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Effects of multi-species swards on dry matter production and the incidence of unsown species at three Irish sites

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Recent ecological research provides evidence that an increased number of plant species in natural grasslands is associated with increased biomass productivity, and provides a wide range of other ecosystem benefits. This suggests that increases in species diversity in agricultural ecosystems may similarly lead to increased benefits. The work reported below was part of the COST 852 Agrodiversity experiment, carried out at 34 sites across Europe. In Ireland, the effects of four-species grass-clover mixtures on herbage production, species persistence and unsown species suppression at three sites over multiple years, were investigated under growing conditions that were intensive relative to unfertilised natural grassland systems. The design included a range of four-species mixtures and monocultures of perennial ryegrass, timothy, cocksfoot, white clover, red clover and Caucasian clover. Several harvests were taken at each site for two or three years. Species diversity had a strong, persistent and positive effect on overall yield and the yield of sown species, and enhanced resistance to the growth of unsown species. Mixtures generally yielded well when compared with the best monoculture, and sometimes out yielded it. These effects on total yield declined over time but were still important at the end of the experiments. The diversity effects on sown species yield and on resistance to unsown species increased with time. Diversity effects were robust to changes in species composition,

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and persisted for the duration of the experiments across mixtures and over time. Virtually every mixture had a higher yield, and suppressed unsown species better, than monocultures of perennial ryegrass. These patterns were broadly consistent across sites. The persistence of species varied widely and was not consistent across sites.

Keywords: grass-clover mixtures; over-yielding; simplex design; transgressive over-yielding; unsown species suppression

Introduction

Provision of food, fibre and fuel are services delivered by agricultural ecosystems through the interplay of a variety of biological processes. The functioning of agricultural ecosystems is dependent on the activities of a host of biological organisms that provide services such as decomposition, maintenance of soil fertility, provision of clean water, pollination etc. Recent ecological research generally indicates that the provision of ecosystem services is reduced by declines in species diversity (reviewed in Hooper *et al*., 2005; Balvanera *et al*., 2006; Cardinale *et al*., 2007), although much of this research has been conducted in semi-natural grassland systems that are relatively species-rich and nutrient-poor. An interesting corollary, therefore, is whether, in grassland systems, moderate increases in species numbers at low levels of species richness can result in increased ecosystem function at relatively high levels of nutrient availability.

This is particularly pertinent to agricultural ecosystems, where an increase in crop diversity in species-poor agronomic systems might result in improved provision of ecosystem services such as biomass production. While natural grasslands have significant ecological importance in Ireland, there are approximately 4 million ha of intensively or semi-intensively managed grasslands which provide food, fibre and fuel for human consumption. Other services provided by agro-ecosystems will be important to varying degrees, and include yield stability over space and time, forage quality, unsown species suppression, reduction of pests and diseases and/or reduced susceptibility to them, inhibition of nitrogen losses to air and groundwater, stimulation of soil biodiversity and consequent effects on nutrient cycling. The multifunctional nature of agro-ecosystems highlights the importance of a full assessment of the value of diversity in agronomic systems.

Traditionally, agronomic science suggests that, compared to more species-rich mixtures, species-poor mixtures or even monocultures of high-yielding species will maximise yield under productive and fertilised conditions. Although the maximisation of yield is often the dominant reason for use of monocultures, other advantages of agricultural monoculture systems include the synchronisation of farm management and the simplified harvesting associated with a single crop type. Whether increased crop diversity in species-poor agronomic systems could improve the provision of ecosystem services (Hooper *et al*., 2005) remains largely untested due to the rarity of multi-species agronomic experiments that use more than two species. Experiments have largely used two-species legume-grass or legume-crop combinations (Vandermeer, 1989; Federer, 1999;

Gibson *et al*., 1999; Connolly, Wayne and Bazzaz, 2001). A review of results from trials with diverse mixtures (Sanderson *et al*., 2004) showed that there were indications that productivity of pastures increased with increasing numbers of productive species in some trials.

Biological diversity is increasingly recognised as a key component for the sustainability of managed ecosystems, and as an important adaptation strategy against agricultural risks in an uncertain future (Millenium Ecosystem Assessment, 2005). But to what extent can sward diversity contribute to intensively managed systems? The increasing cost of nitrogen fertiliser is putting pressure on the profitability of pasture-based systems, causing a re-evaluation of the use of fertiliser nitrogen on farms and a search for lower-cost alternatives. Forage legumes offer the potential to lower fertiliser costs on farms and may offer a more sustainable option for pasture-based production, not just economically but also in terms of other environmental impacts. Recent studies (Kirwan *et al*., 2007; Helgadóttir *et al*., 2008; Lüscher *et al*., 2008; Frankow-Lindberg *et al*., 2009; Nyfeler *et al*., 2009) indicate that four-species grass-clover mixtures can deliver strong and persistent diversity effects under low to moderate levels of fertiliser nitrogen input. A remarkable result in Nyfeler *et al*. (2009) shows that these mixtures at moderate fertiliser nitrogen input (150 kg/ha) can yield as well as the best monoculture at high nitrogen input (450 kg/ha) over 3 years. There are also strong theoretical reasons for predicting that multi-species mixtures have a higher probability of coping with environmental heterogeneity than monocultures, but tests of such predictions have been rare (but see Wacker *et al*., 2008; Isbell *et al*., 2009 and Wittebolle *et al*., 2009 for non-agronomic examples). Culleton, Murphy and O'Keeffe (1986) found no significant differences between the yield of monocultures and that of mixtures of two to three varieties of perennial ryegrass (*Lolium perenne*), but did find greater temporal stability in the yield of mixtures. Stability of output is a key requirement in agronomic systems and the fluctuation in clover content in the sward is regarded as a major difficulty with low input clover-based systems in Ireland. What is not so well established is the extent to which any production or stability benefit of mixtures is closely related to particular levels of clover content in the sward.

