvest: Total DM yield was higher in swards with perennial ryegrass than in swards with timothy/meadow fescue in the first year (Table 3). Total DM yield was higher in swards with Atlantis than in swards with Betty, Lea and Dalfia in the first year, while swards with Dalfia had higher DM yield than Betty and Lea in the second year. Timothy/meadow fescue plants were taller than the perennial ryegrass plants in the second year (Table 3). Perennial ryegrass had lower NDFD, concentration of CP and NDF than the timothy/meadow fescue grass plants in the first year, but in the second year, NDFD was higher in perennial ryegrass plants. During the first year, grass plants in swards with Atlantis had highest concentration of CP and NDF than the grasses with other varieties (Table 3). In the second year, grass plants in swards with Dalfia had higher NDF concentration than swards with Betty and Lea (Table 3).

<u>Third harvest:</u> During the second year, swards with perennial ryegrass had higher total DM yield than swards with timothy/meadow fescue (Table 4). During the first year, total DM yield was higher in swards with Atlantis and with Dalfia than in swards with Betty. Timothy/meadow fescue grass plants were taller than perennial ryegrass plants in both years (Table 1 and 4). During the second year, grass plants in swards with Dalfia were taller than the grass plants with Calisto (Table 4). Timothy/meadow fescue had lower NDFD, but higher concentration of CP and NDF than the perennial ryegrass in both years (Table 1 and 4).

<u>Annual dry matter yield</u>: Total annual DM yield differed according to clover variety and grass mixture in the first year, but not in the second year (Table 5). During the first year, swards with Atlantis gave higher DM yield than swards with Betty (p = 0.049), while swards with other varieties did not differ from these two swards. Total annual DM yield was higher with perennial ryegrass

than with timothy/meadow fescue (p < 0.001) in the first year. Atlantis contributed highest red clover DM yields in both years. In the second year, it did not differ from Dalfia in this regard (data not shown).

4. Discussion

In the present study, we have addressed challenges related to the disproportional content of red clover between first and later harvests of mixed swards, and have investigated whether varietal differences in phenological and morphological characteristics can be exploited to overcome the disproportionality.

In our first hypothesis, we suggested that during spring growth, the proportion of red clover in grass-clover mixed swards would be higher with early than with late maturing red clover varieties. Based on MSC recordings in our study, there were no differences between the varieties regarding phenological stage of development at first harvest, so the six varieties that were selected did not provide the span in earliness needed to test this hypothesis. Although the positive correlation between red clover proportion and MSC scores across varieties at first harvest can not be interpreted as a causal relationship, it provides a basis for further exploration of our idea of increasing the red clover proportion in mixed swards by selecting red clover varieties with higher phenological rate of development during spring. There may be other varieties or germplasm available that are earlier and have higher phenological rate of development in spring than the ones investigated here, which later enable us to explore this issue further.

Red clover proportion in swards with Atlantis and Calisto differed in the first harvest in both years (34 versus 22% of DM as average over two years). Those varieties were both among the earliest ones according to MSC scores in later cuts, which is another fact that does not support the

hypothesis that developmental rate is important for red clover competitiveness and dominance in spring growth. The difference in yield proportion between them might have been related to differences in physiological or morphological characters not directly linked to phenology as this is expressed by MSC, or to number of established and/or surviving plants in the sward. In previous studies, characters like branching pattern, branch weight and bud formation rate have been shown to be directly related to yield and persistency of red clover in pure and mixed stands, with no direct link to rate of phenological development (Cnops et al., 2010, Van Minnebruggen et al., 2012, 2014). In a recent study of Hoekstra et al. (2018), there even seemed to be some relationship between persistency and earliness linked to architectural types of red clover.

Our study further indicated that the observed differences in clover proportion in the yield to some extent were related to establishment success or single plant survival. The correlation between plant density in the sward and proportion of final yield was, however, significant only for first cuts and across varieties. Because plant density did not differ according to variety, we conclude that the red clover proportion of first cut yields were affected both by single plant survival rates and other variety specific characteristics.

In this regard, it is not correct to exclude links to red clover phenology, either, because there might have been differences in rate of development that comparisons of scores on the MSC scale did not reveal. The MSC expresses the average of individual shoot stages weighted for the number of shoots in each stage, giving an equal importance to young (small) and old (bigger) shoots (Kalu and Fick, 1981; Ohlsson and Wedin, 1989). The MSC scores do not reveal differences in rate of development of the generation of shoots at most advanced stages, which may be more important for competitiveness with grasses and for clover yield than the numerous small and vegetative shoots in the lower layers of the canopy.

Based on the expected competitiveness of the grass species, we suggested in our initial set of hypotheses that the proportion of red clover would be higher in swards with timothy and meadow fescue than in swards with perennial ryegrass. In the first year, this was true at all harvests and for all clover varieties. A similar trend was observed during spring in a previous study by Hakala and Jauhiainet (2007). In spring 2015, however, the perennial ryegrass swards were severely set back by ice encasement and low temperatures the previous winter, due to its low tolerance to harsh winter conditions (Höglind et al. 2008). Perennial ryegrass did not recover enough during the growing season to fully re-establish and was thus less competitive against red clover in the second year. This also led to a more equal content of clover in the three harvests compared to the previous year. Relative to the previous year, the higher clover biomass production did not compensate for the 52% reduction in total yield of perennial ryegrass. In the timothy/meadow fescue swards, however, with 23% lower grass production in 2015 relative to 2014, increased clover growth compensated for the reduction. The rather low CP concentration (11% of DM, corresponding to 1.8% N) in timothy/meadow fescue at first cut in 2015 indicates N limitation for the grass component (Bélanger and Richards, 1997; Duru et al., 1997), which would have benefitted the clover and explain the higher and more equal clover proportion between the harvests for that sward type.

Red clover varieties differed in forage quality. In agreement with findings reported by Hetta et al. (2004), there was an inverse relationship between phenological advancement and digestibility. Our findings of positive relationships between phenological advancement, stem to leaf ratio and NDF concentrations were also in agreement with previous findings (Buxton 1996; Sanderson and Wedin 1989). These results support our third hypothesis that forage quality of early and late red clover varieties differs at the same time of harvest, with early varieties having lower feed quality than the

late varieties. In our study, red clover NDFD was higher in the first harvest as compared to second and third harvest, similar to the previous studies (Belyea et al., 1999; Drobna and Jancovic 2006) and related to a lower stem to leaf ratio.

