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## **Analysis of existing experiments to deliver enhanced understanding of sward dynamics in MSS**

Rosemary Collins, Michael T. Abberton, Bernadette Julier, Francois Gastal,  
Olivier Huguenin-Elie, Sebastien Husse, Nicole Wrage

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# MultiSward

## Multi-species swards and multi scale strategies for multifunctional grassland-base ruminant production systems

Grant agreement number: FP7-244983

**Collaborative Project**  
**SEVENTH FRAMEWORK PROGRAMME**

Priority: Food, Agriculture and Fisheries, Biotechnology

### **Deliverable D2.4**

***Analysis of existing experiments to deliver  
enhanced understanding of sward dynamics in  
MSS***

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## Glossary and Definitions

ANOVA	Analysis of variance
C	Carbon
CE	Multisward Common Experiment
Ci	Chicory ( <i>Cichorium intybus</i> )
CP	Crude protein in forage
CT	Condensed tannins in forage
DM	Dry matter
DOMD	Digestible organic matter content of forage
FA	Tall fescue ( <i>Festuca arundinacea</i> )
LDMC	Leaf dry matter content
MSS	Multispecies swards
N	Nitrogen
PPO	Polyphenol oxidase enzyme in forage
PRG	Perennial ryegrass ( <i>Lolium perenne</i> )
RC	Red clover ( <i>Trifolium pratense</i> )
RGR	Relative growth rate
RGRD	Difference in species' relative growth rates
SLA	Specific leaf area
WC	White clover ( <i>Trifolium repens</i> )
WSC	Water soluble carbohydrate in forage

## Summary

**Objectives:** The objective of this Deliverable was to examine some current experimentation being carried out by partners in 'MultiSward' on sown multispecies swards (MSS), with the aim of (1) describing the extent of species dynamics in a range of experiments; (2) demonstrating the effects of species dynamics on important MSS properties (in this case forage quality) using one experiment as an example; (3) examining different approaches to the analysis of species dynamics.

**Rationale:** MultiSward partners were requested to send information and data on species dynamics in current experiments involving sown MSS. A literature review was carried out which focused on factors influencing species dynamics in MSS and experimental approaches to analysing this. Three experiments were chosen to demonstrate the extent of species dynamics in MSS and to show possible approaches to analysing species dynamics. The effects of species dynamics on sward nutritive quality were examined in detail for one of these experiments. Because species dynamics influence many properties of MSS, including ecosystem services, it was considered important to identify their effects on other aspects being researched in the MultiSward project. Therefore, links between this Deliverable and other MultiSward Workpackages are highlighted in **bold**.

**Teams involved:** ART (Switzerland), AU-IBERS (UK), INRA (France), PULS (Poland), UGOE (Germany), UMB (Norway)

**Geographical areas covered:** Conclusions are considered to be applicable to MSS across all regions of Europe.

# 1. Background

## ***Introduction***

Relationships between biodiversity and ecosystem function are central issues in plant ecology and are not yet fully understood, although the capacity of levels of biodiversity to affect ecosystem function has been recognised for a long time (Hector and Bagchi, 2007). Recent interest has been stimulated by the realisation that sustained losses of biodiversity could have a major impact on ecosystem functioning and on the services supplied to agriculture.

In multispecies swards (MSS), the ‘diversity effect’ has been defined as the excess of mixture performance over that expected from component species’ monoculture performances, the species identity effect (Loreau, 1998). The benefits of diversity can derive from interspecific interactions (eg. niche partitioning and facilitation) among species in a community (Kirwan *et al.*, 2009). Diversity has two components: species richness, or the number of species in a given area, and species evenness, or how well distributed abundance or biomass is among species within a community (Wilsey and Potvin, 2000). Until recently, the relevance of diversity-function relationships to intensively managed grassland was largely untested due to the scarcity of agronomic experiments using more than two species, and it was difficult to determine just how many plant species would be needed to produce maximum diversity effects (Hector and Loreau, 2005). It has been suggested that a highly species-rich system (> 30 species) may not necessarily meet farmers’ primary objective of producing high yields in productive and stable environments, although it may be advantageous in highly heterogeneous environments serving a multifunctional role (Sanderson *et al.*, 2004), eg. in alpine pastures. In less challenging agro-ecosystems it would clearly be useful in practical terms if the diversity effect did not rely on highly species-rich communities, but instead could be obtained with a mixture of a few species, well adapted to the appropriate environmental conditions (Kirwan *et al.*, 2007; Lüscher *et al.*, 2008). By applying the modelling framework described below, Kirwan *et al.* (2007) addressed a number of fundamental questions in agronomic diversity-function research. The results showed: (1) consistent positive effects of diversity on the annual total yield (sown plus unsown species) of plots originally sown with an equal proportion of all species (centroid); (2) these positive effects of diversity were consistent over a range of productivity from 1.3 to 13.6 t ha<sup>-1</sup>, and a spatial range from Greece to Iceland; (3) the diversity effects persisted over three harvest years at virtually all sites (Helgadóttir *et al.*, 2008).

In perennial forage systems it would be desirable for MSS to demonstrate sufficient temporal stability for the benefits of diversity to persist for several years. It would also be a considerable practical advantage if the benefits were robust to changes in the relative abundance of component species (J. Finn, pers. comm.). Difficulties in maintaining well balanced mixtures and the tendency to lose key species in MSS (Guckert and Hay, 2001) are major reasons for the prevalence of grass monocultures in temperate agriculture (although ease of management and responsiveness to fertilisation are important contributory factors). The initial species choice and subsequent sward management are likely to play important roles in determining species trajectories over time in MSS, and developing management practices that establish and maintain balanced MSS to optimise productivity through diversity effects presents a major challenge for research in grassland agronomy. The objective of this Deliverable was to examine some current experimentation being carried out by partners in ‘Multisward’ on MSS, with the aim of (1) describing the extent of species dynamics in a range of experiments; (2) demonstrating the effects of species dynamics on important MSS properties (in this case the nutritive value of forage) using one experiment as an example; (3) proposing an approach to the analysis of species dynamics based on the way in which pairs of species interact.

### **Experimental approaches to analysing MSS**

It has been suggested that maintaining an even distribution of competitive forage species in grassland mixtures, rather than maintaining a high species number, would be important for producing diversity effects (eg. reducing weed invasion) (Tracy *et al.*, 2004). However, studies on diversity and ecosystem functioning have generally used experimental designs that ignore or confound the important components of diversity (species evenness, richness and identity) (Connolly *et al.*, 2001; Schmid, 2002). Most of these studies have focused on the number (richness) or composition of species or functional groups in the community, whilst ignoring the active determinant of species' relative abundance (evenness). These fundamental questions in agronomic diversity-function research were recently addressed in COST Action 852 using a novel experimental and modelling framework (Kirwan *et al.*, 2007). The experimental design ('simplex') allowed separate analysis of the effects of the components of diversity (species evenness, richness and identity) on community function and structure in a range of four-species mixtures (comprising two legumes and two grasses) with widely varying relative species proportions and monocultures sown at two densities (Kirwan *et al.*, 2007). An experimental and modelling framework was developed which allowed separate estimates to be made of the contributions of interactions in species mixtures by manipulating relative abundance. The modelling approach used by Kirwan *et al.* (2007) was based on the proposition that the success with which pairs of species in a mixture contribute to ecosystem services such as primary productivity and resistance to weed invasion would depend on their initial relative abundance in the mixture and on their propensity to interact. This approach is currently being refined to provide more insight into the mechanisms driving diversity effects (Connolly *et al.*, 2010).

Based on the above considerations, a **Common Experiment (CE)** has been established in a number of sites participating in the Multisward project: IBERS- Aberystwyth; ART-Tänikon; ALP-Posieux; INRA-Rennes; PULS-Brody and Szelejewo; UMB-Ås. Details of the experimental set up and measurements at each site have already been described in Deliverable 2.2. Briefly, the CE involves the implementation of cutting and grazing managements on MSS. One important element of the CE has been the establishment at many sites (ART, IBERS, PULS and UMB) of a series of plots of four forage species arranged according to a simplex design. Using this design a wide range of species proportions can be analysed in a statistically efficient way. Grazed plots sown with the same species as the simplex plots but a reduced range of species proportions are used in ART, ALP, IBERS and INRA. Plots at all sites except ALP were sown in 2010, therefore providing one year's yield data for analysis in this document. Aspects of species dynamics in these plots are discussed in section 3 (2) 'Multisward Common Experiment'.

In an alternative approach to studying dynamics in MSS, plant traits have sometimes been considered as field indicators instead of the actual species composition of the plant communities. In this approach, the response of plant species to variation in resources is predicted according to a classification based on functional parameters directly linked to growth and development. This can encompass a range of traits like leaf sizes, canopy heights, or rooting depths (Díaz and Cabido 2001). For example, Ansquer *et al.* (2009) described how plant functional traits could determine the properties of MSS (including species dynamics) eg. by enabling resource capture in resource-rich or -poor environments, or by facilitating competitive dominance by some species. Four key plant traits were identified: specific leaf area (SLA), leaf dry matter content (LDMC), leaf N content and leaf C:N ratio. These traits allow species to be located on a resource capture/use-availability axis. For example, species adapted to poor resource availability (low growing species) have a low SLA and N content, and a high LDMC and leaf C:N ratio (Louault *et al.*, 2005). Plant height or specific shoot height are considered to be the most relevant traits indicating capacity for competitive dominance because they express an ability to capture light. They respond strongly to management intensity. Flowering time is also related to plant growth rates and the

capture and use of nutrients, and these latter variables are correlated with leaf traits. It responds to the frequency of defoliation (Louault *et al.*, 2005), and is closely related to herbage growth pattern (Duru *et al.*, 2005). Ansquer *et al.* (2009) found that plant functional traits in multispecies grassland tended to converge within particular managements (nutrient additions, defoliation regimes), thus producing uniformity in local plant community structure and composition. A recent study has successfully used a plant functional trait approach to model the seasonal dynamics of biomass production and sward nutritive quality in permanent grassland (Michaud *et al.*, 2012).

Plant functional traits could be used to inform the choice of species for sown MSS in order to obtain consistent levels of overall biomass production and sward nutritive value. The selection of small numbers of species with appropriate functional characteristics can potentially deliver all (or most) of the level of ecosystem function associated with higher levels of species richness. Informed by such principles, the choice of species for use in MSS can be strategically designed to include traits that maximise niche complementarity and interspecific interactions to improve resource utilisation and yield of above ground biomass (J. Finn, pers. comm.). This is discussed further in section 2 (3) 'Functional traits'.