Among mechanisms that may combine to produce positive effects of such sward diversity are niche differentiation (van Ruijven and Berendse, 2003), nitrogen transfer from legumes to grasses (e.g. Harper, 1977; Spehn *et al*., 2002; Kahmen *et al*., 2006; Nyfeler *et al*., 2009), facilitation involving lower incidence or severity of pests and diseases in mixtures (Zhu *et al*., 2000) and selection of a particular species that comes to dominate mixtures and which may also give highest monoculture yields.

The work reported herein was carried out in Ireland as part of an EU supported international project under the aegis of EU Concerted Action 852 (Kirwan *et al*., 2007; Helgadóttir *et al*., 2008), and included an evaluation of the effects of four-species mixtures (2 grass and 2 clover species) on herbage production and species persistence under relatively intensive growing conditions across a wide range of environments. Intensively managed grasslands in many European countries have developed towards single species monoculture of high yielding/high value grass species that are managed at high levels of fertiliser nitrogen and stocking rate or with frequent harvesting (Wilkins *et al*., 2002). Interest in mixtures (mainly of grass and clover), even under relatively intensive management levels, has persisted. There remains, however, a need to test systematically the performance of monocultures and mixtures under a range of environmental conditions and management intensities, to test the effect of the relative abundance of the species in a mixture, and, consequently, test which mixture composition produces the largest diversity effects.

Grass-clover swards sown in Ireland are mostly two-species mixtures of perennial ryegrass and white clover (*Trifolium repens*). White clover is included in the seed mix for its ability to fix atmospheric nitrogen and provide high quality herbage, allowing a reduction in fertiliser nitrogen use and increased individual animal performance. It may be beneficial to include other pasture species with different growth and persistence characteristics in an attempt to improve the yield and stability of the sward. Possible options for Irish pastures include cocksfoot (*Dactylis glomerata*: a slow establishing and persistent grass), timothy (*Phleum pratense*: a slow establishing and high quality grass), red clover (*Trifolium pratense*: a fast establishing but non-persistent legume) and Caucasian clover (*Trifolium ambiguum*, also known as kura clover: a slow establishing and persistent legume). Any advantages gained from growing these species in a multi-species mixture are likely to depend on their relative proportions in the seed mix.

An experimental and modelling framework, which has been developed recently (Kirwin *et al.,* 2007), allows separate estimation of the contributions of interactions in species mixtures by experimentally manipulating species relative abundance. Compared to comparisons of monocultures and mixtures in which each species is represented in equal proportions, this methodology can identify when mixture performance is maximal at some other combination of species proportions (e.g., O'Hea, Kirwan and Finn, 2010). This framework was applied in an experiment repeated at three sites in Ireland, using mixtures consisting of four agronomic species (two grasses and two clovers), to address basic questions in agronomic diversity-function research: (i) can positive interactions between important agronomic species result in higher yields from mixtures than expected from the performance of the individual species sown as monocultures; (ii) can species diversity affect resistance to the growth of unsown species; (iii) are diversity benefits stable over time, sites and species' relative abundance?

Materials and Methods

Experimental design, sites and measurements

The experiment was carried out at three sites in Ireland, all attached to Teagasc, the Irish Agriculture and Food Development Authority: Johnstown Castle (JC) (52°18' N, 6°30'W, 53 m above sea level), Athenry (AT) (53°17'N, 8°44'W, 40 m above sea level) and Moorepark (MP) (52°8'N, 8°16'W, 48 m above sea level). At each experimental site cultivars of two grass and two clover species were chosen such that one of the grass and one of the clover species was faster establishing than the other. The grasses used at Johnstown Castle were *Lolium perenne* cv. Lacerta (Lp) and *Dactylis glomerata* cv. Accord (Dg) and the clovers were *Trifolium pratense* cv. Merviot (Tp) and *Trifolium repens* cv. Milo (Tr). For Athenry and Moorepark the grasses were *Lolium perenne* cv. Spelga and *Phleum pratense* cv. Motim (Pp) and the clovers were *Trifolium repens* cv. Avoca and *Trifolium ambiguum* cv. Endura (Ta).

The experimental layout followed a simplex design (Kirwan *et al*., 2007; Cornell, 2002) with four monocultures and 11 mixtures of the four species sown at two levels of overall sowing density (low being 60% of high). The 11 mixtures consisted of four mixtures dominated in turn by each species (sown proportions of 70% of dominant and 10% of each of the other species), six mixtures dominated in turn by pairs of species (40% of each of two species and 10% of the other two) and the centroid mixture (25% of each species). Species proportions at sowing were based on proportions of seed mass considered appropriate for monocultures for each species at the site. There was no replication; estimation was based on regression methods and inference was based on the residual variation around the regression model fitted. At Johnstown Castle an additional 18 plots, comprising monocultures and a selection of the mixtures above, were used to assess total yield.

A common protocol was established for plot management. Plots were hand sown in 2003 at Johnstown Castle (seeding rate: 35, 25, 10 and 15 kg/ha for monocultures of Lp, Dg, Tp and Tr, respectively, at the high density) and in 2005 at Athenry and Moorepark (seeding rate: 24, 22, 21 and 50 kg/ha for monocultures of Lp, Pp, Tr and Ta, respectively, at the high density). The plots were harvested five times per annum at Johnstown Castle for 2004, 2005 and 2006 and seven times at each of the other sites in 2006 and 2007. At sowing, phosphorous (P), potassium (K) and nitrogen were applied at 0, 0 and 30 kg/ha at Johnstown Castle and at 40, 0, 0 kg/ha for the other two sites. Annual fertiliser nitrogen inputs of 150, 75 and 100 kg/ha were applied in equal aliquots of 30, 10.7 and 14.3 kg/ha at Johnstown Castle, Athenry and Moorepark, respectively, before each regrowth. For estimation of total annual aboveground dry matter and botanical composition, including unsown species, each plot was cut to a height of 5 cm at each harvest at Johnstown Castle and to 4 cm at Athenry and Moorepark. Grab samples were separated for botanical composition at harvests 1, 3 and 5 at Johnstown Castle, and at Athenry and Moorepark for all harvests in 2006 and at harvests 1, 3, 5 and 7 in 2007. Subsamples of the cut material were dried at 65 °C to constant weight at Johnstown Castle and at 40 °C for 48 h at the other two sites and the dry matter percentage calculated and applied to the total weight of the cut material to determine the aboveground dry matter. Average botanical composition in the separated samples was used to estimate the overall botanical composition of annual biomass. Plot sizes (and area sampled) were 16 (6), 10 (6.4) and 10 (6.4) m^2 at Johnstown Castle, Athenry and Moorepark, respectively.

