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Using legume-based mixtures to enhance the nitrogen use efficiency and economic viability of cropping systems

by

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1. ABSTRACT

As costs for mineral fertilisers rise, legume-based leys are recognised as a potential alternative nitrogen source for crops. Here we demonstrate that including species-rich legume-based leys in rotations helps to maximise synergies between agricultural productivity and other ecosystem services. By using functionally diverse plant species mixtures, these services can be optimised and fine-tuned to regional and farm-specific needs. Replicated field experiments were conducted over three years at multiple locations, testing the performance of 12 legume species and 4 grass species sown in monocultures, as well as in a mixture of 10 of the legumes and all 4 grasses (called the All Species Mix, ASM). In addition, we compared this complex mixture to farmer-chosen ley mixtures on 34 sites across the UK.

The trials showed that there is a large degree of functional complementarity among the legume species. No single species scored high on all evaluation criteria. In particular, the currently most frequently used species, white clover, is outscored by other legume species on a number of parameters such as early development and resistance to decomposition. Further complementarity emerged from the different responses of legume species to environmental variables, with soil pH and grazing or cutting regime being among the more important factors. For example, while large birdsfoot trefoil showed better performance on more acidic soils, the opposite was true for sainfoin, lucerne and black medic. In comparison with the monocultures, the ASM showed increased ground cover, increased above-ground biomass and reduced weed biomass. Benefits of mixing species with regard to productivity increased over time. In addition, the stability of biomass production across sites was greater in the ASM than in the legume monocultures. Within the on-farm trials, we further found that on soils low in organic matter the biomass advantage of the ASM over the Control ley was more marked than on the soils with higher organic matter content. Ecological modelling revealed that the three best multifunctional mixtures all contained black medic, lucerne and red clover.

Within the long term New Farming Systems (NFS) rotational study, the use of a clover bi-crop showed improvement to soil characteristics compared to current practice (e.g. bulk density and water infiltration rate). Improvements in wheat yield were also noted with respect to the inclusion of a clover bi-crop in 2010, but there was evidence of a decline in response as the N dose was increased. Cumulatively, over both the wheat crop and the spring oilseed rape crop, the clover bi-crop improved margin over N. The highest average yield response (≈9%) was associated with the ASM legume species mix cover cropping approach.

2. SUMMARY

2.1. Introduction

The demands for increasing food productivity from farmland are often seen to be in conflict with non-farmed habitats (Phalan *et al*., 2011; Godfray, 2012, Tscharntke *et al.,* 2012; Gabriel *et al.,* 2013). At the same time, a similar conflict is seen to arise on farmed land itself, where crop productivity may not always easily be reconciled with the provision of other ecosystem services such as biodiversity conservation, flood amelioration, carbon storage or cultural benefits. In a three year study we asked if it is possible to design a part of the agricultural ecosystem for simultaneous optimisation of productivity and other ecosystem services such as biodiversity conservation.

Our approach was to use functionally diverse species-rich leys with nitrogen fixing legumes in the rotation. Nitrogen is a key nutrient to achieve acceptable yields and crop quality (Smil, 2011). Due to globally rising costs of mineral nitrogen fertiliser and concerns over the negative environmental impact of anthropogenic nitrogen (Canfield *et al*., 2010), agricultural policy makers, farmers and scientists are increasingly paying attention to the use of leguminous plants as an alternative source of nitrogen (Crews & Peoples, 2004). Legumes, through their symbiosis with rhizobacteria are able to fix aerial nitrogen and provide it in a form that is readily available to plants (Long, 1989).

After growing legumes in a rotation, nitrogen accumulated in the plants' above-ground and below-ground residues is broken down by microbial activity and released for the take up by the following crop. This use of legumes for fertility-building in the rotation is adopted in farming systems where the use of mineral nitrogen fertiliser is either too expensive, or, as in organic agriculture, not permitted (Watson *et al*., 2002). Because of its function as the main nutrient provider, this stage in the rotation is of central importance for organic (and also increasingly non-organic) farming systems. Furthermore, legume-based leys provide additional benefits for soil management through soil cover and reduced soil disturbance.

Depending on climate and soil and the suitability of the land for arable production, the ley phase on organic farms can vary in duration from short term (12-18 months) to long term (around 5 years), but typically the ley is kept for about 18 months to 3 years. In Europe, organic farmers most frequently use grass-clover mixes for their leys, with white clover *(Trifolium repens*), red clover (*T. pratense*) being popular legume species and perennial ryegrass (*Lolium perenne*) and Italian ryegrass (*L. multiflorum*) as commonly chosen grass species. Thus, currently used leys are relatively species-poor. The potential for nitrogen fixation by these leys is high, but the establishment of simple grass-clover leys is sometimes sub-optimal as a result of the cool, moist conditions required by white and red clover species.

In addition, the current practice of using red or white clover or other easily decomposable legumes in fertility building leys can lead to an asynchrony between the release of nutrients following incorporation of the green manure and the demands of the following crop (Crews & Peoples, 2005; Cook *et al*., 2010; Dabney *et al*., 2010; Campiglia *et al*., 2011). One way to improve the efficiency of the rotational system is to combine several legume species in a mixture, including a number of slower growing species, that would result in a more complex residue structure with a better nutrient release profile.

In a three year study across multiple locations in the UK, we studied if ley performance and the provision of multiple ecosystem services can be simultaneously improved by exploiting the functional diversity of various legume species. In particular, we studied productivity in the ley and in the following crop, weed suppression, decomposition and nitrogen losses, as well as pollination and the provision of invertebrate resources. We further adapted an electronic rotation planning tool for farmers to incorporate more complex species mixtures. Finally, we tested the agronomic effects of the diverse species cover crop mixture in a large conventional rotation experiment, measuring the yield response of various cash crops and economic performance of the rotation.

2.2. Materials and methods

The project was organised in 6 work packages. In replicated trials (WP1), various legume and grass species were tested in monocultures and in an All Species Mixture (ASM), containing 10 legume species and 4 grass species. Eco-physiological modelling (WP2), building on data from the replicated field trials, established which legume species would grow best together in simplified legume-based mixtures (LBM). In on-farm participatory trials (WP3) the ASM was grown on 34 sites across the UK and was compared to a farmer-chosen control ley. Further modelling, using the DNDC model, investigated nitrogen dynamics for selected legume and grass species (WP4). WP5 adapted an existing decision support model for rotational planning to assess the effect of legume-based mixtures on soil fertility. In WP6, the ASM was tested in a large-scale rotation field trial under conventional management.

2.2.1. Measuring performance of species in monocultures and mixtures

We set up replicated field trials at six sites across the UK, evaluating various legume and grass species in monocultures and in an All Species Mixture (ASM) (Tables S1 and S2). In these experiments we trialled 18 treatments. Twelve legume species and four grass species were each grown singly as monocultures; in addition, two treatments were reserved for the All Species Mixture, which was grown with and without *Rhizobium* inoculation. At all six trial locations, the experiments were sown in spring 2009 (Table S1). All trials were laid out as one-factorial randomised complete block designs with three replications. Seed lots of the four clover species, the vetch, the lucerne and one of the All Species Mix treatments were inoculated with rhizobial preparations before sowing, with 1% substrate per seed weight. The locations, plot sizes and sowing dates are listed in Table S2. Trial sites were distributed over a large geographical area within the UK.

Table S1. Legume and grass species included in the trials: Scientific and common name; inoculation and seed rate (in kg/ha) in the monoculture plots (Monoc.) and in the All Species Mix (ASM).

				Seed rate (kg/ha)	
Abbreviation	Scientific name	Common name	Inoc.*	Monoc.	ASM
AC	*Trifolium hybridum*	Alsike clover	C	10	1.25
BT	*Lotus corniculatus*	Birdsfoot trefoil	-	12	2.5
BM	*Medicago lupulina*	Black medic	L	15	2.5
CC	*Trifolium incarnatum*	Crimson clover	-	18	2.25
IR	*Lolium multiflorum*	Italian ryegrass	-	33	1
LT	*Lotus pedunculatus*	Large birdsfoot trefoil	-	12	2.5
LU	*Medicago sativa*	Lucerne	L	20	2.5
MF	*Festuca pratensis*	Meadow fescue	-	25	1.25
MP	*Lathyrus pratensis*	Meadow pea	V	75	3.25
PR	*Lolium perenne*	Perennial ryegrass	-	33	2.5
RC	*Trifolium pratense*	Red clover	C	18	2.5
SF	*Onobrychis viciifolia*	Sainfoin	-	80	5
TY	*Phleum pratense*	Timothy	-	10	0.5
WC	*Trifolium repens*	White clover	C	10	1.5
SC	*Melilotus alba*	White sweet clover	L	18	-
WV	*Vicia sativa*	Winter vetch	V	100	-

* Inoculated with clover inoculant (C), lucerne inoculant (L) or vetch inoculant (V).

Table S2. Details of replicated trials: locations, plot sizes and sowing dates (2009)

Site	Barrington	Duchy	IBERS	Rothamsted	SAC	Wakelyns
North coordinate	51°49'52''	50°13'38''	52°25'48''	51°48'38''	57°11'06''	52°21'37''
West coordinate	1°40'12''	5°18'23''	4°01'22.1''	0°22'02''	2°12'45''	-1°21'09''
Altitude (m)	150	42	29	114	109	51
Plot width (m)	1.5	1.5	2	2	1.5	1.2
Plot length (m)	10	5	8	5	12	10
Sowing date	20 Apr	24 Apr	23 Apr	15 Apr	13 May	29 Apr

At all sites a number of eco-physiological and agronomic traits was measured several times, including seedling emergence and establishment seedling biomass, plant height, biomass, crop cover, weed cover, above-ground crop biomass, weed biomass, flowering time, C:N ratio, root biomass, lignin and polyphenol content of the above-ground residues. In addition, at one site (Rothamsted Research), the number of pollinators and phytophagous insects supported by the different species was also recorded. To assess the after-effects of the legume species in the rotation, i.e. after incorporation of the ley plots into the soil, mineral nitrogen was measured in soil samples pre- and post-incorporation of the legumes and grasses. In addition, a cereal was grown at two sites for yield assessment (winter wheat, sown in autumn 2010 at Rothamsted and spring wheat, sown in spring 2011 at Wakelyns). Yield of the following cereals was quantified in 1 m^2 quadrats in each plot.

2.2.2. Modelling competition and functionality of species mixtures

A simulation model of inter-plant competition was used to predict the performance of the legumes when grown in different species mixtures. The model used daily weather data to calculate the radiation intercepted by each species in the canopy and temperature dependent photosynthesis and growth rates. The model was validated using external datasets and was used to predict the biomass of possible legume mixtures as well as the biomass proportions of their component species. Using regression analyses, plant traits were related to various functions (Violle *et al.*, 2007); these were weed suppression, yield of the following crop, regrowth, soil fertility and biodiversity value.

The competition model was then used to predict the community weighted mean of different plant traits for various mixtures; this aggregated trait of the mixture is the weighted average of the traits of individual mixture components. The weights are generated by the competition model (see above) by calculating the different species' proportions in the mix. Community weighted means of the traits can then be used to predict the function values of any mixture. Finally, an aggregated function of agronomic productivity was calculated as the harmonic mean of the values for weed suppression, yield of the following crop, regrowth and soil fertility.

2.2.3. Assessing a complex species mixture in on-farm trials

To determine the effect of increased plant diversity under a range of environmental and management conditions, and to measure individual species performance when growing in a complex ley mixture, the All Species Mixture (ASM) was assessed on 32 organic and 2 non-organic farms across the UK. Seed of the ASM was provided for a 0.5 ha strip which was sown next to or within a control ley. The species composition of the control ley was chosen by each farmer individually. Per farm, management for the ASM and the control ley were identical. Once per year (in 2009, 2010 and 2011) the ASM and control ley were assessed for the presence and cover of individual sown and weed species on each farm. At the last assessment in 2011, biomass samples were taken from both the ASM and the control ley. Soil samples from the farms were taken at the beginning and at the end of the trialling period, i.e. in 2009 and 2011.

2.2.4. Modelling nitrogen dynamics

Measurements of key soil and plant-based properties for the Craibstone site at SAC in Scotland were used for validation of UK-DNDC (DeNitrification-DeComposition) for nitrous oxide emissions and N-leaching losses for selected individual species. The UK-DNDC is a process-based dynamic model comprising five interacting sub-models describing thermal-hydraulic conditions, aerobic decomposition, denitrification, fermentation and plant growth processes. The latter also contains sub-routines to account for agronomic practices such as crop rotation, tillage, fertiliser addition and biomass cutting. In conjunction with locality-specific measured weather data, carbon and nitrogen biogeochemistry in agricultural systems was modelled for daily time steps.

2.2.5. Adapting a rotation planning tool to include species mixtures

The FBC model (Fertility Building Crops (Model), see Cuttle (2006)) is a simple empirical model designed for use by organic growers to help design cropping rotations to make efficient use of N to maximise crop production and minimise losses. In this project, a new version of the model, FBC-LINK, was developed to describe N release following cultivation of the LBMs developed, including changes to simulate the effects of high lignin and polyphenol content species included as a means of manipulating N dynamics. The FBC-Link version of the FBC model includes the following mixtures: (1) Mix A (RC+WC+LU+BM); (2) Mix A + PR; (3) Mix B (WC+LU+BM+SF); (4) WC + PR (see table S1 for abbreviations).

The method of estimating N accumulation by different leys in the previous model was unsuitable for the more complex LBMs and was replaced by a calculation of N accumulation for the specific combination of ley type, management, yield and species composition described by the user. The calculations involved an initial estimate of biomass production and N, lignin and polyphenol contents and an estimate of the proportion returned to the soil in residues. The amount of N accumulated in ley residues was calculated as the difference between the initial N input and the amount mineralised between the residue input dates and end of the ley. A further change was to modify the algorithm used to determine N mineralisation from ley residues to take account of their lignin and polyphenol contents. The revised algorithm was used to estimate N mineralisation from ley residues but the methods of calculating N release from arable crop residues and of modelling N uptake and losses during the subsequent arable phase were not changed in this project.

2.2.6. Testing cover crop mixtures in conventional rotations

Research was undertaken by NIAB TAG (Morley, Norfolk) within the long term New Farming Systems (NFS) study (supported by The Morley Agricultural Foundation and The JC Mann Trust). The NFS study facilitated the comparison of the ASM against standard practice and other cover crop approaches (specifically a brassica cover crop and a clover bi-crop) at a range of nitrogen (N) doses. Cover crops were sown in late August or early September and destroyed and incorporated in the spring. The legume bi-crop was a small leaf white clover (sown August 2007 then allowed to naturally regenerate each season). All rotations grow wheat every second year, the year between is a break crop. The study was fully replicated (4 replicates) and used large plots (to facilitate the use of typical farm practice). The distinct plot areas remained in position throughout the project. While drilling dates and inputs varied with season, activities were in keeping with local best practice appropriate for the prevailing conditions. The soil series was Ashley, soil texture was sandy loam and shallow non-inversion establishment systems were used throughout.

2.3. Results

2.3.1. Characterisation of legume species

Summarising the results from WP1, Table S3 gives an overview of the performance of different legume species tested in the field trials across various performance criteria. Our approach was to condense all measured variables into seven distinct functions: (1) early development, (2) general productivity, (3) weed suppression, (4) flowering, (5) value as a pre-crop, (6) resistance to decomposition and (7) performance of the following crop. As shown in Table S3, WC, RC, BM, BT and LU are generally among the best species with regard to most criteria. If all seven functions are weighted equally, these five species score very similar average ranks (between 7.9 in WC and 9.4 in RC). MP and WV are consistently among the worst species (with average ranks below 3), while AC, SF and SC are in the middle range.

Table S3. Ranks of species performance (WP1); high ranks show high performance; empty cells indicate lack of sufficient data.

Criterion	AC	BM	BT	CC	LT	LU	MP	RC	SC	SF	WC	WV
Early development	5	6	2	12	1	11	8	10	9	7	3	4
Productivity	7	9	8	6	1	10	3	12	4	5	11	2
Weed suppression	7	11	8	3	5	9	1	12	4	6	10	2
Flowering	6	11	8	8	2	2	1	6	2	2	8	1
Pre-crop value	5	8	10	3	7	12	2	11	4	6	9	1
Resistance to decomposition	5	4	9	1	10	3		7	6	8	2	
Perform. following crop	7	9	10	2	4	11	1	8	3	6	12	5
Average rank	6.0	8.3	7.9	5.0	4.3	8.3	2.7	9.4	4.6	5.7	7.9	2.5

As is also evident from Table S3, there is a large degree of functional complementarity among the species. This means that no single species scored high on all criteria and that the currently most frequently used species, WC, is outscored on a number of parameters (in particular, early development and resistance to decomposition) by other legume species. Further complementarity emerged from the different responses of legume species to environmental variables, with soil pH and grazing or cutting regime being among the more important factors. For example, while LT showed better performance on more acidic soils, the opposite was true for SF, LU and BM.

2.3.2. Benefits of mixing species

By comparing a complex species mixture (the ASM) with the average of its component species when grown in separate monocultures, we were able to establish the effects resulting from the interactions among the different species in the mixture. In comparison with the monocultures, the mixture showed increased ground cover, increased above-ground biomass and reduced weed biomass. Benefits of mixing species with regard to productivity increased over time. In addition, the stability of biomass production across sites was greater in the ASM than in the legume monocultures.

Within the on-farm trials, we further found that on soils low in organic matter the biomass advantage of the ASM over the Control ley was more marked than on the soils with higher organic matter content.

2.3.3. Output of ecological modelling

A number of significant relationships between plant traits and different agronomic and ecological functions were quantified using regression analysis: numbers of phytophagous insects and rate of re-growth were related to specific leaf area (leaf area / leaf weight), abundance of pollinators to flowering traits and weed suppression to height and seed size. Yield of the following cereal was significantly related to biomass of the previous legume species.

These relationships were used to predict the agronomic function and biodiversity function of the individual legume and grass species and of different mixtures. Analyses showed that mixes containing both LU and WC score highly regarding the agronomic productivity function. For multifunctional mixtures, i.e. those fulfilling additional functions besides agronomic productivity, the overall performance was generally improved by including a third or fourth legume species.

The three best multifunctional mixtures all contained BM, LU and RC. The additional inclusion of CC in such a legume-based mix will then increase the value for pollinators because of its early flowering time. Importantly, the growth pattern of CC as an annual species is complementary to LU, BM and RC so that a mix of these species increases resource use efficiency and reduces interspecific competition.

2.3.4. Modelled nitrogen dynamics

Predictions of annual nitrous oxide emissions from soil supporting the leguminous species during the ley phase were approximately three-times greater than those associated with the grass species. Similarly, the annual amount of nitrogen lost through leaching was at least 4 times greater for legume plots than the grasses. Furthermore, the amount of nitrogen lost through leaching showed a greater dependency on legume species with losses from WC and RC being 2 – 3 times greater than those from either BM or LU.

Nitrous oxide emissions in the calendar year after the ley phase (i.e. the spring barley year) were very similar irrespective of the ley phase species grown. However, in agreement with predictions for the ley phase the leaching losses showed some relationship to the choice of ley phase species with greater loss occurring after leguminous species than after the grasses.

2.3.5. Decision support model for rotation planning

The method of estimating the amount of N accumulated by the leys was able to take account of the type of ley, its duration, management (cutting, grazing, mulching), growth category (vigorous, moderate, poor) and variations in species composition.

The modified mineralisation algorithm incorporating the effects of lignin and polyphenols, described changes in mineralisation rates in line with the limited information available from the literature. Increasing concentrations of lignin or polyphenol in residues progressively reduced calculated rates of N mineralisation or at high concentrations resulted in immobilisation of mineral N.

The modelled effects of different lengths of ley, types of management and yield on N accumulation and N release were generally in line with expectations. However, in the absence of suitable data for calibrating the model, the more specific predictions of the relative performance of the LBMs cannot be taken as a reliable indication of how the mixtures would perform in reality. The model suggested that both mixtures A and B would accumulate more N than the simple grass/clover ley. Replacing the RC in Mix A with SF (Mix B) appeared to have little effect on the modelled N accumulation and N release. Including ryegrass with Mix A reduced the estimate of N accumulation by the ley, with a similar reduction in the amount mineralised after cultivation. For all leys, the amounts of N accumulated during the ley phase appeared to have a greater influence on the predicted N release to following crops than did the post-cultivation mineralisation rate.

2.3.6. Legume cover crops in conventional rotations

The use of a clover bi-crop showed improvement to soil characteristics compared to current practice. Assessment of bulk density in year 3 (2010 – winter wheat) indicated reductions from

1.17 g/cm^3 to around 1.04 g/cm^3 at depths of 20cm, which was associated with greater water infiltration rates (from 0.50 mm/minute to 1.17 mm/minute). Improvements in wheat yield were also noted with respect to the inclusion of a clover bi-crop in 2010. Within the yield responses there was evidence of a decline in response as the N dose was increased. The average yield response over all N doses from a clover bi-crop was ≈8%.

Considering the wider rotational response, averaged over a break crop and winter wheat cycle, findings follow a similar pattern to that seen in the wheat crop in 2010. Cumulatively, over both the wheat crop and the spring oilseed rape crop, the clover bi-crop improved margin over N, although again the response varied in relation to N dose. Over this cycle, yield responses above current standard practice were apparent for the brassica cover crop and ASM legume mix cover crop. The highest average yield response (≈9%) was associated with the ASM legume species mix cover cropping approach. Incremental N doses appeared to have less of an influence on the yield responses for the ASM legume species mix and the fodder radish cover crop approaches compared to that observed with the clover bi-crop.

2.4. Conclusions and implications

The **characterisation of legume species** conducted in this study has generated new knowledge on the specific responses of legumes to environmental and management conditions, and the relative merits of various species for the use in a range of farming systems. It has shown that the range of currently used species is relatively narrow and often restricted to just two species (WC and RC); several other species such as BM, BT, CC, LU and SF show great potential to increase the productivity and provision of ecosystem services across the UK. For unlocking this potential however, it is necessary to invest in breeding programmes, to conduct further agronomic research into optimised management and to intensify knowledge transfer with regard to best practice.

In addition, the study showed that **mixing different legume** species in the ley has several advantages: it suppresses both early and late weeds; it extends forage availability for key pollinator species; and it increases stability of performance. In addition, mixing species may help to increase the reliability of establishing the ley; further, by producing residues with different residue profiles (carbon to nitrogen ratios and polyphenol and lignin contents) mixtures also provide opportunities for modifying decomposition rates to improve the synchrony between nitrogen release following incorporation and crop nitrogen demand. Finally, widening the range of legume species increases opportunities to build short term leys into rotations on conventional farms.

The **ecological simulation model** used in this study has shown the potential to generate a range of optimal mixtures that deliver multiple functions. This includes new varieties of legumes, or even new species; if their plant traits are measured, their functions can be determined and their performance in potential mixes can be predicted. Therefore, the combination of the simulation model and relationships between plant traits and ecosystem functions provides a powerful tool for exploring the performance of a large number of mixtures in terms of the delivery of multiple services. A number of observations from the **rotational planning model** (FBC-Link model) may be relevant to the development and use of LBMs and manipulation of N release. However, the primary requirement for completing the FBC-Link model would be the provision of the data needed to parameterise the model, most of which would be available from the field trials and laboratory mineralisation studies required for any further development of these legume-based mixtures.

With regard to the inclusion of leguminous plants into **conventional rotations**, it seems probable that the yield responses in the wheat crop arising from the clover bi-crop may have been partly associated with clover N fixation and partly with soil conditioning (possibly linked to changes to clover root structure over time). However, the variation observed in soil and yield responses between cover cropping approaches suggests the need for more research to improve understanding and consistency of performance. The cumulative margins over standard practice across the wheat and spring oilseed rape crops for the cover cropping approaches averaged around £84/ha for the clover bi-crop and the legume mix cover crop and around £69/ha for the brassica cover crop. However, margin over N responses in relation to the clover bi-crop varied markedly in relation to N dose, with greater margin over N apparent at lower N doses. The costs for establishing and managing a cover crop would be in keeping with these figures. While improvements to techniques and species selection should reduce the costs of delivering a cover crop, it is likely that cover crops would only cover their cost based on these margins. It should also be remembered that benefits may accrue as the systems mature and changes to input costs and environmental schemes will also influence financial returns. Hence, while these positive benefits are encouraging, they should be treated with some caution and further development of the systems and approaches would seem prudent. This reinforces the importance and value of long term farming system research.

3. TECHNICAL DETAIL

3.1. Introduction

3.1.1. Background

Increased profitability across the arable sector requires systems with improved nitrogen (N) use efficiency and reduced adverse environmental impacts, which are also more resilient to climatic fluctuations. The cost of bagged mineral N fertiliser is rising (HGCA, 2007) primarily due to rising oil costs. Diffuse pollution from agricultural land accounted for 70% of nitrate (NO_3) in fresh water in England (Defra, 2002) prior to the introduction of Nitrate Vulnerable Zones in 2002 and still poses a significant risk (Nicholson *et al*., 2011; Howden *et al*. 2013). Minimising NO_3 leaching is a requirement of the Water Framework Directive and the Nitrates Directive, and under the Kyoto agreement the UK has undertaken to reduce greenhouse gas emissions (including nitrous oxide (N_2O)). To satisfy these constraints and commitments, there is a pressing need to develop agricultural systems that rely less on inputs of mineral N while simultaneously reducing N losses to the environment. A successful system will be adaptable enough to operate within the context of future global climate change, which is likely to result in greater climatic fluctuations across the country.

Many successful low-input and organic systems rely on a legume-based fertility-building ley phase as an alternative to mineral N applications, and there is increasing interest in applying these systems more widely. Incorporation into soil (ploughing-in) is the first stage in making the accumulated fertility within the residues of the legume(s) and grasses available to the following crop. However, significant N losses occur post-incorporation (Francis *et al*., 1994) as a result of the asynchrony between the flush of N released and the N demands of the subsequent crop. The rate, timing and quantity of N release from the plant residues are partly driven by the residue composition (Cadisch *et al*., 1998). As this depends on the species present in the ley phase, there is limited potential to manipulate the residue composition of the commonly used rye grass/white clover mixtures. However, exploiting the variation in residue composition by using a complex mixture containing a large and diverse range of species offers greater opportunities to influence residue inputs, potentially reducing N losses and increasing productivity of the following crop. In addition, species-rich mixtures may have increased quantity and stability of biomass production, reduced N losses as well as other important benefits (Scherer-Lorenzen *et al*., 2003; Tilman *et al*., 2006).

3.1.2. Aims and objectives

This project explored how recently developed ecological theories of the efficiency and stability of resource use in natural plant communities can be applied to improve N use within UK arable farming systems. It aimed to create a legume based mixture (**LBM**) that can have significantly improved resilience in fertility building and N release dynamics compared to the traditional grass/clover mixtures, over a range of environmental conditions. By studying the growth parameters of individual legume species and grasses in field trials, and the mixture of all trial species (the all species mixture - **ASM**) in participatory trials, the project sought to understand a) the potential and mechanisms by which a *designed* LBM may increase the profitability of UK arable systems, and b) to provide robust data on which to base the development of commercial seed mixes.

This addresses Defra Sustainable Arable LINK R&D priorities to 1) promote sustainable, diverse, modern and adaptable farming, 2) lessen environmental impacts of the arable sector and 3) enhance biodiversity and the rural environment.

The objectives of the present study were:

- to characterise relevant physical and physiological characteristics of candidate legume species;
- to test the ASM for stable agronomic performance across the UK climatic gradient under a range of soil, rotational and management conditions;
- to develop a LBM, using a model-based approach, based on data from objective 1 and 2;
- to estimate nitrate leaching and N_2O emissions using a modelling approach;
- to incorporate the findings from objectives 1 and 2 into the Fertility Building Crops (FBC) decision support model (see Cuttle 2006);
- to assess the performance of the ASM in a high-input crop rotation, and its consequent effect on unit production cost and soil characteristics.

3.1.3. Scope

There were amendments to the scope initially planned for the project due to funding being awarded for 36 months instead of the 5-years originally requested. As the choice of species to be included in the final LBM could not be made until near the end of the 3-year project there was no field testing of the chosen LBM (as envisaged in the original proposal) and therefore little direct information of its performance to support the model development. The LBMs recommended here and the modelling (including the FBC-Link model) are therefore primarily serving as a proof of concept and the modelling output values regarding the LBMs are untested against any field data.

3.2. Materials and methods

3.2.1. Overview

The project was organised in 6 work packages. The experimental backbone of the study was a combination of replicated field trials and on-farm trials. In the replicated trials (WP1), various legume and grass species were tested in monocultures and in an All Species Mixture (ASM), containing 10 legume species and 4 grass species. Eco-physiological modelling (WP2), building on data from the replicated field trials, established which legume species would grow best together in simplified legume-based mixtures (LBM). In the on-farm participatory trials (WP3) the ASM was grown on 34 farms across the UK and was compared to a farmer-chosen control ley. Further modelling, using the DNDC model, investigated nitrogen dynamics for selected legume and grass species (WP4). WP5 adapted an existing decision support model for rotational planning to assess the effect of legume-based mixtures on soil fertility. In WP6, the ASM was tested in a large-scale rotation field trial under conventional management.

3.2.2. Selection of legume and grass plant species for trialling

To provide insurance against failure and to combine desirable traits of different species, leys are often sown with mixtures of plants (e.g. two clover and one grass species). To compose optimal species mixtures, a useful selection criterion is the functional complementarity of the different species with the aim of minimising functional redundancy. Following this idea, we collected data on the ecological and agronomic traits of 22 legume species from the literature: *Anthyllis vulneraria* L., *Lathyrus pratensis* L., *Lotus corniculatus* L., *Lotus pedunculatus* Cav., *Lupinus albus* L., *Lupinus angustifolius* L., *Lupinus luteus* L., *Medicago lupulina* L., *Medicago sativa* L., *Melilotus albus* Medik., *Onobrychis viciifolia* Scop., *Trifolium alexandrinum* L., *Trifolium hybridum* L., *Trifolium incarnatum* L., *Trifolium pratense* L., *Trifolium repens* L., *Trifolium resupinatum* L., *Trifolium subterraneum* L., *Trigonella foenum-graecum* L., *Vicia hirsuta* (L.) Gray, *Vicia lathyroides* L., *Vicia sativa* L.

The ecological characteristics were analysed using a Principle Components Analysis (PCA) to quantify the functional space occupied by the chosen legume species and identify candidates for screening that were functionally different. If a species was intolerant of any two of three specific factors (i.e. cold, mowing or autumn sowing), it was excluded. Further selection criteria included practical aspects such as seed availability for the species in the UK. This process resulted in a short list of 12 legume species supplemented by four grass species (*Festuca pratensis, Lolium perenne, L. multiflorum, Phleum pratense*). All four grass species as well as 10 of the 12 tested legume species were combined in an All Species Mixture (ASM) (Figure 1; Table 1), which was tested in both WP1 and WP3.

Table 1. Legume and grass species included in the trials: Scientific and common name; variety, seed rate (in kg/ha), seed weight (Thousand Kernel Weight, TKW in g) and seed rate in the monoculture plots (Monoc.) and in the All Species Mix (ASM).

					Seed rate (kg/ha)		
Abbreviation	**Scientific name**	**Common name**	**Variety**	**Inoc.***	**Monoc.**	**ASM**	**TKW**
AC	*Trifolium hybridum* L.	Alsike clover	Dawn	C	10	1.25	0.7
BT	*Lotus corniculatus* L.	Birdsfoot trefoil	San Gabrielle	-	12	2.5	1.2
BM	*Medicago lupulina* L.	Black medic	Virgo Pajberg	L	15	2.5	1.6
CC	*Trifolium incarnatum* L.	Crimson clover	Coutea	-	18	2.25	3.1
IR	*Lolium multiflorum* Lam.	Italian ryegrass	Teana	-	33	1	2.9
LT	*Lotus pedunculatus* Cav.	Large birdsfoot trefoil	Maku	-	12	2.5	1
LU	*Medicago sativa* L.	Lucerne	La Bella de Campagnola	L	20	2.5	2.4
MF	*Festuca pratensis* Huds.	Meadow fescue	Rossa	-	25	1.25	2.14
MP	*Lathyrus pratensis* L.	Meadow Pea	no variety	V	75	3.25	153
PR	*Lolium perenne* L.	Perennial ryegrass	Orion	-	33	2.5	2
RC	*Trifolium pratense* L.	Red clover	Merviot	C	18	2.5	1.8
SF	*Onobrychis viciifolia* Scop.	Sainfoin	Esparsette	-	80	5	19.2
TY	*Phleum pratense* L.	Timothy	Dolina	-	10	0.5	0.32
WC	*Trifolium repens* L.	White clover	Riesling	C	10	1.5	0.5
SC	*Melilotus alba* Medik.	White sweet clover	no variety	L	18	-	2.3
WV	*Vicia sativa* L.	Winter vetch	English Vetch	V	100	-	41

*Inoc.: Inoculation prior to sowing with Clover inoculum (C), Lucerne inoculum (L) and Vetch inoculum (V).

3.2.3. Field characterisation of legume and grass species (WP1)

In the replicated field trials there were 18 treatments. Twelve legume species and four grass species were each grown singly as monocultures; in addition, two treatments were reserved for the All Species Mixture, which was grown with and without *Rhizobium* inoculation. The densities of the species in the ASM were calculated as the appropriate proportions of the monoculture sowing densities. At all six trial locations, the seed was sown in spring 2009 (Figure 4).

All trials were laid out as one-factorial randomised complete block designs with three replicates. Seed lots of the four clover species, the vetch, the lucerne and one of the All Species Mix treatments were inoculated with rhizobial preparations before sowing at a rate of 1% substrate per seed weight for each species. These commercially-available inoculants comprised a carrier of sterile peat containing a polymer adhesive (to increase adhesion of the inoculant to the seed coat) and strains of Rhizobia appropriate to the plant species (Legume Technology Ltd, Nottinghamshire, UK). The locations, plot sizes, sowing dates and sowing equipment are listed in Table 7. Trial sites were distributed over a large geographical area within the UK (Figure 3).

Figure 1. Seed of the All Species Mix (ASM)

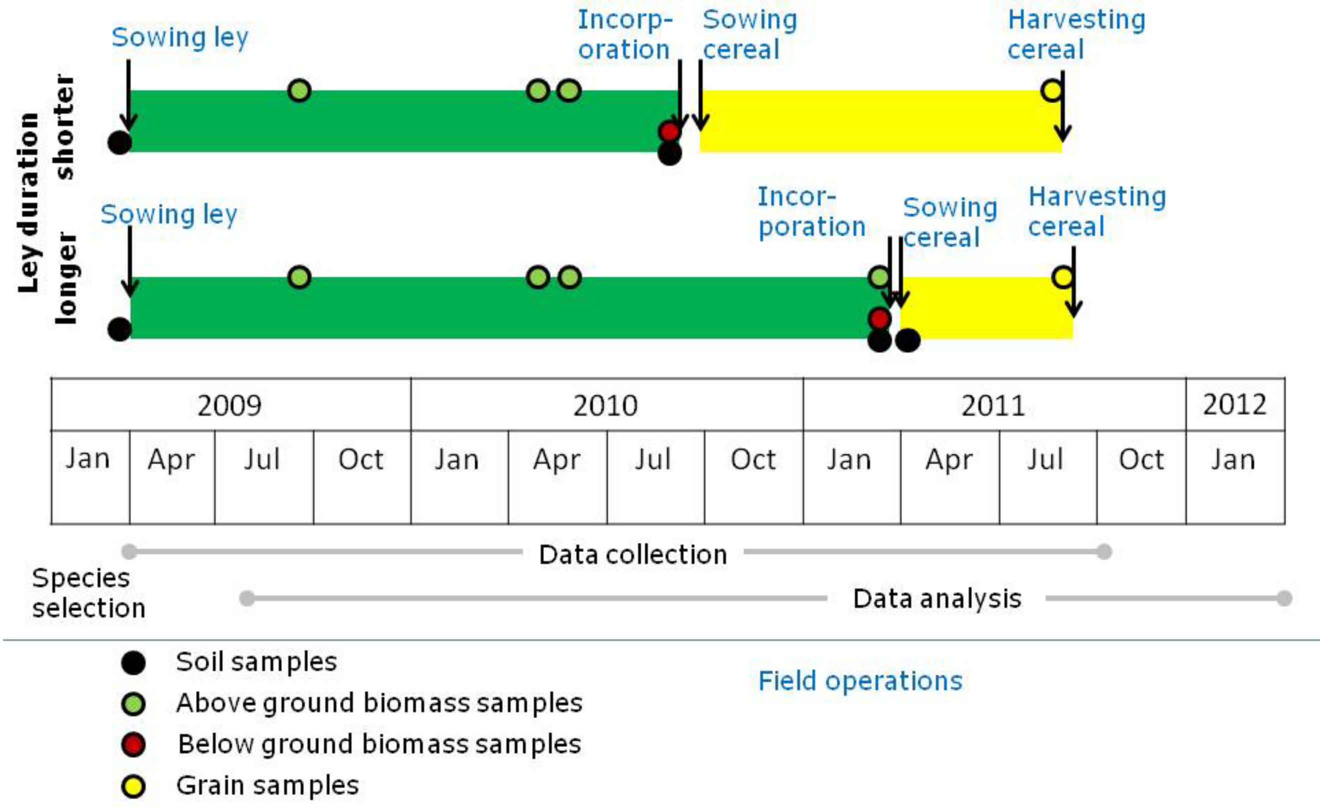

Figure 2. Schedule of field activities and measurements in WP1

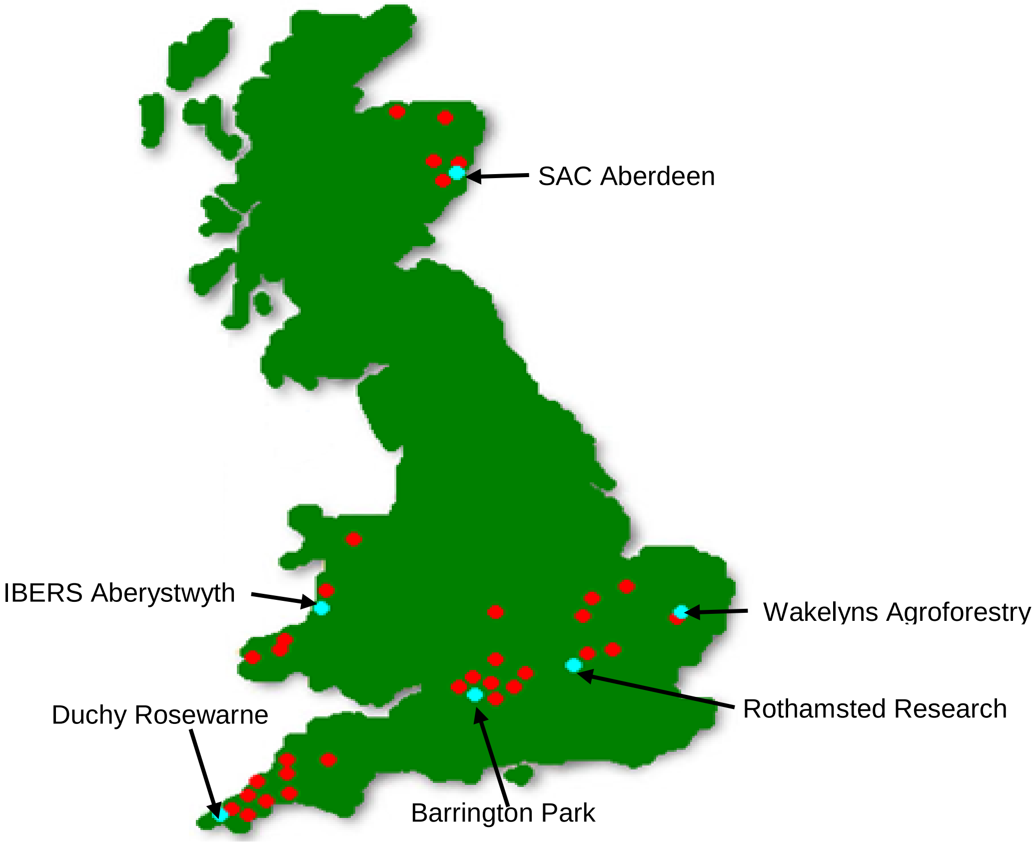

Figure 3. Map of 34 trial sites, shown in red and hub sites, shown in light green.

Table 2. Details of replicated trials: locations, plot sizes, sowing dates and pre-crops

Site	**Barrington Park**	**Duchy (Rosewarne)**	**IBERS Aberystwyth**	**Rothamsted Research**	**SAC Aberdeen**	**Wakelyns Agroforestry**
Abbreviation	B	D	I	R	S	W
North coordinate	51°49'52.2"	50°13'38.2"	52°25'48.1"	51°48'38.6"	57°11'05.6"	52°21'36.7"
West coordinate	1°40'12.3"	5°18'23.0"	4°01'22.1"	0°22'02.4"	2°12'45.1"	-1°21'09.2"
Altitude (m)	150	42	29	114	109	51
Plot width (m)	1.5	1.5	2	2	1.5	1.2
Plot length (m)	10	5	8	5	12	10
Sowing (date 2009)	20 Apr	24 Apr	23 Apr	15 Apr	13 May	29 Apr
First mowing (date 2009)	24 Jun	14 Jul	20 Jul	05 Aug	23 Jul	27 Jul
Previous crop	Winter barley*			Fallow	Spring barley*	Potatoes
Biomass sampling dates						
2009	-	18 Aug	1 Sep	5 Oct	20 Aug	24 Aug
2010 (1)	-	20 Apr	-	15 Apr	13 May	28 Apr
2010 (2)	-	18 May	21 Sep	13 May	11 Jun	28 May
2011	-	13–18 Apr	Mar	-	Apr	Apr

*stubble left overwinter


Figure 4. Sowing the WP1 trial at Wakelyns (left) and at Barrington Park (right)

Weed and crop assessments

Weed and crop species were assessed for cover repeatedly during the trial, using the Braun-Blanquet scale with seven classes (0: species not present; +: only isolated individuals present; 1: <5% cover; 2: 5 to ≤25%; 3: 26 to ≤50%; 4: 51 to ≤ 75%; and 5: 76 to 100% cover). In the replicated trials, cover was estimated 11 times at one of the sites (Barrington Park). Cover estimates were done either on a per plot basis or with two 0.25m^2 sectioned quadrats per plot.

In the on-farm (participatory) trials, weed and crop assessments were carried out with a 0.25m^2 sectioned quadrat. Four such areas were assessed within each treatment, i.e. both in the ASM and the control ley, resulting in eight assessment points per farm and date. At least 10m were left between any two assessment points.

In some cases, weeds could not be identified to individual species level and were therefore assessed as a species-group, e.g. 'docks' included *Rumex crispus* L., *R. obtusifolius* L. and hybrid *R. x pratensis (R. crispus* x *obtusifolius)* Mert. & W.D.J. Koch. Where differentiation was possible, the indication was that *R. obtusifolius* was the most dominant form. Volunteer crops encountered in weed assessments were excluded from further analysis.

Eco-physiological parameters

The six replicated trial sites were used to measure the detailed eco-physiological parameters listed below, which were required for modelling inter-plant competition (WP2):

Establishment and early growth: A 0.25m^2 fixed quadrat was located on each plot and assessed twice a week following drilling to monitor the emergence of the legume species. Counts continued until no further plants emerged. Following the end of the emergence counts, at weekly intervals five individual plants from each plot were cut at ground level and the dry weight recorded after drying at 80^{o}C overnight.

Height: Ten times in each growing season (five occasions before mowing and five post-mowing) five measurements of height were taken on each plot. Height was measured from the ground to the top of the canopy without moving or disturbing the plants. In Year 1, the first measurement was taken in the same week as the final establishment assessment and then every two weeks. Measurements were then taken immediately after mowing and every two weeks until five post-mowing assessments had been completed. The same protocol was followed in Year 2; the first assessment was done at a similar time to Year 1.

Re-growth: Recovery of the sward after mowing was assessed by taking five sequential above-ground biomass samples from a 0.25m^2 quadrat in each plot and measuring dry weight after removing weeds. In the final sample, weed dry weight was also recorded. The location of the quadrats was marked with pegs to avoid sampling the same area twice. On the final re-growth sample date, the species in the ASM were separated out before drying and weed dry weight was also assessed.

Proportional biomass and residue composition: Immediately prior to incorporation in the second year of the experiment, above-ground biomass samples from three 0.25m^2 quadrats were taken in each plot and combined. For the monocultures, the weeds were removed and the weed dry weight recorded following drying at 80°C until weights were constant. The legumes were then dried at 40°C for 48 hours, milled and sent for chemical analysis. A similar protocol was followed for the ASM except that the mixture was separated into its component species before drying. At the same time as the above-ground samples, three soil cores (3cm diameter and 20cm depth) were taken from each plot and the roots washed out for determination of below-ground biomass.

Additional measurements at Rothamsted: In addition to the protocols described above, extra measurements were taken at Rothamsted to further parameterise the model of inter-plant competition. Each time an early growth sample was taken, the plants were separated into leaves, stems and flowers to assess partitioning functions. A separate experiment was also done at Rothamsted, growing the legume species in individual pots in a sand bed to parameterise early growth following the protocol described elsewhere (Storkey, 2004). Finally, in June 2009, a vortis sample was taken from each plot to assess the invertebrate communities using the legumes as a resource.

Residue analysis

Prior to drying at 100˚C, a sub-sample of above-ground biomass was removed from each sample and dried at 35–40˚C in a forced-air oven for 48 hours or until samples had reached constant weight. Dried samples were then milled to pass a 1mm mesh size. Lignin was calculated as Acid Detergent Fibre using a sulphuric acid solution of cetyltrimethyl ammonium bromide (Palm & Rowland, 1997). The Folin-Ciocalteu reagent was used for the colorimetric determination of polyphenols (Palm & Rowland, 1997).

Environmental variables

Immediately before sowing, soil samples were taken on all trial sites, including participatory farms. Soil cores (2.5 to 4 cm diameter, depending on availability) were taken to a depth of 15 to 20cm (plough depth) following a ‘W’ configuration across the field. Sub-samples of 300g were then air dried and analysed for soil texture (percentage sand, silt and clay), soil organic matter (%), soil pH, and available phosphorus, potassium and magnesium (mg/l). For a more detailed description of soil analytical methods see Appendix 4.2, p. 188. Geographic coordinates (latitude, longitude and altitude) of all sites were obtained from publicly available digital maps.

Data analysis

All statistical analyses were performed with the programme R, version 2.14.1 (R Development Core Team 2012). For multiple comparisons among different species, not all legume and grass species were compared against each other as this would unnecessarily have reduced testing power. Instead, several sets of comparisons were performed, as follows. All legume species were collectively compared with all grass species (t-test); within the grasses, all species were compared against PR using the Dunnett-test; and within legumes, species were compared against WC, again using the Dunnett-test. PR and WC were chosen as reference species as they are the species currently most often used in fertility building leys (Table 4, page 30).

Finally, within the ASM, the inoculated ASM was compared to the non-inoculated ASM; and the average of the two ASM against the expected average of the monocultures (see section 3.3.7.1).

For many of the measured response variables, there was large variability of the values between the different research sites; in fact, differences among species within sites were often lower in magnitude than between-site differences. In the graphical representation of the data, individual values were normalised to each site’s maximum value (i.e. expressed as % of the site maximum), so that sites with overall high values would not have a higher influence on species means than values from other sites. However, the statistical analysis was based on the original data, with mixed models using site (research hub) as random factor and species as fixed factor.

3.2.4. Ecophysiological modelling (WP2)

Modelling competition between species

The model of inter-plant competition was based on that reported previously (Storkey & Cussans, 2007). Early growth was modelled as an exponential function of effective day degrees (combining temperature and light). After the onset of competition, growth was modelled on a daily time step using a calculation of the proportion of incident radiation absorbed by each species in the canopy. This was done by dividing the canopy into layers and modelling competition for light from plant heights and distribution of foliage. Photosynthetic rate was calculated on the basis of light captured plus leaf temperature and assimilate allocated to different plant organs according to partitioning functions modelled against photothermal time (Figure 5). There were not sufficient resources within the project to collect data on rooting depth, and therefore competition for below-ground resources was not included in the model. The impact of cutting was modelled using a function describing the vertical distribution of stem and leaf material in the canopy. Cutting height was input into the model and the proportion of green area removed was calculated. Re-growth was modelled using the light use efficiency measured in the monocultures to parameterise the light competition function in the multi-species canopy.

Designing optimal, multi-functional legume mix

The assessments from the research hubs generated data on a range of functions or services delivered by the legumes including early productivity (before first cut), re-growth, weed suppression and support of phytophagous invertebrates. In addition, the yield of cereal crops following the legumes was quantified at four sites: Rothamsted, SAC, Wakelyns and IBERS. The parameterisation of the inter-plant competition model also generated a database of plant traits for all the legume species, these included specific leaf area, C:N ratio, height, leaf:stem ratio, leaf N and seed weight. In addition, data were also available on residue composition in terms of the amount of lignin, polyphenols and C:N ratio. An analysis was done to identify relationships between plant traits and functions using all sub-sets regression. These relationships were used to predict the function of different legume mixtures using the community aggregated value for different traits based on the proportional biomass (p) output by means of the inter-plant competition model:

$$\mathrm{trait}_{\mathrm{agg}} = \sum_{i=1}^{n} \mathrm{p}_{\mathrm{i}} \times \mathrm{trait}_{\mathrm{i}}$$

To evaluate different mixtures, a standard management scenario was used: sowing on 15th April, emergence 7 days later and cutting at 10cm on 31st July. Monocultures and all possible binary mixtures were modelled using weather data from two contrasting sites (Rothamsted and IBERS) and all possible three species mixes were modelled using data only from Rothamsted. In addition, the best three species mix was tested with the addition of a fourth species. Densities were calculated as the average observed at all hub sites for the monocultures or the relevant proportion in the mixes.

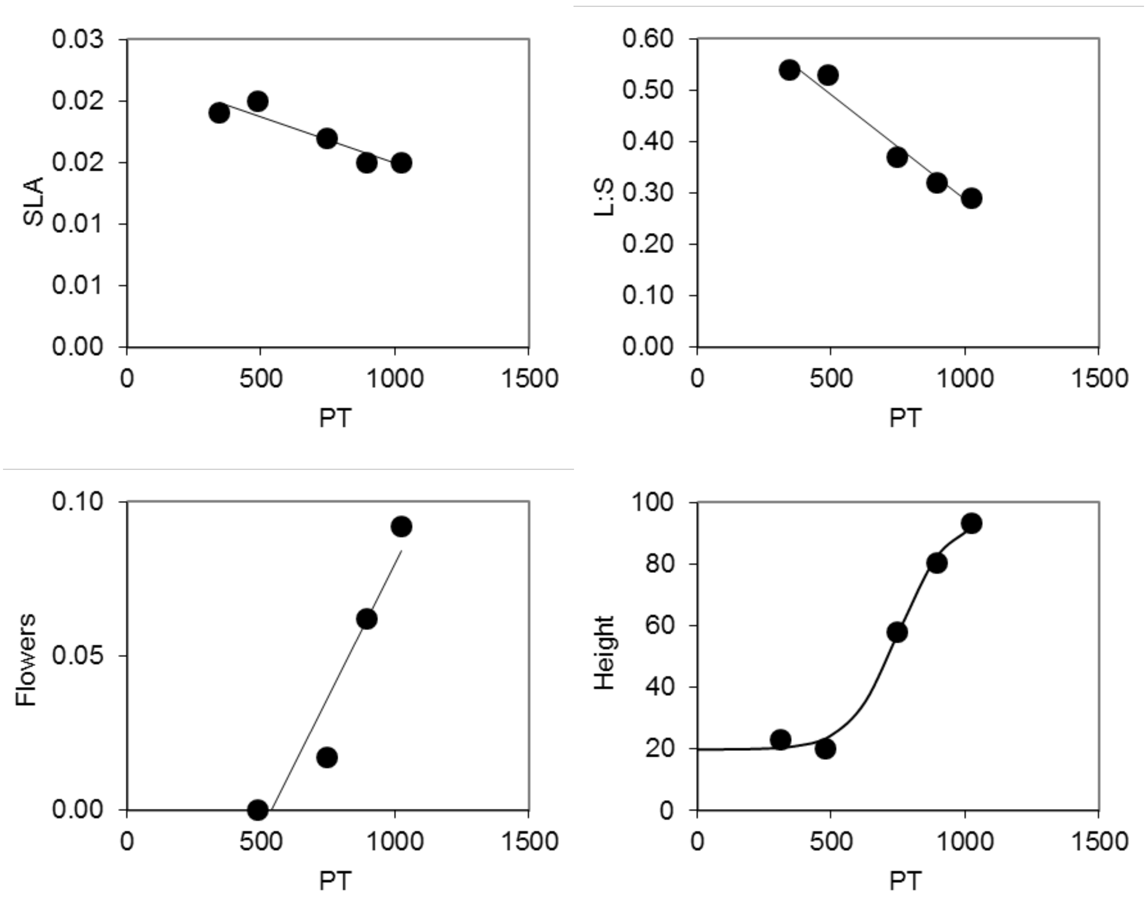

Figure 5. Example dataset (Sainfoin, Onobrychis viciifolia) of ecophysiological parameters used to populate the model of inter-plant competition; specific leaf area (SLA g leaf m-2), Leaf:Stem ratio, Proportion of flowers by dry weight (%) and maximum height (cm); PT = photothermal time. Similar datasets were generated for each legume species.

3.2.5. Participatory field trials with legume mixtures (WP3)

Methods overview

In addition to the replicated plot trials (WP1), the All Species Mixture (ASM) was also sown by 32 organic and 2 non-organic farmers across the UK (Figure 3, page 21). These participatory trials are also called on-farm trials throughout this report. Seed of the ASM was provided for a 0.5 ha strip which was sown next to or within a control ley (Figure 6). The species composition of the control ley was chosen by each farmer individually (Table 4, also see section 3.3.3 below).

Per farm, management for the ASM and the control ley were identical. Most farmers sowed the leys in spring 2009, while some delayed sowing until later in 2009 (Table 4). Once per year (in 2009, 2010 and 2011) the ASM and control ley were assessed for the presence and cover of individual sown and weed species on each farm. At the last assessment in 2011, biomass samples were taken from both the ASM and the control. Soil samples from the farms were taken at the beginning and at the end of the trialling period, i.e. in 2009 and 2011.

The experimental approach of this work package served two aims. First, it allowed us to determine the effect of increased plant diversity under a range of environmental and management conditions. Second, by analysing the presence and cover data of individual plant species, it was possible to measure their performance when growing in a complex ley mixture, again under a wide range of conditions.

Field trial sites: location and set up

The participatory on-farm field trials were un-replicated, i.e. on each farm there was only one ASM strip and one control ley next to the ASM. The field trials were conducted over 3 years at 34 commercial farms in the UK (Figure 3). A multi-species mixture of legumes and grasses (the All Species Mixture "ASM", (Figure 1; Table 1) was sown at a total seed rate of 31kg/ha in 0.5 ha strips alongside farmer-chosen control leys (Table 4). The ASM was inoculated with a *Rhizobia* substrate to the same specifications as in the ASM used in the replicated plot trials (see WP1, section 3.2.3, page 20).

The farms cover a wide range of soil conditions and farm management practices (Table 3); but management of the ASM and the control ley was identical within farms (except for one site (WAF), where the timing of mowing in 2010 was different in the two treatments.