### ***Species dynamics and ecosystem properties***

In addition to their obvious effects on biomass production, changes in the species composition of MSS over time also affect ecosystem properties such as the temporal distribution of production, yield stability, forage quality, animal intake, weed invasion, resilience to disturbance, carbon storage, water acquisition and reduction of N losses to the environment (see references in Wrage *et al.*, 2011). Some of these issues are explored in more detail in **Multisward WP 3**, and are thus outside the scope of this document, but the effects of species dynamics on aspects of forage quality in MSS are dealt with in **Multisward WP 2**, and are discussed section 3 (1) of this report. In grassland agriculture, the nutritive quality of forage is of the same agronomic importance as biomass production. There is great potential for species with complementary forage quality traits to be grown in mixtures designed to provide high quality forage for animal consumption.

A first example concerns the digestibility and energetic value of forage. One of most decisive advantages of white clover is that the rate of decline of nutritional quality throughout the plant-ageing process is far less than for grasses. Digestibility and voluntary intake of grasses decrease by 20 g/kg and 0.2 kg DM/day per week respectively, whilst their decline is two times less for white clover. Peyraud (1993) reported digestibility higher than 0.75 after 7 weeks regrowth or at flowering stage during the first growth. Under grazing the difference in DM intake between pure grass pastures and white clover-grass pastures increases with increasing age of regrowth. Ribeiro-Filho *et al.* (2003) showed that herbage intake declines by 2.0 kg DM/day on PRG pastures compared to 0.8 kg on mixed pastures. This makes mixed pastures easier to manage than pure grass pastures, because regrowth intervals can be increased without adverse effect on forage quality and energy supply. Herbage quality will be monitored in sites of Multisward CE where plots are grazed.

Another example concerns forage chemical components that will increase the efficiency of incorporation of forage-N in the animal rumen. Examples of forage chemical components that would help to achieve this aim are high protein (CP) levels from legumes in combination with high sugar (WSC) levels from grasses (Dewhurst *et al.*, 1996), appropriate levels of condensed tannins (CT) from tanniferous legume species (Kingston-Smith and Theodorou, 2000), and functional levels of the protein-protecting enzyme polyphenol oxidase (PPO) from legumes such as red clover (Lee *et al.*, 2004). Predicting the concentrations of these substances in grasses and legumes growing together in MSS is not an easy task, however, as values are affected by factors such as management, variety, grass heading date, seasonal effects and species dynamics. Similarly, estimating plant maturity, and therefore herbage quality, is not a straightforward issue in MSS, and a combination of



species in a mixture can result in a spread of pasture maturity through the year. Consequently, accurate descriptions of the link between variation in herbage quality and phenological development of MSS via modelling would be of great practical significance in planning harvest schedules to optimise herbage quality (Sanderson and Wedin, 1989). An additional factor to consider is that excessive development of structural tissue in pastures can be prevented by grazing, although other factors such as diet selection by animals also determine the herbage composition and, consequently, forage quality of the sward (Machado *et al.*, 2007). The effects of grazing on forage quality and plant species dynamics are therefore key factors to take into account in considering the design and management of MSS, and these are being addressed in the CE. An experiment is currently being conducted at IBERS, Aberystwyth in which four forage species combined in differing proportions are being analysed for biomass productivity in conjunction with forage quality parameters (see section 3 (1) of this report). The large effects of species dynamics on forage quality are very evident in these mixtures.

## **2. Factors influencing species dynamics in MSS**

It is recognised that changes in species dynamics are linked to a range of factors which are discussed below.

### ***Species relative growth rates***

Change in community composition can be expressed in many ways, including the percentage contribution of each species to total biomass, or percentage of total count from point quadrat data. Beyond such descriptions of species dynamics, however, the question of what actually drives changes in plant communities is an important one. For example, does the initial biomass of a species in a mixture predict its subsequent performance; and to what extent do co-occurring species affect and interact with each other? One way of looking at the way in which mixtures change in composition over time is to analyse species' Relative Growth Rates (RGR) and use these to predict the outcome of competition between species *i.e.* to ask the question 'which species gains?' (Connolly and Wayne, 2005; Ramseier *et al.*, 2005). This type of approach has the potential to elucidate how interactions between plant species in mixtures determine the structure of the community, although it is understood that aspects other than simple biomass accumulation may also affect the achievement of equilibrium between species (eg. leaf area index, grass tiller density, root biomass etc.). The 'RGRD' approach examines differences in species' RGRs over time, and is therefore concerned with measuring relative changes in species abundance. It focuses directly on the question of outcome: over an interval of time, is one species gaining in a mixture relative to others? (Connolly and Wayne, 2005). 'Gain' here means 'net gain', because biomass can be lost due to factors such as herbivory, senescence etc. I propose that this approach to analysing species interactions in MSS is worth considering in the Multisward project. It may be particularly appropriate in the Common Experiment, in which not only will differences in species' RGR be observed, but these may interact with the cutting/grazing and high-N/moderate-N treatments. **A preliminary attempt was carried out to use the concept of RGRD to analyse data from an MSS experiment in IBERS, Aberystwyth (see section 3 (1) of this report).**

### ***Management effects***

In agricultural grassland initial levels of species diversity are manipulated by the imposed management, and species richness and evenness are in dynamic equilibrium with the management (Wrage *et al.*, 2011). Management effects can potentially override the diversity effects produced by species mixtures eg. via applications of fertilisers, or by grazing. However, Nyfeler *et al.* (2009) found that the application of even very high rates of N-fertiliser (450 kgN/ha/yr) did not prevent diversity effects from occurring in the Swiss site in the COST 852 experiment. **The effects of grazing/cutting and high-N/moderate-N applications on diversity and species dynamics in MSS are analysed in the CE (see section 3 (2) of this report).**

### ***Functional traits***

Several studies have pointed out the larger impact of species identity or functional diversity than species number on primary production (references in Wrage *et al.*, 2011), and the principle of combining functionally complementary species in agricultural grassland mixtures has been used effectively in Switzerland for many years (Suter, 2010). In the MultiSward CE, mixtures were strategically designed by using a functional group approach to select forage species for inclusion. Functional diversity in mixtures is not necessarily described only by the presence or absence of legumes. Thus, in the CE, in addition to a 'N-fixing' vs 'non-N-fixing' contrast, there is also a root depth contrast: 'shallow-rooted' vs 'deep-rooted'. It was hypothesised that this functional diversity would promote optimal levels of species complementarity in the mixtures, because functionally similar plant species are likely to compete for the same resources. However, it has also been proposed that legume/grass mixtures exhibit *inherent* fluctuations in species balance. For example, Schwinning and Parsons (1996) described an intrinsic oscillatory component of variation in grass/legumes systems which, together with external stimulants of legume yield variation, interacts to produce a random element to species balance. The key to understanding the source of yield variability in grass/legume mixtures is the difference in these functional groups' response to N in the soil. Grasses supply their demand for N solely by uptake of mineral N in the soil; if soil N levels are low, then the growth rate of grasses will be slow. In contrast, legumes supplement soil mineral N uptake with N-fixation and can thus maintain high growth rates in low soil N environments. The contrasting responses of grasses and legumes to the availability of soil N provides one explanation for their sustainable coexistence: at low soil N legumes have a growth advantage and should exclude grasses; at higher soil N grasses have the advantage and should exclude legumes. Thus, in conditions of stable soil N Schwinning and Parsons (1996) argued that these species could not coexist. However, soil N level is a highly dynamic variable that not only affects plants, but is also affected by them. Thus, in conditions of low soil N the dominance of legumes causes N to accumulate in the soil, which eventually promotes dominance of the grass component (the so-called 'paradox of enrichment'). Schwinning and Parsons (1996) proposed that this negative feedback control of soil N status maintains the competitive balance between grasses and legumes in mixed swards. However, the control of soil N is a relatively slow process, and their models showed that grasses and legumes can oscillate in dominance for many years before an equilibrium is reached. **This highlights the importance of results from long-term experiments on MSS eg. the experiment sown in 2003 in INRA, Lusignan (see section 3 (3) of this report).**

### ***Genetic change over time***

Programmes of forage germplasm improvement usually result in synthetic varieties derived from intercrossing between small numbers of parental genotypes. However, many of the major components of temperate grasslands are outbreeding, heterozygous perennial species. This means that they contain considerable within-population genetic variation.

Consequently, although a successful forage variety released in the EU must satisfy the requirement of distinctiveness, uniformity and stability (as well as value for cultivation and use), it will nevertheless be composed of genetically distinct individuals (Humphreys *et al.*, 2011). Because of this, within-species variation in traits is an important aspect of grassland diversity. Given the high degree of genetic variation present in outbreeding forages, genetic change will inevitably occur in pastures over time and this is likely to affect species interactions and dynamics. In sown pastures the establishment year is characterised by high genotype mortality rates and a decrease in variability among the survivors (Charles, 1961). In swards with stable managements, further natural selection processes occur after establishment eg. by directional selection acting to stabilise relevant population trait values around an optimum, thereby gradually reducing population variability still further (Hill, 1990). Countering the tendency towards reduced intraspecific variability over time, species diversity itself has been shown to promote genotypic diversity in grassland mixtures (Adams and Vellend, 2011). It has been suggested (Allard and Adams, 1969) that selection in mixed populations favours the survival of genotypes that are good competitors and at the same time good neighbours – presumably resulting in mixtures in which component species have reached an equilibrium. The identification of traits conferring such ‘ecological combining ability’ in mixture components could be speeded up considerably by the use of molecular techniques. **The complex issue of genetic change in MSS is being addressed in MultiSward WP2 (Task 2.2) using marker-based approaches to measure allelic shifts over time in, for example, plant architecture traits. Such traits are likely to be highly important in determining the outcome of plant competitive interactions in MSS.**

### **3. Analysis of existing MultiSward experiments**

#### **3 (1) IBERS-Aberystwyth, UK: ‘Biomass production and forage quality in MSS’**

In order to affect biomass production in MSS, it seems intuitive to suppose that a component species must be present in significant quantities. Thus, an efficient strategy may be to mix a low number of species of comparable competitive ability to produce equilibrated mixtures *i.e.* mixtures with a high level of species evenness (Lüscher *et al.*, 2011). This experiment focuses on the role of species evenness in determining the productivity and nutritive value of MSS. To achieve this, evenness in a group of four temperate forage species was manipulated to create eight mixtures: four near-monocultures (low evenness) and four near-centroids (high evenness). The expectation was that the largest diversity effect would be found in the near-centroids, in which interspecific interactions would have the greatest opportunity to be expressed. The component species were selected on the basis of their potential contribution to sward nutritive quality and reduction of rumen N-inefficiency, rather than on the basis of biomass productivity alone (particularly in the case of Lotus).

32 field plots (6 m<sup>2</sup>) containing eight multispecies mixtures (comprising different sown proportions of perennial ryegrass (PRG) (high sugar variety cv. AberDart), timothy (cv. S48), red clover (cv. AberRuby) and *Lotus corniculatus* (cv. Leo)) were established in 2008 in a randomised block design with four replicates (Table 1). The selected species provided these contrasts: (a) legumes vs. grasses; (b) within the grasses, high sugar vs. low sugar trait; (c) within the legumes, tannin containing vs. non-tannin containing trait.