The varietal differences in scores on quality characters were, however, small compared to the principal difference between grasses and red clover. Even if more competitive clover types would have a slightly lower CP content and lower NDFD compared to the traditional ones, their contribution to dilute the rather high NDF content of especially first cut yields of timothy/meadow fescue would be substantial. An increase in clover proportion from 10 to for instance 30% in first cuts would have decreased the NDF content from 58 to 54% of DM, with a NDF concentration in grass and clover of 60 and 40 % respectively. Such a reduction would benefit feed intake by high yielding dairy cows (Mertens, 1994).

5. Conclusion

The red clover varieties investigated in the present study did not provide the span in earliness needed to test our hypothesis of a positive relationship between clover rate of phenological development and clover proportion in spring growth of mixed swards. Still, their developmental rate as expressed by MSC and their feed quality differed in summer regrowth after an early first cut. This was a novel finding that supports that there may be other red clover varieties or germplasm available with higher phenological rate of development in spring which later enable us to explore these issues further. Clover proportion of first cut yields was related to other variety specific characteristics and clover winter survival, and was lower in vital swards of perennial ryegrass than in swards with timothy and meadow fescue. The study did however not reveal any other effective means than grass N deprivation to equalize the clover proportion between harvests.

Acknowledgements

This study was part of the AGROPRO project (<u>www.agropro.no</u>), financed by The Research Council of Norway (Norges Forskningsråd, grant agreement number 225330) and the Norwegian Institute of Bioeconomy Research (NIBIO, strategic Institute programme (SIS), project number 130175). The authors would also like to acknowledge and thank Anna Langerud for her assistance in organizing the experimental work.

References

Abberton, M.T. and Marshall, A.H. (2005). Progress in breeding perennial clovers for temperate agriculture. *The Journal of Agricultural Science*, 143,117-135.

Bakken, A.K., Lunnan, T., Hoglind, M., Harbo, O., Langerud, A., Rogne, T.E. and Ekker A.S. (2009). Mer og bedre grovfor som basis for norsk kjøtt- og mjølkeproduksjon. Bioforsk Rapport, vol. 4(38), ISBN nr. 978-82-17-00483-7.

Bélanger, G. and Richards, J.E. (1997). Growth analysis of timothy grown with varying N nutrition. *Canadian Journal of Plant Sciences*, 77, 373-380.

Belyea, R., Restrepo, R., Martz, F. and Ellersieck, M. (1999). Effect of year and cutting on equations for estimating net energy of alfalfa forage. *Journal of Dairy Science*, 82, 1934-1949.

Buxton, D.R. (1996). Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology*, 59, 37-49.

Cnops, G., Rohde, A., Saracutu, O., Malengier, M. and Roldán-Ruiz, I. (2010).
Morphological and molecular diversity of branching in red clover (Trifolium pratense). In
Huyghe C. (Eds.), *Sustainable use of Genetic Diversity in Forage and Turf Breeding*.
Springer, Dordrecht.

CSSC, (1998). The Canadian System of Soil Classification. Soil Classification Working Group, 3rd ed. CSSC, Ottawa, (pp. 187).

Davies A. (1988). The growth of grass swards. In Jones M.B. and Lazenby A. (Eds.), *The grass crop: the physiological basis of production*. London, UK: Chapman & Hall, 85-127.

Drobna, J. and Jancovic, J. (2006). Estimation of red clover (*Trifolium pratense L.*) forage quality parameters depending on the variety, cut and growing year. *Plant soil Environment*, 52, 468-475.

Duru, M., Lemaire, G. and Cruz, P. (1997). Grasslands. In G. Lemaire (Eds.), *Diagnosis of the nitrogen status in crops* (pp. 59-72). Verlag Berlin Heidelberg, Germany, Springer.

Eriksen, J., Askegaard, M. and Søegaard, K. (2014). Complementary effects of red clover inclusion in ryegrass– white clover swards for grazing and cutting. *Grass and Forage Science*, 69, 241–250.

Fystro, G. and Lunnan, T. (2006). Analysar av grovfôrkvalitet på NIRS (Analyses of forage quality by NIRS), In A. Ø. Kristoffersen (Eds.), *Proceedings of the Plantemøtet Østlandet 2006*, Bioforsk FOKUS, 1, 180-182.

Gustavsson, A. M. (2011). A developmental scale for perennial forage grasses based on the decimal code framework. *Grass and Forage Science*, 66, 93-108.

Hakala, K. and Jauhiainen, L. (2007). Yield and nitrogen concentration of above- and belowground biomasses of red clover cultivars in pure stands and in mixtures with three grass species in northern Europe. *Grass and Forage Science*, 62, 312–321.

Halling, M. A., Topp, C. F. E. and Doyle C. J. (2004). Aspects of the productivity of forage legumes in Northern Europe. *Grass and Forage Science*, 59, 331-344.

Halling, M.A., Hopkins, A., Nissinen, O., Paul, C., Tuori, M. and Soelter U. (2002). Forage legumes–productivity and composition. In R.J. Wilkins and C. Paul (Eds.), *Legume Silages for Animal Production-LEGSIL*, 234, 5-15.

Hay, R.J.M. and W.F. Hunt (1989). Competition from Associated Species on White and Red Clover in Grazed Swards. In: G. C. Marten, A. G. Matches, R. F. Barnes, R. W. Brougham, R. J. Clements and G. W. Sheath, (Eds.), *Persistence of Forage Legumes*, ASA, CSSA, SSSA, Madison, WI. p. 311-326. doi:10.2134/1989.persistenceofforagelegumes.c22. Hetta, M., Gustavsson, A.M., Cone, J.W. and Martinsson, K. (2004), In vitro degradation characteristics of timothy and red clover at different harvest times. *Acta Agriculturae Scandinavica, Section A. Animal Science*, 54, 20-29.

Hoekstra N.J., De Deyn G.B., Xu Y., Prinsen R. and Van Eekeren N. (2018). Red clover varieties of Mattenklee type have higher production, protein yield and persistence than Ackerklee types in grass–clover mixtures. *Grass and Forage Science*, 73, 297-308.

Høgh-Jensen, H. and Schjoerring J.K. (2001). Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys. *Soil Biology and Biochemistry*, 33, 439-448.