Soil types were well- to moderately-drained Brown Earth with a fine loamy texture at Johnstown Castle, Brown Earth with a stony loam to silt loam texture at Athenry and Acid Brown Earth with a sandy loam to loam texture at Moorepark. Mean climate data for each site are given in Table 1.

Trifolium ambiguum requires a specific strain of N-fixing rhizobia (*Rhizobium leguminosarum* biovar *trifolii* strain ICC148) which, unlike the root-nodule bacteria that fix nitrogen for Tp and Tr, is not present in Irish soils. This strain of rhizobium was inoculated onto the Ta seed immediately before sowing.

Data analyses

Total yield (annual dry matter yield), sown yield (annual dry matter yield of sown species), and unsown yield (annual dry matter yield of unsown species) were analysed at each site over three

Table 1. Mean monthly and annual total rainfall (mm) and average monthly and annual air temperature (°C) at Johnstown Castle (JC), Athenry (AT) and Moorepark (MP) over the course of the experiments

Site	Variable	Month												Annual
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
JC[a]	Rainfall	78	48	87	67	78	43	67	69	97	159	78	81	952
	Temp.	6.7	5.7	6.9	8.5	11.0	14.5	15.7	15.6	14.5	11.7	8.5	7.5	10.6
AT[b]	Rainfall	66	47	71	30	86	55	74	99	79	75	79	123	885
	Temp.	5.9	5.4	6.2	9.7	11.0	14.7	16.0	15.0	14.0	11.6	7.9	6.8	10.4
MP[c]	Rainfall	148	116	97	70	84	68	65	90	97	126	109	137	1207
	Temp.	4.8	5.1	6.3	7.9	10.1	12.7	14.9	15	12.9	10.1	7.4	6	9.5

[a] Average of 2004, 2005 and 2006.

[b] Average of 2006 and 2007 at Birr 75 km from Athenry.

[c] Average of 2006 and 2007.

years at Johnstown Castle and two years at Athenry and Moorepark. A modelling approach (Kirwan *et al*., 2007), was extended to analyse effects across years. Transgressive overyielding (mixtures performing better than the best monoculture) was investigated using a non-parametric method detailed in Kirwan *et al*. (2007). The annual percentage contribution of unsown species (100*(Total yield-sown yield)/total yield) was assessed for each mixture and monoculture.

Model of diversity effects in species mixtures

In a mixture the initial expectation is for constituent species to contribute to plot response by scaling their monoculture performance (identity effects) by their relative proportion in the mixture. Additional contributions due to interspecific interactions may arise and these are called the diversity effect (D). The following linear model was used to describe this system;

$$y = \sum_{i=1}^{4} \beta_i P_i + \gamma M + \sum_{\substack{i,j=1\\ i<j}}^{4} \delta_{ij} P_i P_j + \varepsilon$$

y is a response from a mixture (or monoculture), β_i is the identity effect for the i^{th} species (an estimate of its performance in monoculture at average sowing density), P_i is the sown proportion of the i^{th} species, M is the overall sown density of the mixture, scaled to have zero mean and γ measures the effect of density. d_{ij} represents the interaction effect for the combination of species i and j and ε is a random term assumed normally distributed with mean zero and variance σ^2. The average identity effect (ID) in a mixture at density M is a simple function of species sown proportions. $ID = \sum_{i=1}^{4} \beta_i P_i + \gamma M$. Diversity effects are produced by interactions among the species in a mixture. The contribution of pairwise interactions was characterised as $D = \sum_{\substack{i,j=1\\ i<j}}^{4} \delta_{ij} P_i P_j$ where δ_{ij} is the pairwise interaction coefficient between species i and j. The δ_{ij} reflect the potential of the two species to interact and this potential is scaled by the relative abundance of the two species (P_i and P_j) to give the contribution ($\delta_{ij} P_i P_j$) of that interaction to the response. The δ_{ij} may be positive, indicating complementarity, selection or facilitation, or negative, indicating competitive suppression. There may be patterns among the δ_{ij} (Kirwan *et al*., 2007, 2009) relating to various biologically-driven scenarios. Here two such possibilities are considered. All the interaction terms may have the same value ($\delta_{ij} = \delta$ for all i and j), essentially the evenness (E) model in

Kirwan *et al.* (2007). The second model is based on the two functional groups, grasses and legumes. This functional group model assumes that all four pairwise interactions between species from different functional groups (a grass and a legume) have the same strength (δ_{bfg}) and pairwise interactions between species within a functional group (e.g. a grass with a grass) also have the same strength (δ_{wfg}). Of course, interactions could be more complex, and involve more than two species. These models were extended to test whether diversity effects persist over time and include all experimental years in a repeated measures analysis that assumes an unstructured covariance matrix over time (using Proc MIXED of SAS (2005)). Each site was analysed separately and predictions for each year were made from the model fitted in the repeated measures analysis. Models are compared using likelihood ratio tests (Pawitan, 2001).