**Table 1.** Composition of mixtures presented as proportions of the standard monoculture sowing rate for each species. Standard monoculture sowing rates used were: Lotus = 10 kg ha<sup>-1</sup>; red clover = 10 kg ha<sup>-1</sup>; PRG = 20 kg ha<sup>-1</sup>; timothy = 20 kg ha<sup>-1</sup>.

Mixture	Evenness	Lotus	Red clover	PRG	Timothy
1	Low	0.91	0.03	0.03	0.03
2	Low	0.03	0.91	0.03	0.03
3	Low	0.03	0.03	0.91	0.03
4	Low	0.03	0.03	0.03	0.91
5	High	0.34	0.22	0.22	0.22
6	High	0.22	0.34	0.22	0.22
7	High	0.22	0.22	0.34	0.22
8	High	0.22	0.22	0.22	0.34

After sward establishment no further weeding was carried out on the plots. The experiment was managed under a locally-realistic conservation cutting regime *i.e.* 3 cuts per year (late spring, mid-summer, late summer) at a height of 4 cm. The plots received a total of 90 kg N/ha/yr applied in three equal doses. Harvested biomass was subsampled and separated into its sown and unsown species components, oven-dried at 80°C and weighed. Representative sub-samples were also collected from each plot at each harvest, oven dried at 60°C and finely milled in preparation for standard chemical analyses of the major forage quality parameters. This discussion focuses on DOMD (digestible organic matter content) and relationships between concentrations of WSC (water soluble carbohydrate) and CP (crude protein). Results were analysed by ANOVA, focusing on species evenness effects on annual dry matter yield, and on mixture effects for forage quality. Results of sward yield are presented in Table 2 for 2009 (Year 1), 2010 (Year 2) and 2011 (Year 3). Forage quality results are presented in Table 3 for 2009 and 2010 (2011 results not yet available).

**Table 2.** Annual sown species yield (kg ha<sup>-1</sup>)

Evenness	Year 1	Year 2	Year 3
Low	10869	5397	6144
High	14235	6582	7085
F prob.	0.002	0.046	0.03

**Biomass production:** The yield of both low and high evenness mixtures decreased sharply after Year 1 but remained stable in subsequent years. High evenness mixtures were significantly more productive in all years. Over the three years of the experiment the yield of unsown species increased substantially (36, 297 and 1194 kg ha<sup>-1</sup> in Years 1, 2 and 3), but did not differ significantly between the evenness treatments, except in Year 2 in which it was lower in the high evenness mixtures.

**Table 3.** Forage quality parameters (% DM) for each mixture

**Year 1**

Forage quality	Mixture								F prob.
<i>Cut 1</i>	1	2	3	4	5	6	7	8	
DOMD	58.82	59.07	61.03	56.85	60.21	58.05	58.69	59.38	0.284
WSC	8.58	6.65	13.59	6.91	6.55	6.86	6.64	6.36	<0.001
CP	9.82	12.2	9.51	9.24	11.86	11.11	11.26	12.43	0.002
<i>Cut 2</i>									
DOMD	63.3	63.57	64.76	63.98	63.7	64.4	63.9	62.07	0.086
WSC	4.16	5.13	8.99	6.92	5.34	5.74	5.72	4.99	<0.001
CP	16.91	16.21	14.1	14.76	16.58	16.12	16.0	16.12	0.002
<i>Cut 3</i>									
DOMD	62.62	67.32	64.7	58.96	66.37	65.68	66.06	63.93	0.072
WSC	9.44	7.03	13.07	10.64	9.38	9.22	9.89	10.75	<0.001
CP	16.69	18.54	13.91	13.31	16.58	17.64	16.18	16.09	<0.001

**Year 2**

Forage quality	Mixture								F prob.
<i>Cut 1</i>	1	2	3	4	5	6	7	8	
DOMD	66.17	64.39	65.85	64.41	64.22	64.37	64.67	64.52	0.236
WSC	7.78	9.15	13.31	10.24	14.29	11.14	13.69	13.37	0.030
CP	16.52	13.67	12.33	13.67	12.44	12.62	11.94	11.66	<0.001
<i>Cut 2</i>									
DOMD	66.92	64.43	64.58	63.85	65.53	64.61	65.31	65.51	0.020
WSC	7.25	7.0	9.07	8.27	7.77	7.4	7.89	8.35	0.176
CP	18.59	15.8	14.8	15.34	16.44	15.72	16.16	15.94	0.017
<i>Cut 3</i>									
DOMD	60.78	61.27	58.57	58.56	61.06	60.75	59.5	62.64	0.002
WSC	6.59	7.5	9.09	8.03	8.72	7.99	8.46	7.26	0.007
CP	15.59	15.02	12.91	13.23	14.38	14.23	13.77	15.33	0.016

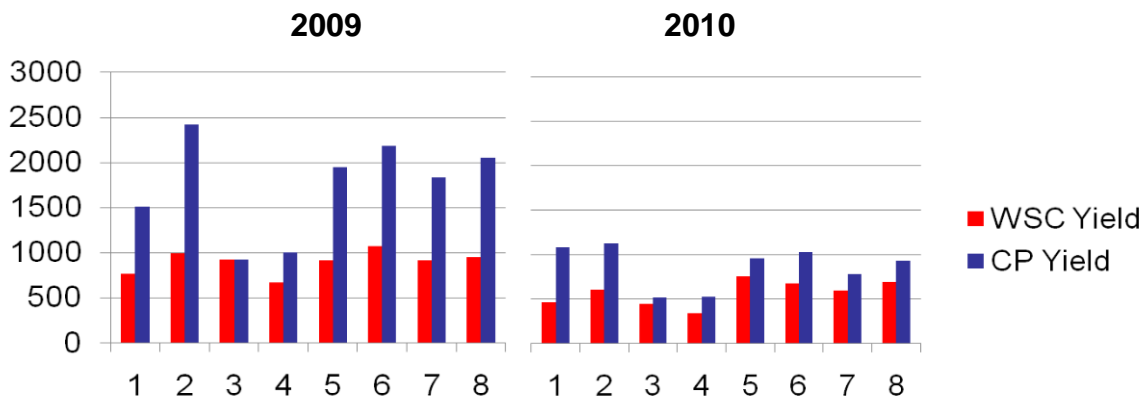
*Forage quality:* DOMD is a measure of the % of forage that can be utilised by animals. In Year 1 there was no significant effect of mixture on DOMD at any harvest. The overall mean values in cuts 1, 2 and 3 were 59.38%, 63.71% and 63.93% respectively. In Year 2 the mixtures did not differ in DOMD in cut 1 and the overall mean was 64.8%. In cut 2 there was a significant mixture effect, with the highest value (66.9%) found in the Lotus near-monoculture (mixture 1), and the lowest (63.8%) in the timothy near-monoculture (mixture 4). DOMD values were substantially lower in all mixtures in cut 3 and there was a significant

mixture effect. The highest value (62.6%) was in mixture 8 and the lowest in mixture 4 (58.5%). Overall, the DOMD values reported here are low - expected average values for a monoculture of PRG cv. AberDart in Aberystwyth would be about 71%.

There were significant differences between mixtures for herbage % CP in all harvests in Year 1. Highest levels were found in mixtures containing a high proportion of legumes, whilst mixtures dominated by grasses (3 and 4) contained the lowest amounts. There were significant differences between mixtures in herbage % WSC in all harvests. This parameter was strongly influenced by the presence of the high sugar PRG cv. AberDart, and was therefore highest in mixture 3. The second grass in the experiment, timothy, was chosen to provide a functional contrast to the high sugar PRG, as this species is naturally relatively low in WSC. This contrast was evident in the results, in which lower WSC levels were observed in mixture 4 (dominated by timothy). The ratio between WSC and CP in forage is important in terms of ruminant nutrition. The ideal ratio in terms of balancing the supply of energy and protein to the ruminant has been estimated as around 2.4:1, but this was not achieved by any treatment in either Year 1 or Year 2. In both years the highest ratios were observed in mixture 3 in cut 1, showing the benefit of the high sugar trait. However, all other mixtures and cuts were deficient in WSC due to the low yield of the high sugar PRG relative to the high yield of timothy.

Another way of analysing forage quality data such as % WSC and CP in each cut is to combine it with DM yields of each cut in order to produce values of annual substance yield – this integrates the effects of botanical composition and forage quality into one parameter. The results of this approach are presented in Fig.1.

**Fig 1.** Treatment effects (Treatments 1-8) on WSC and CP expressed as annual yield (kg/ha) for 2009 and 2010

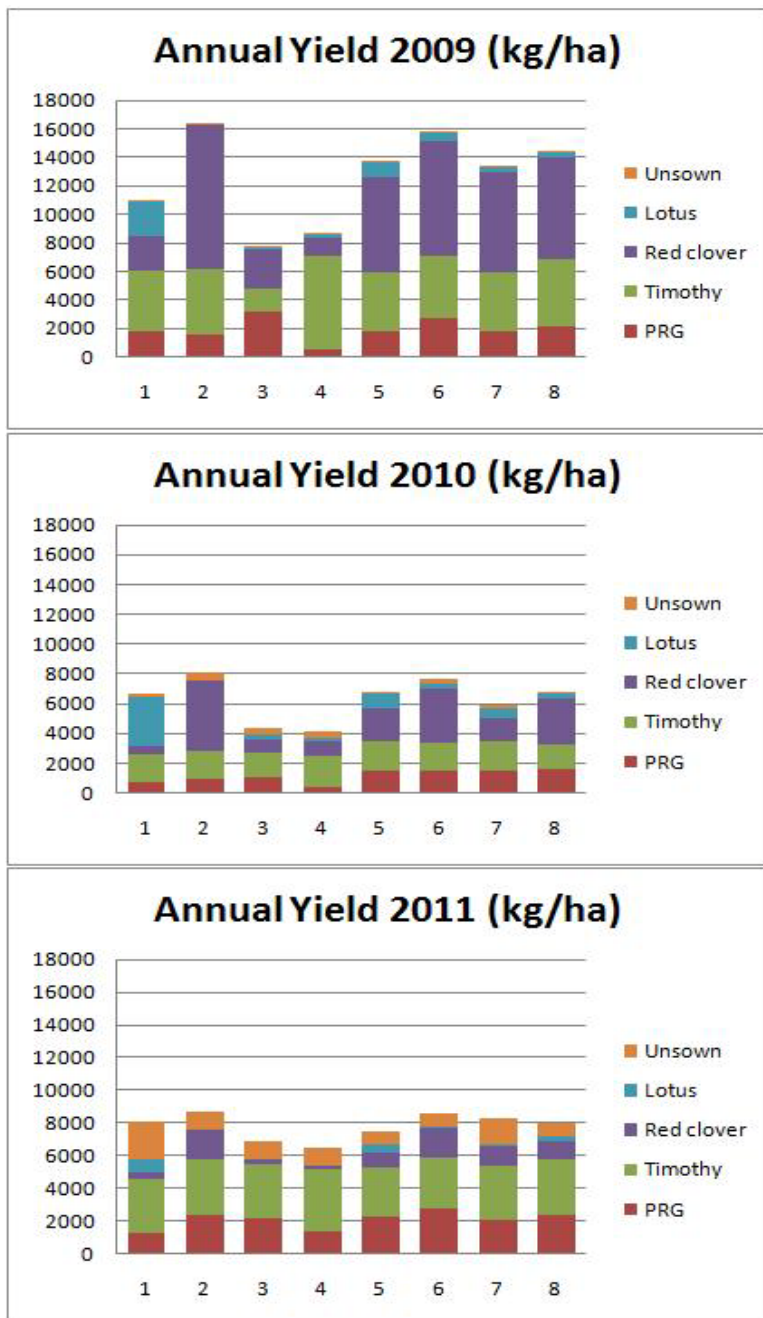


It is clear from these results that despite the high WSC content of the high sugar PRG, the actual amount of WSC provided by mixtures containing this grass was low because the grass itself was low-yielding, although it was persistent. Although all eight treatments in this experiment contain the same four species, they differ substantially in terms of forage quality and productivity.

