Performance and quality of tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) and mixtures of both species grown with or without white clover (*Trifolium repens* L.) under cutting management

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Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is of increasing interest in Western Europe, mainly because of its good drought resistance and high yield potential compared to perennial ryegrass (*Lolium perenne* L.). Important drawbacks of tall fescue are its lower digestibility and voluntary intake compared to perennial ryegrass. Mixtures of both grass species might combine the advantages of both species, and species interactions may eventually lead to transgressive overyielding. We compared the agronomic performance of tall fescue, perennial ryegrass and mixtures of both species grown either as pure grass swards or in association with white clover (*Trifolium repens* L.). Mixtures of tall fescue and perennial ryegrass differed in the proportion and ploidy of the perennial ryegrass component. Yield, feeding quality and botanical composition were measured in the three years after the sowing year. We found significant effects of the ploidy of the ryegrass variety and of the proportion of ryegrass seeds in the initial seed mixture on the botanical composition of the mixtures. Nevertheless, all mixtures were dominated by tall fescue at the end of the experiment. No overyielding of the mixtures compared to the pure swards was found but feeding quality showed to be intermediate to that of the pure swards and mixtures proved to have a better drought resistance and a better feeding quality than monospecific swards of Lp and Fa respectively.

Introduction

More dry summer spells are expected in North West Europe due to climate change (IPCC, 2007). As perennial ryegrass (*Lolium perenne* L.; **Lp**), currently by far the most important forage grass species in North West Europe both for cutting and grazing management, is relatively sensitive to drought stress (Norris, 1982), interest in species like cocksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.; Fa) and *Festulolium*, which are known to have a relatively good drought resistance, is increasing (Gilliland *et al.*, 2010; Graiss *et al.*, 2011; Mosimann *et al.*, 2010; Reheul *et al.*, 2011; Surault *et al.*, 2007). The deeper rooting and higher root biomass may explain the higher drought resistance of Fa compared to Lp (Willman *et al.*, 1998; Van Eekeren *et al.*, 2010; Durand and Ghesquière, 2002). Tall fescue is not only more drought resistant than Lp, it also has a higher yield potential than Lp (Gilliland *et al.*, 2010; Wilman and Gao, 1996; Van Eekeren *et al.*, 2010; Pontes *et al.*, 2007).

The major constraints of Fa are its lower voluntary intake and its lower digestibility than Lp. In a Dutch study, intake and milk production of dairy cows fed with fresh Fa was lower than that of cows fed with fresh Lp (Lutten and Remmelink, 1984).

An ideal grass species or grass sward for intensive dairy or beef production combines the excellent forage quality of perennial ryegrass and the drought resistance and persistence of tall fescue. *Festulolium* genetically combines the advantages of *Festuca* and *Lolium* in a single species (Thomas *et al.*, 2003; Humphreys *et al.*, 2012). Another option is to combine the advantages of both species by sowing a seed mixture of both species. While in *Festulolium* a monospecific sward is created, the strategy of mixing *Festuca* and *Lolium* species results in a bispecies sward which may have both advantages and disadvantages. A potential shift in composition in time may be disadvantageous while interspecific interactions may have positive yield effects (Huyghe *et al.*, 2012). Multi-species swards can lead to overyielding of two kinds. Transgressive overyielding occurs when the yield of the mixed sward is higher than that of the highest yielding monospecific sward; non-transgressive overyielding occurs when the yield of a mixture is greater than expected based on the weighted average of the monospecific swards (Drake, 2003). Particularly in mixtures combining grasses and legumes, transgressive overyielding driven by the N fixation of the leguminous crops, is well described (Nyfeler *et al.*, 2009). Interaction between grass species can lead to positive biodiversity effects (Pontes *et al.*, 2012). Two different mechanisms can explain this biodiversity effects: a selection effect or a complementarity effect (Loreau and Hector, 2001). The former describes the probability of including a highly productive species in a randomly selected species rich mixture. The latter is based on the assumption that species rich communities better exploit available resources owing to plant functional traits (e.g. rooting depth, plant habitus). Pontes *et al.* (2012) found that both mechanisms were acting together in grass species mixtures grown on nutrient rich soils.

Literature supports the idea that Fa and Lp have complementary functional traits. First, rooting depth and biomass of Fa is higher than that of Lp (Wilman *et al.*, 1998; Van Eekeren *et al.*, 2010). Second, seasonal growth pattern of Fa and Lp is different (Gilliland *et al.*, 2011).

Pontes *et al.* (2012) performed a principal component analysis on seven functional traits measured on different grass species. Fa and Lp differed mainly on the first axis, which separated tall plant species with coarse roots and high root biomass (Fa) from small species with thin roots (Lp).

In contrast to the study of Pontes *et al.* (2012), the aim of the present study was not to identify the underlying mechanisms explaining the interaction found in grass species mixtures, but to proof the concept that the complementary traits of Fa and Lp would lead to mixtures of Fa and Lp that are more tolerant against drought than pure Lp and have a better feed quality than the pure Fa swards. Several mixtures of Fa and Lp were compared with monospecific swards of both species under two managements.