Table 3. Details of participatory trials: Geographic coordinates and soil properties of the trials sites (explanatory notes: Table 5)

Farm Nr	Research Hub	Soil Texture	Sand	Silt	Clay	pH	P	K	Mg	OM	Elevation (m)	North	West
1	Duchy	Clay	31	24	45	7.5	68.2	330	87	4.5	42	50.2273	5.3064
2	Duchy	Clay	22	30	48	5.8	23.8	135	53	4.9	132	50.3956	4.4840
3	Duchy	Clay	19	37	44	6.3	22.0	180	60	5.5	144	50.5701	4.4527
4	Duchy	Clay Loam	44	30	26	6.4	9.6	158	75	5.4	53	50.2136	4.9700
5	Duchy	Clay Loam	40	34	26	7.1	27.6	174	56	6.6	34	50.5120	4.7991
6	Duchy	Clay Loam	50	21	29	6.1	43.6	158	84	4.2	172	50.7699	3.9022
7	Duchy	Clay	24	32	44	6.3	12.8	106	60	4.0	86	50.3422	4.6602
8	Duchy	Silty Clay Loam	20	50	30	6.2	11.0	93	41	7.5	115	50.4325	4.9098
9	Duchy	Sandy Loam	75	15	10	6.4	12.6	163	66	5.1	135	50.6511	4.4274
10	Elm Farm	Silty Loam	16	61	23	7.1	20.4	95	53	2.6	164	51.4411	1.9016
11	Elm Farm	Clay Loam	43	38	19	7.4	47.4	134	44	3.4	125	51.3803	1.5344
12	Elm Farm	Silty Clay	18	36	46	7.7	17.2	224	71	3.6	135	51.7323	1.9393
13	Elm Farm	Clay Loam	38	37	25	7.8	17.0	241	47	5.5	125	51.7461	1.7340
14	Elm Farm	Clay Loam	21	51	28	7.9	20.8	244	53	2.8	142	51.4381	1.9596
15	Elm Farm	Clay Loam	46	33	21	7.2	31.4	185	51	3.3	99	51.4505	1.1610
16	Elm Farm	Clay Loam	32	42	26	8.0	21.0	110	35	8.2	162	51.5183	1.4572
17	Elm Farm	Clay Loam	29	42	29	7.6	28.4	123	42	3.8	170	51.3156	1.5193
18	Elm Farm	Clay Loam	41	40	19	6.0	33.6	77	63	2.6	52	51.4966	1.0584
19	WAF	Clay Loam	39	33	28	6.7	34.2	201	103	NR	0	52.5215	0.1629
20	WAF	Clay	28	31	41	7.6	38.2	441	424	NR	1	52.6306	-0.3452
21	WAF	Clay Loam	43	22	35	8.2	16.8	247	61	NR	45	52.1412	0.0492
22	WAF	Clay	35	24	41	7.2	18.4	282	187	4.5	15	52.4106	0.2220
23	WAF	Clay	22	20	58	7.4	31.6	122	58	NR	51	52.3602	-1.3526
24	WAF	Clay	42	21	37	6.6	30.4	336	108	NR	73	52.3671	1.4132
25	IBERS	Silty Clay	7	47	46	4.9	19.2	67	62	NR	70	52.0456	4.5998
26	IBERS	Clay Loam	50	30	20	4.8	14.6	69	42	NR	26	51.0327	4.5520
27	IBERS	Sandy Loam	77	12	11	6.2	16.2	89	161	NR	56	52.6293	4.0839
28	IBERS	Clay Loam	32	41	27	5.9	18.4	170	121	6.5	85	51.8063	5.0682
29	IBERS	Silty Loam	7	58	35	4.9	21.2	131	65	NR	309	53.0107	3.6467
30	SAC	Clay Loam	38	41	21	5.7	18.0	103	80	8.3	120	57.5508	2.3001
31	SAC	Loamy Sand	77	16	6	6.3	34.0	110	73	2.4	20	57.6712	3.2752
32	SAC	Clay Loam	43	38	19	6.2	30.4	212	90	9.2	194	57.3107	2.3083
33	SAC	Sandy Loam	58	29	13	5.8	94.2	179	171	7.8	109	57.1849	2.2125
34	SAC	Sandy Loam	45	39	16	5.5	34.0	213	77	8.0	97	57.2813	2.1325

Table 4. Management details for participatory trials (WP3): Grazing, mowing and composition of control ley (notes: Table 5)

Nr	Sowing date	Grazing	Mowing	RC	WC	IR/PR	other species	sampling date
1	17/09/2009	x	yes	X	X	PR	AC,BT,CF,CH,MF,SB,SF,SP,TF,TY,WSC,YW	25/07/2011
2	20/04/2009	S	yes		X	IR/PR	-	06/04/2011
3	02/04/2009	x	yes	X	X	IR/PR	-	NA
4	26/09/2009	x	yes	X		IR/PR	-	07/04/2011
5	16/08/2009	x	yes	X		IR/PR	-	31/03/2011
6	22/04/2009	x	-	X	X	IR/PR	-	NA
7	April 2009	S	yes	X		IR	-	09/02/2011
8	End of April	S	yes		X	IR/PR	-	19/07/2011
9	03/06/2009	x	yes		X	IR/PR	-	NA
10	14/04/2009	B	yes	X	X	PR	AC,BM,BT,CC,CF,CH,MF,RG,SB,SF,SP,T,YW	26/04/2011
11	02/04/2009	S	no	X	X	PR	BM,BT,CF	20/06/2011
12	June 2009	S	-		X	IR/PR	BT	07/04/2011
13	Start of April	S	-	x	X	-	-	NA
14	09/09/2009	B	yes			IR/PR	-	11/04/2011
15	01/04/2009	S	no		X	IR/PR	BT,CF,MF	24/03/2011
16	Start of April	S	-	X	X	IR/PR	-	25/03/2011
17	April 2009	S	no		X	IR/PR		07/04/2011
18	01/07/2009	x	yes	X	X		CC,LU	28/03/2011
19	01/05/2009	x	yes		X		AC,BM	14/02/2011
20	04/04/2009	x	yes	X			-	NA
21	01/04/2009	x	yes	X	X	PR	AC,LU	14/04/2011
22	01/10/2009	S	yes	X			BM	11/04/2011
23	28/04/2009	x	yes		X		AC,BM,CH	13/04/2011
24	22/04/2009	x	yes				LU	04/03/2011
25	23/04/2009	B	yes	X		IR	-	Spring/summer 2011
26	11/05/2009	S	yes		X		-	Spring/summer 2011
27	21/04/2009	B & S	yes	X	X		-	Spring/summer 2011
28	12/05/2009	B & S	yes		X		-	Spring/summer 2011
29	05/05/2009	S	yes		X		-	Spring/summer 2011
30	10/04/2009	B	no		X	PR	TY	10/02/2011
31	18/04/2009	x	yes	X		PR	-	10/02/2011
32	01/05/2009	S	no	X		PR	-	14/02/2011
33	14/04/2009	S	no	X	X	PR	-	22/02/2011
34	20/04/2009	B	no	X	X	PR	TY	14/02/2011

Table 5. Explanatory notes for Table 3 and Table 4

Table	Heading	Explanation
Table 3	Research Hub	Research partner responsible for farm trial
	Sand	% Sand in soil
	Silt	% silt in soil
	Clay	% clay in soil
	pH	Soil pH
	P	Available phosphorus (mg/L) in soil
	K	Potassium (mg/L) in soil
	Mg	Magnesium (mg/L) in soil
	OM	Organic matter in soil (%)
	Elevation	Altitude of site in m
	North	Latitude, metric degrees North
	West	Longitude, metric degrees West
Table 4	Sowing date	Sowing date of trial (ASM and control ley)
	Grazing	S: Sheep, B: Bovine; x: no grazing
	RC	Red clover used in control ley
	WC	White clover used in control ley
	IR/PR	Italian ryegrass/Perennial ryegrass used in control ley
	Other species	Other legume and grass species used in control ley
	Sampling date	Sampling date for biomass prior to incorporation of the ley

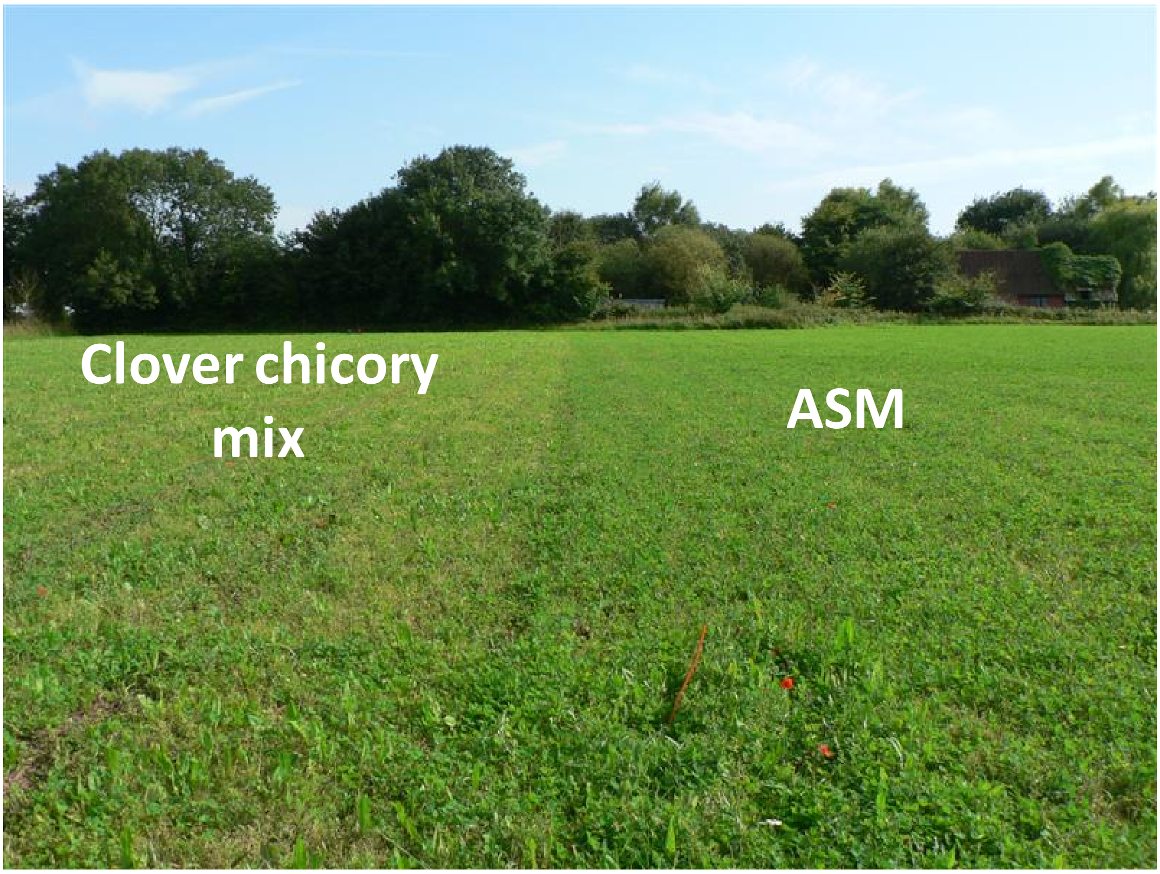

Figure 6. Participatory t**rial site at Wakelyns Agroforestry, Suffolk, August 2010.**

Plant assessments

The percentage ground cover, according to the Braun-Blanquet scale (Braun-Blanquet, 1932; Podani, 2006) of each component crop and weed species was determined in four 0.25m^2 subplots per plot in spring of each of the three trial years. Plant cover was recorded even if it was unobservable from above (i.e. below the canopy of neighbouring plants), allowing total ground cover scores of >100%. Assessments were made at intervals of no less than 10m across plots. Because of variation in sowing dates, according to regionally typical practice, and due to some leys being incorporated into the soil before the end of the project, only 22 of 34 farms were assessed in all three years: a subset of 30 farms was assessed in 2009; 29 farms in 2010 and 29 farms in 2011.

Weed assessments

Weeds were assessed on the same sampling quadrats together with the sown species again using the Braun-Blanquet scale as above. As in WP1, some individual plants could not be identified to species level and were assessed as a species-group. Volunteer crops, such as potato (*Solanum tuberosum*), wheat (*Triticum aestivum*) and oats (*Avena sativa*), which were encountered in weed assessments were excluded from further data analysis.

Plant biomass sampling

In spring or autumn 2011 (Table 4), prior to the scheduled incorporation dates of the trials, above-ground biomass was sampled from four subplots per treatment from within a 0.25m^2 sectioned quadrat (Figure 7). Above-ground plant matter (including crop and weeds) was cut at ground level, and oven dried at 100˚C to determine dry weight.

Figure 7. Biomass sampling in the ley on an organic farm in Berkshire, 2011

Soil sampling

Soil samples were collected prior to sowing in 2009 at each trial location, again in 2011 before incorporation (Table 4), and, where trials had been incorporated, once after emergence of the following crop. Samples were taken from the top 20cm of the soil profile in a W shape across plots with sampling points 2–4m apart. A 300g subsample was taken for analysis at Natural Resource Management Ltd analytical laboratories.

Organic matter was determined using the wet oxidation Walkley Black colorimetric method. Mineral nitrogen was determined using 2M potassium chloride as the extracting solution. Available phosphorus was determined according to Olsen; available potassium was extracted using Molar ammonium nitrate and K concentration was determined by flame photometry. Available magnesium was extracted using Molar ammonium nitrate and Mg concentration was determined using atomic absorption spectroscopy (also see Appendix 4.2, p. 188).

Ley management record and farmer perceptions

Prior to the start of the field trials, farm managers at the 34 trial sites were questioned about their current use of legumes, and their expectations on the performance of the ASM and its component species. The farmers were visited again at the end of the trial and asked about their experiences of managing and general opinions on the performance of the ASM and its component species. Information on ley management (e.g. cutting and grazing regime) was also obtained from the farmers.

Data analysis

All statistical analyses were performed with the programme R, version 2.14.1 (R Development Core Team, 2012). To evaluate the performance of individual legume species in leys under different environmental and management conditions we used the Braun-Blanquet data collected in the All Species Mixture (ASM) on the participatory farms. With this approach we explored how individual legume species grow in complex mixture (i.e. in the ASM), rather than in monocultures (as was studied in WP1). We analysed four measurements:

(1) The proportion of farms on which the species was present (termed “*farm presence*”); for the balanced subset of farms on which assessments were done in all three years, presence in 3 years on all 22 farms corresponds to 100% (i.e. the species was present in all 3 x 22 = 66 observations).

(2) The proportion of 0.25m^2 quadrats on which the species was present (termed “*quadrat presence*”); because observations were made on four quadrats on each farm in each year, this measure is more fine grained than the previous one.

(3) The *percentage cover* as assessed with the Braun-Blanquet data; this measure is likely to be more subjective and more noisy than the presence data because cover estimates were collected by different observers, but it is also more relevant to general productivity.

(4) The development of cover over the years measured as slope of cover; this can be seen as a measure of *persistence* of species.

The presence of species on farms or on individual quadrats was analysed using generalised linear mixed effects models with binomial error structure, with farm treated as a random factor. When data was analysed over three years, year was treated as an additional random factor. Cover data on the Braun-Blanquet scale was transformed into cover percentage (Table 6) and then log-transformed to reduce skew.

Table 6. Transformation of Braun-Blanquet scores into percentage cover

Braun-Blanquet score	Upper boundary	Lower boundary	Transformed value (% cover)
5	100	75	87.5
4	75	50	62.5
3	50	25	37.5
2	25	5	15
1	5	1	3
+	1	0.5	0.75
r	(0.5)	(none)	0.1

For statistically testing the differences between the various species we used two scenarios. In the first scenario, all species were compared against white clover as a species that is currently very frequently sown as a ley species (for evidence, see section 3.2.3.5). In the second scenario we determined whether there were any significant differences among the less used species (i.e. without red and white clover), in particular between the best (maximum performance) and the remaining species, i.e., for each performance parameter we used the best species as the control against which the others were compared. This second scenario explores the potential of various legume species when the aim is to diversify beyond the use of white and red clover. For both scenarios a Dunnett test was used (many to one control).

For analysing the response of species to soil conditions, a multiple regression analysis was used on the species presence data. Full models containing percentage of sand, pH, available P, K and Mg as explanatory variables and the presence of individual legume species as the response variable were reduced by stepwise removal of non-significant terms (Burnham & Anderson, 2002; Kirchner *et al*., 2011). The best model was chosen on the basis of the lowest value of Akaike's Information Criterion (AIC) (Knight, 1985). Cumulative Akaike weights were then calculated to determine the relative importance of explanatory variables (Burnham & Anderson, 2002; Kirchner, *et al*., 2011).

3.2.6. Modelling nitrogen dynamics (WP4)

Outline of work undertaken

Measurements of key soil and plant-based properties for the Craibstone site at SAC in Scotland (part of WP1; see Figure 3) were used for validation of UK-DNDC (DeNitrification-DeComposition) for nitrous oxide emissions and N-leaching losses for selected individual species.

DeNitrification-DeComposition (DNDC) model

Nitrate leaching and N_2O emissions for the Craibstone site were estimated using the UK version of the DeNitrification-DeComposition (UK-DNDC) process-based model, a modified version of DNDC. It is a dynamic model comprising five interacting sub-models describing thermal-hydraulic conditions, aerobic decomposition, denitrification (Li *et al*., 1992), fermentation and plant growth processes (Li *et al*., 1994). The latter also contains sub-routines to account for agronomic practices such as crop rotation, tillage, fertiliser addition and biomass cutting. In conjunction with locality-specific measured weather data, carbon and nitrogen biogeochemistry in agricultural systems is modelled for daily time steps. The DNDC model was originally developed to model greenhouse gas emissions from agricultural soils in the USA (Li *et al*., 1992). Furthermore, the UK version continues to be developed with recent updates incorporating the “anaerobic balloon”, enabling nitrification and denitrification to occur simultaneously within a soil profile (Li *et al*., 2004) and revised water drainage and N-adsorption equations for improved predictions of N-leaching (Li *et al*., 2006). Under the Intergovernmental Panel on Climate Change (IPCC) definitions of N_2O emission estimation methodologies suitable for the compilation of national greenhouse gas inventories, DNDC is a “Tier 3” approach, i.e. one which requires high resolution input parameters, but with the advantage of reduced uncertainty in the output.

Input parameters

Climate files were prepared from weather data collected by the weather station at Craibstone (57° 11.2’ N, 02° 12.8’ W, 700m from the field site). Descriptors of the physico-chemical properties of the soil, e.g. texture, % clay, pH, bulk density and porosity, were either measured as part of the project or were based on pre-existing data for the site. Data on the saturated hydraulic conductivity and the water release characteristics were obtained from Papadopoulos (2007), the latter being used to estimate the water-filled pore space at field capacity and permanent wilting point after fitting of the van Genuchten function (van Genuchten, 1980).

Modelling was conducted for a Legume Based Mixture (LBM) as emerging from the ecological modelling work of this project (see Results from WP2, section 3.3.2.2, p. 87), *viz.* mixture of BM, LU, RC and WC. Crop parameters for the four legume species plus two grasses as non-nitrogen fixing species for comparison were based on the default parameters contained within DNDC for lucerne and grass. The smaller growth habit (Stace, 1991) of the two clover species was specified by reducing the maximum potential biomass by 40% and the nitrogen fixation efficiency was estimated using field data collected as part of the project. The three years of grass/clover ley set-aside were specified as non-legume hay and the optimised parameters of Dietiker *et al*. (2010) were used for winter wheat, oilseed rape and winter barley. The parameters for spring barley were the same as those for winter barley with the exception of potential grain yield and the thermal degree day development index which were reduced by 20%.

Where the timings of agronomic operations such as ploughing, sowing, biomass cutting, harvesting and fertiliser application (where applicable) were unknown, a standard crop diary was used based on what would be considered typical for the UK. Similarly, the fertilisation of small grain crops in the stabilisation phase was based on the recommended values of McBain and Curry (2009). Fertiliser application was always split with 40kg N ha^{-1} being applied 4-6 weeks after sowing for spring crops and in early spring for winter crops followed by the balance at a later date.

Modelling approach

Prior to the LegLink trial two successive, conventionally produced, spring barley crops had been grown (2007 and 2008). Both crops were conventionally produced and received 100kg N ha^{-1} and 70kg ha^{-1} each of P and K per year, a standard herbicide regime, but no fungicides. Thereafter, three years of grass-clover ley set-aside (2004-2006) followed. To maximise model performance six repetitions of a 5-year rotation sequence “typical” for Scotland (Hay *et al*., 2000), comprising winter barley, oilseed rape, winter wheat and two successive years of spring barley were run using the crop specific and management parameters described above. The purpose of this “run-in” sequence was to stabilise the soil C and N pools within the model before the years of interest.

Field measurements of spring barley grain yield and soil mineral nitrogen content were used to validate the model. The root mean-square error (RMSE) was calculated as a comparator of model performance for the predictions of spring barley grain yields and soil mineral nitrogen after each of the chosen ley-phase species. The RMSE is a deviation statistic, expressed in the same units as the values from which it is derived, representing the mean distance between the predicted and measured values (Kobayashi & Salam, 2000). Legume species vary considerably, not only in the amount of biomass produced, but also in the efficiency with which they fix nitrogen (Brockwell *et al*., 1995). Hence, the sensitivity analysis focussed on the effect of altering the nitrogen fixation capacity of lucerne, as a representative legume. The values for the nitrogen fixation capacities used for the sensitivity analysis were obtained from a random number table (Rohlf & Sokal, 1995).

3.2.7. Decision support model for rotation planning (WP5)

The Fertility Building Crops model (FBC model) developed in DEFRA Project OF0316 is an empirical model that describes N availability, crop uptake and N losses in organic farming rotations (Cuttle, 2006). A feature is that it only requires input data that would be readily available to a commercial grower. This inevitably limits the accuracy of the outputs, but accepting less accuracy allows a simpler approach to be adopted for the modelling. The FBC model is written as a Microsoft Excel workbook based on the Stix model developed in Defra Project NT2501. It operates on a monthly time-step to estimate N mineralisation from crop residues using a simple algorithm based on the C:N ratio of the residues to divide their N content into Fast_N, Med_N and OM_N components, which are converted to mineral N at a fast, medium and slower 'background' rate over fixed periods of degree-days. This is a mathematical division and the fractions are not intended to correspond to any specific chemically or biologically definable forms of soil N. The model simulates the cropping rotation for a single field. As outlined in Figure 8, it assumes the rotation starts with an initial ley period followed by an arable cropping phase. The model describes N release from the ley residues for up to five years after cultivation of the ley and from additional inputs of crop residues and manures during this cropping phase. Crop yields are estimated on the basis of the mineral N available in the soil, which also determines N losses during the arable phase.

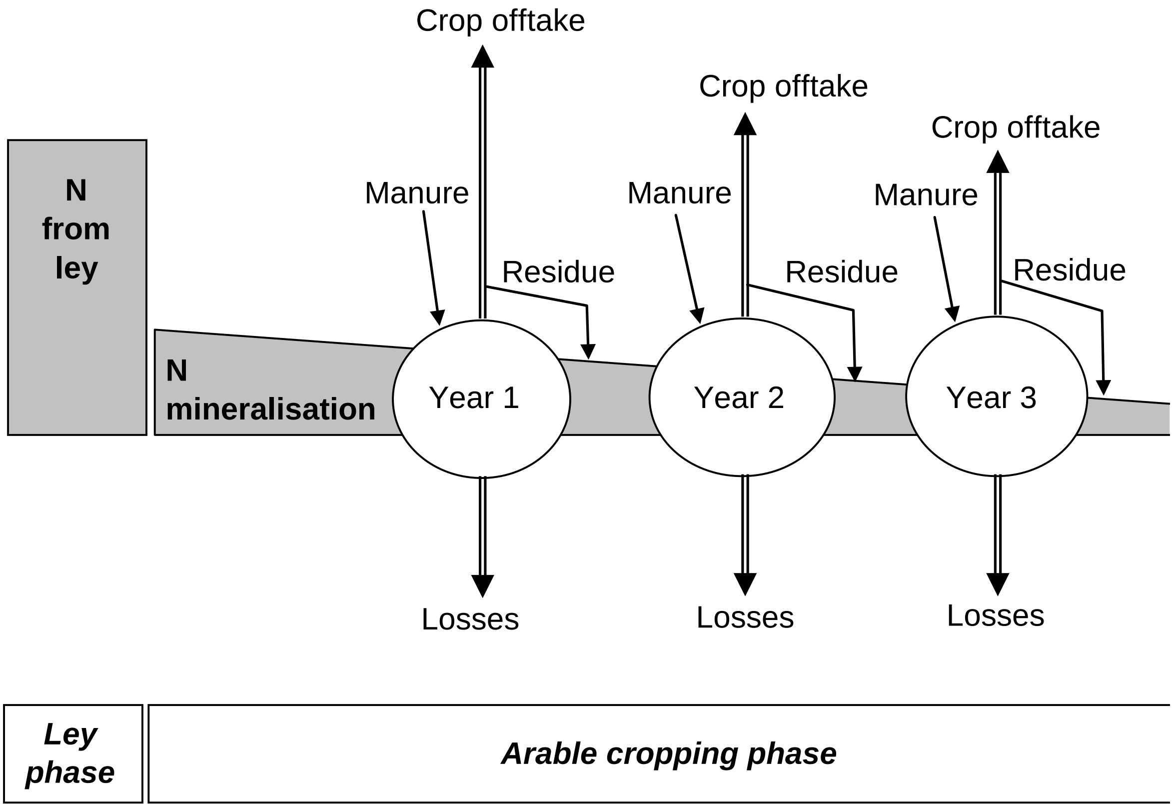

Figure 8. Outline of the main processes simulated in the FBC model.

Such a model describing the behaviour of the LBMs developed in this project would be of particular value in facilitating their adoption by farmers and growers who would initially be unfamiliar with these novel mixtures and how best to maximise their contribution to improved N efficiency within crop rotations. Changes that were required to adapt the FBC model for use with the LBMs were:

(i) to change the way the model determines the amount of N accumulated during the ley phase;
(ii) to modify the method of calculating N mineralisation to include the effects of high-lignin and high-polyphenol content residues;
(iii) to redesign the data input screen to allow the user to enter the additional information required to describe the performance of the more complex LBMs in the ley.

Parts of the model development were dependent on information from WP1 and WP3, much of which was not available until the final stages of the project. Waiting for the completed results would have left insufficient time to complete the modelling work before the project end-date and it was sometimes necessary to rely on incomplete data. In particular, decisions about the composition of the LBMs to include in the model had to be made before the final recommendations from the project were decided and these were therefore made on the basis of what were the favoured candidate species at that time. The new version of the model, referred to as FBC-Link, allows the user to select from the following four options:

- Mix A (RC + WC + LU + BM)
- Mix B (SF + WC+ LU + BM)
- Mix A + PR
- WC + PR

Red clover in Mix A is replaced by sainfoin in Mix B to examine the effect of including a polyphenol-rich species in the mixture. The third option allows the user to examine the effect of including ryegrass in Mix A. The simple clover/grass mixture is included as a more familiar reference and to provide continuity with the previous FBC model. Due to the different way the LegLink version of the model estimates N accumulation by the ley, it was not possible to retain the other legume options that were provided in the earlier model.

Estimation of N accumulation by the ley

The original FBC model allows the user to select from a limited range of legume monocultures or single legume/grass mixtures for the ley phase, together with the proportion of legume, duration of the ley and whether cut, grazed or mulched. Based on this information, the model then selects a value for the amount of N accumulated by the ley from look-up tables of fixed, previously determined values. The range of values is restricted to those included in the tables. This approach is unsuitable for the more complex LBMs where there are more variables to influence the build-up of N during the ley phase and too many possible alternatives to include in look-up tables. The simulations also require more detailed information about the proportions of individual species, especially if some have characteristics that might have a particular effect on N accumulation or the subsequent rate of N release.

The look-up tables in the model were replaced by a subroutine that calculates N accumulation specifically for the ley yield, management and species composition described by the input data. The estimation ignores many of the processes determining the accumulation of N but was considered to be sufficiently accurate in view of the limitations of the input data and the approximations elsewhere in the model. The revised input screen allows the user to enter data for the ley phase for up to four years; with an estimate of total annual growth (described as either poor, moderate or vigorous, corresponding to 7, 10 or 15 t DM ha^{-1}) for each year, the approximate percentage of each species in the ley (on a dry matter basis; for up to six species in the LBM plus weeds) and management (cut with herbage removed, cut and mulched, grazed or a combination of these options). For each year of the ley, the model calculates the dry matter yield for each component species and the quantities of C, N, lignin and polyphenols in each (using typical concentrations stored within the model). Similar calculations are carried out for the root and stubble component using the (root + stubble):herbage ratio for each species. These are then combined to obtain the composition of the total herbage and root + stubble residues for that year. Values for N, lignin and polyphenol concentrations and for (root + stubble):herbage ratios were obtained from the literature and from the WP1 and WP3 field trials where available. However, data were often lacking, especially for roots, and when they were available they were often very variable. The values used in the model are listed in Appendix Table A3 but in view of the many uncertainties involved they should be considered as temporary values.

Although constant values were assumed for N concentration in the legumes, concentrations in grass were determined by the availability of N from the accompanying legumes. The concentration was allowed to vary in the range 1.4 - 3% in herbage and 1 - 2.2% in roots + stubble, with the value calculated from the proportion of legumes, modified by any manure inputs and a factor for whether the top growth was cut, grazed or mulched. The values of the management factors for Year 2 onwards were adjusted to reflect the greater contribution of N from the preceding year. Concentrations for Year 2 onwards were calculated as the average of that and the previous year's values.

It has been reported that lignin and polyphenols start to affect N mineralisation at concentrations above about 15 and 3% respectively (Palm 1995, Palm & Rowland 1997), suggesting that the model should include a threshold below which these do not influence the mineralisation calculations.

However, other studies indicate that the above thresholds may be too high. The majority of crop residues contain low concentrations of polyphenols but they will generally contain more lignin. It is likely that the original algorithm used in the FBC model will to some extent include the effect of this basal lignin content. To allow for this, the model subtracts a 5% threshold value from all lignin concentrations to obtain an 'effective lignin' content. No threshold was applied to the polyphenolic contents.

The model determines the amount of N accumulated during the ley phase by estimating how much N is added to the soil in residues each year and then follows the mineralisation of this N to obtain the amount remaining at the end of the ley. The quantities of herbage N returned to the soil will depend on the type of management. Where herbage is cut and removed, 95% of the herbage N above stubble height is assumed to be harvested and is excluded from subsequent calculations. Where herbage is mulched, it is assumed that 80% is returned to the soil and 10% of the N content is assumed to be volatilised from this material as it lies on the soil surface (Janzen & McGinn, 1991; Larsson *et al*., 1998; De Ruijter *et al*., 2010). Where grazed, 80% of herbage N above stubble height is assumed to be utilised by grazing but 28% of the N is returned to the soil in dung (Thomas, 1992; Whitehead, 1995; McDonald *et al*., 2011). Urine-N is assumed to be either volatilised, utilised in growth, leached or denitrified and does not add to N reserves in the soil other than indirectly through uptake and return of plant residues. Similar estimates were made for the proportions of polyphenols and lignin returned to the soil.

During the ley phase, residues are assumed to be added to the soil on three dates during the year (in mid-May and at the end of July and October) and start to decompose immediately. The N release characteristics of the residue are determined by a simplified version of the algorithm used for the cropping phase of the model to obtain Fast_N, Med_N and OM_N fractions based on the C:N ratio and lignin and polyphenol concentrations (see below). Mineral N release from the Fast_N and OM_N fractions is estimated assuming a zero-order relationship between cumulative mineralisation and degree-days: N release from the Med_N fraction is determined by a similar, first-order relationship. For Fast_N and OM-N, the N mineralised between each residue input date and the end of the input year is subtracted from the initial Fast_N or OM_N to determine the amount remaining at the end of the year (Figure 9). The Fast_N remaining from the three input dates is then combined into a single mineralisation stream that continues for the remainder of the ley period. The total mineralisation is subtracted from the initial Fast_N to determine the Fast_N remaining at the end of the ley; similarly for OM_N. There are similar calculations for the fresh inputs in each of the remaining years of the ley.

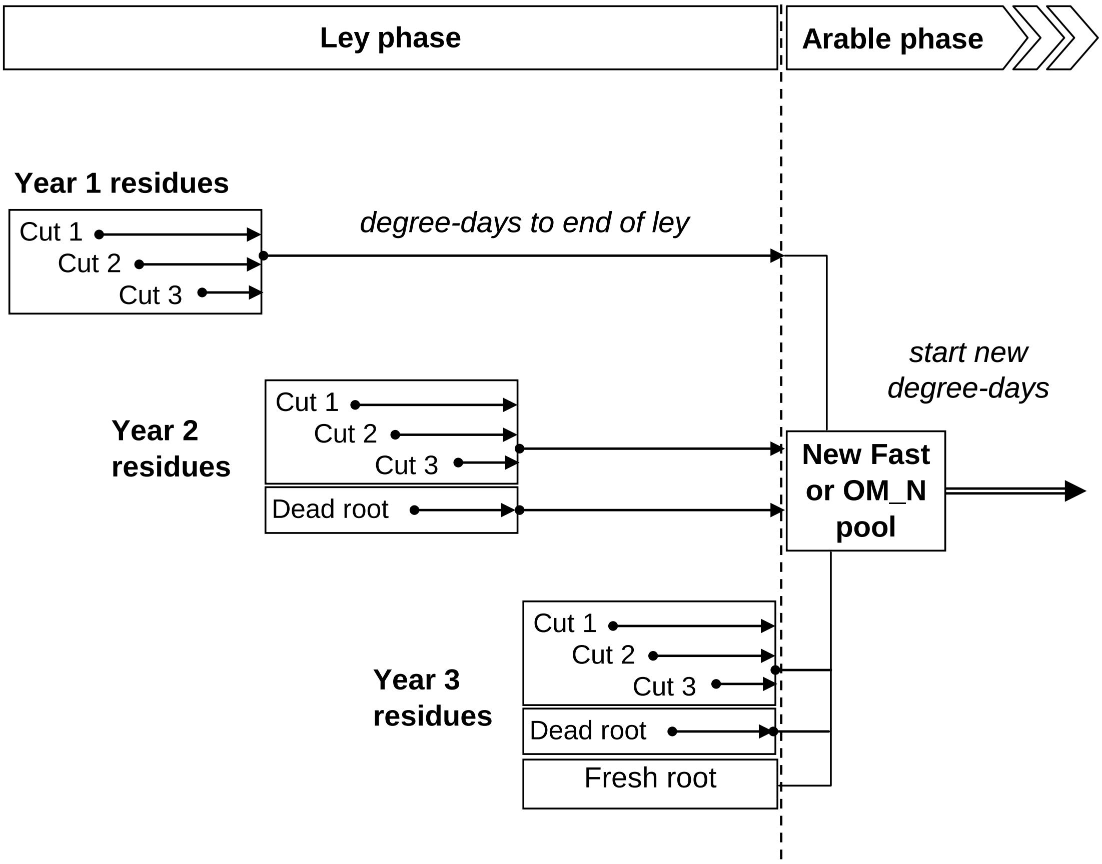

Figure 9. Flow-diagram of the steps in the FBC-Link model to estimate mineralisation of Fast_N and OM_N from residues during the ley and the quantity remaining in these fractions at the end of the ley. Arrows denote separate mineralisation streams that act to reduce the N remaining from the initial residue input.

The mineralisation of N from roots and stubble is modelled in a similar way except that there is only a single input each year at the mid-point of the year or part year. Fine roots are assumed to decompose rapidly and not to contribute to the ley residues. Larger roots and stubble are treated together and are assumed to live for 1 – 2 years. None of the root-N enters the residue pool in the year that the root is produced but 60% is assumed to die and contribute to residues in the second year. The remaining N is assumed to be recycled within the root system. At the end of the ley, the sum of Fast_N remaining from previous years' above- and below-ground residues is added to the Fast_N from the fresh root and stubble added to the soil at cultivation to obtain the total Fast_N from the ley; similarly for OM_N. These are treated as a fresh residue with the degree-day decomposition period restarting at zero.

Release of mineral N from the Med_N fraction is assumed to be described by a first-order, temperature-dependent process. Degree-days between the residue input and cultivation of the ley determine N release during the ley phase and the amount of Med_N remaining at cultivation. However, unlike the Fast_N and OM_N, this is not treated as a single fresh residue at the end of the ley. Instead, the cumulative Med_N release from each year's residues is allowed to continue without interruption into the cropping phase, keeping the residues from each year of the ley as separate N-release streams during the cropping phase.

As the soil is not cultivated during the ley phase it is probable that there would be an additional flush of mineralisation from the previous years' residues when the soil is disturbed by ploughing at the end of the ley (Gurr, 1974; Silgram & Shepherd, 1999). This could be incorporated into the model by transferring a proportion of the Med_N remaining from the years before the final ley year to the Fast_N fraction. This step was not included in the current version of the model because of the absence of any measured data about the mineralisation properties of the LBM residues that could be used to quantify the effect.

The mineral N that is released from these fractions during the ley phase is assumed to be either taken up by the ley, leached or denitrified and does not directly contribute to the soil N at cultivation. The N in the residues added to the soil each year originates from fixation of atmospheric N by the legumes plus uptake of the mineral N from previous residues. A final adjustment is to add a quantity of N directly to the mineral N pool to represent urine-N if the ley has been grazed within 12 months of the cultivation date. A maximum of 30kg N ha^{-1} is assumed if the ley is grazed immediately before cultivation. For longer periods between grazing and cultivation, the amount of urine-N is reduced in proportion to the length of this interval and a variable leaching/uptake factor appropriate to the time of year.

Modification of the mineralisation calculations to include the effect of the lignin and polyphenol content of residues

As the objective was to examine whether the FBC model can be adapted to include the effects of high-lignin or high-polyphenol residues, we started from the existing procedure for estimating N mineralisation in which residue N is divided between Fast_N, Med_N and OM_N fractions (as developed in Defra Project NT2501) as follows:

Fast_N (kg ha^{-1}) = [58.9 – 1.41C/N] x Total N (kg ha^{-1})/100
Med_N (kg ha^{-1}) = 0.9 x [Total N – Fast_N]
OM_N (kg ha^{-1}) = 0.1 x [Total N – Fast_N]

Mineralisation of the Fast_N fraction proceeds as a zero-order reaction over a period of 800 degree-days (equivalent to approximately 3 months). Mineralisation of the Med_N fraction does not start until the end of the Fast_N period and then proceeds as a first-order reaction with an exponential constant (-*k*) selected for 90% of the fraction to be mineralised in 10,000 degree-days (equivalent to approximately 3 years). OM_N mineralisation also proceeds as a zero-order reaction determined by degree-days and a decay constant for the particular soil type. The options for modifying N release characteristics within the model are therefore to include concentrations of lignin and polyphenols with C:N ratio in the division of N between the Fast_N, Med_N and OM_N fractions and/or to vary the degree-day periods over which these fractions are mineralised. Various studies have shown significant correlations between N mineralisation and lignin:N, polyphenols:N or (lignin + polyphenols):N ratios (Fox *et al*., 1990; Handayanto *et al*., 1997; Mafongoya *et al*., 1998; Seneviratne, 2000; Tscherning *et al*., 2006; Nakhone & Tabatabai, 2008); however, there is little consistency about which of these parameters, or C:N ratio, provides the best prediction of N release. In part this can be explained by the different ranges of N, lignin and polyphenols in the materials studied (Myers et al. 1997, Jensen et al. 2005). Vigil & Kissel (1991) reported a closer correlation between mineralisation and N plus lignin:N ratio as separate variables than with the C:N ratio alone. Tian *et al*. (1995) developed a Plant Residue Quality Index that combined terms for C/N and concentrations of both lignin and polyphenols. This index was correlated with N mineralisation rates and with other decomposition related processes.

The significant correlations reported between mineralisation and the simple sum of lignin and polyphenol concentrations in the (lignin + polyphenols):N ratio suggested that the scale of effect of the two factors is broadly similar (when both are expressed in the same units) and it was unnecessary to include a weighting factor in the initial development of an expression for use in the revised model. Where significant correlations occur with mineralisation, both the C:N and (lignin + polyphenols):N ratio are inversely related to N mineralisation rate and their effects can be expected to be additive. The additional factors were therefore included in the algorithm to determine the Fast_N fraction by replacing C/N with either C/N + [L + Pp]/N or C/N + [L + Pp], where [L + Pp] refers to lignin (%) + polyphenols (%). As the OM_N fraction has only a small influence on the shape of the N release curve, its calculation was left unchanged and the lignin + polyphenols factor was only applied to the division between the Fast_N and Med_N fractions. Initial tests indicated that the shape of the response curve produced by the combination of (C/N) + [L+Pp] was most like those described in the literature and this expression was adopted for subsequent tests (see Results, section 3.2.7, p. 37).

In addition to including lignin + polyphenol contents with C:N ratio in the proportioning of the Fast_N and Med_N fractions, the shape of the N release curve can also be adjusted by varying the number of degrees-days over which the Fast_N and Med_N are mineralised. While it has been clearly demonstrated that lignin and polyphenols slow the initial rate of N release, there is uncertainty about the duration of this effect (Palm & Rowland, 1997). Some incubation studies comparing high and low polyphenolic residues show N release curves converging during the course of the incubation while others indicate a more extended inhibition of N release. Current understandings of the processes involved describe the products of lignin breakdown and polyphenol-protein complexes as contributing to the formation of soil humus, suggesting a relatively long turn-over time. There are also indications that polyphenols might have the greatest influence in the early stages of decomposition with the lignin + polyphenols:N ratio being a better indicator of longer-term effects (Oglesby & Fownes, 1992; Palm, 1995; Mafongoya *et al.,* 1998; Trinsoutrot *et al*., 2000; Verkaik *et al*., 2006). Though there is insufficient information to quantify the duration of the effects of lignin and polyphenols, an additional step was included in the model calculations to allow the increasing concentrations of lignin and polyphenols to slow the rate of mineralisation by extending the periods over which Fast_N and Med_N are mineralised. This provided a further mechanism for adjusting the shape of N release curves and for fitting curves to future, measured data. The mineralisation periods were linked to lignin + polyphenol concentrations by a linear relationship that was arbitrarily set to increase the 800 and 10,000 degree-day Fast_N and Med_N periods by 50% at the maximum lignin + polyphenol content (assumed to be 40%). When lignin and polyphenol concentrations are zero, the new method of calculation is effectively the same as in the original FBC model.

In the absence of any measurements of N mineralisation rates for the LBM residues in the LegLink study, the performance of the modified mineralisation procedure in the model was assessed against measured data obtained from the report of a study by Vanlauwe *et al*. (Vanlauwe *et al*., 2005). This provided information about the chemical composition of 30 organic residues from a range of tropical species together with values of N mineralisation determined from a 28-day incubation at 25^{o}C. Concentrations of N in the residues were in the range of 0.6 – 5.3% (C:N ratio 8.3 – 70.9). Corresponding ranges for lignin and polyphenols were 2.5 – 23.7% and 0.8 – 14.8%, respectively. Although it would have been preferable to compare the model predictions against data from studies of temperate rather than tropical species, no suitable datasets were found. A study by Trinsoutrot *et al*. (2000) could potentially provide the required information for a range of temperate crop residues but the raw data were not readily available. In view of the other uncertainties about the decomposition properties of the LBM residues, the data of Vanlauwe *et al.* (2005) were considered sufficiently representative of the effects of lignin and polyphenol contents at this stage of model development.

These changes to the way in which mineralisation was calculated were confined to the estimates of N release from ley residues. Nitrogen mineralisation from other residues during the cropping phase was estimated as in the original FBC model. Other aspects of the FBC simulations that are known to require improvement were outside the scope of the present study and were not changed in the LegLink version.

Changes to the user interface

The model simulations require sufficient information about the management and performance of the ley to be able to estimate how much N is present in the soil at the start of the arable cropping period. Although more information is needed to describe the performance of the multi-species

LBMs than for the simple one- or two-component ley options in the original FBC model, the additional data requirements were kept to a minimum in line with the original concept of producing an easy-to-use model. The two-species mixtures in the original FBC model only required the user to select whether the ley contained a high, medium or low proportion of legume but this is insufficient for more complex mixtures where individual species may differ widely in their contribution to the amount of N accumulated by the ley and to its subsequent mineralisation. The LegLink version of the model therefore requires the user to either enter information about the proportion of each component species in each year of the ley or to select the option of using default values for these proportions. The default values for the proportions of species in each mixture in Years 1 – 4 of the ley are listed in Appendix Table A2. Year 1 proportions were based on the output from the growth model developed in WP2. Proportions in later years were based on the survival of the species in the All Species Mixture and information about their expected persistence from published sources but all should be treated as temporary values, particularly as the same proportions were assumed for all managements, soil types and UK regions. Information from WP3 demonstrates the wide variation that can occur in practice.

Table 7. Input data required to be entered by the user for the LegLink version of the FBC model (only site and ley descriptors shown)

Site description	
UK region	- from a list (used to set weather data)
Annual rainfall	- optional: if set to zero, model uses default for region
Soil type	- from a list
Previous cropping	- select grassland, long-term arable or ley/arable
Ley description	
Type of ley	- Mix A, Mix A + grass, Mix B or clover/grass
Ley sowing date	- sets start of ley period
Ley cultivation date	- sets end of ley period
Date of last grazing	- optional (determines any mineral-N from urine)
For each year of the ley:	
Type of management	- cut, mulched, grazed or a combination of these
% removed by grazing	- % of herbage removed by grazing during the year
Manure applied to the ley	- none, light application or heavy application
Annual yield of ley	- vigorous, moderate or poor (approx. 15, 10, 7 t DM/ha)
% of each species in ley	- up to 6 species + weeds: default or user-entered values

As well as requesting more detailed information about the composition of the ley, other changes were made to increase the range of managements to which the model could be applied. These were designed to increase the flexibility of the model rather than being direct consequences of adapting it for use with the LBMs. The information requested about management of the ley was changed to allow mixed cutting and grazing regimes and different managements in individual years, rather than a single form of management for the full ley period as in the earlier model. The revised input data required to describe the site and ley phase in the LegLink version of the model are listed in Table 7. Other changes from the original organic farming model were the addition of a section to enter information about any inputs of mineral N fertiliser during the cropping phase and a section to allow the user to enter information about irrigation events. The user can skip these sections if not required.

The way the results of the simulation are presented was also changed. Tables of values were replaced by graphs to improve clarity and to avoid giving a false sense of precision. The output now consists of four graphs showing:

- Output Graph 1. Achieved crop yields as a percentage of the potential yield if N was non-limiting
- Output Graph 2. Annual N losses expressed as an equivalent fertiliser cost
- Output Graph 3. Monthly N release from ley and crop residues, manures and soil organic matter
- Output Graph 4. Monthly patterns of N loss compared with the surplus or deficit of soil mineral N

To enable the user to see the effect of adjusting the input data on yields and losses, Graphs 1, 2 and 4 also show the corresponding values from the previous simulation. These are updated each time a fresh simulation is run.

The ease of use of the revised model and clarity of the output graphs were tested at a workshop for farmers and advisors in February 2012. Opinions were collected by circulating a questionnaire following a presentation describing the main features and use of the model.

3.2.8. Legume cover crops in conventional rotations (WP6)

This experiment was undertaken at the NIAB TAG site at Morley, Norfolk and was initiated in autumn 2007 as part of the NIAB TAG New Farming Systems (NFS) study (supported by The Morley Agricultural Foundation and The JC Mann Trust). This research provided a structure to facilitate the comparison of the ASM both against standard practice and other cover crop approaches. The other cover cropping approaches were a clover bi-crop and a brassica cover crop (fodder radish, *Raphanus sativus*). Where adopted cover crops were sown at 10 kg/ha typically in late August or early September and were destroyed and incorporated pre-drilling of the spring crop. The legume bi-crop system in the cover crop study was a small leaf white clover (cv. AberPearl); this was sown is August 2007 and allowed to naturally regenerate each season. Inputs to the bi-cropping system have otherwise been as the ‘current practice’ system.

Treatments were set out as a series of rotational progressions in combination with associated cover cropping practices and nitrogen (N) inputs. All rotations grow wheat every second year, the year between is a break crop. In 2009 the winter oilseed rape in rotation 1 was over-drilled with spring oilseed rape due to establishment issues, otherwise all treatments were as protocol. The distinct plot areas have remained in position since the start of the project. The soil series is Ashley and the soil texture is a sandy loam.

Experimental layout

The experiment is an incomplete factorial design with four replicates. Each main plot is 12m wide and 36m long, however each plot is subdivided into three 12m by 12m areas to deliver the differing N regimes. Further detail of the treatments and the design is presented in Table 8.

Establishment and inputs

The trial area is cultivated each season ahead of crop establishment using a shallow non inversion technique; the specific technique varies according to season and crop but typically targets a 10-15cm cultivation depth. Nitrogen doses were determined according to cropping practice (see Results section 3.3.6). Specific drilling dates vary according to season but crops (and cover crops) are sown in keeping with local best practice and seed rates are appropriate for the prevailing conditions. All inputs are consistent with local best practice and in keeping with guidance followed by NIAB TAG Network members.

Table 8. Treatment and rotational progression details for the 'cover crop' experiment.

System	**Rotation**	**Cropping and harvest year**				
		2008 (Year 1)	**2009 (Year 2)**	**2010 (Year 3)**	**2011 (Year 4)**	**Comments**
1	Winter break	ww	wosr	ww	wbn	A conventional approach that can be used as a benchmark for current systems.
2	Spring break	ww	sosr	ww	sbn	Spring crop approaches that may help maximise the benefits of autumn cover/clover systems.
3	(Un)balanced	sw	sosr	ww	wbn	Spring cropping in years 1 and 2 followed by winter cropping.

Cropping key – ww (winter wheat), sw (spring wheat), wosr (winter oilseed rape), sosr (spring oilseed rape), wbn (winter bean), sbn (spring bean).

Three rotations (as in Table 8 above)

Four management systems:

- Current; rotation 1-3 run as standard with regard to fertiliser inputs and husbandry.

- Legume (clover bi-crop); rotation 1-3 using clover as a legume bi-crop to augment fertiliser.
- Current plus a brassica cover crop (fodder radish); rotation 2 and 3, only with autumn cover crops prior to a spring sown crop.
- Current plus a legume cover crops (ASM legume species mixture); rotation 2 and 3, only with autumn cover crops prior to a spring sown crop.

Three Nitrogen management approaches:

- N doses applied across treatments as a banded dose i.e. each plot 36m x 12m plot is sub-divided into 12m x 12m sub-sections and each sub-section receives one of the following N doses -
- 0% of standard dose for the crop being grown
- 50% standard dose for the crop being grown
- 100% standard dose for the crop being grown

3.2.9. Satellite project: Legume mixtures and pollinators

Ten field sites were chosen from 34 Farm sites participating in the LegLINK project. All chosen sites are within organic farmland and all legume mixtures were sown in 2009. Sites were chosen on the basis of management type, and were either grazed by sheep (between 9-10 ewe/ha) or regularly mown. At three sites, ewe grazing was frequent between February 2010 and September 2010. One site was grazed after being cut for hay in June. One site was cut for hay in June followed by subsequent mowing. All other sites were cut throughout the year, and all cuttings were mulched. Study sites were planted with 0.5ha of the diverse legume mixture (All Species Mix, ASM) within the farmer’s standard legume ley and compared to a control of standard legume ley selected by individual farmers.

A 100m pollinator observation transect, walked at a continuous pace of 15m/min, was undertaken at each legume plot within the sample site in the morning and repeated in the afternoon, between 10:00-17:00, under standard weather conditions as stipulated in the Butterfly Monitoring Scheme (BMS). All pollinators were recorded where possible to recognisable taxonomic unit (RTU) within 2.5m of the transect line. Pollinators not identifiable on the wing were captured for later identification. The flora of each transect was recorded as flower abundance (number of flowers) and species diversity using five 0.5m^2 quadrats placed at random along length of transect, at the start of each visit to study site. Monthly observations were carried out between April and September.

3.3. Results

3.3.1. Field characterisation of legume and grass species (WP1)

Overview

The field trials in WP1 were set up to answer three research questions: (1) What is the agronomic performance of 12 legume and 4 grass species grown in monocultures at six locations across the UK? (2) What is the effect of using a complex species mixture, the All Species Mix, relative to the use of monocultures? And (3), what is the effect of rhizobial inoculation on the All Species Mix?

While this section (3.3.1, pages 49–80) focuses on the results concerning the first question, the other two questions are dealt with in section 3.3.7 (page 122) and 3.3.8 (page 126), respectively. Results are shown in chronological order of the response variables, i.e. from selection of species to establishment of the crop measured at the beginning of the trials, to biomass measurements throughout the growing seasons, to post-incorporation variables determined at the end of the field experiments.

Selection of legume and grass plant species for trialling

The result of the multivariate analysis of published data on 22 legume species is shown in Figure 10.

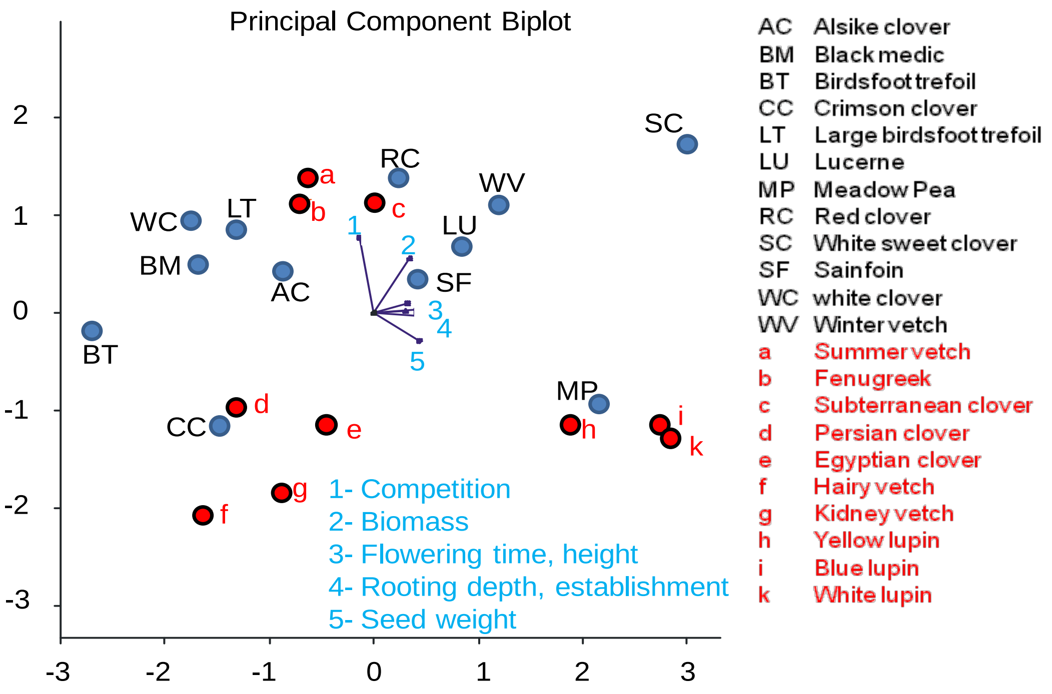

Figure 10. Principal Component Analysis (PCA) biplot for 22 legume species; species selected for field trialling are shown in black font and with blue circles; deselected species are shown in red letters and with red circles. The PCA was based on published data.

Species were selected for inclusion in the field trials so as to minimise redundancy, e.g. if two species were close to each other in the PCA, only one of them was selected for the trials. In addition, selection was based on further criteria apart from the PCA, such as ready availability of seed.

Emergence and early development

With repeated seedling counts of the sown species in the replicated trials, two aspects of crop emergence could be assessed. First, the speed of emergence was determined for each species by calculating the number of days after sowing when a threshold proportion of sown plants had emerged. This threshold was set as 5% of sown plants. By further calculating the number of days that each species deviated from each site average, it was possible to differentiate between slow and fast emerging species across sites, with negative and positive differences indicating fast and slow emergers, respectively. Second, the maximum proportion of sown plants that had emerged at each site was determined.

With regard to the speed of emergence, CC was found to be the fastest species. In comparison with WC, three species (CC, LU and RC) were significantly faster to emerge, whereas one species (LT) was significantly slower (Figure 11). Among the grasses, TY was significantly slower to emerge than PR. As groups of species, the grasses and legumes were not significantly different with regard to the speed of emergence.

The proportion of sown plants that emerged varied among the species (Figure 12) and among sites (Table 9), with Barrington Park and the IBERS site showing the lowest and highest rate of emergence, respectively. Wakelyns was the site with the slowest emergence (Table 9).

RC, CC, LU, MP and SF showed significantly higher emergence rates than WC, with RC showing the highest average rate across the six trial sites (Figure 12). Among the grasses, TY was significantly lower than PR with regard to the proportion of sown plants that emerged, while IR and MF were significantly higher than PR.

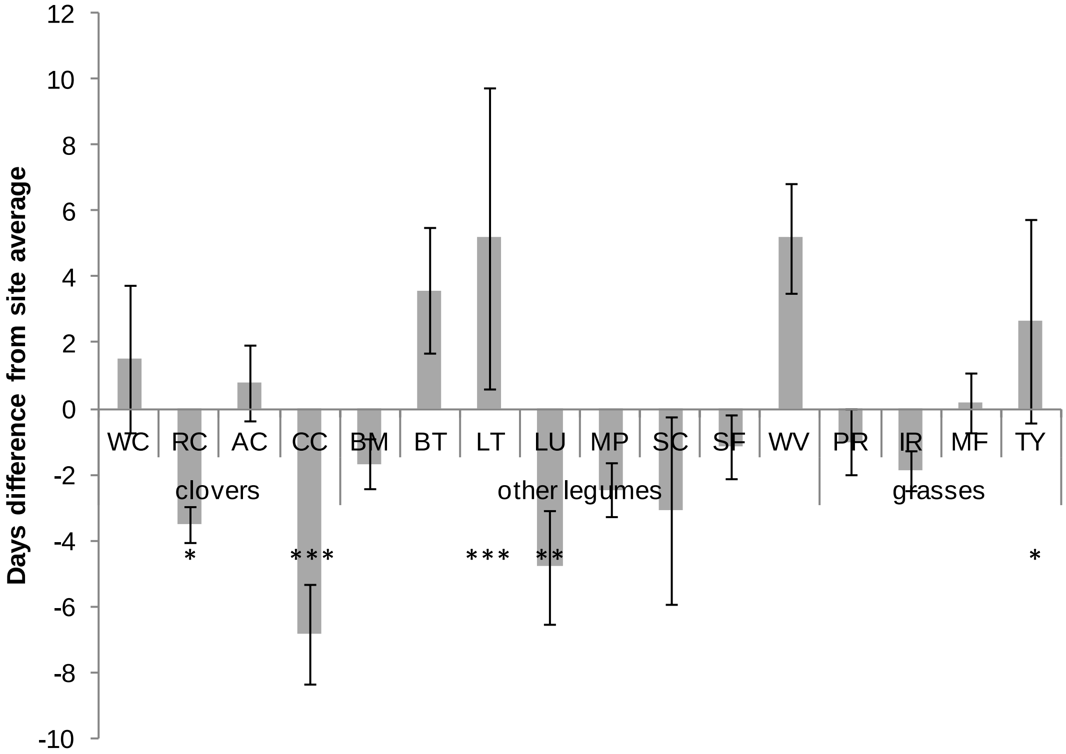

Figure 11. Speed of emergence measured as days when the species reached a threshold proportion of sown plants; the graph shows the differences in days from the site average, with means and standard errors of these differences over all six sites. Negative differences mean fast emergence, positive differences indicate slow emergence. Stars indicate the significance for the comparison of the legume species against WC and of the grass species against PR.