Höglind, M., Hanslin, H.M. and Van Oijen, M. (2005). Timothy regrowth, tillering and leaf area dynamics following spring harvest at two growth stages. *Field Crops Research*, 93, 51–63.

Höglind, M., Jørgensen, M., Østrem, L., Bakken, A.K. and Thorsen, S.M. (2008). Overwintering of timothy and perennial ryegrass in Norway from a climate change perspective. *Grassland Science in Europe*, 13, 203-205.

Kalu, B.A. and Fick, G.W. (1981). Quantifying morphological development of alfalfa for studies of herbage quality. *Crop Science*, 21, 267-271.

Kleen, J., Taube, F. and Gierus, M. (2010). Agronomic performance and nutritive value of forage legumes in binary mixtures with perennial ryegrass under different defoliation systems. *The Journal of Agricultural Science*, 149, 73-84

Kuoppala, K., Rinne, M., Nousiainen, J. and Huhtanen, P. (2008). The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. *Livestock Science*, 116, 171-182.

Mertens, D.R. (1994). Regulation of Forage Intake. In Fahey Jr., G.C. et al. (Eds.), Forage Quality, evaluation and utilization (pp. 450-493). *American society of Agronomy, Madison, WI*.

Moore, K.J., Moser, L.E., Vogel, K.P., Waller, S.S., Johnson, B.E. and Pedersen, J.F. (1991). Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal*, 83, 1073-1077.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E. and Luscher, A. (2011). Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture ecosystem and environment*, 140, 155-163.

Ohlsson, C. and Wedin, W.F. (1989). Phenological staging schemes for predicting red clover quality. *Crop Science*, 29, 416-420.

Pineiro J. and Harris W. (1978). Performance of mixtures of ryegrass cultivars and prairie grass with red clover cultivars under two grazing frequencies: I. Herbage production in the establishment year. *New Zealand journal of agricultural research*, 21, 83-92.

Rasmussen, J., Søegaard, K., Pirhofer-Walzl, K. and Eriksen, J. (2012). N₂-fixation and residual N effect of four legume species and four companion grass species. *European Journal of Agronomy*, 36, 66-74.

Riesinger, P. and Herzon, I. (2008). Variability of herbage production in mixed leys as related to ley age and environmental factors: a farm survey. *Agricultural and food science*, 17, 394-412.

Ross, S.M., King, J.R., Izaurralde, R.C. and O'Donovan, J.T. (2001). Weed suppression by seven clover species. *Agronomy Journal*, 93, 820-827.

Sanderson, M.A. and Wedin, W.F. (1989). Phenological stage and herbage quality relationships in temperate grasses and legumes. *Agronomy Journal*, 81, 864-869.

Seppanen, M.M., Pakarinen, K., Jokela, V., Andersen, J.R., Fiil, A., Santanen, A. and Virkajarvi, P. (2010). Vernalization response of Phleum pratense and its relationships to stem lignification and floral transition. *Annals of Botany*, 106, 697–707.

Soil Survey Staff, (1998). Keys to Soil Taxonomy, 8th ed. USDA, Washington, (pp. 326).

Steinshamn H., Nesheim L. and Bakken A.K. (2016). Grassland production in Norway. *Grassland Science in Europe*, 21, 15-25.

Sturludóttir, E., Brophy, C., Bélanger, G., Gustavsson, A.M., Jørgensen, M., Lunnan, T. and Helgadóttir, Á. (2014). Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Grass and Forage Science*, 69, 229-240.

Van Minnebruggen, A., Rohde, A., Roldán-Ruiz, I., Paepe, K. de., Van Dingenen, J., van Bockstaele, E. and Cnops, G. (2012): Architecture in red clover (*Trifolium pratense*). *Communications in agricultural and applied biological sciences*, 77, 95-99.

Van Minnebruggen, A., Roldán-Ruiz, I., van Bockstaele, E., Haesaert, G. and Cnops, G. (2014). The relationship between architectural characteristics and regrowth in *Trifolium pratense* (red clover). *Grass and Forage Science*, 70, 507-518.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583-3597.

WRB (1998). World reference base for soil resources. World Soil Resources Report 84. ISRIC and FAO, Rome, (pp. 88)

Table 1. Statistics of model with average across two production years (2014-2015). Presented *p* values show two way and three way interactions at each harvest separately. Total dry matter (DM) yield, morphological measurements of red clover (mean stage of phenological development by count (MSC), leaf proportion, plant weight, number of shoots per plant), red clover proportion in the harvested mixture, plant height and nutritional values of red clover and grass (concentrations of crude protein (CP), neutral detergent fibre digestibility (NDFD) and neutral detergent fibre (NDF)).

