MultiSward

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

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Intermediate report on the effect of plant species diversity on the environmental roles of grassland

Merged Deliverables
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D3.1 Progress report on the effect of plant species diversity on the environmental roles of grassland at the field level as input for Task 3.3 and 3.4

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Foreword

In this document, the two initially planned deliverables D2.3 ‘Effect of plant species diversity on the environmental roles of permanent grassland’ and D3.1 ‘Intermediate report on the effect of plant species diversity on the environmental roles of grassland at the field level as input for Task 3.3 and 3.4’ have been merged. The objective of this merging was to produce a reference document dealing with both results from diversity experiments using experimental communities (‘manipulative’ approach) and results from studies based on existing communities of permanent grasslands (‘observational’ approach). The first section of this document focus on studies based on permanent grassland communities and corresponds to the initially planned D2.3 and the second section focuses on diversity experiments with experimental communities and corresponds to the initially planned D3.1.

The MultiSward partners involved in the preparation of this deliverable are:

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- Aberystwyth University - Institute of Biological, Environmental and Rural Sciences (AU-IBERS),
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Summary

Multi-species swards could be a key element in improving delivery of provisioning, regulating and supporting services from grassland-based ruminant production systems. But the relationships between plant species or functional diversity and the delivery of ecosystem services from grassland have been controversially debated during the last decades. One of the objectives of the MultiSward project is to provide adequate evaluation tools (indicators and models) to assess production systems based on the utilization of multi-species swards. Such evaluation tools therefore need to be sensitive to the effects of plant diversity on productivity and other ecosystem services.

The objectives of this report are to review current knowledge about the effects of plant diversity on (i) forage production, sward nutritive value and animal product quality in permanent grassland and sown grassland; (ii) the environmental impacts and ecosystem services of grassland-based forage production at the plot level, to serve as documentation and discussion material for the selection and development of models and indicators for the evaluation of ruminant production systems based on multi-species grasslands. To achieve these objectives, a literature review encompassing agronomic as well as ecological studies on diversity effects was performed, and preliminary results from experiments of the MultiSward project were included when available. The conclusions focus on aspects that could be considered in the development of an evaluation tool.

It is concluded that the results currently available from ecological and agronomic research on the effects of plant diversity on ecosystem functioning clearly show that increased biomass productivity of permanent and sown grassland through the concerted use of plant diversity does not conflict with the delivery of a broad range of services. Improving plant species mixtures for forage production is therefore an option to target a sustainable intensification of grassland-based agricultural production, or a reduction of the environmental burden of forage production through a reduction in agricultural inputs. The apparent effect of plant diversity on biomass productivity of both extensively and intensively managed mown grasslands is well documented and data are available for its quantification. This is not yet the case for grazed pastures. An approach that accounts for plant diversity effects on the emissions of CO₂-equivalents from mineral fertilizer input and mechanization, the emissions of nitrous oxides and the risks of nitrate leaching would help to quantify the value of the diversity-productivity effect and the consequent reduction in the need of agricultural inputs per unit of produced forage. The effects of plant diversity on carbon sequestration and soil quality and stability cannot be quantified yet with the information found during the preparation of this report. The development of an indicator describing the related positive effect of plant diversity on the diversity of pollinator insects would be an important element for the evaluation of multi-specific swards. It is further concluded that functional diversity might be more adequate than species richness as predictor for the delivery of ecosystem services from agriculturally managed grasslands.
1. Introduction

Relationships between plant species or functional diversity and ecosystem functioning of grassland have been controversially debated during the last decades. A large number of studies assessed the effects of one or few diversity criteria on ecosystem processes and reviews on the effects of plant diversity on ecosystem services have been published (e.g. Hooper et al., 2005; De Bello et al., 2010).

Many aspects of the relationship between plant diversity and the delivery of ecosystem services remain controversial (Willig, 2011). This is, for instance, the case for the type and level of plant diversity that could improve the delivery of multiple services from ruminant production systems. While agronomical benefits of grass-legume mixtures over grass monocultures have been recognized for a long time (Sanderson et al., 2004) and extensively implemented into productive agriculture in a few regions (Frankow-Lindberg, 2005; Kessler and Suter, 2005), the simplicity of managing grass monocultures and low prices of mineral nitrogen have in the past postponed the use of multi-species mixtures for forage production in many European countries. Nowadays, the increasing importance of improving the sustainability of production systems and the corresponding development of agri-environment schemes translate into an increased interest in the potential benefits of multi-species forage mixtures (Rochon et al., 2004; Peeters, 2009). One of the main objectives of the FP7 project MultiSward is to evaluate ruminant production systems that are based on the use of multi-species grassland swards. This evaluation requires using models and/or indicators sensitive to the effects of plant diversity on productivity and other ecosystem services of such systems.

The objective of this report is to review current knowledge on the effects of plant diversity on the environmental impacts and ecosystem services of grassland-based forage production at the plot level, to serve as documentation and discussion material for the selection and development of models and indicators for the evaluation of ruminant production systems based on multi-species grasslands. The paper will consider permanent grasslands and temporary (or sown) grasslands. In permanent grassland, species diversity is usually substantially larger. For example, Sanderson et al. (2004) summarised that American grazing lands comprised between nine and 50 species per 1000 m² and European grasslands between 10 and 60 species per 100 m², depending on management intensity and environmental conditions. The paper will also consider nutrient poor conditions (extensive grassland) and nutrient rich grassland in fertilized agricultural systems (intensive grassland).

2. Species diversity and biomass productivity

Because biomass productivity directly or indirectly influences the assessment of the environmental roles of grasslands, the effects of plant species diversity on biomass productivity should briefly be analysed. To date, most experimental work investigating the potential benefits of plant diversity in grassland has dealt with sown grassland, whereas permanent grassland has been relatively neglected (Petersen et al., 2010: MultiSward partners). Every permanent grassland is a unique mixture of plant species differing in phenology, and this complexity makes it difficult to characterise in terms of biomass productivity and nutritive value (Michaud et al., 2012).
2.1. Focus on permanent grassland

As a first step in elucidating the role of plant diversity in permanent grassland it is important to examine the different experimental approaches that have been taken, as the methods used can determine the results obtained.

Traditionally, two experimental approaches to the topic of plant species diversity and ecosystem functioning have been used: in the 'manipulative' approach, plant diversity is manipulated and the effects analysed (eg. Symstad and Tilman, 2001); in the 'observational' approach, comparative studies are conducted in natural plant communities to relate differences in diversity to observed productivity along environmental gradients (eg. Grace et al., 2007; Assaf et al., 2011: Multisward partners). To some extent, the approach taken determines the results obtained. Thus, using the manipulative approach, it was concluded that the productivity of ecosystems increases asymptotically with increasing diversity (Tilman et al., 1997; Hector et al., 1999). This result has been attributed mainly to niche complementarity and facilitation among species (Hooper, 1998; Loreau and Hector, 2001), as well as to the “sampling effect”, i.e. an increased probability of including a highly productive or highly competitive species at higher diversity levels. On the other hand, experiments based on the observational approach show that biodiversity reaches a peak at intermediate productivity levels, which is described by the “hump-backed” model (Grime, 1973). This model indicates that species richness declines in more productive sites due to higher rates of extinction of less competitive species as a result of higher dominance of strong competitors (Grace et al., 2007). The observational approach studies the effect of species diversity on productivity across environmental gradients, where other factors such as soil fertility, climate, disturbance regime, or herbivory are correlated with the observed diversity and productivity levels. Generally, a strong correlation between species richness and productivity was not evident in these experiments. This might be explained by the overwhelming effects of environmental factors which might have masked the diversity effect (Diaz and Cabido, 2001; Schmid, 2002; Hector et al., 2007). Thus, there appear to be some inconsistencies between results obtained from ‘manipulative’ and ‘observational' approaches.

2.1.1. Observational approaches to study the relationship between diversity and productivity

In an alternative experimental approach, biotic and/or abiotic factors would not be correlated with diversity and productivity levels. An example is provided by the experiment of Assaf et al., 2011, in which the relationship between plant diversity and productivity was analysed at two levels: within-sites, in order to minimise the influence of environmental factors; and across sites, to investigate the relationship across environmental gradients. Two different grassland types were selected for the study: species-rich, agriculturally managed grasslands ('managed'), which were managed by cutting or grazing and typically had 9-17 species/m²; and dry, acidic, nutrient-poor, not-managed grasslands ('natural'), with a species richness range of 1-5 species/0.25m² (i.e. maybe around 3-15/m²). They studied the effect of species number and evenness on above- and below-ground biomass production both within and across sites in order to test whether different diversity components would have different relationships with productivity. In the managed grasslands the within site
relationship between species richness and above-ground productivity was significant for two sites out of seven, with a negative correlation. Evenness was positively correlated with above-ground productivity for two sites. The relationship between diversity and below-ground productivity was rather weak, as only one site showed a significant correlation between evenness and below-ground biomass. Across all managed sites, productivity increased linearly with increasing evenness but there was no significant relationship between species richness and productivity (Fig 1.1). In the natural grasslands, no within site relationship between species richness or evenness and productivity was found (Fig 1.2). Across all managed sites, productivity (both above- and below-ground) increased linearly with increasing species richness but no such effect was found for the relationship between productivity and evenness.