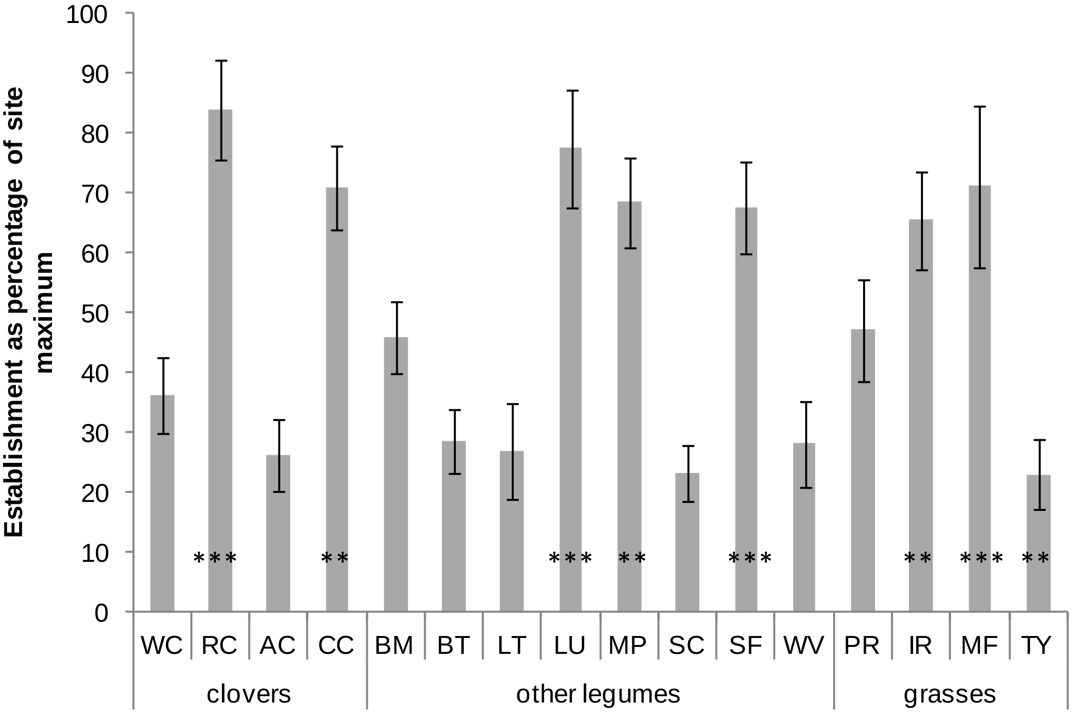

Figure 12. Proportion of sown plants that emerged, expressed as percentage of site maximum; means and standard errors over all six sites. Significance stars as in previous figure.

Table 9. Emergence of legumes and grasses: average, maximum and minimum values across all sites.

		D	**E**	**I**	**R**	**S**	**W**
Proportion of sown plants established	average	36.7	12.0	50.4	20.1	30.3	35.1
	max	70.4	24.3	82.9	46.5	89.8	61.6
	min	6.0	2.2	21.7	4.7	2.7	14.1
Days after sowing reaching the threshold	average	15.5	16.6	4.1	11.1	10.9	21.8
	max	27.1	26.6	13.1	25.2	19.4	32.4
	min	11.2	4.6	1.2	1.7	2.6	16.5

Further, the early development of the different species was assessed with repeated measurements of above-ground biomass of individual seedlings at four sites (data from Barrington Park had to be discarded because of inaccuracies). All the data gathered in these seedling growth measurements was used to build the ecophysiological model in WP2 (section 3.2.4). In addition, two aspects of early development are reported here. Seedling biomass at 60 days after sowing (DAS) was calculated by linear interpolation of biomass data between two dates nearest to, and either side of the 60 DAS date.

In addition, the relative growth rate of the seedlings was calculated for each plot as biomass increase per day per g biomass: $R_{i,j}$ =[(B_j- B_i)/B_i]/(d_j-d_i), where B is the biomass and d_i and d_j are two consecutive dates of the biomass measurements. Seedling biomass measurements were taken more than two times (except at Barrington Park). For each site we then determined the date with the maximum growth rate (averaged across the species). Here we present the growth rate data only from these dates of maximum growth (Figure 13).

The species with the lowest **seedling biomass** was LT; species with high seedling biomass include WV, IR, MP, and SC, with WV having a significantly larger biomass than WC. Also, LT was the species with the lowest **seedling relative growth rate**. The highest relative growth rates were found in SC, AC, and RC; comparing the legume species to WC revealed no significant differences (e.g. the difference between LT and WC was non-significant, p=0.257). However, compared to SC, i.e. the species with the highest relative growth rate, several legume species showed significantly lower relative growth rates (CC, LT, MP, SF and WV).

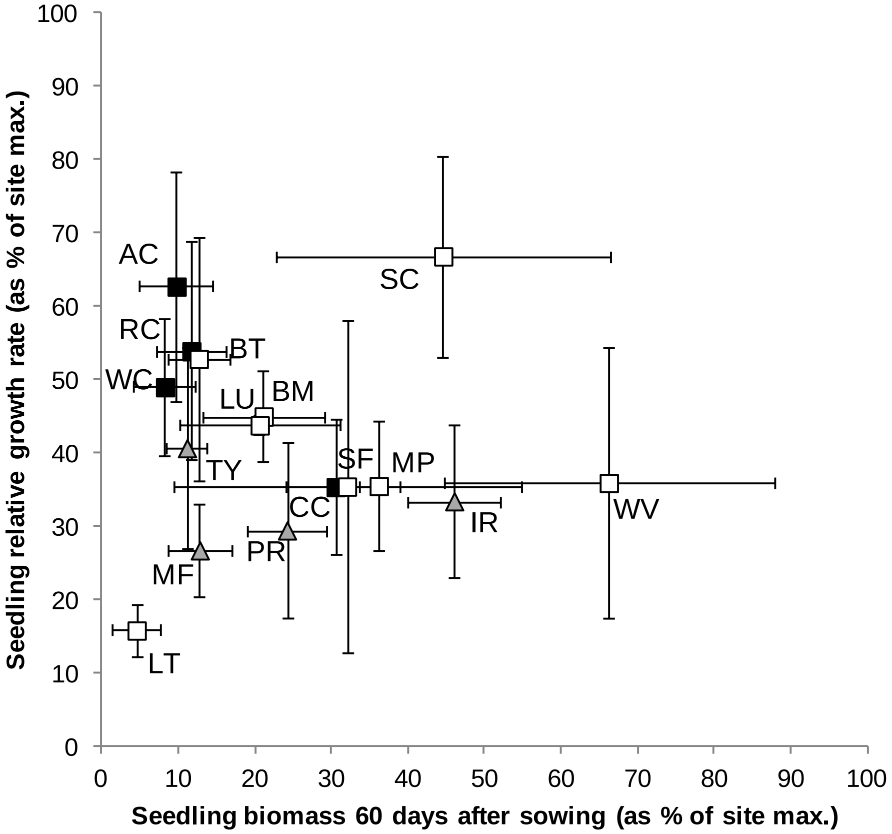

Figure 13. Seedling relative growth rate and seedling biomass; means and standard errors across four sites (Duchy, Rothamsted, SAC, Wakelyns).

Productivity: Plant height, crop cover and biomass

At all research hub sites, **plant height** was measured repeatedly over the first growing season (2009) and at 3 of the sites in the second growing season (see Figure 14 for an example). Plant height can be used to assess the ability of plants to compete for light with other species, a trait that is of high importance for compiling viable species mixtures (see section 3.2.4, page 25 and section 3.3.2, page 85). Also, measuring plant height before and several times after mowing provides insights into a species' response to ley management (such as cutting).

In both 2009 and 2010, plant height ranged widely among the different legume and grass species and among trial sites (Table 10). Figure 15 shows the plant height at the point in time just before the first cut of each of the two growing seasons (2009 and 2010). IR was consistently among the tallest species in both years, while LT was among the smallest. Species that were relatively tall in the second but not in the first year include MF, TY and RC, whereas species with the opposite trend were MP and WV.

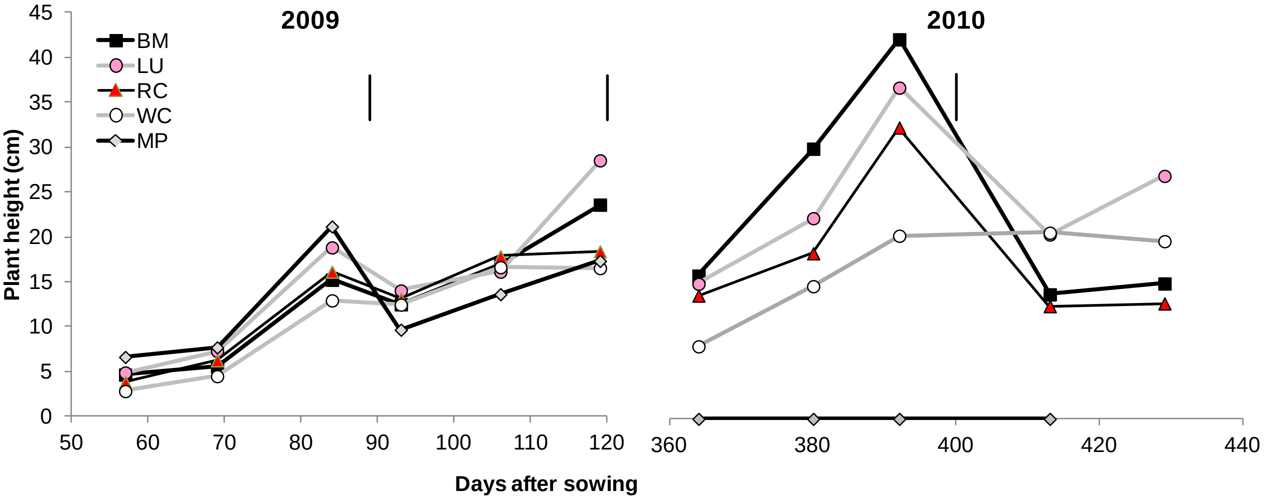

Figure 14. Plant height (cm) of selected legume species at the WP1 trial site in Suffolk (Wakelyns Agroforestry) in 2009 and 2010; vertical bars indicate time of mowing; the second mowing in 2010 (not shown) happened after the last assessment of the season. Note the steep decrease of plant height in MP after the first cut in 2009; after a further cut at 120 days after sowing, MP did not recover anymore and was completely absent from the trial in 2010.

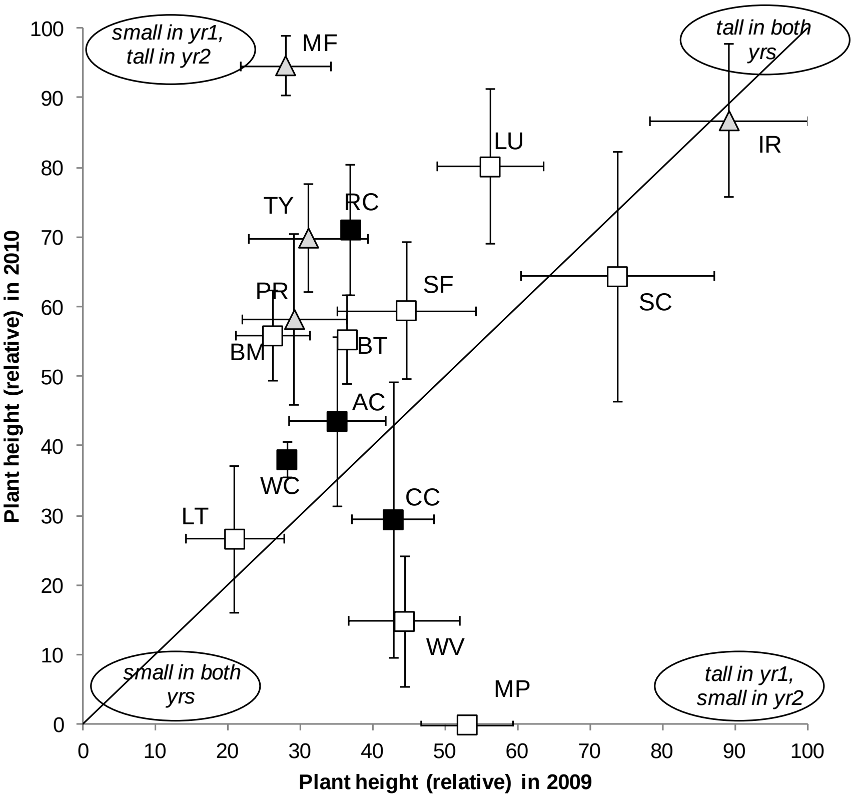

Figure 15. Plant height before the first cut of the season, expressed as percentage of site maximum, in the first year of the trial (x-axis) and in the second year (y-axis), for grass species (triangles), clover species (black squares), and other legume species (open squares); means and standard errors over three sites (Duchy, SAC, Wakelyns) for which height data was recorded in both years.

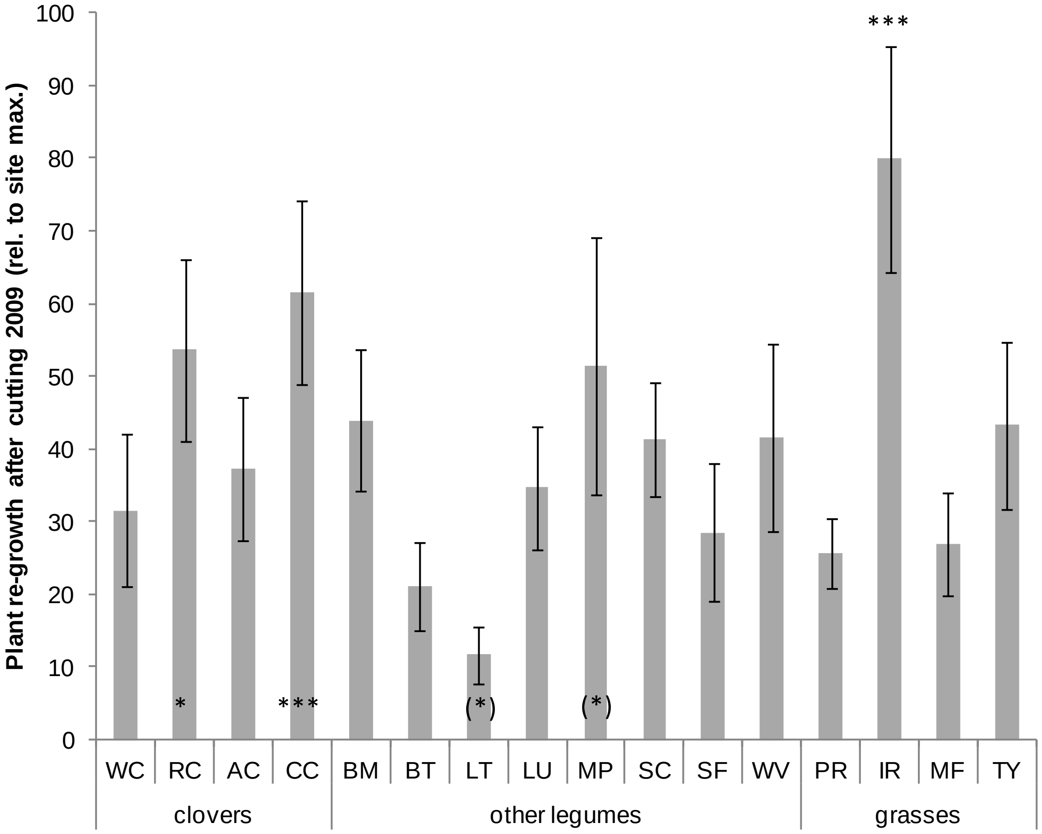

Figure 16. Plant regrowth (measured as cm increase per day) after cutting in the first trial year, expressed as percentage of site maximum; means and standard errors over sites. Stars indicate the significance for the comparison of the legume species against WC and of the grass species against PR.

Regrowth after cutting was measured as the increase of plant length in cm per day after the first cut in the first trial year (Figure 16) and it ranged widely among the trial sites (Table 10). Among the legume species, RC and CC re-grew significantly more than WC after cutting. Among the grass species, regrowth after cutting was consistently very high in IR; however, in year 2, regrowth was very high in MF (data not shown).

Table 10. Averages, maxima and minima of plant height pre-cutting and regrowth after cutting

		D	E	I	R	S	W
Height pre-cut (cm) (2009)	average	28.2	7.2	56.3	58.8	36.6	18.7
	max	68.6	14.4	106.3	102.9	83.1	45.4
	min	5.0	1.9	30.8	14.4	19.6	12.8
Height pre-cut (cm) (2010)	average	48.0	-	-	-	28.4	26.7
	max	75.7	-	-	-	54.7	60.9
	min	0.0	-	-	-	0.0	0.0
Regrowth (cm/day) (2009)	average	0.68	0.25	-	0.60	0.27	1.87
	max	1.85	0.78	-	1.43	0.58	4.69
	min	0.24	-0.03	-	-0.09	0.12	0.74

Crop cover (ground cover by the crop) is an estimated measure of productivity and depends on several factors, such as the germination rate and establishment rates of a species but also on its growth habit, with more erect species tending to have lower cover for the same amount of biomass. Also, crop cover depends strongly on the time of the year. In this study, crop cover was estimated at all six trial sites at the beginning of the second growing season, i.e. in spring 2010. Ground cover at this time therefore also reflects the ability of the species to produce new growth after the winter.

The species with the highest ground cover at this time was WC (Figure 17). The only species that was not significantly lower in crop cover than WC was RC. Among the other legume species BM showed consistently the highest cover, while the lowest values were found in MP and WV.

Overall, the grasses showed a significantly higher ground cover than the legumes. Comparisons within the grass species showed that IR was significantly lower in ground cover than PR, but not MF and TY. There was no significant difference between NM and IM with regard to ground cover.

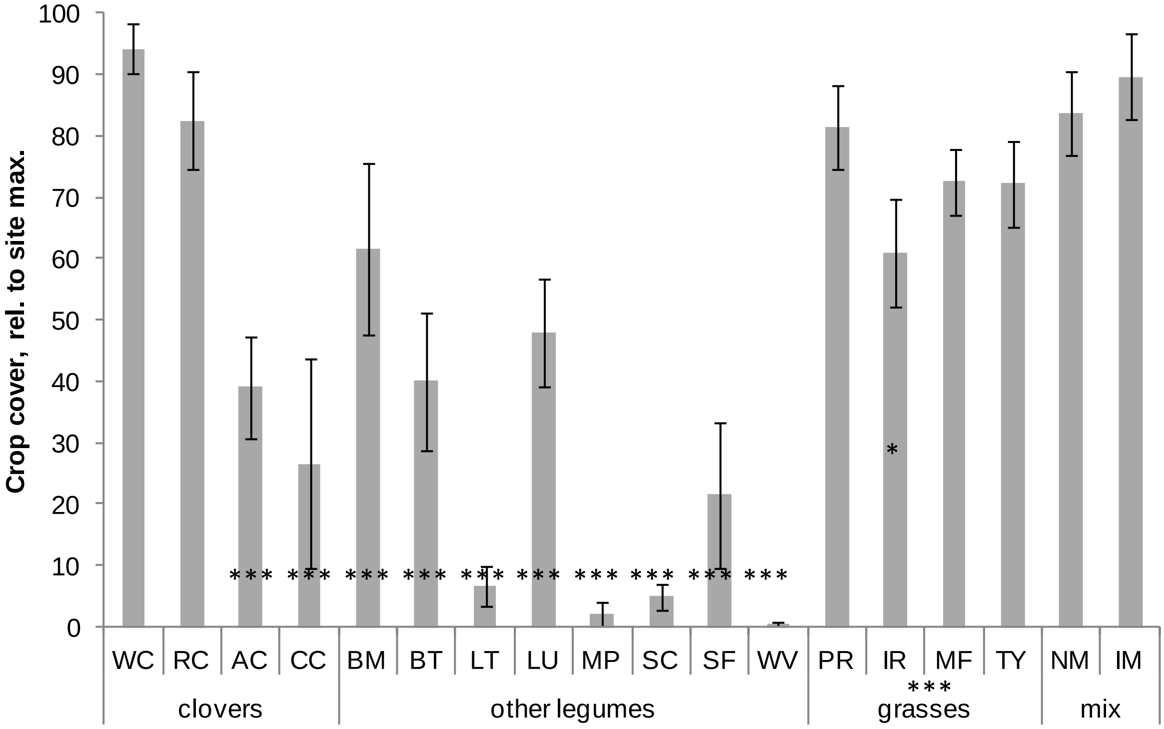

Figure 17. Crop cover after the first 12 months of the ley, in spring 2010, relative to site maximum; means and standard deviations over 6 sites. Stars across the bars indicate the significance for the comparison of the legume species against WC and of the grass species against PR. Stars above the ‘grasses’ label indicate the significance for the comparison between grasses and legumes.

Crop biomass was measured repeatedly at all sites in 2009; data from all dates was used to build the ecophysiological model (see 3.3.2, page 85). Here we present the data from one date for each site, at around 108 days after sowing (DAS; mean = 107.7, s.e. = 2.5, min. = 99, max.= 116), i.e. beginning to mid-August 2009. At this time, the species had regrown for 2–6 weeks after the first mowing.

The comparison between grasses and legumes revealed that collectively, the grass species had a significantly higher biomass than the legume species (Figure 18). Among the legume species, LT, MP, SC, SF and MF were all significantly lower in biomass than WC. While BM tended to have a higher biomass than WC, there was no species that significantly outperformed WC in terms of this biomass assessment in the summer of 2009. Comparisons among the grass species revealed that MF and TY had significantly lower biomass than PR. There was no significant difference between the inoculated and non-inoculated species mixes (NM vs. IM).

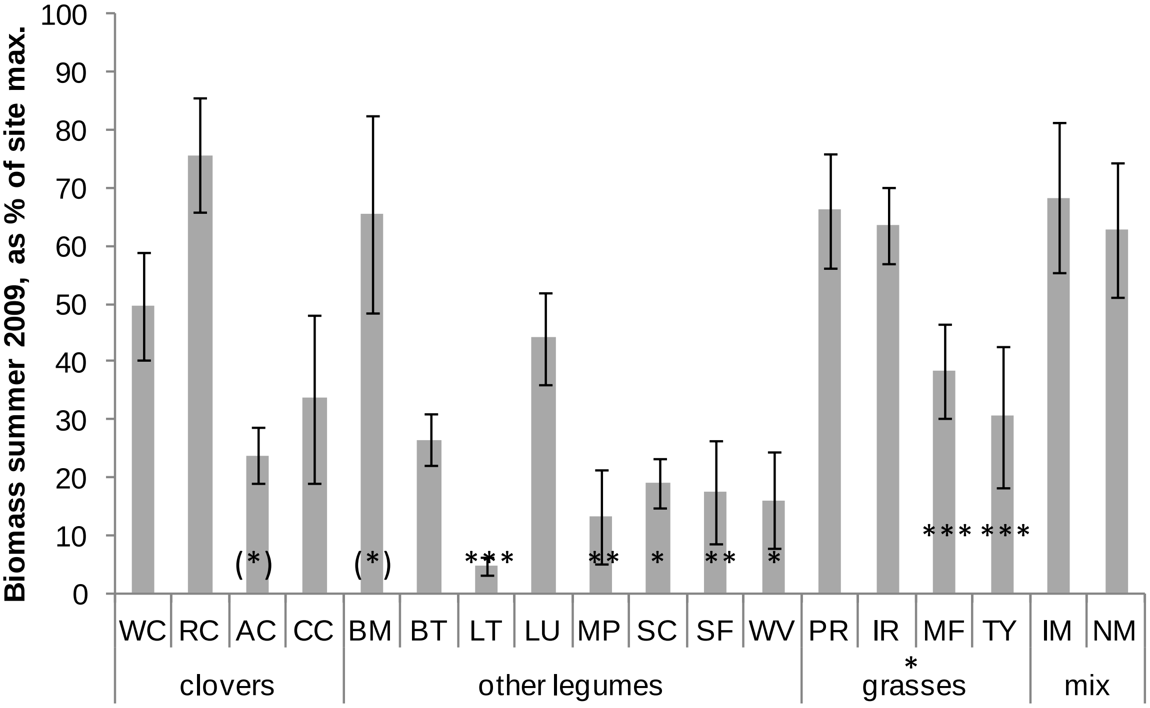

Figure 18. Biomass of legume and grass species in summer 2009, at 108±2.5 days after sowing. Means and standard errors across all 6 field sites. Stars across the bars indicate the significance for the comparison of the legume species against WC and of the grass species against PR. Stars above the ‘grasses’ label indicate the significance for the comparison between grasses and legumes.

An analysis of **stability** was conducted on the crop biomass data to investigate for each species how variable the biomass was among sites. Shukla’s stability variance and Wricke’s ecovalence were calculated for crop biomass from summer 2009 (~108 DAS, see Figure 18) with the *stability.par* procedure from the *agricolae* package in the programme R. This analysis revealed that variance was highest in TY and CC and lowest in BT (data not shown). As there was a tendency for the variance to increase with species means, we also calculated the coefficient of variation

(CV), which expresses variation as standard deviation in relation to the mean. High CVs were associated with low means; the lowest CV was found in IR (43%), the highest in TY (242%); among the legumes, highest CV was found in SF (224%), the lowest in BT (59%).

Competition against weeds

The ability of the different legume and grass species to compete against weeds was indirectly determined by monitoring weed cover at one of the sites, Barrington Park, five times during the trial, and by repeatedly measuring weed biomass at all of the other sites. At Barrington Park, the estimated weed cover first rose sharply after sowing, but then steadily decreased over time (Figure 19). This reflects the appearance of annual weeds after sowing and the subsequent disappearance in response to repeated mowing. Throughout the trial period, the variation in weed cover among the different species was large at the Barrington Park site (Figures 19 and 20). A list of weed species found at Barrington Park can be found in the Appendix.

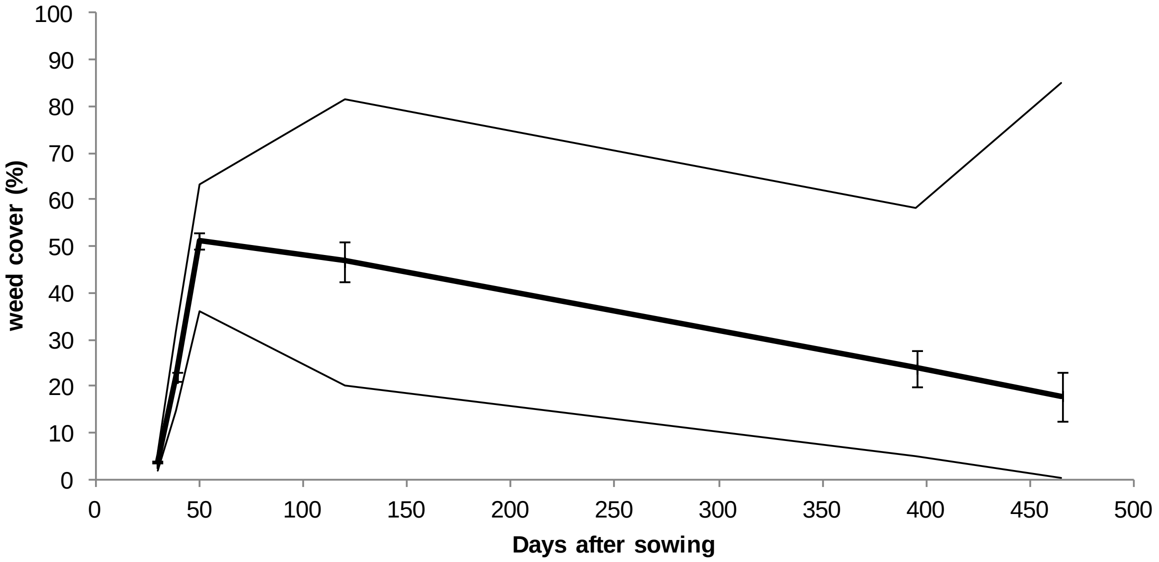

Figure 19. Estimated weed cover (%) at Barrington Park over time; average (bold line, with error bars indicating standard errors) as well as minimum and maximum (thin lines) for each assessment date.

In Figure 20, estimated weed cover at Barrington Park in the first trial year (2009) is plotted against estimated weed cover in the second year. The area in the lower right part of the graph, below the diagonal line, contains points that have a lower weed cover in 2010 than in 2009; all observed data points were either *on* this line (WV) or *below* it, i.e. even those species that showed very low crop cover (e.g. MP) showed a reduction in weed cover over time. The graph also reveals a positive relationship between weed cover in 2009 and weed cover in the following year (linear regression of 2010 weed cover against 2009 weed cover: y= ax+b with a= 0.6472 ± 0.120; b=-6.41 ± 6.082; adjusted R^2 = 0.346; p<0.001).

Among the species with the lowest weed cover in both years is RC. Species with a comparatively low weed cover in the second year are WC and TY. LT and MP show the highest weed cover in the first year.

With regard to weed biomass, there was a strong negative relationship between above-ground crop biomass and weed biomass (Figure 21). Interestingly, all grass species are below the regression line, i.e. their reducing effect on weeds is higher than it would be expected from their above-ground crop biomass. Similarly, some legume species, given their crop biomass show a large weed biomass for what would be expected from the relationship between crop biomass and weed biomass, e.g. in view of its high crop biomass, BM has a relatively low competitive (reducing) effect on weeds.

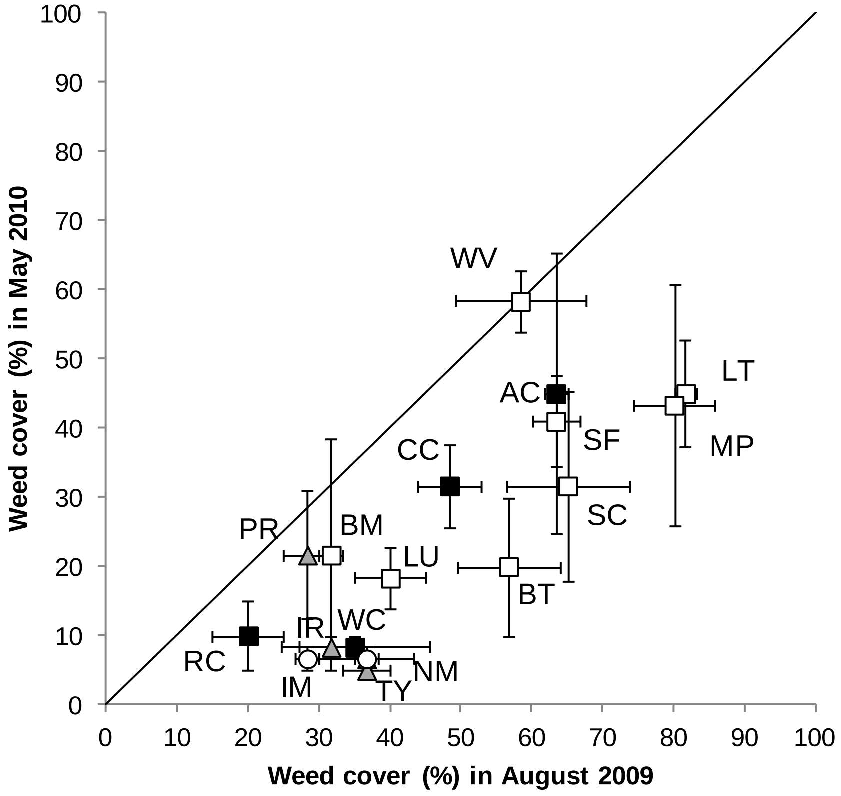

Figure 20. Estimated weed cover (%) at Barrington Park on 17 August 2009 vs. weed cover on 19 May 2010; means and standard errors (3 replications).

Later, when the sampling was repeated in 2010, the regression line was flatter, i.e. the relationship between crop biomass and weed biomass was not as strong as before. However, in spring 2011 (pre-incorporation, data not shown), the picture was similar as before, i.e. as shown in Figure 21.

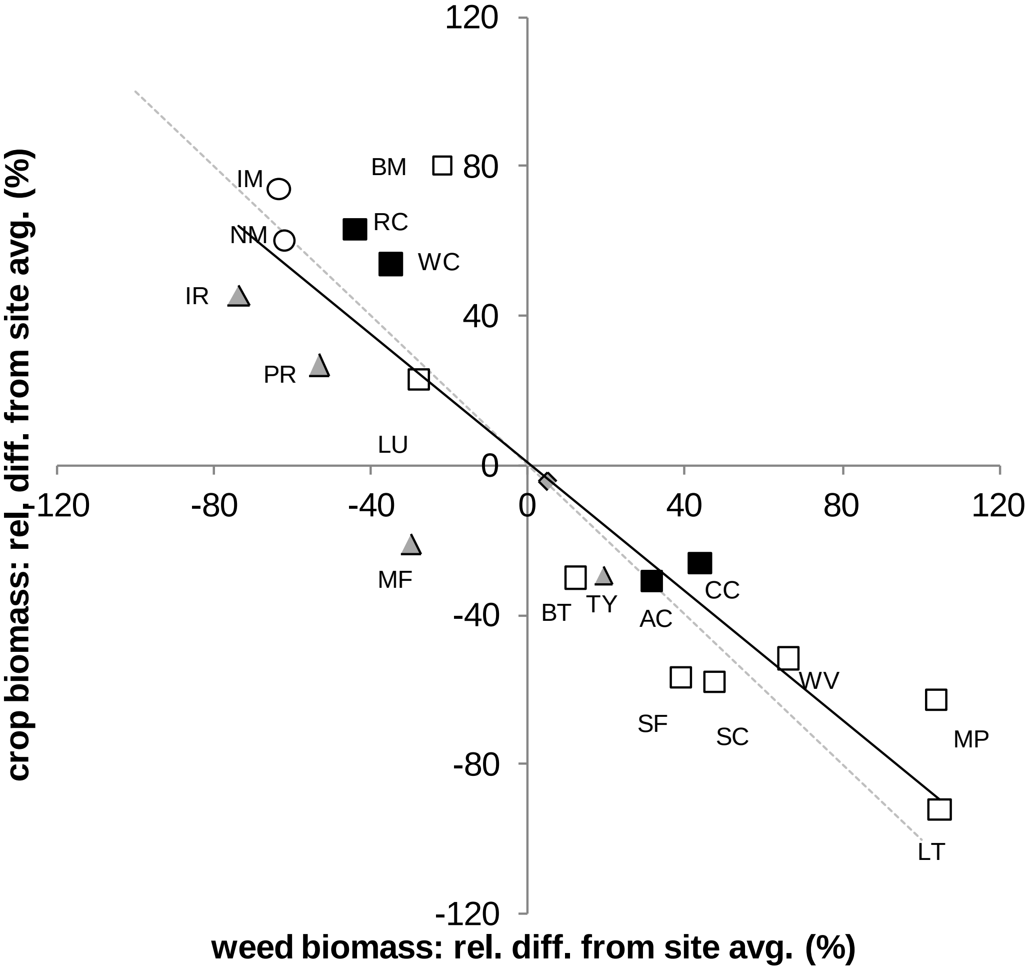

Figure 21. Relationship between weed biomass and crop biomass in early-autumn 2009. Grey diamond: average of monocultures (only ASM components); filled squares: clovers; open squares: other legumes; grey triangles: grasses; open circles: All Species Mix. Black line: regression through all points; broken line: y=-x. Mean of five sites (all except Barrington Park).

In most species, the proportion of weed biomass within the total above-ground biomass did not significantly change over time (Figure 22). This indicates that the characteristics of species shown in Figure 21 were mostly stable over time.

Among the legume species, only MP and SC tended to show an increase in the proportion of weed biomass. However, among the grass species, the proportion of weeds in the biomass significantly decreased in TY from autumn 2009 to spring 2011.

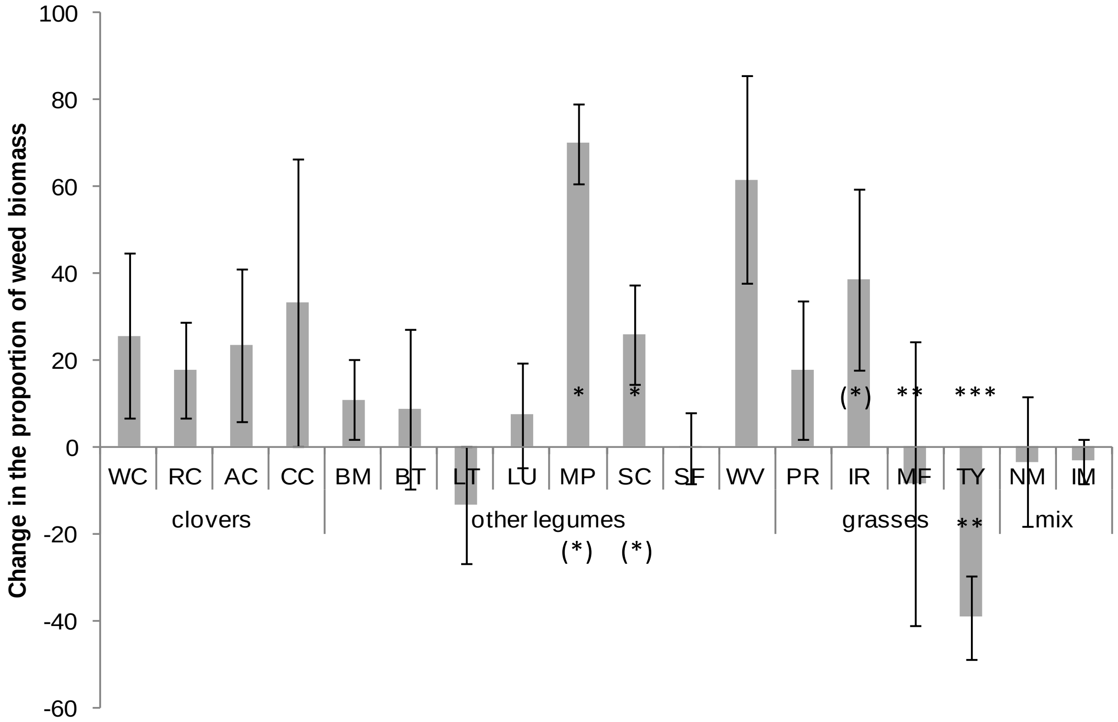

Figure 22. Proportion of weed biomass in total biomass (above-ground): Change from autumn 2009 to spring 2011; means and standard errors across 4 sites. Positive values mean an increase in the proportion of weed biomass in the total above-ground biomass. Significance stars *below* the zero-line indicate whether the change was significantly different from zero; stars *above* the zero-line refer to the difference between legume species and WC or the difference between grasses and PR.

Response of species to simulated grazing

In the replicated plot trials, the response of species to grazing could not be measured directly because of the relatively small plot size. However, grazing was simulated by cutting at a low height (2 cm) in spring 2010, i.e. at the time when animals would be let on to the ley for grazing. The re-growth of the plants was then measured with a second cut at ground level four weeks (28–30 days) after the first cut.

The highest biomass in the first cut was obtained in the two species mixes (Figure 23). Biomass was very low in LT and zero in WV and MP. LU and RC were the only legume species that showed a higher biomass from the first cut than WC. Significantly lower biomass values than in WC were found in LT, MP, SC, and WV. Among the grasses, IR was found to have a higher biomass in the first cut than PR.

After the simulated grazing cut, WC showed a significantly stronger regrowth than all other legume species. WC was followed by the two species mixtures and RC.

In general, the relationship between the biomass in the first and the biomass in the second cut reveals the interplay of two effects. First, those species that have a low biomass in the first cut also possess low resources to grow back after the cut (see blue arrow ‘resource effect’, parallel to the diagonal). Therefore, there is a positive correlation between biomass values of the first and second cut.

Second, there is a species-specific trait of tolerance for being cut low (‘grazing tolerance’), which will depend, among other things, on the height of the vegetative tissue from which the plant can re-grow after being cut. For species with a very low vegetative point, cutting low as in this simulated grazing experiment will be less detrimental to subsequent regrowth than in species with a higher regrowth point. Separating this effect from the resource effect, grazing tolerance can then be conceived as being perpendicular to the ‘resource effect’ (see green arrow).

For example, given its biomass in the first cut, LU would have been expected to grow back more than, e.g., SF, which had a much lower biomass in the first cut than LU, but had a very similar regrowth as LU in the second cut. Thus, the results can be interpreted as LU being a species with lower ‘grazing’ tolerance than SF.

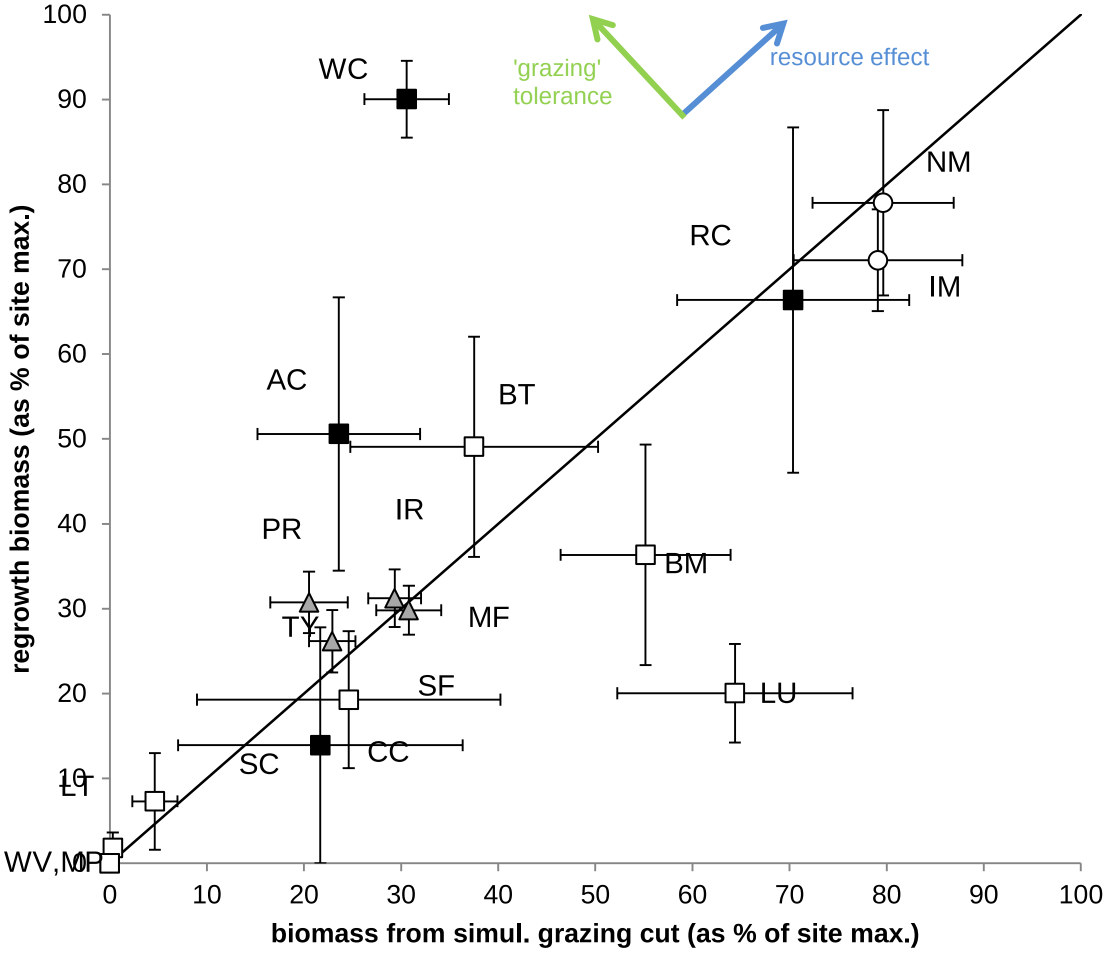

Figure 23. Biomass from a simulated grazing cut and biomass re-grown after the cut; means and standard error across four sites (Duchy, Rothamsted, SAC, Wakelyns). Filled squares: clovers; open squares: other legumes; grey triangles: grasses; open circles: All Species Mix.

Flowering

Flowering of legume species is of relevance because of the potential value of flowers as nectar and pollen sources for insects such as bees and bumblebees (Hymenoptera: Apidae), or butterflies (Lepidoptera). Flowering was scored in the replicated trials repeatedly during the first and second year of the trial. At each scoring date, it was noted whether there were no flowers yet, flowers were present, or flowering had ceased. No quantitative data on the number of flowers or the amount of flowering was recorded. The data is therefore analysed in terms of phenology only, i.e. the beginning and end of flowering time of the different species. Sufficient data for analysis was available from 3 and 2 sites from year 1 and 2, respectively. (Year 1: SAC, Wakelyns and Rothamsted; year 2: SAC and Wakelyns).

Plant phenology strongly depends on latitude and altitude of a location, with higher latitudes (such as the Scottish site, compare Table 2, p. 22) and higher altitudes showing a later start and earlier end of the growing season. To make the different locations more comparable to each other so that a joint analysis of flowering data from all locations would reveal relative differences between the legume species more clearly, we aligned the phenological dates of all sites to a common point in time. In particular, WC was selected as a reference species (see section 3.2.3, p. 25) and the start of flowering of WC was aligned in time for all sites so that the corrected start of WC flowering at all sites was on the same date, namely the date when it had occurred at the earliest site (Rothamsted in year 1 and WAF in year 2, see blue arrows in Figures 24 and 25, respectively).

In year 1 (Figure 24), flowering of most species started much later than in year 2 (Figure 25). Also, in year 1 there was much less pronounced variation of the start of flowering time among the legume species than in year 2, when the start of flowering was more drawn out over time. The opposite was the case for the end of flowering, i.e. the end of the flowering fluctuated more in year 1 than in year 2.

In year 1, the latest species to start flowering was RC. In year 2, the earliest species to flower were CC and BM whereas SF, SC, LU and LT flowered relatively late. BM was the species with the longest flowering period in year 2. Notably, despite its early emergence (Figure 11, page 51), CC was not the earliest species to flower in the first trial year.

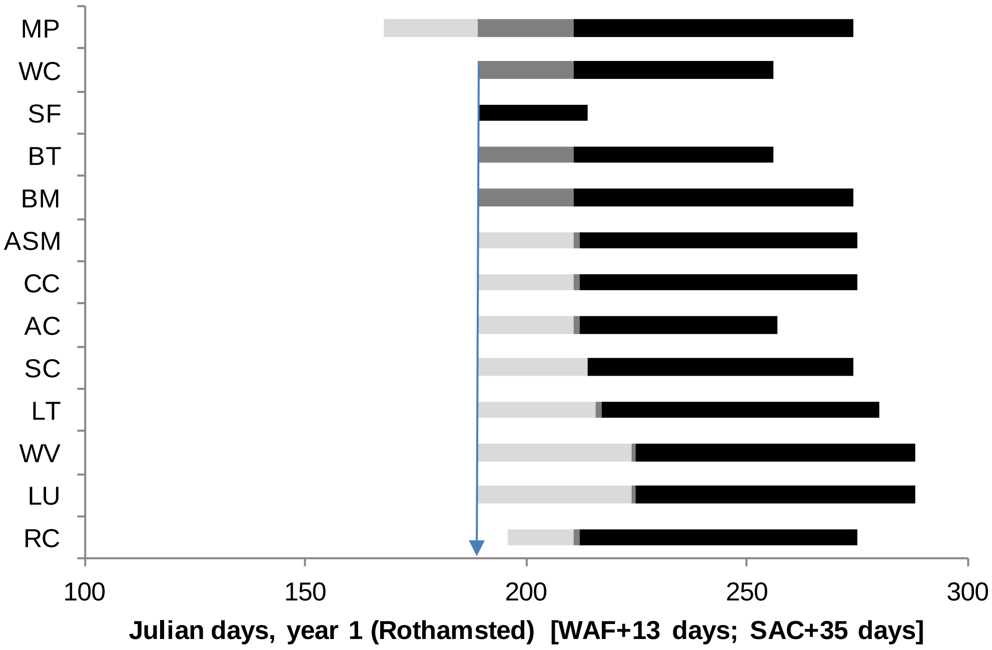

Figure 24. Flowering time of legume species, data from Rothamsted, Wakelyns and SAC, aligned to match the beginning of WC flowering at Rothamsted (later at WAF by 13 days and at SAC by 35 days); where the light grey bar starts is the earliest start date for flowering among the sites; where the light grey bar ends is the latest start of flowering among the sites; similarly, the start and end of the black bar dark show the earliest and latest end of flowering among the sites. The dark grey bar in the middle therefore shows the time when the species were flowering at all sites. Start of flowering of WC indicated by vertical arrow.

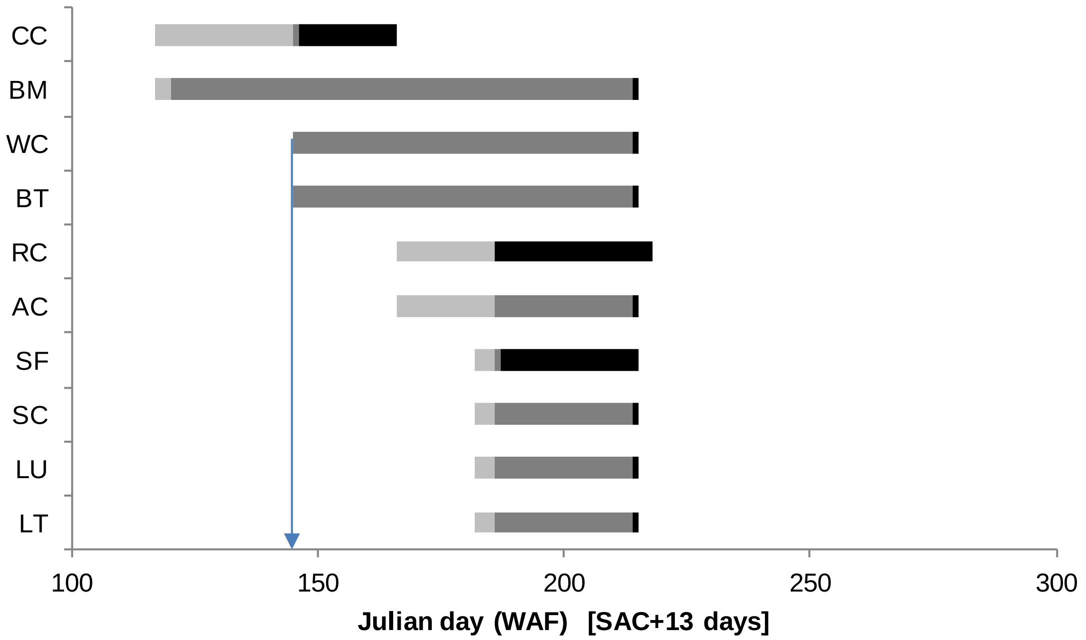

Figure 25. Flowering time of legume species, data from Wakelyns and SAC, aligned to match the beginning of WC flowering (later at SAC by 13 days); otherwise see caption of previous figure.

Pre-incorporation biomass and composition of plant residues

To determine the quantity and quality of plant material to be incorporated into the soil, biomass samples were taken in 2011. Plant samples were from both above-ground and below-ground (see Methods, section 3.2.3, p. 20). In the above-ground samples, the plant material was analysed for lignin, polyphenols, carbon and nitrogen.

Overall the grasses showed higher above-ground and below-ground biomass than the legume species. Among the legume species, above-ground biomass was significantly higher in BM than in the reference species WC (Figure 26). The lowest pre-incorporation biomass values were found in WV, MP, CC and SC. However, because of high variability these species were not significantly different from WC. No significant differences in above-ground biomass were found among the four grass species. The highest average of the pre-incorporation biomass was found in one of the ASM (non-inoculated).

Stability analysis of the above-ground biomass data revealed a positive correlation between variances and means, i.e. species with high means across sites tended to have high variances as well. The opposite was the case for the coefficient of variation (CV), which was larger in species with smaller means. The smallest CVs were found in PR and TY (68% and 71%, respectively) while SF and AC showed the highest CVs (317% and 201%, respectively). Among the legume species, WC had the lowest CV (110%) but BM, with a substantially higher mean (Figure 26), had a similar CV (124%).

The pre-incorporation biomass samples were also used to assess the ability of the different species to compete against weeds by determining the weed biomass as a proportion of total biomass. This revealed significantly lower weed biomass proportion in the grasses than in the legumes (Figure 27). The lowest weed biomass proportion was found in the two ASMs. In line with crop biomass results (Figure 26), MP, SC and WV showed a significantly higher weed biomass proportion than WC.

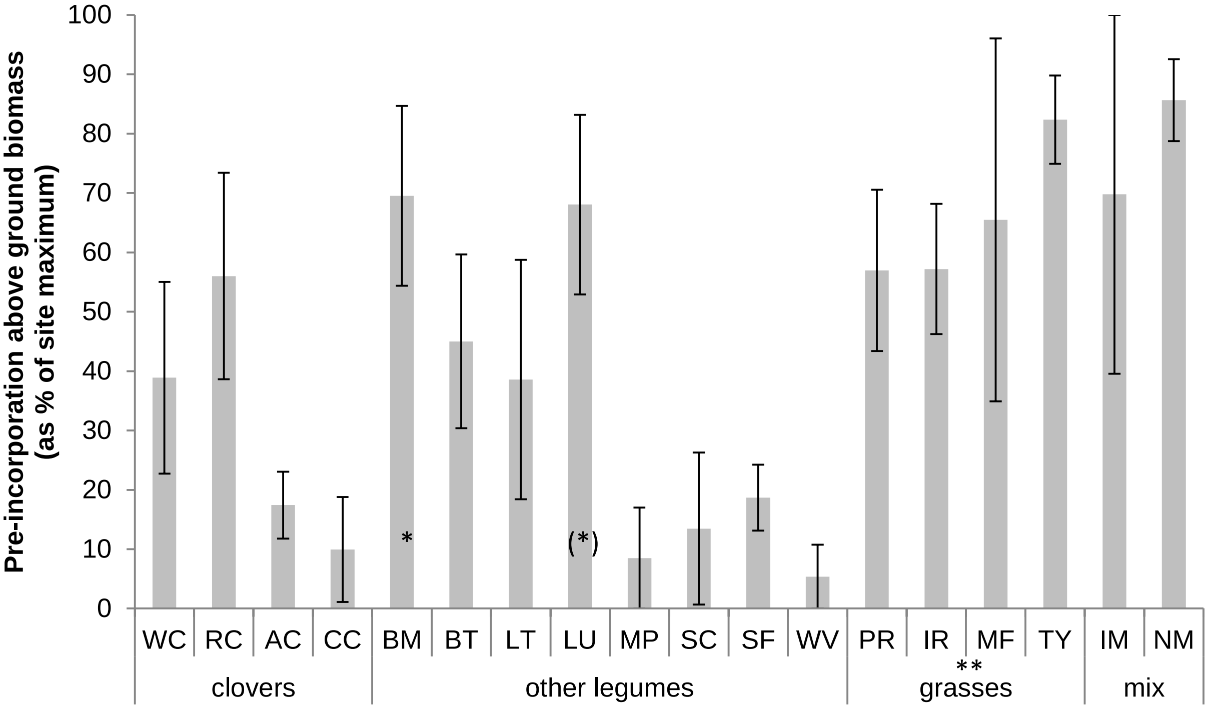

Figure 26. Pre-incorporation biomass (above-ground), as % of site maximum for different legume and grass species and for the inoculated and non-inoculated All Species Mix; mean and standard error across four sites (Duchy, IBERS, SAC, Wakelyns). Stars indicate statistical significance of the comparison between all grass species vs. all legume species (on axis title above 'grasses' label); of legume species against white clover on bottom of bars (further tests among grass species, and among mixes) were not significant.

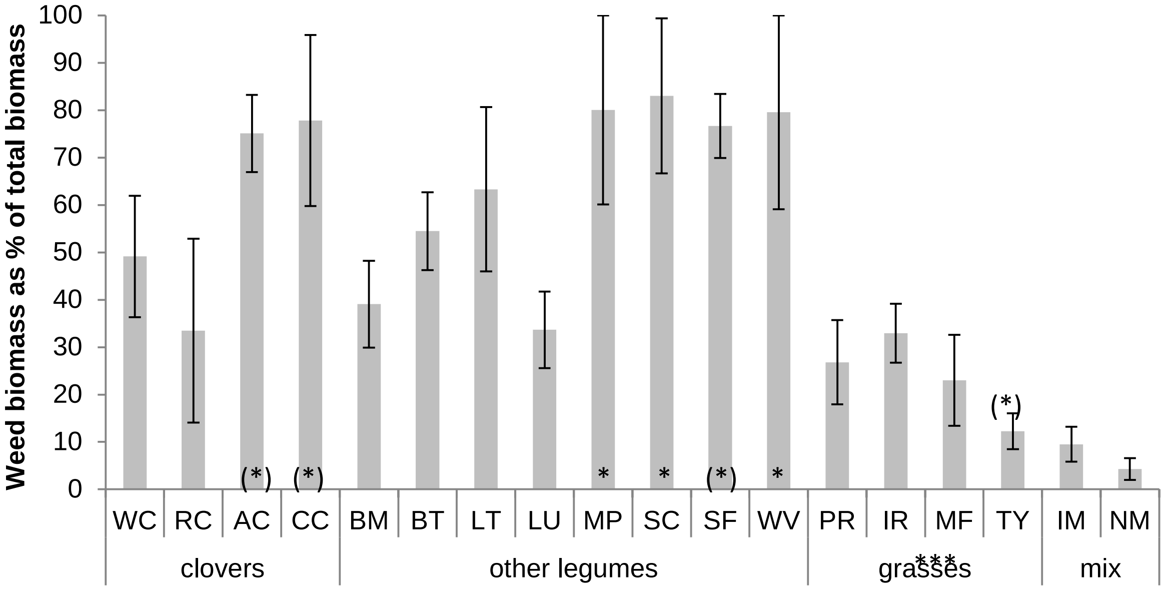

Figure 27. Pre-incorporation proportion of weed biomass as percentage of total above-ground biomass for different legume and grass species and for the inoculated and non-inoculated All Species Mix; mean and standard error across four sites (Duchy, IBERS, SAC, Wakelyns). Stars indicate statistical significance of the comparison between all grass species vs. all legume species (on axis title above 'grasses' label); of legume species against white clover (on bottom of bars); and among grass species (above bars), differences among mixes were not significant.