As Wilman and Gao (1996) showed that swards sown with a seed mixture of Fa and Lp in which both species were equally represented (on a seed mass basis) were dominated by Lp, we used seed mixtures with a smaller proportion of Lp.

As we suspected potential differences in the functional traits between diploid and tetraploid perennial ryegrass, owing to differences in both tiller density and tiller dimensions, we used a diploid and a tetraploid variety.

As the grassland management influences the interaction between species in mixtures (Surault *et al.*, 2006), two management regimes were applied: plots were either fertilized with a high mineral N dose or with a low mineral N dose. In the latter case white clover (*Trifolium repens* L.; **Tr**) was added to the seed mixture in order to make this management more relevant for practise. Yield, botanical composition and quality parameters were followed for three successive years after the sowing year.

Our research hypotheses were that:

(i) Mixtures of Lp and Fa combine the advantages of both species: a better drought resistance than Lp in drought periods and an overall better digestibility than Fa.

(ii) The positive interactions between the grass species lead to transgressive overyielding with a larger effect under low mineral N fertilization compared to high mineral N fertilization.

(iii) The ploidy of the ryegrass variety and the initial proportion of the ryegrass component influence botanical composition of the mixtures and hence the interaction between both species.

Materials and Methods

Trial design

The trial was sown in April 2009 on a sandy loam soil in Merelbeke, Belgium. The land had been used for three years as arable land before the trial was established. The soil was analysed in 2007 (0-30 cm): the pH_{KCl} of the soil was 5.5, the soil organic matter content was 1.4 % and P and K content were 14 and 8 mg (kg dry soil)$^{-1}$ respectively. The trial consisted of two adjacent subtrials; a "**pure grass trial**" and a "**grass-clover trial**". Both trials were randomized complete block designs with three replicates; plot size was 7.8 m² and the gross area of each trial was 252 m². In the pure grass trial, monocultures of Fa *cv.* 'Castagne' (Fa), intermediate heading diploid Lp (Lp2) *cv.* 'Plenty' and intermediate heading tetraploid Lp (Lp4) *cv.* 'Roy' were compared with mixtures of Fa and Lp. Mixtures were created by substituting 1/4 or 1/8 of the Fa seeds by Lp2 or Lp4 seeds (Table 1). The proportion of Lp in the seed mixture was kept at a low level to prevent an early domination of Lp in the emerging swards owing to the slow establishment of Fa. Two different proportions were considered in order to study the effect of the composition of the seed mixture on the resulting swards. In order to prevent a variety effect rather than a ploidy effect, we used a diploid and a tetraploid Lp variety bred from the same germplasm. Sowing densities were 1500 germinating seeds m^{-2} in all cases. Annual fertilization was 300 kg N ha^{-1}, 11kg P ha^{-1} and 270 kg K ha^{-1}. The concept of the grass-clover subtrial was in line with the concept of the pure grass trial, but all seeding densities were supplemented with 700 germinating seeds m^{-2} (approx. 5 kg ha^{-1}) of 'Merwi' a medium leafed white clover variety. Annual fertilisation was 165 kg N ha^{-1}, 32 P ha^{-1} and 258 kg K ha^{-1}. Throughout the text the abbreviations indicated in Table 1 will be used to identify the different sward compositions in the trials. In the establishment year, dicot weeds were removed using herbicides (MCPA + clopyralid + fluroxypyr) in the pure grass trial and by hand weeding in the grass clover trial. As a consequence the abundance of unsown species in the plots was very low.

Measurements

Five cuts were harvested both in 2010, 2011 and 2012 (Table 2). The first cut was taken when the pure Lp swards reached a dry matter yield (DMY) of *circa* 3000 kg DM ha^{-1}. After the first cut, a harvest interval of *circa* 6 weeks was imposed. Due to the rainy weather during the last decade of April and the beginning of May 2012 (only two days without precipitation between April 20^{th} and May 10^{th}), the spring harvest was delayed in 2012. Plots were mown and weighed with a Haldrup (Haldrup, Logstor, Denmark) plot harvester. At each cut, samples of ± 150 g were taken on the harvester for determination of the crude protein content (CPC) and the *in vitro* organic matter digestibility (IVOMD). Samples were dried for 24h at 60°C and ground (Brabender shear mill) to pass a 1mm sieve. The near infrared reflectance spectroscopy (NIRS) spectra of the ground samples were collected with a Foss NIRSystems 5000 (FOSS-NIRSystems, Silver Springs, MD, USA) and ISIscan 2.85.1 software (Infrasoft international, Port Mathilda, PA, USA). The NIRS equation for CP was based on 396 grass and grass-clover samples that had been analysed by the Kjeldahl method. The NIRS equation for IVOMD was based on 396 grass and grass-clover samples analysed according to Tilley and Terry (1963). In 2010 and 2011, the 20 samples that were spectrally most distant from the calibration samples were analysed using the reference methods for CP and IVOMD in order to expand the calibration library. Ten supplementary samples were chosen at random, and analysed using the reference methods for validation of the equations for CP and IVOMD. The root mean square errors of cross validation were below 0.44 % and 0.16 % for CP and IVOMD content respectively; root mean square errors of prediction (RMSEP) were below 0.38% and 1.72% for CP and IVOMD content respectively; the biases (the mean error between the wet chemistry lab value and the NIRS value) were below -0.02 % and -0.28 % for CPC and IVOMD content respectively.