Figure 1. The relationship between diversity (species richness and evenness) and productivity (above- and below-ground) across sites (Assaf et al., 2011).

Fig. 1.1 Managed grassland

Fig. 1.2 Natural grassland
The analysis at the across-sites level allows a comparison of these results with the ‘observational’ approach, as both of them investigate the diversity-productivity relationship across environmental gradients. The results obtained from the managed grasslands showed no general positive diversity-productivity relationship when the diversity measure was species richness. Complementarity between species might not increase with increasing number of species in the relatively species rich managed permanent grasslands (i.e., there is species redundancy). This is confirmed by the positive species richness-productivity relationship in the natural (not managed) grassland. Moreover, species richness did not behave according to the hump-backed model of Grime (1973). The explanation for this might be that obtaining the hump-shaped relationship requires extreme environmental conditions to be included in the surveyed plots (stress factors at one end and conditions that favour high productivity at the other end). A lack of these extreme conditions would produce a diversity-productivity relationship that is located at the centre of the hump-shaped curve.

The relationship between diversity and productivity in the managed grasslands varied according to the diversity measure used. The positive relationship between evenness and productivity at across-site level (and to a lesser extent at within-site level) indicates that evenness may be more related to ecosystem functions than species richness, at least in relatively species rich grasslands. It has previously been hypothesized that evenness might be more related to the productivity and nutrient cycling of ecosystems than number of species (Wilsey and Potvin, 2000). Ecosystems with evenly distributed species might use resources more efficiently, both spatially and temporally. Compared to evenness, species richness is greatly influenced by subordinate species that have low abundance, with negligible effects on productivity or other ecosystem functions.

At the within-site scale, all the natural sites and several managed sites showed no significant relationship between plant diversity and above-ground productivity. Therefore, the type of the diversity-productivity relationship could be influenced by the existing environmental conditions. Again these results are not in agreement with those obtained from ‘manipulated’ grasslands, where an asymptotic increase in productivity with increasing species richness has previously been found.

These results indicate that the relationship between diversity and productivity is different for managed, species rich, productive grasslands and nutrient poor, unproductive natural grasslands. A single general diversity-productivity relationship does not properly characterise natural vegetation, thus biotic and/or abiotic factors might affect this relationship significantly. Future experiments should be continued in natural vegetation and the investigated sites should be pre-classified according to their differences in ecological, compositional, or environmental characteristics (Assaf et al., 2011).

2.1.2. Effect of the scale at which sampling is carried out on the relationship between diversity and productivity

The scale at which sampling is carried out has also been shown to be a possible determinant of the results obtained in diversity-productivity experiments. An example is given by T. From, N. Wrage and J. Isselstein (Multisward partners)
(pers. comm.) working on plots established in the experiment described below. In this component of the experiment, yield sampling and vegetation surveys were carried out at four spatial scales: 0.16m$^2$, 1m$^2$, 9m$^2$ and 225m$^2$ (Fig. 2).

Figure 2. Species diversity-productivity relationships at four different spatial scales (From Tatiana et al., unpublished).

There was a trend towards decreasing yield with increasing species number at the 0.16m$^2$, 9m$^2$ and 225m$^2$ scales, but no trend at the 1m$^2$ scale (Fig. 23). Samples grouped by scale size showed dissimilarities in vegetation composition: plots of 9m$^2$ and 225m$^2$ tended to have more species contributing to yield than did the smaller plots. Thus, sampling on a larger scale may underestimate plant identity effects. The conclusion to be drawn from this experiment is that the relative importance of species identity for productivity is scale dependent. This is in agreement with observations for semi-natural permanent grasslands submitted to different management practices (site of Theix, see next section), showing a positive relationship between species number and aboveground biomass in small scale observations (0.02m$^2$), while at larger scales this was not confirmed (Gross et al, 2009).

2.1.3. Manipulative approaches to study the relationship between diversity and productivity

Other experiments also concluded that species richness is not the major determinant of biomass productivity of species rich permanent grassland.
In an example of a ‘manipulative’ experiment on permanent grassland (a semi-natural, nutrient-poor permanent grassland in the Solling Uplands, Germany), three different sward types under four different managements were created by applying selective herbicides (Petersen et al., 2010: Multisward partners). The sward types were: untreated control sward (Co), dicot-reduced (Dic-) and monocot-reduced (Mon-); the managements were: two levels of fertilisation (none (x) and 180/30/100 kg ha\(^{-1}\) NPK (NPK); two levels of utilisation (one cut (1) and three cuts (3) per year). The resulting 12 treatments were harvested for DM yield, botanical composition (Fig. 3) and forage quality (see section below for discussion of forage quality results).

**Figure 3. Sum of DM yields in 2009 according to treatments. Annual means denoted by the same letters are not significantly different (P<0.05) (Petersen et al., 2010).**

The 12 treatments resulted in widely spread annual DM yields, ranging from 5.5 t ha\(^{-1}\) (Mon- 3x) up to 12.2 t ha\(^{-1}\) in the Co 3 NPK treatment. Within managements there were no significant effects of sward type on yield, although the proportions of grasses, forbs and legumes did differ. The main factor influencing yield was fertiliser application, which also interacted with cutting frequency. Sward type accounted for less than 1% of the yield variance. A relationship between species number (on average 13-19 species per 9m\(^2\)) and productivity could not be established – possibly due to the narrow range of species richness. It might be possible to produce a closer relationship between these parameters if the actual proportions of plant functional groups were used as continuous variables.

Further examples of the ‘manipulative’ approach of analysing permanent grassland come from experiments being conducted at two sites in semi-natural grassland in the Auvergne, France (Theix and Laqueuille). Starting from an initially uniform state, different levels of grazing pressure (Laqueuille), and perturbation and fertiliser application (Theix) were imposed on plots over a number of years. The effects on plant community structure, and its links with above-ground herbage production, were measured. For the Theix site, herbage production was measured in 2010 on three occasions and for the Laqueuille site 5 to 7 times a year between 2003 and 2010. At
both sites botanical composition was measured by the point quadrat method (Louault et al., 2005: Multisward partner) and plant traits were analysed at the community level in order to characterise the diversity on a functional basis. Three traits in particular were shown to capture functional variations across land use treatments: reproductive height ($H_{rep}$), leaf dry matter content (LDMC) and specific leaf area (SLA), and these were measured on the most abundant species in each plot. A stepwise regression procedure was carried out to identify the ability of diversity (species richness, family groups) or trait (LDMC, SLA, $H_{rep}$) variables to explain herbage production across managements. Under the nutrient availability gradient herbage production was significantly affected by two traits (SLA and LDMC) and the contribution of forbs. These gave a significant value of $R^2 = 0.87$ for the regression with herbage production. Under the perturbation gradient, only the contribution of legumes was of predictive value for herbage production ($R^2 = 0.32$). Over both gradients, annual herbage production was significantly predicted ($R^2 = 0.83$) by two traits (LDMC (-ve) and SLA (+ve)), plus legume contribution (-ve) and species richness (-ve). Thus, it was concluded that plant functional traits were better overall predictors of herbage production than the number of plant species. In Laqueuille, mean ‘potential herbage production’ was significantly affected by the grazing treatments, being higher in the intensive and fertilised plots than in the extensive treatment. A stepwise multiple regression of annual herbage production (2003 to 2010) with climatic factors, functional traits and legume contribution showed that the best predictive variables were LDMC (-ve), species richness (-ve) and legume contribution (+ve) ($R^2 = 0.83$).

### 2.2. Focus on temporary grassland

Numerous biodiversity experiments that varied plant species richness in nutrient-poor grassland systems showed a positive effect of species richness on biomass production (Hector et al. 1999; Hooper and Dukes 2004; Roscher et al. 2005; Weigelt et al. 2009; Spehn et al. 2005; Tilman et al. 2002; van Ruijven and Berendse 2005; Marquard et al. 2009, Hooper et al. 2005). These experiments were conducted with communities sown with species usually found in extensively managed agricultural grasslands and containing from a single to numerous (60) plant species. Under nutrient poor conditions, more diverse plant communities are expected to acquire more of the limiting growth resources and/or to transform them more efficiently to biomass than less diverse plant communities (as reviewed e.g. in Hooper et al., 2005; Loreau, 2000). This was attributed to greater utilisation of total niche space due to niche differentiation of species, positive interspecific interactions, and higher probability of containing highly productive species (e.g. Cardinale et al., 2007). In fertilized and nutrient rich grassland systems these benefits of plant species richness might not be that relevant for production. Firstly, higher levels of nutrient availability might reduce the benefits of the higher nutrient acquisition expected under nutrient-poor conditions. Secondly, because in agriculture the best performing monoculture can be selected for cultivation, multi-species swards have to perform not only better than the average of all the species grown in monoculture, but at least as well as the most profitable monoculture in order to be advantageous for agricultural production. Moreover, a high level of species richness cannot be maintained under intensive grassland management because of the competitive exclusion of many grassland species under these conditions (Faurie et al., 1996; Guckert and Hay 2001). But
because functional groups with contrasting, complementary traits can be chosen to design agricultural mixtures, significant mixture effects on productivity might be achieved with a low number of species (Lüscher et al., 2008).