The net effect of interspecific interactions on yield may be negative, although this is unusual. The net effect of positive interspecific interactions may lead to overyielding, where the performance in mixture exceeds that expected from the average monoculture performance, or even to transgressive overyielding (Trenbath, 1974), where mixture performance exceeds that of the highest yielding monoculture. The non-parametric test of transgressive overyielding (Kirwan *et al*., 2007) relies on the probability of observing the patterns of transgressive overyielding in mean responses in the data under the null hypothesis that it does not occur.

Results

Results for the simple model with average interaction effect

The diversity effect for total yield was positive and significant for each year at each site (Table 2). It was lower in later years than in year 1 at all sites ($P<0.05$, except at Moorepark), but did not decline between the second and third years at Johnstown Castle. At Johnstown Castle, monoculture total yields generally declined across years for all species, generally significantly so in the third year. This pattern was not repeated at Athenry where the total monoculture yield for two grasses and one clover increased or at Moorepark where total yield increased over years for all species, with most of these changes being significant. There was no general pattern to total monoculture yields among species, with total yields for Lp and Tr being neither consistently higher nor lower than other species across sites or times.

For sown yield, the diversity effect was positive and highly significant for each year at each site (Table 2). It was higher in all cases for sown yield than for total yield. The diversity effect increased between years 1 and 2 at all sites, significantly at Johnstown Castle and Moorepark and declined, between years 2 and 3 at Johnstown Castle ($P=0.001$). At Johnstown Castle, the sown yield for monocultures declined across years for all species, generally significantly so, and very greatly for both clover species. At Johnstown Castle sown yields of Lp were lower than for Dg, significantly so in the first two years. At Athenry sown monoculture yields for the two grasses increased, and declined for the two clovers (some significant effects) and at Moorepark sown species yield increased over years for all species, all being significant except for Ta. Sown monoculture yields for the slower establishing clover (Tr at Johnstown Castle and Ta at Athenry and Moorepark) were always lower than for any other species, significantly so in Johnstown Castle compared with both grasses and for Tp in

Table 2. Identity and diversity effects estimated from the evenness model for total dry matter (DM) yield (t/ha), and for the yield of DM from sown species (sown yield; t/ha) for each year at three sites

Item	Site by year						
	Johnstown Castle			Athenry		Moorepark	
	2004	2005	2006	2006	2007	2006	2007
				Total yield[a]			
Species[b]							
Lp	12.5	12.4	8.9	4.7	7.6	5.8	10.7
Dg	15.1	14.6	10.5				
Pp				5.2	8.7	6.7	11.4
Tp	14.5	12.5	8.4				
Tr	11.3	12.6	7.9	10.3	8.4	8.6	10.2
Ta				6.8	10.1	6.3	10.4
s.e.d.[c]	0.63	0.68	0.64	1.41	1.02	0.62	0.40
Diversity effect[d]	4.3***	2.0***	2.4***	3.5***	2.2**	2.7***	2.2***
				Sown yield			
Species							
Lp	10.3	7.6	7.8	4.0	5.0	4.5	7.9
Dg	15.3	13.0	8.8				
Pp				4.7	7.2	5.8	9.9
Tp	10.1	2.5	1.6				
Tr	4.8	2.3	1.0	8.1	5.9	5.7	7.2
Ta				3.9	3.7	2.3	2.8
s.e.d.	1.24	1.93	1.29	1.44	1.19	1.06	1.10
Diversity effect	7.2***	9.2***	6.7***	5.0***	5.4***	4.6***	6.0***

[a] The total number of plots at Johnstown Castle was 48 for total yield and 30 for sown yield.

[b] Species are *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Trifolium pratense* (Tp), *Trifolium repens* (Tr), *Phleum pratense* (Pp) and *Trifolium ambiguum* (Ta). The values in the row are predicted monoculture yields at mean density.

[c] For the predicted monoculture means.

[d] The diversity effect is the additional estimated effect in the evenness model at the centroid, compared to the average monoculture. The evenness model assumes that all pairwise interaction coefficients are equal.

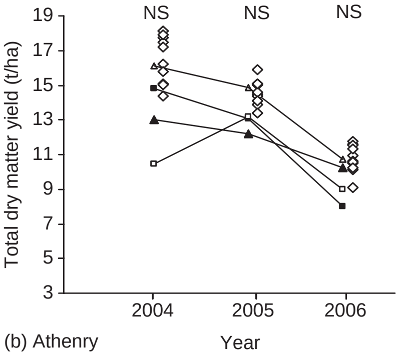
the first year. At Athenry, Ta sown yields were significantly lower than Tr in 2006 and than Pp in 2007, and at Moorepark were generally significantly lower than all other species. At Johnstown Castle sown yield of Tp was lower ($P<0.05$) than either grass species in the second and third year.

The suppressive power of mixtures is exemplified by the plots of yields, sown yields and average percentages of unsown species for each year and across all years at each site for each mixture and each monoculture (Figures 1–4). The suppressive effect was consistent across all 11 mixtures, being approached only by the monoculture Dg (Johnstown Castle) and Pp (Athenry and Moorepark).

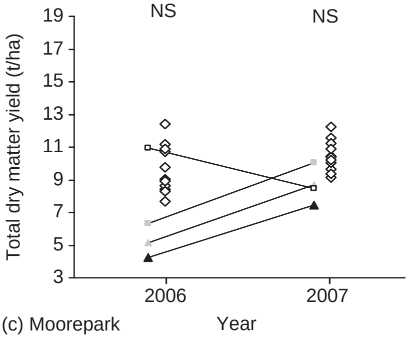
Comparison of models

Three models were compared for total yield and sown yield, using the simple evenness model just discussed and also the functional group and all-pairwise models. The evenness model fit best for total yield at Johnstown Castle and for both yield responses at Athenry, and the separate