The contributions of individual species to annual DM yield are shown in Fig. 2. Immediately apparent from the graphs is the poor persistence of both of the legume species – Lotus, in particular, disappeared from most treatments by Year 3 (2011). Yields of red clover had also decreased sharply in all treatments by Year 3. Conversely, yields of timothy and PRG in Year 3 were not very different from their levels in Year 1. It is clear that the yield of any individual species was strongly influenced not just by its relative abundance in the mixture, but by the identity of its major competitor.

Overall, the results of this experiment raise some important issues relating to the performance of MSS: (a) there were clear productivity gains in high evenness mixtures in terms of sown species yield, and (b) these persisted over time; but (c) increased resistance to unsown species invasion was not a stable characteristic of these mixtures; and (d) the nutritive value of harvested forage did not differ markedly between the two levels of species evenness, but, rather, depended on the identity of the component species. It is clear, then, that the species identity, as well as species diversity, in the mixtures had the strongest effect on their forage quality. Changes in the species composition of MSS over time (*i.e.* species dynamics) are therefore of great interest to farmers who want to obtain the maximum nutritive benefit from MSS, in addition to yield *per se*. The following section deals with species dynamics in relation to biomass productivity.

**Fig 2.** Annual yields (kg DM/ha) in eight treatments, showing the yield contributions of component species.



As discussed in the introductory section of this document, one way of analysing species dynamics in MSS is to use the concept of RGRD:

$$\text{RGR}_i \text{ is the average relative growth rate of species } i \text{ during the growth period}$$

$$= \ln(W_i/w_i)/t$$

where  $t$  is the duration of the growth period,  $w_i$  is the initial mean biomass and  $W_i$  is the mean yield of the species at the end of the growth period. For our purposes, yields of a species refer to its mean annual DM yield at the end of Years 1, 2 and 3 of the experiment. The experimental period was designated as 1 year, resulting in two periods: 1 =Year 1 to Year 2; 2 =Year 2 to Year 3. If the average RGR is the same for two species in a stand over a period of growth, RGRD = 0, so neither species gains and their relative yield does not change. Thus, if one species is initially twice as abundant as the other it will be twice as abundant at the end of the period. If RGRD > 0, however, the species with the higher RGR gains proportionally more than the other species. The RGRD procedure was carried out for pairs of species in each of the 8 treatments in this experiment for 2 time periods (RGR<sub>1</sub> and RGR<sub>2</sub>). Note that the pairwise procedure carried out here is a preliminary attempt to explore the concept and its usefulness in explaining species dynamics in MSS. Further analysis (involving modelling, as proposed by Connolly and Wayne, 2005 and Ramseier *et al.*, 2005) would be required if the idea were to be adopted by Multisward participants. A worked example for PRG vs red clover is given in Table 4.

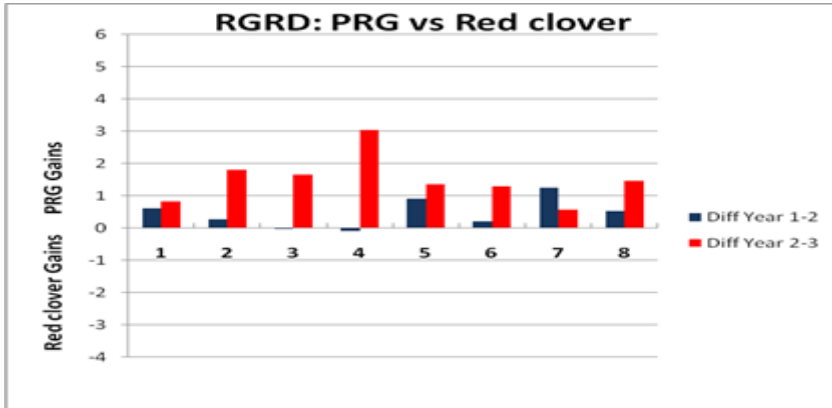
**Table 4** Values of RGR for perennial ryegrass and red clover, and the difference between them in each treatment during two growth periods.

Treatment	Growth period 1			Growth period 2		
	PRG	Red clover		PRG	Red clover	
	RGR <sub>1</sub>	RGR <sub>1</sub>	Diff	RGR <sub>2</sub>	RGR <sub>2</sub>	Diff
1	-0.89	-1.51	0.62	0.55	-0.27	0.82
2	-0.49	-0.77	0.28	0.89	-0.92	1.81
3	-1.13	-1.13	0	0.74	-0.92	1.66
4	-0.27	-0.18	-0.08	1.2	-1.84	3.04
5	-0.21	-1.12	0.91	0.44	-0.91	1.36
6	-0.58	-0.78	0.20	0.59	-0.71	1.30
7	-0.23	-1.48	1.25	0.33	-0.25	0.58
8	-0.31	-0.84	0.53	0.42	-1.04	1.47



**Fig. 3 (a-f)** Differences in RGR (based on mean annual DM yields) between pairs of species for 2 growth periods (Year 1 – Year 2, and Year 2 – Year 3).

(a)



In the first growth period both species showed negative growth in all treatments, but because PRG growth rates were usually less negative they show on the graph as relative gains. In the second growth period PRG growth rates increased, whereas those of red clover remained negative. Thus, PRG’s relative gains against red clover tended to be greater over time.

(b)

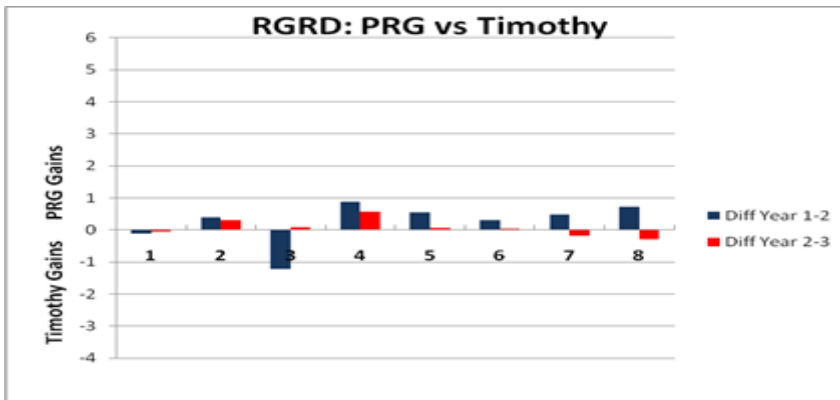


Fig. 3b shows the graphical output from RGRD analysis of PRG vs Timothy. Differences between the two grasses were smaller than those in the first (grass vs legume) comparison, and tended to be greater in the first growth period.

(c)

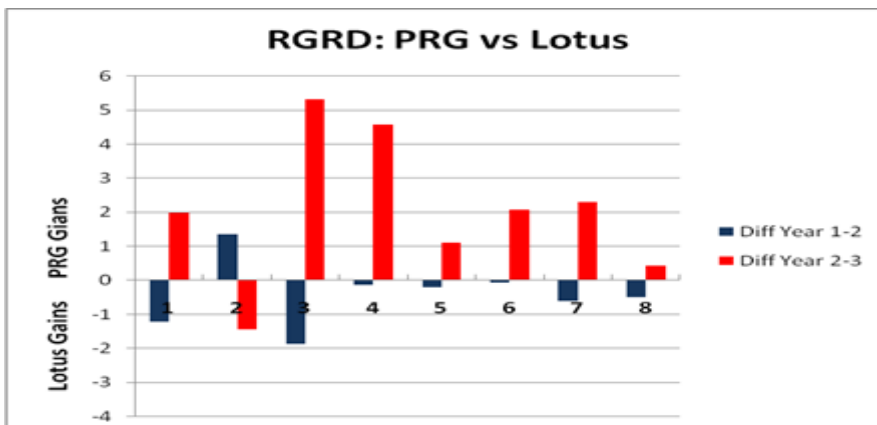
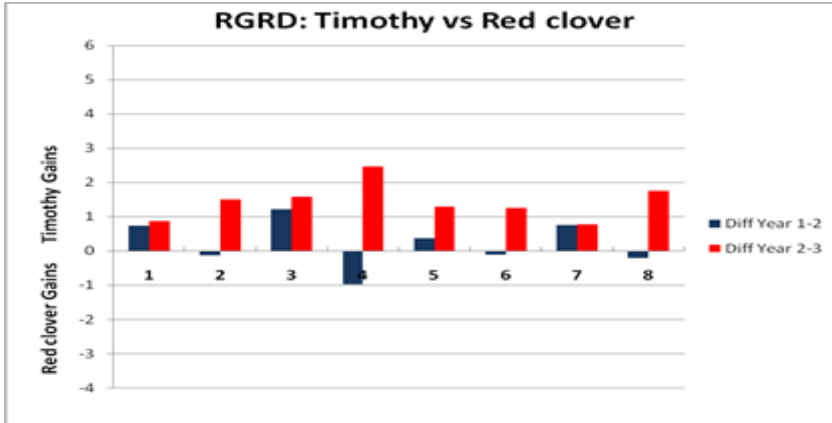


Fig. 3c shows RGRD for PRG vs Lotus and some interesting features emerge. Firstly, the competitive balance between these species was strongly influenced by treatment. Secondly,

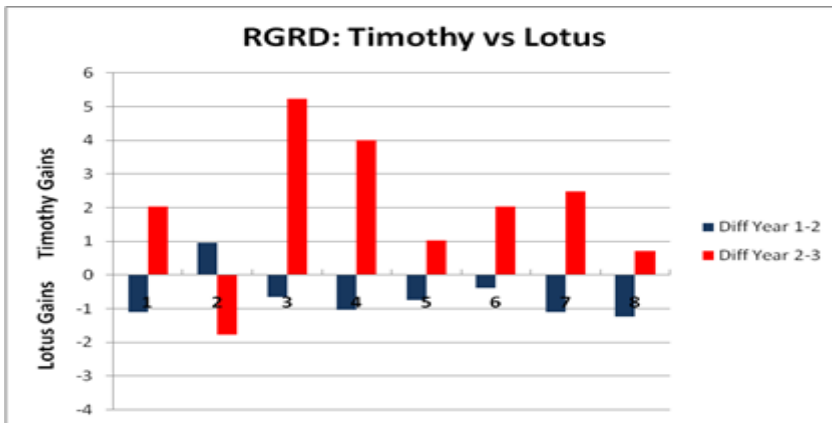
in some treatments the competitive balance reversed in successive growth periods. Overall, however, PRG tended to gain against Lotus over time.