Clover	Total		Red clover						CP (0) of D	A)	NDF	<u> </u>	NDFD	<u></u>
variety	yield (kg ha ^{.1})	MSC	Leaf Prop.	Plant weight (g)	Shoots plant ⁻¹	Clover share	(cm) Red clover	grass	Red clover	grass	Red clover	grass	Red clover	grass
First h	arvest													
Effects, p-	value													
Y	0.006	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.02	0.004	<.001	<.001	<.001
GM	NS.	NS.	NS.	0.009	NS.	0.029	NS.	<.001	NS.	NS.	<.001	<.001	NS.	<.001
$\mathbf{Y}\times\mathbf{G}\mathbf{M}$	<.001	NS.	NS.	0.001	NS.	< .001	NS.	0.001	NS.	0.016	NS.	<.001	NS.	0.001
V	NS.	NS.	NS.	NS.	NS.	0.008	0.04	NS.	<.001	NS.	<.001	NS.	<.001	NS.
$\mathbf{Y}\times\mathbf{V}$	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	0.05	NS.	NS.	NS.	0.045	NS.
$GM \times V$	NS.	NS.	NS.	0.04	0.05	NS.	NS.	NS.	NS.	0.045	NS.	NS.	NS.	NS.
$\begin{array}{c} Y\times GM\\ \times V \end{array}$	NS.	NS.	NS.	0.04	NS.	NS.	NS.	NS.	NS.	NS.	0.047	NS.	NS.	NS.
Second	l harves	st												
Effects, p-	value													
Y	<.001	NS.	0.05	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
GM	0.053	NS.	NS.	NS.	NS.	0.012	0.002	0.001	NS.	<.001	<.001	<.001	NS.	0.013
$\mathbf{Y}\times\mathbf{G}\mathbf{M}$	0.018	NS.	NS.	NS.	NS.	<.001	NS.	<.001	NS.	<.001	<.001	0.003	NS.	<.001
V	0.001	<.001	0.03	0.015	NS.	0.001	<.001	0.004	<.001	NS.	<.001	0.005	<.001	NS.
$\mathbf{Y}\times\mathbf{V}$	0.013	NS.	NS.	NS.	NS.	0.001	0.05	0.013	0.01	0.001	<.001	<.001	0.001	NS.
$GM \times V$	NS.	NS.	NS.	NS.	0.05	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.
$Y \times GM \times V$	NS.	0.048	NS.	NS.	NS.	NS.	NS.	0.018	NS.	0.033	0.06	0.009	NS.	0.007
Third	harvest													
Effects, p-	value													
Y	<.001	NS.	NS.	<.001	NS.	<.001	<.001	<.001	<.001	0.012	<.001	NS.	<.001	<.001
GM	0.008	NS.	NS.	NS.	0.026	0.019	NS.	<.001	0.001	<.001	0.005	<.001	0.044	<.001
$\mathbf{Y}\times\mathbf{G}\mathbf{M}$	0.051	NS.	NS.	NS.	NS.	NS.	NS.	<.001	NS.	0.025	NS.	NS.	NS.	NS.
V	0.014	<.001	<.001	NS.	NS.	NS.	0.001	NS.	<.001	NS.	<.001	0.007	<.001	0.02
$\mathbf{Y}\times\mathbf{V}$	NS.	NS.	0.033	NS.	NS.	0.03	0.011	0.02	<.001	NS.	0.003	NS.	0.008	NS.
$GM \times \mathbf{V}$	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.
$Y \times GM \times V$	NS.	NS.	NS.	NS.	NS.	0.035	NS.	0.036	NS.	NS.	NS.	NS.	NS.	NS.

Y= year, GM= grass mixture, V= variety, NS.= non-significant.

Table 2. First harvest of red clover/grass-mixtures 2014-2015. Total dry matter (DM) yield, morphological measurements of red clover (mean stage of phenological development by count (MSC), leaf proportion, plant weight, number of shoots per plant), red clover proportion in the harvested mixture, plant height and nutritional values of red clover and grass (concentrations of crude protein (CP), neutral detergent fibre digestibility (NDFD) and neutral detergent fibre (NDF)).

Clover	Total		Re	ed clover			Height	<u>`</u>	СР		NDF		NDFD	
variety	DM						(cm)		(% of D	M)	(% of D	M)	(% of NI	OF)
	yield	MSC	Leaf	Plant	Shoots	Clover	Red	grass	Red	grass	Red	grass	Red	grass
	(kg na 1)		Prop.	weight (g)	plant*	snare	ciover		clover		clover		ciover	
2014	-						I				L			
Mean valu	es over co	mpanio	n grass											
Betty	5559	1.62	0.85	1.0	2.8	0.08^{ab}	27	64	26 ^a	12	35°	58	77 ^a	68
Lea	5491	1.81	0.72	1.4	3.4	0.10 ^{ab}	25	57	22 ^{bc}	12	37 ^{ab}	57	71 ^{bc}	68
Ilte	5567	1.71	0.87	1.4	2.7	0.12 ^{ab}	26	63	24 ^{ab}	12	35°	59	74 ^{ab}	68
Atlantis	5514	1.66	0.78	1.9	2.7	0.14 ^a	31	63	21°	12	36 ^{bc}	58	69°	68
Dalfia	5455	1.82	0.79	1.9	2.9	0.09 ^{ab}	26	62	22 ^{bc}	13	37 ^{ab}	58	67°	69
Calisto	5385	1.57	0.83	0.8	2.6	0.05 ^b	28	66	23 ^{bc}	13	37 ^a	58	72 ^{bc}	69
Mean valu	es over re	d clover	variety											
Per. Rye	5873ª	1.68	0.82	1.1	2.7	0.08 ^b	26 ^b	58 ^b	23	12	36 ^b	53 ^b	72	71ª
Tim+MF	5118 ^b	1.72	0.79	1.7	3.0	0.12 ^a	29ª	66ª	23	13	36 ^a	62ª	71	66 ^b
Effects, p-	value													
V	NS.	NS.	NS.	NS.	NS.	0.008	NS.	NS.	0.001	NS.	<.001	NS.	<.001	NS.
GM	<.001	NS.	NS.	NS.	NS.	0.003	0.04	<.001	NS.	NS.	<.001	<.001	NS.	<.001
GM x V	NS.	NS.	NS.	NS.	0.03	NS.	NS.	0.085	NS.	NS.	0.032	NS.	0.363	NS.
2015														
Mean valu	es over co	mpanio	n grass											
Betty	5795	2.39	0.57 ^{ab}	12.1	6.1ª	0.46^{ab}	58 ^b	85	21 ^a	12	36 ^b	53	65 ^{ab}	72
Lea	5929	2.46	0.52 ^{bc}	14.7	4.9 ^{ab}	0.53ª	61 ^{ab}	83	19 ^b	12	36 ^{ab}	55	64 ^{bc}	72
Ilte	5870	2.2	0.67ª	14.2	6.7ª	0.47^{ab}	56 ^b	84	21 ^a	12	34°	53	66 ^a	72
Atlantis	5588	2.12	0.52 ^{bc}	17.0	4.4 ^b	0.54ª	66ª	81	18°	11	34°	54	62 ^{dc}	72
Dalfia	5458	2.18	0.43°	13.0	4.0 ^b	0.48^{ab}	65ª	77	17°	12	36 ^{ab}	55	58°	73
Calisto	5633	2.27	0.47 ^{bc}	12.5	4.4 ^b	0.38 ^b	62 ^{ab}	81	18°	11	37 ^a	53	60 ^{de}	71
Mean valu	es over re	d clover	variety											
Per. Rye	5490 ^b	2.38	0.52	16.6ª	5.1	0.53ª	61	72 ^b	19 ^b	12ª	35 ^b	48 ^b	62	$78^{\rm a}$
Tim+MF	5934ª	2.17	0.53	11.2 ^b	5.0	0.42 ^b	62	92ª	19 ^a	11 ^b	36 ^a	$60^{\rm a}$	63	66 ^b
Effects, p-	value													
V	NS.	NS.	<.001	NS.	0.036	0.027	0.017	NS.	<.001	NS.	<.001	NS.	<.001	NS.
GM	0.005	NS.	NS.	0.028	NS.	<.001	NS.	<.001	0.045	0.003	<.001	<.001	NS.	<.001
GM x V	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.