A second sample was taken for the determination of the botanical composition of the harvested material. At least 1% of the fresh yield of each plot (corresponding to weights between 150 and 300 g) was separated by hand into the different sown species and the fresh and dry weights were recorded. As the presence of unsown species in the harvested material was rare, they were not taken into account in the botanical composition of the harvested material.

Meteorological data were obtained from an official meteorological station at approximately 3 km from the experimental site. The standard precipitation index (SPI) was calculated for the experimental site for every month using a time scale of two months (Aper *et al.*, 2012). The SPI is a probability index of abnormal wetness and dryness, based on long term meteorological data for a particular location (McKnee *et al.*, 1993). Months with an SPI below -1 or -1.5 are classified as moderately or severely dry respectively. Based on these criteria we identified three dry spells during the experimental period. A first dry spell occurred in the spring - early summer of 2010: 83 mm of rainfall instead of 157 mm normally from

April 1st till June 30th (See appendix Table A.1) resulting in a SPI below -1.6 in May and June 2010 (Table 3). The second growth and half of the third growth of 2010 fell in this drought period. A second dry spell occurred in the spring of 2011: 34 mm of rainfall instead of 135 mm normally from March 1st till May 20th (See appendix Table A.1), resulting in a SPI of below -2.3 in April and May 2011 (Table 3). The first growth and half of the second growth of 2011 fell in this drought period. Finally, August-September 2012 was dry: 74 mm of rainfall instead of 150 mm normally in the two months (See appendix Table A.1), resulting in a SPI below -1 in September (Table 3) and affecting the fourth and a part of the fifth growth of 2012. The winters and summers of 2010, 2011 and 2012 were relatively wet, so the dry spells did not dramatically hamper grass growth. Two cold winter periods occurred: December 2010 was the coldest December month since 1950, with freezing temperatures below -10 °C; in February 2012, it was freezing for 15 successive days, reaching a minimum of -15°C. No excessive heat periods occurred during the experiment. Nevertheless, the year 2011 was the warmest since the start of the Belgian records of meteorological data in 1833.

Data analysis

Analyses of variance were computed using the aov() function in R (R Development Core Team, 2011). Data with and without clover were analysed separately, using a model for a randomized complete block design (Crawley, 2007). The different sward compositions were considered as a fixed factor with seven levels, replications as a random factor with three levels. Multiple comparison of means between the mixtures was done using the TukeyHSD() function. For the anova of the proportion of Fa in the grass DMY, there were only four levels as the monospecific swards contained either 0% or 100% Fa. A supplementary anova was performed to model the effect of the initial proportion of Lp seeds in the seed mixture and the ploidy of the Lp component on the Fa proportion in the DMY. Analysis of covariance (ANCOVA) was performed to model functional relationship between Fa content in the mixtures and total DMY or IVOMD. The analysis was done with the lm() function, and the minimum adequate model (*i.e.* the lowest degree polynomial that minimised the Akaike's information criterion) was selected with the step() function (Crawley, 2007).

Results

Effect of mixing Fa and Lp on DMY

In the pure grass trial, Fa overyielded both Lp2 and Lp4 every year (Table 4). The yield gap between Fa and Lp grew every year: Fa yielded 13 %, 22 % and 35 % more than Lp2 in first, second and third year after the year of establishment respectively. Over the three years, Fa had a 23 % higher yield than Lp2. Only mixture 1/8Lp2 overyielded Lp in each year. In 2012, all mixtures overyielded Lp, but the mixtures never overyielded Fa. Over the 15 cuts in the three growing seasons, only 1/8Lp2 had a yield that was not significantly lower than that of Fa. Hence, no transgressive overyielding was found in the mixtures of Fa and Lp. Regression of the Fa content (FaC) on the DMY indicated that the effect of mixing the grass species was mostly additive. The minimal adequate model describing the effect of FaC and year on total DMY was a linear model for 2010 and 2011 with a different intercept but a common slope for both years. The DMY of the mixtures increased with 28.5 kg DM ha^{-1} for each extra % point in FaC. Hence, the DMY of the mixtures was not higher than the weighted average DMY of the monospecifc swards in 2010 and 2011. For 2012, the quadratic term was significant, but the shape of the polynomial was convex (Figure 1), suggesting a lower yield of the mixtures than the weighted average DMY of the monospecific swards.

In the cuts preceded by drought periods (SPI < -1), the yield of Fa was generally superior to that of Lp. In the 3rd cut of 2010, taken shortly after the end of the drought period in 2010, all mixtures were overyielding Lp, but they were overyielded by Fa on their turn. At the end of the drought period in the spring of 2011, Fa and the mixtures 1/8Lp2 and 1/8 Lp4 were overyielding Lp, but the yield of 1/8Lp4 was lower than that of Fa. In the fourth cut of 2012, Fa and 1/8Lp2 were overyielding Lp (See appendix Table A.2, for yield details per cut).