(d)



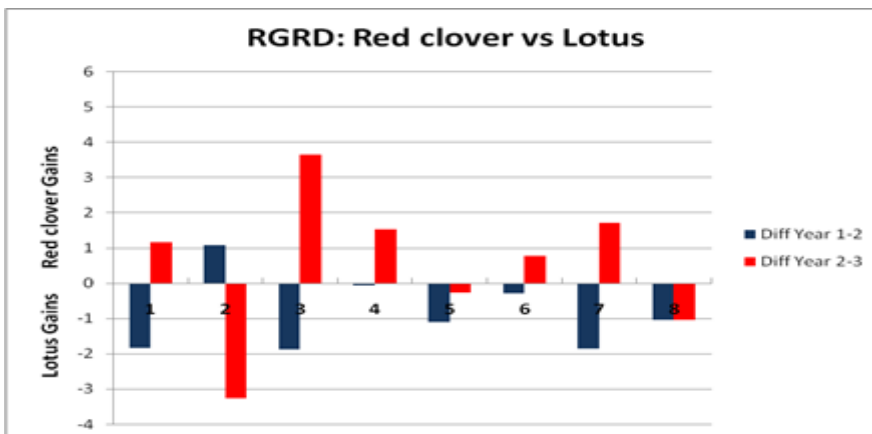
Interactions between timothy and red clover were similar to those found for PRG and red clover.

(e)



The effects of treatment and time on interactions between timothy and Lotus were almost identical to the pattern observed in the PRG vs Lotus analysis (*i.e.* Lotus tending to gain in growth period 1 before being outyielded in the next growth period).

(f)



Treatment and time both had a strong effect on the outcome of interactions between red clover and Lotus.

In the graphs presented here the scale of the RGRD axis was kept the same for all pairwise analyses so that the strength of the species' effects on each other could be readily compared. Temporal effects on species dynamics emerged clearly, with very different patterns of RGRD between the two growth periods observed in most species combinations. The exception was the PRG vs timothy combination, in which RGRD values were within the -1 to +1 range in all treatments in both years. All other combinations of grass/legume and legume/legume showed much larger fluctuations in RGRD over time and with treatment. Connolly and Wayne (2005) suggest that equilibrium points, in which RGRD values would be around zero, may take several years to emerge, and the results presented here would support this proposition. In particular, interactions of all other species with Lotus in this experiment tended to involve large and fluctuating values of RGRD. This experiment will carry on until autumn 2012, so another growth period will become available to add to the analysis. Of course, it would also be possible to use the RGRD approach to analyse species dynamics *within* each year and this would assist in identifying key times in sward development eg. how much influence does species interaction have in the establishment phase compared to other times?

**3 (2) IBERS, UK; INRA, France; Agroscope, Switzerland; UMB, Norway; PULS, Poland: *Multisward Common Experiment (Grazed, Cut-control and Simplex plots)***

Full details of the experimental design and hypotheses being tested in the CE are provided in Deliverable 2.2, available to consult on the Multisward website. The various components of the CE were sown in the above sites in 2010, meaning that data for this document are only available for the establishment year. Not all CE components are being carried out by all sites. Therefore, the preliminary analysis in this document deals with species dynamics in the only two sites in which all three components were sown – Aberystwyth (IBERS) and Tänikon (ART). Table 5 shows the treatments imposed in the grazed and cut-control plots in the CE.

**Table 5** *Treatments imposed in the Multisward Common Experiment*

Treatment	PRG	FA/Ci*	WC	RC	N fertiliser	Reps
A	1	0	0	0	low	3
B	2/3	0	1/6	1/6	low	3
C	1/2	1/6	1/6	1/6	low	3
D	1	0	0	0	high	4
F	2/3	1/3	0	0	low	3

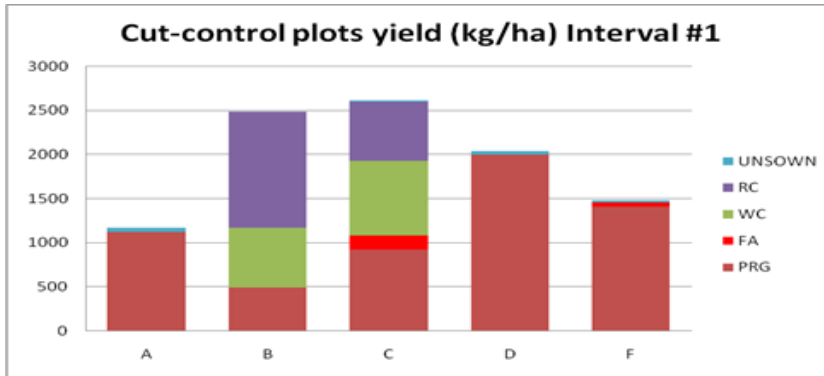
Fractions refer to the monoculture sowing rate in common agronomic use at each site.

\*In some sites FA was replaced by Ci

In the IBERS site, the botanical composition of DM yield was obtained from Grazed and Cut-control plots on two occasions in 2011 (Fig 4 a-d). Yields from Grazed plots were obtained just prior to introducing the animals to the plots, and Cut-control plots were sampled at the same time.

**Fig. 4 (a-d).** Species dynamics in cut-control and grazed plots for two grazing intervals (#1 = August; #2 = September) in IBERS in 2011.

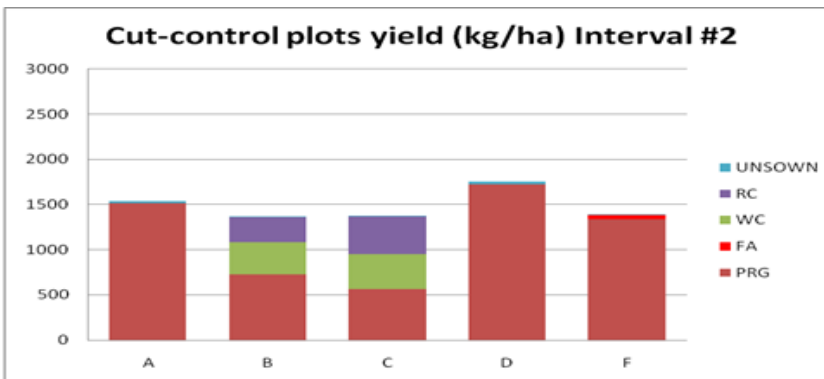
(a)



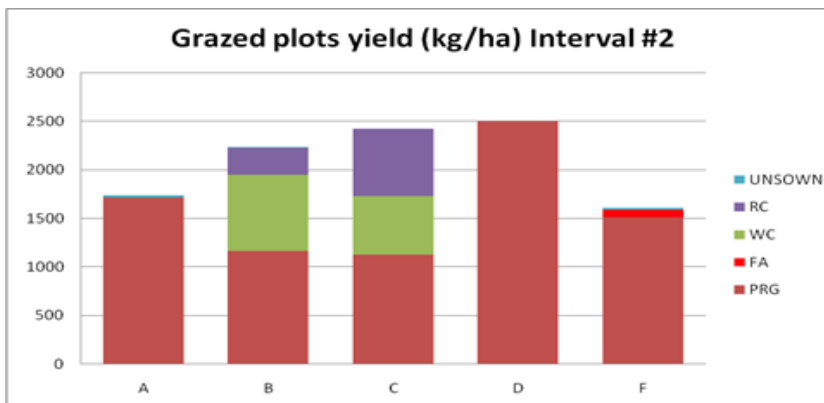
(b)



(c)



(d)



It is evident from these results that species proportions changed considerably in the two intervals and that there also appeared to be interactions with defoliation management. The RGRD approach described previously would potentially identify the species interactions that were most affected by time and management.

The third component of the CE consists of a much wider range of species proportions with plots arranged in a simplex design (unreplicated). These plots are managed under cutting and are defoliated at the same time as the Cut-control and grazed plots. They add a much wider range of species proportions than would be feasibly managed under grazing, and so provide extra information for the CE. The simplex plots receive the same level of external N as the low-N cut-controls and grazed plots.

**Table 6.** Simplex plot profiles. Proportions relate to monoculture sowing rates in common agronomic use at each site.

Plot Profile	Seeding density	PRG	FA/Ci	WC	RC
1	High	0.66	0.11	0.11	0.11
2	High	0.11	0.66	0.11	0.11
3	High	0.11	0.11	0.66	0.11
4	High	0.11	0.11	0.11	0.66
5	High	0.25	0.25	0.25	0.25
6	High	0.5	0.5	0	0
7	High	0.5	0	0.5	0
8	High	0.5	0	0	0.5
9	High	0	0.5	0.5	0
10	High	0	0.5	0	0.5
11	High	0	0	0.5	0.5
12	High	1	0	0	0
13	High	0	1	0	0
14	High	0	0	1	0
15	High	0	0	0	1
16	Low	0.66	0.11	0.11	0.11
17	Low	0.11	0.66	0.11	0.11
18	Low	0.11	0.11	0.66	0.11
19	Low	0.11	0.11	0.11	0.66
20	Low	0.25	0.25	0.25	0.25
21	Low	0.5	0.5	0	0
22	Low	0.5	0	0.5	0
23	Low	0.5	0	0	0.5
24	Low	0	0.5	0.5	0

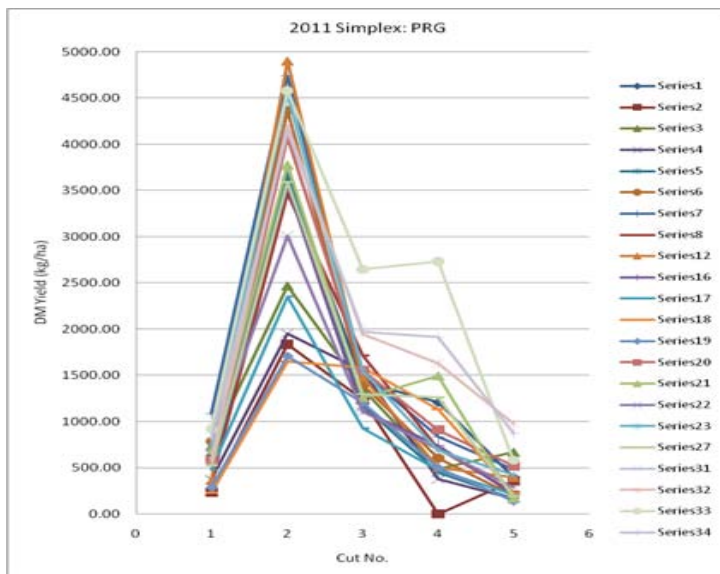
25	Low	0	0.5	0	0.5
26	Low	0	0	0.5	0.5
27	Low	1	0	0	0
28	Low	0	1	0	0
29	Low	0	0	1	0
30	Low	0	0	0	1

In addition to these basic plots, some sites installed extra treatments eg. in ART-Tänikon, 3 plots of PRG under high-N and 3 each of monoculture WC and RC under zero-N.