Red clover proportion = clover dry matter share of total biomass dry matter, NS.= non-significant, Per. Rye = perennial ryegrass, Tim+MF= timothy + meadow fescue, V= variety, GM= grass mixture.

Table 3. Second harvest of red clover/grass-mixtures 2014-2015. Total dry matter (DM) yield, morphological measurements of red clover (mean stage of phenological development by count (MSC), leaf proportion, plant weight, number of shoots per plant), red clover proportion in the harvested mixture, plant height and nutritional values of red clover and grass (concentrations of crude protein (CP), neutral detergent fibre digestibility (NDFD) and neutral detergent fibre (NDF)).

Clover	Total	Red clover					Height (cm)		CP (% of DI	M)	NDF (% of DI	<u> </u>	NDFD (% of NDF)		
,	yield	MSC	Leaf	Plant	Shoots	Clover	Red	grass	Red	grass	Red	grass	Red	grass	
	(kg ha' 1)		Prop.	weight (g)	plant	share	clover		clover		clover		clover		
2014															
Mean valu	es over co	mpanion g	rass												
Betty	3139 ^{bc}	1.63°	0.90ª	3.4	2.5	0.32 ^{bc}	35 ^b	40^{ab}	19 ^a	9 ^{bc}	38 ^d	57 ^{bc}	56ª	70	
Lea	3196 ^{bc}	1.80 ^{bc}	0.81^{ab}	4.2	2.5	0.29 ^{bc}	35 ^b	39 ^b	20 ^a	9 ^{bc}	38 ^d	56 ^{bc}	56ª	71	
Ilte	3375 ^{abc}	1.82 ^{bc}	0.83 ^{ab}	5.1	2.3	0.40 ^b	40 ^b	42 ^{ab}	19 ^a	10 ^b	38 ^{cd}	58^{ab}	55ª	70	
Atlantis	3656ª	2.53ª	0.58 ^b	8.1	1.9	0.54ª	47ª	46 ^a	17 ^b	11 ^a	41ª	60 ^a	50 ^b	71	
Dalfia	3062°	2.32 ^{ab}	0.61 ^{ab}	5.9	2.4	0.27°	36 ^b	41 ^{ab}	17 ^b	9°	39 ^{bc}	55°	51 ^b	70	
Calisto	3418 ^{ab}	2.16^{abc}	0.68 ^{ab}	3.4	2.0	0.36 ^{bc}	41^{ab}	43 ^{ab}	18 ^b	9 ^b	41 ^{ab}	57 ^{bc}	50 ^b	70	
Mean valu	es over red	l clover va	riety												
Per. Rye	3450ª	2.03	0.78	4.4	1.9	0.29 ^b	36 ^b	41	18	9 ^b	38 ^b	54 ^b	53	69 ^b	
Tim+MF	3166 ^b	2.06	0.7	5.7	2.7	0.43ª	42ª	43	18	11 ^a	40 ^a	60 ^a	53	72ª	
Effects, p-	value														
V	<.001	0.001	0.013	NS.	NS.	<.001	<.001	0.033	<.001	<.001	<.001	<.001	<.001	NS.	
GM	<.001	NS.	NS.	NS.	NS.	<.001	<.001	NS.	NS.	<.001	<.001	<.001	NS.	<.001	
GM x V	NS.	0.085	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	0.09	NS.	
2015															
Mean valu	es over co	mpanion g	rass												
Betty	2379 ^{bc}	1.8 ^{bc}	0.71	7.9 ^{ab}	3.3	0.52	47 ^{bc}	51 ^{ab}	21ª	14	36 ^{bc}	54 ^{bc}	63 ^{ab}	79	
Lea	2284°	1.7°	0.75	5.7 ^b	2.9	0.51	44 ^c	46 ^b	20^{ab}	14	36 ^{abc}	53°	65ª	81	
Ilte	2571 ^{abc}	2.1^{abc}	0.68	11.6 ^{ab}	3.8	0.59	56 ^{ab}	54 ^{ab}	20 ^{abc}	15	35°	56 ^{ab}	63 ^{abc}	81	
Atlantis	2891 ^{ab}	2.1^{abc}	0.71	10.6 ^{ab}	3.5	0.56	60 ^a	55 ^{ab}	19 ^{bc}	14	35 ^{bc}	56^{abc}	62 ^{bcd}	79	
Dalfia	2976ª	2.4ª	0.58	13.9ª	4.0	0.6	57ª	60 ^a	18°	14	37 ^a	57 ^a	60 ^d	79	
Calisto	2617 ^{abc}	2.2 ^{ab}	0.64	6.8 ^b	2.7	0.5	57ª	56ª	20^{abc}	14	36 ^{ab}	56 ^{abc}	61 ^{cd}	80	
Mean valu	es over red	l clover va	riety												
Per. Rye	2599	2.1	0.68	10.3	3.6	0.57ª	53	49 ^b	19	14	35 ^b	51 ^b	63	82ª	
Tim+MF	2641	2	0.68	8.6	3.2	0.52 ^b	55	59ª	20	14	36 ^a	59ª	62	77 ^b	
Effects, p-1	value														
V	0.003	0.001	NS.	0.003	NS.	0.048	0.001	0.005	<.001	NS.	<.001	0.001	<.001	NS.	
GM	NS.	NS.	NS.	NS.	NS.	0.021	NS.	<.001	NS.	NS.	0.006	<.001	NS.	<.001	
GM x V	NS.	NS.	NS.	NS.	NS.	NS.	NS.	0.038	NS.	NS.	NS.	NS.	NS.	NS.	

Red clover proportion = clover dry matter share of total biomass dry matter, NS.= non-significant, Per. Rye = perennial ryegrass, Tim+MF=

timothy + meadow fescue, V= variety, GM= grass mixture.

Table 4. Third harvest of red clover/grass-mixtures 2014-2015. Total dry matter (DM) yield, morphological measurements of red clover (mean stage of phenological development by count (MSC), leaf proportion, plant weight, number of shoots per plant), red clover proportion in the harvested mixture, plant height and nutritional values of red clover and grass (concentrations of crude protein (CP), neutral detergent fibre digestibility (NDFD) and neutral detergent fibre (NDF)).