In the grass-clover trial, only in the year 2011 significant differences in the DMY of the sward compositions were found: Fa + Tr and 1/8Lp2 + Tr were overyielding both Lp2 + Tr and Lp4 + Tr. Over the 15 cuts in the three growing seasons, the yield of Fa + Tr was 20 % higher than that of Lp2 + Tr. Only the grass species mixture 1/8Lp2 + Tr had a DMY that was significantly higher than that of Lp + Tr (Table 5).

In the cuts taken towards the end of drought periods (PSI < 1), the effect of the sward compositions on the performance was similar to the pure grass trial: Fa + Tr was mostly overyielding Lp + Tr; but the performance of the grass species mixtures with clover was rarely significantly better than that of Lp + Tr. (See appendix Table A.3, for yield details per cut).

Botanical compositions of mixtures

In both trials, FaC in all mixtures was lower than the sown proportion in the first year after sowing but it increased over the years (Table 6). Over the three years, in the pure grass trial, the FaC was significantly lower in mixtures with an initial Lp proportion of 1/4 than 1/8 ($p < 0.001$) and in combination with Lp2 than Lp4 ($p < 0.001$); no interaction was found between both factors ($p = 0.31$).

Over the three years in the grass clover trial, the effect of ploidy of the Lp component on FaC was not significant ($p = 0.051$), but the effect of the initial proportion of Lp in the mixtures was significant ($p < 0.001$): the higher the FaC in the initial seed mixtures, the higher the FaC. No interaction was found between both factors ($p = 0.63$).

In both trials, FaC increased stepwise after each harvest year with large differences between the last autumn cut of the previous year and the first cut of a succeeding year. Although FaC mostly decreased after the first cut in the pure grass trial, its change within the years was limited compared to the progress between the years (Figure 2a and 2b). In the grass clover trial, there was a decreasing trend in the FaC in the total DMY within each year (Figure 2b); the trend was inversed to the evolution of the clover content in the harvested dry matter (TrC) (See appendix Figure A.1)

The TrC was not significantly affected by the companion grass species or grass species mixture. Both for grass species and mixtures, the clover content in the harvested material was higher in the second year (19.9 % - 26.3 %) than in the first year (18.8 % - 22.5 %), but it decreased again in the third year (11.7 % - 15.3 %). Within each year, there was a clear seasonal pattern: low clover content in spring, steeply increasing in the summer and decreasing again in the autumn and winter (see appendix Figure A.1). In the third cut of 2010, towards the end of a drought period, this pattern was disturbed: TrC increased more strongly in combination with Lp than in combination with Fa: the TrC increased from 18, 14 and 18 % for Fa, Lp2 and Lp4 respectively in the second cut to 23 % for Fa and 30 % for Lp2 and Lp4 in the third cut.

Effect of mixtures on feeding quality

In the pure grass trial, IVOMD of Fa and all mixtures of Fa and Lp was significantly lower than that of Lp in every year (Table 7). Particularly in the first two cuts of 2010 and the first cut of 2012 differences between Fa and Lp2 were high: 11 % points, 18 % points and 14 % points respectively. For every year, a linear negative effect was found of the FaC on the IVOMD (Figure 3). Hence, the mixture 1/4Lp4, the mixture with the lowest FaC, had the highest IVOMD. The IVOMD of this mixture was in every year significantly higher than that of Fa. Where all the mixtures had a higher IVOMD than Fa in 2010, only the mixture 1/4Lp4 had a higher IVOMD than Fa in 2012 as a result of the increasing FaC in the mixtures.

The presence of white clover did not change substantially differences in IVOMD in the harvested biomass. Indeed, the difference in IVOMD between Fa + Tr and Lp + Tr was from the same order of magnitude as in the pure grass trial: 5% points at least. All grass species mixtures with Tr had a significantly lower IVOMD than Lp, but the mixtures 1/4Lp2 + Tr and 1/4Lp4 + Tr with the lowest FaC had a significantly higher IVOMD than Fa + Tr in 2010 and 2012 (Table 8).

In none of the years and in none of the trials, significant differences were found in the CPC between the grass species or the mixtures (Table 7 and Table 8).

The significant DMY differences, resulted in significant differences in nitrogen yield (NY). In the pure grass trial, the NY of Fa and 1/8Lp2 was significantly higher than that of Lp in 2011 and 2012 (Table 7). In the grass clover trial, Fa + Tr had a higher NY than Lp + Tr in 2011; NY of the grass species mixtures with clover was intermediate (Table 8).

Discussion

The higher yield potential and the lower digestibility of Fa compared to Lp were confirmed in this trial: over three years, Fa had a 23 % higher yield than Lp. In agreement with the trial of Wilman and Gao (1996), the yield difference between the two species increased with sward age; the increase was mainly due to decreasing yields of Lp with time. Mixtures did not lead to transgressive overyielding; the presumed complementarities of both species apparently did not lead to selection nor to complementarity effects in our trials with very good growing conditions. Organic matter digestibility was consistently lower for Fa than Lp; the difference was comparable to the data reported by Lutten and Remmelink (1984). Using the standard precipitation index to define dry periods, we found a clear evidence for the better drought resistance of Fa compared to Lp.