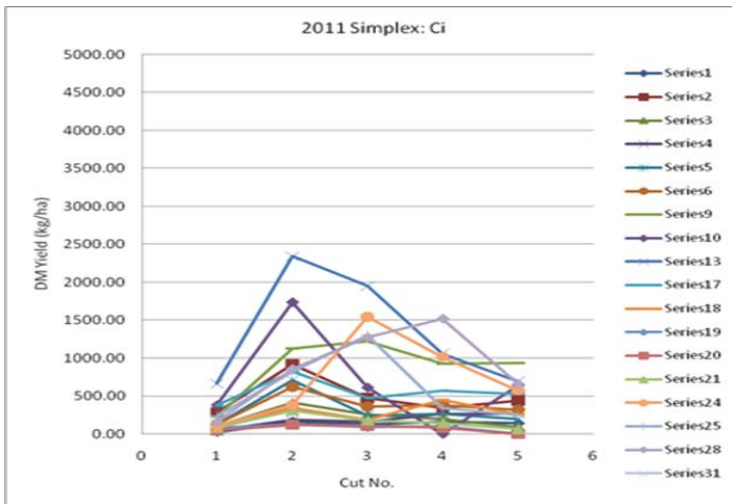
Species proportions are measured in the simplex plots at regular intervals in the growing season, so species dynamics can be assessed. Here, preliminary results for 2011 are presented in graphical form from the site at ART-Tänikon (Fig. 5 a-d). The plot profiles are represented by 'Series #', and all DM yields are fixed to the same scale. The purpose of showing the graphs is not to allow a detailed analysis of species dynamics for the mixture components, but rather to demonstrate the overall pattern of changes in species abundance during the year.

**Fig. 5 (a-d).** Species dynamics in simplex plots from 5 cuts in ART- Tänikon in 2011.

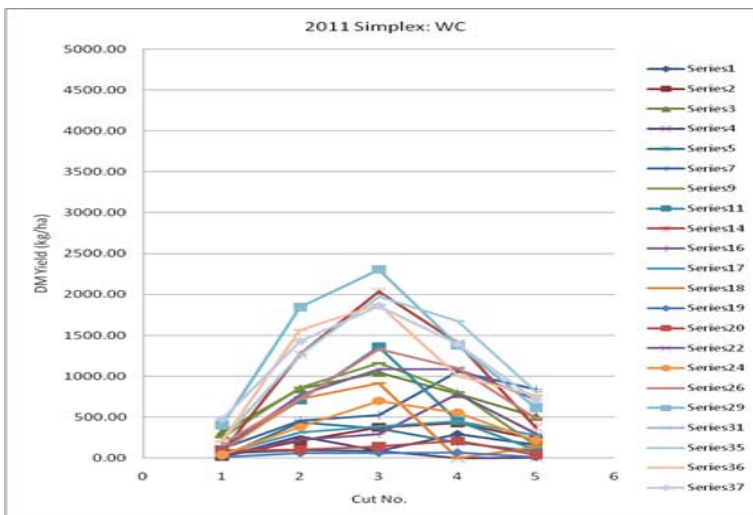
**(a) Perennial ryegrass**



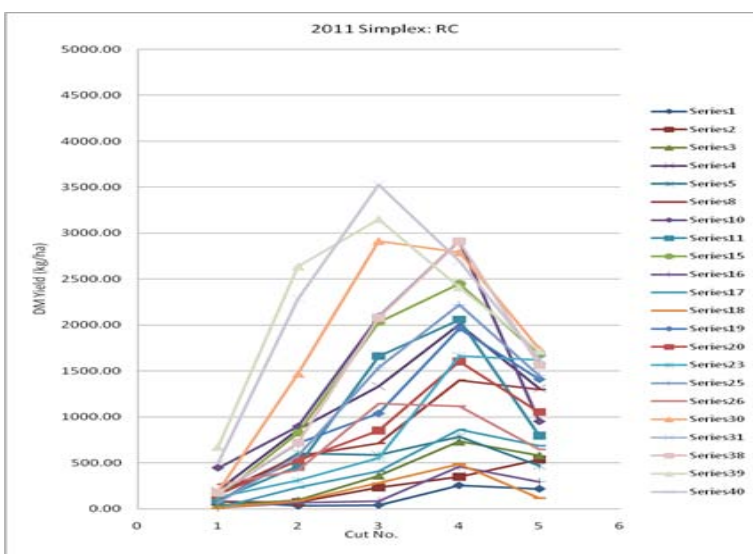
**(b) Chicory**



**(c) White clover**



**(d) Red clover**



These graphs provide a striking visual impression of the differences in patterns of the species' productivity over a growing season, and also of the large effect of simplex plot profile on this (*i.e.* in the same harvest there can be a 10-fold difference in the yield of a species in different treatments). When growth data are presented in this way a high degree of temporal separation in the seasonal productivity patterns of, for example PRG and red clover, becomes evident, whereas the yields of chicory and white clover tend to be more evenly distributed throughout the growing season. PRG and red clover are also higher-yielding than the other two components of these mixtures, so we can see that by incorporating species with a range of productivities and high degree of temporal separation of productivities, high-yielding and stable MSS can be achieved.

### 3 (3) INRA-Lusignan, France 'Analysis of species dynamics in long-term MSS under different cutting and nitrogen managements'

Two major management constraints are often encountered by productive grassland: nitrogen fertilisation and cutting frequency. The objectives of this experiment were:

- To study the change in botanical composition of the sward with N fertilisation and cutting frequency,
- To study the effect of the number of species on the agronomic value of the swards,
- To study the effect of N fertilisation and cutting frequency on forage yield and forage quality in the swards.

The experiment consisted of 25 mixtures based on 13 perennial forage species (8 grasses, 5 legumes) (Table 7). The number of species in the mixtures was between 1-8. Two nitrogen fertilisation treatments (low: 60 kg N/ha/year on grasses, 0 kg N/ha/year for grass-legumes vs. high: 180 kg N/ha/year on grasses, 30-50 kg N/ha/year for grass-legumes) and two cutting frequencies (frequent: every 25-30 days, and infrequent: every 45-50 days) managements were applied. The experiment was sown in 2003 and continued until 2010.

The design was clearly focused on some species (*Lolium perenne*, *Dactylis glomerata*, *Festuca arundinacea*, *Trifolium repens*, and to a lesser extent *Phleum pratense*, *Festuca rubra* and *Trifolium pratense*). Among them, the five grass species were also studied in pure stands. The other species only appeared in some swards and were not present in pure stands. Thus, the design is not balanced, so all types of analyses were not possible.

**Table 7.** Composition of the 25 mixtures

Sward	Type	RGA	Lh	DAC	FEL	FEP	FLE	PAT	FRO	Ms	TB	TV	MIN	LOT
1.1	G	X												
1.2	G			X										
1.3	G				X									
1.4	G					X								
1.5	G								X					
2.1	G	X		X										



2.2	G		X	X									
2.3	G	X		X									
2.4	GL	X								X			
3.1	G	X	X	X									
3.2	GL	X	X							X			
4.1	G	X	X	X	X								
4.2	GL	X	X	X						X			
4.3	GL				X		X	X					X
5.1	G	X	X	X	X			X					
5.2	GL	X	X	X	X					X			
5.3	GL	X	X	X						X		X	
5.4	GL	X	X	X					X		X		
5.5	GL	X	X	X							X		X
5.6	GL	X	X				X			X	X		
6.1	GL	X	X	X	X			X		X			
6.2	GL	X				X	X	X		X	X		
7.1	GL	X	X	X						X	X	X	X
7.2	GL	X	X			X	X	X		X	X		
8.1	GL	X	X		X	X	X	X		X	X		

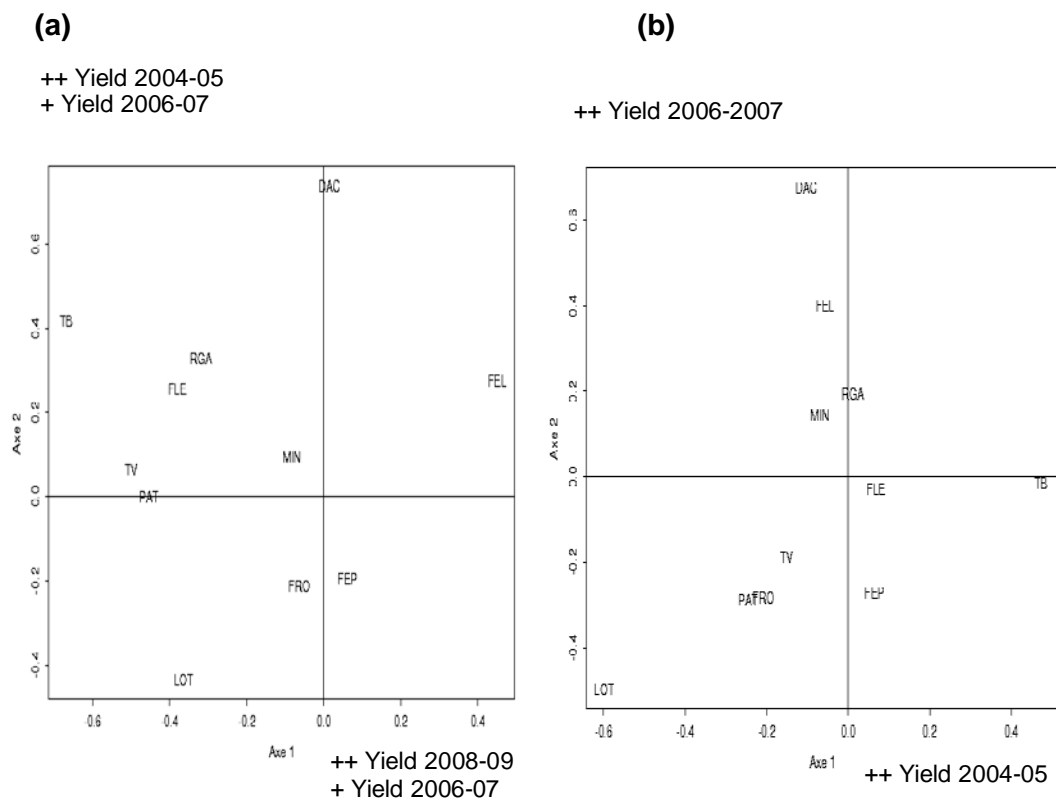
RGA: *Lolium perenne*, Lh: *Lolium hybridum*, DAC: *Dactylis glomerata*, FEL: *Festuca arundinacea*, FEP: *Festuca pratensis*, FLE: *Phleum pratense*, PAT: *Poa pratensis*, FRO: *Festuca rubra*, Ms: *Medicago sativa*, TB: *Trifolium repens*, TV: *Trifolium pratense*, MIN: *Medicago lupulina*, LOT: *Lotus corniculatus*

Twice a year (spring and autumn), the botanical composition of the swards sown with a mixture of species was determined. A sample was harvested (0.2 m<sup>2</sup>) and the species were manually separated, dried and weighed. The non-sown species component was also measured. Forage production varied a lot in the different years, and with the imposed management. It was average in the first three years (2004, 2005, 2006), but very high in 2007 and 2008. In 2009 and 2010, the yield was low, and the difference between swards was more restricted.