(a) Johnstown Castle



(b) Athenry



(c) Moorepark

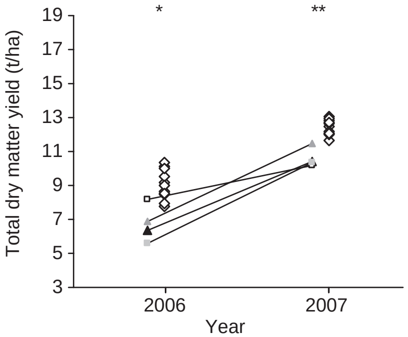
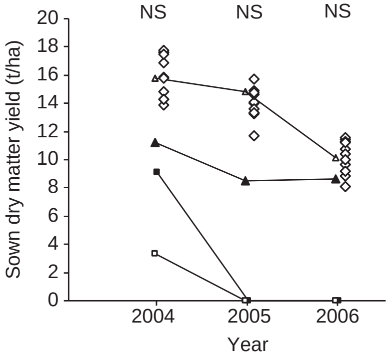
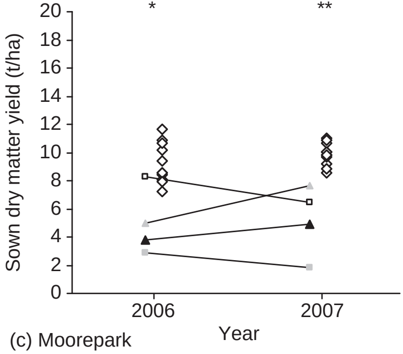


Figure 1. Total annual yield of dry matter (t/ha) for 11 mixtures and four monocultures for each year at Johnstown Castle, Athenry and Moorepark based on averages of raw data. Also indicated is significance of test for transgressive overyielding using the method in Kirwan et al. (2007). For clarity mixture points are offset and monoculture points are joined over years. (Plotting symbols are ◇ Mixtures, ▲ Lp, △ Dg, ▲ Pp, ■ Tp, □ Tr, ■ Ta.)

(a) Johnstown Castle



(b) Athenry



(c) Moorepark

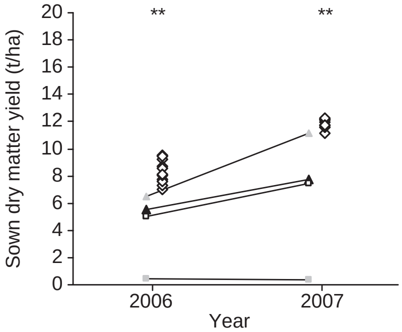


Figure 2. Total annual yield (dry matter) of sown species (t/ha) for 11 mixtures and four monocultures for each year at Johnstown Castle, Athenry and Moorepark based on averages of raw data. Also indicated is significance of the test for transgressive overyielding using the method in Kirwan et al. (2007). For clarity mixture points are offset and monoculture points are joined over years. (Plotting symbols are ◇ Mixtures, ▲ Lp, △ Dg, ▲ Pp, ■ Tp, □ Tr, ■ Ta.)

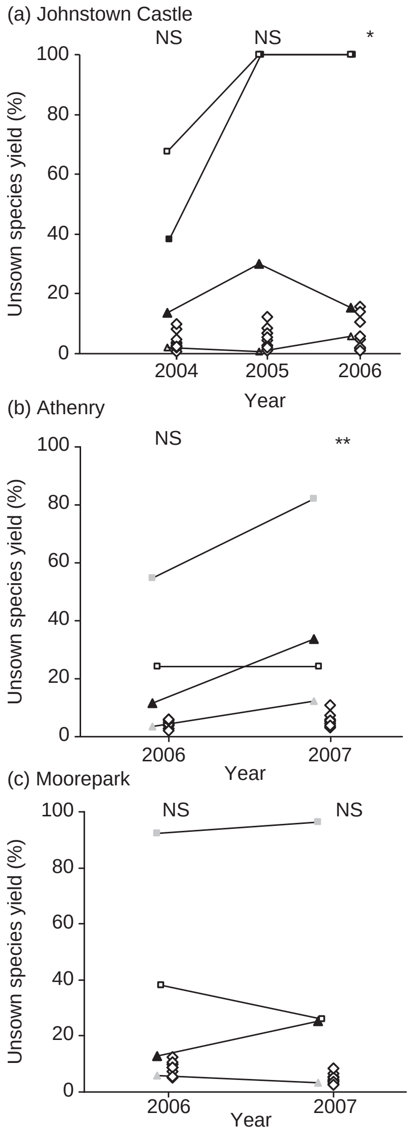


Figure 3. Unsown yield, as percentage of total annual yield, for 11 mixtures and four monocultures for each year at Johnstown Castle, Athenry and Moorepark based on averages of raw data. Also indicated is significance of the test for transgressive underyielding using the method in Kirwan et al. (2007). For clarity mixture points are offset and monoculture points are joined over years. (Plotting symbols are ◊ Mixtures, ▲ Lp, △ Dg, ▲ Pp, ■ Tp, ☐ Tr, ■ Ta.)

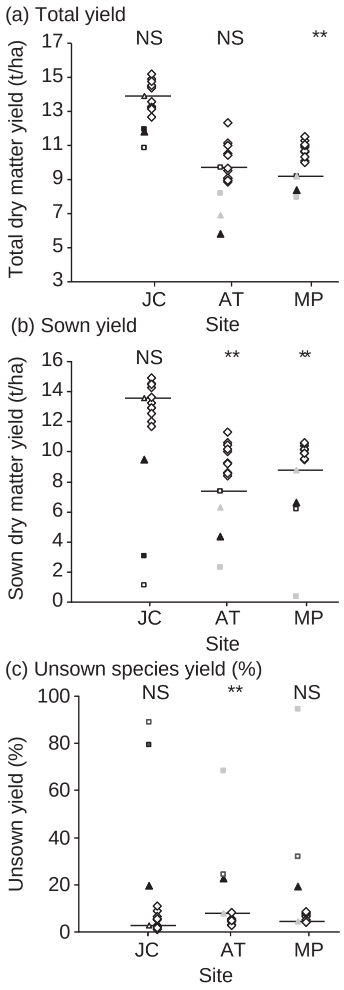


Figure 4. Total annual yield of dry matter (t/ha), sown yield (t/ha) and unsown yield, averaged over all experimental years as a percentage of total yield, averaged over all experimental years for 11 mixtures and four monocultures at Johnstown Castle, Athenry and Moorepark based on averages of raw data. Also indicated is significance of the test of transgressive overyielding/underyielding using the method in Kirwan et al. (2007). Horizontal dashed line indicates the highest average monoculture yield (panels (a) and (b)) and the lowest average unsown yield percentage for monoculture (panel (c)). (Plotting symbols are ◊ Mixtures, ▲ Lp, △ Dg, ▲ Pp, ■ Tp, ☐ Tr, ■ Ta.)