In both trials, mixtures of Fa and Lp expressed a limited superiority to Lp in dry periods: the higher the FaC in the mixtures, the more they outyielded pure Lp swards. The yield of the mixtures with low FaC was lower than Fa under drought. Although the SPI indicated severe drought periods, plant growth was not really hampered, most probably because of the absence of severe summer droughts. As the drought periods in 2010 and 2011 occurred in spring we hypothesize that there was still enough plant available water in the soil to ensure grass growth, even if SPI was indicating severe drought. SPI is uniquely related to probability and gives no information on plant available water.

Higher N yields were found for Fa and some mixtures of Fa and Lp compared to Lp. We presume that the deeper rooting of Fa than Lp (Willman *et al.*, 1998; Van Eekeren *et al.*, 2010) resulted in a better exploration of the soil profile, and that the longer leaf life span of Fa than Lp (Lemaire *et al.*, 2009) resulted in a lower build-up of soil organic matter under Lp than under Fa.

Increase of FaC in our experiment mainly occurred between the last cut of a previous year and the first cut of the following year. Gilliland *et al.* (2011) found that Fa (*cv.* 'Barolex') had a higher productivity per degree centigrade and per unit of photosynthetic active radiation than a late Lp variety (*cv.* 'Portstewart') in the spring period under low temperature and light intensity. These phenomena may explain the sharp increase of FaC in the first growth of each growing season in our experimental period. We have no data on photosynthetic efficiency of early nor of intermediate Lp varieties, so it remains speculative to consider that early Lp varieties might compete better with Fa in spring and hence temper the dominance of Fa in Fa + Lp mixtures. However experiments by Suter *et al.* (2012) support this hypothesis. They found that in mixtures containing *Dactylis glomerata* and Lp, substitution of a late Lp variety by an early Lp variety in the mixtures resulted in a five times higher Lp proportion in the mixtures (30 % *versus* 6 %).

The IVOMD of the mixtures was found to be additive. It could be predicted by the weighted average composition and the weighted average IVOMD of the Fa and Lp components. We found the largest differences in IVOMD between Fa and Lp in the first cut of each year. The reason may lay in the differential development of the Fa and Lp component combined with the sharp increase of FaC during early spring growth. Indeed, we used intermediate Lp varieties heading approximately 14 days later than the Fa variety. As harvest dates were oriented according to the development of Lp swards, the development of Fa in both the pure swards as in the mixtures was ahead of the Lp development resulting in a lower quality. To solve this problem the first cut should be taken earlier, or one should use mixtures with components with a better corresponding development, *e.g.* by using earlier heading Lp varieties or later heading Fa varieties. Indeed heading dates of actual Fa varieties (found on the French variety list), fall within the range of early Lp varieties (Haquin, 2012). Using varieties with a more even development may prevent the sharp increase of FaC during spring growth improving the quality of the early harvested forage. If later on in the growing season, the FaC is increasing, it may form a buffer against drought with less effect on quality.

Initially our grass swards contained approximately 50% Fa in 1/4Lp and 60% Fa in 1/8Lp to evolve up to approximately 75% and 90% respectively at the end of the third year. This trend was similar both under a high N application and a low N application + white clover. Although the effect of the composition of the seed mixture on the FaC decreased every year, it remained significant in the third year.

What can one do to strive for a stable, evenly distributed species composition? Enhancing the Lp component in the seed mixture? Wilman and Gao (1996), used a seed mixture in which both species were equally represented; this led to swards that were dominated by Lp from the first year till the third year after sowing. Probably a Lp composition in the seed mixture between 1/4 and 1/2 (*e.g.* 1/3) may be a good try.

Changing the cutting regime? Surault *et al.* (2006), found that Fa increased under high compared to low N fertilization (250 kg N ha^{-1} *versus* 60 kg N ha^{-1}) and under infrequent compared to frequent cutting (every 45 days *versus* 25 days). So an extra cut (*e.g.* 6 instead of 5) may delay the dominance of Fa and improve the quality of the forage simultaneously.

Tetraploid perennial ryegrass competed better with Fa than diploid perennial ryegrass, resulting in a lower FaC in the pure grass mixtures with Lp4 than in the pure grass mixtures with Lp2. The well known better early vigour of tetraploid seedlings may explain this ploidy effect. From this perspective, the use of tetraploid Lp varieties seems to be advantageous. Unfortunately mixtures with tetraploid Lp had a lower drought tolerance than mixtures with a diploid Lp component.

So to prevent or to delay a Fa dominance it is provisionally recommended to use a tetraploid Lp variety in a proportion between 1/4 and 1/2 of the seed mixture and to apply a frequent cutting management. Hence there are conflicting interests. Striving for an evenly and stable species composition does not take the full advantage of a better drought tolerance. The question is what is really most important: the drought tolerance or the stable composition? Under grazing conditions, the latter may prevail; under zero grazing, there are many options to mix forages to produce rations with a balanced feeding value.

However if there is no transgressive overyielding, the meaning of mixtures may be questioned. One could grow either pure stands of Fa and of Lp and mix them later in the ruminants rations. Under grazing a productive, drought tolerant sward with an acceptable feeding quality remains an important goal which makes species mixtures meaningful.