The contribution of the presence of each species to yield was analysed with a canonical analysis of correlations (Fig. 6). The yield variables that were used corresponded to the sums of the annual yield of two successive years. Depending on the treatment, the species that contributed to the yield components were different. The first two axes represent, respectively, the yield in the first years of the trial and the yield in the last years of the trial. *T. repens* contributed positively to the yield in the first years. *D. glomerata* was strongly

associated with annual yield from 2004 to 2007. *F. arundinacea* contributed to the yield in the last years. The presence of *L. corniculatus* was always associated with low yields. In some cases the cutting treatment affected the strength of species' contributions to yield eg. the yield of red clover (*T. pratense*: TV) in early and later years was adversely affected by more frequent cutting; conversely, the yield of perennial ryegrass (RGA) was more evenly distributed over the experiment under frequent cutting. The early yield of *F. arundinacea* (FEL) was negatively affected by frequent cutting, but its yield subsequently recovered. This type of multivariate approach has the potential to be useful in the MultiSward CE to compare species' yield responses to cutting and grazing, although currently we do not have enough data points to do this. This is one of the major points of interest of this experiment.

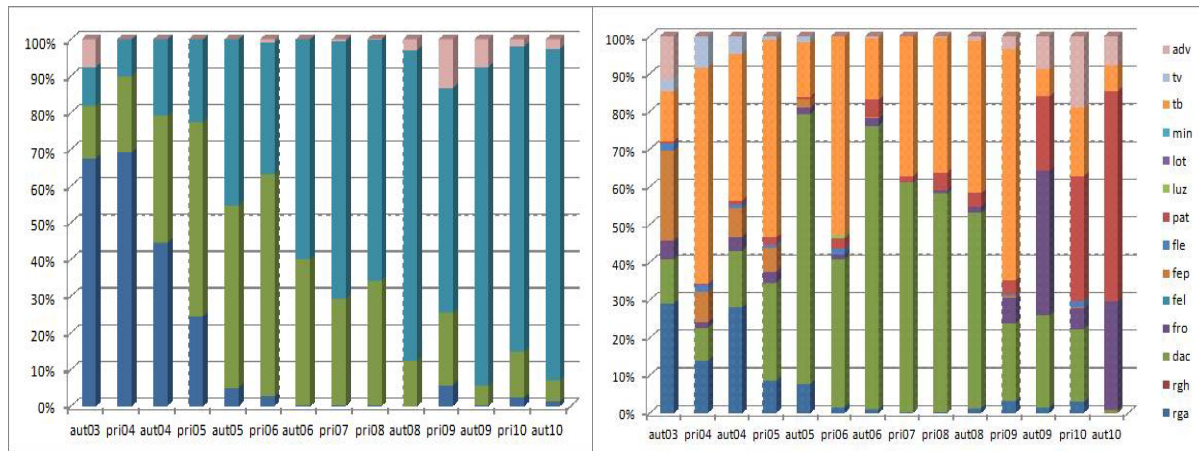
**Fig. 6.** Canonical analysis of correlations between the presence of the species at sowing and forage yield. Yield was considered as the sum of 2004 and 2005, the sum of 2006 and 2007 and the sum of 2008 and 2009. (a) Infrequent cutting and high N fertilisation; (b) Frequent cutting and high N fertilisation. The contribution of the variables to the axes is indicated.



RGA: *Lolium perenne*, DAC: *Dactylis glomerata*, FEL: *Festuca arundinacea*, FEP: *Festuca pratensis*, FLE: *Phleum pratense*, PAT: *Poa pratensis*, FRO: *Festuca rubra*, TB: *Trifolium repens*, TV: *Trifolium pratense*, MIN: *Medicago lupulina*, LOT: *Lotus corniculatus*.

To study the change in botanical composition of the sward with N fertilisation and cutting frequency, graphs of the frequency of each species were drawn as a function of time for each sward in each treatment. Then, the frequency of each species was modelled by using a logistic model (Logistic procedure in SAS). The residuals of the regression were plotted against the observed data. The quality of the model adjustment was tested with a deviance test.

**Fig. 7.** Species proportions measured in spring (pri) and autumn (aut) 2003-2010 for mixtures 3.1 (3 grasses; left) and 8.1 (6 grasses/2 legumes; right). Species abbreviations as for Fig. 6 (adv = unsown).



For each sward, the frequency of the species was represented (Fig. 7). It appeared that the fate of each species followed the same tendency whatever the other species that composed the sward and the management, for example:

- High frequency after sowing but rapid decrease (*L. perenne*)
- Frequency relatively high after sowing, frequency kept high during several seasons but late decrease (*D. glomerata*, *T. repens*)
- Low frequency after sowing but a high rate of expansion (*F. arundinacea*)
- Very low latent period after sowing but late expansion (*F. rubra*, *Poa pratensis*)

The model included the effects of time, with powers up to 5, the frequency of cutting, the nitrogen level and the initial sowing rate of the considered species. Each factor was tested. For example, for *L. perenne*, the model included time, cutting frequency and the initial rate of sowing of this species in the swards (Table 8), but nitrogen fertilisation had no significant effect in the model. Depending on the species, the models included several levels of power of times. The effect of frequency of cutting was significant for all species except *F. arundinacea* and *T. repens*. The level of nitrogen fertilisation was significant for all species but *L. perenne*. The effect of the initial rate of each species was significant for all species but *T. repens* (Table 9).

**Table 8.** Model for the change in *L. perenne* as a function of time.

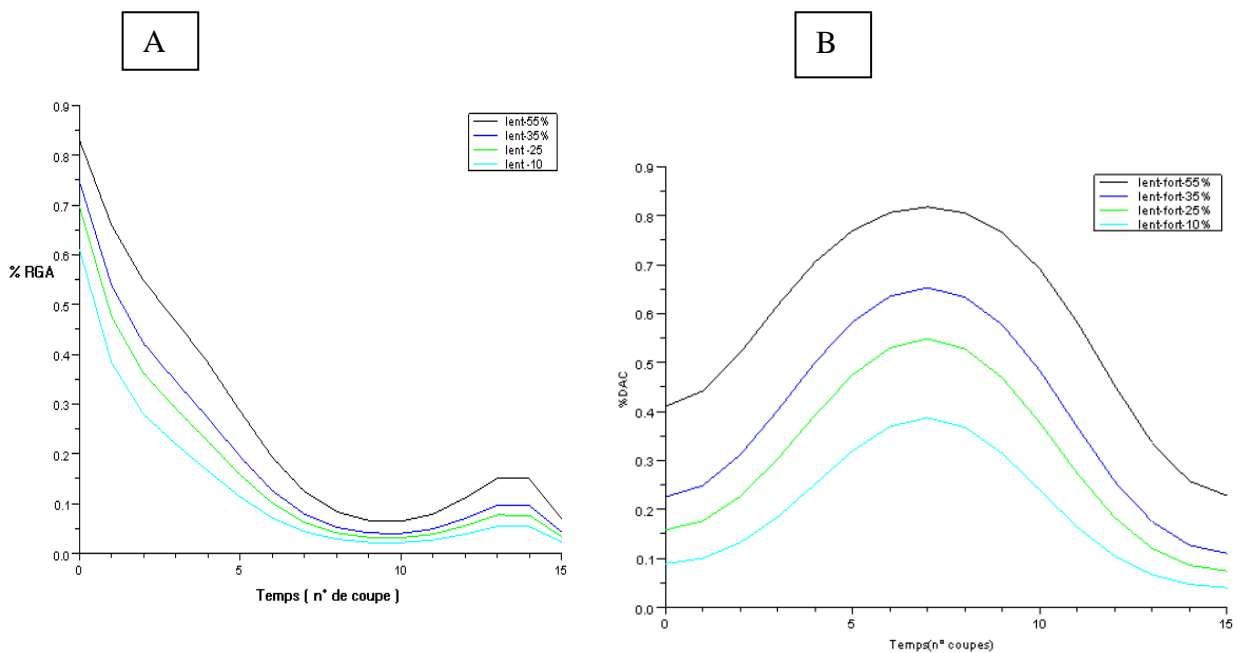
Effect	DF	Wald	Pr > Chi-sq
time	1	39.5936	<.0001
time*time	1	32.5640	<.0001
time*time*time	1	50.4977	<.0001
time*time*time*time	1	70.2299	<.0001
time*time*time*time*time	1	86.1068	<.0001
Frequency of cutting	1	75.7241	<.0001
Initial rate of <i>L. perenne</i>	1	548.8400	<.0001

**Table 9.** Significant effects of the models for each species

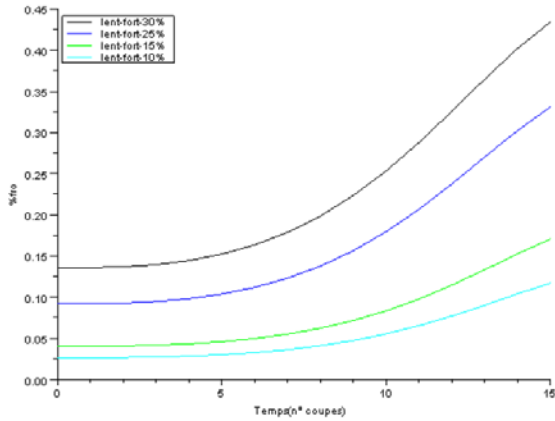
Species	Significant effects
<i>L. perenne</i>	t t <sup>2</sup> t <sup>3</sup> t <sup>4</sup> t <sup>5</sup> frequency_of_cutting initial_Lp
<i>D. glomerata</i>	t <sup>2</sup> t <sup>3</sup> t <sup>4</sup> frequency_of_cutting nitrogen initial_Dg
<i>F. rubra</i>	t <sup>3</sup> t <sup>4</sup> frequency_of_cutting nitrogen initial_Fr
<i>F. arundinacea</i>	t t <sup>2</sup> t <sup>3</sup> nitrogen initial_Fa
<i>P. pratensis</i>	t <sup>2</sup> t <sup>3</sup> frequency_of_cutting nitrogen initial_Pp
<i>T. repens</i>	t t <sup>4</sup> nitrogen

The curves describing the evolution of the main species are given in Fig. 8. *L. perenne* frequency declined with time, even if it represented a large proportion of harvested forage in the first years. *D. glomerata* was moderately frequent after sowing but represented up to 80% of harvested forage in 2007-2008, then declined abruptly. *F. arundinacea* was infrequent during the first year of the trial, then it developed progressively, reaching up to 80% of forage production in 2010. *F. rubra* frequency followed the same scheme than *F. arundinacea*, but its development was later (2008-2009) and less important. The increase in *F. rubra* frequency coincides to the decrease in frequency of *D. glomerata*. The frequency of *T. repens* was high after sowing and remained high during several years but suddenly declined in 2008.