Clover variety	Total DM	Red clover			0		Height (cm)	~ \	CP NDF (% of DM) (% of DM)			M)	NDFD (% of NDF)		
	yield (kg ha ⁻¹)	MSC	Leaf Prop.	Plant weight (g)	Shoots plant ⁻¹	Clover share	Red clover	grass	Red clover	grass	Red clover	grass	Red clover	grass	
2014				(6/											
Mean values over companion grass															
Betty	1947 ^b	1.41 ^b	1.00ª	3.4	3.8	0.53 ^{ab}	33 ^b	32	22ª	14°	35 ^b	56 ^{ab}	59ª	78	
Lea	2161 ^{ab}	1.59 ^b	0.92ª	4.6	3.9	0.48 ^b	35 ^b	31	22ª	15 ^{abc}	36 ^b	56 ^{ab}	59ª	79	
Ilte	2080 ^{ab}	1.57 ^b	0.92ª	4.5	4.0	0.55 ^{ab}	37 ^b	35	22ª	16 ^a	35 ^b	56 ^{ab}	60 ^a	80	
Atlantis	2267ª	2.06ª	0.61 ^b	6.8	2.7	0.59ª	52ª	33	21 ^b	16 ^{ab}	38ª	57ª	55 ^b	81	
Dalfia	2266ª	2.23ª	0.54 ^b	5.2	3.8	0.50 ^{ab}	53ª	32	19°	14 ^{bc}	38ª	55 ^{bc}	51°	80	
Calisto	2150 ^{ab}	2.06ª	0.56 ^b	4.1	4.3	0.54 ^{ab}	49ª	32	20 ^{bc}	15 ^{abc}	38 ^a	54°	53 ^{bc}	80	
Mean valu	es over re	ed clover v	ariety												
Per. Rye	2167	1.8	0.78	4.2	4.1	0.50 ^b	43	31 ^b	21 ^b	14 ^b	36 ^b	52 ^b	56	81ª	
Tim+MF	2123	1.85	0.74	5.3	3.4	0.57ª	43	34 ^a	21ª	16 ^a	37 ^a	59ª	56	79 ^b	
Effects, p-	value														
v	0.02	< 0.001	< 0.001	NS.	NS.	0.048	< 0.001	NS.	<.001	0.005	<.001	<.001	<.001	NS.	
GM	NS.	NS.	NS.	NS.	NS.	<.001	NS.	0.03	0.011	<.001	0.003	<.001	NS.	0.001	
GM x V	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	
2015															
Mean valu	es over co	ompanion	grass												
Betty	2340	1.54°	0.80^{ab}	7.8	4.5	0.63 ^{ab}	47	45 ^{ab}	20^{ab}	14	38 ^{ab}	55	61ª	81	
Lea	2453	1.60 ^{bc}	0.82ª	7.3	3.8	0.60^{ab}	50	49 ^{ab}	20 ^{ab}	14	38 ^{ab}	55	62ª	83	
Ilte	2455	1.67 ^{bc}	0.83ª	9.0	3.8	0.63 ^{ab}	51	45 ^{ab}	21ª	15	38 ^b	56	62ª	82	
Atlantis	2409	2.23ª	0.62 ^{ab}	11.2	3.6	0.67 ^{ab}	52	49 ^{ab}	20 ^b	15	38 ^b	56	61ª	83	
Dalfia	2626	2.18ª	0.57 ^b	11.3	3.7	$0.70^{\rm a}$	59	52ª	19°	15	39 ^a	56	58 ^b	83	
Calisto	2464	1.97 ^{ab}	0.71 ^{ab}	7.8	3.4	0.54 ^b	50	40 ^b	20^{ab}	14	38 ^b	54	61ª	82	
Mean valu	es over re	ed clover v	ariety												
Per. Rye	2558 ª	1.9	0.73	9.3	4.1ª	0.61	52	39 ^b	20 ^b	14 ^b	38	51 ^b	60	84 ^a	
Tim+MF	2357 ^b	1.83	0.72	9.1	3.5 ^b	0.64	51	55ª	20 ^a	15 ^a	38	59ª	61	82 ^b	
Effects, p-	value														
V	NS.	<.001	0.001	NS.	NS.	0.05	NS.	0.042	<.001	N.S	0.016	NS.	0.002	NS.	
GM	0.003	NS.	NS.	NS.	0.016	NS.	NS.	< 0.001	0.001	0.001	NS.	<.001	NS.	<.001	
GM x V	NS.	NS.	NS.	NS.	NS.	0.031	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	

Red clover proportion = clover dry matter share of total biomass dry matter, NS.= non-significant, Per. Rye = perennial ryegrass, Tim+MF= timothy + meadow fescue, V= variety, GM= grass mixture.

Table 5. Total annual dry matter yield of clover/grass-mixtures. Total dry matter (DM) yield (kg ha⁻¹ year⁻¹) is presented as an average across the companion grass and red clover variety in both production years.

Variety	2014 annual DM yield (kg ha ⁻¹)	2015 annual DM yield (kg ha ⁻¹)
Mean values over	companion grass	
Betty	10646 ^b	10515
Lea	10849 ^{ab}	10667
Ilte	11023 ^{ab}	10897
Atlantis	11438 ^a	10889
Dalfia	10784 ^{ab}	11061
Calisto	10954 ^{ab}	10714
Mean values over	red clover variety	
Per. Rye	11490ª	10648
Tim+MF	10408 ^b	10933
Effects, p-value		
V	0.049	NS.
GM	<.001	NS.

NS.= non-significant, Per. Rye = perennial ryegrass, Tim+MF= timothy + meadow fescue, V= variety, GM= grass mixture.



Figure S1. Weather data of experimental site from a nearby (200 m) field weather station at the same research farm NIBIO Kvithamar. Weather data is shown as a mean daily soil temperature (0 C) at 5cm depth, air temperature (0 C) 2m above the soil surface, snow cover (cm) and precipitation (mm).