To assess if the benefits of diversity are also valid for nutrient-rich, intensively managed grasslands, a multi-site grassland experiment over 32 sites across Europe has recently been performed (COST action 852, Kirwan et al., 2007). In this experiment, strong yield benefits of mixing grasses and legumes were achieved, as well as benefits from grass-grass and legume-legume interactions (Kirwan et al., 2007). These benefits occurred with mixtures of only four species and prevailed over a wide range of climatic conditions and a fairly wide range of nitrogen fertilization levels (Lüscher et al., 2008; Nyfeler et al., 2009). At the Swiss site, the average mixture yield was higher than the average monoculture yield for all 5 yearly regrowths, but the difference was larger for the first regrowth of the season. The mixing effects even led to transgressive overyielding, that is, biomass yield of the mixture was higher than that of the highest yielding monoculture (Trenbath, 1974). For the first experimental year, the transgressive overyielding was 12% averaged over the 20 mid-European and north-European sites. This corresponded to a diversity effect of 40% more yield compared to the average yield of the monocultures. This experiment also showed the importance of the relative abundance of the different species to achieve highest yield. At the Swiss site of this multi-site experiment, the positive interactions between nitrogen fixing legumes and non-fixing grass species contributed to a significantly larger extent to the increase in biomass productivity than the grass-grass and legume-legume interactions (Nyfeler et al., 2009). This is in agreement with results from other studies (Li et al., 2007; Spehn et al., 2002; Temperton et al., 2007). The grass-grass and legume-legume interactions had nevertheless a significant positive effect on yield. In an experiment using two non-fixing forb species (Cichorium intybus L. and Plantago lanceolata L.) in combination with grasses and/or legumes, Landert (2011) also observed a positive effect of mixing non-fixing species belonging to contrasting functional groups: the grass-forb mixtures yielded more than the 2-species mixtures containing only grasses or only forbs. Contrastingly, no positive mixing effects on biomass production were observed for the mixture containing forbs and legumes but no grasses (Fig.4). These experiments show that the increase of productivity in multi-species sward is linked to different aspects of diversity like the species or functional group richness, the identity and number of the functional groups forming the community and the relative abundance with which they are present in the sward (evenness; e.g. Hooper et al., 2005; Lanta and Leps 2006; Marquard, Weigelt et al. 2009; Wilsey and Potvin 2000).

The common experiment of MultiSward uses mixtures combining the four following functional groups: 1) non-fixing and shallow rooting, 2) non-fixing and deep rooting, 3) fixing and shallow rooting and 4) fixing and deep rooting (one species for each of the functional groups). Preliminary yield results are already available for the first experimental year at two sites. The preliminary trends indicate an increase in biomass production from one to two and from two to four species in the sward, as well as a predominant role played by the legumes, in agreement with the results of the COST action 852 (Kirwan et al., 2007; Nyfeler et al., 2009). It is noteworthy some moderately fertilized mixtures achieved a higher yield than the heavily fertilized monocultures of perennial ryegrass (Fig.5).
Figure 4. Biomass production of mixtures containing each of two species belonging to the same functional group “Grasses”, “Legumes” or “Forbs” compared to the biomass production of mixtures encompassing four to six species belonging to two or three functional groups. (From Landert, 2011).

Figure 5. Effect of the botanical composition (presence of legumes, number of species) on the biomass production of mown temporary grasslands in the MultiSward common experiment for the first experimental year. 1 sp nf = 1 species, non-fixing; 1 sp f = 1 species fixing; 2 sp nf = 2 species, non-fixing; 2 sp nf/f = 2 species, non-fixing and fixing; 2 sp f = 2 species, fixing; 4 sp nf/f = 4 species, non-fixing and fixing; Lp hi N = Lolium perenne at high N fertilization

All the experiments cited above have been performed under mowing. Because grazing modifies the structure of the plant cover and the evolution of the botanical composition, as well as symbiotic nitrogen fixation (Menneer et al., 2004) and possibly other aspects of inter-specific plant interactions, the apparent effect of plant diversity on primary biomass productivity is expected to be different under grazing than under mowing. Grass-clover pastures are believed to be less productive than highly fertilized grass pastures (Peyraud et al., 2009). At similar level of nitrogen fertilization, mixtures can perform better than grass and legume pure stands also under grazing (Deak et al., 2004 as in Sanderson et al., 2007). In a diversity experiment under grazing with mixtures of 2 to 9 species, Sanderson et al. (2005)
observed a higher biomass production with the complex than with the 2-species mixtures during the experimental year with dryer weather conditions but not during the year with higher water availability. The importance of the identity of the species used in the sown pastures was shown by Deak et al., 2007. Preliminary results from the MultiSward common experiment under grazing indicate that moderately fertilized 4-species grass-clover mixtures could rich levels of primary biomass productivity similar to highly fertilized *Lolium perenne* pure stands. The variability between the replicates was nevertheless large.

### 2.3. Conclusion

On species rich permanent grasslands there is no general positive diversity-productivity relationship. Species richness may be too large to find strong effects of diversity on productivity (Wrage et al., 2011). In these grasslands, species richness is greatly influenced by subordinate species that have low abundance and negligible effects on productivity. Species evenness may be more related to ecosystem functions and to the productivity than the number of species (Wilsey and Potvin, 2000). Strong positive relationships are found in grasslands that are characterised by a relatively low number of species, although some increase in biomass production was still found between communities sown with 16 or 60 species (Weigelt et al., 2009). Under nutrient poor conditions, more diverse plant communities are expected to acquire more of the limiting growth resources and/or to transform them more efficiently to biomass than less diverse plant communities. For temporary grassland, there is a general positive and asymptotic diversity-productivity relationship even under nutrient rich conditions. These benefits can occur with mixtures of only four species and prevail over a wide range of climatic conditions provided that functional groups with contrasting, complementary traits are chosen. These results can be attributed mainly to niche complementarity and facilitation among species. Thus, under mowing and at equal level of nitrogen fertilization, multi-species swards using well-balanced proportions of grass and legume species outperform both grass and legume monocultures under a wide range of environmental conditions and management intensities. However, data are still too scarce to draw conclusion of a consistent positive effect of plant diversity on biomass productivity for intensively grazed pastures.

### 3. Species diversity, forage nutritive value quality and quality of animal product

#### 3.1. Forage nutritive value

Animal performance is a result of herbage intake and quality. Therefore forage produced must contain sufficient energy and other nutritional components to be of agronomic use.

Some data are available on forage quality from permanent grassland plots manipulated to achieve contrasting species compositions (Petersen et al., 2010) (Table 1). The treatments are described above (see 2.1.3, Fig. 3). In contrast to yield, forage quality was influenced by sward composition. Mixtures rich in legumes and
forbs (Mon-) tended to contain higher amounts of crude protein (CP). The difference is important, especially in July for non fertilized swards. During this period the CP content in forage was below 11%, which might limit dairy cow performance (Peyraud and Astigarraga, 1998), whereas MON- treatment reached 13%. Obviously CP was significantly raised by fertiliser application for all swards. In September the dicot-reduced mixtures had higher amounts of acid detergent fibre (ADF) than other mixtures. Levels of neutral detergent fibre (NDF) were relatively high in all mixtures: ranging from 475 g kg\(^{-1}\) in Mon- 3_NPK mixtures up to 680 g kg\(^{-1}\) in Dic- 1_x swards (data not shown). CP contents were lower and ADF contents higher in the swards receiving one cut compared to those cut three times.

Table 1. Means and SD of forage nutritive value parameters (g kg\(^{-1}\) DM) influenced by the different experimental treatments (as described above). * indicates significant differences from the reference level (Co 3_x or Co 1_x). (*P<0.05, **P<0.01,***P<0.001) (Petersen et al., 2010).

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<td>Co3x</td>
<td>166 ± 13.1</td>
<td>174 ± 9.1</td>
<td>147 ± 1.9</td>
<td>270 ± 6.3</td>
<td>333 ± 7.2</td>
<td>291 ± 1.4</td>
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<td>Co3NPK</td>
<td>232 ± 12.4***</td>
<td>155 ± 13.3***</td>
<td>135 ± 0.6*</td>
<td>252 ± 6.3*</td>
<td>337 ± 11</td>
<td>307 ± 1.7***</td>
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<tr>
<td>Dic-3x</td>
<td>158 ± 7.2**</td>
<td>107 ± 10.8</td>
<td>134 ± 0.9**</td>
<td>271 ± 10.3</td>
<td>337 ± 6.6</td>
<td>303 ± 1.5**</td>
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<td>328 ± 13</td>
<td>310 ± 1.4***</td>
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<tr>
<td>Mon-3x</td>
<td>171 ± 15.8***</td>
<td>128 ± 10.7***</td>
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<td>177 ± 14.9***</td>
<td>151 ± 1.1</td>
<td>253 ± 8.8*</td>
<td>316 ± 9.4**</td>
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On temporary grassland, the nutritional advantage of white clover over grasses is well established (Peyraud, 1993; INRA 2007). Voluntary dry matter intake and digestibility are higher for white clover. But 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. In particular, Peyraud (1993) and Delaby and Peccatte (2003) reported digestibility higher than 0.75 after 7 weeks regrowth or at flowering stage during the first growth. Under grazing, the beneficial effects of mixed grassland including white clover on animal intake and performances have been demonstrated. Mixed grassland steadily increased DM intake and milk yield (on average 1.5 kg day\(^{-1}\)) whatever the level of herbage allowance (Wilkins et al., 1994; Ribeiro Filho et al., 2003 and 2005). Herbage intake declined less rapidly than for pure grasses. This makes mixed pastures easier to manage than pure grass pastures.