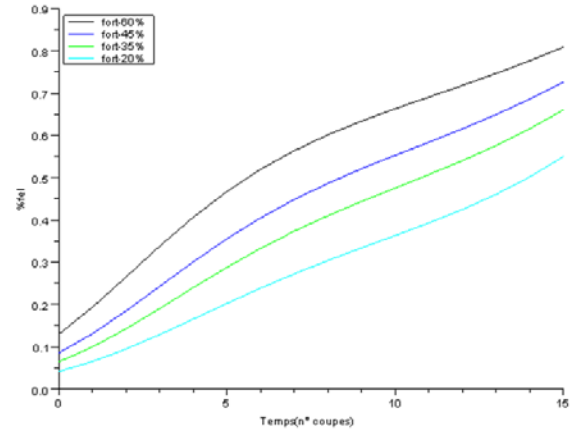
**Fig. 8.** Change in species composition as a function of time for different management practices and initial sowing rates. A = *L. perenne*; B = *D. glomerata*; C = *F. rubra*; D = *F. arundinacea*; E = *T. repens*



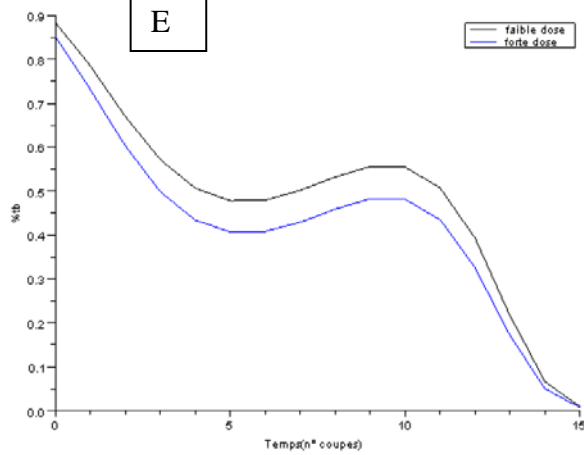
C



D



E



## Modelling of forage yield in sward composed of a mixture of species

We have seen that it is possible to model the frequency of each species over time. The next question is: is it possible to predict forage yield over time? One problem arises from the fact that forage yield is affected by climatic conditions, in addition to species composition of the sward. So “time” that covers a range of effects (drought, temperature...) is one factor of forage yield. To overcome this problem, we have expressed the yield of a sward as a ratio of the yield of sward 1.1 (pure *L. perenne*). This sward, as the others, was influenced by the various pedoclimatic factors that affect forage yield. We have chosen to model the forage yield of mixed stands using as regression factors the yield of the species cultivated as pure stands, time, and the percentage of the other species. We have restricted the analysis to *L. perenne*, *D. glomerata*, *F. arundinacea* cultivated as pure stands (swards 1.1, 1.2 and 1.3), as mixtures of 2 species (swards 2.1 and 2.3) or as a mixture of the 3 species (sward 3.1). The model is thus:

and this was used on each treatment. The parameter  $p(\text{time})$  indicates that time was introduced as time,  $\text{time}^2$ ,  $\text{time}^3$ .

As a result, the  $R^2$  of the regressions varied from 0.41 to 0.82. The regressions usually do not contain any time factors (Table 10). It means that the yield of a sward composed of a mixture of species is well predicted by the proportion of each species and the yield of the species included in the mixture. The effects of interactions between species that could have an effect on both the frequency of the species and on their yield are low compared to the potential yield and the frequency of each species.

**Table 10.** Regression models to explain the yield of swards composed by several species.

*I* = infrequent cutting; *F* = frequent cutting.

Mixture	Treatment	Factors	$R^2$
2.3 (Lp + Fa)	I, N+	_____	0.75
	I, N-	_____	0.73
	F, N+	_____, %Fa	0.72
	F, N-	_____	0.63
2.1 (Lp + Dg)	I, N+	_____	0.82
	I, N-	_____, %Dg	0.53
	F, N+	_____, $t^3$	0.52
	F, N-	_____	0.49
3.1 (Lp + Dg + Fa)	I, N+	_____, _____	0.77
	I, N-	_____, _____	0.49
	F, N+	_____, _____	0.74
	F, N-	_____, %Fa	0.49

*Lp*: *Lolium perenne*, *Dg*: *Dactylis glomerata*, *Fa*: *Festuca arundinacea*

This trial, carried out for 8 years, contributes new information about the agronomic value of MSS:

- On average, the number of species has a positive effect on the forage production of the sward. It is as if the forage production of a sward depended on the presence of species that were able to give a high production under specific conditions. The presence of a legume species, here mainly *Trifolium repens*, contributed to achieving a high yield, especially when N fertilisation is low. Some species have a high level of forage production (*Festuca arundinacea*, *Dactylis glomerata*), but other species are able to produce significantly in some occasions.

- Some swards have a relative forage production that increases with time, and are more suitable for long-term grasslands, even if their forage production is not very high in the first years. Other swards are more productive in the first years, but their productivity declined afterwards.
- Some species are able to survive in highly productive swards, even if they contribute little to forage production at these times. Then, when some productive species disappeared, these species recovered and contributed significantly to forage production. We observed this feature for *F. rubra*, *Phleum pratense*, *Poa pratensis* in the trial.
- The rapid disappearance of *L. perenne* was expected because the climatic conditions in Lusignan generate intense drought stress, especially when it is grown with more vigorous species such as *D. glomerata* and *F. arundinacea*. Contrastingly, the late disappearance of *D. glomerata* and *T. repens* was not expected. *D. glomerata* is usually able to survive for longer. For *T. repens*, it is possible that the rare surviving plants could recover after one or two years, especially on the non-N- fertilised treatment because the grasses will be less persistent there.
- The fate of the different species in this trial is related to the choice of sward composition and to the pedoclimatic conditions during the period of time of the trial. It is not possible, using this trial, to recommend any mixtures for different regions. Anyway, taking into account the growth ability of the different species, general rules can probably be established: the highly productive species tend to contribute a lot to the forage production, but this high contribution can be delayed if the species has a slow establishment; the species with low persistency will disappear rapidly, even if they are able to produce a lot when they are present. Their disappearance can be speeded up when they are sown with highly vigorous species. Species with a high persistency, that sometimes have under-optimal forage yield, are able to fill up to space left by the low-persistency species. These highly persistent species will thus be able contribute a consistent forage yield after many year of production.
- The yield of a sward composed of a mixture of species and expressed as a ratio of the yield of one of its species is mainly explained by the yield of the other species cultivated in pure stand and by the frequency of this later species. The interactions between species probably have limited effects.
- The conclusions obtained after 8 years are different of that obtained after 3-5 years. If the objective is to increase the persistency of the grasslands, it is important to extend the duration of the trials.

## 4. Conclusions

**Extent of species dynamics:** In one experiment (the Multisward CE: experiment 3(2)) data were only available for one year, so analysis was limited to within-year species dynamics. In the other experiments, data were available for three years (experiment 3(1)), and eight years (experiment 3(3)). Over all three time scales significant changes in the contribution of species to total biomass over time in MSS were evident. This illustrates the complexity of species dynamics in MSS: understanding them requires knowledge of the growth patterns of species over time, how these may be modified by co-occurring species, variation in the responses of genotypes within species, and the effects of genetic change over time within genomes. Species dynamics are influenced by uncertainty in biotic and abiotic events, variability, unpredictability, temporal and spatial variability, and a cumulative effect of many plant to plant events (Greiner, 2011

<http://www.personal.psu.edu/mef5268/blogs/portfolio/2011/04/autecology-population-dynamics-community-assembly-and-plant-association.html>).

It may well be the case that there is an intrinsic oscillatory element to interactions between grasses and legumes driven by dynamics in soil N pools.

**Effects of species dynamics:** Species dynamics were shown in experiment 3(1) to have profound effects on the properties of MSS – in this case the nutritive value of the harvested herbage. Effects on forage quality were mediated through the impact of species dynamics on biomass production in component species eg. reduced yields of CP were observed in Year 2 due to lower biomass yields of the legume species. Thus, it is clear that whilst designing MSS to include species with desirable forage quality traits is potentially useful, the agronomic usefulness of such mixtures will ultimately be determined by changes in the relative abundance of species over time. Forage quality parameters will be an important component of the measurements carried out on the MultiSward CE.

**Analytical approaches:** “Questions about the eventual outcome of competition have not been sufficiently differentiated from questions regarding how much neighbouring species affect each other and the mechanisms through which this occurs” (Connolly *et al.*, 2001). This statement is pertinent to the analysis of species dynamics in MSS, in which the issue we seek to analyse is how the present species composition arose, and in which direction it is likely to develop. The RGRD approach described in section 3 (1) (*i.e.* asking the question ‘which species gains’) can provide an answer to both these questions. While the answer to the “which species gains” question provides information about species’ relative performances in a given mixture, however, it does not necessarily provide information about the causes underlying species’ performances. More specifically, it tells us very little about the extent to which the relative success of one plant species was caused by any interactions with another species (Connolly *et al.*, 2001). One species in a pair may be out-performing the other in terms of biomass accumulation, but may be deriving nutrients and water from different soil strata and thus not ‘competing’ with it directly. To assess how plant species affect one another we generally need to measure more than relative performance in one mixture - we need to compare the performance of a target species across a range of mixtures. This is the idea behind the simplex experimental design used in the MultiSward CE. In experiment 3(3) the modelling approach assumed that component species in the mixtures were not interacting *i.e.* one of the regression factors was the yield of the species cultivated as pure stands. However, it is possible that the experimental design used here did not include a sufficiently wide range of species proportions to allow this assumption to be made. It is recommended that the RGRD approach is applied to results from the MultiSward CE in order to maximise their impact. The use of multivariate statistics (as described in experiment 3 (3)) to explore species’ contributions to biomass production over time should also be considered.

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