Below-ground biomass (i.e. root biomass) was significantly higher in the grasses than in the legumes (Figure 28); however, there were no statistically significant differences in root biomass among the different legumes species, among the grass species or between inoculated and non-inoculated ASM.

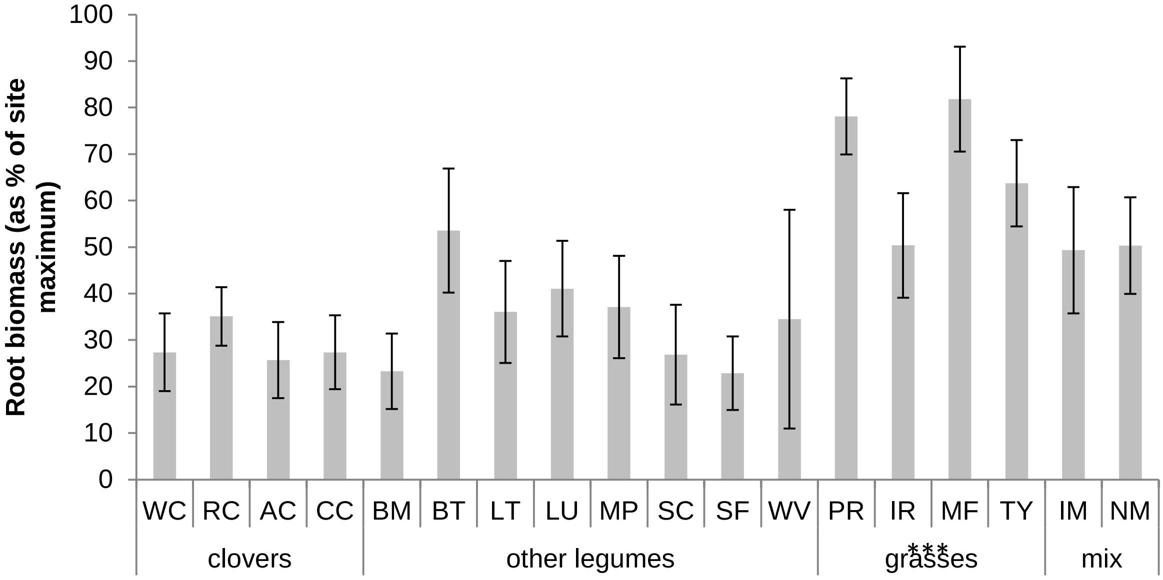

Figure 28. Pre-incorporation root biomass of different legume and grass species and of the inoculated and non-inoculated All Species Mix, expressed as percentage of site maximum; mean and standard error across five sites. Stars indicate statistical significance of the comparison between all grass species vs. all legume species (on axis title above 'grasses' label); further comparisons (among grass species, among legume species, and among mixes) were not significant.

The plant residue composition, determined in 2011 in the replicated plot trials, varied among individual legume and grass species (Figures 29 to 31) and among sites (Table 11). Overall, the legumes showed a greater lignin content than the grasses (Figure 29) as well as lower C:N ratios (Figure 31).

Among the grass species, none of the species was significantly different from PR in terms of lignin content (Figure 29) and polyphenol content (Figure 30), however, MF showed a significantly higher C:N ratio than PR (Figure 31). Among the legumes all non-clover species were significantly higher in lignin content than WC (Figure 29). Regarding polyphenol content, RC and SF showed significantly higher values than WC (Figure 30). Also, BT, LT and SC showed significantly higher C:N ratios than WC (Figure 31). MP and WV were not sampled for residue composition because not enough plant material was growing on the trials.

Differences between inoculated and non-inoculated All Species Mix were not significant regarding residue composition.

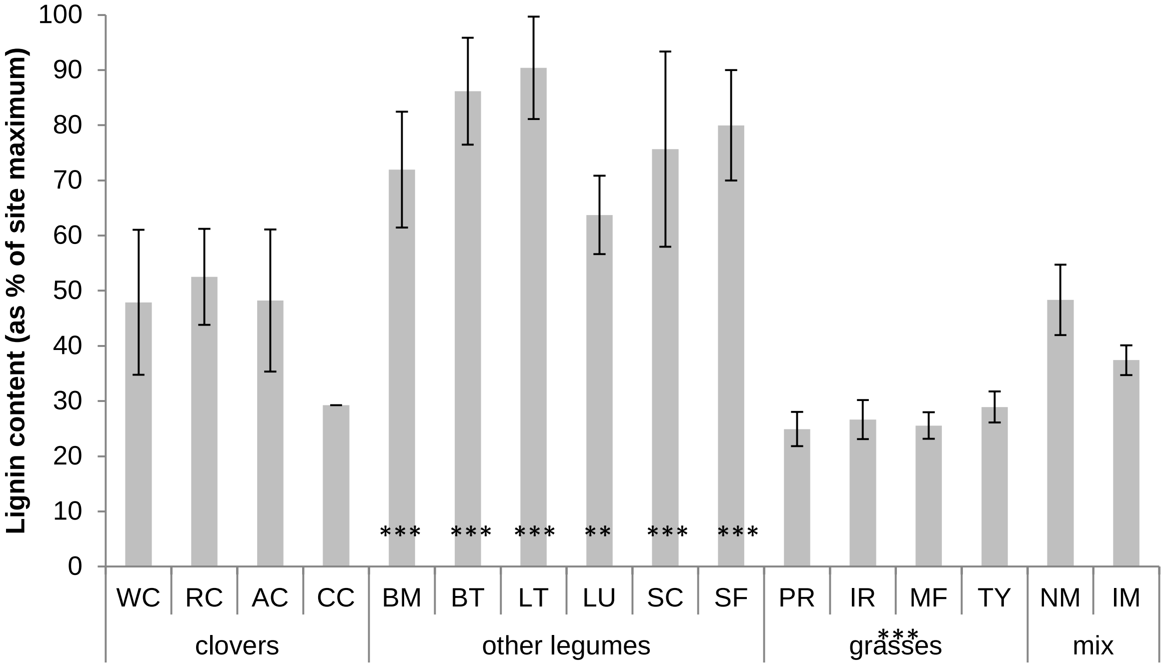

Figure 29. Lignin content of different legume and grass species and of the inoculated and non-inoculated All Species Mix, expressed as percentage of site maximum; mean and standard error across five sites. Significance stars indicate statistical significance of the following comparisons: all grass species vs. all legume species (on axis title above ‘grasses’ label); and legume species vs. white clover (Dunnett test) (at bottom of bars). Further comparisons (among grass species and among mixes) were not significant.

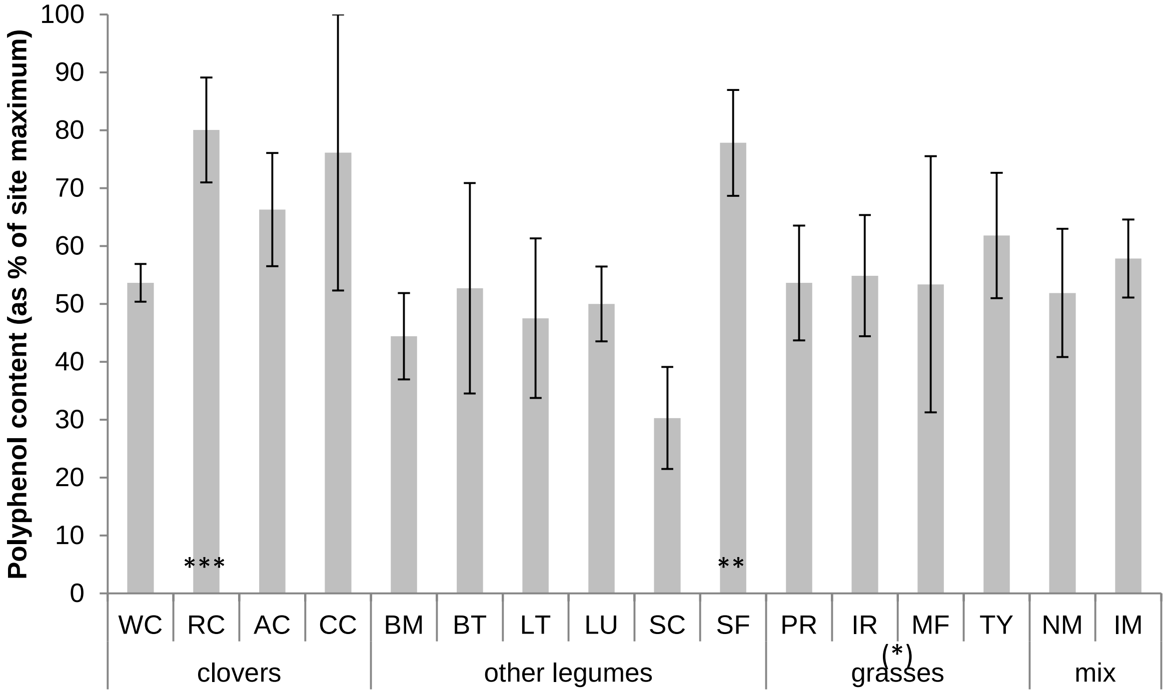

Figure 30. Polyphenol content of different legume and grass species and of the inoculated and non-inoculated All Species Mix, expressed as percentage of site maximum; mean and standard error across five sites. Significance stars indicate statistical significance of the following comparisons: all grass species vs. all legume species (on axis title above 'grasses' label); and legume species vs. white clover (Dunnett test) (at bottom of bars). Further comparisons (among grass species and among mixes) were not significant.

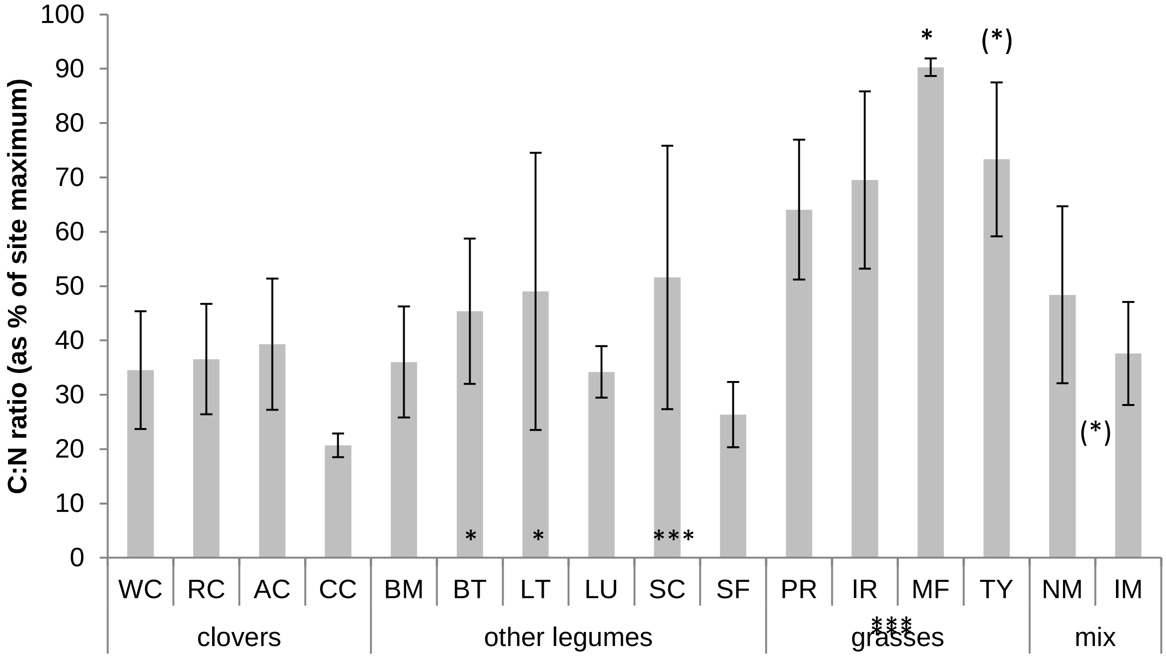

Figure 31. C:N ratio of different legume and grass species and of the inoculated and non-inoculated All Species Mix, expressed as percentage of site maximum; mean and standard error across five sites. Significance stars indicate statistical significance of the following comparisons: all grass species vs. all legume species (on axis title above 'grasses' label); legume species vs. white clover (Dunnett test) (at bottom of bars); grass species against perennial ryegrass (above bars) and among mixes (between NM and IM bars.

Regarding lignin content and C:N ratios, there was large variation among sites (Table 11), with the SAC site showing the highest average lignin values, Rothamsted showing the highest C:N ratios. Smaller variability among sites was observed for polyphenol contents.

Table 11. Lignin, polyphenol and C:N in plant residues: average, minimum and maximum values for each site.

		Site				
		Duchy	**IBERS**	**Rothamsted**	**SAC**	**Wakelyns**
Lignin (%)	average	4.24	2.21	6.52	10.94	8.87
	maximum	9.36	3.81	13.46	21.17	17.22
	minimum	1.89	1.15	3.22	3.25	4.18
Polyphenol (%)	average	0.21	0.26	0.20	0.15	0.12
	maximum	0.29	0.32	0.38	0.31	0.26
	minimum	0.18	0.17	0.12	0.06	0.06
C:N ratio	average	12.1	10.2	28.8	18.2	20.8
	maximum	23.3	13.2	75.4	35.1	74.4
	minimum	7.3	6.5	9.7	8.0	9.7

We also observed a negative relationship between nitrogen content and lignin content in the above-ground plant residues (Figure 32). Among legume species, greatest variability of N content was found in LU. In CC, there was a low lignin content in relation to what would be expected for the N content of this species (see dark red squares in the following figure). The opposite is true for SF (see orange squares), which showed a relatively high lignin content given its N content.

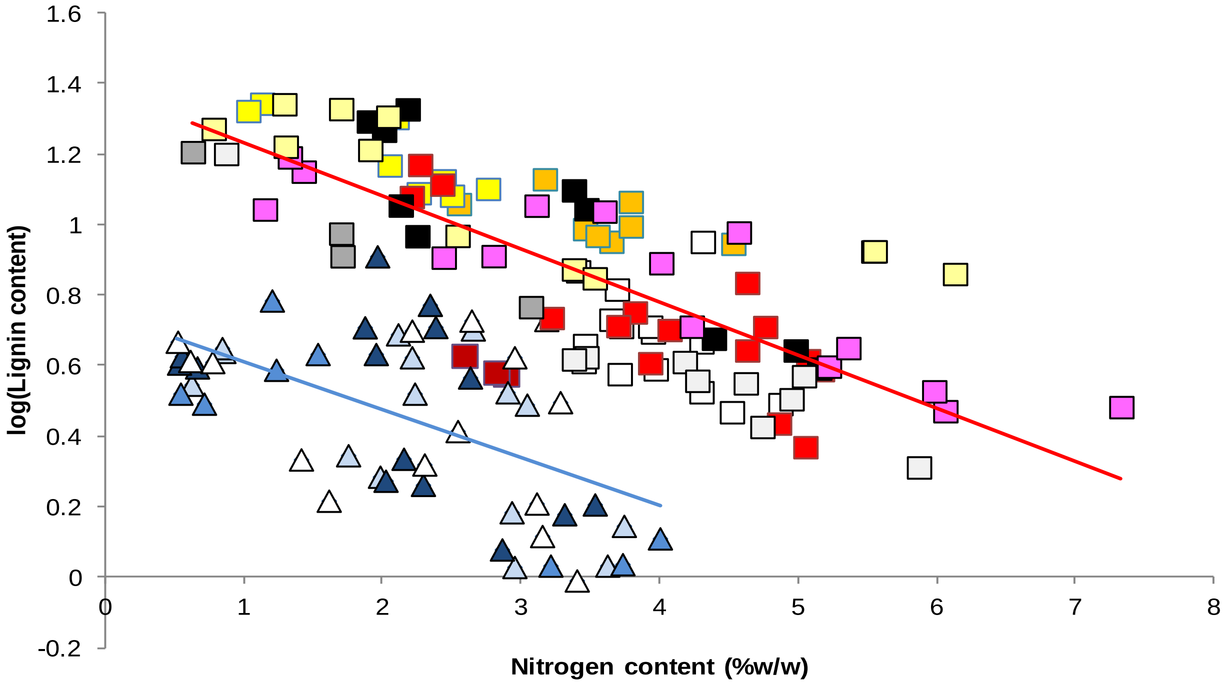

Figure 32. Lignin content ((log)ADL) vs. Nitrogen content: Squares and the red trendline represent legume species, triangles and the blue trendline represent grasses. Colour codes for squares. AC (bright grey), BT (bright yellow), BM (black), CC (dark red), LT (yellow), LU (magenta), RC (red), SC (dark grey), SF (orange), WC (white); for triangles: IR (white), MF (blue), PR (light blue), TY (dark blue).

Soil water content and soil nitrogen pre-incorporation

Prior to incorporation, soil water content varied little among the sites (Table 12). There was no significant difference among the legume species, or among the grass species with regard to soil moisture (Figure 33). However, collectively the grass species showed significantly higher soil moisture than the legumes.

With regard to available nitrogen before incorporation, none of the legume species showed significant differences in comparison with WC (Figure 34). The grasses had significantly lower available N than the legumes. The soil under the grass species showed lower nitrate contents than under the legume species but similar ammonium contents (Figure 35).

Table 12. Soil moisture and soil nitrogen pre-incorporation, site averages and ranges

		Duchy	**Rothamsted**	**SAC**	**Wakelyns**
Water content (%)	average	14.4	16.4	-	19.4
	max	15.4	17.4	-	20.0
	min	13.0	15.0	-	18.9
Nitrate (mg/kg)	average	4.6	8.9	11.8	10.0
	max	8.9	16.2	19.3	13.0
	min	1.8	1.1	5.0	7.6
Ammonium (mg/kg)	average	6.1	4.9	8.2	2.3
	max	8.0	10.4	10.5	4.8
	min	4.0	3.3	5.4	0.7
Available N (kg/ha)	average	42.5	54.9	80.2	49.2
	max	61.8	104.6	113.8	65.8
	min	27.0	18.6	51.9	35.1

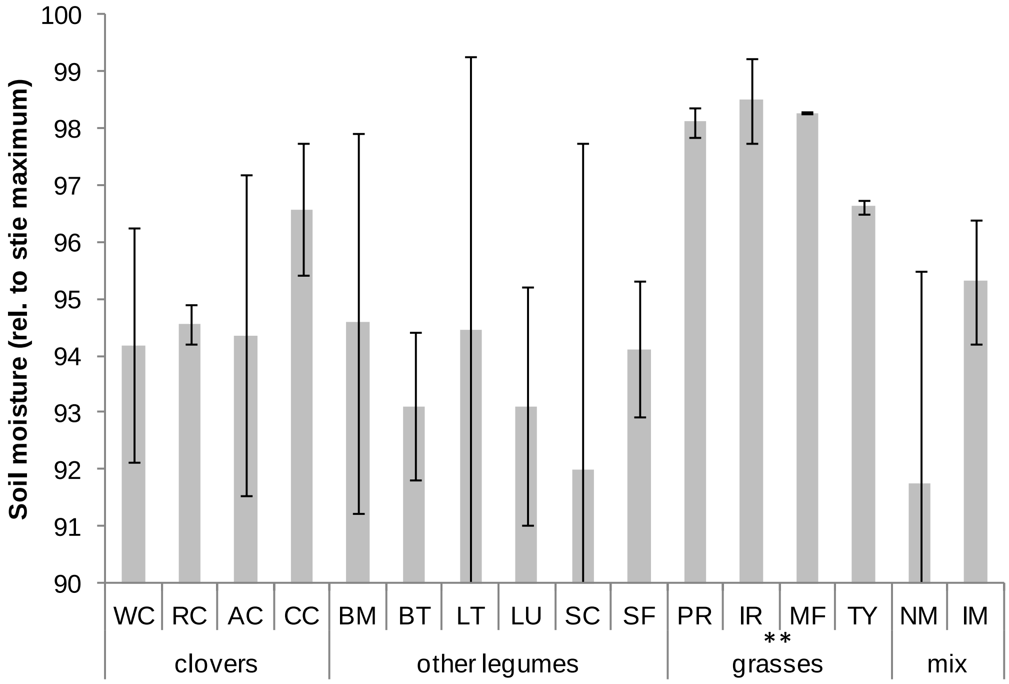

Figure 33. Pre-incorporation water content in the soil for species, relative to site maximum. Means and standard error over three sites (Wakelyns, Rothamsted, Duchy). Note that the y-axis is cut off at 90%. Grasses were significantly different from legumes. No other comparisons were significant.

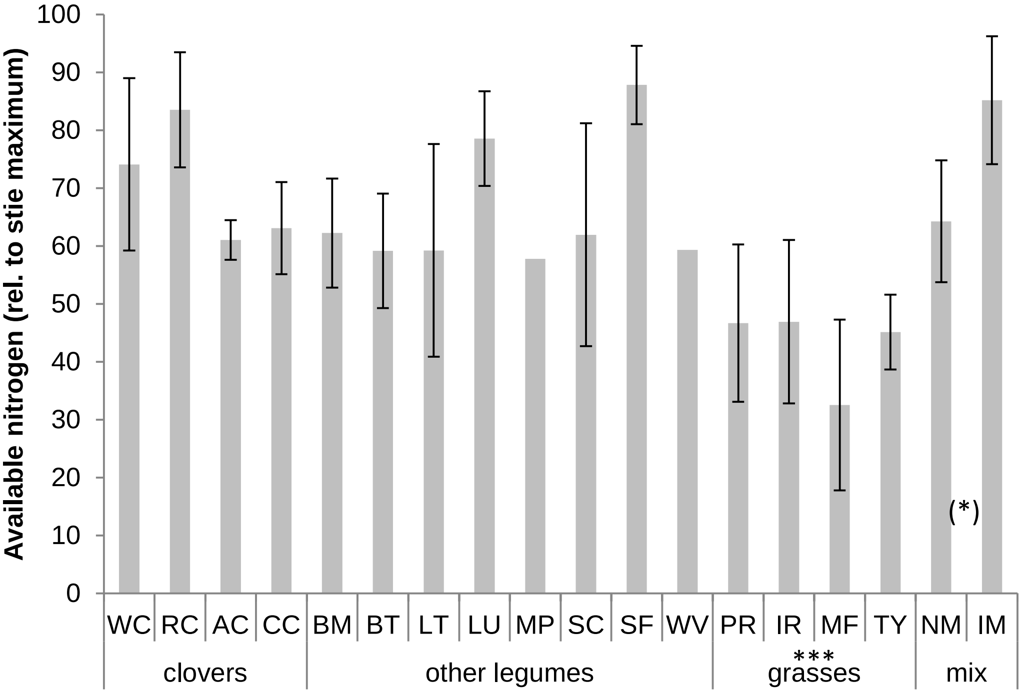

Figure 34. Available mineral nitrogen in the soil (nitrate + ammonium), pre-incorporation, expressed as percentage of site maximum; means and standard error over four sites (Rothamsted, Wakelyns, SAC, Duchy).

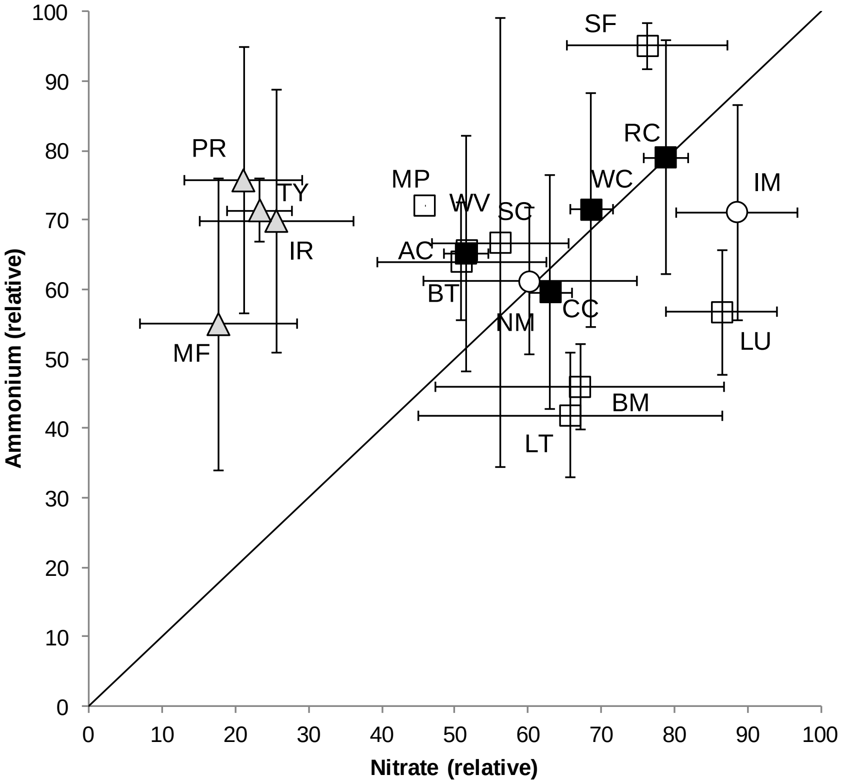

Figure 35. Pre-incorporation nitrate and ammonium content in the soil, expressed as percentage of site maxima, for grass species (triangles), clover species (black squares), other legume species (open squares) and mixtures (circles); means and standard errors. Abbreviations of species see page 188.

Soil water content and soil nitrogen post-incorporation

After incorporation (compare Figure 2, p. 21), the difference between legumes and grasses with regard to soil moisture, as observed before incorporation, had disappeared (Figure 36). As before, the available nitrogen was not significantly different between WC and any other legume species (Figure 37) but ranged widely among sites (Table 13). Among the grass species, soil under MF was found to have significantly lower available nitrogen than PR (Figure 37).

Table 13. Soil moisture and nitrogen post-incorporation, site averages and ranges

		Duchy	**SAC**	**Wakelyns**
Water content (%)	average	18.7	-	13.0
	max	19.3	-	14.1
	min	17.3	-	12.0
Nitrate N (mg/kg)	average	8.6	7.0	20.9
	max	11.4	10.7	28.1
	min	5.4	4.5	14.8
Ammonium N (mg/kg)	average	4.5	-1.7	1.14
	max	6.2	6.7	4.66
	min	3.5	-3.5	0.43
Available N (kg/ha)	average	52.4	21.4	88.0
	max	63.1	66.1	116.2
	min	36.6	3.8	62.6

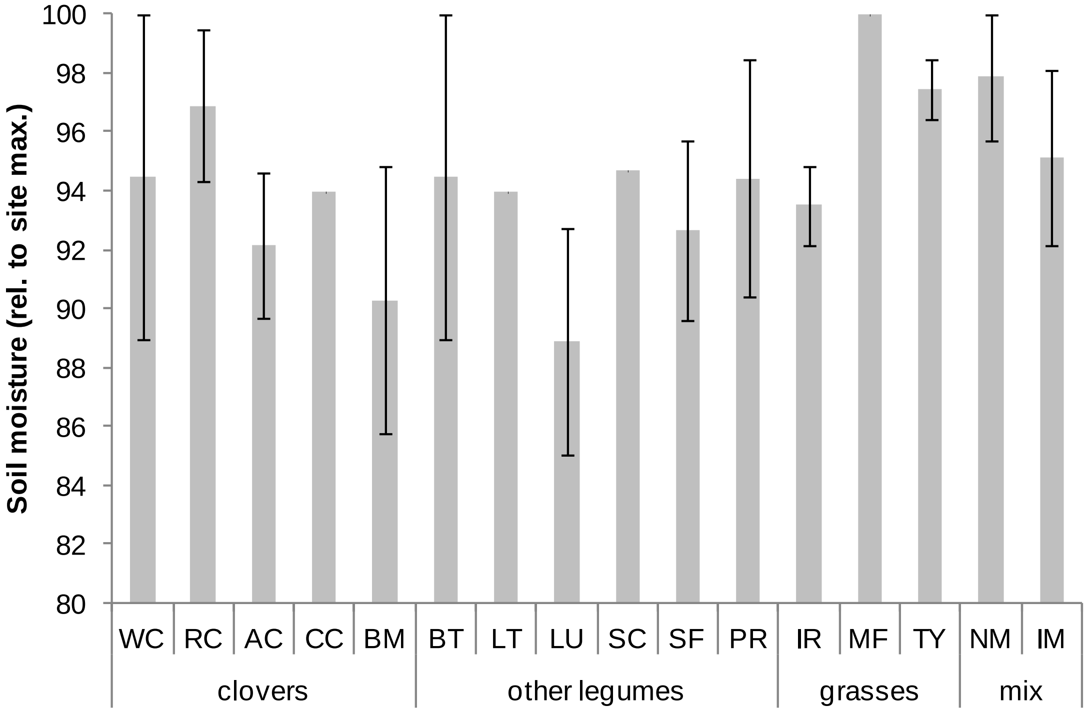

Figure 36. Post-incorporation water content in the soil for legume and grass species and the ASM, relative to site maximum. Means and standard error over three sites (Wakelyns, SAC, Duchy). None of the comparisons were statistically significant.

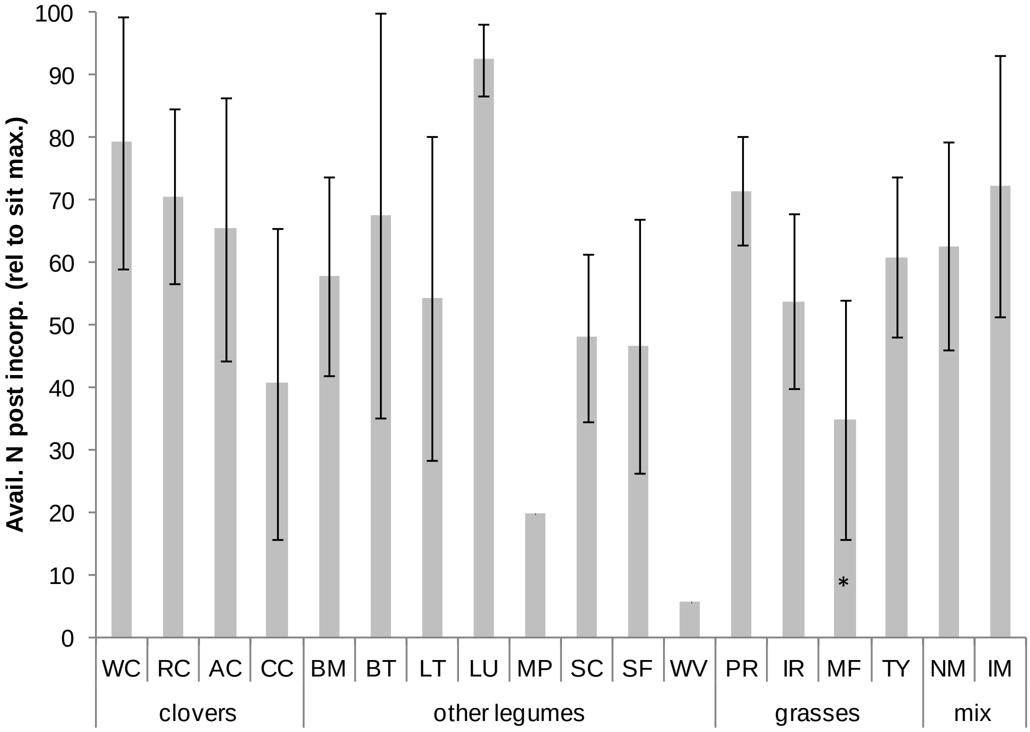

Figure 37. Available mineral nitrogen in the soil (nitrate + ammonium), post-incorporation, expressed as percentage of site maximum; means and standard error over three sites (Wakelyns, SAC, Duchy). MF was significantly lower than PR. No other comparisons were statistically significant.

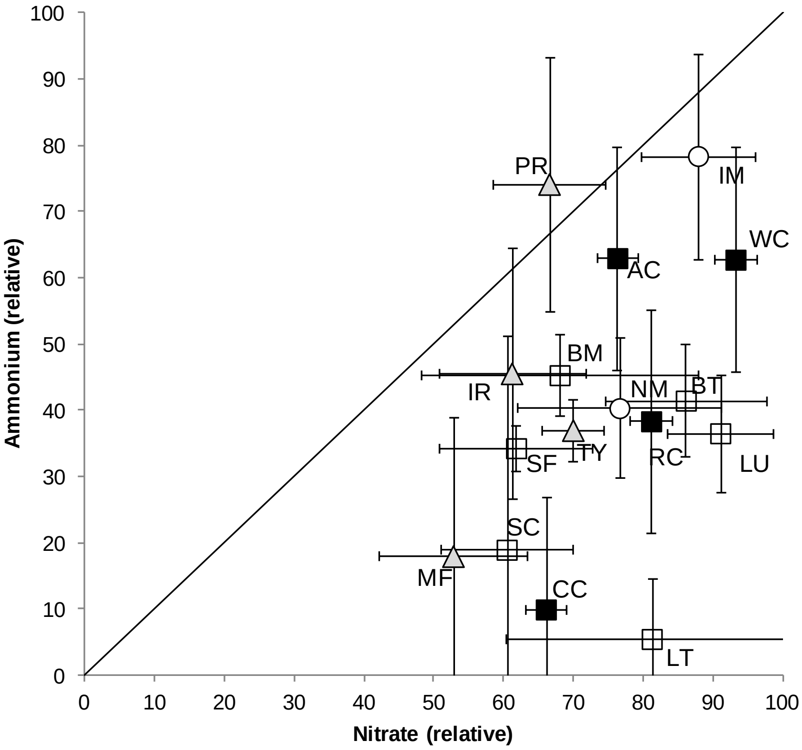

Figure 38. Post-incorporation nitrate-N and ammonium-N in the soil, expressed as percentage of site maxima, for grass species (triangles), clover species (black squares), other legume species (open squares) and mixtures (circles); means and standard errors over 2 sites (Wakelyns and Duchy).

Yield and yield components of the following cereal crop

At three of the sites, cereals were sown after incorporation of the legume, grass and mixture plots (spring wheat at Wakelyns, winter wheat at Rothamsted and spring barley at SAC). Contrary to expectations, grain yields were not significantly higher after grasses than after legumes (p=0.136). When all sites were considered together, there were no significant differences between PR and any other grass species or between WC and the other legume species (Figure 39).

Generally, agreement of yield data among the sites was better between the Rothamsted and Wakelyns trial than between SAC and the other two sites. At Rothamsted and Wakelyns, highest grain yields were observed after WC, but at SAC grain yields were highest after WV.

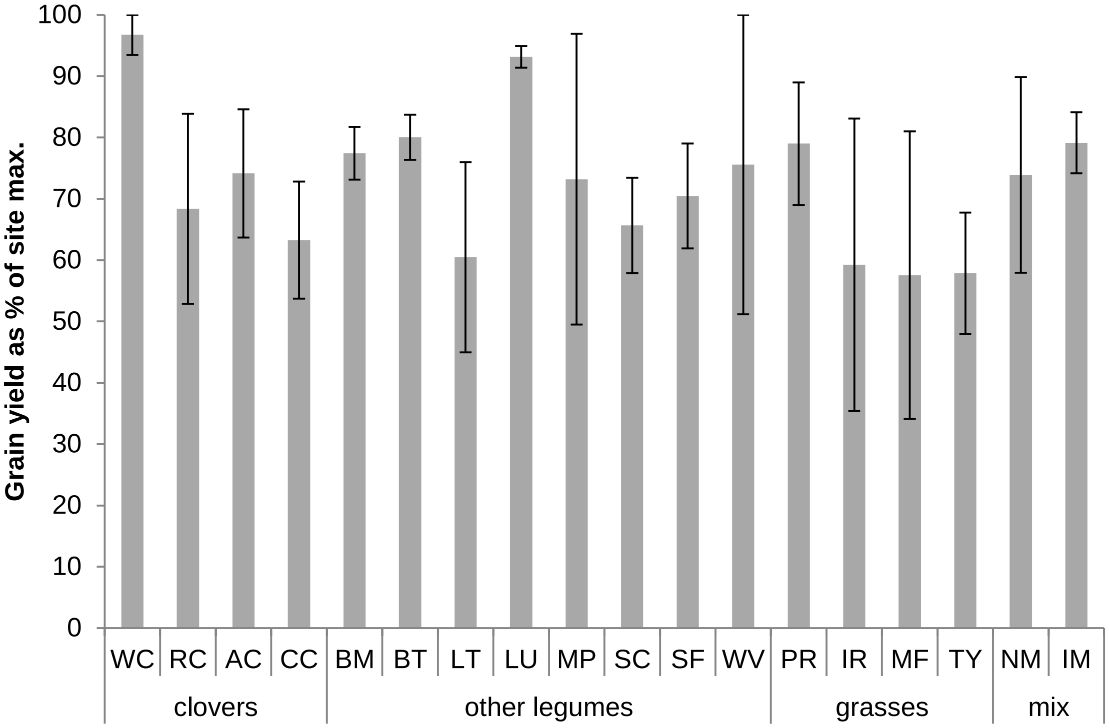

Figure 39. Grain yield of following crop; means and standard errors over 3 sites (Wakelyns, Rothamsted, SAC).

In the Rothamsted trial there was a significant positive correlation (Adj. R^2 = 0.596, p<0.001) between grain yield and the legume biomass (cumulated over the last three sampling dates before sowing the cereal, 5/10/2009, 15/4/2010 and 13/5/2010) (Figure 40).

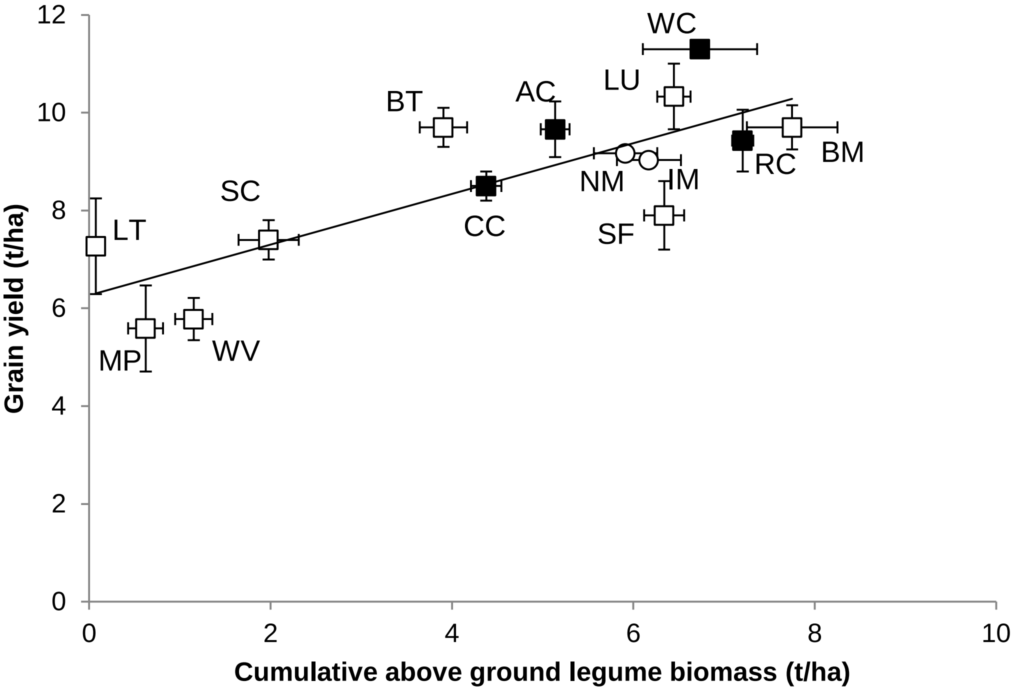

Figure 40. Grain yield of winter wheat vs. cumulative above-ground biomass of preceding legumes at Rothamsted. Shown are grass species (triangles), clover species (black squares), other legume species (open squares) and mixtures (circles); means and standard errors over 3 replicates.

Yield components of the grain yield were affected by legume species (Figure 41), with the cereal showing the highest head densities after WC, AC and RC while high thousand grain weights (TGW) were observed in LU and BT.

Figure 41 also illustrates two different mechanisms in which the preceding legume species affected the yield components; more productive species such as WC and LU tended to result in both higher head density and higher TGW than legume species with low productivity such as LT, MP and WV (see blue arrow).

However, once this productivity aspect is taken into account, it is evident that some species (such as WC) result in a comparatively low TGW and high head density, while other species (such as LU) show the opposite effect. As TGW is determined later in the season than head density, this finding suggests that softer species with lower lignin content, such as WC, are decomposed and release nitrogen earlier in the season than harder species with higher lignin content such as LU or BM (green arrow in Figure 41, also see Figure 29).

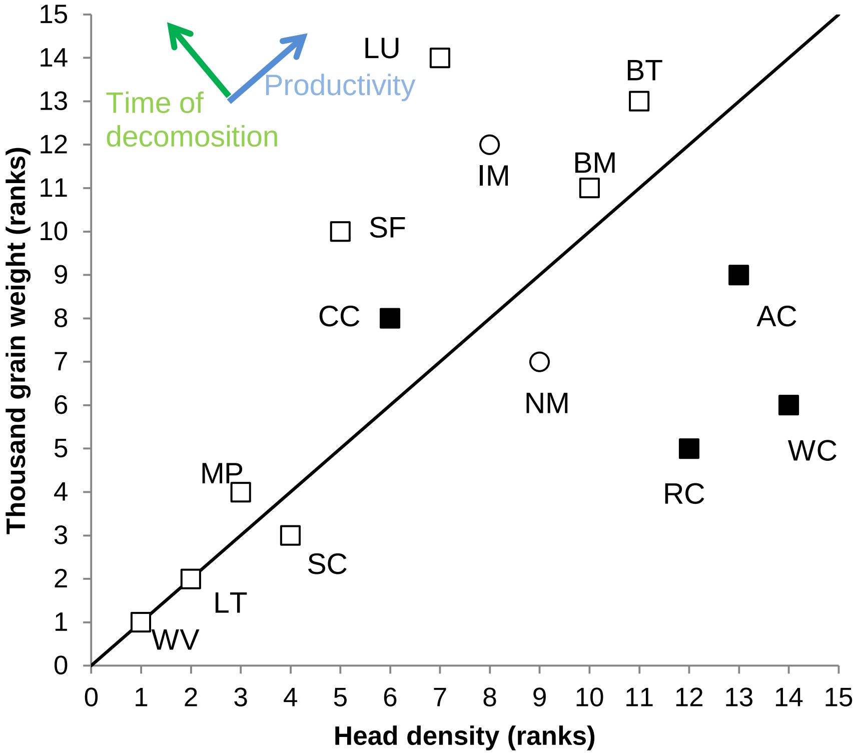

Figure 41. Head density and thousand grain weight of winter wheat following various legumes and the All Species Mix at Rothamsted.

At SAC, there was no correlation between pre-incorporation soil nitrate content and grain yield (Figure 42). However, there was a significant positive correlation between soil nitrate content and plant growth of the cereal crop during the elongation phase, mid-June to mid-July (Adj. $R^2 = 0.285$, df = 52, $p<0.001$) (Figures 42 and).

The nitrogen content in the harvested grain correlated strongest with nitrate content in soil samples taken 66 days before harvest (4 July) (Figures 42 and 44).

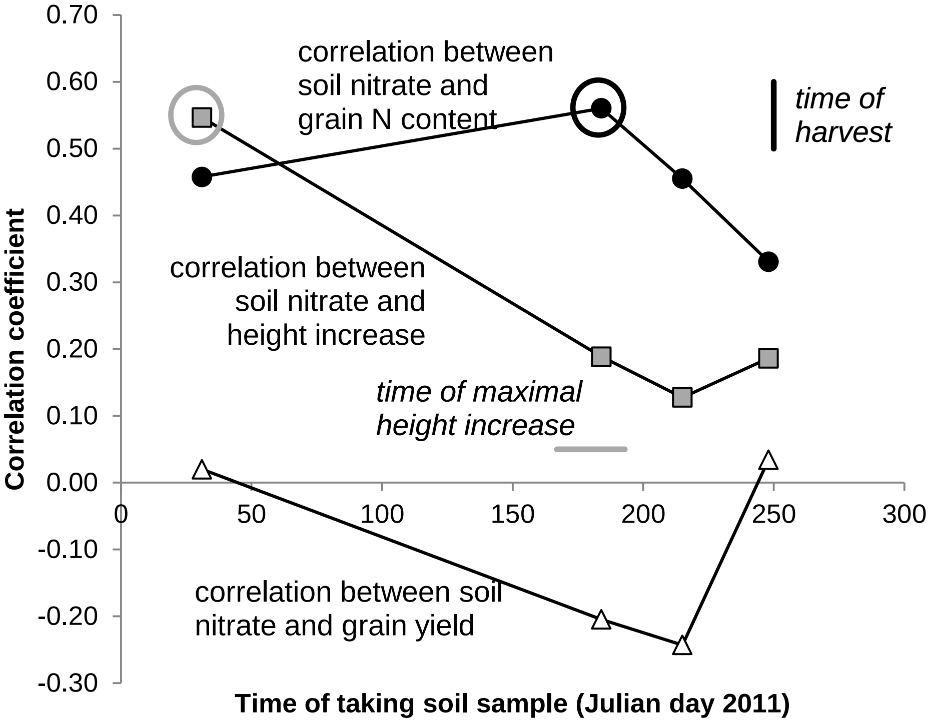

Figure 42. Correlation coefficients between nitrate content in soil samples taken four times over the growing season and three other variables, namely plant height increase in mid-summer (grey squares); grain yield (white triangles); and grain nitrogen content (black circles); data from SAC. Encircled points refer to the next figures.

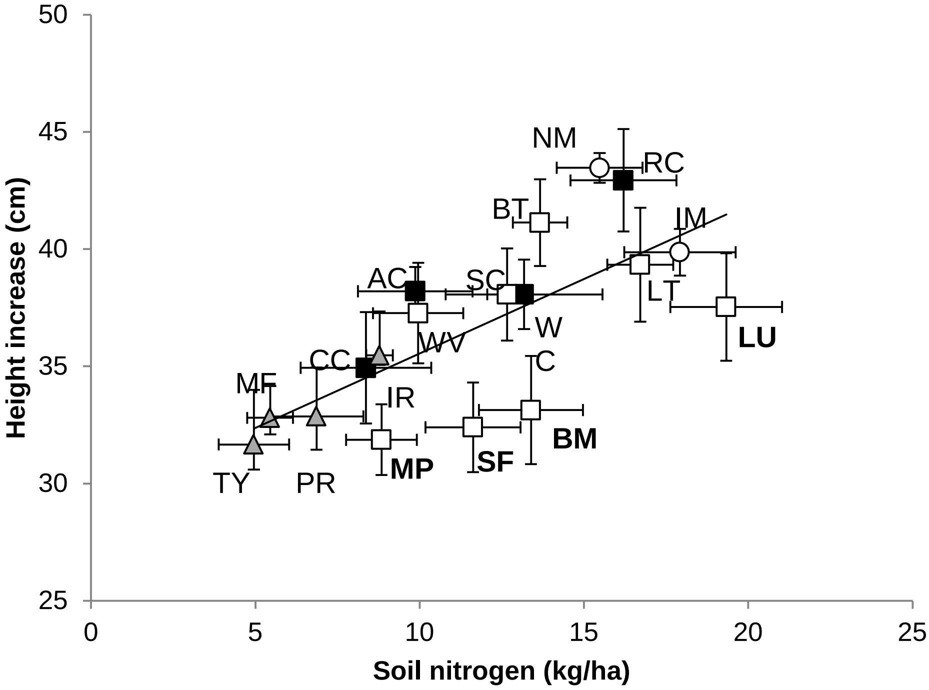

Figure 43. Soil nitrogen content at SAC in February 2011 against height increase in spring barley from mid-June to mid-July, after incorporation of various legume and grass species. The graph corresponds to the correlation represented by the grey encircled point in the previous figure. Significant deviations of species from the trendline are labelled in bold.

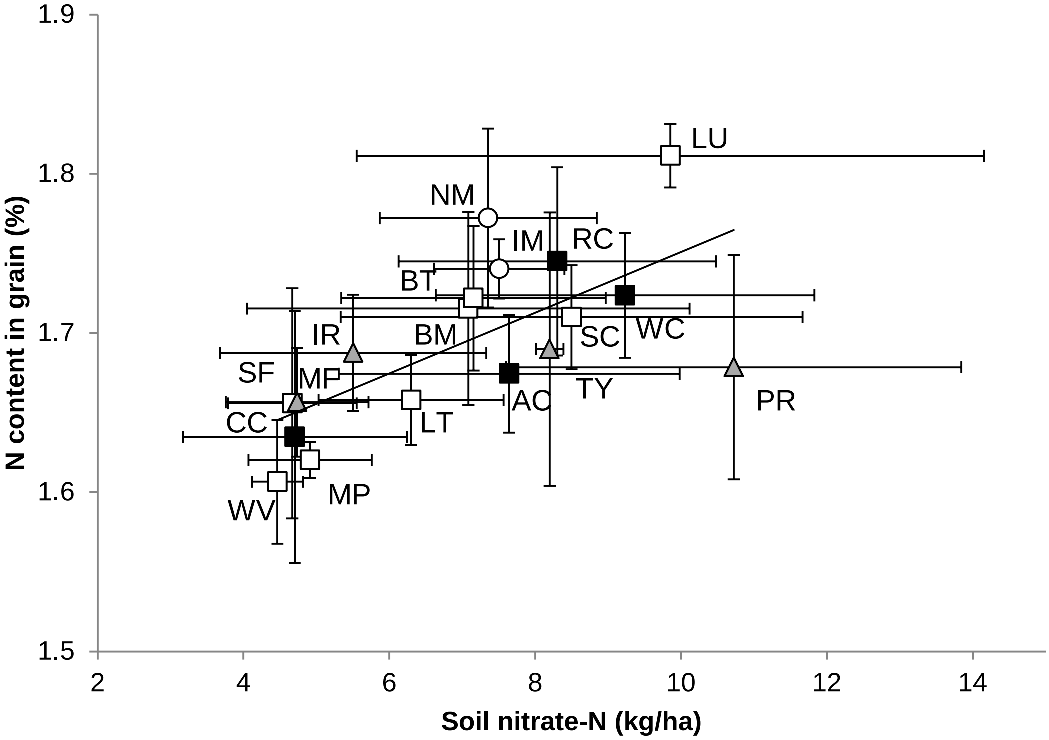

Figure 44. Soil nitrogen content at SAC in July 2011 against nitrogen content in harvested grain in spring barley, after incorporation of various legume and grass species. The graph corresponds to the correlation represented by the black encircled point in Figure 42.

Composition of the All Species Mix

At three sites, the component species of the ASM were identified as far as possible to species level and their dry matter biomass determined in spring 2011. Within the ASM, grasses showed a higher proportion of the biomass than would be expected from the densities at which the plants were sown in 2009 (Figure 45). In addition, there was a gradient of increasing grass proportion in the biomass from the West to the East.

Further, in comparison with the plant densities at sowing, the biomass proportion at the end of the trial showed a simplified species composition within the ASM, with three to five species dominating the mixture (Figure 46). Interestingly however, the identity of the most dominant species varied among the three sites.

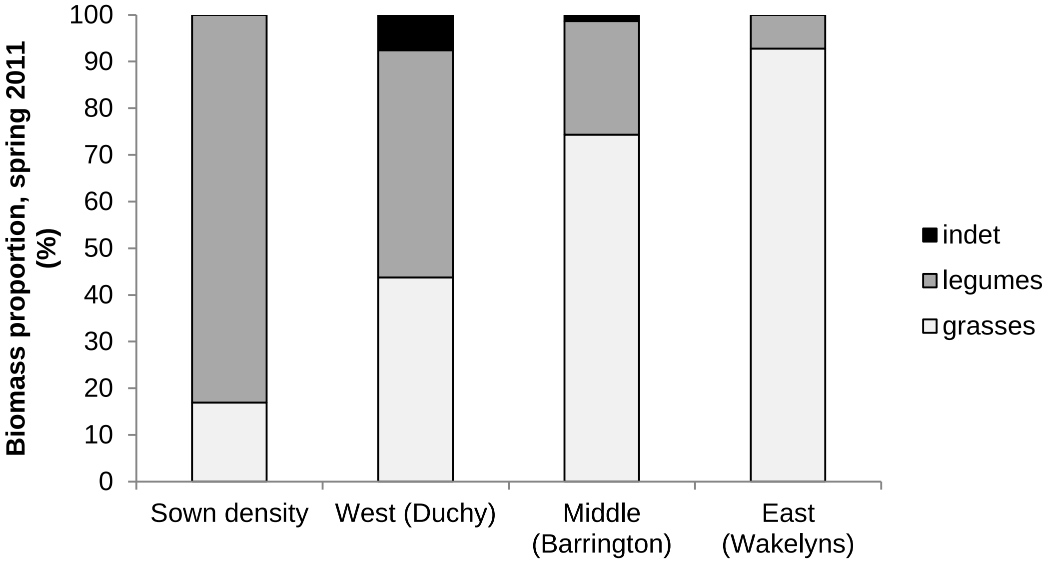

Figure 45. Legume and grass proportions of the biomass within the inoculated ASM in spring 2011

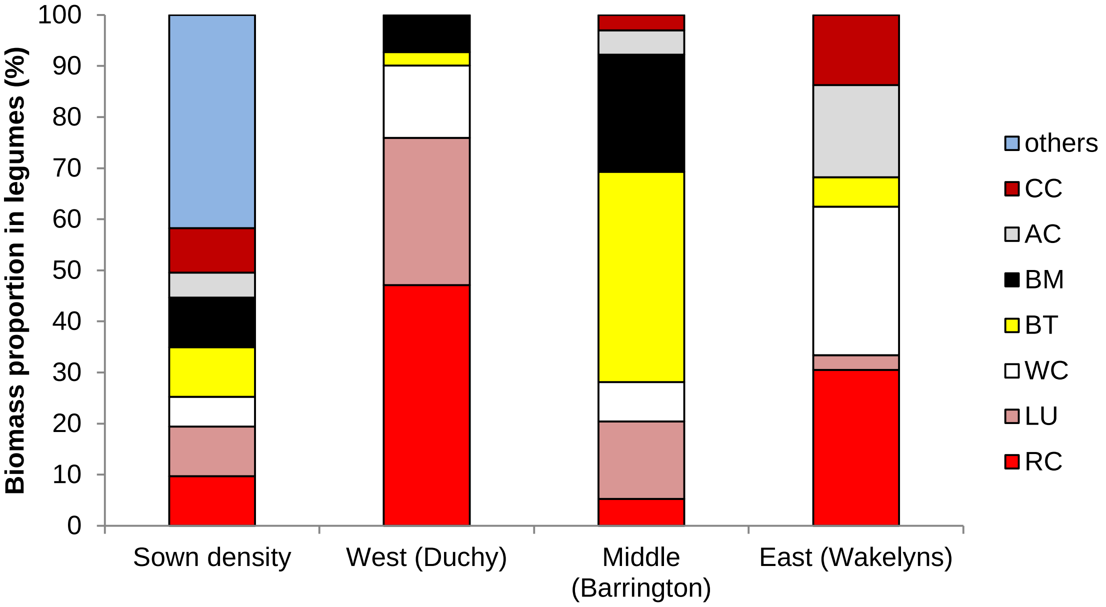

Figure 46. Biomass proportion of different species within legume biomass in the ASM, spring 2011

Summary of global species characterisation

Summarising the results from WP1, Table 14 gives an overview of the performance of different legume species tested in the field trials across various performance criteria. Our approach was to condense all measured variables (as reported in the previous sections) into seven distinct functions: (1) early development, (2) general productivity, (3) weed suppression, (4) flowering, (5) value as a pre-crop, (6) resistance to decomposition and (7) performance of the following crop. For each function, performance values were averaged across all variables pertaining to the function (as detailed below); then species were ranked according to these averages.

The **early development** rank was obtained from the average of four variables: speed of emergence (as days difference from site average, Figure 11), proportion of sown species emerged (Figure 12), seedling biomass at 60 days (Figure 13) and seedling relative growth rate (Figure 13).

Variables going into the **productivity** rank were plant height in spring 2009 (Figure 15), plant height in spring 2010 (Figure 15), plant height regrowth in 2009 (Figure 16), crop cover in spring 2010 (Figure 17), crop biomass in August 2009 (Figure 18), crop biomass regrowth in 2010 before and after a low cut (simulated grazing) (Figure 23), persistence in the ASM as biomass proportion in 2011 (Figure 46).

Weed suppression was assessed with three variables: weed cover in August 2009 and in spring 2010 (Figure 20) and the change in the proportion of weed biomass 2009–2011 (Figure 22). **Flowering** was assessed with two variables, using an earliness index (high= early) and a flowering length index (high= long) (Figures 24 and 25).

The pre-crop value used four variables for ranking the species: pre-incorporation above-ground biomass (Figure 26), pre-incorporation below-ground biomass (Figure 28), available N in the soil before incorporation (Figure 34) and available N after incorporation (Figure 37).

Ranks for resistance to decomposition were determined using three variables: lignin content (Figure 29), polyphenol content (Figure 30) and the C:N ratio (Figure 31). **Performance of the following crop** was assessed using three variables: plant height (Figure 43), grain yield and grain protein content of the following crop.

As shown in Table 14, WC, RC, BM, BT and LU are generally among the best species with regard to most criteria. If all seven functions are weighted equally, these five species score very similar average ranks (between 7.9 in WC and 9.4 in RC). MP and WV are consistently among the worst species (with average ranks below 3) while AC, SF and SC are in the middle range.

Table 14. Ranks of species performance (WP1); high ranks show high performance; empty cells: no or not enough data available.