Multi-species pastures with grasses and legumes have been shown to be of great interest for ruminant feeding. Recently, a five year experiment compared two multi-species swards: the first, ‘Swiss melange’, was the mixture recommended by ADCF for use in Swiss lowlands and contains foxtail, red fescue, meadow fescue, common meadow grass, perennial rye grass and white clover. The second, ‘Loire valley’,...
contains tall fescue, timothy, perennial ryegrass, red clover, white clover, hybrid clover and birds-foot trefoil (Delaby et al., 2010). The nutritive value of both mixtures was high on average during the year and the Loire valley mixture had a slight advantage in spring and autumn. The high nutritive value observed during autumn reaffirms the nutritional potential of autumn grazing, especially when using multi-species swards.

Table 2. Chemical composition and nutritive value (standard sheep digestibility measures) of two multi-species swards according to the season and the age of regrowth (from Delaby et al., 2010).

<table>
<thead>
<tr>
<th></th>
<th>Mean (33-68 days)</th>
<th>Spring (28-65 days)</th>
<th>Early Summer (30-63 days)</th>
<th>Late Summer (37-63 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (%DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swiss</td>
<td>14.8</td>
<td>12.4</td>
<td>13.2</td>
<td>15.6</td>
</tr>
<tr>
<td>Loire Valley</td>
<td>16.0</td>
<td>14.1</td>
<td>13.7</td>
<td>16.7</td>
</tr>
<tr>
<td>Digestibility</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swiss</td>
<td>72.0</td>
<td>70.7</td>
<td>71.4</td>
<td>69.4</td>
</tr>
<tr>
<td>Loire Valley</td>
<td>73.3</td>
<td>75.2</td>
<td>71.8</td>
<td>68.6</td>
</tr>
<tr>
<td>Intake (g DM/kg LW0.75)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swiss</td>
<td>72.9</td>
<td>57.7</td>
<td>77.3</td>
<td>81.3</td>
</tr>
<tr>
<td>Loire Valley</td>
<td>78.8</td>
<td>66.1</td>
<td>79.1</td>
<td>85.1</td>
</tr>
</tbody>
</table>

The effect of multi-species grassland, combining several grasses and legumes, on animal nutrition (herbage intake, digestibility, milk yield) has to our knowledge never been quantified under grazing. The common experiment of MultiSward will use mixtures combining grasses, legumes and forbs for grazing.

3.2. Quality of animal products

Important effects of plant species diversity on product quality and animal health have been found. Recent studies have demonstrated that dairy products from grazing ruminants have a composition thought to be beneficial to human health, compared to that from animals fed concentrate diets. Grazing, compared to indoor feeding, results in different fatty acid composition in milk and meat and in the organoleptic properties of animal products (Coulon and Priolo, 2002). Pasture is associated with higher content of some unsaturated fatty acids beneficial to human health (i.e. linolenic acid and conjugated linoleic acid-CLA) and a decrease of saturated fatty acid (palmitic acid in particular) (Martin et al., 2004). The concentration of unsaturated fatty acids in milk has been found to increase linearly as the amount of fresh forage was increased (Dhiman et al., 1999; Couvreur et al., 2006). Levels of vitamin A, β-carotene and vitamin E are also higher in milk from cows fed pasture than in the milk of cows fed corn silage, or a concentrate rich diet or hay (Martin et al., 2004). The global antioxidant status of milk reveals higher antioxidant protection in milk form pasture,
although its content of unsaturated fatty acids is higher (Martin et al., 2004). Similarly, grazing compared to indoor fattening results in a different fatty acid composition (higher proportions of linoleic and linolenic acid), darker and redder meat with better sensory qualities and an increased shelf life (references in Wragge et al., 2011: Multisward partner).

Grassland botanical composition can affect fatty acids composition of milk fat as suggested by the high CLA content in milk (Collomb et al., 2001) from highlands, which are highly diverse grasslands, compared with lowlands. Similarly milk CLA was higher with diets containing silage of semi natural grassland than with those containing silage of ryegrass monocultures (Fievez et al., 2002). Moloney et al. (2008) concluded from a review of several experiments that more diverse pastures produced milk with increased C18:3n-3 and polyunsaturated fatty acid concentrations, whereas the saturated fatty acid concentrations were in most cases reduced. Lucas et al (2003) also reported a higher CLA and vitamin E content in highland cheeses than in lowland cheeses. Tornambe et al (2010) reported a higher proportion of linolenic acid in milk of cows grazing a species-rich permanent pasture (50 species) compared to a permanent grassland (31 species) and a temporary grassland (17 species), but no difference for CLA or for the content of vitamins A and E. These authors pointed out that phenological stage had a much more important effect on milk fatty composition than did species diversity. In temporary grassland, grass species or cultivar have minor effects on fatty acid composition of milk fat (Delagarde and Peyraud, 2002).

Fraser et al. (2009) (Multisward partner) conducted grazing experiments with different breeds on improved permanent pasture (ryegrass/clover) and semi-natural rough grazing on *Molinia caerulea* dominated swards. Their results indicated a greater influence of the sward type on meat quality than the breed when beef cattle were produced in less favoured areas. Under rough grazing, loin steaks contained more vitamin E and had a lower lipid oxidation.

Certain plants may also have health benefits for the animals. For example, some legumes (eg. *Lotus* spp.) contain condensed tannins that potentially result in increased production of milk and wool, improve the lambing percentage and reduce bloating risk as well as the burden of intestinal parasites. In addition, adding tannin-rich legumes to animal diets may decrease rumen methanogenesis, and thus the production of the potent greenhouse gas methane. As reducing methane production during rumination also means decreasing energy losses by the animals, this is interesting from a production point of view as well. So far, however, the importance of diverse grasslands in this respect is not well quantified and not completely understood (Wragge et al., 2011).

It can be concluded that pasture contributes to the production of healthy milk and meat, and that the botanical composition of grassland can modulate the response, the highest benefits being observed in milk from highlands. The effect of multi-species grassland with mixtures of grasses, legumes and to some extend forbs on milk composition compared to intensively managed grasses monocultures needs to be quantified.
4. Species diversity, air quality and climate change

4.1. Greenhouse gas emissions

Because agricultural inputs such as mineral fertilizer are associated with negative environmental impacts (e.g. Gruber and Galloway, 2008), the maintenance of productivity while decreasing agricultural inputs through the use of multi-species swards could improve the sustainability of forage production systems (Weigelt et al., 2009; Nyfeler et al., 2011). Moreover, some environmental costs of forage production do not increase linearly with an increase in yield, as shown for the consumption of non-renewable energy needed for harvesting. Increasing the yield through beneficial mixture effects would therefore reduce the mechanization-related CO₂-emissions per unit of forage produced. In their life cycle analysis, Nemecek et al. (2005) calculated the amount of non-renewable energy resources and the amount of CO₂-equivalent emitted for silage production on intensively managed permanent grasslands fertilized with slurry. Using their data we calculated for an intensive silage production under favorable pedo-climatic conditions that if a yield increase of 18 % per surface area is achieved without increasing fertilization, this would provoke an increase of only 9 % of non-renewable energy resources used for the production of silage. This corresponds to a reduction of 90 kg CO₂-Equivalent (-7 %; global warming potential over 100 years according to IPCC 2007) per tons of forage produced for the higher yielding field compared to the lower yielding one.

Weigelt et al. (2009) studied the biomass production of mixtures with a range of species richness for 3 levels of nitrogen fertilization in a low intensity management system (2 annual cuts). In their study the mixtures with legumes produced even more than the mixtures without legumes and additional fertilization of 100 kg N ha⁻¹ yr⁻¹. While symbiotic N₂ fixation from photosynthetic carbon is ‘greenhouse gas neutral’, an equivalent of greenhouse gas emissions of 3.3 and 8.6 kg CO₂-Equivalent (global warming potential over 100 years according to IPCC 2007) is calculated per kg of urea-N and ammonium nitrate-N, respectively, for production and transport to the regional storehouse, by the database ecoinvent data v2.1 (ecoinvent Centre, 2008). Saving 100 kg of mineral N therefore corresponds to a reduction of 330 to 860 kg CO₂-Equivalent of greenhouse gas emissions from production and transport. Because fertilization accounts for more than half of the total amount of CO₂-Equivalent emitted during forage production (including N₂O and CH₄ emissions; Nemecek et al., 2005), reducing N fertilization would significantly decrease the global warming potential of forage production.

Another possibility for estimating the fertilizer N saving potential of grass-clover mixtures would be to estimate the amount of nitrogen symbiotically fixed by the legumes growing in mixture with grasses. This is a well studied area and different models have been proposed for such estimations (Liu et al., 2011). The effect of the relative abundance of legumes on the amount of symbiotically fixed nitrogen is described by Nyfeler et al. (2011). From the data of this study it is obvious that the yield increase per unit of symbiotically fixed nitrogen is strongly influenced by the relative abundance of the legumes in the sward (Fig. 6). At low relative legume abundance, the increase in dry matter yield with increasing legume abundance is steeper than the increase in the total amount of fixed nitrogen. But at high relative legume abundance, the total amount of symbiotically fixed nitrogen stays high with a further increase in legume abundance, while the yield markedly decreases (Fig. 7; Nyfeler et al., 2011; Nyfeler et al., 2009). Using estimates of the amount of
symbiotically fixed nitrogen to estimate the apparent nitrogen fertilizer replacement value of the clover would therefore result in an underestimation at low legume proportions and in an overestimation at high legume proportions (Fig. 6).