Conclusion

Regarding our research hypotheses we can conclude that:

1. Swards sown with mixtures of Fa and Lp have a higher drought resistance than pure Lp swards, and a better digestibility than pure Fa swards.
2. No transgressive overyielding was found with the mixtures; the interaction between Fa and Lp was similar under high and low N fertilization.
3. The Fa proportion in the seed mixture and ploidy influence the botanical composition and hence the characteristics of mixed swards until the three years after sowing. The FaC in the mixtures was lower in combination with tetraploid Lp than diploid Lp.

In the absence of transgressive overyielding, mixing Fa with Lp guarantees a forage production in dry periods with a reasonable forage quality. More research is needed to find optimal mixtures composed with Lp and Fa varieties leading to a relative dominance of Lp in the spring and Fa in the summer guaranteeing productive swards with a reasonable forage quality and a good drought tolerance.

Table 1 Sowing density (number of germinating seeds m^{-2}) for *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both grass species with or without *Trifolium repens* (Tr).

Sward compositions	Tall fescue	Diploid perennial ryegrass	Tetraploid perennial ryegrass	White clover
Pure grass trial:				
Fa	1500	0	0	0
Lp2	0	1500	0	0
Lp4	0	0	1500	0
1/8Lp2	1312	188	0	0
1/8Lp4	1312	0	188	0
1/4Lp2	1125	375	0	0
1/4Lp4	1125	0	375	0
Grass-clover trial:				
Fa+Tr	1500	0	0	700
Lp2+Tr	0	1500	0	700
Lp4+Tr	0	0	1500	700
1/8Lp2+Tr	1312	188	0	700
1/8Lp4+Tr	1312	0	188	700
1/4Lp2+Tr	1125	375	0	700
1/4Lp4+Tr	1125	0	375	700

Table 2 Harvesting dates in 2010-2012.

Cut	2010	2011	2012
1	27 April	2 May	11 May
2	2 June	14 June	18 June
3	19 July	18 July	25 July
4	24 August	22 August	5 September
5	4 October	10 October	15 October

Table 3 Standardized Precipitation Index (SPI) calculated for the experimental site in Merelbeke, Belgium during the experimental period, for every month using a time scale of two months.

Month	Standard precipitation index		
	2010	2011	2012
January	-0.12	-0.19	1.3
February	-0.51	0.11	-0.41
March	0.04	-1.58	-1.26
April	-0.62	-2.32	0.17
May	-1.62	-2.52	1.14
June	-1.77	-0.82	0.68
July	-1.23	0.6	1.01
August	1.4	0.62	0.23
September	2.2	-0.06	-1.02
October	0.88	-0.9	0.14
November	0.73	-2.52	-0.06
December	0.44	0.37	1.13

Table 4 Dry matter yield (kg ha^{-1}) for *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both species (indicated in Table 1) in three successive years (2010-2012).

	1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4	Fa	Lp2	Lp4	Sign.[A]
2010	14319^{ab}	14600^{ab}	15363^{a}	14934^{ab}	15487^{a}	13746^{bc}	13046^{c}	***
2011	13085^{ab}	13170^{a}	14321^{a}	13716^{a}	14357^{a}	11798^{bc}	11555^{c}	***
2012	14974^{a}	15301^{a}	16958^{a}	15131^{a}	16988^{a}	12550^{b}	11499^{b}	***
Total	42379^{c}	43070^{bc}	46643^{ab}	43781^{bc}	46832^{a}	38094^{d}	36099^{d}	***
Relative	90.5	92.0	99.6	93.5	100.0	81.3	77.1	

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

Table 5 Dry matter yield (kg DM ha^{-1}) of *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both grass species in association with *Trifolium repens* (Tr) (indicated in Table 1) in three successive years (2010-2012).

	1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4	Fa	Lp2	Lp4	Sign.^{A}
				+ Tr				
2010	13067	12655	14123	12789	14733	12212	11562	NS
2011	14378^{abc}	14132b^{cd}	15326^{ab}	14377^{abc}	15994^{a}	12446^{d}	12981^{cd}	**
2012	16262	15820	17107	16479	16975	15037	13952	NS
Total	43707^{ab}	42606^{ab}	46556^{a}	43640^{ab}	47701^{a}	39695^{b}	38496^{b}	***
Relative	91.6	89.3	97.6	91.5	100.0	83.2	80.7	

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

Table 6 *Festuca arundinacea* content (FaC) (%) in the dry matter yield of swards sown with mixtures of *Festuca arundinacea* and *Lolium perenne* (indicated in Table 1) in three successive years (2010-2012). Data are weighted averages over all cuts (indicated in Table 2).