Criterion	WC	RC	AC	CC	BM	BT	LT	LU	MP	SC	SF	WV
Early development	3	10	5	12	6	2	1	11	8	9	7	4
Productivity	11	12	7	6	9	8	1	10	3	4	5	2
Weed suppression	10	12	7	3	11	8	5	9	1	4	6	2
Flowering	8	6	6	8	11	8	2	2	1	2	2	1
Pre-crop value	9	11	5	3	8	10	7	12	2	4	6	1
Resistance to decomposition	2	7	5	1	4	9	10	3		6	8	
Perform. following crop	12	8	7	2	9	10	4	11	1	3	6	5
Average rank	7.9	9.4	6.0	5.0	8.3	7.9	4.3	8.3	2.7	4.6	5.7	2.5

It is also evident that there are trade-offs between the different performance criteria. In particular, early development and productivity are both negatively correlated with resistance to decomposition (correlation coefficients on the ranks as presented in Table 14: -0.52 and -0.46, respectively). Also, there is no species that scores among the three best across all evaluation criteria, highlighting the functional complementarity of the different legume species.

Further evidence for the complementarity among the species comes from looking at the way productivity developed over time (Figure 47). Whereas in some species (such as CC and SC) biomass (relative to the site maximum) declined between 2009 and 2011, other species such as TY showed significant increases over time. Although many species did not show slopes significantly different from zero, it is notable that all grass species showed positive slopes, which is consistent with the general increase of proportion of grass biomass in the ASM (Figure 45); at the same time, all clovers showed negative slopes.

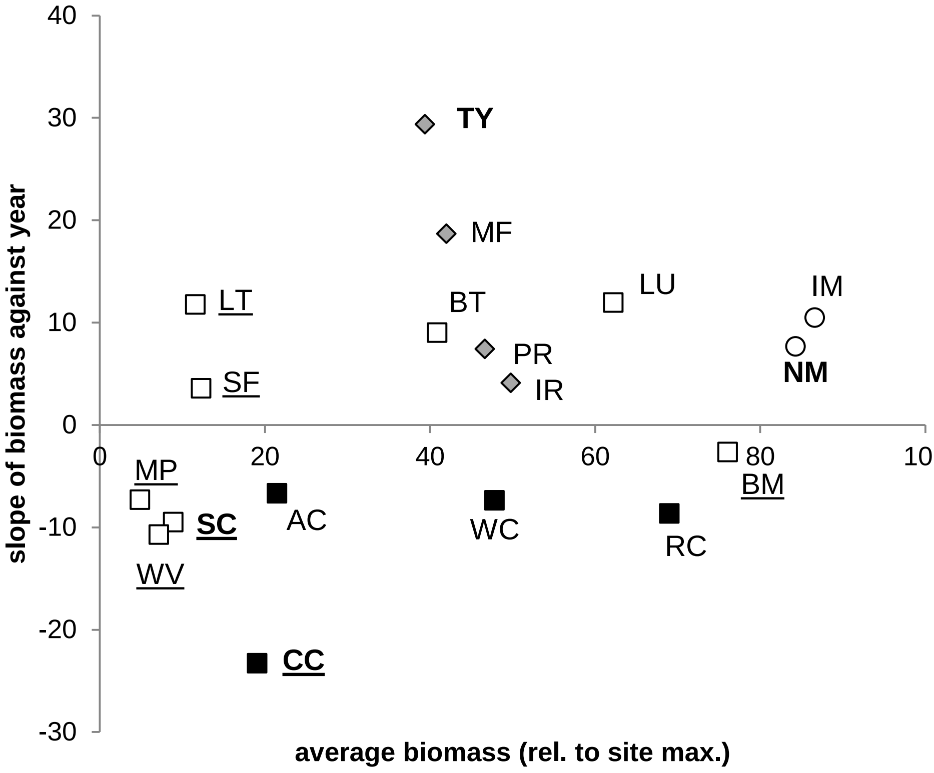

Figure 47. Above-ground biomass averaged over 3 sites (Duchy, Wakelyns, SAC) and over three years (2009, 2010 and 2011) against the biomass development over time (slope against year). Bold labels indicate slopes with p<0.1; underlined labels indicate legume species with significantly different average biomass values compared to WC.

Also, early development (speed of emergence) correlated negatively (r=-0.40) with the slope of biomass over time, i.e. species that emerged relatively *slowly* tended to *increase* their biomass (relative to the site maximum) over the following two years; an interesting exception is LU, which emerged quickly (Table 14) and showed a positive (albeit non-significant) slope. Similarly, the slowest grass to emerge (TY) showed the strongest increase of biomass over time. Interestingly, the All Species Mixes showed the maximum average biomass and, in the case of NM, significant biomass increases over time.

We further analysed the relationship between seed weight and other variables to investigate potential factors underlying the observed trade-offs. While there was a *positive* correlation between seed weight (measured as the logarithm of the thousand kernel weight) and seedling biomass 60 days after sowing (Adj. R^2=0.458, p=0.0093, df = 10), there was a significant *negative* relationship between seed weight and regrowth after simulated grazing (Adj. R^2=0.340, p=0.0274, df = 10) (Figure 48). Similar negative relationships were observed for other productivity parameters (e.g. crop cover in spring 2010, (Adj. R^2=0.305, p=0.0367, df = 10). In other words, while species with large seeds tended to perform well very early during the trials and high seed weight provided resources for early growth, their later productivity was compromised and species with lower seed weights performed relatively better.

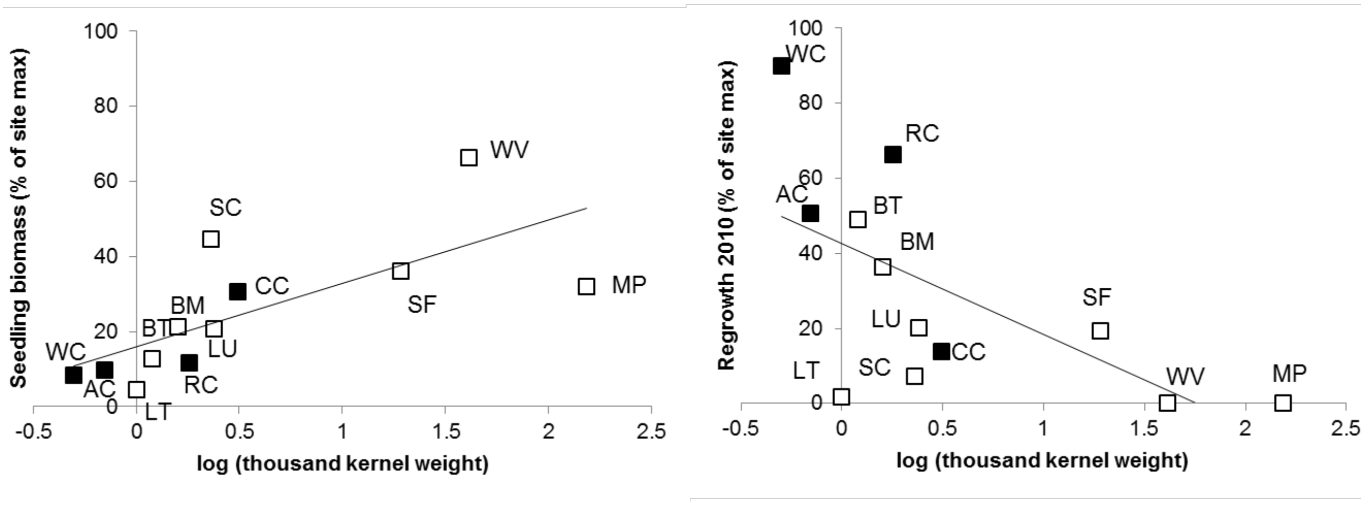

Figure 48. Relationship between seed weight (log thousand kernel weight, x-axes) and seedling biomass (left) and biomass regrowth after low cut in 2010 (right).

Regional differentiation of species productivity

We finally tested whether the performance of individual species was significantly different at the six research trial sites by comparing the data of individual sites to the overall average across all sites with a two-factorial ANOVA.

According to this analysis there were not many significant interactions between species and site for the variables tested (Table 15). In fact, the frequency of interactions was not higher than would be expected by chance (there were 19 interactions between site and species with p<0.1 out of 392 possible cases = 4.8% of cases; there was no variable in which the frequency of site by species interactions was higher than 10%.) Notably there were no negative deviations from the average across sites with $p<0.05$, i.e. at no site did any species significantly deviate from the overall average. This indicates that the results are relatively robust across the trial sites and that the ‘global’ results shown above (in section 3.3.1) are representative.

Table 15. List of interactions between species and site for which P<0.1 for biomass variables

Variable	Species	Site	P-value	change	possible interaction cases
Seedling rel. growth rate	WV	D	0.077	+	60
Biomass Summer 2009	CC	S	0.021	+	102
	TY	E	0.038	+	
	PR	D	0.061	-	
	IM	E	0.074	-	
Biomass (Sim. grazing 1)	RC	R	0.073	-	65
	BT	W	0.023	+	
	IM	W	0.083	+	
Biomass (Sim. grazing 2)	BM	W	0.017	+	65
	NM	W	0.039	+	
	LU	W	0.092	+	
Crop cover spring 2010	SF	D	0.087	-	100
	PR	I	0.024	+	
	RC	I	0.071	+	
	TY	I	0.017	+	
	WC	I	0.098	+	
	CC	R	0.006	+	
	SF	R	0.096	+	
	CC	W	0.078	-	

3.3.2. Ecophysiological modelling (WP2)

MP established poorly at a number of the sites and did not recover after mowing. This species was, therefore, excluded from many of the analyses presented here. Other large seeded species (SC, SF, CC and WV) had high absolute growth rates early in the season (Table 16). However, because they were also tall and had their growing points largely above the level of cutting, these species had slow rates of re-growth. In the case of CC (an annual), any secondary growth post-mowing was from seed. More prostrate species, including BT, BM, WC and AC, had the highest rates of re-growth and were also more resistant to grazing.

Table 16. Contrasting functions delivered by 11 legume species (excluding Meadow pea, L. pratensis) measured at six research hubs (table quotes averages across all research hub sites).

Species	Weeds g/m2	Re-growth (g/g/d)	Invertebrate numbers	Early growth (g/plant/d)	Autumn crop yield (t/ha)	Spring crop yield (t/ha)
AC	175.85	0.0213	534	0.010	9.66	2.16
BT	128.63	0.0187	444	0.009	9.68	2.33
BM	73.88	0.0192	507	0.010	9.69	2.17
CC	161.90	0.0123	413	0.025	8.69	1.78
LT	207.20	0.0095	399	0.002	7.28	1.89
LU	79.33	0.0176	342	0.015	10.37	2.76
RC	84.28	0.0206	442	0.010	9.49	1.94
SC	128.60	0.0103	388	0.037	7.38	2.06
SF	135.68	0.0084	299	0.029	7.90	2.14
WC	93.78	0.0226	506	0.005	11.31	2.77
WV	183.13	0.0102	601	0.080	5.75	*

Modelling competition between species

The simulation model of inter-plant competition, based on parameters measured in species monocultures, successfully predicted the relative proportions of the different species in the ASM (Figure 49). Before mowing, the model predicted that the mixture would be dominated by the more competitive species early in the season, particularly CC which has a large seed and is relatively tall. Less competitive species, for example LT, were only present as a very low proportion of overall biomass. After mowing, the model predicted that the mix would be dominated by BM, LU, RC and WC. This reflected what was observed in the field, although the competitive balance between WC and RC was shifted more in favour of the latter species than was predicted by the model. The model was not designed to simulate a second season of growth (including overwinter dynamics) and the final mixture composition appeared to indicate a reduced proportion of BM and WC. However, BM was observed to die back at Rothamsted after mowing in the late summer of the second year in contrast to other sites where it persisted and WC also continued to be a dominant component in the mix at other sites.

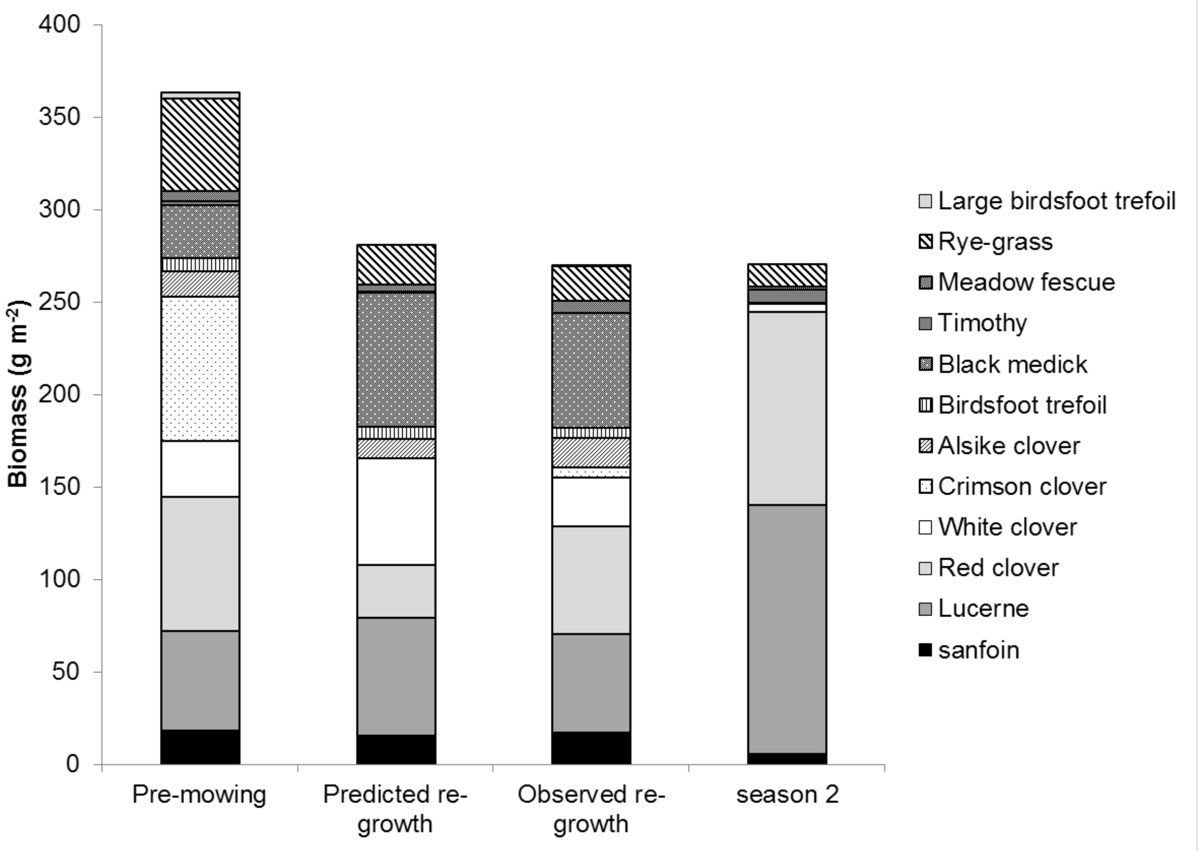

Figure 49. Predicted biomass of component species in ASM at Rothamsted immediately prior to mowing and at the final re-growth sample compared to observed re-growth data and biomass immediately prior to incorporation in the second year.

Designing optimal, multi-functional legume mix

Variance between the legume species in their functionality (in terms of weed suppression, early growth, re-growth, support of phytophagous invertebrates and yield of the following crop) was significantly explained using different combinations of traits (Table 17, Table 18). It is important to note that legume biomass was an important co-variate in the optimum models of weed suppression and yield of the following crop. These functions were used as the basis for calculating the multi-functionality of different combinations of legumes. The model of inter-plant competition was first used to calculate relative biomass of the component species in different combinations of legumes (e.g. Figure 50). This output was then used to calculate the aggregated trait values for the different mixes and input into the regression equations predicting the relationship between traits and functions. An index of multi-functionality for each mix was derived by assigning each mix a rank for each function and then adding the reciprocals of the rankings. Ranking in terms of polyphenol and lignin content of the residues were also included in this index assuming that higher levels would be desirable to slow down the rate of decomposition of the mulch.

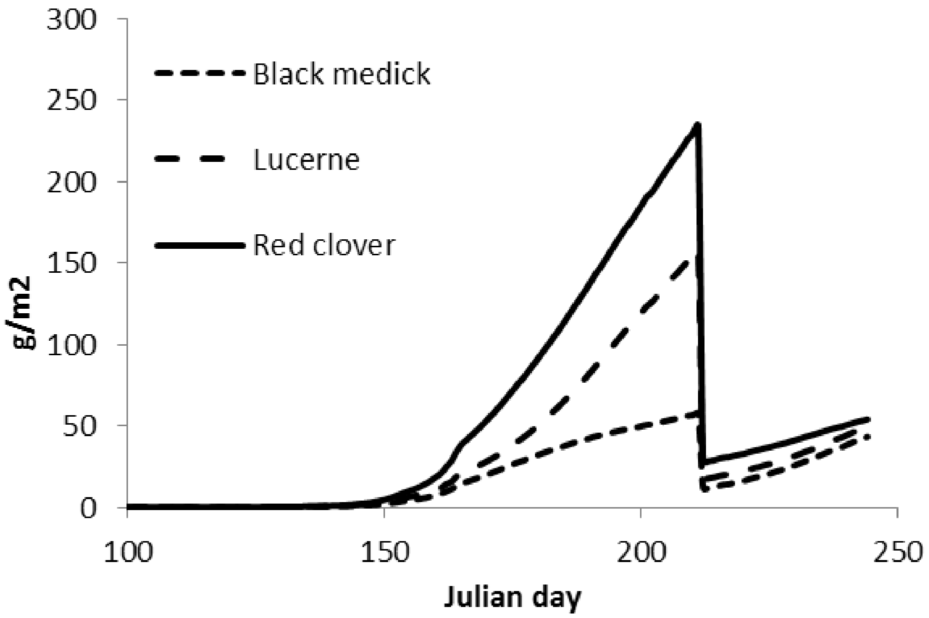

Figure 50. Example of model output with a 3-species mix to calculate proportional biomass.

Table 17: Plant functional traits measured on the legumes and used to derive relationships with functions.

Species	**SLA* (m^2/g)**	**C:N early season**	**Height (cm)**	**L:S**	**Leaf N (%)**	**Ln seed weight**	**Lignin****	**Residues Polyphenols****	**C:N****
AC	0.033	13.95	50.53	0.35	4.62	-7.00	3.75	0.24	9.70
BT	0.025	16.70	45.53	0.41	4.34	-6.58	7.94	0.24	14.26
BM	0.030	13.11	41.33	0.35	4.25	-5.65	11.34	0.16	20.87
CC	0.026	25.48	53.93	0.22	3.46	-5.39	3.94	0.20	13.99
LT	0.024	15.71	14.43	0.51	3.40	-7.22	13.46	0.27	19.30
LU	0.024	17.61	73.87	0.27	4.69	-6.17	8.00	0.16	14.35
RC	0.029	14.18	59.20	0.32	4.94	-6.19	4.91	0.30	10.82
SF	0.017	18.17	93.20	0.41	4.22	-3.83	9.32	0.38	12.34
WC	0.032	14.23	28.40	0.34	4.48	-7.20	5.34	0.17	12.27
SC	0.023	15.06	102.87	0.38	5.06	-5.87	7.81	0.15	22.06
WV	0.026	12.54	79.67	0.36	5.07	-2.96	*	*	*

*SLA: specific leaf area

** not based on data from all sites

Table 18. Relationships between combinations of plant traits and functions derived from all subsets regression.

Service	Sites	%var	p-value	Function
Early productivity (g/plant/d)	1,3,4,5	71.4	<0.001	y = 0.1034 + 0.01414 * Seed
Regrowth (g/g/d)	1,2,3,4,5	68.1	0.011	y = 0.03146 - 8.53e-4 * C:N -0.0476 * L:S - 0.002527 * Seed
Weed suppression	1,3,4,5	94.5	<0.001	y = 0.0027 +3.86e-5 * biomass + 0.0014 * Leaf N - 6.5e-4 * Seed -0.358 * SLA
Invertebrates	1	89.2	<0.001	y = -59 + 433 * L:S +44.95 *Seed +23197 * SLA
Winter crop yield	1	88.7	<0.001	y = 12.47 + 0.00732 * biomass – 0.1995 * Residue C:N – 9.07 * polyphenols
Soil fertility	1	78.3	<0.001	y = 164.7 + 16.25 * Seed
Spring crop yield	3,5		NS	
Pollinators	1		NS	

SLA: specific leaf area; NS: not significant; Sites: 1:, 2:, 3:, 4:, 5:

As a result of these intrinsic trade-offs, no one species could deliver high productivity both before and after mowing, high weed suppression and a complex residue structure. When the monocultures were compared with binary mixes, therefore, the mixes out-performed the monocultures. This was true using both the weather data from IBERS and Rothamsted; the best performing mixtures were generally similar although the order varied between the sites. The top two mixtures at both sites were BM + RC and LU + WC (Figure 51).

Generally, including a third and fourth species also improved the multi-functionality of the mixes with the best mix (at Rothamsted) being BM + LU + RC. Including WC made little difference but would improve the resilience of the mix. Including rye-grass decreased the index of functionality but does not take account of possibly enhancing N fixation.

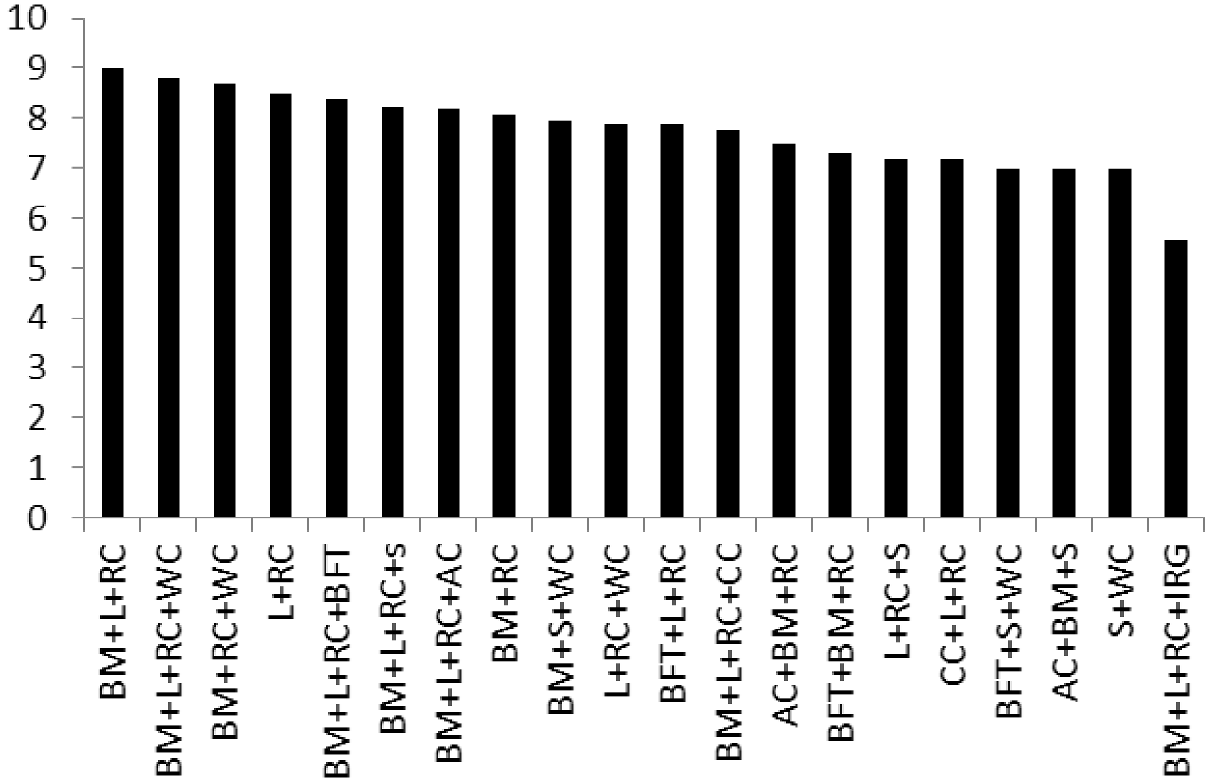

Figure 51: Score of the top 2-way, 3-way and 4-way mixtures according to ecological modelling

3.3.3. Participatory field trials with legume mixtures (WP3)

General performance of legume species within a complex mixture

In the figures below we present the results with the species ordered by decreasing performance in the first parameter (*farm presence*, Figure 52, also see section 3.2.5, page 33 on Data analysis); i.e. throughout the results section of this work package the order of species in the graphs is always the same.

When all years are considered together, the presence of legume species within the All Species Mix (ASM) on the participatory farms was highest in WC, followed by RC; however, the difference between WC and RC was not significant. These two species are followed by a group of four species (AC, BT, LU, BM) with intermediate presence; lowest values were found for MP (Figure 52).

When looking at presence of species in the quadrats over the three individual years (Figure 53), WC and RC are strongest performers shortly after establishment of the ley in year 1, but even more so in the following two years. CC shows a high quadrat frequency in the first year, but decreases strongly in the second as expected for an annual species. As for the presence on farms, a middle range is occupied by AC, BT, BM, LU, LT and SF, whereas very low performance was observed in MP. In 2010 and 2011, stronger differences are found than in 2009. In 2011, there is marked decrease of presence in the quadrats for LU.

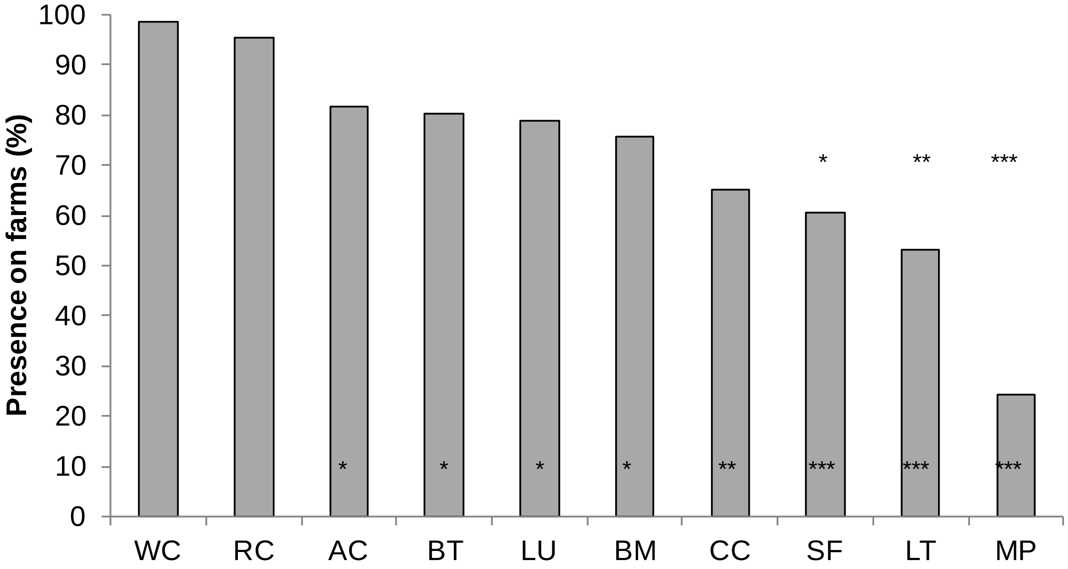

Figure 52. Presence of legumes over three years (2009–2011) on a balanced set of 22 farms. The lower row of stars indicates the significance of the difference between individual species and WC (the best performer). The upper row indicates the significance of the difference between individual species and the maximum after WC and RC (in this case: AC). The maximum value (100%) corresponds to 3 x 22 = 66 observations. (Dunnett test, *: $p<0.05$, **: $p<0.01$; ***: $p<0.001$).

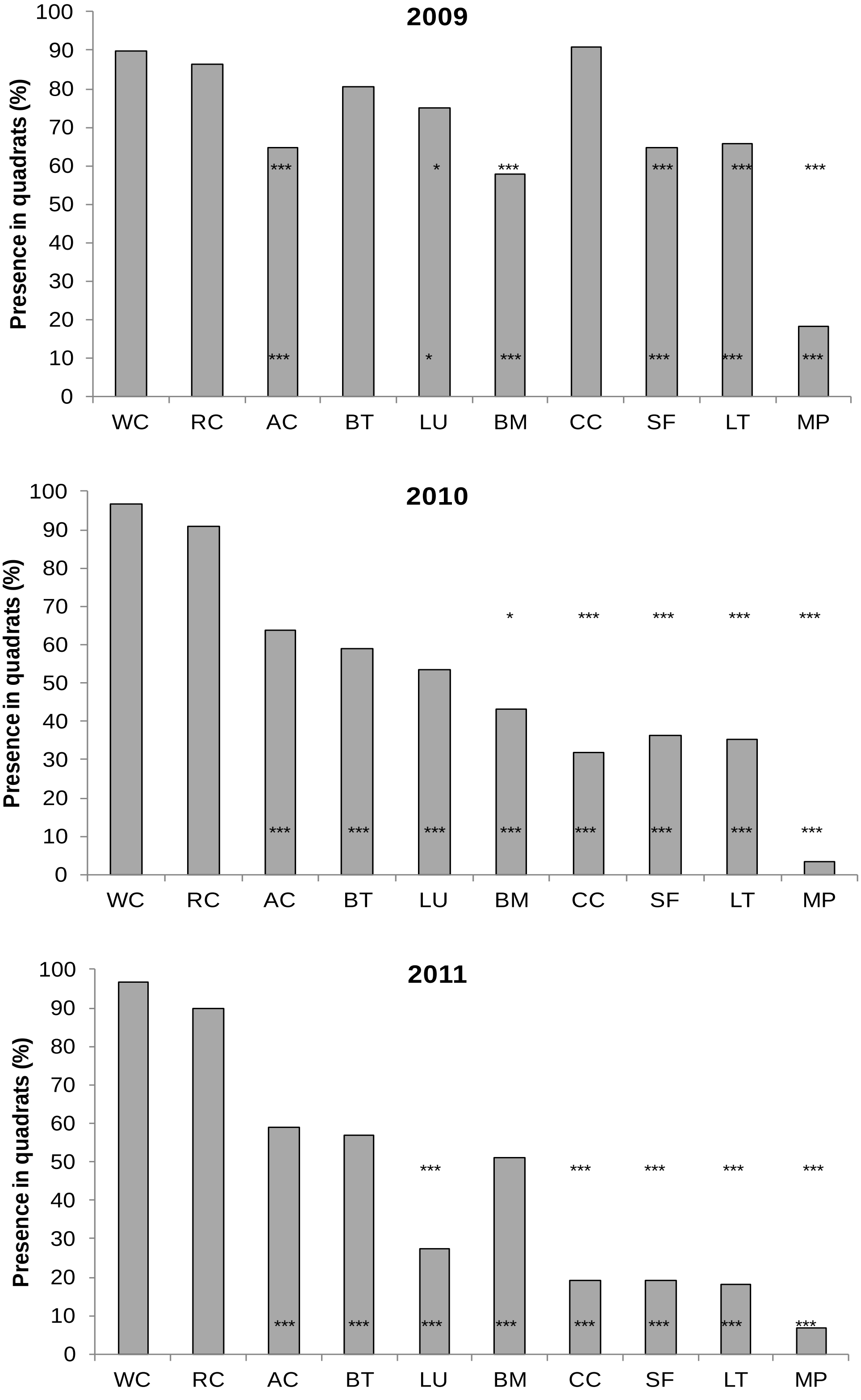

Figure 53. Presence of legume species in 3 years on a balanced set of 22 farms in 4 sampling quadrats. The lower row of stars indicates the significance of the difference between individual species and WC. The upper row indicates the significance of the difference between individual species and the maximum when WC and RC are taken out of the dataset e.g. in 2009 this maximum is found in CC. The maximum presence (100%) corresponds to 4 x 22 = 88 observations. (Dunnett test; *: p<0.05, **: p<0.01; ***: p<0.001)

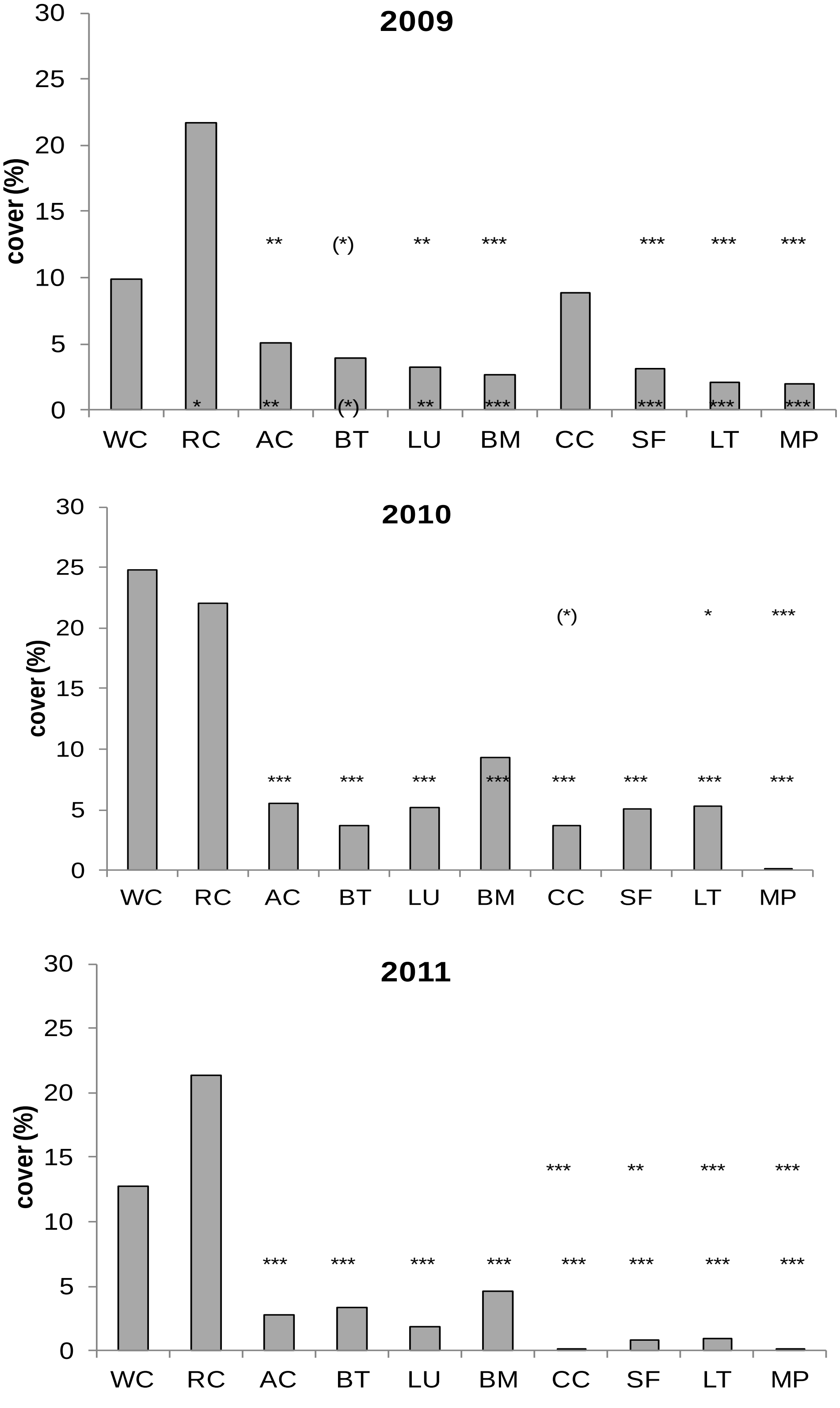

Figure 54. Estimated ground cover of 10 legume species in the All Species Mix across 22 farms in the UK. Error bars show standard errors. The lower row of stars indicates the significance of the difference between individual species and WC. The upper row indicates the significance of the difference between individual species and the maximum when WC and RC are taken out of the dataset. (Dunnett test; *: $p<0.05$, **: $p<0.01$; ***: $p<0.001$)

Crop cover (Figure 54) shows similar results to the quadrat presence data, with WC and RC showing highest values in all three years; CC performing well in the first year and then decreasing in crop cover and AC, BT, LU and BM being in the middle range. Lowest values were again observed for MP. Analysing the development of ground cover over time (i.e. a measure of the persistence of species, Figure 55) reveals that CC, SF and MP are the only species for which ground cover significantly decreases over time. In general there is large variation between farms regarding this parameter.

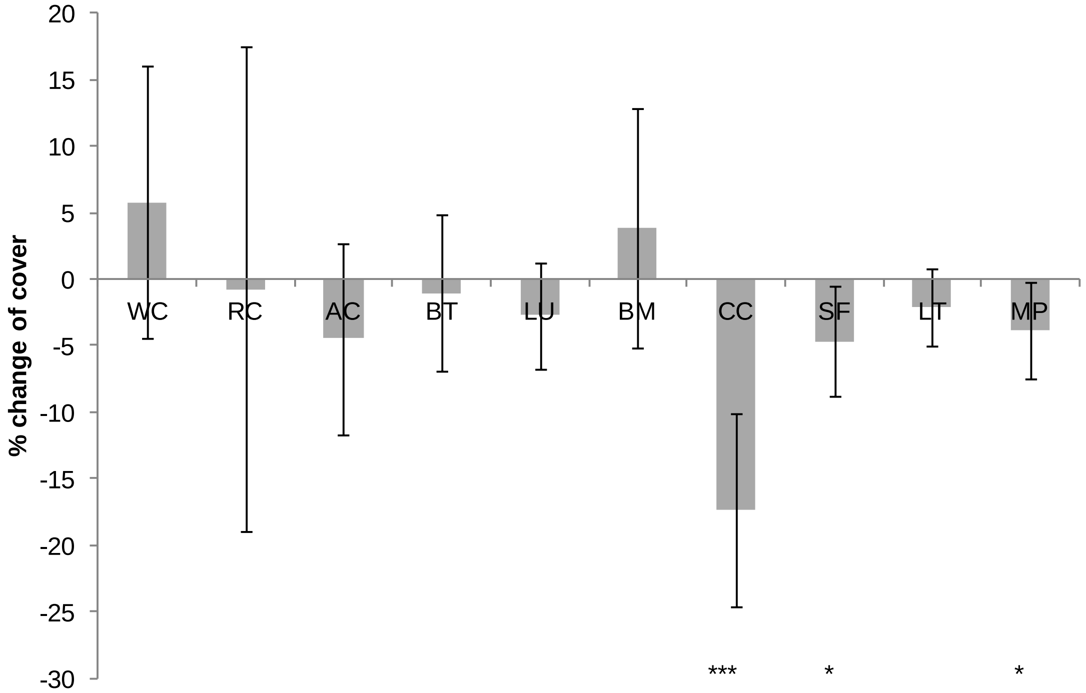

Figure 55: Change of ground cover over three years in 10 legume species growing in the All Species Mix on 22 farms across the UK. Error bars show standard errors. Stars show whether the slope of cover over years is significantly different from zero (t-test, p<0.05, ***: p<0.001).

The performance of individual legume species in the ASM across the participatory farms, measured by the performance indicators presence on farm, presence in the sampling quadrats, and ground cover is summarised in Table 19.

Consistently well-performing species are WC and RC, with WC being more reliable than RC in terms of presence while RC produces significantly higher early ground cover than WC. Generally the different performance indicators show good agreement; a notable exception is CC, with high early ground cover but poor persistence.

Table 19. Ranks of 10 legume species growing in the All Species Mix on 22 farms across the UK. Lower ranks indicated better performers.

Species	Farm presence	Quadrat presence	Ground cover	Early ground cover	Persistence
WC	1	1	2	2	1
RC	2	2	1	1	3
AC	3	4	4	4	8
BT	4	3	6	5	4
LU	5	5	7	6	6
BM	6	6	3	8	2
CC	7	7	5	3	10
SF	8	8	8	7	9
LT	9	9	9	9	5
MP	10	10	10	10	7

Response of legume species to growing regions, environmental and management variables

Regional differences between individual legume species in the ASM were analysed by comparing the presence of the legume species in the sampling quadrats for the four main regions of the study (North, South, East and West; Figures 56 and 57 below, Table 3 in Methods). AC and LT showed their highest presence in the West, whereas BM was performing best in the South; LU and SF were growing best in the East. BT, CC, WC and RC showed no significant influence of the region.

For this dataset, regional differences in the performance of the species cannot be attributed to any single factor (such as specific climate or soil conditions) because environmental conditions are confounded with management factors (such as grazing regime, which will tend to reflect climatic conditions). For example, while the Western farms will tend to be characterised by a moister climate more than the Eastern farms, the Western farms were also all grazed, whereas none of the Eastern farms were grazed.

However, for a subset of farms from two regions (North and South) where there was at least one grazed and at least one non-grazed site in the balanced subset of farms, significant effects of grazing on legume presence in the quadrats were observed. LU was performing significantly better on the non-grazed sites within region (presence in 67% of quadrats on non-grazed sites vs. 41% on grazed sites, p=0.0073). CC showed a similar pattern (non-grazed: 67%; grazed: 42%; p = 0.0092). The opposite effect was observed for BM (non-grazed: 42%; grazed: 61%; p= 0.031). All other species showed no significant responses to the grazing factor.

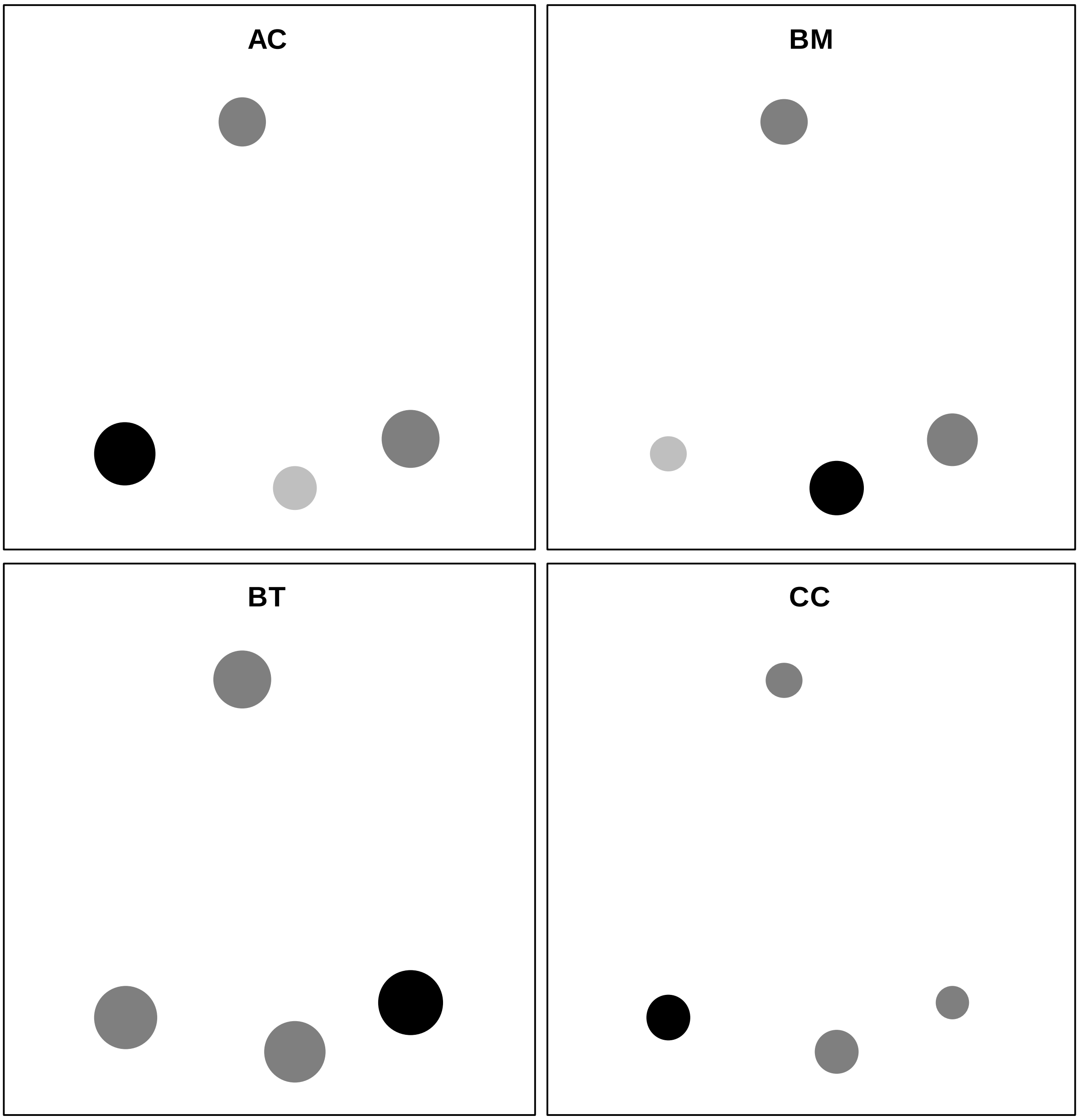

Figure 56. Presence of legume species in sampling quadrats growing in the All Species Mix on 22 participatory farms across the UK, broken down for the four main regions of the study. The centre of the circle shows the geographic centre of the sampled farms; the area of the circle corresponds to proportion of quadrats in which the species was found to be present. Black circles: region with the maximum presence value. Light grey circles: region with significantly smaller presence value than the maximum. Dark grey: region not significantly different presence value than the maximum (Dunnett test).

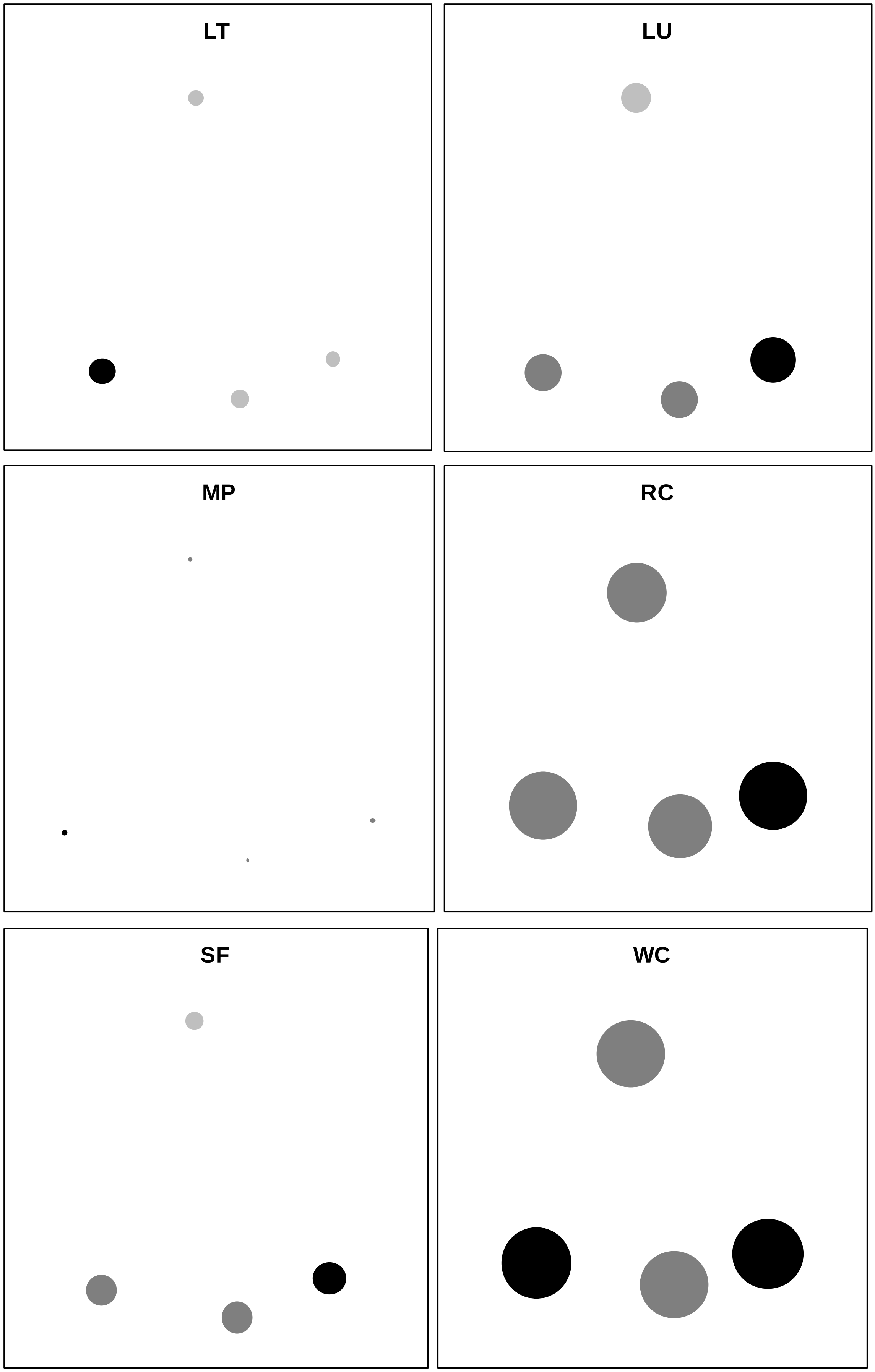

Figure 57. Presence of legume species in sampling quadrats growing in the All Species Mix on 22 participatory farms across the UK, broken down for the four main regions of the study, continued. For further explanations see previous figure.

The multiple regression analysis on the soil parameters revealed that the most important factor for determining legume presence was potassium (K) (Table 20). Unexpectedly, there was a consistent negative correlation between K and the presence of many of the legume species, most notably in WC, RC and BT and LT (Table 21, Figure 58).

The second most important factor for the legume species was pH; interestingly, the effect of pH became gradually more important over time (Table 20). For most species, higher soil pH was associated with larger presence values; when considering all years together, this effect was strongest in BM (Table 21, Figure 59). For some species (WC, RC, LU, MP) a high sand content was associated with a greater presence values in the first year.

Table 20. Ranks of the importance of various soil parameters as predictors for legume presence across participatory farms in the UK. Low numbers show high importance. Importance values were determined via cumulative Akaike weights in the model reduction process (Burnham & Anderson 2002).

Importance	Sand	pH	P	K	Mg
all years	5	2	4	1	3
2009	2	5	3	1	4
2010	4	3	2	1	5
2011	5	1	4	3	2

Table 21. Influence of various soil parameters on legume presence in the ASM. Since the factors were not manipulated experimentally, results need to be taken with caution. +: positive correlation, -: negative correlation. (+): 0.05< p<0.1. ++: r>0.4; --: r<-0.4. Empty cells represent non significant effects. Best models selected on the basis of AIC. Year 1 is 2009, Year 3 is 2011; All = Year 1 to year 3.

Factor	**Sand**			**pH**			**P**			**K**			**Mg**		
year	**all**	**1**	**3**	**All**	**1**	**3**	**all**	**1**	**3**	**All**	**1**	**3**	**all**	**1**	**3**
WC		+				+	-		+	--	-	--	+		+
RC		+			+	+					--	--			+
AC	-		-		--	+				-		-	+		+
BT						+			-		--	-	--		
LU		+				++		-	-	(+)	-	++		+	
BM				++		++	(+)	+		(-)		-			+
CC															
SF				+		++	--	--			-				+
LT				--		--		+	-	--	--				+
MP	(+)	+					(-)			(-)			(+)		

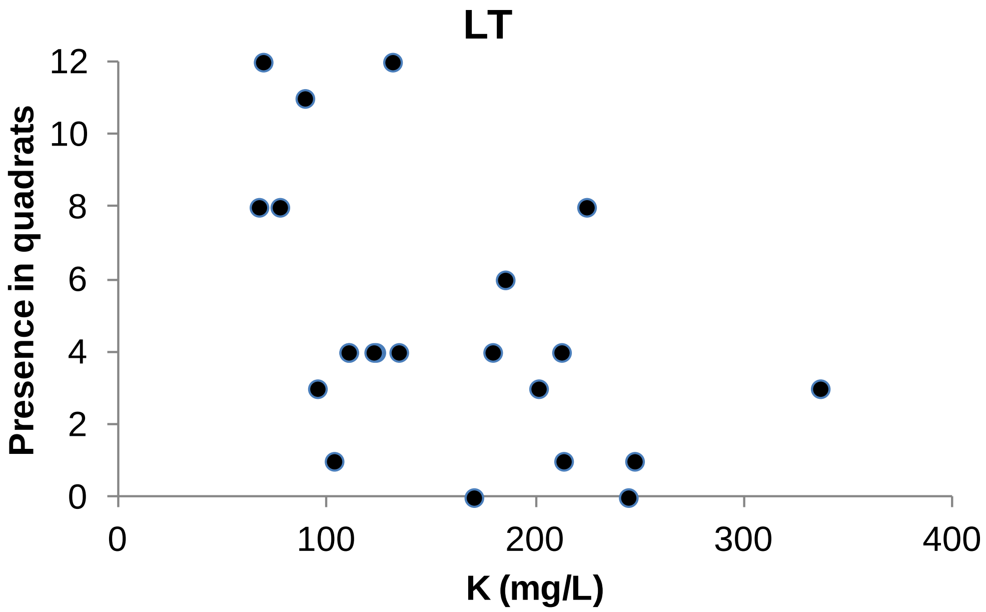

Figure 58. Presence of Large birdsfoot trefoil in sampling quadrats vs. potassium (mg/L) (all years). Maximal number of observations is 3 years x 4 quadrats =12. Each dot represents one farm.

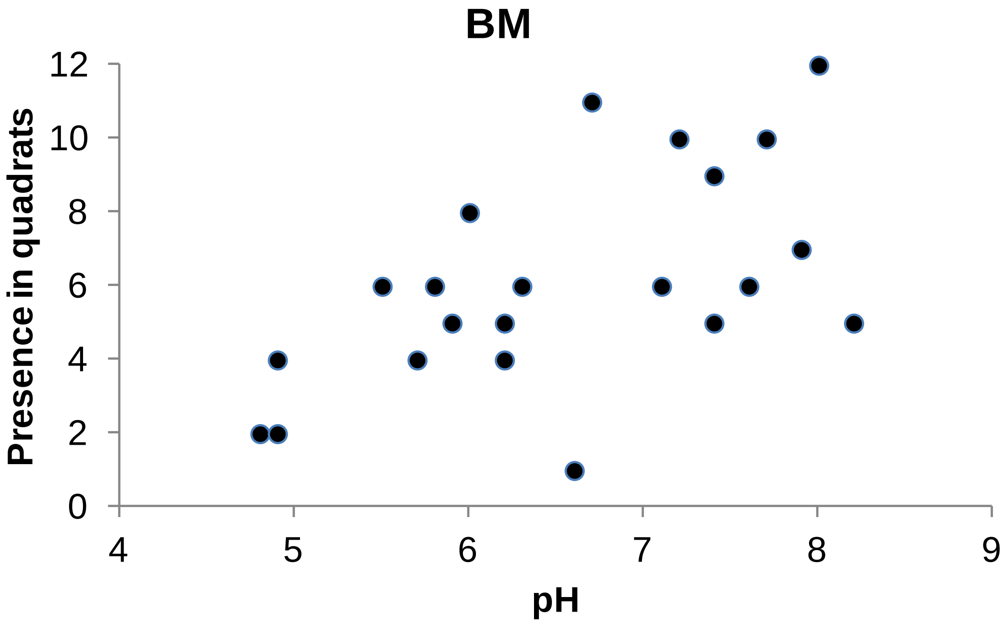

Figure 59. Presence of Black medic in sampling quadrats vs. soil pH (all years). Maximal number of observations is 3 years x 4 quadrats =12. Each dot represents one farm.

Species diversity of sown legumes and weeds in the ASM

***Legume** diversity*

The number of legume species *sown* was much higher in the ASM (10) than in the control (3.3 ± 0.7 species; mean ± standard error, compare Table 4, page 30). Over time, the legume species richness in the ASM decreased from 8.5 species per farm in 2009 to 6 species per farm in 2011.

The Northern (Scottish) farms showed a significantly stronger decrease in the number of legume species over time than the other three regions ($p < 0.0032$ for North vs. East; $p < 0.001$ for North vs. West and North vs. South; Tukey-test). Also, within the non-Scottish farms there was a tendency for species diversity to be lower the farther North the site was situated ($p=0.108$); this effect was significant ($p=0.044$) when the AIC-best model was used (Burnham & Anderson, 2002).

Weed community composition in on-farm trials

In the first year of the ley (2009), the most frequently encountered weed species were chickweed (*Stellaria media*), sowthistle (*Sonchus arvensis*), and field speedwell (*Veronica persica*) (Tables 22 and 23). These species are very common and typical annual weeds of arable fields. In the second year of the ley, almost all annual species decreased in frequency. Conversely, some perennial species such as dandelion (*Taraxacum officinale agg*.) and creeping thistle (*Cirsium arvense*) increased in frequency over time.

Factors affecting weed species richness

In total, 63 weed species were recorded on the organic leys (both ASM and control leys combined). With a total of 56 weed species found in the first year of the ley, the species richness was twice as large as in second one, when only 28 species were recorded. Similarly, the number of weed species *per farm* was 11.9 ± 1.6 and 3.8 ± 0.7 in the first and second year respectively (average ± standard error). The total number of weed species found on each farm, in both years together, ranged from 3 to 27.

Weed species numbers between the first and the second years of the study were uncorrelated (Adjusted R^2= 0.079, $p=0.137$), i.e. farms with a higher number of weed species in the first year did not necessarily tend to have a higher species number in the second year as well. Also, weed diversity did not correlate with the crop species diversity in the ley (Adjusted R^2 = 0.007, $p=0.247$), indicating that increasing the number of species within in a ley mixture does not compromise the conservation of wild farmland plants.

Interestingly, on farms where soil samples showed relatively high organic matter contents (i.e. values above the average of 5.3%), a significantly lower number of weed species was found than on farms with below-average organic matter contents (9.2 ± 1.6 vs. 20.0 ± 2.6 species, $p<0.01$, df=10). As expected, the number of weed species was lower in the more Northern farms than further South.