Figure 6. Effect of the relative legume abundance on the forage yield gained per unit of symbiotically fixed nitrogen as compared with grass monoculture for intensive forage production under favorable pedo-climatic conditions and a fertilization of 150 kg N ha\(^{-1}\) yr\(^{-1}\) (from Nyfeler et al., 2009 and 2011).

Reducing the need of nitrogen fertilizer per unit of forage produced could also help reducing N\(_2\)O emissions. Carter and Ambus (2006) showed that a very small fraction (3 \(10^{-6}\)) of symbiotically fixed nitrogen is directly lost as N\(_2\)O (while the nitrogen fraction lost from mineral nitrogen applications is in the range of 1%; Cardenas et al., 2010). Correspondingly, the IPCC guidelines for inventories of greenhouse gases emissions assume direct N\(_2\)O emissions related to symbiotic fixation to be negligible for grassland. Nevertheless, clover species usually have a lower C/N ratio compared to grasses (Whitehead, 2000) and the decomposition of clover plant residues can enhance nitrification-denitrification processes (e.g. Rochette and Janzen, 2005).
Increased clover relative abundance could therefore have an indirect negative impact on N$_2$O emissions.

The N$_2$O emissions were measured in some experiments. In the monocultures and mixtures used by Nyfeler et al. (2009), mixed swards emitted less N$_2$O than legume monocultures, at a rate comparable to or lower than the default emission factors of the IPCC emission inventory methodology. The quantity of N$_2$O losses per unit of forage produced was within the same range for the grass-clover mixtures and the grass monocultures (unpublished). Results available from Teagasc (MultiSward partner) also indicate that direct N$_2$O emissions from symbiotic nitrogen fixation and indirect emissions from the decomposition of clover residues are very small compared to fertilization-linked N$_2$O emissions. In this study, N$_2$O fluxes were measured weekly with the static chamber method from October 2009 to September 2010 on a grass monoculture fertilized with 226 kg N ha$^{-1}$ yr$^{-1}$ (mineral) and a grass-clover mixture fertilized with 86 ha$^{-1}$ yr$^{-1}$ (58 kg mineral-N + 28 kg slurry-N) grazed at the same stocking density. Other treatments included an unfertilized, mown grass monoculture and an unfertilized, mown grass-clover mixture. No difference in N$_2$O emissions was detected between the unfertilized grass monoculture and the unfertilized mixture. In the study of Li et al (2011), the measured N$_2$O losses from the fertilized grass monoculture tended to be higher than those from the grass-clover mixture with reduced level of fertilization. Klumpp et al. (2010; MultiSward partners) tested whether high (35 ± 4 %) and low (19 ± 4 %) clover abundance affects N$_2$O emissions of a permanent, fertilized (157 N ha$^{-1}$ yr$^{-1}$) grassland. In this study too, N$_2$O emissions were not significantly different between clover abundance treatments, but showed a tendency for increased N$_2$O emissions from the low clover treatment, presumably due to shifts in plant/soil competition for N. From a study with experimental communities grown under controlled conditions, Niklaus et al. (2006) concluded that “N$_2$O emissions increased in the presence of legumes”, which is in contradiction to the studies described above. Nevertheless, in this study, mixtures with legumes and non-legumes did not consistently show higher N$_2$O emissions than mixtures without legumes. Taking into account the fertilization-linked N$_2$O emissions, it therefore seems reasonable to consider that the partial replacement of fertilization with symbiotic fixation from clover is an effective way of lowering N$_2$O emissions from productive temporary grasslands. Nevertheless, longer term studies are still needed to assess the impact of this strategy on N$_2$O emissions from soils under permanent grassland as the longer term effect of legume-containing associations on soil nitrogen pools might differ from their short term effect (Oelmann et al., 2011).

As discussed above, grassland productivity can also be improved by plant diversity in the absence of legumes. In an experiment in which the number of grass and forb species in the community was increased from 1 to 8, Van Ruijven and Berendse (2003) showed that the observed increase in biomass productivity with increasing plant species richness was partly due to increased nitrogen-use efficiency in the species rich swards (i.e. reduction in leaf nitrogen content with constant productivity). Such effects of diversity could reduce N$_2$O emission by reducing both the pool of inorganic nitrogen in the soil and the C/N ratio of the plant residues. Also cutting / defoliation frequency promotes short term N$_2$O emissions (Kammann et al., 1998), which supports the idea that the cutting regime may play an important role in annual N$_2$O emissions from grasslands through competition for inorganic N (Tallec and Klumpp 2011 in prep.).
Other studies showed the preponderant influence of the water-filled pore space in the soil on \( \text{N}_2\text{O} \) emissions (see Flechard et al., 2007; Klumpp et al., 2010). Diversity effects on biomass production could alter the quantity and timing of water uptake by the cover which in turn could influence water-filled pore space and consequently \( \text{N}_2\text{O} \) emissions. No data has been found on this potential effect. Under grazing, increased biomass productivity would result in a higher stocking rate and the subsequent increased soil compaction due to treading (Cuttle, 2008) would have a negative effect on \( \text{N}_2\text{O} \) emissions (Luo et al., 2010). The effect of plant diversity on \( \text{N}_2\text{O} \) emission under grazing has to our knowledge only been quantified by indirect studies, analyzing denitrification and nitrification with respect to plant species composition (Patra et al., 2006; Le Roux et al., 2003).

### 4.2. Carbon storage in the soil

The effect of plant diversity on carbon sequestration is of special interest in connection with efforts to mitigate climate change. Lal (2004) estimated the soil organic carbon (SOC) sequestration potential to be 0.01-0.3 Gt C year\(^{-1}\) on the 3.7 billion ha of permanent pasture worldwide. The European project “GreenGrass” showed, from 9 European sites, that the permanent pastures were carbon sinks of an average intensity of 2.7 t eq CO\(_2\) ha\(^{-1}\) yr\(^{-1}\) (i.e. 0.7 t of C ha\(^{-1}\) yr\(^{-1}\)), which is comparable with that of temperate forests (Soussana, 2005). The collective scientific expertise led by INRA (Arrouays et al., 2002) had established that a lengthening of the duration of the temporary pasture or a moderate intensification of the permanent pasture was accompanied by an increased storage of carbon in the first 30 cm of the soil of 0.5 t C/ha/an during the first 20 years. Conversely, the ploughing of grassland and conversion into arable crops, is accompanied by a fast destocking of carbon (-1 t C/ha/year) and therefore the long term SOC sequestration will depend of the live span of the grassland. This section only considers the role of plant diversity in changing the potential of SOC storage under grassland during the period grassland vegetation cover the soil.

In the Jena experiment, Steinbeiss et al. (2008) found increasing carbon storage in 0-30 cm depth with sown species richness on an alluvial soil without an influence of root biomass. Only in the top 5 cm annual root biomass production played a role in increasing SOC storage, whereas between 20 and 25 cm soil depth the proportion of the functional group of tall herbs had a larger effect on SOC stocks. Fornara and Tilman (2008) conducted a similar experiment on sandy soil with the result of higher species richness increasing soil C accumulation by its effect on root biomass (net soil C accumulation of around 1 t ha\(^{-1}\) yr\(^{-1}\) under the covers with the highest root biomass compared to no C accumulation under the covers with the lowest root biomass, over the 12 years of the experiment). They attributed high root biomass production and thus enhanced soil carbon storage to niche complementarity between C4 grasses and legumes. Klumpp and Soussana (2009) studied the role of plant functional traits for above- and belowground C-fluxes and found root and rhizome traits controlling belowground C sequestration through their influence on decomposition rate of belowground litter. Leaf traits affecting aboveground primary production did not have an influence on soil C sequestration. Nevertheless, Prescott (2010) conclude in his review on forest ecosystems that litter quality has the most direct influence on decay rates and that decomposition is influenced by the leaf dry matter content and the specific leaf area of the plants. The functional identity of the cover might therefore
influence terrestrial carbon storage. In a further field experiment aiming at restoring grassland diversity, de Deyn et al. (2011) show increasing SOC sequestration through cessation of fertilizer use, but no effect of species richness. Indeed, the difference in species number in this study was only 2-4 species compared to 1-60 in the two other experimental studies. A promotion of the legume *Trifolium pratense* L. over two years especially increased soil carbon storage. Szanser et al. (2011) found that decomposing litter from a mixture of species led to a higher increase of carbon content in the substrate than litter from single species. Litter-feeding soil invertebrates interact with the plant species composition of the litter to modulate rates of decomposition and soil fauna therefore co-determine the magnitude of plant diversity on litter decomposition (Hattenschwiler and Gasser, 2005). On the other hand, microbial diversity has been found not to be related to the rate of CO2 emission in agricultural soils (Levine et al., 2011).