Phenological stage	Code	Description	Numerical
		_	value
Leaf stage	V0	First leaf visible	1.00
-	V1	First leaf fully developed	1.07
	V2	Second leaf fully developed	1.23
	V3	Third leaf fully developed	1.40
	V4	Fourth leaf fully developed	1.57
	V5	Fifth leaf fully developed	1.73
	V6	Sixth leaf fully developed	1.90
Stem elongation stage	E0	Stem elongation started	2.00
	E1	First internode longer than 1 cm	2.10
	E2	Second internode longer than 1 cm	2.30
	E3	Third internode longer than 1 cm	2.50
	E4	Fourth internode longer than 1 cm	2.70
	E5	Fifth internode longer than 1 cm	2.90
Reproductive stage	R0	Flower bud emergence	3.00
	R1	First flower bud visible	3.10
	R2	First individual flower stalk emergence	3.30
	R3	Pollen visible on first flower	3.50
	R4	First flower head	3.70
	R5	Second flower	3.90
Seed formation stage	S0		4.00

Table S1. Phenological stages and their numerical indices and description for quantifying developmental stage of red clover. Adopted from Moore et al. (1991) describing mean stage of count (MSC) of perennial grasses.

Moore, K.J., Moser, L.E., Vogel, K.P., Waller, S.S., Johnson, B.E. & Pedersen, J.F. (1991). Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal*, 83, 1073-1077.

Table S2. First harvest of red clover/grass-mixtures average values across the two production years (2014-2015). Total dry matter (DM) yield, morphological measurements of red clover (mean stage of phenological development by count (MSC), leaf proportion, plant weight, number of shoots per plant), red clover proportion in the harvested mixture, plant height and nutritional values of red clover and grass (concentrations of crude protein (CP), neutral detergent fibre digestibility (NDFD) and neutral detergent fibre (NDF)).

Clover	Total		Rec	l clover	,		Height		СР		NDFD		NDF	
variety	DM						(cm)		(% of DI	м)	(% of NE	DF)	(% of DI	VI)
	yield (kg ha⁻¹)	MSC	Leaf Prop.	Plant weight (g)	Shoots plant ⁻¹	Clover share	Red clover	grass	Red clover	grass	Red clover	grass	Red clover	grass
Mean valu	ies over com	panion gr	ass and ye	ar										
Betty	5677	1.98	71	6.6	4.4	0.27 ^{ab}	43	75	23ª	12	71ª	70	35 ^{bc}	55
Lea	5710	2.12	62	8.1	4.2	0.31 ^{ab}	43	70	21 ^b	12	67 ^b	70	36 ^{ab}	56
Ilte	5719	1.95	77	7.8	4.6	0.29 ^{ab}	41	74	23ª	12	70 ^a	70	34 ^d	56
Atlantis	5552	1.87	65	9.4	3.5	0.34ª	49	72	20 ^b	12	65 ^b	70	35 ^{cd}	56
Dalfia	5457	2.02	60	7.5	3.5	0.28 ^{ab}	45	69	20 ^b	13	63°	71	36 ^{ab}	56
Calisto	5510	1.91	65	6.6	3.5	0.22 ^b	45	73	20 ^b	12	66 ^b	70	37 ^a	55
S.E	84	0.15	4	1	0.3	0.02	1.5	1.5	0.3	0.3	0.6	0.6	0.22	0.4
Mean valu	ies over red	clover var	iety and ye	ear										
Per. Rye	5682	2.00	67	9	3.9	0.31	43	65	21	12	67	75	35	51
Tim+MF	5526	1.94	66	7	4.0	0.27	45	79	21	11	67	66	36	61
S.E	66	0.06	0.03	0.6	0.2	0.01	0.82	1.8	0.3	0.2	0.5	0.4	0.1	0.2
Effects, p-	value													
Y	0.006	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.02	<.001	<.001	0.004	<.001
GM	NS.	NS.	NS.	0.009	NS.	0.029	NS.	<.001	NS.	NS.	NS.	<.001	<.001	<.001
$\boldsymbol{Y}\times\boldsymbol{G}\boldsymbol{M}$	<.001	NS.	NS.	0.001	NS.	< .001	NS.	0.001	NS.	0.016	NS.	0.001	NS.	<.001
V	NS.	NS.	NS.	NS.	NS.	0.008	0.04	NS.	<.001	NS.	<.001	NS.	<.001	NS.
$\mathbf{Y}\times\mathbf{V}$	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	0.05	NS.	0.045	NS.	NS.	NS.
$GM \times V$	NS.	NS.	NS.	0.04	0.05	NS.	NS.	NS.	NS.	0.045	NS.	NS.	NS.	NS.
$\begin{array}{l} Y\times GM\\ \times V\end{array}$	NS.	NS.	NS.	0.04	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	0.047	NS.

S.E = standard error, red clover proportion = clover dry matter share of total biomass dry matter, Per. Rye = perennial ryegrass, Tim+MF=

timothy + meadow fescue, Y= year, GM= grass mixture, V= variety, NS.= non-significant.

Table S3. Second harvest of red clover/grass-mixtures average values across the two production years (2014-2015). Total dry matter (DM) yield, morphological measurements of red clover (mean stage of phenological development by count (MSC), leaf proportion, plant weight, number of shoots per plant), red clover proportion in the harvested mixture, plant height and nutritional values of red clover and grass (concentrations of crude protein (CP), neutral detergent fibre digestibility (NDFD) and neutral detergent fibre (NDF)).