Table 3. Likelihood ratio tests among three models for total yield and sown yield based on repeated measures analysis of annual data at three sites. Shown for each model is –2Log Likelihood (–2LL). Tests of the difference between the values of –2LL for each model and associated P values are provided

Site	Statistic	Model[a]			Model		
		E	FG	AP	E	FG	AP
			Total yield[b]			*Sown yield*	
Johnstown	–2LL[c]	**397.2**	393.1	379.2	307.4	304.1	**275.5**
	Test	E v FG	E v AP	FG v AP	E v FG	E v AP	FG v AP
	DF diff[d]	3	15	12	3	15	12
	Diff	4.1	18.0	13.9	3.3	31.9	28.6
	P value	0.251	0.263	0.307	0.348	0.007	0.005
Athenry	–2LL	**176.3**	170.9	163.8	**229.3**	226.0	220.1
	Test	E v FG	E v AP	FG v AP	E v FG	E v AP	FG v AP
	DF diff	2	10	8	2	10	8
	Diff	5.4	12.5	7.1	3.3	9.2	5.9
	P value	0.067	0.253	0.526	0.192	0.513	0.658
Moorepark	–2LL	109.7	106.3	**82.8**	184.8	184.5	**134.7**
	Test	E v FG	E v AP	FG v AP	E v FG	E v AP	FG v AP
	DF diff	2	10	8	2	10	8
	Diff	3.4	26.9	23.5	0.3	50.1	49.8
	P value	0.183	0.003	0.003	0.861	<0.000	<0.000

[a] E = evenness (same interaction coefficient for all interactions), FG = functional group (pairwise interaction coefficients between species from different functional groups are equal and pairwise coefficients between species within functional groups are equal), AP = all pairwise interactions are unique.

[b] The total number of plots at Johnstown Castle was 48 for total yield and 30 for sown yield.

[c] LL = log likelihood for the model; best model is indicated in bold.

[d] Models all include the interaction of year with the other model terms and comparisons between models (e.g. evenness vs. functional group) test for the additional diversity effects and their interactions with years. Thus, the degrees of freedom for comparing models (DF diff) is the number of interaction effects being tested in comparing the two hierarchical models, e.g. the 3 d.f. for evenness vs. functional group is because the larger model (functional group) has one extra parameter for each of three years.

interaction model for both yield responses at Moorepark and for sown yield at Johnstown Castle (Table 3). Examination of the individual coefficients from the all-pairwise model for the Moorepark responses for each year showed that although coefficients were not equal they were consistent in direction within each year (or not significantly different if of opposite sign; data not presented). In the interests of a clear presentation the simple evenness model (E) is shown in all cases (Table 2), supplemented by plots of averages of the raw data for each mixture and monoculture (Figures 1–4).

Transgressive overyielding

The total yield, sown yield and the unsown yield (as a percentage of total yield computed from the raw data) are shown for each mixture and monoculture at each site for each year (Figures 1–3) and for each site overall (Figure 4). Also shown are the results of the test for transgressive overyielding proposed in Kirwan *et al.* (2007). Several mixtures exceeded the total yield of any monoculture in each site and year and there was significant evidence for transgressive overyielding at Moorepark (Figures 1 and 4). A similar result was obtained for yield of sown species and the evidence for transgressive overyielding was significant at both Athenry and Moorepark (Figures 2 and 4). All mixtures consistently suppressed unsown species across years at all sites (Figure 3) and overall (Figure 4). Significant transgressive overyielding was evident in percentage unsown species yield in year 3 at Johnstown Castle and in the second year at Athenry (Figure 3). There was a very wide range in the suppressive ability of grasses and clover in monoculture. Transgressive overyielding for total and unsown species yields and transgressive underyielding for unsown species proportions, was universally significant for mixtures compared with ryegrass monoculture. Virtually every mixture yielded better, frequently by a considerable amount, and suppressed unsown species more, than Lp in monoculture

Species contribution to sown species yield

The percentage contribution of sown species to total yield, for monocultures and mixtures, calculated from the raw data, showed great variation across systems, years and sites (Table 4). At Johnstown Castle, Dg contribution remained high over time in monoculture and this species

Table 4. Percentage of sown species dry matter in the total yield for each site for each year in monocultures and mixture (based on raw data). For mixtures, the average, maximum and minimum percentages across all mixtures are shown for each species