When looking at the effect of management on soil C storage, rich organic soils are more likely to act as CO2 sources compared with mineral soils. Rogiers et al. (2008) investigated the C-balance of grassland on rich organic soil in the Swiss Alps that was drained in the past. The site was a net source of CO2 during all three years of the study, which was reinforced by hay harvests and grazing. Grass cutting and grazing reduces leaf area and thus degrades assimilative capacity just after the event. This results in a net carbon loss (Rogiers et al., 2008; Zeeman et al., 2010). Disturbance can lead to a higher herbage production but at the same time lower the C accumulation in the soil (Klumpp and Soussana, 2009). On the other hand, manure applications and grazing animals provide an input of C to the ecosystem. Soussana et al. (2007) showed that grassland which is only grazed can function as CO2 sinks, whereas cutting leads to carbon exports which are not compensated by carbon import through usual amounts of manure.

Thus, plant diversity showed a positive effect on terrestrial carbon storage in a few diversity experiments. But carbon storage is also influenced by the organic matter content of the soil, as well as decomposers and agricultural management, which are themselves related to plant diversity. A deeper insight on these interactions should be available before to conclude on the effects of plant diversity on carbon sequestration.

5. Species diversity and water quality

5.1. Nitrate leaching

Forage production on grasslands is generally associated with a lower risk of nitrate leaching than arable crop production (e.g. Strebel, 1989; Hansen, 2000; BAFU, 2009). However, intensive management and heavy nitrogen fertilization greatly increases this risk (e.g. Barraclough et al., 1983; Vertes et al., 2007). Legislation and policies throughout Europe limit nitrogen fertilization levels to reduce this problem and comply with the EU Nitrate Directive. These limits are sometimes considered as too low by representatives of agriculture (Schröder et al., 2007) and in this context, grass-legume swards are becoming increasingly interesting. The botanical composition and diversity of grassland influence the risks of nitrate leaching through their influence on numerous aspects: these include biomass production – and consequently nutrient uptake and seepage rate –, mineralization, symbiotic nitrogen fixation, rooting depth and timing of nitrogen uptake.
By increasing biomass production as compare to monocultures, multi-species swards increase the sink for nitrogen and might therefore reduce the quantity of nitrates \( (\text{NO}_3^-) \) present in the soil during the growing season (Hooper and Vitousek, 1998; Tilman et al., 2002, Maestre and Reynolds 2007, Oelmann et al., 2007; Roscher et al., 2008;) on mown grassland. The reduction of nutrient leaching under multi-species swards is then related to a higher nutrient uptake from the soil, respectively a more fully exploited soil inorganic nitrogen (Tilman et al., 1996; but see Von Felten et al., 2009) and some evidence for a reduced risk of nitrate leaching with a higher number of plant species in the communities has been observed (Bingham and Biondini, 2007).

5.1.1. Effect of the traits of the species composing the community

The traits of the species composing the community have an important influence on the risk of nitrate leaching. Multi-species swards usually associate species with differing rooting strategies. Differences in root architecture between the different species of the community modify the spatial use of soil nitrogen (Kahmen et al., 2006). This spatial complementarity in nitrogen acquisition (Jumpponen et al. 2002; Personeni and Loiseau, 2005) might allow for nitrogen uptake in deeper soil layers, thereby improving utilization of leaching prone nitrate. An indication for nitrogen uptake at deeper soil layers by *Cichorium intybus* L. than by *Lolium perenne* L. is given by Frankow-Lindberg and Dahlin (2010). Jumpponen et al. (2002) showed with an experiment using \(^{15}\text{N}\) injected at 5 and 20 cm soil depth that competition between species can modify spatial differences in nitrogen uptake: in monoculture *Achillea millefolium* L. extracted \(^{15}\text{N}\) mainly at 5 cm depth while *Festuca ovina* L. extracted at both 5 and 20 cm soil depths. When grown in mixture, *Achillea millefolium* increased its nitrogen acquisition from 20 cm soil depth whereas for *Festuca ovina* the nitrogen acquisition at 20 cm soil depth was decreased compared to the situation with intra-specific competition only.

Beside of spatial complementarity, differences in growth pattern (phenology) across the year might also contribute to a reduction in the risk of nitrate leaching: this complementarity in time between the species might allow the nitrogen mineralized in the soil throughout the growing season to be more fully utilized by the sward. As mentioned in the discussion on \( \text{N}_2\text{O} \) emissions, plant diversity effects on biomass production might influence water uptake by the plant cover and therefore water seepage below rooting depth. This could influence the amount of nitrates leaching into waterways. In an experiment with *Dactylis glomerata* L., *Lolium perenne* L., *Trifolium repens* L. and *Cichorium intybus* L., Skinner (2008) observed however that including the deep-rooting forb *Cichorium intybus* in the mixtures improved the water use efficiency of the cover, but not the uptake from water at deeper soil layers (75 cm).

5.1.2. Effect of legumes and their capability of fixing atmospheric Nitrogen

The capability of symbiotically fixing atmospheric nitrogen and the presence and abundance of legumes in the sward, besides the spatial and temporal complementarity of the species composing the community, might also influence soil inorganic nitrogen and leaching potential more than species richness (Hooper and Vitousek 1998; Scherer-Lorenzen et al., 2003; Palmborg et al., 2005). While Loiseau
et al. (2001) measured an only slightly elevated amounts of leached NO$_3^-$ under grass-legume mixtures as compared to grass monocultures (4, 9 and 63 kg N ha$^{-1}$ yr$^{-1}$ for grass monocultures, grass-legume mixtures and legume monocultures, respectively), Scherer-Lorenzen et al. (2003) reported a significantly higher nitrate leaching under grass-legume mixtures than under grass monocultures (2, 20 and 70 kg N ha$^{-1}$ yr$^{-1}$ for grass monocultures, grass-legume mixtures and legume monocultures, respectively), in spite of a significantly higher biomass production in the mixtures than in the monocultures. Nyfeler et al. (2011; MultiSward partner) studied the acquisition of nitrogen resources by legumes and grasses in a three-years field experiment on intensively managed swards fertilized with either 50, 150 or 450 kg N ha$^{-1}$ yr$^{-1}$. The species proportions of *Lolium perenne* L., *Dactylis glomerata* L., *Trifolium pratense* L. and *Trifolium repens* L. were varied to investigate the effects of species proportion (0, 3, 10, 25, 40, 50, 70, 90, 100% sowing proportion) on nitrogen yield, as well as on nitrogen acquisition from symbiotic and non-symbiotic sources (soil and fertilizer). Grass-legume interactions stimulated the acquisition from both symbiotic and non-symbiotic sources. The grass-clover mixtures thus took up at least as much nitrogen from the soil and fertilizer as the grass monocultures did, although the clover provided large amounts of nitrogen to the system through symbiotic fixation. This was true for mixtures with a relative clover abundance of up to 50-60 %, corresponding to the range of clover abundance where biomass yield was maximized but where total nitrogen yield was slightly lower than at maximum. Correspondingly, the measurements of the nitrate content in the soil water showed that the risk of nitrate leaching was low as long as the relative abundance of clover was not very high (less than 80 % clover) and the fertilization was not above 150 kg N ha$^{-1}$ and yr$^{-1}$. High-yielding forage production system using symbiotic nitrogen fixation and low levels of nitrogen fertilization therefore do not increase the risk of nitrate leaching during the utilization period of the grassland compared to grass monoculture systems relying on heavy nitrogen fertilization.

Nevertheless, only few studies assessed the changes in nitrate content in the soil under mixtures containing legumes during more than two to three years. In the longer term, soil nitrate content could return to levels similar than under fertilized grass monocultures due to the mineralization of nitrogen rich legume residues. Rapid root turnover of the legume plants could be an important source of nitrogen input into the soil. In multi-species swards however, two processes might maintain the content of mineralized nitrogen in soil water at low levels. Firstly, the companion grasses are efficient competitor for nitrogen (Nyfeler et al., 2011) and consume most of the nitrogen available in the upper soil layer during the growing season. Secondly, nitrogen input in the system through symbiotic fixation is reduced when the availability of inorganic soil nitrogen increases (Soussana et al., 2002; Nyfeler et al., 2011). But Fornara and Tilman (2008) showed for 12-years old grasslands that diversity increased nitrogen accumulation in the soil. It has to be expected that this will in time provoke an increase in nitrogen release by mineralization. This is supported by the findings of Oelmann et al. (2011): these authors showed that the initial decrease in NO$_3^-$ content in the soil achieved by the better use of soil resources by multi-species swards compared to monocultures disappeared within 5 years after mixture establishment. For multi-species grasslands containing legumes and utilized as permanent grassland, the effect of plant diversity on soil nitrate content is therefore probably small but this remains to be elucidated.
In grazed systems, using studies from Europe and New Zealand, Ledgard et al. (2009) showed that nitrate leaching from grazed grass monocultures and grass-clover mixtures increases with increasing total nitrogen input, independently from the origin of the nitrogen input (fertilizer or symbiotic fixation). In grazed systems, this is nevertheless mainly due to the increase in biomass production with higher nitrogen inputs and the consequent increase in stocking rate and deposition of animal excreta (Laurent et al., 2000), and is therefore not an assessment of the direct risk of increased inorganic nitrogen in the soil due to the input of symbiotically fixed nitrogen. At similar levels of total nitrogen inputs, Humphreys et al. (2008, MultiSward partners) also found no differences in nitrate concentration in soil water at 1 m soil depth between grass-clover pastures and pastures with almost no clover. In this experiment however, no relation between total N input (from 205 to 400 kg N ha\(^{-1}\) yr\(^{-1}\)) and the nitrate concentration in soil water could be observed. Based on the results of Hooda et al. (1998), Ledgard et al., (1999) and Peyraud and Delaby (2006), Peyraud et al. (2009) come to the conclusion that “mixed pastures reduce the risk of nitrate leaching primarily because they cannot sustain the same level of stocking than highly fertilized grasses pastures”. Preliminary results from the MultiSward common experiment nevertheless indicate that some moderately fertilized 4-species grass-clover mixtures could reach the biomass production of highly fertilized \textit{Lolium perenne} monocultures under grazing. At the field level, the biomass production–stocking rate–dejections relationship can also influence nitrate leaching by influencing the repartition of excreta deposition within the growing season: because clover growth is shifted toward summer compared to grass growth (Lüscher et al., 2005), grass-clover mixtures generally allows for more grazing days during summer than does grass monocultures. With grass-clover mixtures, more dejection are thus deposited during the second half of the growing season than with grass monocultures, which could negatively affect nitrogen leaching at the plot level.