Factors affecting weed cover in on-farm trials

There were no strong effects of any of the measured environmental variables (soil parameters, geography, and management) on either the total weed cover or on problem weeds.

Table 22. Weed species in year 1 and 2 of the ley: Presence on number of farms and on number of squares, sorted by number of squares in 2009 on which the species was present: Presence on 10 or more quadrats.

Presence on 10 or more quadrats	**No. Farms (out of 21)**		**No. Quadrats (out of 168)**	
Species	**2009**	**2010**	**2009**	**2010**
Stellaria media	15	7	82	15
Sonchus (arvensis)	10	0	38	0
Veronica persica	8	0	35	0
Persicaria maculosa	11	0	33	0
Ranunculus repens	9	5	32	13
Viola arvensis	9	2	32	7
Spergula (arvensis)	6	0	26	0
Veronica spec.	5	4	26	6
Chenopodium album	8	1	23	2
Poa annua	7	0	21	0
Lamium (purpureum)	6	2	20	3
Myosotis arvensis	7	1	20	1
Sinapis arvensis	5	4	20	5
Anagallis arvensis	5	0	19	0
Tripleurospermum maritimum	4	0	19	0
Capsella bursa-pastoris	5	0	18	0
Galeopsis tetrahit	4	0	17	0
Polygonum spec.	4	0	16	0
Rumex spec.	8	5	16	11
Anthemis arvensis	4	2	15	7
Convolvulus (arvensis)	5	1	15	1
Fallopia convolvulus	5	0	15	0
Papaver rhoeas	4	2	14	4
Polygonum aviculare	3	0	14	0
Taraxacum officinale	5	8	13	22
Galium aparine	3	1	12	1
Cirsium arvense	5	2	9	12
Cerastium (fontanum)	2	3	8	5
Elytrigia repens	1	1	8	3
Achillea millefolium	2	1	4	6
Aphanes arvensis	1	2	3	11

Table 23. Weed species in year 1 and 2 of the ley: Presence on number of farms and on number of squares, sorted by number of squares in 2009 on which the species was present: presence on fewer than 10 quadrats

Presence on fewer than 10 quadrats	No. Farms (out of 21)		No. Quadrats (out of 168)	
Species	**2009**	**2010**	**2009**	**2010**
Senecio vulgaris	4	0	9	0
Alopecurus myosuroides	3	0	9	0
Vicia hirsuta	1	0	8	0
Geranium spec.	4	1	5	1
Avena fatua	2	0	5	0
Kickxia elatine	2	0	5	0
Plantago major	2	0	5	0
Sisymbrium officinale	2	0	4	0
Matricaria recutita	1	0	4	0
Brassica napus	1	0	3	0
Legousia hybrida	1	0	3	0
Veronica arvensis	2	1	2	6
Mentha arvensis	2	0	2	0
Glebionis segetum	1	0	2	0
Kickxia spuria	1	0	2	0
Aethusa cynapium	1	0	1	0
Fumaria officinalis	1	0	1	0
Lactuca serriola	1	0	1	0
Lapsana communis	1	0	1	0
Matricaria discoidea	1	0	1	0
Matricaria matricarioides	1	0	1	0
Odontites vernus	1	0	1	0
Poa trivialis	1	0	1	0
Senecio jacobea	1	0	1	0
Urtica urens	1	0	1	0
Poa spec.	0	1	0	8
Cirsium vulgare	0	2	0	5
Cichorium intybus	0	1	0	4
Arabidopsis thaliana	0	1	0	2
Daucus carota	0	1	0	1
Sherardia arvensis	0	1	0	1

Analysis of plant residue parameters

Across the 13 farms in which plant residue properties were measured in the ASM and the control ley, the total polyphenol content in the plant samples was higher in samples with low lignin content, i.e. there was a significant negative relationship between log-transformed lignin content and the polyphenol content (Figure 60) (Adj. R^2= 0.247, p<0.001, df=106).

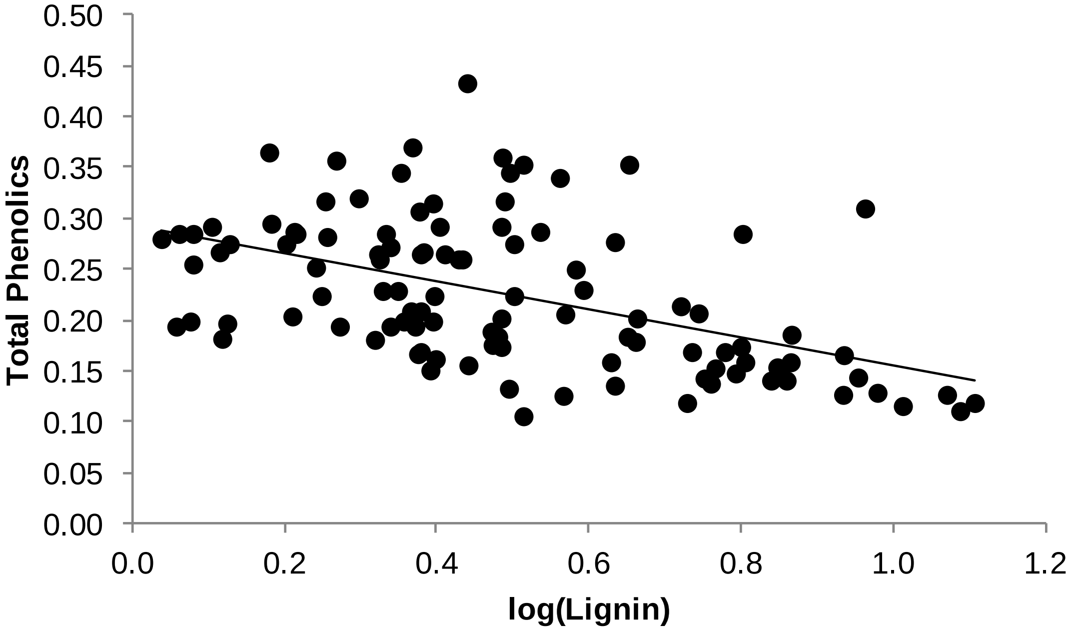

Figure 60. Relationship between Lignin content and phenolic content.

None of the soil parameters had a significant influence on the plant residue characteristics. However, generally there was an effect of geographic region on polyphenol and lignin content, with the samples from the Western region (n=6 farms, Duchy Hub) being significantly higher in polyphenol and lower in lignin content (Table 24).

Table 24. Regional differentiation regarding plant residue composition in the ASM; means ± standard deviation. The p-values indicate the statistical significance of the difference between the two regions, West and Central.

Parameter	West		Central		p-value (Region)
Phenol	0.27	± 0.05	0.18	± 0.05	0.0002 ***
C:N	13.27	± 2.93	12.95	± 2.75	0.8110 ns
ADL	2.63	± 0.97	4.85	± 2.78	0.0215 *

Composition of above-ground plant residues

Compared to the Control ley, the ASM was significantly lower in polyphenol content; however, absolute differences in the polyphenol content were small (Table 25). There were no significant effects of ley type (ASM vs. Control) on the C:N ratio or the lignin content of the plant samples.

Table 25. Residue properties in ASM compared to control ley, means ± standard deviation

Treatment	**Phenol**		**C:N**		**ADL**	
ASM	0.22	±0.07	13.0	±3.6	4	±2.8
Control	0.23	±0.07	13.2	±2.9	3.6	±2.2
p-value (treatment)	0.06		0.9		0.42	
p-value (Farm)	<0.001		0.0938		<0.001	
p (Treatment) LinMixed	0.0355		0.901		0.407	

Further data analysis using community weighted averages showed that, regarding the development over time, grasses have most influence on the residue composition of the ley mixtures, with the C:N ratio increasing over time, as grasses (with their higher C:N ratios) increase in dominance.

Soil nitrogen pre- and post-incorporation of the ley

Regarding soil nitrogen pre- and post-incorporation, differences between ASM and control were not significant (Table 26). On 4 of the tested farms, the ASM showed higher, while the other 4 farms showed lower, pre-incorporation content of available nitrogen in the soil. Among the 8 farms, pre-incorporation available N ranged from 33.0 to 136.4 kg N/ha (median 92.8, mean 84.6 kg N/ha). On those farms where both pre- and post-incorporation nitrogen was measured in the soil, the post-incorporation was always lower than pre-incorporation.

Interestingly, the variability of nitrogen between farms was markedly lower in the ASM than in the Control ley, but it was difficult to establish if this would be an effect of plant diversity or an effect of the different farms, or of using differences among the control leys.

Table 26. Nitrogen in soil samples pre- and post-incorporation of the ley.

		Dry matter %w/w	**Nitrate N mg/kg**	**Ammonium N mg/kg**	**Available N kgN/ha**
Pre (n= 8)	Control	79.9	12.4	8.3	82.7
	ASM	81.1	13.6	8.0	86.5
Post (n=3)	Control	72.5	22.4	7.8	120.8
	ASM	73.5	25.7	4.9	122.7
Diff. Post-Pre (n=4)	Control	3.05	1.7	-13	-45.5
	ASM	6.7	7.3	-18	-41.25

3.3.4. Modelling nitrogen dynamics (WP4)

Model performance (validation)

The grain yield of the spring barley crop (cv. Westminster) grown after N-fixing ley species was predicted with greater accuracy than was the yield after grasses (Table 27) where predictions following both grass species were only 47 – 61 % of that actually measured.

Table 27. Spring barley grain yield (t ha-1) after two years of fertility-building ley composed of either nitrogen-fixing or non-fixing (grass) species. The measured values are the mean of three replicate plots and standard deviations are given in brackets.

	BM	**LU**	**RC**	**WC**	**PR**	**TY**
Measured yield	4.5	5.5	5.0	5.4	5.3	4.1
(t ha^{-1})	(0.57)	(1.10)	(0.68)	(1.22)	(0.54)	(1.68)
Predicted yield	4.2	5.1	5.2	5.0	2.5	2.5
(t ha^{-1})						
RMSE	0.53	0.96	0.58	1.06	8.67	9.00

In the field, soil mineral nitrogen declined by approximately 70 % between the initial measurements made before ploughing and those collected from under the mature spring barley crop and at harvest (Figure 61). Whilst the initial measurements showed variation of between 20 and 25 %, subsequent measurements were more variable with the majority of coefficients of variation ranging between 55 and 105 % after a legume-based ley and 25 and 100 % after a grass-based ley. With respect to the predictions of soil mineral nitrogen the RMSE values reflect the variability of the field measurements and ranged between 7.49 and 10.16.

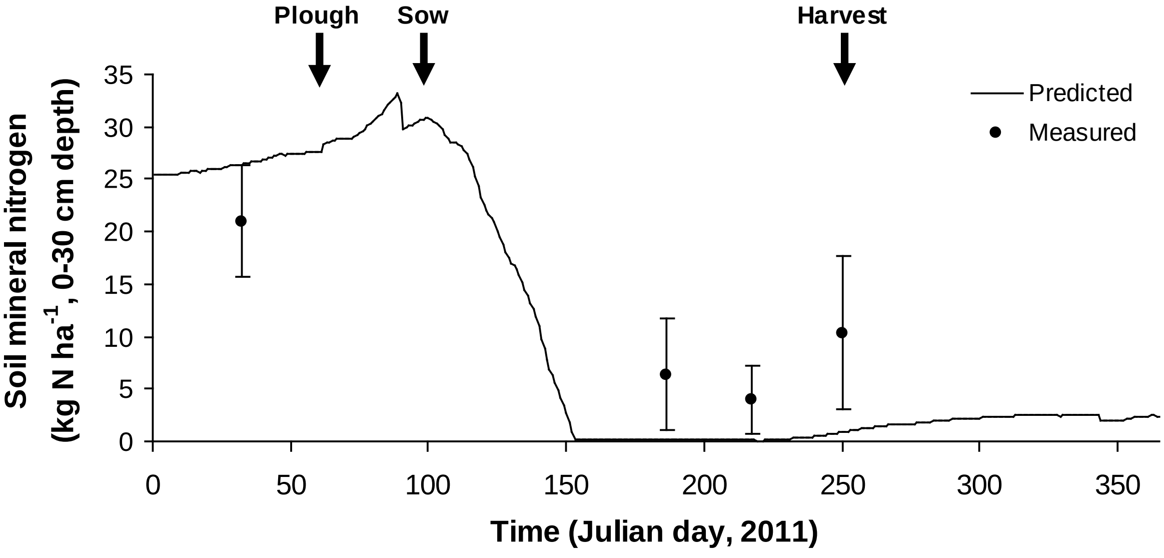

Figure 61. Soil mineral nitrogen as measured in and predicted for plots previously supporting white clover. Measured values are the mean of three replicates. Error bars indicate standard deviations.

Fertility building ley phase nitrogen inputs (predicted)

A greater proportion (35–40 %) of the nitrogen added in biomass from both RC and WC was fixed nitrogen, whereas in BM and LU it was only 25% (Table 28). For non-nitrogen fixing (grass) species the contribution of fixed nitrogen (via free-living N-fixing bacteria) to the nitrogen content of the biomass from the grasses was very small (1%).

Table 28. The predicted amounts of nitrogen supplied by the cut biomass and through nitrogen fixation by the ley-phase species. Values are the mean of two years and standard deviations are given in brackets.

	BM	**LU**	**RC**	**WC**	**PR**	**TY**
N in litter and cut biomass (kg N ha^{-1})	105 (20.3)	155 (33.0)	126 (2.6)	126 (2.7)	18 (2.7)	18 (2.7)
N fixation (kg N ha^{-1})	25 (4.6)	41 (7.5)	50 (0.6)	44 (4.2)	0.2 (0.06)	0.2 (0.06)

Nitrous oxide emissions and nitrogen leaching (predicted)

Predictions of annual nitrous oxide emissions from soil supporting the leguminous species during the ley phase were approximately three times greater than those associated with the grass species (Table 29). Similarly, the annual amount of nitrogen lost through leaching was at least 4 times greater for legume plots than the grasses. Furthermore, the amount of nitrogen lost through leaching showed a greater dependency on legume species with losses from the two species of clover being 2 – 3 times greater than those from either BM or LU. Nitrous oxide emissions in the calendar year after the ley phase (i.e. the spring barley year) were very similar irrespective of the ley phase species grown. However, in agreement with predictions for the ley phase, the leaching losses showed some relationship to the choice of ley phase species with greater loss occurring after leguminous species than after the grasses.

Table 29. The predicted annual nitrous oxide emissions and the annual amount of nitrogen lost through leaching during the ley-phase and subsequent spring barley crop. Values for the fertility-building ley phase are the mean of two years and standard deviations are given in brackets.

	BM	**LU**	**RC**	**WC**	**PR**	**TY**
	Fertility-building ley phase calendar years					
N_2O emissions (kg N ha^{-1} yr^{-1})	2.6 (2.40)	3.1 (3.03)	3.3 (3.29)	3.0 (2.98)	1.1 (0.69)	1.1 (0.69)
N leaching (kg N ha^{-1} yr^{-1})	19 (1.3)	29 (2.3)	63 (1.1)	50 (7.6)	5 (0.1)	5 (0.1)
	Spring barley calendar year					
N_2O emissions (kg N ha^{-1} yr^{-1})	0.7	0.6	0.6	0.6	0.6	0.6
N leaching (kg N ha^{-1} yr^{-1})	5.9	8.0	8.9	8.2	1.9	1.9

Even though a WC ley phase did not result in the greatest spring barley grain yield in the SAC trials, based on the predicted nitrous oxide emissions for the spring barley year, the intensity of nitrous oxide emissions per unit grain yield was least after a white clover ley phase (Table 30).

Table 30. Ranked spring barley nitrous oxide emission intensities (g N2O-N kg-1 grain).

Rank	Legume species		Grass species	
1	WC	0.113	PR	0.111
2	LU	0.115	TY	0.145
3	RC	0.121		
4	BM	0.139		

Sensitivity analysis

Alterations to the nitrogen fixation capacity of the ley phase legume revealed direct relationships between nitrogen fixation capacity, the amount of nitrogen fixed and the amount of nitrogen returned to the soil in the cut biomass. The effects of increased nitrogen inputs via cut biomass were more pronounced for the amount of nitrogen lost through leaching than for nitrous oxide emissions during the ley phase (Figure 62).

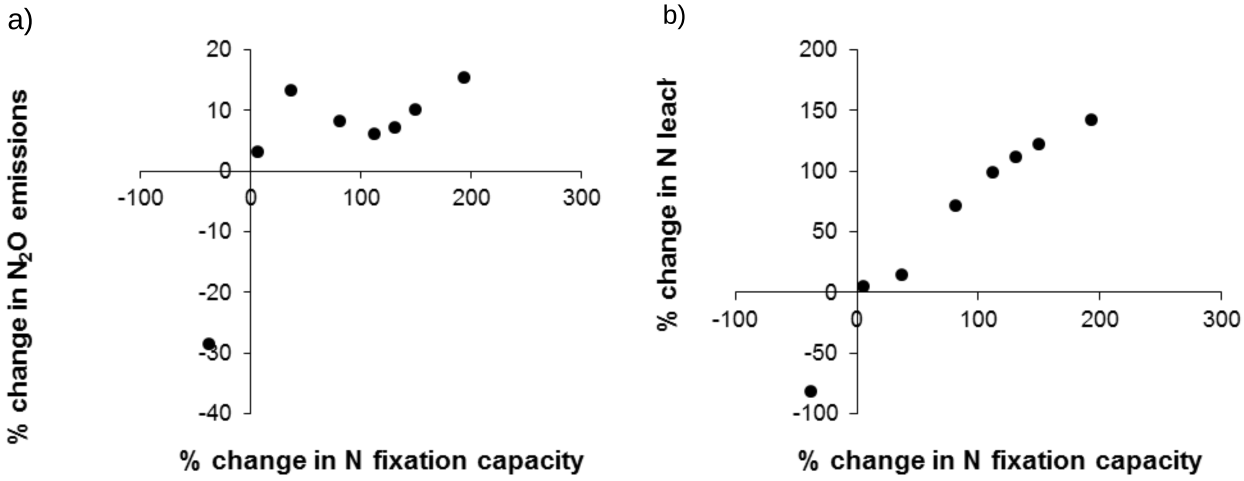

Figure 62. The effect of nitrogen-fixation capacity on a) nitrous oxide emissions and b) nitrogen leaching during the ley phase.

3.3.5. Decision support model for rotation planning (WP5)

Estimation of nitrogen accumulation by the ley

The modelled estimates of the amount of nitrogen (N) accumulated for the different leys and management practices are summarised in Figure 63 to Figure 65. These were obtained from model runs in which the region was set to North-West England and North Wales and soil type to Medium soils. Leys were assumed to be sown at the beginning of March and cultivated either 12, 24, 36 or 48 months later. The default values were used for the proportion of species in the ley. The amounts of N accumulated by the four types of ley included in the model are shown in Figure 63, in this case, assuming the leys are managed by mulching. For all types of ley the predicted rate of N accumulation diminished with increasing length of the ley phase, particularly for the clover/grass ley where the model indicates there is little benefit of extending the ley beyond three years. Both Mix A and Mix B leys appeared to accumulate more N than the clover/grass ley. However, the difference could be greater than that shown. The values in the figure were obtained from model runs where the growth category was set as moderate in each year of the ley and while this is reasonable for clover/grass leys, yields of Mixtures A and B would be expected to be greater and more likely to correspond to the vigorous growth class, with corresponding increases in the amount of N accumulated by the ley. This is evident in Figure 64 where Mix A, growing vigorously and managed by mulching, was estimated to accumulate over 600 kg N ha^{-1}. As would be expected, the quantities accumulated were shown to become progressively smaller for moderate and poor yielding leys.

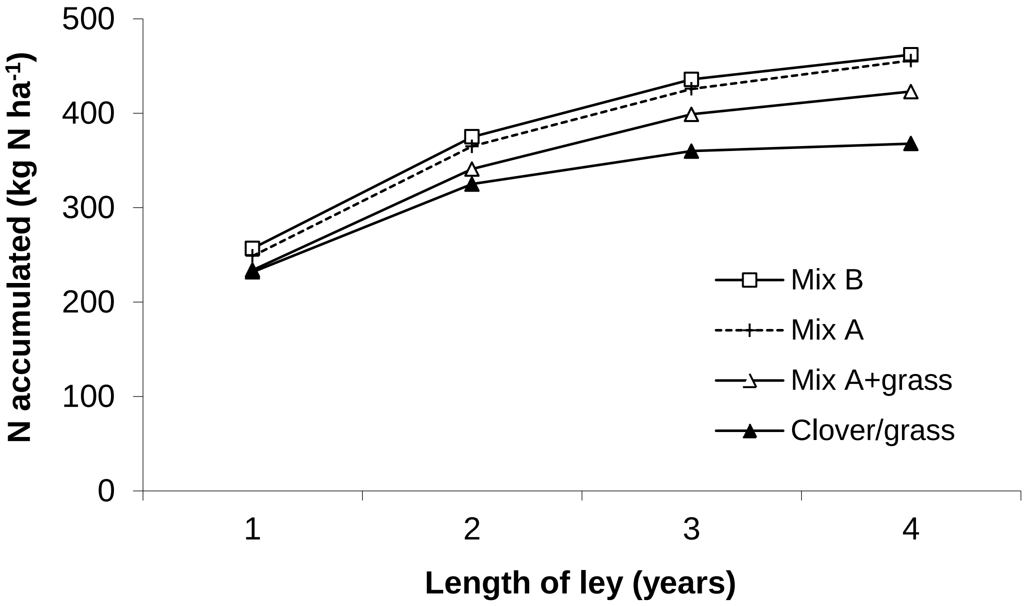

Figure 63. Model estimates of the total N accumulated by 1 – 4 year leys, comparing the different types of LBM and clover/grass leys (all mulched, of moderate growth and assuming the default proportions of species in the ley).

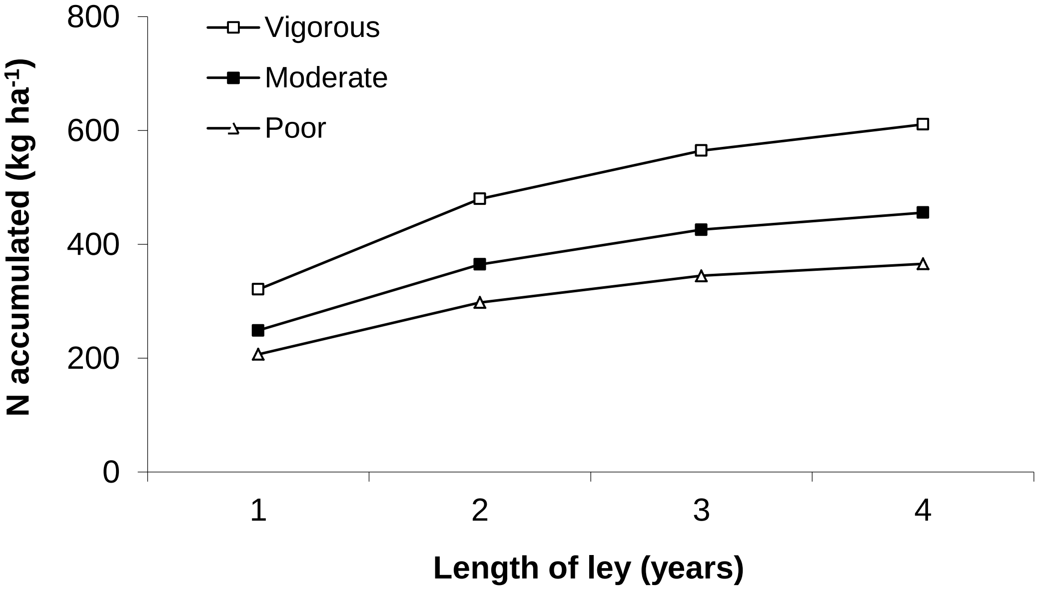

Figure 64. Model estimates of the total N accumulated by 1 – 4 year leys, comparing the effect of poor, moderate and vigorous growth classes (all Mix A, mulched and assuming the default proportions of species in the ley).

The model predicts that adding grass to Mix A reduces the amount of N accumulated by the ley by about 6 – 7% (Figure 63). Replacing the red clover in Mix A with sainfoin in Mix B had very little effect on N accumulation. The chemical composition of red clover and sainfoin residues was set in the model so that both had the same N and lignin concentration and only differed in polyphenol content. The amount of N accumulated would also be influenced by small differences in the default proportions of these two species in the respective mixes.

The effects of different management approaches are shown in Figure 65. With the assumptions used in setting the parameters within the model, a 1-year grazed ley was estimated to accumulate 20% more N than under cutting and this increased to 40% more for a 4-year ley. Returning the cut herbage as a mulch rather than removing it from the field was estimated to increase N accumulation by 90 and 140% for 1 and 4-year leys, respectively. With the mulched ley, the amount of N accumulated continued to increase from Year 3 to 4, whereas there was little further increase after Year 3 for the cutting and grazing managements.

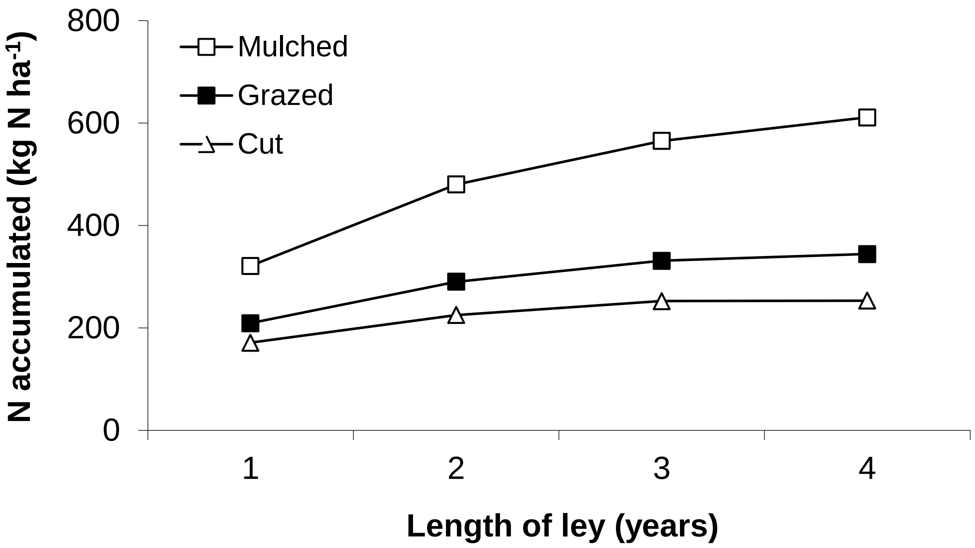

Figure 65. Model estimates of the total N accumulated by 1 – 4 year leys, comparing the effect of cutting, grazing and mulching managements (all Mix A, of vigorous growth and assuming the default proportions of species in the ley).

Modification of mineralisation calculations to include the effect of the lignin and polyphenol content of residues

Initial tests examined the alternatives of including lignin and polyphenol concentrations with C/N in the algorithm to determine the Fast_N fraction as either (C/N) + [L+Pp]/N or as (C/N) + [L+Pp]. When included as [L + Pp]/N, increasing concentrations of lignin and polyphenols caused much greater reductions in the N mineralisation rate of low N residues than of those with higher N concentrations. The second expression produced a more uniform response across a range of N concentrations, which appeared to be in closer agreement with the general response suggested by various published studies. This form of the expression was therefore adopted for use in the LegLink model but further evidence is needed to confirm that it is the better option.

In the revised procedure, increasing concentrations of lignin and polyphenols also influence the rate of mineralisation by extending the degree-day periods over which the Fast and Med_N fractions are mineralised. Figure 66 shows the effect of this additional factor on estimates of cumulative N mineralisation. For clarity, the mineralisation component of the model was run using a constant average temperature throughout the simulation to remove the seasonal fluctuations from the mineralisation curve. Mineralisation starts at the beginning of Month 3 when the ley is ploughed.

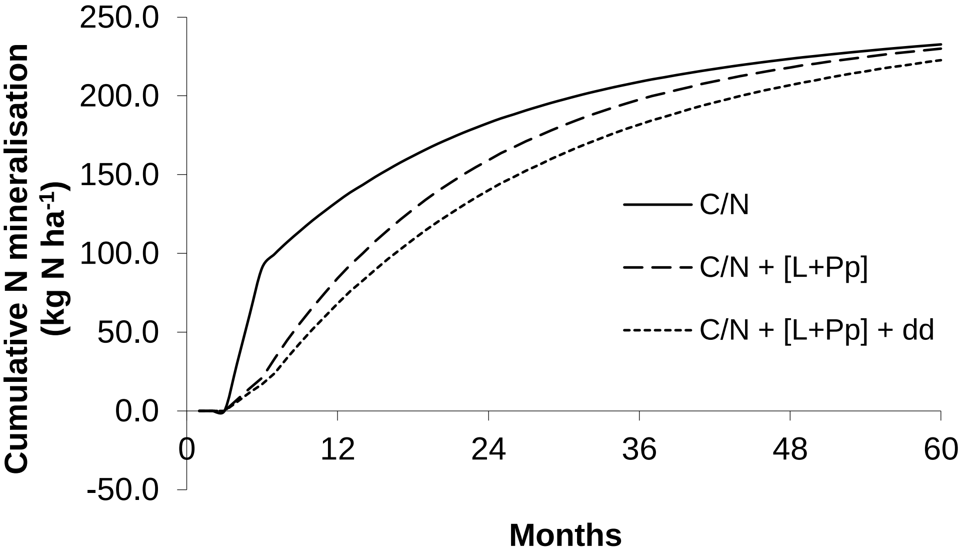

Figure 66. Comparison of cumulative N release from the ley where Fast_N, Med_N and OM_N fractions were calculated using either C/N alone, C/N + [L+Pp] or C/N + [L+Pp] + adjustment to the Fast_N and Med_N degree-day periods. Degree-days used in the calculations assumed a constant daily temperature, with the ley cultivated at the start of Month 3. Residues were assumed to contain 250 kg N ha-1 at the start of the decomposition with concentrations of 2.5% N and 25% lignin + polyphenols.

The effect of including [L + Pp] with C/N in the apportioning of N between the Fast_N, Med_N and OM_N fractions (but not increasing the degree-day decomposition periods) is to reduce Fast_N and slow the initial rate of N release relative to the original method using C/N alone. In the later stages, however, mineralisation proceeds more rapidly with the revised method so that the two lines converge around Month 60. When the [L + Pp] factor is also included as a modifier of the degree-day decomposition periods the rate of mineralisation is further reduced and there is less compensation in the later stages. As a result, the cumulative mineralisation is still less than that determined by the original method at Month 60. This final method, with [L+Pp] influencing both the N fractionation and the mineralisation periods, was adopted for the LegLink version of the FBC model and all subsequent references refer to mineralisation determined in this way.

Figure 67 shows the effect of increasing lignin + polyphenol concentration, which progressively reduced the rate of N release to the extent that at the highest concentration (40%) there was an initial 7 month period of net N immobilisation rather than mineralisation. Compared with residues containing no lignin or polyphenols, concentrations of 10, 20, 30 and 40% reduced cumulative N mineralisation 12 months after the end of the ley by 9, 29, 50 and 72%, respectively. These simulations assumed that the residue provided 250 kg N ha^{-1} and contained 2.5% N.

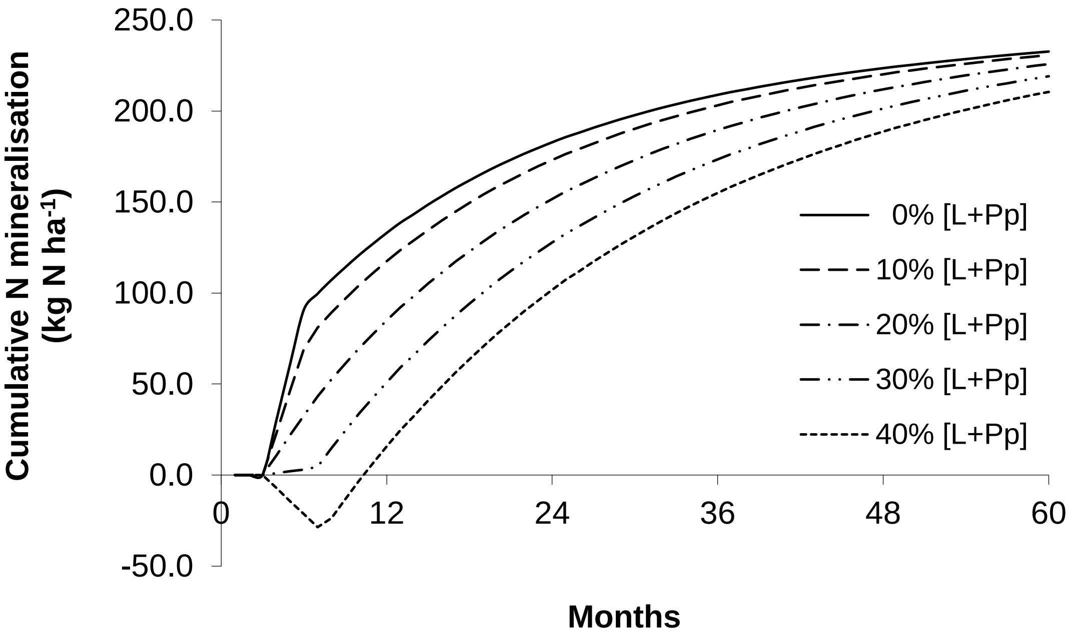

Figure 67. Effects of increasing concentrations of lignin + polyphenols on cumulative N release from ley residues where the Fast_N, Med_N and OM_N fractions were calculated using the revised factor C/N + [L + Pp] + adjustment to the Fast_N and Med_N degree-day periods. Degree-days used in the calculations assumed a constant daily temperature, with the ley cultivated at the start of Month 3. Residues contained 250 kg N ha-1 at the start of the decomposition and 2.5% N.

The results of comparing estimates of N mineralisation determined using the mineralisation component of the FBC-Link model with measured values from the dataset obtained from Vanlauwe *et al*. (2001) are shown in Figure 68. There was a close correlation between the model's estimates of cumulative mineralisation after 12 months and the results of the short-term incubations reported in the paper (R^2 0.8609, $p \leq 0.01$). Although inclusion of lignin and polyphenolic factors with C:N ratio in the estimation of mineralisation explained slightly more of the variation than did estimates based on the C:N ratio alone (R^2 0.7778, $p \leq 0.01$), the improvement was not statistically significant.

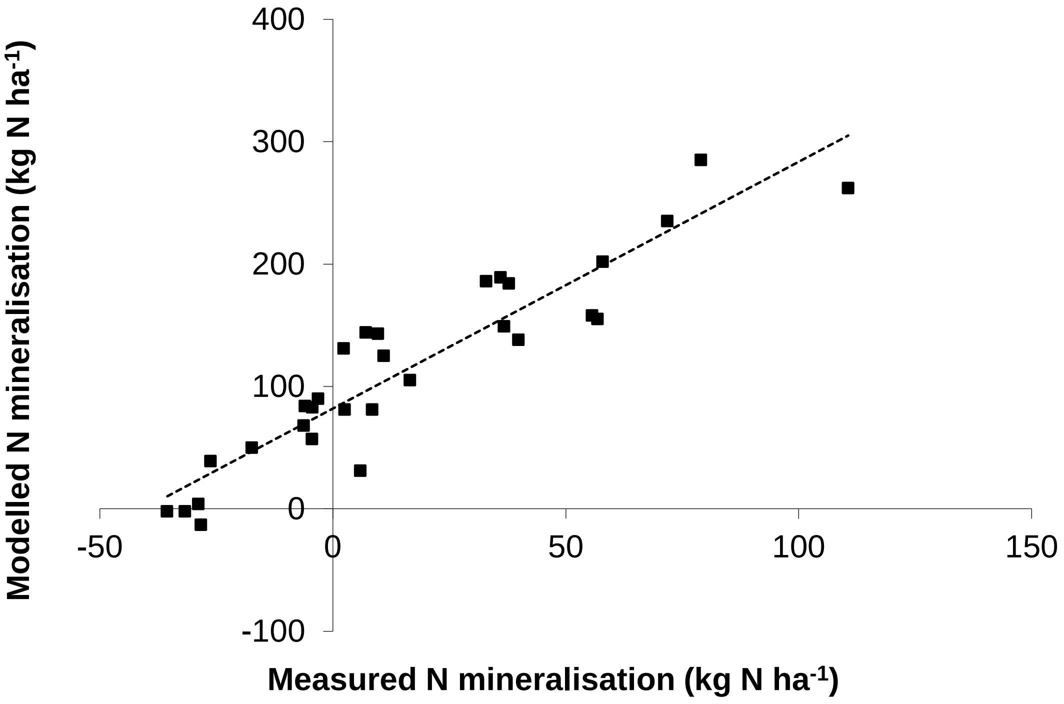

Figure 68. Comparison of measured and modelled estimates of N mineralisation using data from Vanlauwe et al. (2005).

The effects of the different ley mixes, type of management and productivity of the ley on cumulative N mineralisation in the 12 months following cultivation of the ley are summarised in Figure 69 and Figure 70.

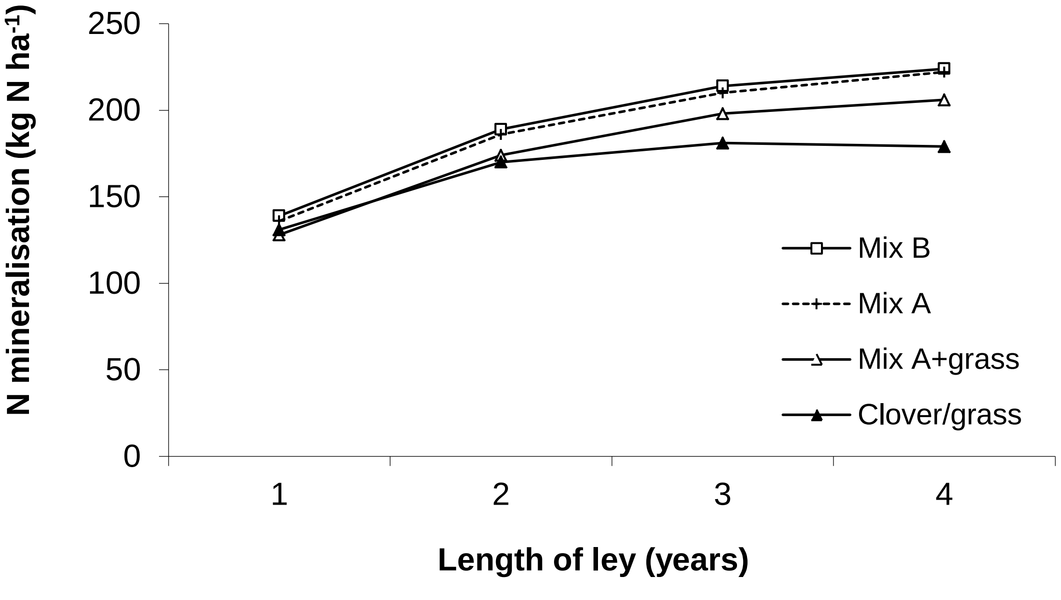

Figure 69. Model estimates of cumulative N mineralisation from 1 – 4 year leys in the 12 months following cultivation, comparing the different types of LBM and clover/grass leys (all mulched, of moderate growth and assuming the default proportions of species in the ley).

These figures are directly comparable with Figure 63 Figure 65 showing the estimates of the quantities of N accumulated by the ley. The two sets of graphs have very similar forms, reflecting the almost constant proportion of the accumulated N mineralised in the 12 months after cultivation. The model predicted that approximately 55% of the N accumulated by 1-year leys was mineralised in the following year, reducing slightly to 49% for 4-year leys. Although less N was mineralised from Mix A with ryegrass than from Mix A alone (Figure 69), this can be attributed to the similar differences in the amounts of N accumulated by the two mixtures (Figure 65) rather than to any direct effect of the grass residues on N mineralisation. Similarly, the lower mineralisation from the clover/grass ley mirrors the lower N accumulation by this type of ley. The presence of the high polyphenol species, sainfoin, in Mix B had little effect on N accumulation or on N mineralisation compared with Mix A. Differences between the amounts of N mineralised from leys of vigorous, moderate and poor growth and from leys under different managements in Figures 70 and 71 are also largely explained by differences in the amounts of N accumulated by the leys.

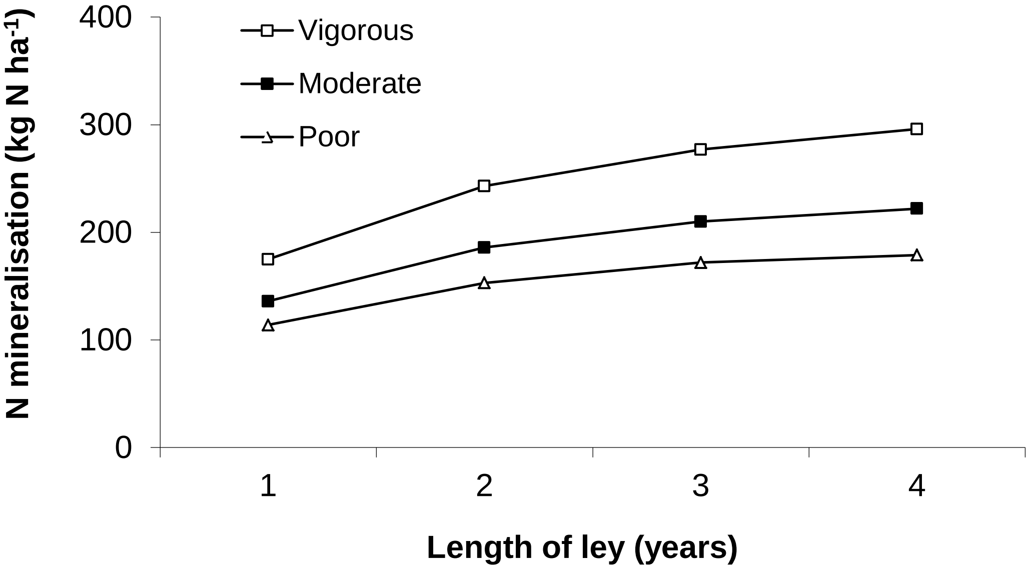

Figure 70. Model estimates of cumulative N mineralisation from 1 – 4 year leys in the 12 months following cultivation, comparing the effect of poor, moderate and vigorous growth of the ley (all Mixture A, mulched and assuming the default proportions of species in the ley).

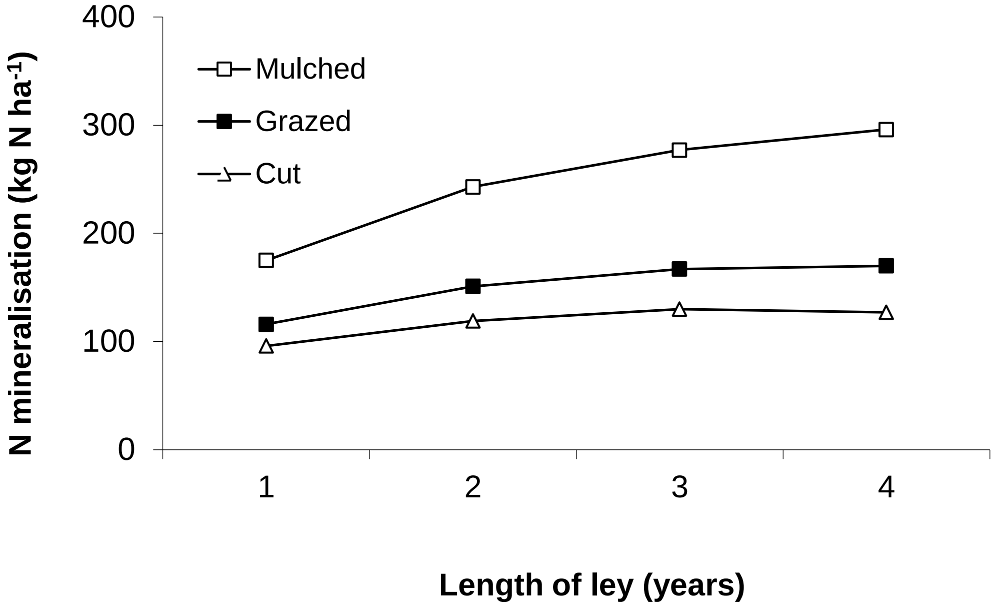

Figure 71. Model estimates of cumulative N mineralisation from 1 – 4 year leys in the 12 months following cultivation, comparing the effects of cutting, grazing and mulching managements (all Mixture A, of vigorous growth and assuming the default proportions of species in the ley).

Changes to the user interface

At the workshop for farmers and advisors, questionnaires were circulated at the start of the on-screen presentation and nine completed forms were received (representing about half of those still present at this stage of the meeting). Of those completing the questionnaire, 8 felt there was a need for a model such as this and one was uncertain. All replied 'yes' when asked if the process of entering data into the model was logical and easily understood. All but one respondent believed that the information needed by the model was readily available to farmers/growers. All found the main results graphs of crop yield and N losses easy to understand but only five replied that they provided the right information; three were uncertain and one would have preferred the N losses to be expressed in kg N ha^{-1} rather than as equivalent fertiliser costs. All preferred to have the results in a graphical form rather than as tables. When asked if the additional graph showing N mineralisation from different sources in the soil and the more complicated graph linking N losses with N surpluses and deficits in the soil were easy to understand and whether the information in these figures was of value, 7 replied 'yes' to both questions and two were uncertain.

Additional comments on the forms referred to the need to ensure that the model output was accurate; that it would be useful to be able to enter measured values of soil mineral N to set the initial soil content; and that the model would be particularly valuable for farmers/growers new to organic farming and lacking experience.

3.3.6. Legume cover crops in conventional rotations (WP6)

Yields from each individual season are set out in Table 31 to Table 34. Long term average yield responses over project years 1–4 are presented in Table 35 as a percentage of the mean yield for each season and averaged across all seasons. In both the standard N input scenario and when averaged across all N input regimes, the current practice winter cropping approach gave the highest yields.

Table 31. Yield responses in 2008 in winter wheat (t ha-1). Comparing current practice to cover cropping approaches with three nitrogen (N) regimes.

Rotation and System	Zero N	50% N	100% N	Average
Winter breaks (current)	5.46	7.99	10.56	8.00
Winter breaks (clover bi crop)	5.63	7.68	10.20	7.84
Spring breaks (current)	4.89	7.98	10.57	7.81
Spring breaks (clover bi crop)	5.26	7.56	10.34	7.72
Unbalanced (current)	3.81	5.14	7.44	5.46
Unbalanced (clover bi crop)	1.63	2.59	3.71	2.64
Spring break (radish cover crop)	5.50	7.86	10.71	8.02
Unbalanced (radish cover crop)	5.03	7.00	8.72	6.92
Spring break (ASM mix cover crop)	5.14	7.42	10.55	7.70
Unbalanced (ASM mix cover crop)	4.25	5.54	6.95	5.58
LSD (t/ha)	0.95	1.00	0.91	
CV (%)	13.4	9.8	7.5	
Sig	P<0.0001	P<0.0001	P<0.0001	

Note - Analyses are presented for the individual fertiliser dose regimes. When all regimes are analysed collectively an LSD of 1.03 t/ha, a CV of 10.7% and a significance of P<0.0001 were applicable.

Table 32. Yield responses in 2009 in spring oilseed rape (t ha-1). Comparing current practice to cover cropping approaches with three nitrogen (N) regimes.

Rotation and System	Zero N	50% N	100% N	Average
Winter breaks (current)	0.80	1.34	1.69	1.28
Winter breaks (clover bi crop)	0.80	1.51	1.47	1.26
Spring breaks (current)	0.66	1.15	1.30	1.04
Spring breaks (clover bi crop)	0.71	1.06	1.55	1.11
Unbalanced (current)	0.76	1.21	1.54	1.17
Unbalanced (clover bi crop)	0.57	1.10	1.19	0.95
Spring break (radish cover crop)	0.84	1.22	1.38	1.15
Unbalanced (radish cover crop)	0.85	1.49	1.73	1.36
Spring break (ASM mix cover crop)	0.87	1.36	1.62	1.28
Unbalanced (ASM mix cover crop)	0.97	1.44	1.52	1.31
LSD (t/ha)	0.24	0.28	0.35	
CV (%)	21.7	15.0	16.1	
Significance	NS (P = 0.114)	P = 0.02	P = 0.09	

Note - Analyses are presented for the individual fertiliser dose regimes. When all regimes are analysed collectively an LSD of 0.318 t/ha, a CV of 19.0% and a significance of P<0.001 were applicable. In 2009 the winter oilseed rape in rotation 1 (see Table 8, Page 47) was over-drilled with spring oilseed rape.

Table 33. Yield responses in 2010 in winter wheat (t ha-1). Comparing current practice to cover cropping approaches with three nitrogen (N) regimes.

Rotation and System	Zero N	50% N	100% N	Average
Winter breaks (current)	5.00	7.42	9.70	7.37
Winter breaks (clover bi crop)	5.42	7.80	9.96	7.73
Spring breaks (current)	4.36	6.81	9.14	6.77
Spring breaks (clover bi crop)	5.26	7.52	9.67	7.48
Unbalanced (current)	4.09	6.80	8.95	6.61
Unbalanced (clover bi crop)	5.79	7.42	8.95	7.39
Spring break (radish cover crop)	4.62	7.35	9.03	7.00
Unbalanced (radish cover crop)	4.05	6.74	8.88	6.56
Spring break (ASM mix cover crop)	4.14	6.59	8.52	6.42
Unbalanced (ASM mix cover crop)	4.56	7.27	9.53	7.12
LSD (t/ha)	0.79	1.02	1.18	
CV (%)	11.64	9.77	8.69	
Significance	P = <0.001	NS (P = 0.27)	NS (P = 0.40)	

Note - Analyses are presented for the individual fertiliser dose regimes. When all regimes are analysed collectively an LSD of 0.98 t/ha, a CV of 9.87% and a significance of P<0.001 were applicable.

Table 34. Yield responses in 2011 in beans (t ha-1). Comparing current practice to cover cropping approaches with three nitrogen (N) regimes.

Rotation and System	**Zero N**	**50% N**	**100% N**	**Average**
Winter breaks (current)	1.27	1.22	2.07	1.52
Winter breaks (clover bi crop)	1.30	1.49	1.39	1.39
Spring breaks (current)	1.41	1.40	1.12	1.31
Spring breaks (clover bi crop)	1.12	1.43	1.08	1.21
Unbalanced (current)	1.47	1.36	1.31	1.38
Unbalanced (clover bi crop)	1.39	1.45	1.55	1.46
Spring break (radish cover crop)	1.47	1.32	0.92	1.24
Unbalanced (radish cover crop)	1.20	1.54	1.62	1.45
Spring break (ASM mix cover crop)	1.56	1.42	1.39	1.46
Unbalanced (ASM mix cover crop)	1.30	1.14	1.32	1.25
LSD (t/ha)	0.538	0.605	0.561	
CV (%)	27.0	30.0	27.7	
Significance	NS (P = 0.85)	NS (P = 0.94)	P = 0.02	

Note - Analyses are presented for the individual fertiliser dose regimes. When all regimes are analysed collectively an LSD of 0.54 t/ha, a CV of 27.7% and a significance of P= 0.274 (NS) were applicable.

Table 35. Long term average yield responses over project years 1–4 of the cover crops experiment within the NFS programme. Yields are presented as a percentage of the mean yield for each season and averaged across all seasons.

Rotation and system	**Relative yield return**			
	Zero N	**50% N**	**100% N**	**Average**
Winter breaks (current)	78	106	147	110
Winter breaks (clover bi crop)	81	115	129	108
Spring breaks (current)	73	103	119	99
Spring breaks (clover bi crop)	73	103	125	100
Unbalanced (current)	71	93	116	93
Unbalanced (clover bi crop)	64	86	99	83
Spring break (brassica cover crop)	81	105	117	101
Unbalanced (brassica cover crop)	73	109	130	104
Spring break (legume mixture cover crop)	80	105	129	105
Unbalanced (legume mixture cover crop)	76	97	116	96

With regard to the use of clover bi-crops, this approach has resulted in notable improvements in soil characteristics compared to current practice. Assessment of bulk density in cropping year 3 (2010 – winter wheat) indicated bulk density reductions from 1.17 g/cm^3 to around 1.04 g/cm^3 at depths of 20cm. This was associated with improvements in water infiltration rates (measured over a 20 minute period using a Minidisc Infiltrometer; Decagon Devices Inc.) from 0.50 mm/minute to

1.17 mm/minute. Marked improvements in crop yield were also noted with respect to the inclusion of a clover bi-crop in 2010.

Yield responses and associated impacts on margin over N cost are presented in Figures 72 and 73. While responses were apparent at all N doses there was clear evidence of a decline in response as N dose increased (with an associated reduction in clover ground cover in response to increasing N dose); the full (100%) N dose applied in this season was 200 kg/ha N. The average yield response over all N doses from a clover bi-crop was around 8%.

Considering the wider rotational response, data for the clover bi-crop for the spring and unbalanced rotational approaches, averaged over a break crop (spring oilseed rape in year 2 – 2009) and winter wheat (year 3 – 2010) cycle, are presented in Table 36. Yields are presented as a percentage of the mean yield for each season and averaged across both seasons (note the full N dose in the spring oilseed rape crop was 100 kg/ha N). Yield and margin data follow a similar pattern to that seen in the wheat crop in 2010, and cumulative margin over N data for the average yield responses presented in Table 36 are depicted in Figure 73.

Cumulatively, over both the wheat crop and the spring oilseed rape crop, the clover bi-crop improved margin over N compared to standard practice by on average £84/ha, although, again the response varied in relation to N dose.

Table 36. Yield response data averaged over a break crop (spring oilseed rape in year 2 – 2009) and winter wheat (year 3 – 2010) cycle. Yields are presented as a percentage of the mean yield for each season and averaged across both seasons.

Rotation and system	**Relative yield return**			
	Zero N	**50% N**	**100% N**	**Average**
Spring breaks (current)	59	97	119	92
Spring breaks (clover bi crop)	67	98	134	100
Spring break (brassica cover crop)	68	103	122	98
Spring break (legume mixture cover crop)	66	104	129	99
Unbalanced (current)	61	99	128	96
Unbalanced (clover bi crop)	65	99	114	92
Unbalanced (brassica cover crop)	64	110	136	104
Unbalanced (legume mixture cover crop)	73	112	132	106
Average (current)	60	98	124	94
Average (clover bi crop)	66	98	124	96
Average (brassica cover crop)	66	107	129	101
Average (legume mixture cover crop)	70	108	130	103

The brassica cover crop and ASM legume mix cover crop were also included in each of the spring and unbalanced rotational approaches; the associated yield data are also presented in Table 36. Yield responses above current standard practice were apparent for both spring-sown cover cropping approaches. The highest average yield response (around 9%) was associated with the ASM legume species mix cover cropping approach. Incremental N doses appeared to have less of an influence on the yield responses for the ASM legume species mix and the fodder radish cover crop approaches compared to that observed with the clover bi-crop. However, the marked improvements in soil bulk density and water infiltration rates recorded with the clover bi-crop approach were not apparent with the ASM legume species mix and the fodder radish cover crop systems. Associated impacts on cumulative margin over N across both wheat and spring oilseed rape crops are presented in Figure 73; again all cover crop systems improved margin over N compared to standard practice, with an average improvement of around £77/ha. These responses excluded the cost of the cover crop establishment.

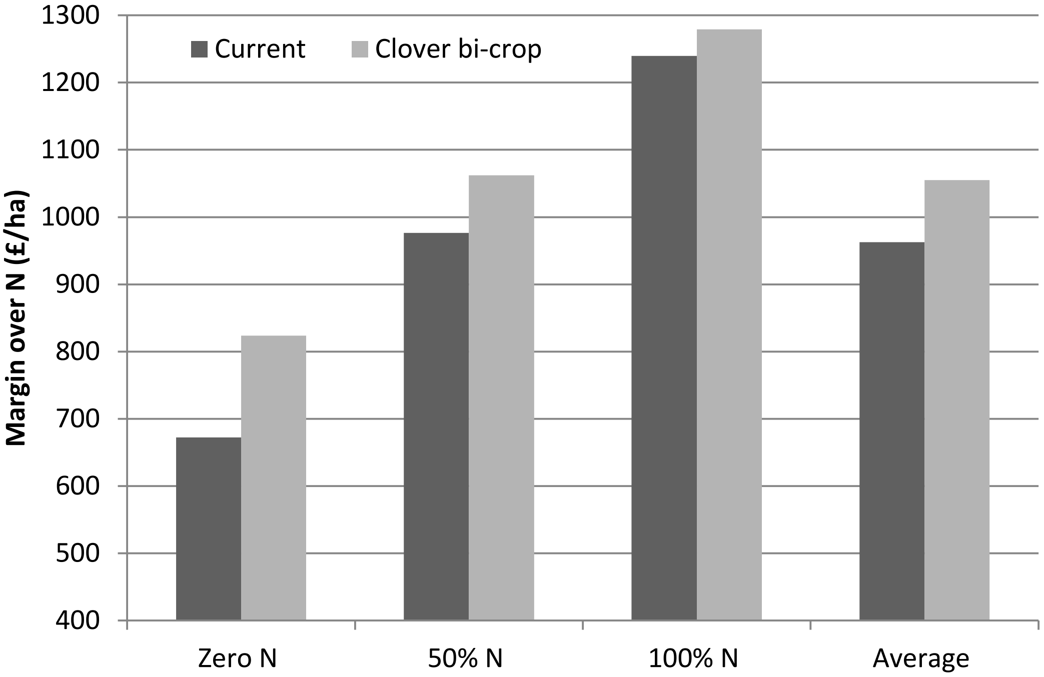

Figure 72. Margin over nitrogen (N) response in winter wheat comparing current practice to the inclusion of a white clover bi-crop. Data from NFS 'cover crop' experiment 2010. Based on £150/t for winter wheat and £0.75 kg N.