	1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4	Sign.^{A}	1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4	Sign.^{A}
								+ Tr		
2010	50.4^{b}	39.6^{c}	64.4^{a}	55.1^{ab}	***	32.4^{b}	28.7^{b}	47.0^{a}	45.6^{a}	**
2011	67.9^{b}	53.9^{c}	82.8^{a}	70.6^{b}	***	44.5^{ab}	38.3^{b}	56.2^{a}	48.7^{ab}	*
2012	79.8^{ab}	69.4^{b}	88.6^{a}	85.8^{a}	**	67.6^{a}	60.8^{b}	77.1^{a}	77.0^{a}	*

A: Significance of the anova model with the four sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

Table 7 *In vitro* organic matter digestibility (IVOMD), crude protein content (CPC) and N yield (NY) for *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both grass species (indicated in Table 1) in three successive years (2010-2012). Data are weighted averages over all cuts (indicated in Table 2).

		1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4	Fa	Lp2	Lp4	Sign.[A]
2010									
IVOMD	(%)	77.2^{bc}	78.1^{b}	74.8^{c}	75.9^{c}	72.7^{d}	80.5^{a}	81.4^{a}	***
CPC	(%)	12.8	12.6	12.7	13.0	12.5	12.9	12.9	NS
NY	(kg N ha^{-1})	293.4^{ab}	294.8^{ab}	313.4^{a}	311.7^{a}	309.9^{a}	283.6^{ab}	270.1^{b}	*
2011									
IVOMD	(%)	72.6^{e}	74.1^{c}	71.3^{ef}	72.7^{d}	70.4^{f}	76.0^{b}	77.9^{a}	***
CPC	(%)	13.4	13.0	13.2	13.3	13.3	13.7	13.5	NS
NY	(kg N ha^{-1})	281.1^{abc}	274.6^{abc}	303.4^{a}	291.3^{ab}	305.6^{a}	258.3^{bc}	250.7^{c}	***
2012									
IVOMD	(%)	71.1^{bc}	72.5^{b}	70.8b^{c}	70.5^{bc}	69.8^{c}	77.6^{a}	79.1^{a}	***
CPC	(%)	12.1	11.8	11.7	12.2	11.9	12.1	12.0	NS
NY	(kg N ha^{-1})	288.4^{c}	288.5^{c}	317.0^{ab}	293.9^{bc}	324.3^{a}	242.4^{d}	220.9^{d}	***

A: Significance of the anova model with the four sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

Table 8 ***In vitro*** **organic matter digestibility (IVOMD), crude protein content (CPC) and N yield (NY) for** ***Festuca arundinacea*** **(Fa), diploid and tetraploid** ***Lolium perenne*** **(Lp2, Lp4) and mixtures of both grass species in association with** ***Trifolium repens*** **(Tr) (indicated in Table 1) in three successive years (2010-2012). Data are weighted averages over all cuts (indicated in Table 2).**

		1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4 +Tr	Fa	Lp2	Lp4	Sign.[A]
2010									
IVOMD	(%)	77.7^{b}	78.7^{b}	76.6^{bc}	77.5^{bc}	75.3^{c}	81.1^{a}	81.3^{a}	***
CPC	(%)	15.9	16.2	15.8	15.9	15.7	14.8	15.9	NS
NY	(kg N ha^{-1})	332.1^{a}	328.8^{a}	357.0^{a}	324.9^{a}	368.0^{a}	288.7^{a}	294.1^{a}	*
2011									
IVOMD	(%)	72.3^{cd}	73.1^{c}	71.0^{e}	72.0^{def}	70.1^{f}	76.7^{b}	77.8^{a}	***
CPC	(%)	17.5	16.9	17.3	16.8	16.9	17.7	17.7	NS
NY	(kg N ha^{-1})	402.3^{ab}	381.2^{bc}	424.0^{a}	385.7^{bc}	431.1^{a}	352.9^{c}	366.8^{c}	***
2012									
IVOMD	(%)	71.6^{b}	72.2^{b}	70.7^{b}	71.0^{b}	69.9^{b}	76.5^{a}	77.3^{a}	***
CPC	(%)	14.3	14.2	14.5	14.6	14.5	15.0	14.9	NS
NY	(kg N ha^{-1})	373.2	360.1	393.9	383.1	394.1	360.4	331.9	NS

A: Significance of the anova model with the four sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

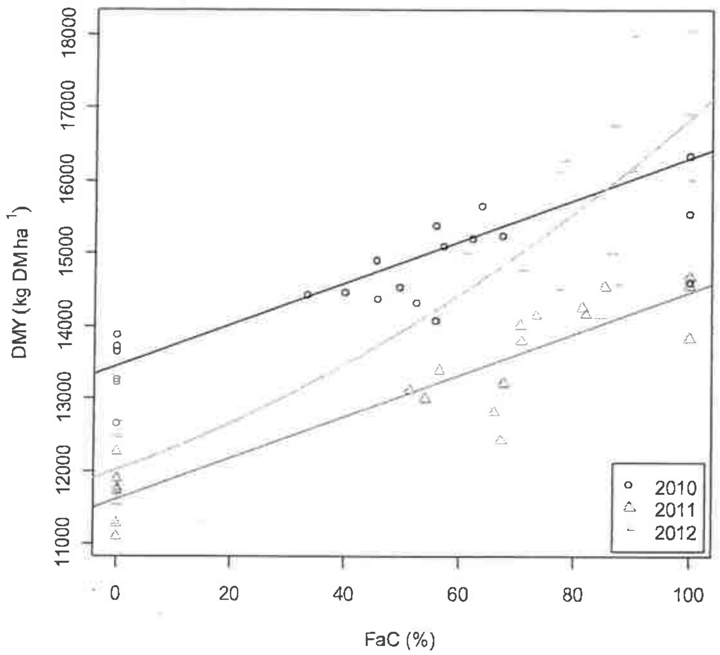

Figure 1 Regression of the dry matter yield (DMY) on the *Festuca arundinacea* content (FaC) in mixtures of *Festuca arundinacea* and *Lolium perenne* (2010: y = 13460 + 28.5x, 2011: y = 11624 + 28.5x, 2012: y = 12023 + 28.5x + 0.20x²; R^2 = 83 %).