It can be concluded that in the short term (temporary grasslands) and under mowing, improved resource utilization in multi-species swards has a reducing effect on the risk of nitrate leaching during the presence of grassland cover on the field. In the long term or under grazing, however, the fixing–non-fixing mixing effects on biomass production, respectively on the need of nitrogen fertilization, should be considered as neutral regarding the risk of nitrate leaching.

5.2. Herbicides

Diffuse herbicide losses are a major source of pesticide contamination of surface water (Chelme-Ayala et al., 2007). The quantities of herbicides utilized on intensive grasslands are usually much less than on arable crops. For instance Nemecek et al. (2011) calculated with 0.5 kg ha\(^{-1}\) yr\(^{-1}\) active herbicide matter for conventional intensive grass production and with 1.8, respectively 2.1 kg ha\(^{-1}\) yr\(^{-1}\) for winter wheat and rape in their scenario analysis of the environmental impact of crop and forage production in Switzerland. Nevertheless, Müller et al. (2006) indicated a recommended herbicide application on pastures of 1 to 1.6 kg ha\(^{-1}\) active matter in New Zealand, and contamination of surface runoff water from grassland has been observed in some cases (Müller et al., 2006; Cessna and Elliott, 2004). Different diversity experiments delivered evidences that plant species of functional diversity enhances the resistance of grassland to weed invasion both in extensively (Crawley et al., 1999; Tilman et al., 2002; Van Ruijven et al., 2003) and in intensively (Connolly
et al., 2009; Frankow-Lindberg et al., 2009) managed grasslands. In the COST action 852 multi-site experiment, the difference in the incidence of unsown species was more pronounced between the clover monocultures and the mixtures than between the grass monocultures and the mixtures (Lüscher et al., 2008). This higher resistance to weed invasion could reduce the utilization of herbicides in production systems based on multi-specific swards. Preliminary observations on the MultiSward common experiment nevertheless indicates that the proper establishment of multi-species sward requires a weed-free soil at sowing and that broad-leaved dock plants (*Rumex obtusifolius* L.) are able to establish well from the soil seed bank in establishing multi-species temporary grasslands. Moreover, the incertitude regarding the estimation of the influence of herbicide applications on the aquatic eco-toxicity potential of forage production is still large: for instance, broad-leaved dock regulation by plant-by-plant herbicide application was estimated to be less than 1 % of the aquatic eco-toxicity potential of intensive forage production by the EDIP97 assessment method (Hauschild and Wenzel, 1998) in the production scenarios of Nemecek et al. (2011) but would have been estimated to represent more than 30 % of it by the CML01 method (Guinée et al., 2001). The effect of the observed higher resistance to weed invasion of mixtures on the impact of agricultural grassland management on water quality is therefore still difficult to quantify and might be marginal.

6. Species diversity and soil quality and stability

The composition and diversity of the plant cover might influence important aspects of soil stability because it modifies the characteristics of the root system of the grassland, rhizodeposits and the soil biota (Eisenhauer et al., 2011). Both the roots and the soil biota play an important role in the aggregation of soil particles (Amezketa, 1999), which in turn affects the hydrological and the mechanical properties of the soil. Evidences of positive effects of plant diversity on soil processes were observed by Milcu et al. (2010) who showed a stabilizing effect of plant diversity on root biomass, microbial biomass and microbial respiration. In their study on a tallgrass prairie, Porazinska et al. (2003) nevertheless did not observe any relationship between species richness and the biotic and abiotic soil characteristics they studied. Plant diversity might influence the spatial distribution of the roots in the soil (Schenk et al., 1999) and because it enhances aboveground biomass productivity, it potentially affects belowground plant biomass and root density (Balvanera et al., 2006, but see Gastine et al., 2003 and Bessler et al., 2009). The important role of root biomass in the prevention of soil erosion has been discussed by Gysseels et al. (2005) and the influence of root morphology on this process by Reubens et al. (2007) and De Baets et al. (2007). But evidences of positive effects of grassland plant diversity on soil stability are scarce. Miller and Jastrow (1990) and Pohl et al. (2009) reported positive diversity effects on the stability of soil aggregates. In his PhD thesis on the relationships between plant diversity and soil stability in disturbed alpine ecosystems, Pohl (2010) concluded that “the resistance of topsoil to water-induced erosion at disturbed alpine sites depends not only on the degree of vegetation cover, but also on the presence of a high number of plant species that are highly diverse in functional traits above and below ground.” Although a positive effect of plant diversity on soil quality and stability can be expected, a quantification of
these processes in term of for instance lower erosion risks can to date not be derived from the literature for agriculturally managed grasslands.

7. Species diversity and biodiversity of other taxas

7.1. Plant species richness

The structure and composition of the plant cover as food resource and habitat-modifying factor influence the diversity of other taxa. The conservation of this biodiversity is at the same time an objective per se and a support to ruminant production systems, as different animal, fungi and microorganism taxa deliver supporting services to forage production (Le Roux et al., 2008). Plant diversity impacts on soil organisms (e.g. Hooper et al., 2000; Eisenhauer et al., 2010) might influence nutrient cycling and consequently grassland productivity and effects on air and water quality. The apparent consequences of the direct and indirect effects of plant diversity on these services are discussed in the respective chapters and will not be further discussed in this chapter. We focus here on the effects of plant diversity on the diversity of arthropods.

Numerous studies have shown that extensification of grassland management increases both plant and animal diversity (e.g. Aviron et al., 2009). Butterflies and ground beetles (Aviron et al., 2009; Kampmann et al., 2008), as well as bee species (Batáry et al. 2010; Albrecht et al., 2007) showed a significant higher species diversity in extensive, species-rich grasslands than in more intensively managed, species-poorer grasslands. But this relation seems much weaker for grasshoppers (Kampmann et al., 2008) and spiders (Aviron et al., 2009). The current knowledge about the combined effects of agricultural management and plant diversity on the diversity of arthropod species has been implemented in SALCA-BD, a biodiversity assessment tool for life cycle assessments based on an evaluation of the agricultural activities at the field level (Jeanneret et al., 2009; Jeanneret et al., 2007). Nevertheless, most arthropod species have a dispersal ability much larger than a farmland field. Land utilization, management intensity and structure at the landscape level therefore strongly influence the differences in arthropod diversity observed between more or less intensively managed grassland plots (Batáry et al. 2010; Knop et al., 2011). Landscape structure is of outmost importance for arthropod needing more than one type of habitat during their life cycle, like a large number of butterfly species (Hanski and Thomas, 1994) and increasing grassland quality could promote such species only if the other landscape elements necessary for the completion of their live cycle are available in sufficient quantity and quality. The size and density of the plant populations attractive to pollinators or herbivores also influences the number of species found on a grassland plot (Rathcke, 1983; Dauber et al., 2010).

In all these studies however, the effects of management and the effects of plant diversity are confounded. Studies that distinguish plant diversity from agricultural management and assess how grassland plant diversity alone influences arthropod diversity are scarce. In a diversity experiment on a tallgrass prairie of Minnesota, Haddad et al. (2001) collected insects and terrestrial arthropods and found a positive relationship between plant species richness and total insect species richness. This relationship was strong and positive for herbivores and predators, but weak for both parasitoids and detritivores. Within the herbivores, plant species richness had a
significant positive effect on sucking and seed- or pollen-feeding herbivore species richness, but no effect on the diversity of chewing and boring herbivores.