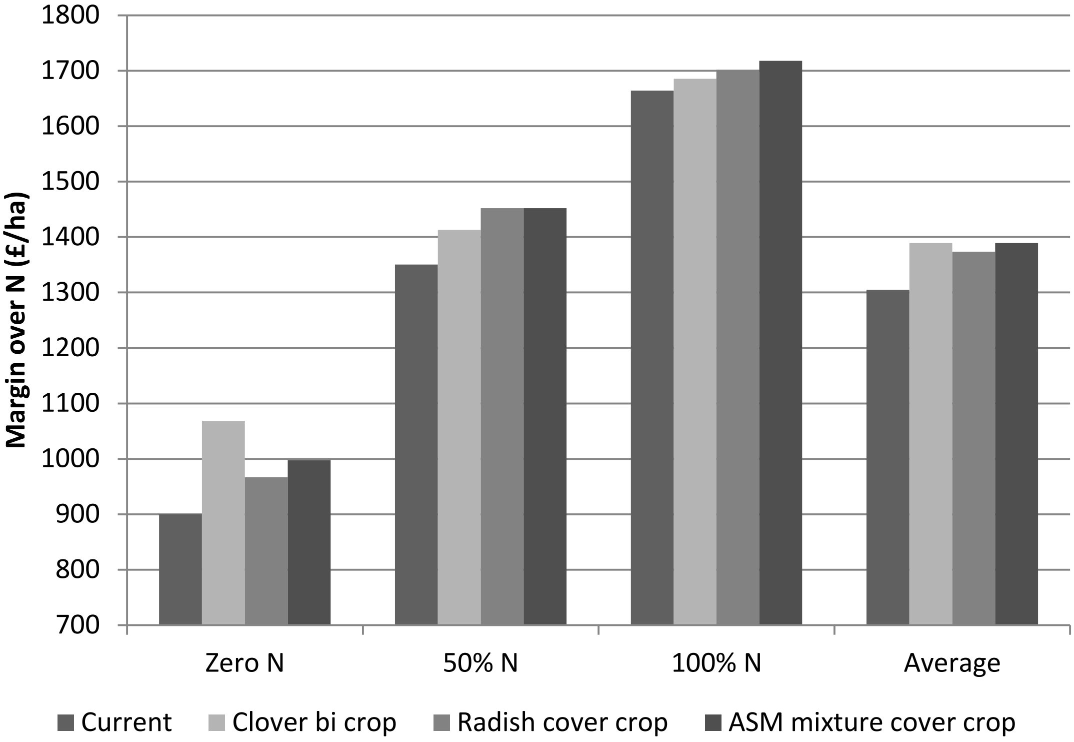

Figure 73. Cumulative margin over nitrogen (N) response data comparing current practice to a white clover bi-crop, a brassica cover crop or a legume mixture cover crop. Responses were recorded over a break crop (spring oilseed rape in year 2 – 2009) and winter wheat (year 3 – 2010) cycle. Based on £150/t for winter wheat, £375/t for oilseed rape and £0.75 kg N.

3.3.7. Assessing the effects of mixing species

From the literature, it is known that there is a positive relationship between grassland productivity and species diversity (Fornara & Tilman, 2009). Mixing species that are functionally complementary can improve resource use efficiency and productivity of the plant community in comparison with species monocultures (Hector, 1998). The effects of mixing species on productivity and other parameters are a result of the interaction between the mixed species. In other words, if there was no interaction, and thus no effect of mixing the different species, it would be expected that the mixture behaves in the same way as the average of its component species. The benefits or detrimental effects of mixing can then be assessed simply by the difference between the performance of the mixture and the average performance of the component monocultures.

In WP1 we studied a complex mixture of forage plant species, the ASM, alongside the component species in monoculture, which offered the opportunity to assess the effects of species diversity. In addition, in WP3 we compared the ASM with farmer-chosen control mixtures which, on average, had a lower species diversity that the ASM.

Comparison of ASM and monocultures in replicated trials

The effect of mixing species was assessed by comparing the two All Species Mix (ASM) entries to the average of the data of all the component species. First, for each replicate *r*, we calculated the average *a* of data from inoculated and non-inoculated mix

$$a_r = \frac{1}{2}[c(NM)_r + c(IM)_r]$$

where *c* is the value of the measured variable (such as biomass or crop cover), NM is the non-inoculated ASM and IM is the inoculated ASM. To calculate the average of the monocultures, we followed two approaches. On the one hand, we calculated the average *m* as

$$m_r = \frac{1}{n}\sum_{i=1}^{n} c_{ir}$$

where n is the number of species that were used in the ASM, *r* is the replicate (block), running from 1 to 3, *i* is the index of the species running from 1 to *n.* Alternatively, we calculated a *weighted* average of monocultures as

$$\widetilde{m_r} = \frac{1}{n}\sum_{i=1}^{n} c_{ir}\, w_i$$

where the weight w_i is the proportion of plants in the mixture as determined by the sowing densities of species within the ASM, so that $\sum_{i=1}^{n} w_i = 1$. For each block *r*, the effect *d* of mixing the species was then calculated as the relative difference between ASM and monocultures:

$$d_r = \frac{a_r - \widetilde{m_r}}{\widetilde{m_r}} \text{ x } 100\%$$

This relative difference d_r is displayed in Figure 74. The statistical analysis to test the significance of the difference between *a* and *m* was carried out using a linear mixed effects model with site as a random factor.

As Figure 74 shows, the effect of mixing species had several beneficial effects. The ASM had a higher above-ground biomass and crop cover than the (weighted) average of the monocultures, and also had a lower weed biomass. Interestingly, there was also an effect on the plant residue composition; in particular, the ASM had a significantly lower lignin content than would have been expected from the (weighted) average of its components.

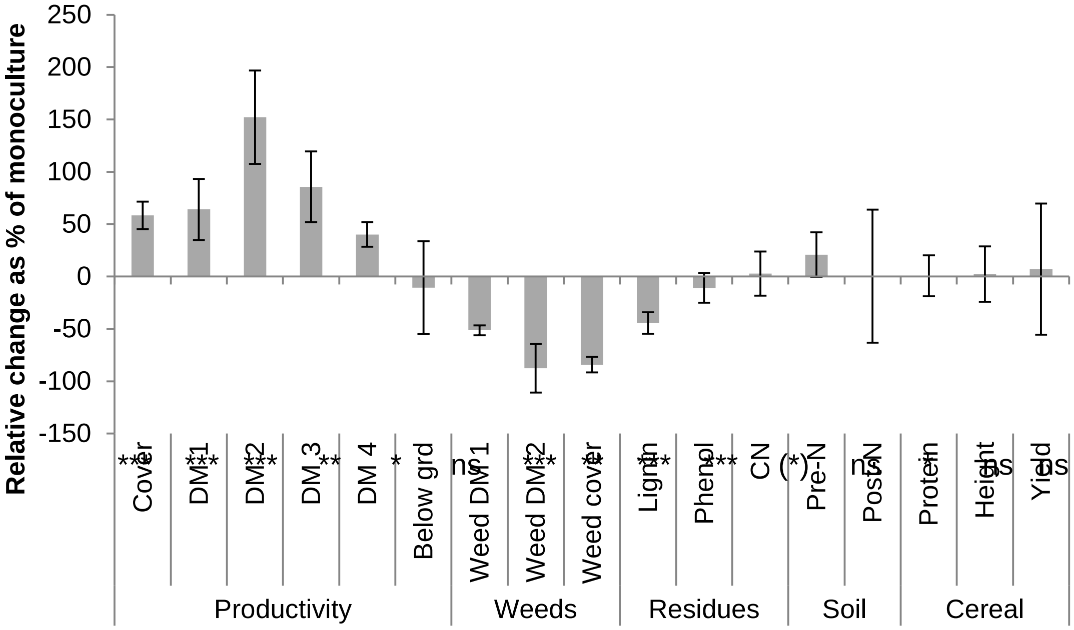

Figure 74. Effect of mixing species in the All Species Mix (ASM), expressed as relative difference between ASM and monoculture; Cover = crop cover in spring 2010; DM1 = crop biomass in summer 2009; DM2 = biomass 2010 before simulated grazing cut, DM3 = regrowth biomass after simulated grazing cut; DM4 = pre-incorporation above-ground biomass; Below grd = pre-incorporation below-ground biomass; Weeds: DM1 = Weed biomass late summer 2009; DM2 = weed biomass spring 2011; cover = weed cover in spring 2010; Residues: lignin content, phenolics content and C:N ratio of above-ground plant residues; Soil: available N in soil pre-incorporation and post-incorporation; Cereal: plant height, grain yield and grain protein content of following crop.

While the ASM also showed a higher content of plant-available nitrogen in the soil before incorporation, this effect was not observed after incorporation. No significant effects of mixing the species could be found in the following crop.

The comparison of ASM and the unweighted average of the monocultures showed generally similar results and is summarized in the Appendix.

To investigate the development of the mixing effects over time, we analysed crop cover at the Barrington Park trial site, where 9 assessments had been conducted over the trial period. The ASM had a higher crop cover than the average of all its components when they were grown in monocultures; in addition the advantage of the ASM over the monocultures grew over time (Figure 75). This effect was even observed when the ASM was compared with the average of the best five monocultures.

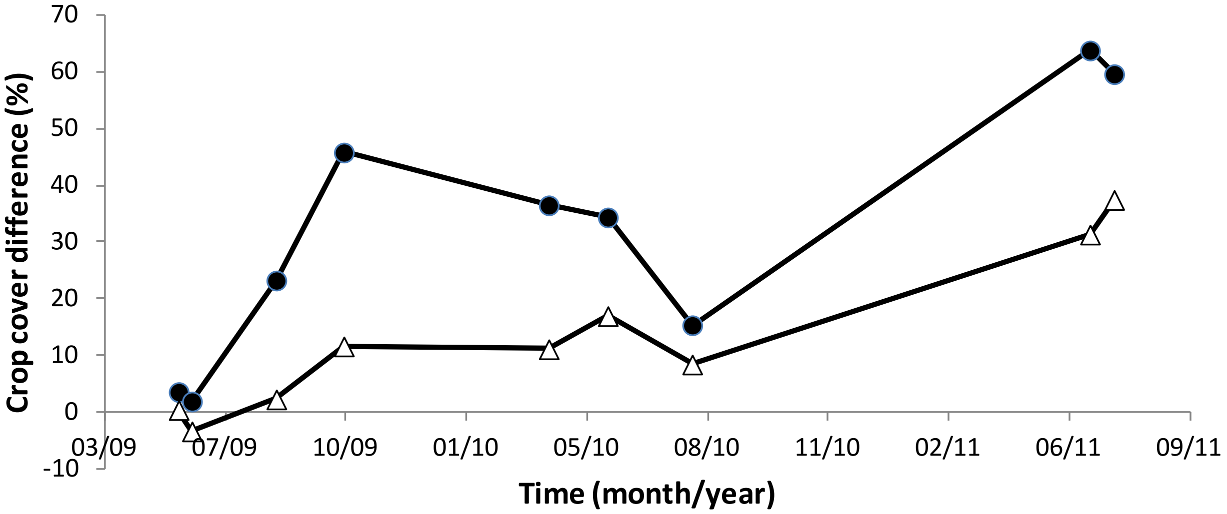

Figure 75. Absolute difference d in crop cover (%) of the All Species Mixture over the average crop cover % of several single species grown in monocultures (see eqn.). Filled circles represent the difference when all single species are included in the monoculture average; open triangles represent the difference when only the five best species (RC, WC, BM, PR and TY) are included.

Comparison of ASM and control ley in participatory trials

At the end of the trial period in 2011, before incorporation of the ley, biomass samples were taken from both the ASM and Control ley in the participatory trials. In a mixed effects model using region as the random factor, the biomass in the ASM was significantly greater than biomass in the Control ley (t-test, p=0.0204; Figure 76).

Interestingly, the absolute difference between ASM and Control was negatively correlated with the soil organic matter content (Figure 77), i.e. on soils poor in organic matter the advantage of the ASM over the Control was more marked than on the soils with higher organic matter content. None of the other environmental variables was found to influence the difference between ASM and Control ley. Also, the number of species sown in the control ley was of no consequence for the difference between ASM biomass and Control ley biomass (p= 0.221).

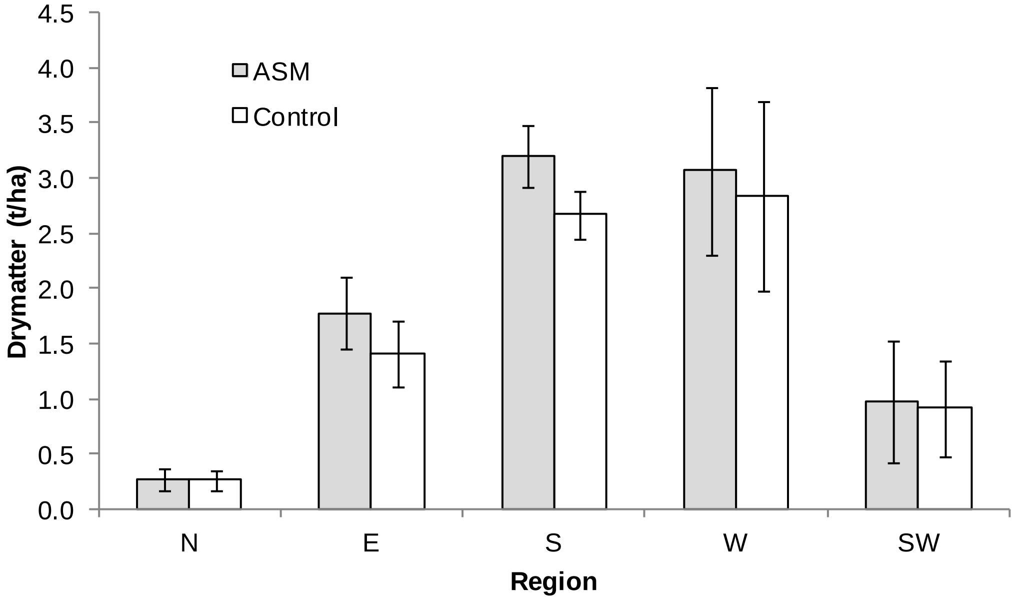

Figure 76. Biomass (dry matter, t/ha) of All Species Mix (ASM) and control ley broken down by study region, total 29 farms. The graph shows means and standard errors. N= North, E= East, S=South, W=West (Wales), SW= Southwest (Cornwall). The ASM biomass is significantly greater than the Control biomass (t-test, p=0.0204).

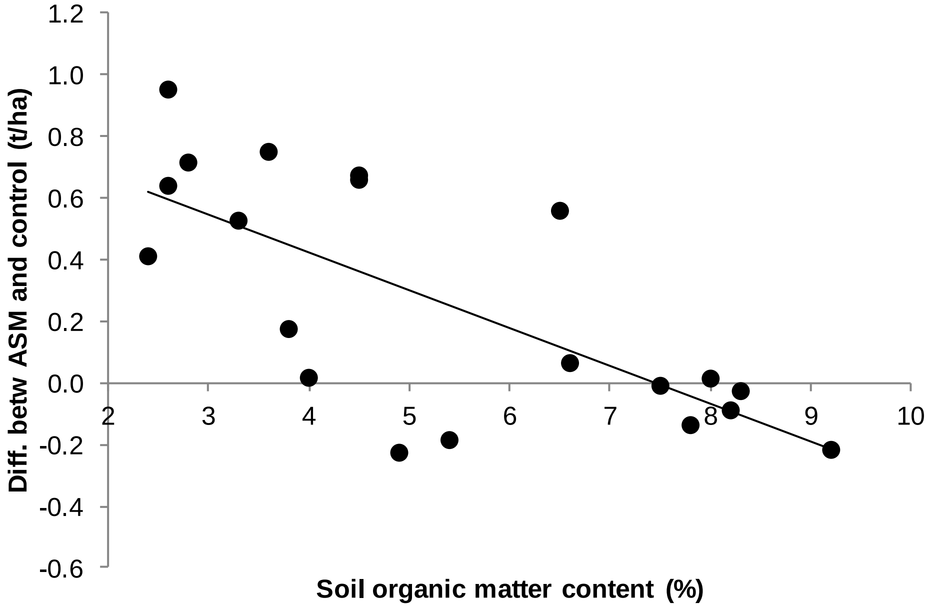

Figure 77. Difference between biomass (dry matter, t/ha) of All Species Mix (ASM) and control depending on soil organic matter content. The negative relationship between the two variables is significant (mixed effects model with region as random factor, p=0.0018).

3.3.8. Effects of inoculation with Rhizobia

In WP1, inoculation of the ASM did not significantly affect any of the measured variables. Even when all these non-significant trends were considered together, no consistent advantage of inoculating the ASM was observed.

There is some indirect evidence for an effect of inoculation which comes from crop cover estimates at the Barrington Park trial (Figure 78). Here, the cover of individual species within the inoculated and non-inoculated ASM was assessed. While the species that were not inoculated within the ASM (e.g. rye grasses or BT), show lower cover in the inoculated than in the non-inoculated ASM (i.e. they are below the diagonal line), WC is located above the line, i.e. its cover is higher in the inoculated ASM (although this is not significant).

Generally, there is a very dynamic picture, with some species strongly increasing over time (LU), while other species are decreasing (BM).

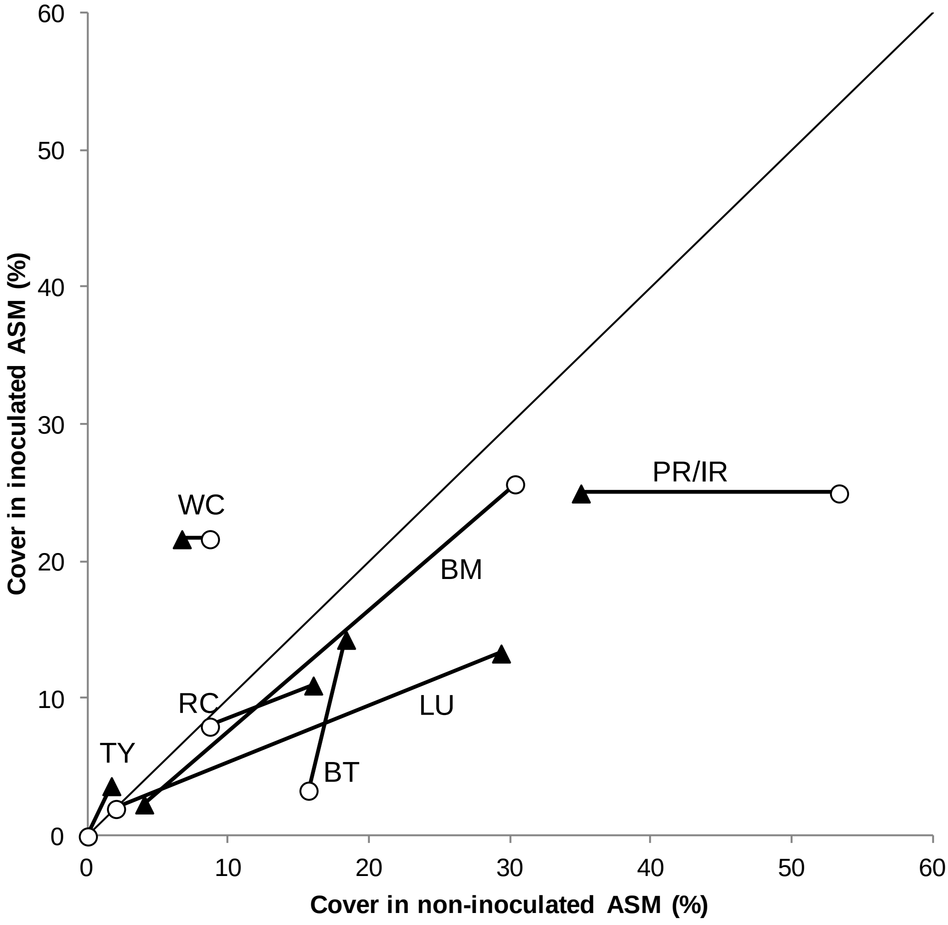

Figure 78. Crop cover of different legume and grass species within the inoculated All Species Mix (y-axis) and the non-inoculated All Species Mix (x-axis) at Barrington Park on 23 June (open circles) and 13 July 2011 (filled triangles).

3.3.9. Satellite project: Legume mixtures and pollinators

In total 3,231 individual pollinators were recorded throughout the season, of which bumblebee (*Bombus* species) comprised 57.9% (1,795 individuals). Of the bumblebee species, *B. lapidarius* was the most abundant (n=1,252 individuals). Other pollinating species were less abundant: butterflies (n=293), solitary bees (n=243), hoverflies (n=553), honeybees (n=139), and 'other' species (n=208). The pollinator assemblage changed throughout the season (Figure 79) differing significantly with observation point (P=<0.001) but not with treatment. The ASM mixture tended to have a greater number of flowers (per m^2) than the control leys, both in the early season (observation point 2), and late season (observation point 4), although, overall differences in floral assemblage were not significant (P=0.082).

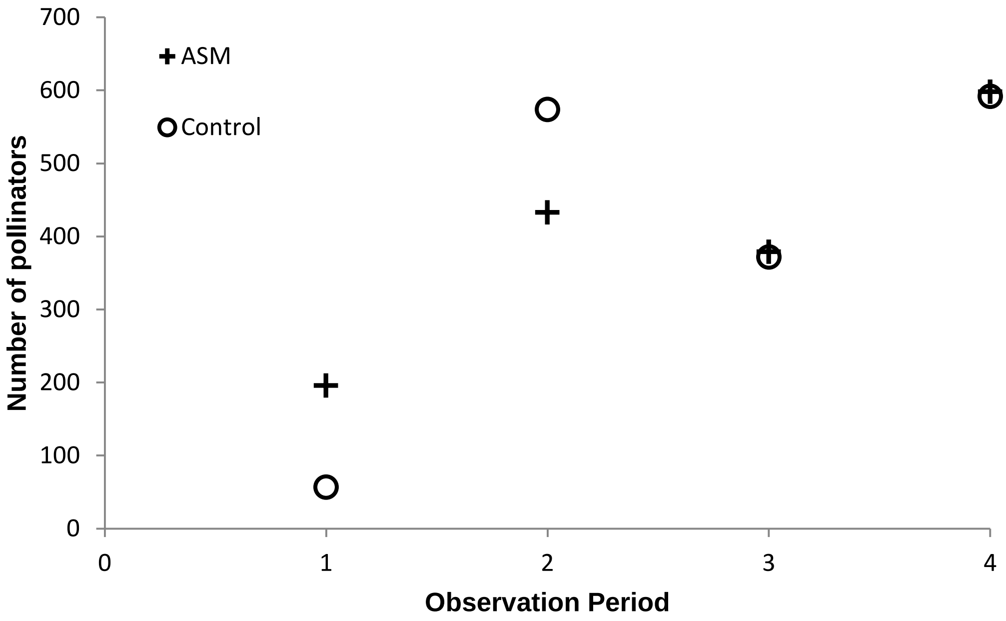

Figure 79. Number of pollinators recorded at differing ley types throughout the flowering season. Time point: 1 (May–June); 2 (June–July); 3 (July); 4 (August).

The total number of flowers differed significantly between grazed ($9.0 \pm 3.9 m^{-2}$) and non-grazed sites ($30.5 \pm 5.3 m^{-2}$, P=0.002). The overall mean number of pollinators recorded on non-grazed sites ($23.2 \pm 3.7 m^{-2}$) was greater than grazed sites ($8.0 \pm 5.3 m^{-2}$), though the difference was not significant (P=0.074). The lack of significant differences for pollinators is likely to be due to the fact that management of grazed sites was not constant throughout the season. Sheep were removed from grazed sites for a two week period in June in two farms (time period 3) to allow the regeneration of the ley, and this allowed mass flowering of white clover.

Bumblebee species generally showed a preference for un-grazed plots during June and July (mean number of bumblebee species, un-grazed plots= 23.2±4.6, grazed plots= 7.7±2.6) although this was not a significant (P= 0.084). During July (observation point 4), when grazing intensity was reduced, the trend was even less pronounced (P= 0.567).

In the non-grazed sites, pollinator group dominance varied significantly throughout the flowering season. The ASM mixture supported a significantly more bumblebees (P=0.046) and hoverflies (P=0.011) early in the season (observation point 2), and hoverfly (P=0.041) and solitary bee species (P<0.001) late in the season (observation point 5). The control sites supported a larger number of hoverfly species in mid-season (P=0.014, observation period 3) (Table 37).

Table 37. Mean abundance of major pollinator groups on different ley types at different time points throughout the flowering season: 2=May, 3=June, 4=July, 5=August. S. E. D. Indicates standard error of the difference, P values indicate relative significance between the ASM and Control leys.

Pollinator Group	Time Point	2	3	4	5
Bumblebees	Mean ASM	4.9	28.9	4.6	13.0
	Mean Control	0.7	37.4	4.4	25.7
	S.E.D	0.7	0.3	0.5	0.6
	P value	0.046	0.418	0.488	0.428
Butterflies	Mean ASM	1.9	2.6	3.3	7.7
	Mean Control	0.4	1.3	0.8	11.2
	S.E.D	1.2	0.3	0.4	0.3
	P value	0.210	0.760	0.085	0.742
Solitary bees	Mean ASM	3.6	0.7	4.1	6.8
	Mean Control	2.8	1.5	2.0	0.0
	S.E.D	0.5	0.4	0.6	0.0
	P value	0.601	0.121	0.334	0.001
Hoverflies	Mean ASM	2.9	1.7	1.4	18.3
	Mean Control	0.5	13.1	0.0	3.1
	S.E.D	0.5	0.6	0.0	0.7
	P value	0.011	0.014	0.001	0.041

3.4. Discussion

3.4.1. Characterisation of legume species (WP1 & WP3)

As shown in WP3, white clover (WC) and and red clover (RC) are the most popular legume species that are currently used by organic farmers in fertility building leys (Table 4, page 30). A crucial question is therefore whether there is any scope for using alternative legume species which might offer any advantages over these species.

In WP1, we characterised 10 legume species alongside WC and RC in field trials across the UK. These trials revealed that WC, with respect to many performance variables (e.g. with respect to biomass in the first year), was either the best one among the 12 tested legume species, or there were no significant differences between WC and other legumes. At the same time however, WC was *not* the best species with regard to other variables in the replicated plot trials, such as speed of emergence, and overall biomass production.

In the following sections, we provide species profiles for all tested legume species in alphabetical order to highlight their respective advantages and disadvantages, using both information from the extensive literature and results from the project field trials. Results obtained for the four grass species are not discussed here.

AC - Alsike Clover (Trifolium hybridum)

In comparison to other legume species, Alsike clover (AC) came out as mid-range in many respects, i.e. often its **productivity** was not as high as that of WC and RC, but still better than some other legume species such as MP, WV, SC or LT (e.g. regarding crop biomass, weed biomass or crop cover). It was notable that the species showed relatively high seedling relative growth rates in the replicated field trials. It has been praised for its ability to suppress weeds (because of its growth habit) (Zimmermann, 1958), but this was not borne out by the present study, which showed it to have higher weed cover than the WC and RC (Figure 20, p. 59; Figure 21, p. 60).

AC was further characterised by good **grazing tolerance** and a good presence on the farms in the All Species Mixture (ASM), but it also showed relatively low **persistence**, indicating that it was not as competitive as WC and RC in the ASM. This echoes general observations that AC is relatively short-lived and persists in swards for only about two years, though it has the ability to regenerate from seed (Frame *et al*., 1998).

Normally, a **seed rate** of 2–5 kg/ha is recommended for AC in seed mixtures (Frame *et al*., 1998); and 7kg/ha (Kahnt, 2008) or 9–14 kg/ha (Zimmermann, 1958) when sown on its own. In the current study, a seed rate of 1.25kg/ha was used in the ASM. This comparatively low rate was mainly chosen because of the large number of species in the ASM, but the comparison with the normally recommended rate indicates that for higher productivity of this particular species the seed rate in a (less complex) mixture might need to be adjusted upwards. As AC shows a small seed weight (between RC and WC), a shallow **sowing depth** of 10–15mm is recommended (Frame *et al*., 1998). Since the species does not spread vegetatively (in contrast to WC), the **establishment** phase is crucial for the productivity of AC. In the replicated field trials, establishment rates of AC were at the low end (26±6%, mean ± s.e., n=6 sites, compared to 50.6% averaged over all other legume species), which may explain part of the species' mid-range performance.

Trials with a following cereal crop indicate that AC was similar to WC and RC in being relatively early to **release nitrogen** to the cereal (Figure 41, page 78), although the evidence for this is only indirect. In terms of **root biomass** and **residue** profile (polyphenol and lignin content as well as C:N-ratio) AC was not significantly different from WC. AC originates from Northern Europe; therefore, the species is said to be better adapted to northern conditions, but in our study, we did not observe better performance on the Scottish sites than in England or Wales (Figure 56, p. 96). The minimal **germination temperature** has been given as 2°C (Kahnt, 2008) or 5°C (Hartmann and Lunenberg, 2013). In terms of **soil pH**, normally the species is recommended for conditions that are too acidic for growing RC or LU. In accordance with this, we found a negative correlation between soil pH and AC presence in the farm trials in the first study year, i.e. AC tended to show higher presence on more acidic soils with a lower pH; however, this relationship was lost over the subsequent two years of the study. Also, among the six sites of the replicated field trials, establishment rates of AC were highest at IBERS (52%), which showed the lowest soil pH (5.9). It is therefore possible that AC might tolerate both more acidic and more alkaline conditions than many other clovers (Smetham, 1973a; Hartmann *et al*. ca., 2009). It is generally recognized that AC can tolerate **heavier soils** than RC (Zimmermann, 1958). In accordance with this, we found RC, but not AC, responded positively to the sand content in the soil in the year of establishment (Table 21, p. 98). AC is more self-tolerant than RC (Zimmermann, 1958).

Regarding **water requirements**, AC is often recommended for relatively wet conditions (Frame *et al*., 1998; Wright, 2001; Hartmann *et al*. ca., 2009), but is also said to be able to tolerate both wet and dry conditions (Smetham, 1973a). In contrast to this, however, greenhouse experiments showed AC to have moderate flooding tolerance, being similar to RC in this respect, and tolerating flooding for not as long as WC (Heinrichs, 1970). Kahnt (2008) estimates that while AC requires more water than RC, it is not different from WC in terms of both water requirements and drought tolerance. With respect to water requirements of AC, the picture therefore remains inconclusive at the moment. AC is currently mainly used to complement other species in **mixtures** (Frame *et al*., 1998; Kahnt, 2008); it has been noted for its compatibility with RC and TY (Frame *et al.,* 1998). The growth habit of AC means that heavy stocking reduces productivity (Smetham, 1973a). Though AC is considered to be similar in **nutritional value** to RC (Frame *et al*., 1998), it needs to be noted that AC can be toxic for horses (Zimmermann, 1958; Nation, 1989; Wright, 2001) and, because of its bitter taste, is not recommended to be fed on its own (Zimmermann, 1958; Hartmann *et al*. ca., 2009).

BM - Black medic BM (Medicago lupulina)

Black medic (BM), sometimes also known as yellow trefoil, is a **summer annual**, **biennial** or **short-lived perennial** species (Clapham *et al*., 1987). It is native to the UK with a vast distribution found throughout Europe, Asia, North Africa and the United Sates. It is prevalent in disturbed habitats and is a common weed in fields and gardens. BM can be used as a fodder crop, a green manure and also as a hay crop. Out of 34 farms participating in this research project, only 5 chose to include BM in the control mix (Table 4, p. 30). The species is morphologically very diverse. BM has a procumbent **growth habit** with a thin wiry taproot (Hartmann *et al*. ca., 2009).

BM **flowers** from April to August (Clapham *et al*., 1987); plants can flower within six weeks of emergence and may initiate new flowers throughout the growing season. In line with this, the present study showed that BM had a relatively long flowering period. In fact, it was the species with the longest flowering period in year 2 (Figure 25, p. 64), thus indicating that it is an important source of pollen and nectar for insects. Although self-pollination takes place readily in BM, it is far less productive than cross-**pollination**. BM is visited by bumblebees and honey bees (Kozuharova, 2000). **Seeds** germinate readily and seedlings can emerge at any time of year (Turkington *et al*., 1978). In the growing season, seedlings may emerge from immature seeds that germinate beneath the parent plant. Premature germination allows BM to produce more than one generation in a year (Bond *et al*., 2007). Scarification promotes germination and the level of germination has been found to increase from 13 to 98% following scarification (Grime *et al*., 1981). Like many other legumes, BM is slow to **establish** and is not very competitive during the establishment phase (Wallace, 2001); however, BM has also been characterised as a fast developing species (Hartmann *et al*. ca., 2009). Compared with the other legumes species, the present study found BM to be mid-range in terms of early development (Table 14, p. 82).

Looking at **productivity**, crop cover as measured at the start of the second growing season was highest in RC and WC, but amongst the other legume species BM consistently showed the highest cover (Figure 17, p. 56), thereby reflecting a good ability to put on new growth after the winter. Also, BM showed high values of **crop biomass** when measured in the early autumn of the first trial year (Figure 18, p. 57). Finally, above-ground biomass prior to incorporation into the soil in 2011 was significantly higher in BM than in the reference species WC (Figure 26, p. 66). These results are in contrast to the general view that BM is characterised by low yields (Hartmann *et al*. ca., 2009). BM also showed good **weed** suppression (Figure 21, p. 60), although it was not as good as RC and WC in this respect; notably, BM showed a lower competitive (reducing) effect on weeds than would be expected from its high crop biomass. When sown as part of the complex mixture (ASM) in the on-farm trials, BM showed significantly lower crop cover than WC (Figure 54, p. 93); however, BM showed good **persistence** in the sward, which is in contrast to the recommendation to sow BM for one-year leys only, rather than two years (Hartmann *et al*. ca., 2009).

With regard to its **residue** profile, BM had a high lignin content (Figure 29, p. 68), while its polyphenol content and C:N ratio were similar to the values observed in the reference species WC. From modelling in WP4, lower **N fixation** was found in BM than in WC, but also lower **leaching** of N than in WC (Table 29, p. 106). **Grain yield** of the following cereal was not significantly different between BM and WC (Figure 39, p. 76). This is in line with a study comparing the effect of green manures on grass seed production, where MF and TY were not affected in different ways by BM vs. WC (Slepetys *et al*., 2011). In a related study, BM did not influence mycorrhiza colonisation or nutrient uptake of a following flax crop, except under drought conditions, where flax biomass was reduced by 38% and the total uptake of N, P, Zn and Cu was decreased following BM (Turmel *et al*., 2011).

In terms of **soil conditions** BM has a preference for fine, well-drained soils, low in organic matter with a pH from 6.0 to 7.5 (Kahnt, 2008). For most species in this study higher **soil pH** was associated with larger presence values (Table 21, p. 98); indeed, BM was the species, where this effect was strongest among all investigated legumes (Figure 59, p. 99). BM is said to prefer **warm** and dry conditions (Hartmann *et al*. ca., 2009); this may explain its relatively good performance in the current study, with dry and warm springs both in 2009 and 2010. BM is more **winter hardy** than RC and LU; only after spring sowing it is sensitive to frost (Hartmann *et al*. ca., 2009). In a weed community BM decreased in frequency when **N-fertiliser** was applied; it was also characterised by low competitive ability within a barley crop (Pysek & Leps, 1991). Nevertheless, BM is occasionally used with WC in **undersowing** (Hartmann *et al*. ca., 2009). **Regionally**, in terms of presence in sampling quadrats it was found that BM performed best in the South and worst in the West (Figure 56, p. 96). However these regional differences cannot be attributed to any single factor because environmental conditions are confounded with management factors.

The simulated grazing trials showed that BM had substantially lower tolerance to being cut at low height than WC (Figure 23, p. 62). However, at the same time, BM performed significantly worse on non-grazed than on **grazed** sites in the participatory on-farm trials (see section 3.3.3.2).

Potential factors to explain these contrasting results are that (a) climate or soil conditions may have confounded the on-farm results; (b) BM can tolerate treading damage (Hartmann *et al*. ca., 2009), which was not simulated in the replicated trials and (c) that BM is characterised by a bitter taste (LfL), which could lead to it being deselected by grazing livestock. One reason why BM is part of seed mixtures is its relatively low seed price (Hartmann et al. ca., 2009). It is recommended to have a three year **break** between successive BM crops (Hartmann *et al*. ca., 2009). BM has been suggested for **phytoremediation** following contamination of soil with heavy metals (Amer *et al*., 2013).

To **conclude**, in the replicated plot trials, BM showed significantly better performance than WC with regard to several variables, namely, pre-incorporation above-ground biomass, overall biomass production and length of flowering. According to the findings of this study, BM therefore seems to be a species that is currently underutilised.

BT - Birdsfoot trefoil (Lotus corniculatus)

Birdsfoot trefoil (BT) is a native plant in Europe, growing on a vast area ranging from Northern Russia through to West Africa (Frame *et al*., 1998). Its growth habits are diverse ranging from erect to prostrate. BT is self-incompatible and **pollinated** by honey bees as well as bumble bees such as *Bombus lapidarius* and *B. terrestris* (Jones *et al*., 1986; Rasmussen & Brødsgaard, 1992); the flowering is indeterminate. BT has a long tap **root**, almost going as deep as LU, but shows a larger lateral root spread than LU.

The species is known for its relatively weak seedling **emergence** and slow **establishment**, and the experiments conducted in the present study confirmed that BT was slow to establish, slower than many other legumes tested (such as CC, LU, RC), with LT and WV being similar (Figure 11, p. 51); interestingly, however, it was not significantly slower than WC in this study. BT also showed a relatively low establishment rate (Figure 12, p. 51).

Regarding many **productivity** parameters BT was not significantly different from WC, e.g. seedling biomass and seedling relative growth rate (Figure 13, p. 53), plant re-growth after cutting in first year (Figure 16, p. 55), above-ground biomass in the first summer, pre-incorporation biomass and pre-incorporation proportion of weed biomass. However, BT showed a substantially and significantly lower crop cover than WC after the first winter. In comparison with other legume species, it was mid-range in terms of weed cover and weed biomass. In the on-farm trials, BT decreased in frequency over time within the ASM, and showed a significantly lower presence on farms and in sampling quadrats than WC (Figure 53, p. 92), as well as a lower ground cover. Compared to LT, the other *Lotus* species, BT showed consistently higher productivity on all measured parameters; this is in line with recent research conducted on low fertility soils in Wales (Marshall *et al*., 2013). This is in line with results from some, but not all, trials in England and Wales reported by Hopkins *et al*. (Hopkins *et al*., 1998), where in some cases LT was shown to yield higher than BT, depending on year, *Lotus* variety, and companion grass species.

Among the legumes, BT ranked highest in terms of **root biomass**, but it was not significantly different from WC in this respect (Figure 28, p. 67). Regarding the plant **residue** profile, BT showed a significantly higher lignin content than the clover species tested, a similar polyphenol content as WC, but a higher C:N ratio than WC. Among the legume species tested, BT was only surpassed by LT in terms of resistance to breakdown (Table 14, p. 82). Accordingly, in the cereal following the legumes, the relation between cereal head density and thousand grain weight (Fig. 40) indicated that BT was likely to be associated with a slower release of nitrogen than WC or RC (Figure 41, p. 78). The species was similar to WC in terms of soil N pre- and post-incoroporation and ranged in the upper third in terms of **grain yield of following cereal**, though there were no significant differences between BT and WC in this respect.

While Frame *et al.* (1998) describe BT as being adapted for acid infertile soils, we observed a mildly positive correlation between the presence of BT on farms and **soil pH** (Table 21, p. 98), though only in the third year of the study. Hopkins *et al*. (1998) regard BT as being more tolerant than WC to soils of extreme pH values.

BT has a relatively wide tolerance regarding various environmental conditions and the present study supports this finding in that no significant regional differentiation was observed across the UK (Figure 56, p. 96); the wide adaptation of BT also refers to its **water requirements**: while BT has been reported to be more drought tolerant than LT and LU (Petersen *et al.,* 1992), it is also better yielding than LU, WC and RC under poor drainage conditions and more flooding tolerant than LU (Barta, 1986). BT is tolerant of **saline** soils (Frame *et al*., 1998). However, the species requires high **sunlight** levels and is not shade tolerant: it needs at least 50 % of normal daylight during early seedling growth (Frame *et al*., 1998).

In the present study, BT was found to be reasonably tolerant to being cut to a low height, but less so than WC (Figure 23, p. 62). These results are in agreement with Brummer and Moore (2000) who found that WC showed higher **tolerance to grazing** by beef cattle than BT. Regarding **forage value**, BT is a non-bloating legume (Majak et al. 1995), containing condensed tannins; larger plants were found to contain higher tannin concentrations (Briggs & Schultz 1990). Also, there is considerable variation regarding the content of condensed tannins among different BT varieties (Marshall, et al. 2013). BT has been reported to be highly acceptable to livestock (Frame, et al. 1998), and controlled rotational grazing is recommended.

In **summary**, BT is a legume species with moderate yield levels which is relatively weak in the establishment phase. However, it provides good forage value as a non-bloating species, and high adaptability to relatively poor soil conditions, as well as slower breakdown after incorporation.

CC - Crimson Clover (Trifolium incarnatum)

Crimson clover (CC) is a **winter-annual** species. Native to Europe where it is grown as a winter annual for forage (Knight & Hollowell, 1973; Knight, 1985), CC can also be used as a summer annual for green manure in cooler, northern latitudes (Hoveland & Evers, 1995). CC is self-fertile and **pollinated** by bumblebees (see picture) and honey bees (Blake & George, 1958); in the present study it was the first legume species to **flower** in year 2 (Figure 25, p. 64), but not in year 1. The species has relatively **large seeds**; this may explain why it showed the strongest **early development** among the tested legume species, a finding confirmed in previous studies (Evers, 1999; Kahnt, 2008). CC showed a mid-range **plant height** in year 1, and was then among the shorter species in year 2 (Figure 15, p. 54). While in the literature, plant height of CC is given at 30–60 cm (Knight *et al.*, 2008) or 50–100 cm (Kahnt, 2008), this study found the maximum height of CC to range between 17 cm and 61 cm, with average of 46 cm (6 sites, year 1).

Among the legumes, it showed a very strong **regrowth** after the first cut (cm increase per day), being significantly higher than WC (Figure 16, p. 55). After 12 months, however, in spring 2010, crop **cover** was found to be low. Similarly, **biomass** production was good in year 1 (e.g. not being significantly different from that of WC), but decreased over the course of the three study years. Within the ASM in the on farm trials, CC showed relatively high **crop cover** in the first year; it then self-seeded and produced second generation on many farms in year 2, but disappeared in year 3. Thus, CC showed a relatively low **persistence** and its frequency significantly decreased over time (Figure 55, p. 94). This phenological patterns explains why CC was mid-range regarding **weed** cover in year 1 (summer), however, prior to incorporation in the third year, it showed a relatively high weed biomass.

Yields of 3-6 t/ha were achieved in the USA when plant development was at the pre-50% bloom stage (Hoveland & Evers, 1995). Kahnt (2008) reports CC yields of 4–8t/ha dry matter for Germany.

CC is characterised by a high production of soft **seed** (Bennett, 1959) and rapid softening of hard seed, with hard seeds having an impermeable seed coat which prevents absorption of moisture to initiate germination. Therefore, germination of CC seed can occur in summer rather than in the autumn, thus limiting optional, timely self-reseeding (Hoveland & Evers, 1995). The minimal germination temperature of CC was given as 3°C (Kahnt, 2008).

With regard to **residue properties**, the present study showed CC to have the lowest **lignin** rate among the legumes, as well as a low C:N ratio; its polyphenol content was not different from WC. It was characterised by a medium amount of available N in the soil post-incorporation, with large variability among sites (Figure 37, p. 75). With regard to **grain yield** of a following cereal, CC was mid-range, similar to RC and it tended to be lower than WC, though differences between CC and WC in this respect were not significant. According to its residue profile, CC was considered to have a low resistance to decomposition (Table 14, p. 82); however, this was not supported by the results of the grain yield components of the following cereal at Rothamsted where results indicated relatively slow decomposition in comparison to the other three clover species (Figure 41, p. 78). Pre-incorporation, CC showed a similar **root** biomass as the other clovers (Figure 28, p. 67).

With regard to **nitrogen fixing** ability, a pure-sown stand was found to fix 155 kg N/ha (Brink, 1990). Kahnt (2008) reports 150–220 kg N /ha in above-ground parts of CC. If used as green manure, maximum N release (73 kg/ha) from CC was achieved at late-bloom stage (Ranells & Wagger, 1992).

CC is adapted to a wide range of **soil and climatic conditions**, more so than other frequently used annual forage legumes. It is thought not to grow on poorly-drained (Knight & Hollowell, 1973), or saline, soils (Kahnt, 2008) and it is not tolerant to shade. The target **soil pH** for CC is given as 6.0–7.0 (FAO, 2000) or 5.5–6.5 (Kahnt, 2008). This medium range is in line with the current study, where no significant correlation between soil pH and presence of CC on the farms was found. However, in a previous study in a no-till system, CC was found to have reduced dry matter yields at pH 5.7 compared to pH 6.2 (Duck & Tyler, 1987). CC will grow on soils of low fertility but benefits from good soil **phosphorus** status (Knight & Hollowell, 1973). In the current study, however, we did not find any significant correlation between soil phosphorus and CC presence on the farms. The species is subject to chlorosis on strongly alkaline soils because of associated iron deficiency (Knight & Hollowell, 1973). CC shows low to medium tolerance to **drought** (Kahnt, 2008).

CC is considered to be only moderately **winter hardy**; Kahnt (2008), basing his assessments on experience in Germany, considers it to be survive temperatures down to -5°C. Accounts from the USA vary, with one study reporting CC to be killed at 10°F (-12.2°C), while another report saw it survive 0°F (-17.8°C) (Knight & Hollowell, 1973); Evers (1999) from the USA found it not be a winter hardy species (though more than arrowleaf and rose clovers). Interestingly, after establishment, CC makes more growth at low temperatures than many other clovers (Knight & Hollowell, 1973).

The optimum **sowing depth** is given as 8–12 mm or 10–15 mm (Kahnt, 2008) with a light but firm soil cover. **Seed rate** is recommended at 10–20 kg/ha or 24–45 kg/ha (Kahnt, 2008) when sown as a monocrop, with a lower rate (10kg/ha) used when sown in mixture with grass (Kahnt, 2008), though in mixtures with PR, 25–30kg/ha have been recommended (Zimmermann, 1958). It is compatible for mixtures with brassicas such as rape and mustard (Zimmermann, 1958). Early maturity makes it highly suitable for **no-tillage** rotations e.g. with maize where corn grain and silage yields and corn N uptake were found to be consistently higher following CC than no cover crop (Holderbaum *et al*., 1990). Probably because of susceptibility to clover diseases, it is recommended to have 3 break years in between growing CC (Kahnt, 2008).

In **mixtures**, CC is usually combined with various grasses such as PR but also wheat and rye; a good companion legume species is RC (Knight & Hollowell, 1973).

CC is known to show relatively poor regrowth after **grazing**, which is confirmed in this study (Figure 23, p. 62). It is recommended that close grazing should be avoided in winter so as not to affect spring growth or seed production adversely. Calyces are covered with stiff hairs which may cause digestive upsets if the sward is grazed during flowering (Zimmermann, 1958). CC is considered to be suitable for **hay and silage** especially at leafy growth stage because it provides protein rich forage. However, cutting at mature growth stage when hairs of stems and flower heads have become hard and tough can be harmful to livestock. Cutting of CC crop sown in September is recommended for the end of May (Kahnt, 2008). CC is associated with risk of bloat.

In **summary**, CC is an annual species characterised by a vigorous establishment phase and high forage production. It shows adaptability to a wide range of soils.

LT - Large Birdsfoot trefoil (Lotus pedunculatus)

Large Birdsfoot trefoil (LT, *Lotus pedunculatus*, syn. *L. uliginosus*) can clearly be placed among the legume species with slowest growth and lowest **productivity**. It was the species with the lowest seedling biomass, and also showed the lowest **seedling relative growth rate**. LT showed low values for **crop cover** and, in terms of **plant height** was among shortest species in year 1, and among the shorter ones in year 2 of the replicated field trials. Thereby, the project results correspond with several studies that document *Lotus* species as having weak seedling emergence as well as slow **establishment** (Beuselink & McGraw, 1984; Frame *et al*., 1998).

In parallel with such findings, LT (along with MP) had the highest **weed cover** in the first year of this project and was among the most weed-infested species in the second year (with only WV being poorer). *Lotus* swards are often invaded by broad-leaved weeds during establishment due to their non-aggressive early growth and *Lotus* species are known to grow poorly under shade (Frame *et al*, 1998), which indicates another disadvantage of slower emergence and growth rates which may allow them to be easily outcompeted by other species. The slow establishment and poor competitive ability of *Lotus* species has undoubtedly led to them being overlooked by the farming sector for sown pastures (Frame *et al*., 1998). LT also showed lowest **re-growth** after cutting in the first year.

Among the legumes, LT was found to have the lowest presence in the on-farm trials; in the ASM it was outcompeted by stronger species over time, i.e. its presence in sampling quadrats tended to decrease from year 1 to year 3. On the farms, LT showed a significantly better performance in the Southwest of England than in the other three **regional** clusters (Figure 57, p. 97).

In terms of the **plant residue profile**, LT showed a significantly higher C:N ratio than WC, and in this respect it was similar to BT and SC. Among the legumes, LT had the highest lignin content. Possibly because of its low overall productivity, LT showed the lowest **grain yield in subsequently sown cereals**, though even differences between LT and the best performing legume, WC, were statistically significant.

The response of LT to **soil pH** was different from other legume species; in the on-farm trials we observed a negative correlation between presence of LT and soil pH. This is in agreement with the literature, e.g. assessing LT as being more tolerant of soil acidity than BT (Floate *et al*., 1989). With regard to other nutrients, LT has higher P use efficiency than WC (Hart & Collier, 1994) and is more tolerant of Mn than LU.

LU - Lucerne (Medicago sativa)

Globally, lucerne (LU), which is also called alfalfa, is one of the most important legumes species. **Originating** in the Vavilov's Near Eastern centre, LU had reached Italy about 200 BC; while its use had seen a strong decline in Europe by the 4th century, and was re-introduced later into Europe from North Africa, and reached England in the mid-17th century (Frame *et al*., 1998). Currently, it is grown on a vast scale in temperate regions around the world. LU is closely related to a more winter hardy species, yellow lucerne (*Medicago falcata* L.); the hybrid of the two is the variegated sand lucerne (*M. media* Pers.). LU is an upright **perennial** with a strong deep **taproot**, which helps to make nutrients available from great depths and conveys good drought tolerance to the plant. Depending on soil type, the roots of LU usually go down to 2–4 m (Frame *et al*., 1998). LU is mainly **pollinated** by honey bees and bumblebees (Palmer-Jones & Forster 1965), but is also visited by several other bee species (Strickler & Freitas, 1999; Liu *et al*., 2008). With regard to pollination efficiency, it was shown that pollination by bumblebees (*Bombus terristris*) led to higher seed yields of LU than pollination by honey bees (Cecen *et al*., 2008). In the current study, LU was the latest to stop **flowering** in first year; although it thereby extended the provision of vital nectar resources for pollinators in the season, it was outscored on other flowering criteria (early nectar provision and length of flowering) by most other legume species (Table 14, p. 82).

In comparison with the other legume species, LU was relatively **tall**; measurements indicated it to be the second tallest of the species tested in the first year (behind SC) and the tallest legume species in the second growing season (Figure 15, p. 54), although at individual sites, other species such as SF grew taller than LU (Table 17, p. 88). Among the legumes, LU showed a high pre-incorporation **biomass**, tending to be higher than WC (Figure 26, p. 66). In line with this high productivity, LU has been called "the highest-yielding of the temperate forage legumes" (Frame *et al*., 1998). However, LU was not significantly better than WC with regard to **plant re-growth** after cutting (Figure 16, p. 55) and biomass in summer 2009 (Figure 18, p. 57); also, WC had a significantly higher **crop cover** after winter (Figure 17, p. 56). On balance however, LU was characterised by very high productivity both at later stages in the field experiments (i.e. in years 2 and 3), and regarding its early development (in the first season), when it outperformed all other legume species except CC (Table 14, p. 82). In line with its high productivity and its tall height, results averaged across the six research hubs indicated that LU was highly effective at suppressing **weeds** (Figure 21, p. 60).

However, despite its high productivity, LU did not become the dominant species over time when sown as part of the **mixture** (ASM) in the on-farm trials, at least not on average (Figure 53, p. 92). This may have been due to its low tolerance of grazing and low soil pH (see below). However, this hypothesis is not entirely supported by biomass measurements in the ASM grown in the replicated field trials, where the proportion of LU biomass was lowest on a high pH soil (Figure 46, p. 81), indicating that more complex, and possibly more site specific mechanisms were responsible for the performance of LU within the mixture. An alternative explanation for these results is the competitiveness against grasses which is considered to be low in LU (Sheldrick *et al*., 1995). LU increased its proportion relative to the seed density (2.5kg/ha) at two out of three sites (Figure 46, p. 81), and became dominant in the ASM on a further site (Figure 49, p. 87). In the ecological modelling, LU was found to be part of 7 of the 10 best multifunctional mixes (Figure 51, p. 90).

Regarding the plant **residue** contents, LU showed relatively high lignin content (significantly higher than WC and RC) (Figure 29, p. 68); its polyphenol content and C:N ratio pre-incorporation were moderate and not significantly different from that of WC (Figures 30 and 31). Based on the residue profile and the effect on the grain yield components, LU was assessed to be relatively slower to **decompose** than WC (Figure 41, p. 78), but was still ranked as having a relatively low resistance to decomposition (Table 14, p. 82). Accordingly, LU showed high **nitrate** content in soil pre- and post-incorporation (Figures 35 and 38). With regard to the **grain yield** of a cereal sown after incorporation of the legume species, LU ranked second after WC, but differences between the two species were not significant (Figure 39, p. 76). LU is among legume species for which experimental evidence exists of a positive effect on **N fixation** from the presence of grasses in the sward (Roscher *et al*., 2011). In comparison with other legumes species (RC, BT, CC), LU is reported to have high N fixation ability (Heichel, 1987).

In the WP1 trials which generated the above results, LU was sown at a **seed rate** of 20 kg/ha. This is high relative to commercially recommended rates of 8 kg/ha (Cotswold Seeds, 2013), but is more in line with other recommendations of 15kg/ha (Kahnt, 2008), 20 kg/ha (Sheldrick *et al*., 1995) or up to 34 kg/ha (Frame *et al*., 1998). Increased seed rates can be used to compensate fungal seedling diseases. **Variety** choice is seen to be crucial to avoid *Verticillium* wilt (Sheldrick *et al*., 1995). The recommended **sowing depth** of LU seed is given as 10-20 mm (Cotswold Seeds, 2013) or 15 mm (Frame *et al*., 1998). LU can be **undersown** into barley (Moffat, 1998), spring wheat (Sheldrick *et al*., 1995), or maize (Lane, 1998), or it can be established on its own. Mature

LU roots release chemicals (medicarpins) which are toxic to germinating LU seeds; therefore, a failed LU crop should not be re-sown except when roots are still young (Sheldrick *et al*., 1995).

LU shows adaptation to a wide range of climatic and **soil** conditions (Janson, 1982) but shows stronger regrowth from frequent harvesting when grown in dry seasons and dry climates (Janson, 1982). In line with the drought tolerance of LU reported in the literature, the present study found LU to be positively responding to higher **sand** content of the soil in the establishment year (2009), which was characterised by a relatively dry spring.

The presence of LU on the farms participating in this study also showed a positive correlation with higher **soil pH**, but this became only evident in year 3 (Table 21, p. 98), with LU being unaffected by soil pH in the previous two seasons. However in the third year, the effect of pH was dramatic, as the frequency of LU in sampling quadrats was almost 5 times higher on sites with a pH>6.0 than on sites with pH<6.0 (46.4% vs. 9.4%). This is in accordance with reports that nodulation of LU cannot occur below a pH of 6.2 (Sheldrick *et al*., 1995). Also, it is known that at pH levels below 5, LU starts to be affected negatively by Al and Mn toxicity (Frame *et al*., 1998). While a soil pH range for LU growth of 6–7 has been suggested (Kahnt, 2008), the present study found that in the third growing season, the highest frequencies of LU in sampling quadrats were only observed when soil pH was above a pH of 7 (i.e. between 7.2 and 8.2, with the latter value being the maximum pH included in the study). LU differed from the other legume species tested in that there was a positive correlation between **K** content in the soil and presence (Table 21, p. 98).

LU is considered as an excellent element of **forage** for spring calving (Jagusch 1982) and dairy cows (Lane, 1998; Phipps *et al*., 1998), though it carries the risk of **bloat**. LU hay is also fed to racehorses, but British climate conditions are thought to be risky for production of hay from LU (Sheldrick *et al*., 1995). At the same time, LU is said to be difficult to ensile because it is low in soluble carbohydrates (Sheldrick *et al*., 1995). Four cuts can be achieved per season, with the first recommended at the mid-bud stage (Sheldrick *et al*., 1995).

In the present study, LU was characterised by a relatively low **grazing** tolerance, i.e. when cut low, there was only poor regrowth. This confirms previous research having found that its yield is significantly reduced by frequent cutting of the plant or cutting at immature stages (Janson, 1982), and LU is normally not grazed except when growth has ceased after the first frost in winter (Sheldrick *et al*., 1995). The nutritional value of LU as a forage is likely to be highest during the early stages of regrowth; thereafter, its leaf to stem ratio and digestibility decrease rapidly (Jagusch, 1982; Sheldrick *et al*., 1995).