Site	System	Year	Species[a]					
			Lp	Dg	Pp	Tp	Tr	Ta
Johnstown Castle	Monoculture	2004	86.3	97.8		61.9	32.5	
		2005	70.0	99.5		0.0	0.1	
		2006	84.6	94.3		0.0	0.1	
	Mixture average	2004	26.5	53.3		10.4	6.3	
		2005	5.6	89.3		0.1	0.1	
		2006	4.4	90.0		0.0	0.2	
	Mixture maximum	2004	45.0	88.8		37.4	16.3	
		2005	18.2	98.1		0.3	0.3	
		2006	15.2	98.9		0.2	1.5	
	Mixture minimum	2004	4.9	27.9		2.1	0.8	
		2005	0.7	77.1		0.0	0.0	
		2006	0.1	78.9		0.0	0.0	
Athenry	Monoculture	2006	88.4		96.5		75.6	45.2
		2007	66.3		87.8		75.9	17.7
	Mixture average	2006	21.0		29.2		45.4	0.3
		2007	26.0		43.4		25.0	0.1
	Mixture maximum	2006	38.3		49.5		55.0	1.6
		2007	39.4		59.5		31.2	0.2
	Mixture minimum	2006	8.9		12.9		31.8	0.0
		2007	16.5		21.9		16.7	0.0
Moorepark	Monoculture	2006	87.0		94.0		62.0	7.6
		2007	74.6		96.7		74.1	3.7
	Mixture average	2006	18.9		49.0		23.5	0.2
		2007	23.9		56.5		14.8	0.2
	Mixture maximum	2006	50.7		70.0		40.0	0.6
		2007	41.8		67.8		23.3	1.7
	Mixture minimum	2006	4.9		30.4		8.3	0.0
		2007	13.6		44.8		5.7	0.0

[a] See footnote to Table 2.

dominated mixtures. A similar pattern held for Pp at Athenry and Moorepark but the dominance in mixtures was not so pronounced as for Dg at Johnstown Castle. The contribution of Lp at Johnstown Castle was relatively stable in monoculture but declined greatly in mixture over time. At the other two sites Lp declined somewhat in monoculture and it retained its position in mixture. Tr was virtually absent in the second and third years in monoculture and mixture at Johnstown Castle. It was relatively stable at both Athenry and Moorepark in monoculture and declined somewhat over time in mixture but was present in appreciable quantity in all mixture systems. Tp almost disappeared over time at Johnstown Castle. At both Athenry and Moorepark Ta had a low contribution and declined over time in monoculture and was almost absent in mixture.

Discussion

With respect to the three questions addressed in this study: (i) species diversity had a strong, persistent, positive effect on overall yield and the yield of sown species over a 3-year period; transgressive overyielding effects were sometimes observed but when the effect was not significant, most mixtures generally still performed relatively well when compared with the best monoculture, (ii) species diversity increased the ability of the sward to resist the growth of unsown species and (iii) these effects on total yield declined over time but were still important at the end of the experiments. The effects on the total yield of sown species and on ability to suppress unsown species increased with time. Diversity effects were robust to changes in species composition and this persisted for the duration of the experiments. Virtually every mixture yielded higher and suppressed unsown species better than the monocultures of perennial ryegrass (Lp). These patterns were broadly consistent across sites but may have been due to different mechanisms. The persistence of species varied widely and was not consistent across sites.

The hypothesis that the positive diversity effects observed in natural grasslands would also be observed in mixtures of more productive pasture species has been supported by evidence from all three sites. This confirms results at other sites in the COST Agrodiversity experiment (Kirwan *et al.*, 2007; Helgadóttir *et al.*, 2008; Lüscher *et al.*, 2008; Frankow-Lindberg *et al.*, 2009; Nyfeler *et al.*, 2009). The mixtures at Athenry and Moorepark retained a clover component through the two years of the experiment (minimum Tr across mixtures was 17% at Athenry and 6% at Moorepark) and the mechanisms for the diversity effects were likely dominated by niche differentiation and facilitation. The virtual absence of clover from Johnstown Castle and the dominance of Dg after the first year (minimum of 77% Dg across all mixtures in the second year and 79% in the third) suggest that the dominance of mixtures by the more productive grass species (selection effect) may be a large contributor to the diversity effect at that site.

The existence of strong diversity effects on yield and unsown species suppression that persist over time agrees with the results of other agronomic studies with similar species (Frankow-Lindberg *et al.*, 2009; Nyfeler *et al.*, 2009). The gradual increase in diversity effect observed in experiments on species from natural systems (Cardinale *et al.*, 2007) was not found here where strong diversity effects were observed from year 1. The robustness of the diversity effects across a wide range of mixtures agrees with results from analysis of one year's data from the COST 852 Agrodiversity experiment across 28 sites (Kirwan *et al*., 2007) and studies over several years (Frankow-Lindberg *et al*., 2009; Nyfeler *et al*., 2009). The diversity effects for total and sown species yield seem to be strong, even with quite small relative proportions of some species in the mixture. This is a very important issue for pasture management; the requirement from these results would be to focus on preserving species in the mixture above a low threshold level rather than at tightly prescribed levels that may be very difficult to maintain in a very dynamic system.

The failure of Tr and Tp at Johnstown Castle may be due partly to the high fertility of the soil, but this effect on Tr was not observed at Athenry or Moorepark. The failure of Ta to establish well in any mixture or monoculture at Athenry or Moorepark, despite inoculation with the appropriate rhizobium strain, was consistent with previous studies (e.g. Black, Moot and Lucas, 2006a). The likely reasons for this failure include its very slow rate of seedling development (Black, Moot and Lucas, 2006b). Difficulties inhibiting the establishment and persistence of Ta need to be resolved before it can be of benefit to Irish pastures.

A feature of the results at all three sites was the level of productivity achieved by virtually all mixtures relative to monocultures and their consistent ability to suppress unsown species over several years in spite of changing sown species composition (even though different mechanisms may be occurring, as discussed above). While transgressive overyielding is regarded as rare with natural and semi-natural grasslands (Hooper and Dukes, 2004; Cardinale *et al*., 2007; Lanta and Leps, 2007) it has been reported with higher-yielding swards at low species richness (Jolliffe and Wanjau, 1999; Roscher *et al*., 2005; Kirwan *et al*., 2007; Frankow-Lindberg *et al*., 2009; Nyfeler *et al*., 2009). (There are technical difficulties with assessing transgressive overyielding in widely used methods that can bias against finding evidence of its presence (Schmid *et al*., 2008).) The occurrence of transgressive overyielding at Athenry and Moorepark may be associated with the presence of nitrogen fixing clovers in the sward, even at quite low levels. The superiority of virtually all mixtures to Lp monoculture in yield and unsown species suppression at all sites and years at the levels of fertiliser nitrogen application used is a signal feature of these experiments that is of great practical relevance in Irish conditions.