Three studies on the effects of plant diversity on diversity, respectively performance, of arthropods have been conducted on the Jena diversity experiment. The study of Ebeling et al. (2008) focussed on insect pollinators which have a preeminent function for agriculture (Klein et al., 2009). They observed until 13 visiting pollinator species (honey bees, bumble bees and solitary bees) per plots and found a positive effect of the number of flowering plant species (grasses were present in the sown species, but not counted as flowering species) on the number of pollinator species visiting the plots. The increase in the average number of visiting species with increasing flowering plant species was marked until 9 flowering plant species but was saturating above this level of plant diversity. The Jena experiment is managed extensively with two cuts per year. Under more intensive management, the benefit of plant diversity on pollinator diversity could be reduced by the reduction in the number of plant species and individuals reaching flowering stage between two utilisations. On the same diversity experiment, Sabais et al. (2011) assessed the effects of plant species richness on the diversity of springtails (Collembola) and found a close relationship between the number of plant species and both the species richness and the density of springtails four years after the establishment of the experimental plant communities. The third study (Specht et al., 2008) assessed the effects of plant diversity on one generalist herbivore (a grasshopper species). They did not find a significant relationship between the number of plant species growing on the plots and the performance of this generalist species. They observed higher survival rates of the grasshoppers foraging on mixtures composed of the two functional groups “legumes” and “forbs” than for the one foraging on either only legumes or forbs. Nevertheless the grasshoppers foraging on grass-only swards showed the highest survival. In a diversity experiment on permanent grassland, in which selective herbicides were used to reduce dicot populations in grazed plots (see section 1, chapter 1.1), the Grassland Science team of the University of Goettingen (MultiSward partner) assessed the number of grasshopper species and their abundance supported by these different swards. Preliminary results didn’t show a significant difference between the species-poor grass-dominated sward and the species-richer sward containing dicots. For specialist insect herbivores, a high grassland plant diversity might even have a negative effect on their abundance because the relative abundance of the specific plant species used as food-resource decreases with increasing plant diversity (eg. Otway et al., 2005).

It can be concluded that grassland plant diversity at the field level alone does not have a significant positive effect on the diversity of arthropods as a single group. This is because the effects of plant diversity depend on the biology and ecology of the different arthropod species and on the habitat structure at the landscape level. Nevertheless, an increase in plant diversity at the field level has been shown to favour the diversity of pollinator species and Collembola. For the pollinators, this positive effect was already observed within a relative small increase in plant diversity (1 to 9 species of flowering plant species). In ruminant production systems, the supporting role of grassland for pollination of agricultural and natural plant populations, as well as decomposition processes in the soil (nutrient cycling; see also chapter 3.2), could therefore be enhanced by multi-specific grasslands compare to swards containing only grasses.
7.2. Species composition or functional diversity

Plant species richness is only one characteristic of cover and others, like species composition and plant functional diversity, might also play an important role in ecosystem processes and derived ecosystem services (Balvanera et al., 2006). The importance of these different components of diversity for the observed effects of diversity on ecosystem processes is still controversially debated. Various studies emphasized the importance of species composition, respectively the presence of a species or species group with particular functional traits (Tilman, 1997; Hooper and Vitousek 1997; Mokany et al., 2008; see also De Bello et al., 2010, for a review). Mixing species belonging to specific functional groups has also been the approach followed by agronomists for the development of forage species mixtures (e.g. Sanderson et al., 2004). Other studies showed a predominant effect of functional diversity on observed diversity effects (Diaz and Cabido, 2001; Petchey et al., 2004; Diaz et al., 2007; Cadotte et al., 2011). Nevertheless, Balvanera et al. (2006) found that for the numerous studies included in their meta-analysis (including 252 grasslands), the effects of manipulating the functional composition of the communities was only slightly larger than the effects of manipulating species richness. Moreover, Hector et al. (2011) recently re-assessed results from a range of grassland experiments and concluded that “counter to earlier predictions, species richness and composition are of similar importance for primary production in grassland biodiversity experiments”, and Eisenhauer et al. (2011) also found that plant species richness rather than the presence of key plant functional groups affects soil biota on the longer term. Even so, for intensive ruminant production systems, evaluating the impact of functional diversity rather than species richness might be better adapted, because in such systems, the botanical composition of the swards often reflects a concerted selection of species widely differing in their functional traits. Under these conditions, large diversity effects can be achieved with as few as 4 species (Kirwan et al., 2007). Moreover, the presence and abundance of legumes plays a major role on the nitrogen related environmental impact of forage production as previously discussed. Functional diversity might therefore be more adequate than species richness as a predictor of the delivery of ecosystem services in the agricultural systems studies by MultiSward.

8. Conclusion

Currently available results from ecological and agronomic research on the effects of plant diversity on ecosystem functioning clearly show that increased biomass productivity of grassland through the concerted use of plant diversity in grassland-based ruminant production systems is not conflicting with the delivery of a broad range of services. Improving plant species mixtures for forage production is therefore an option for achieving sustainable intensification of grassland-based agricultural production, or a decrease in the environmental burden of forage production through a reduction of agricultural inputs. Taking a closer look at the results from experimental grassland studies, it becomes obvious that observed diversity effects are most pronounced with species numbers increasing from one to two or four. Many studies have found that 90% of the productivity effect was reached with five species (Roy, 2001). In permanent grassland, however, species diversity is usually substantially
larger (10 to 30 or more species per 100 m² depending on management intensity and environmental conditions). Thus, species richness may be too large in permanent grassland to find strong effects of diversity on productivity. Accordingly, we need to focus instead on the number of dominant species which contribute to biomass production. We further conclude that functional diversity might be more adequate than species richness as predictor for the delivery of ecosystem services from agriculturally managed grasslands.

A small potential production benefit may not be sufficient to convince farmers to protect diversity in their grasslands. However, several other services of diversity are also of importance to farmers, eg. increased stability of production, resilience to environmental changes, improved use of nutrients and water, or influences on product quality. Here as well, more research is needed under realistic agricultural conditions to better understand the magnitude of these effects. Although in experimental plots more species have been found to be necessary for multiple ecosystem services, species numbers in permanent grassland might already be high enough to allow such multifunctionality. Evaluation of the effects of multi-species grassland swards on multiple environmental impacts and ecosystem services requires the development of models and/or indicators sensitive to the effects of plant diversity. The effects of plant diversity on biomass productivity of both extensively and intensively managed mown grasslands are well documented. But this is not yet the case for some ecosystem services like the protection against soil erosion or for the influence of grazing on these diversity effects. This will impact on the precision level that can at present be achieved with model- and/or indicator-based assessments. This review shows following possibilities for the integration of current knowledge in assessments of plant diversity effects at the plot level in agricultural grassland systems:

**Biomass production**

**Intensive grassland:** The plant diversity effects on aboveground biomass production for simple grass-legume mixtures managed under frequent mowing could be quantified based on the results of the COST Action 852 (Kirwan et al., 2007; Connolly et al., 2009; Frankow-Lindberg et al., 2009; Nyfeler et al., 2009). Alternatively, the nitrogen fertilizer replacement value of the symbiotic nitrogen fixation by the legumes could be estimated using Nyfeler et al. (2011) and models for the estimation of symbiotic nitrogen fixation (e.g. Liu et al., 2011) for comparing biomass productivity at different levels of nitrogen fertilization.

**Extensive grassland:** The plant diversity effects on aboveground biomass production of extensively managed mown grassland could be quantified based on the results of ecological studies on grasslands (Cardinale et al., 2007; Weigelt et al., 2009; Hector et al., 1999; Roscher et al., 2005; Hooper et al., 2005; Marquard et al., 2009).

**Pastures:** For intensively grazed pastures, data are still too scarce to draw conclusions about consistent positive effects of plant diversity on biomass productivity.

**Animal product quality**

It is well established that grazing contributes to the production of healthy milk and
meat and that the botanical composition of grassland can modify the composition of
the product. The highest benefits in term of nutritional value are reported for milk from
highlands.

Air quality and climate

Emissions of CO\textsubscript{2}-equivalent from fertilizer input and mechanization: estimation of the
plant diversity effects through the estimation of biomass productivity, respectively
reduction of fertilizer inputs and the calculation of the emissions of CO\textsubscript{2}-equivalent
per unit of forage produced using existing indicator systems and models (reviewed in
the MultiSward Milestone 3.2).

Nitrous oxide emissions: estimation of the plant diversity effects through the
estimation of biomass productivity, respectively reduction of fertilizer inputs and
calculation of the N\textsubscript{2}O emissions per hectare or per unit of forage produced using
existing indicator systems and models. Current knowledge does not support the
hypothesis that the presence and abundance of legumes is associated to higher N\textsubscript{2}O
emissions.

Carbon storage in the soil: Some evidence of positive plant-diversity-driven root
biomass effects on carbon storage in the soil exists, but a deeper insight on
interactions between the organic matter content of the soil, decomposer activity,
agricultural management and plant diversity should be obtained before conclusions
can be reached on the effects of plant diversity on carbon storage.

Water quality

Nitrate leaching: estimation of the plant diversity effects through the estimation of the
import-export nitrogen balance taking into account fertilization, grazing animals and
symbiotic nitrogen fixation, and then assessment of the risk of nitrate leaching using
existing evaluation models and import-export nitrogen balance as input variable. This
would assume that the long term risk of nitrate leaching from grasslands depends on
the quantity of nitrogen input but not on its source (fertilizer or symbiotic fixation).

Herbicides: The positive plant diversity effect on the resistance to weed invasion
should be taken into account if the indicator systems or models chosen for the
evaluation ascribe a large impact of herbicide applications on the effect of forage
production on water quality.

Soil quality and stability

Soil stability and erosion: It can be considered that higher plant diversity positively
influences soil stability and therefore reduces the risk of soil erosion. Nevertheless,
this diversity effect can to date not be quantified for agriculturally managed
grasslands. This effect could therefore only be taken into account in a qualitative
assessment.

Biodiversity

Arthropod diversity: Positive effects of plant diversity on the diversity and abundance
of pollen-feeding herbivores and decomposers. Because of the relevance of pollination in agricultural production, the evaluation of the effects of plant diversity on pollinator species would be important.

9. References


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