Clover Total variety DM			Rec	d clover	,		Height (cm)	0	CP (% of D	M)	NDFD (% of NDF)		NDF (% of DM)	
	yield (kg ha ⁻¹)	MSC	Leaf Prop.	Plant weight (g)	Shoots plant ⁻¹	Clover share	Red clover	grass	Red clover	grass	Red clover	grass	Red clover	grass
Mean valu	ies over com	panion gr	ass and ye	ar										
Betty	2760 ^b	1.73 ^b	80 ^a	5.7	2.9	0.42 ^b	41 ^{bc}	45 ^{ab}	20 ^a	12	59 ^{ab}	75	37 ^b	55 ^b
Lea	2741 ^b	1.76 ^b	78^{ab}	5.0	2.7	0.40 ^b	39°	43 ^b	20 ^{ab}	12	60ª	76	37 ^b	55 ^b
Ilte	2973 ^{ab}	1.93 ^b	75 ^{ab}	8.4	3.0	0.49 ^{ab}	48^{ab}	48^{ab}	19 ^{abc}	12	59 ^{ab}	75	37 ^b	57^{ab}
Atlantis	3274ª	2.32ª	65 ^{ab}	9.4	2.7	0.55ª	54ª	50ª	18°	12	56°	75	38 ^a	58ª
Dalfia	3019 ^{ab}	2.34ª	60 ^b	9.9	3.2	0.44 ^b	47 ^{abc}	50 ^a	18°	11	56°	74	38 ^a	56 ^{ab}
Calisto	3018 ^{ab}	2.17 ^{ab}	66 ^{ab}	5.1	2.4	0.43 ^b	49 ^a	49 ^{ab}	18 ^{bc}	12	55°	75	38 ^a	56 ^{ab}
S.E	84	0.13	4	1	0.3	0.02	1.54	1.5	0.28	0.2	0.6	0.6	0.2	0.4
Mean valu	ies over red	clover vari	iety and ye	ar										
Per. Rye	3025	2.05	73	7.3	2.7	0.43	44	45	19	11	58	76	37	53
Tim+MF	2904	2.07	69	7.1	2.9	0.48	48	51	19	12	57	74	38	59
S.E	42	0.06	0.03	0.7	0.2	0.02	0.9	0.7	0.1	0.2	0.2	0.6	0.2	0.3
Effects, p-	value													
Y	<.001	NS.	0.05	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
GM	0.053	NS.	NS.	NS.	NS.	0.012	0.002	0.001	NS.	<.001	NS.	0.013	<.001	<.001
$\mathbf{Y}\times\mathbf{G}\mathbf{M}$	0.018	NS.	NS.	NS.	NS.	<.001	NS.	<.001	NS.	<.001	NS.	<.001	<.001	0.003
V	0.001	<.001	0.03	0.015	NS.	0.001	<.001	0.004	<.001	NS.	<.001	NS.	<.001	0.005
$\mathbf{Y}\times\mathbf{V}$	0.013	NS.	NS.	NS.	NS.	0.001	0.05	0.013	0.01	0.001	0.001	NS.	<.001	<.001
$GM\times \mathrm{V}$	NS.	NS.	NS.	NS.	0.05	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.
$\begin{array}{c} Y\times GM\\ \times V \end{array}$	NS.	0.048	NS.	NS.	NS.	NS.	NS.	0.018	NS.	0.033	NS.	0.007	0.06	0.009

S.E = standard error, red clover proportion = clover dry matter share of total biomass dry matter, Per. Rye = perennial ryegrass, Tim+MF= timethy \downarrow meadow from V = verify NS = non significant

timothy + meadow fescue, Y= year, GM= grass mixture, V= variety, NS.= non-significant.

Table S4. Third harvest of red clover/grass-mixtures average values across the two production years (2014-2015). Total dry matter (DM) yield, morphological measurements of red clover (mean stage of phenological development by count (MSC), leaf proportion, plant weight, number of shoots per plant), red clover proportion in the harvested mixture, plant height and nutritional values of red clover and grass (concentrations of crude protein (CP), neutral detergent fibre digestibility (NDFD) and neutral detergent fibre (NDF)).

Clover	Total		Re	d clover			Height		СР		NDFD		NDF	
variety	DM						(cm)		(% of D	M)	(% of NI	DF)	(% of D	M)
	yield (kg ha⁻¹)	MSC	Leaf Prop.	Plant weight (g)	Shoots plant ⁻¹	Clover share	Red clover	grass	Red clover	grass	Red clover	grass	Red clover	grass
Mean valu	ies over com	panion gr	ass and ye	ar										
Betty	2144 ^b	1.48 ^b	90ª	5.6	4.2	0.58	40 ^b	38	21^{ab}	14	60 ^{ab}	80	37 ^{cd}	55
Lea	2307 ^{ab}	1.60 ^b	87ª	6.0	3.9	0.54	42 ^b	40	21 ^{ab}	14	61ª	81	37 ^{cd}	56
Ilte	2268 ^{ab}	1.62 ^b	87ª	6.8	3.9	0.59	44 ^b	40	22ª	15	61ª	81	36 ^d	56
Atlantis	2338ª	2.15ª	62 ^b	9.4	3.2	0.63	52ª	41	20 ^b	15	58 ^{bc}	82	38 ^{bc}	56
Dalfia	2446ª	2.21ª	56 ^b	8.2	3.8	0.6	56 ^a	42	19°	15	54 ^d	82	39 ^a	55
Calisto	2307 ^{ab}	2.02ª	64 ^b	5.9	3.9	0.54	50 ^{ab}	36	20 ^{bc}	14	57 ^{cd}	81	38 ^{ab}	54
S.E	84	0.13	4	1.1	0.3	0.02	1.54	1.5	0.3	0.2	0.6	0.6	0.2	0.4
Mean valu	ies over red	clover vari	iety and ye	ar										
Per. Rye	2363	1.85	75	6.8	4.1	0.55	48	35	20	14	58	82	37	52
Tim+MF	2241	1.84	73	7.2	3.5	0.61	47	44	21	15	59	80	38	59
S.E	43	0.04	0.02	0.6	0.2	0.02	1.5	1.1	0.2	0.2	0.5	0.2	0.2	0.5
Effects, p-	value													
Y	<.001	NS.	NS.	<.001	NS.	<.001	<.001	<.001	<.001	0.012	<.001	<.001	<.001	N.S
GM	0.008	NS.	NS.	NS.	0.026	0.019	NS.	<.001	0.001	<.001	0.044	<.001	0.005	<.001
$\mathbf{Y}\times\mathbf{G}\mathbf{M}$	0.051	NS.	NS.	NS.	NS.	NS.	NS.	<.001	NS.	0.025	NS.	NS.	NS.	NS.
V	0.014	<.001	<.001	NS.	NS.	NS.	0.001	NS.	<.001	NS.	<.001	0.02	<.001	0.007
$\mathbf{Y}\times\mathbf{V}$	NS.	NS.	0.033	NS.	NS.	0.03	0.011	0.02	<.001	NS.	0.008	NS.	0.003	NS.
$GM \times V$	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.
$\begin{array}{l} Y\times GM\\ \times V \end{array}$	NS.	NS.	NS.	NS.	NS.	0.035	NS.	0.036	NS.	NS.	NS.	NS.	NS.	NS.

S.E = standard error, red clover proportion = clover dry matter share of total biomass dry matter, Per. Rye = perennial ryegrass, Tim+MF=

 $timothy + meadow \ fescue, \ Y= year, \ GM= \ grass \ mixture, \ V= variety, \ NS= non-significant.$