The reduced burden of unsown species in mixtures and the persistence of this effect over time, observed here, generally matches the findings in studies with similar species (Kirwan *et al*., 2007; Frankow-Lindberg *et al*., 2009; Nyfeler *et al*., 2009) and has been discussed in some detail in Frankow-Linberg *et al*. (2009). In the experiments reported here, only monocultures of Dg at Johnstown Castle and Pp at Moorepark achieved the same level of suppression of unsown species as virtually all mixtures, and otherwise the invasion of monoculture species was very marked. Monocultures of Tr and Tp at Johnstown Castle and Ta at Moorepark were almost completely overwhelmed by unsown species even in the first harvest year. The suppression of unsown species by mixtures relative to monocultures is the reason for the very high levels of the diversity effect for sown species, since the dry matter yield of sown species in monocultures is reduced due to strong incidence of unsown species for three out of the four species. This is also likely to enhance the quality of forage from mixtures as it is less diluted by low quality unsown species.

The best diversity model varied across the model forms proposed by Kirwan *et al.* (2009), varying between site and across response variables (Table 3). At Moorepark, where a separate pairwise interaction effect was suggested for each response the only consistency in the interaction coefficients tended to be that of sign, the coefficients for yield being generally positive. These data do not provide evidence for the patterns in interaction effects within and between functional groups observed by others (van Ruijven and Berendse, 2003; Hector *et al.*, 2007; Frankow-Lindberg *et al.*, 2009).

The persistent and increasing (Athenry and Moorepark) diversity effects on total and sown yield across time and the relatively high performance of all mixtures relative to monocultures in these systems managed at relatively low nitrogen levels, supports the third hypothesis that diversity benefits show stability over time, site and species' relative abundance and have important practical implications. In an experiment with similar grass-clover mixtures Nyfeler *et al*. (2009) show that the diversity effect at relatively low nitrogen levels was sufficient for mixtures to yield as well as the best monoculture species at nitrogen inputs of 450 kg/ha. Given the desirability of reducing the cost of fertiliser nitrogen inputs, the practical benefit of this diversity effect is clear and should be explored further under grazing as well as cutting conditions. The best system to use under grazing or cutting will depend on several factors and not just on the efficiency of nitrogen fixation. For example, mixtures give higher yields than monoculture clovers and also have advantages in terms of suppression of unsown species.

Hector and Bagchi (2007) have argued that consideration of several ecosystem services may be required to assess the importance of diversity; the presence of different sets of species may be necessary for the delivery of different services. This is a strong argument for the use of mixtures in the development of agriculture. This can place demands on research, multiple ecosystem processes may need to be considered in parallel; this could include such factors as yield, decomposition, nutrient losses to the environment, pollination services and resistance to unsown species. Assessing the full contribution of plant diversity may also require detailed study of its impact on the abundance of organisms at a range of trophic levels and their relationship with decomposition, nutrient leaching and availability.

The results here on the large contribution of diversity appear to contradict conventional understanding about agricultural swards subjected to intensive management and high fertiliser levels, in which one species is often recommended on the basis that it outperforms mixtures. Why is this so? During the past decades such experiments were done at very high nitrogen levels where the diversity effects were eliminated over time as is seen in Nyfeler *et al*. (2009) at a fertiliser nitrogen level of 450 kg/ha, but the comparison with the most productive mixed swards at lower nitrogen levels shows that mixtures at lower nitrogen inputs can perform as well a monocultures at high levels of fertiliser nitrogen. Comparisons were often not made under a design in which the comparisons just mentioned could be made. The stability of the clover fraction in mixtures has always been problematic but the current work and Kirwan *et al*. (2007) show that the diversity effect is robust over quite a wide range of clover inclusion in swards and so stability of content may not be as critical as thought. Also, comparisons made at realised rather than sown levels of species can be misleading, ignoring carry-over effects of mixtures even after the relative abundance of species has changed greatly, or, as at Johnstown Castle, where the mixture effect still persisted although the clovers had disappeared from the swards. Perhaps also highly controlled experiments lead to conditions in which light becomes the limiting resource for plant growth. Competition for light is a positive feedback process, eventually favouring the dominance of species that convert light energy into above-ground biomass most efficiently (Schulte, Lantinga and Struik, 2003). This may explain/favour the recommendation of perennial ryegrass monocultures or simple 2-species mixtures of perennial ryegrass with white clover in many intensive pasture systems.

Looking to the future, it seems clear that species mixtures can deliver benefits to forage yield that are of significant magnitude, temporally persistent and spatially consistent at a geographical scale. An emerging challenge that faces both agronomists and ecologists is: what are the specific biological mechanisms that underpin these benefits of diversity? There are several well-known mechanisms by which diversity effects may be achieved. These include variations among different species in root and canopy architecture (e.g. Black *et al*., 2009) that allow improved capture of available resources by more species-rich communities (complementarity), as well as inter-specific interactions that result in reduced incidence and transmission of pests and diseases in mixtures (facilitation e.g. Zhu *et al*., 2000). In general, mixtures would also be expected to cope better with environmental heterogeneity (Sanderson *et al*., 2004; Wacker *et al*., 2008; Wittebolle *et al*., 2009; Hector *et al*., in press). This could range from small-scale heterogeneity in nutrient availability and soil conditions, to the ability to recover from severe weather or management conditions. Finally, there is the question as to how the grazing animal will affect these processes and the extent to which the benefits from mixing can also be recovered under large animal grazing conditions. Investigating and resolving the degree to which such mechanisms contribute to diversity effects promises to be an exciting challenge!

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