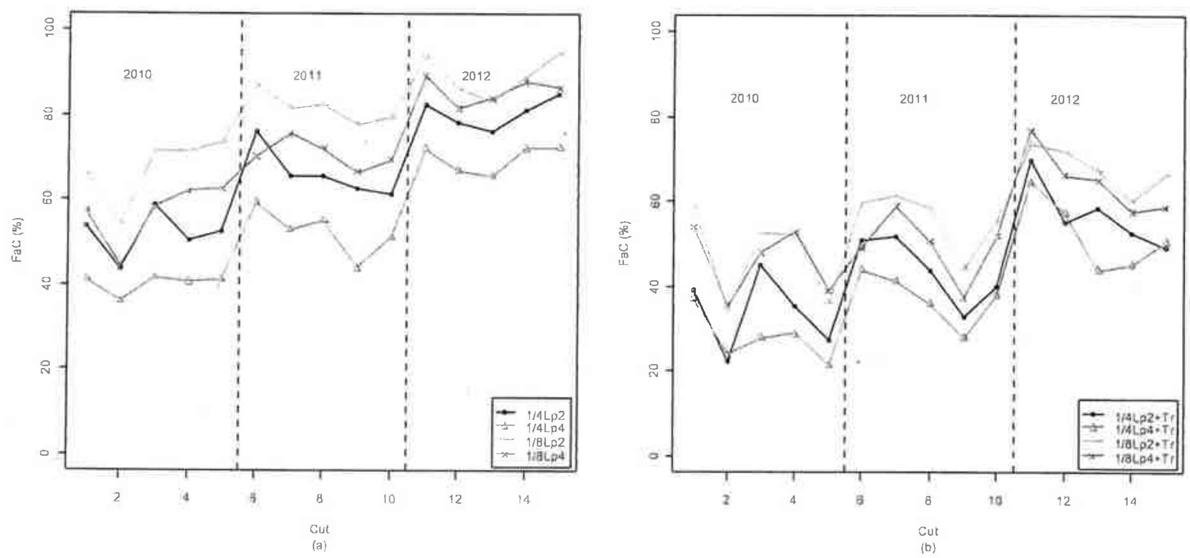

Figure 2 *Festuca arundinacea* content (FaC) in the dry matter yield (DMY) over 15 cuts in three successive years (2010-2012) in four mixtures of *Festuca arundinacea* and *Lolium perenne* (a) in pure grass trial and (b) in grass clover trial (mixture codes indicated in Table 1).

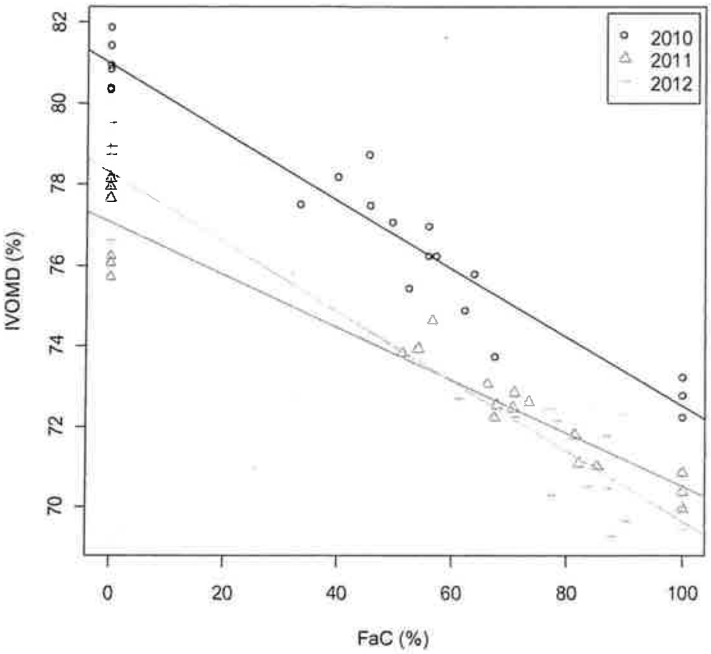

Figure 3 Regression of the weighted average *in vitro* organic matter digestibility (IVOMD) on the *Festuca arundinacea* content (FaC) in mixtures of *Festuca arundinacea* and *Lolium perenne* (2010: y = 81.0 – 0.084x, 2011: y = 77.1 – 0.065x, 2012: y = 78.3 – 0.084x; R^2 = 95 %).

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