Summarising, LU showed high productivity and high pre-crop value, but to deliver its potential depends on appropriate conditions such as a relatively high soil pH, and it does not tolerate grazing. However, out of the 12 farms that did not graze the ley and had a soil pH of at least 6.0, only 3 farms included LU in their control ley. Thus, there seems to be potential to increase the use of LU in fertility building, especially for longer-term leys (3–4 years).

MP - Meadow pea (Lathyrus pratensis)

Meadow pea (MP) was one of the species with the lowest performance in this study. With a relatively large seed size, the species showed a high **seedling biomass** and also a relatively high **plant height** in first trial year. However, after being cut several times, MP almost completely disappeared from the trial plots; for example, it showed very low **crop cover** after 12 months and compared to the other legume species, biomass of MP was very low in year 1. As a consequence, MP also showed poor **weed suppression** and even a significant increase of weed biomass from year 1 to year 2, in contrast to the trend of weed biomass reduction over time which was observed in most other tested legumes in this study. This weed increase in MP plots carried on into the final trial year, with high weed biomass at the time prior to incorporation.

Because of the very low biomass or even complete absence of plants from some of the trial plots, the **residue profile** of this species could not be determined. Probably because of low biomass production, MP showed low **nitrate content in the soil** pre-incorporation as well as low available nitrogen post-incorporation. When grown in a mixture (in the ASM) in the on-farm trials, MP was among the legume species with the lowest **presence** on the farms and in the sampling quadrats; this may be due to a relatively low seed rate, or a consequence of a possibly inappropriate sowing depth in the trials of this study, but may have also been to do with the relatively low competitiveness of the species or its inability to recover after cutting.

The literature on MP as a sown species is scarce, with many sources on forage legume not making any reference to the species at all. While Schlipf (1898) mentions the species as being recommended to be sown as a forage plant, Schneider (ca. 1914) already states that the species has not been widely adopted, referring to the higher productivity of LU and RC.

RC - Red clover (Trifolium pratense)

Both in the UK and internationally, Red clover (RC) is one of the most important legume species for forage production and fertility building. As for WC, there is an extensive literature on RC (Smetham, 1973a; Smith *et al*., 1985; Sheldrick *et al*., 1995; Frame *et al*., 1998; Boller *et al*., 2010; Gaudin *et al.,* 2013) and the species is well known to UK farmers. In fact, out of 34 farms participating in this study, 20 chose to include RC in the control ley (Table 4, p. 30), making RC the second most popular legume species after WC in this study. RC is a short-lived perennial with an upright **growth habit**, and it originates in South-East Europe and Asia Minor (Smith *et al.,* 1985). It was introduced to England in the middle of the 17th century (Smetham, 1973a; Evans, 1976). RC is almost completely self-sterile and is pollinated by various species of bumblebees as well as honeybees (Bohart, 1957; Hawkins, 1961; Free, 1965; Gurr, 1974; Dupont *et al*., 2011). Regarding **flowering** time, we observed that RC generally started later than WC (Figures 24 and 25).

Seed size is relatively small in RC, as in the other clover species. Accordingly, we found **seedling** biomass of RC to be relatively low in the current study, but it was comparable to the other clover species (Figure 13, p. 53); also, the seedling relative growth rate of RC was not significantly different from WC (Figure 13). However, because of faster emergence, RC ranked better than WC in terms of overall early development (Table 14, p. 82).

Plant **height** of RC was moderate in year 1, but high in year 2, substantially and significantly higher than WC (Figure 15, p. 54). In the first year, plant **regrowth** after cutting was significantly higher in RC than in WC (Figure 16, p. 55). Also, in the summer of the first trial year, RC showed the overall highest **biomass**, though it was not significantly different from WC (Figure 18, p. 57); other studies have reported biomass in the first growing season to be higher in RC than in WC, and to be comparable to that of LU (Cormack, 1996). Similarly, Knight *et al*. (2008) claim that RC is generally higher yielding than WC. Crop **cover** after winter was similar in RC and WC (Figure 17, p. 56).

RC showed very low **weed** cover in year 1 and year 2 (Figure 20, p. 59) and very low weed biomass (Figure 21); among the legumes, RC was only outperformed by the mixtures (ASM) in this respect. Regarding many parameters measured pre-incorporation (i.e. in 2011), RC was not different from WC, e.g. crop biomass (Figure 26, p. 66); weed biomass (Figure 27, p. 66); root biomass (Figure 28, p. 67) and plant available N; however, RC was notable for high soil **nitrate** content.

With regard to the **residue** profile, the lignin content of RC was similar to that of WC and lower than other non-clover legumes (Figure 29); but RC showed significantly higher polyphenol content than WC (Figure 30). Grain yield of the following cereal (Figure 39) was not significantly different between RC and WC; in contrast to this, a previous study found that organic wheat had higher grain yield following RC than after WC or LU; this effect was significant in one out of two trial years (Cormack, 1996).

In the ecological modelling, RC was part of the optimal multifunctional 2-, 3- and 4-species **mixtures**; in fact, the best 8 mixes all contained RC (Figure 51, p. 90).

In the on-farm trials, as part of the complex species mixture (ASM), RC was not significantly different from WC in terms of **presence** on the farms (Figure 52), presence in quadrats (Figure 53) or cover in the second and third year of the trial (2010 and 2011); but it was significantly higher than WC in first year (Figure 54, p. 93). RC showed the greatest **variability** in terms of change in ground cover over the course of the three years (Figure 55, p. 94). In the on-farm trials, RC was not as **persistent** as WC (Table 19, p. 95), confirming that RC is typically planned for two year leys (Kahnt, 2008) and not recommended to be used for permanent swards (Smetham, 1973a).

RC is regarded to be highly adaptable to a wide range of **soil conditions** but it does not tolerate "excessively wet or acid soils" (Frame *et al*., 1998). In the present study, RC was found to respond positively to increased **soil pH** in a multiple regression analysis (Table 21). As a single variable, however, soil pH was not significantly correlated with RC presence. In the literature, ranges of 6.0–6.5 (Frame *et al*., 1998), 6.0–7.5 (Knight *et al*., 2008), 5.8–7.5 (Sheldrick *et al*., 1995) or 5.4–7.0 (Kahnt, 2008) are given for RC growth; the optimum was given as 6.0 (Kahnt, 2008) or "around 7" (Zimmermann, 1958). However, this study did not confirm that RC performance suffers below a soil pH of 6.0. For example, in the first year of the on-farm trials, the frequency of RC in the sampling quadrats was almost equal for sites with a soil pH above 6.0 (80%) and below 6.0 (78%). We therefore conclude that within the range of 4.8–8.2 (the extremes within the current study), soil pH may not be the main constraint for RC growth. RC is intolerant of **saline** soils and good **drainage** is required for RC (Kahnt, 2008); in line with this, we found a positive correlation between sand content in the soil and RC presence in the ASM (Table 21). RC develops deep tap **roots** with root depth reported to range between 60 and 150 cm (Kahnt, 2008). This root systems convey **drought resistance** to RC (Knight *et al*., 2008), which is considered to be superior toWC (Smetham, 1973a). At the same time, RC has been reported to dry out the soil (Zimmermann, 1958); however when comparing the different legume species, this is not supported by the current study, which found soil moisture both before (Figure 33, p. 72) and after (Figure 36, p. 74) incorporation not to be consistently lower in RC than in the other legumes. RC has a moderate to high **root biomass** (30–60dt/ha) (Kahnt, 2008). In this study, RC did not show a significantly different root biomass from WC (Figure 28, p. 67). RC is considered to possess good **frost** tolerance (Knight *et al*., 2008), which improves with plant age (Frame *et al*., 1998). The minimum temperature for germination has been given as 1°C (Kahnt, 2008). Higher dry matter yields of RC have been reported for mountain regions (**altitudes** of 500–700 m) in comparison with lowland areas (160–360 m); this observation is ascribed to a positive effect of high humidity on RC (Leto *et al*., 2004). In the current study no effect of altitude was found on RC performance (presence or ground cover) but, except for one farm, sites were all less than 200 m above sea level. When BM, LU, RC and WC are compared, the highest **N fixation** was found in RC (Table 28, p. 106), but also the highest N losses (i.e. leaching and N_2O) (Table 29). Accumulated N contents of 260–420 kg/ha per year have been reported from elsewhere (Kahnt, 2008).

Sowing depth for RC is recommended as 10–15 mm (Frame *et al*., 1998). The recommended **seed rate** is 18 or 20–25 kg/ha in a monocrop (Kahnt, 2008); or 14–24kg/ha (Zimmermann, 1958) and 15–20 kg/ha in mixtures; as with the other legume species, the seed rate in the ASM was below these recommendations (Table 4, p. 30). In comparison with other legume species, RC has been reported to be particularly suitable for **direct drilling** ("sod seeding") (Seguin, 1998) and for **undersowing** (Kahnt, 2008), although there is a danger that in wet years RC outgrows the cereal (Sheldrick *et al*., 1995). **Inoculation** is often not carried out in Europe (Frame *et al*., 1998) and is considered unnecessary (Sheldrick *et al*., 1995) since natural populations of *Rhizobium* are present, but it inoculants are commercially used outside Europe (Rodríguez Blanco *et al*., 2010). **Diseases** (clover rot, *Sclerotinia trifoliorum*) and nematodes (the stem eelworm *Heterodera trifolii*) are regularly mentioned as constraints for RC production (Smetham, 1973a; Sheldrick *et al*. 1995; Frame *et al*., 1998; Knight *et al*., 2008); RC is therefore considered to be self-intolerant, requiring a 4–6 years break (Zimmermann, 1958; Kahnt, 2008). RC makes good **silage and hay** (Kahnt, 2008). However, **grazing** lush stands of RC carries a risk of bloat (Sheldrick *et al.,* 1995). RC can also cause problems with regard to fertility of ewes, as the isoflavine formononentin present in RC has an estrogenic effect (Mu *et al*., 2009) and interferes with the ewes' reproductive cycle (Adams, 1990; Shackell *et al*., 1993). With its more upright growth, RC showed a lower tolerance to being cut at a low height than WC (Figure 23, p. 62), confirming reports of RC to respond to close grazing with reduced yields (Smetham, 1973a).

Summarising, RC is a well appreciated and highly productive legume species and is suitable for a wide range of environmental conditions.

SC - White sweetclover (Melilotus alba)

White sweetclover (SC) is a biennial plant that can grow up to 2 m high. It is indigenous to central Europe, eastern Mediterranean countries and northwest Asia, and was introduced to many other temperate regions. It is mainly used in North America, including use under irrigation, though its use there has declined in recent years. However, in some regions of North America it is considered a weed (Klemov & Raynal, 1981; Kline, 1984) and also in Europe volunteers can be problematic (Kahnt, 2008), although the ability of SC to spread naturally is relatively poor.

SC is the most commonly grown species of the genus, followed by yellow sweetclover (*Melilotus officinalis*) (Smith & Gorz, 1965; Miller & Hoveland, 1995). SC is an important coloniser in disturbed areas, often found on stony ground such as roadsides and railways (Turkington *et al*., 1978; Klemov & Raynal, 1981). It has a very strong **taproot** (Kahnt, 2008), which conveys high drought tolerance. It can tolerate occasional **burning** (Kline, 1984) and is considered to be suitable for amelioration of soils (Kahnt, 2008).

SC is self-fertile, and it is **pollinated** mainly by honey bees. **Breeding** objectives in the past were low coumarin content, increased forage yield, improved characteristics for grazing, and better disease and pest resistance, but currently there is very little breeding work with this plant.

Because of concerns regarding forage value (see below), SC was not included in the ASM, and therefore was not part of the on-farm experiments. For this reason, all results reported here for SC only refer to its performance in monoculture plots.

SC is said to have moderate **seedling** vigour, with a spring to autumn growth season and to show vigorous growth in the year after establishment and where previous autumn management has allowed plants to develop strong root systems with good carbohydrate and N reserves. This was only partly confirmed in the current study. With a medium seed size, SC showed a relatively high seedling biomass in this study and high seedling relative growth rates (Figure 13, p. 53; Figure 48, p. 84). Regarding **plant height**, it was the tallest legume species in the first trial year (Figure 15, p. 54), and it was among the tallest species in the second year. However, compared to other legume species it showed low **biomass** in the first summer (Figure 18, p. 57) and low **crop cover** after the first 12 months. The ability of SC to compete with **weeds** is said to be poor to moderate during early establishment but it improves with time as the legume canopy develops. While this assessment on early competitiveness was generally confirmed in the current study (Figure 20, p.

59), the species also showed a high weed biomass (as a proportion of total biomass) at the end of the experiment in 2011 (Figure 27, p. 66).

SC is adapted to a wide range of **soils**. It tolerates heavy clays to light sands and does especially well on calcareous soils (Duke, 1981). It responds to high fertility, particularly of P and K and tolerates saline soils. While SC tolerates soil **pH** from 4.8–8.2, with the mean of 50 cases being 6.7 (Duke, 1981), the optimum pH range for sweetclover (including white and yellow sweetclover) is given as 6.5–8.0 (USDA, 2000). If soil pH is less than 6, it is recommended to lime soils well in advance of seeding. This view is partly supported by the present study; at the trial site (IBERS) where soil pH was lowest (5.9), SC biomass in the first summer (2009) was poor (11.7% of the site's maximum), but it was even poorer at another site (Barrington Park) where the soil pH was 7.8.

SC is intolerant of **shade**, and has a low tolerance of **flooding**. There is a high degree of drought tolerance once established, though yellow sweetclover is considered to be even more drought tolerant. SC is a **winter hardy** species and tolerates from 0.9–16.0 dm of **precipitation**, with the mean of 54 cases being 7.8 (Duke, 1981). Because of its taproots SC might be expected to mine **nutrients** from deeper soil strata.

Seed crops of SC are cut and windrowed when about half the seed pods have become brown to black (Smith & Gorz, 1965); seed loss can be substantial because of weak attachment of pods and pod shatter during combining. Average seed yields are about 225 kg ha^{-1} (Miller & Hoveland, 1995).

SC requires seed **inoculation** by an effective strain of *Rhizobium meliloti* for successful establishment and performance. The lack of a specific inoculant in the current study may explain why the species underperformed on some sites. For establishing SC, a well-cultivated, uniform and firm seed bed is required. Seed is usually drilled or broadcast directly after conventional seedbed cultivations, but can also be sown under a cereal cover crop. Optimum **seed depth** is 15–20 mm. Direct drilling into the existing grass sward can also be practised. Although usually sown in spring, SC can also be sown in early autumn. **Seed rates** are 9–12 kg/ha for scarified seed when sown pure, but reduced to 4–5 kg/ha if sown in mixture with grasses such as PR. While Duke (1981) states that SC should be sown at 11–25 kg/ha, Kahnt (2008) recommends 25–30kg/ha. In the present study, SC was sown at 18 kg/ha in monoculture plots.

Regarding its **residue profile**, the current study showed that SC had a high **lignin** content (significantly higher than WC and RC) and a high C:N ratio; this is in line with a study comparing yellow sweet clover with RC, which showed that the sweetclover had lower N content and higher cell wall and lignin contents in the stems, but higher N contents in the leaves (Wivstad, 1997). With regard to the resistance to decomposition, SC was ranked mid-range (Table 14, p. 82).

In terms of **forage production,** hay yields of up to 7–8 t/ha are considered to be achievable. In the UK, one study found that SC yielded 9.4 and 5.5 t ha^{-1} for first and second seasons, respectively (McEwen & Johnston, 1985). While SC is regarded to have a high nutritive value at the vegetative stage of growth when grazed, at pre-flowering stage for silage, and at early-flowering stage for hay, (2008) found it to have low **forage** value. SC has good acceptability after stock have become used to the bitter taste caused by the coumarin content of the forage. SC carries bloat risk and produces an anti-coagulant from the coumarin, which can cause ‘bleeding disease’ – hence the past development of low-coumarin cultivars. Defoliation by grazing is recommended to be lax in late autumn of the establishment year in order to allow sufficient root development.

Summarising, SC is a species of moderate productivity. It is drought tolerant, winter hardy and tolerant of alkaline soils. It is suitable for forage conservation, can be grazed and can provide good forage yields.

SF – Sainfoin (Onobrychis viciifolia)

Sainfoin (SF) is a **perennial** legume of South Central Asian origin, native to unimproved chalk grassland. It was introduced to Europe in the 15th century and has been cultivated widely as a forage crop throughout Europe, Asia and Western North America. It was widely grown in Britain in the 18th and 19th century but has since dramatically declined in importance in comparison to other legumes such as LU (Hill, 1998). Indeed, only 5 farms participating in this project included SF in the control mix (Table 4, p. 30). The name sainfoin is of French origin and means safe or healthy hay, referring to its historical usage to treat sick livestock (Frame *et al*., 1998), and possibly to its non-bloating nature (see below). In comparison to the other legumes included in the ASM, it has relatively large, spiny seeds with tough coats that can result in hard **seed** dormancy. It has a long taproot which confers very good **drought resistance** (Sheldrick *et al.,* 1995; Kahnt, 2008), exceeding that of LU (Hill, 1998). Among the legume species tested, it showed the lowest specific leaf area (Table 17, page 88). **Pollination** is mainly by bumble bees and other bees (Richards & Edwards, 1988; Ricciardelli d'Albore & Roscioni, 1990) and in temperate regions it flowers from June–August.

Establishment can be slow (Cormack, 1996), as borne out by the current results showing biomass in the first year was relatively low. However, once established SF can readily produce biomass in favourable conditions; and indeed in the WP1 trials, regrowth after cutting was not significantly different from white clover (WC).

Another relevant factor concerning establishment and general productivity is the **sowing rate**. Whereas the seed rate in a monocrop was given as 140–180kg/ha (Kahnt, 2008), the typical recommended rate for inclusion in a mixture ranges between 12kg/ha (http://sainfoin.eu/farming-sainfoin) and 40–50 kg/ha (Sheldrick *et al*., 1995); Hill (1998) recommends 82kg/ha, when SF is used as a main crop with companion grasses. TY and MF have been suggested as companion grasses (Sheldrick *et al*., 1995).

Due to the complexity of the ASM in terms of the total number of species, the seed rate of SF used within the ASM was much below the recommended rate, with only 5kg/ha (Table 1, p. 19). This may explain why presence of SF in the ASM was consistently lower than in RC and WC (Figure 53, p. 92). As noted for other species, greater productivity of SF in a less complex mix may require a higher seed rate than that used in the ASM. The recommended **sowing time** for SF is spring to early summer, with later sowing time (e.g. late July/August) being regarded as too late for seedling establishment before winter (Hill, 1998). This observation is not confirmed by the current study however, as the six farms that did sow the ASM between July and October 2009 did not show a reduced SF cover in the following year (2010), when compared to the farms that sowed the ASM in spring (between April and June 2009).

Whilst SF has a high capacity for growth as a monocrop, **competition effects** can impact on survival when it is grown as part of a **mixture**, particularly competition from grasses (Liu *et al*., 2009). Similar effects were observed in the ASM where SF was outcompeted by stronger species over time, i.e. decreasing presence in quadrats from year 1 to year 3 (Figure 53, p. 92). Due to such effects, the choice of SF **varieties** in fodder mixtures is important, e.g. cv. Esparsette, which was the variety used in the trials of this study, has been shown to give benefits at a lower proportion than other SF varieties in a mixture with a range of legumes (www.sainfoin.eu).

In terms of **productivity**, SF is often compared to LU, with SF yields reported to reach about 70%–80% of LU yields (Frame, 1992; Hill, 1998). In contrast to these figures, the current study showed that in the first year SF biomass was, on average, only 41% of the LU biomass (Figure 18, p. 57). However the relative performance of the two species was highly variable between sites, with SF outperforming LU at Rothamsted, but reaching less than 10% of LU biomass at the Duchy site (for map see Figure 3, p. 21), which may partly be explained by differences in soil properties such as pH (see below). **Weeds** have been mentioned as a problem in SF cropping (Sheldrick *et al*., 1995).

Regarding **persistence** of SF in the sward over time Kahnt (2008) describes this species as the forage legume with the highest longevity; also, it has been observed that “maximum yields often occur 2 to 3 years after sowing” (Sheldrick, et al. 1995). In the trials of the current study, however, SF showed significantly decreased ground cover over the three year trial period, when grown with other legumes and grasses in the ASM (Figure 55).

The **nitrogen fixation** efficiency in SF is relatively poor in comparison to LU, RC and WC (Frame *et al.,* 1998), and the N amount fixed is lower than LU, but mainly because of the lower overall biomass in SF. Compared to LU, SF has a lower **Leaf Area Index** (LAI) (Sheehy & Popple, 1981) and lower efficiency of using sunlight (Frame *et al*., 1998). **Rhizobial inoculation** of SF has been recommended by Frame *et al*. (Frame *et al*., 1998), but Hill (1998) (p. 57) states that it “has not been considered necessary in the UK”.

SF is known to thrive on alkaline soils, with several authors indicating that for good performance of the species, a **soil pH** of 6.0 and above is required (Sheldrick *et al*., 1995; Frame *et al*., 1998; Hill, 1998); in accordance with this assessment, this study found a positive correlation between soil pH and SF presence in the mixture. Kahnt (2008) describes SF as requiring less warmth than LU, which is in line with the observation by Hill (1998) that SF is more **winter hardy** than LU. Good **drainage** is required for SF growth and even seasonal waterlogging should be avoided (Sheldrick *et al*., 1995). SF is reported to respond well to additions of **P and K** (Sheldrick, et al. 1995); in contrast, this study found a significant negative correlation between P content of the soil and SF presence on the farms (Table 21, p. 98).

With regard to the **residue profile** of the species, the lignin content of SF was significantly higher than for WC and RC (Figure 29, p. 68). This may partly contribute to a relatively slower decomposition rate in relation to clover species. In comparison to the other tested legumes, SF had relatively high **polyphenol** content, similar to that of RC (Figure 30, p. 69). In accordance with this finding, the literature indicates that SF contains high levels and high diversity of phenolic compounds.

These, especially in the form of condensed tannins, have implications for the **forage value** of SF as they result in the species’ non-bloating nature (Jones et al., 1994; Hayot-Carbonero *et al*., 2011) and increase protein absorption and **palatability**. Voluntary intake of SF is 10–20% greater than for RC or LU (Sheldrick *et al*., 1995). High palatability of SF was confirmed in a comparative feeding study with sheep (Scharenberg *et al*., 2007). Finally, phenolic compounds in SF also confer **anti-helminthic** properties (Novobilský *et al*., 2011) e.g., a feeding study found that both dried and ensiled sainfoin reduced established populations of parasitic nematodes in lambs (Heckendorn *et al*., 2006). It is recommended not to overgraze SF with sheep in the autumn (Sheldrick *et al*., 1995).

Traditionally, SF was **undersown** in April in spring barley, while direct sowing in May–June has been suggested as a preferred option because of the warmer soil and the weed reducing effect of the false seedbed (Sheldrick *et al*., 1995). Because SF is a host of clover rot, it is recommended not to observe appropriate breaks, i.e. it should not be sown in **rotation** with RC (Sheldrick *et al.,* 1995).

In **summary**, SF is a species with lower productivity than other legumes such as WC, RC or LU, but has other highly beneficial characteristics, as it is (relatively) drought resistant, is highly palatable, does not cause bloat and has anti-parasitic properties when fed to livestock.

WC - White clover (Trifolium repens)

With respect to several variables white clover (WC) was either the best species or no legume species was significantly better than WC in the replicated plot trials; these variables include the above-ground **biomass** in the first year, the **crop cover** after the first winter, the proportion of **weed** biomass, **grazing** tolerance, the **below-ground biomass** prior to incorporation, the content of available nitrogen in the soil pre- and post-incorporation and the grain yield of the following crop. Thereby our results confirm the great value of this most popular species in fertility building leys. In the participatory trials, WC was chosen to be included in the control mixtures by 24 out of 34 farms (Table 4, p. 30).

Within WP3, WC also performed consistently well across the farms. When grown in a complex mixture (the ASM), WC and RC outperformed all other legume species with regard to most performance parameters (overall presence, ground cover, persistence, Table 19, page 95). WC was more reliable than RC in terms of **presence**.

At the same time however, WC was *not* the best species with regard to other variables in the replicated plot trials (WP1), e.g. speed of **emergence** (better in CC), **establishment** rate (better in

RC, CC, LU and SF), length of **flowering** (better in BM), pre-incorporation above-ground **biomass** (better in BM) and overall **biomass** production (better in BM). Further, the analysis of the **residue** composition of the different legume species reveals that WC has relatively low lignin and polyphenol contents as well as low C:N ratios, while other species such as BT and LT would, according to their residue composition, be slower to decompose. Also, within the participatory trials, WC produced lower early ground cover than RC.

This picture of WC being a valuable species that is outperformed by other species with regard to overall biomass production is confirmed by other studies e.g. in two field trials comparing different legume species, accumulated **dry matter yield** of WC was found to be lower than in RC and LU but higher than in SF (Cormack, 1996). Similiarly, in a field trial comparing WC with LU and Subterranean Clover (*Trifolium subterraneum*) dry matter produced was lowest in WC (Frame *et al*., 1998). Its outstanding suitability in grazing systems due to its stoloniferous habit (Beuselink & McGraw, 1984; Frame *et al*., 1998) is confirmed in this study (Figure 23, p. 62). The creeping growth habit of WC may also be a reason why it was found to **invade** many plots in the replicated plot trials.

In the participatory trials across the UK, WC was not significantly influenced by **region**, indicating the general plasticity of this species (or the variety used). Surprisingly, there was a consistent negative correlation between **potassium** content in the soil and the presence of WC (Table 21, Figure 58). This is in contrast to recommendations that farmers need to maintain K levels in the soil in order to let WC “make its proper contribution” (Cuttle, 2006). As in other legume species, an important factor for WC was **soil pH** (Table 20, p. 98) and higher soil pH was significantly associated with larger presence values of WC in the last trial year. Also, a high sand content was associated with greater presence values of WC in the first year.

Seed rates have been given as 10–14 kg/ha (Zimmermann, 1958) or 6.5 kg/ha (Kahnt, 2008).

WV - Winter vetch (Vicia sativa)

Winter vetch (WV) is one of the agriculturally important species where terminology has become slightly entangled. The botanical name (*Vicia sativa* L.) corresponds to several English names, including Common vetch, spring vetch, garden vetch and tare. It can be spring sown or autumn sown as an overwintering crop, and is therefore sometimes called ‘winter vetch’, e.g. by seed merchants (Wilkinson, 2009). On the other hand, the name ‘winter vetch’ can also refer to *V. villosa* (Kahnt, 2008), which is more accurately known as hairy vetch. This article, however, refers to *V. sativa* only, despite the fact that in most trials WV was sown in the spring.

WV is a tufted trailing or climbing **annual species**. It is found as a native plant in hedges and grassy places throughout Europe, West Asia and Africa (Clapham *et al.*, 1987). It can either be cut for fodder or grazed and is usually sown with a cereal to support the weak vines.

WV is a **large seeded** species and the experiments conducted in this study show that it was one of the legume species with a high **seedling biomass**, significantly larger than WC. WV is known to show relatively **slow establishment**, which was confirmed in this study: WV was slower to establish than most other legumes tested (Figure 11, p. 51).

WV along with other large seeded legume species had high absolute **growth rates** early in the season (Table 16, p. 86). However, because they were also tall and had their growing points largely *above* the level of cutting, these species had slow rates of re-growth. The position of the growing point also explains WV's very low **grazing tolerance**. The response to simulated grazing was tested in this study by measuring the above-ground biomass four weeks after the first cut; this was zero in WV (Figure 23, p. 62). In line with this, **plant height** measured just before the first cut of the growing season was found to be relatively tall in the first year but low in the second year (Figure 15, p. 54). Also, WV showed the lowest **crop cover** (ground cover by the crop) at the start of the second growing season among legumes (Figure 17, p. 56); the species had virtually disappeared, reflecting a relatively poor ability to produce new growth after winter.

Consequently, **pre-incorporation biomass** measurements for WV were relatively low (Figure 26, p. 66). These figures were used to assess the ability of the different species to compete against weeds by determining the **weed biomass** as a proportion of total biomass. In line with crop biomass results, WV showed a significantly higher weed biomass proportion than WC. In the second year of the project WV had the highest weed burden among the legume species. Finally, probably due to the low biomass, WV was also found to show low **nitrate** content in the soil prior to incorporation and very low available N levels post-incorporation (Figure 37, p. 75).

Despite these disappointing results, however, WV grew reasonable well at one trial site (Rothamsted). Here, additional measurements to assess the **invertebrate** communities showed that WV supported the highest number of individuals (Table 16, p. 86).

In terms of **soil conditions**, WV is said to prefer well drained moderately calcareous soils (Sheldrick *et al.*, 1995) with a pH of 5.5–7.0 (Kahnt, 2008). Only a relatively short break of 2–3 years is required between successive WV crops (Kahnt, 2008). Recommended **seed rates** for WV are 35kg–70kg/ha (Sheldrick *et al.*, 1995), 70–80kg/ha (Kahnt, 2008) or 62–125kg/ha (Wilkinson, 2009), when sown as a monocrop. WV was not included in the ASM, because of concerns regarding the effect of its tendrils, when the mixture is **undersown** in combinable cereals. However, common vetches can be sown for arable silage; e.g. sown in early autumn with oats and field beans or in spring with barley and peas (Sheldrick *et al*., 1995).

When the results of the field trials were **summarised** across various performance criteria (Table 14, p. 82), both WV consistently scored the lowest (together with MP). The results of this study suggest that WV performs poorly as a ley species in comparison to other legume species.

Critical appraisal of legume profiles in the literature

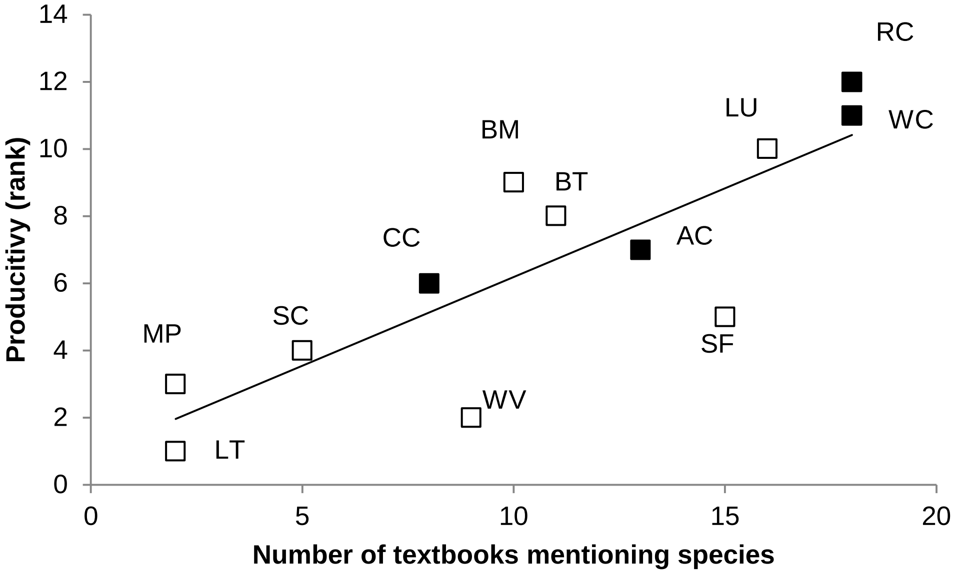

Figure 80. Number of textbooks (out of 18) mentioning legume 12 species and the species' productivity rank as determined in field experiments of Legume LINK (Table 14). Sources: (Zimmermann, 1958; Smetham, 1973b; Robinson, 1977; Lockhart & Wiseman, 1978; Siebeneicher, 1985; Kahnt, 1986; Blake, 1987; Frame, 1992; Neuerburg & Padel, 1992; Lampkin, 1994; Sheldrick *et al*., 1995; Frame et al., 1998; Newman, 1998; Newton, 1999; Freyer, 2003; Kahnt, 2008; Younie, 2012; Hunter ca., 1962).

The descriptions of the legume profiles show that the tested species all have their strengths and weaknesses. However, the attention that the literature has given to various forage legumes mainly reflects differences in their productivity (Figure 80). If a species is less productive, it is less likely to be mentioned in an agricultural textbook. However, this means that species with strengths in other 'disciplines' (such as resistance to decomposition, early development and persistence) are underrepresented in the literature.

It is clear that the literature may predominantly deal with those species that are used on a large acreage and this may in turn be determined to a large degree by a species' productivity. However, there is also the danger that this results in a self-reinforcing development, if producers, seed producers and breeders are influenced by the literature in what to grow and breed. Such a mechanism would impede change and reduce choice. The contents in (future) textbooks and other agricultural literature should therefore be less biased towards productivity and present a greater – truly functional – diversity of legume species.

What this analysis also shows is that BM may currently be underappreciated, and that WV and SF did not perform as well as might be expected from the coverage in the literature, maybe reflecting regional differences, or in fact a true deviation from the apparent focus on productivity (e.g. with regard to SF as a bloat free species).

3.4.2. Ecophysiological modelling, plant traits and functions (WP2)

The simulation model used in this study has shown the potential to generate a range of optimal mixtures that deliver multiple functions. This includes new varieties of legumes, or even new species; if their plant traits are measured, their functions can be determined and their performance in potential mixes can be predicted. Therefore, the combination of the simulation model and relationships between plant traits and ecosystem functions provides a powerful tool for exploring the performance of a large number of mixtures in terms of the delivery of multiple services.

The modelling study also showed that the overall recommendation of which species would be included in a legume based mixture critically depends on the number and identity of functions included in the evaluation. The more functions are included in the modelling the more likely it becomes that optimisation of overall performance can best be achieved by a relatively species-rich mixture. Conversely, including only a few functions resulted in the best mixes being relatively species poor: as species are added to a mixture of two or three species, the overall score is reduced as the added species dilutes the high performance of the other species.

This outcome however, is likely to be an artefact and the result of the fact that the competition model does not include positive interactions between species. Also, the model is only able to model the legume component of the mixture, i.e. interactions between legumes and grasses (see section 3.4.3, page 156) are not included.

A further issue is the relative weighting of functions to produce an overall score for evaluating hypothetical mixtures with the model. Ultimately, the modelling can therefore only give a first indication of overall performance as the relative weighting of different functions needs to be decided by users.

3.4.3. The multiple benefits of mixing species (WP1 & WP3)

In WP1, we found several strong effects resulting from growing various grass and legume species in a mixed stand. By comparing a complex species mixture (the All Species Mix, or ASM) with the average of its component species when grown in separate monocultures, we were able to establish the effects brought about by the species interacting with each other in the mixture. In comparison with the monocultures, the mixture showed an **increased ground cover, increased above-ground biomass and reduced weed biomass**.

Potential mechanisms for the observed effects are manifold, but it is likely that altered nutrient dynamics may have played the major role in the mixture effects. In particular, the interaction between grasses and legumes is probably the most important reason for the increased above-ground crop biomass and reduced weed biomass.

When nitrogen is released from decomposing legume-nodules into the soil, grasses take up this nitrogen (Frame *et al*., 1998), and, as a result, produce more biomass than when grown in the absence of legume species. This effect can outweigh potentially negative effects from competition between the legume and grass species. (In line with these predictions, it was observed in this study that grasses grew taller in the ASM than in the monocultures; however, there was no data collected with regard to this effect.)

In addition, the take up of nitrogen by the grasses also has an effect on the legumes, due to a feedback mechanism in the symbiosis between legumes and Rhizobia: as mineral soil N also acts as an inhibitor for the symbiosis, grasses grown in intimate mixtures with the legumes can reduce the inhibiting effect on further nitrogen fixation. In line with this mechanism, we found that the pre-incorporation mineral nitrogen content in the soil was significantly increased in the mixtures compared to the monocultures. In general the net effect of this increased nitrogen fixation rate per legume plant in the mixture will depend on the relative proportion of grasses and legumes.

Apart from the effects of mixing grasses with legumes, the increased biomass in the mixture (compared to the monocultures) might also be attributable to the mixing of different species within the legumes and within the grasses. Here, mechanisms may include complementary resource use (Hector, 1998; Gross *et al*., 2007) among the different legume, resp. grass species, or pest and disease reduction. However, with the available dataset from WP1, it is not possible to separate the grass-legume interaction from any interactions within the legume or within the grass species. Nevertheless, an indication of beneficial interactions within the legumes (in particular, the possibility of complementary resource use) lies in the phenological complementarity of the different species, with some species being ‘early’ while other species can be characterised as ‘late’ (see above). In addition, analysis of the data obtained in the participatory trials (WP3) showed that the plant biomass in the more diverse ASM was significantly greater than biomass in the Control ley (Figure 76).

Interestingly, the ASM did not show increased below-ground biomass in comparison to the average of the monocultures. However, while this result is in line with previous studies (Gastine *et al*., 2003) it is unclear what would explain the different behaviour between above-ground and below-ground biomass.

On soils poor in organic matter, the advantage of the ASM over the Control leys was more marked than on the soils with higher organic matter content (Figure 77, p. 125). Similar effects of increased resilience as a result of increased diversity have been found in several other studies.

Compared to the Control ley, the ASM was significantly lower in polyphenol content; but absolute differences were small and no significant effects of ASM on the C:N ratio or the lignin content was observed. Further data analysis using community weighted averages showed that regarding the development over time that grasses have most influence on the residue composition of the ley mixtures, with the C:N ratio increasing over time, as grasses increase in dominance.

Over time, the legume species richness in the ASM decreased from 8.5 species per farm in 2009 to 6 species per farm in the third trial year.

Finally, the data from WP1 revealed that early seedling development benefits from high seed weight, while later productivity is reduced in species with higher seed weight. This trade off suggests that a criterion for selecting species in a mixture could be (variable) seed weight, in order to create a species mixture that combines species with both early and late productivity. However, a technical problem arising in this situation is that with more variable seed size it becomes more difficult to drill the seed in one pass.

3.4.4. Modelling nitrogen dynamics (WP4)

The spring barley yields predicted by the DNDC model agree well with those measured after legume-based leys, but not with those measured after grass-based leys. The model predicted soil mineral nitrogen contents under spring barley with less success, particularly later on in the season. A range of agreement has been reported for published validation studies using DNDC, with discrepancies typically occurring when auxiliary inputs for soil and crop parameters are used instead of site-specific measured parameters (Giltrap *et al*., 2010).

The predicted amounts of nitrogen returned to the soil by legumes during the ley phase broadly agree with published values, e.g. 141 – 223 kg N ha^{-1} (Kumar & Goh, 2002) and 50 – 80 kg N ha^{-1}

(Ross *et al*., 2009). However, the predicted values for the grass residues are substantially smaller than the 64 – 72 kg N ha^{-1} reported by Kumah and Goh (Kumar & Goh, 2002), but are closer to the 8 kg N ha^{-1} measured by Ross *et al*. (Ross *et al*., 2009) for rye. Overall, the modelled trends for the grass leys are consistent and reflect well-characterised plant-soil interactions. In comparison with the residues of legumes, grass residues have larger C:N ratios and are therefore liable to immobilise soil N resulting in reduced yield in subsequent crops (Mary *et al*., 1996; Bhogal *et al*., 1997; Kumar & Goh, 2002).

Modelled losses of nitrogen via nitrous oxide emissions and leaching also compare well with the values reported for similar scenarios. Using lysimeter-based measurements Alvarez *et al*. (Alvarez *et al*., 1998) estimated annual losses of nitrogen through leaching to be 35.5 kg N ha^{-1} for white clover and 2.25 kg N ha^{-1} for grass. The annual leaching losses for spring barley are similarly typical for a green-manured spring cereal, e.g. 7.4 kg N ha^{-1} for spring wheat after yellow mustard (Herrera & Liedgens, 2009). Nitrous oxide emissions typically occur when accumulations of nitrate-N coincide with wet conditions, they are therefore episodic in nature and show great variability between both seasons and years (Smith *et al*., 1998), as did the predictions for the ley phase years. Bearing this variability in mind the predicted emissions are similar to emissions of 0.67 – 1.45 kg N ha^{-1} measured over a growing season for lucerne (Rochette *et al*., 2004) (Whitehead *et al*., 1979). The modelled results for the subsequent spring barley were also similar to the annual measurements for a conventionally fertilised crop of 0.8 kg N ha^{-1} reported by (Smith et al., 1998).

The predictions for the grass and leguminous leys and those within the sensitivity analysis suggest that the nitrous oxide emissions associated with a non-nitrogen fixing crop are lower than those associated with nitrogen-fixing crops. The modelled nitrous oxide emissions are relatively insensitive to increased nitrogen fixation capacity because, although nitrogen inputs into soil may be increased, denitrification is limited by factors other than substrate (nitrate) availability and consequently the additional nitrogen is lost from the system by leaching.

3.4.5. Decision support model for rotation planning (WP5)

The FBC-Link model incorporated a revised routine to estimate the amount of N accumulated by the ley. However, the project provided no experimental data about N accumulation or N release that referred directly to the specific LBMs included in the model and could be used to parameterise this part of the model. The current version of the model also contains temporary values for some parameters. The output from this version therefore is not a reliable indicator of the performance of the LBMs or of the capabilities of the completed model.

Although there is little published information directly relevant to the LBMs, the estimates of N accumulation produced by the current version of the model, as summarised above (Figure 63, p.

108 and following figures), are broadly in line with measured values reported in the literature. The FBC-Link estimates of N accumulation by clover/grass leys are also similar to those in the original FBC model that were determined independently using a method based on measurements of the unharvested fractions of fertilised grass swards (Whitehead *et al*., 1990). Measurements of N accumulation and inputs from the literature include values of 371 and 328 kg N ha^{-1} in above-ground material, respectively from red clover and white clover grown for one year and mulched as green manures (Stopes *et al*., 1996). Inputs of symbiotically fixed N in 1-year clover green manures in long-term crop rotation experiments in the UK and Germany were estimated to range from 20 to 350 kg N ha^{-1} (Schmidt *et al*., 1999). In a stockless system in the UK, cut and mulched red clover, white clover, lucerne and sainfoin crops accumulated 502, 408, 497 and 201 kg ha^{-1} of above-ground N, respectively over a two year period (Shackell *et al*., 1993; Cormack *et al*., 2003). Field experiments in Northern Germany compared cropped or mulched red clover, white clover and lucerne swards, either grown alone or with grass. After one growing season the cropped swards where cuttings had been removed had accumulated about 105 kg N ha^{-1} in roots and stubble. In contrast, mulched swards, where there had been no removal of cuttings, left 210–280 kg N ha^{-1} in the mulch, roots and stubble (Loges *et al*., 1999). Similarly, in studies with fertilised grass leys (without legumes) most of the added N was removed in harvested herbage and did not contribute to the build-up of soil N. When cultivated, the residues from 2–6 year leys were predominantly from root material and contained 128–176 kg N ha^{-1} with no significant differences between the different ages. Residues from a 1-year ley contained significantly less N with only 96 kg N ha^{-1} (Christensen *et al*., 2009).

The absence of measured data describing the effect of high lignin and polyphenol materials on N mineralisation from ley residues prevented any parameterisation or quantitative testing of the modified mineralisation routine designed to incorporate these variables. However, the modified method appears to include a sufficient number of variables to provide the flexibility required to be able to fit the output to the range of N release curves expected from these materials. For a simple empirical model such as this, it may be sufficient at this stage to demonstrate that the method of calculation is flexible enough to allow the output to be fitted to whatever pattern of N release is likely to be observed once data from mineralisation studies become available. The significant correlation between modelled estimates of mineralisation and measured values from the dataset of Vanlauwe *et al*. (2005) (Figure 68) suggests that the *relative* responses to changing C:N ratio and lignin and polyphenol concentrations are broadly correct, with the reservation that the test data refer to residues of tropical species that are likely to have different properties from those in the LBMs. Also, the results of short-term incubations may be a poor indicator of longer-term N release under field conditions.

There is insufficient information about the duration of the effects of lignin and polyphenols on N release. Lignin influences residue decomposition through its recalcitrance and production of polyphenols as products of its decomposition. Lignin decomposition kinetics are therefore likely to influence the duration of effects on N release but though widely studied there is still uncertainty about lignin turnover rates in soil (Cook I., 2010). There is also uncertainty about the persistence of the complexes formed between polyphenols and proteins (Dabney *et al*., 2010). Verkaik *et al.* (2006) reported that tannins from New Zealand tree species persisted for up to a year in an incubation study and Nierop *et al*. (2006) found that effects persisted over the 84 day incubation period adopted for their study. With the current settings, the model shows the effects of lignin and polyphenols continuing for longer than in these studies. It is important to understand the fate of the N that is held back from release. If this is not mineralised in a suitable time-scale to be useful to following crops, it will be effectively wasted. Cadisch *et al.* (1998) found no evidence that the N immobilised by high-polyphenolic residues was ever recovered by subsequent crops

A concern with the simple approach adopted for the FBC model and, more particularly, the FBC-Link model is that all forms of residue N are treated as a single pool which is then allowed to mineralise at a rate determined by the average C:N ratio. In reality, the behaviour of residues decomposing together is not simply additive. For example, the dynamics of root decomposition have been shown to differ from those of shoot material added to the soil, not only because of their differing chemical composition but also their position in the soil (Breland, 1994; Puget & Drinkwater, 2001). Treating residues as a single pool may be a necessary approximation in simple models but will involve greater uncertainties when describing the decomposition of more complex, multi-species mixtures. Of particular relevance is the frequently reported finding that N mineralisation from mixed crop residues with contrasting polyphenol contents is often different to that expected from the behaviour of the individual species when incubated separately (Handayanto *et al.,* 1997; Abiven *et al*., 2005; Tscherning *et al*., 2006; Meier & Bowman, 2010).

A further concern about adapting the FBC model is that the LegLink version uses the same basic algorithm to apportion the N in ley residues between the Fast_N, Med_N and OM_N fractions as in the original FBC model. This describes the fate of N from arable crop residues that are incorporated into the soil after harvest whereas much of the organic material added during the ley phase is deposited on to the soil surface without incorporation and can be expected to decompose differently from residues in soils that are tilled each year (Coppens *et al*., 2007). Furthermore, the properties of the residues that have accumulated over several years under a ley will differ from those of fresh residues from an annual crop. The model attempts to adjust for this by allowing the residues to decompose during the course of the ley, the main effect being to reduce the proportion of Fast_N at the end of the ley phase, but this approach needs to be tested against measured data.

Mix B was included in the model to demonstrate the effect of including a high-polyphenol species on the predicted N release following cultivation of the ley. However, results from the current (non-calibrated) model are an indication of how the model behaves rather than of how the mixture would perform in reality. In this mixture, the red clover component of Mix A is replaced by the polyphenol-rich species, sainfoin. Comparison of the N release curves for Mixtures A and B (Figure 63) shows very little difference between the two. This can be attributed to the relatively low concentration of polyphenols in the sainfoin compared with the residues of tropical species shown in other studies to affect mineralisation rates. In addition, the effective concentration would be further diluted by the presence of the other, low polyphenol, species in the mixture. The slightly greater accumulation of N by Mix B was mainly a result of there being a lower proportion of sainfoin in Mix B than of red clover in Mix A, which allowed for a greater proportion of higher N content legumes in Mix B.

The effect of increasing the proportion of sainfoin in the mixture from 27% to 55% and 75% is shown in Table 38 (assuming a 1-year ley with vigorous growth and mulching of the cut herbage). It also shows the effect of increasing the polyphenol content of the sainfoin residues to a concentration similar to the maximum reported in the literature for this species. In these examples, percentages of red clover were equal to those of sainfoin and the percentages of other legumes were the same in both mixtures. Inputs of N were alike for both mixtures and any differences in N accumulation and subsequent mineralisation were due solely to the presence of polyphenols from the sainfoin in Mix B. In the model, the increased concentrations of polyphenols reduced mineralisation during the course of the ley and thereby increased the amount of N accumulated. After cultivation, they reduced the rate at which this N was mineralised, as shown by the percentage of N mineralised in the three or 12 month periods. The increase in the pool of N accumulated during the ley phase offset the reduction in the subsequent rate of mineralisation so that net effect was only a small change in the quantity of N mineralised. Even with 75% sainfoin in Mix B and an increased concentration of polyphenols in the residues, the 3-month mineralisation was only reduced from 70 kg N ha^{-1} for Mix A to 62 kg N ha^{-1} for Mix B.

Table 38. The effects of increasing the proportion of sainfoin (SF) in Mix B on cumulative N mineralisation 3 and 12 months after cultivation of the ley compared with Mix A containing an equivalent proportion of red clover (RC) and the effect of increasing the assumed concentration of polyphenols (%Pp) in the sainfoin residues (herbage residues & root + stubble) as predicted by the FBC-Link model. Values in parentheses are the cumulative mineralisation values expressed as a percentage of the total N accumulated by the ley.

Mix	**%RC**	**%SF**	**%Pp**	**Total N from ley (kg ha^{-1})**	**3-month mineralisation (kg N ha^{-1})**	**12-month mineralisation (kg N ha^{-1})**
A	39	-	-	321	72 (22.4%)	175 (54.3%)
B	-	27	4 & 3	333	72 (21.6%)	178 (53.6%)
A	55	-	-	314	73 (23.2%)	173 (55.1%)
B	-	55	4 & 3	321	69 (21.5%)	172 (53.6%)
B	-	55	10 & 5	332	67 (20.2%)	173 (52.1%)
A	75	-	-	301	70 (23.3%)	166 (55.1%)
B	-	75	4 & 3	311	62 (19.9%)	165 (53.1%)
B	-	75	10 & 5	325	62 (19.0%)	166 (51.1%)

Although FBC-Link is a purely empirical model, some of the processes referred to above are likely to operate in reality and may be relevant to the use of LBMs in practice. In particular, the characteristics of a particular species and its effect on N release will be diluted where it is a single component of a multi-species mixture and secondly, the presence of factors which reduce N mineralisation rates from ley residues will also act during the ley phase, potentially leading to a greater accumulation of N.

Mineralisation rates can also be manipulated by varying the C:N ratio of the residues, for example by including grasses in the ley mixture. However, Ranells & Wagger (1996) reported only slight reductions in N release from grass-legume cover crop residues compared with residues from legume monocultures. How the proportion of grass affects estimates of mineralisation in the FBC-Link model was examined by comparing the ley options of Mix A alone and Mix A with ryegrass added. The effects of increasing proportions of ryegrass are summarised in Table 39, again with model settings corresponding to a 1-year, mulched ley with vigorous growth.

Increasing the proportion of grass reduced the total amount of N accumulated by the ley as the N-rich legume residues were progressively replaced by grass residues of lower N content. The higher C:N ratio of the residues from grass/legume mixtures also reduced N mineralisation during the ley phase, which increased the proportion of N remaining at the end of the ley. However, this increase was small compared with the reduction in N inputs. The net result was a marked reduction in the modelled amount of N accumulated by leys containing an appreciable proportion of ryegrass. There were corresponding reductions in the estimates of N mineralisation following cultivation of the ley. This was largely a result of the reduced amounts of N accumulated during the ley phase as the percentage mineralised after cultivation was unaffected by the proportion of grass except at the highest level.

Table 39. The effects of including increasing proportions of perennial ryegrass (PR) in Mix A on the N concentration of the grass component, total N accumulation by the ley and cumulative N mineralisation 3 and 12 months after cultivation of the ley as predicted by the FBC-Link model. Values in parentheses are the cumulative mineralisation values expressed as a percentage of the total N accumulated by the ley.

		%N in grass		**Total N from ley (kg ha^{-1})**	**3-month mineralisation (kg N ha^{-1})**	**12-month mineralisation (kg N ha^{-1})**
Mix	**%PR**	herbage	root + stubble			
A	0	-	-	321	72 (22.4%)	175 (54.3%)
A+grass	27	2.8	2.1	302	68 (22.4%)	164 (54.4%)
A+grass	45	2.7	2.0	286	64 (22.4%)	156 (54.4%)
A+grass	75	1.8	1.3	206	37 (17.9%)	105 (51.1%)

Neither the inclusion of high polyphenol content residues or addition of grass to the ley mixture reduced the modelled rate of N release as much as was expected from tests of the mineralisation routine alone, indicating that the full model may underestimate their potential for manipulating N dynamics. A likely explanation is that the current model fails to take account of the mixing and disruption of the soil when it is ploughed at the end of the ley. Differences in the modelled mineralisation rate are largely determined by the contribution from the Fast_N fraction but in the current version most of the Fast_N from residues accumulated during longer-term leys will have been mineralised by the time the ley is cultivated and will not influence subsequent mineralisation rates. Including a calculation step to redistribute part of the Med_N to the Fast_N fraction when the ley is ploughed, based on the C:N ratio and lignin and polyphenol content of the residues, would increase the sensitivity of the mineralisation estimates to these parameters; however, the information needed to quantify such an expression was not available.

Alternatively, if the limited response to high polyphenol residues is shown to be realistic, this raises the question of whether it is necessary to include the effects of polyphenols in the model. When the revised mineralisation routine was applied to the dataset from Vanlauwe *et al*. (2005), the inclusion of lignin and polyphenol content as a variable achieved only a small and non-significant improvement in the correlation between modelled and measured values compared with the correlation obtained with the C:N ratio alone, even though the ranges of lignin and polyphenols were broader than those expected for the LBMs. Similarly, Trisoutrot *et al*. (2000) concluded that inclusion of lignin and polyphenol contents with the N concentration or C:N ratio of residues did little to improve simple predictions of soil mineral N dynamics. This may have been because the concentrations of polyphenols in these temperate crop residues were well below those in the tropical residues shown to produce responses in other studies. The polyphenol contents of the residues analysed in the LegLink study were also significantly lower than those shown to influence mineralisation rates.

The modelled responses to increasing proportions of grass in the ley mixture demonstrate a further limitation of the present model. In order to simplify the input data required, the user is asked to describe the performance of the ley as one of three broad yield categories. Subsequent calculations do not automatically adjust for any changes to other input variables that might influence this yield and this can result in comparisons that are based on unrealistic yield data. This can result in simulations based on unrealistic yield data. Even if the user is sufficiently familiar with the model to adjust yields manually, the yield categories only permit very coarse adjustments. For example, the predictions in Table 39 are likely to underestimate the reduction in N accumulation and mineralisation resulting from a high proportion of grass in the ley. All were derived from simulations that assumed vigorous growth and a high yield, which are unlikely to be achieved in the example with a high grass and low legume content. For the ley with 75% grass, it would be more realistic to select the moderate category. This reduced the predicted N accumulation to 157 kg N ha^{-1}. In contrast, moderate proportions of grass are more likely to increase the total yield of the ley through competing for soil N and encouraging the legume component to fix more N from the atmosphere. If, as for the 27% grass example in Table 39, no adjustment is made for this increased yield, the model will predict a reduction rather than an increase in N accumulation compared with Mix A containing no grass.

The changes made to the user interface for the FBC-Link version of the model did not add significantly to the amount of information requested from the user and the feedback from the workshop with farmers and advisors indicated that the data input screens were easily understood. Similarly, presenting the results of the simulations as graphs rather than tables was approved of by all who completed the questionnaire. This change was made to avoid giving a false sense of precision to the results but it was apparent from comments during the meeting that there were unrealistic expectations of what the model can deliver. Its relative simplicity and many approximations are hidden from the user. Future versions should be designed so that the model is seen as providing a general guide to rotation planning rather than providing absolute values.

The primary requirement for any further developments of the FBC-Link model would be to provide the information needed to parameterise the model. Most of this would be available from the field trials and laboratory mineralisation studies that would be needed to fully develop the LBMs for use in sustainable farming systems, irrespective of any further development of the FBC-Link model.

3.4.6. Legume cover crops in conventional rotations (WP6)

Yield responses to the use of cover crops in general were positive; furthermore, differences in responses between cover cropping approaches were also apparent. Considering benefits to soil systems, the clover bi-crop approach resulted in notable reductions in soil bulk density and improvements in water infiltration rates. This did not become apparent until cropping year 3 (2010 harvest) and may have been associated with changes to the root structure or development of the clover during this time. It seems probable that the yield responses in the wheat crop recorded in 2010 may have been partly associated with the N fixation in the clover and partly with soil conditioning effects. While yield responses were apparent to the other cover cropping approaches, associated improvements in soil structure and water infiltration were not recorded. However, the cover crops in the other approaches have only been imposed periodically (only ahead of spring crops) and perhaps further cycles of cover cropping are needed before effects become apparent or can be detected by the assessment methods used.

The positive impacts from the use of cover crops is promising and suggests that such systems may have a role to play in developing bio-sustainable conventional arable cropping practices. The variation observed in soil and yield responses between approaches, however, suggests the need for more research to improve the consistency and resilience of performance and to understand better which approaches are more likely to deliver benefits (or result in problems) in particular scenarios. For example, considering the selection of cover crop species, the brassica species are relatively easy to establish, but can result in volunteers and potentially disease problems in following crops. Alternatively, legume species tend to be more difficult to establish and are generally slower growing. Better understanding of species selection and the development of suitable species mixtures for specific scenarios could improve both performance and accessibility of cover cropping approaches.

The cumulative margins over standard practice across the wheat and spring oilseed rape crops for the cover cropping approaches averaged around £80/ha, around £84/ha for the clover bi-crop and the legume mix cover crop and around £69/ha for the brassica cover crop. However, margin over N responses in relation to the clover bi-crop varied markedly in relation to N dose, with greater margin over N apparent at lower N doses. The costs for establishing and managing a cover crop would be in keeping with these figures. While improvements to techniques and species selection should reduce the costs associated with delivering a cover crop, it is likely that cover crops would only cover their cost based on these margins. However, it should be remembered for all cover cropping systems under consideration benefits may accrue as the systems mature, the choice of specific cover crop species (or species mixtures) should help to maximise any potential benefits, changes to input costs (e.g. fertiliser and fuel) are likely to impact on the return and additional income from appropriate environmental schemes could improve the attraction and add further financial support.

3.4.7. Satellite project: Legume mixtures and pollinators

Preliminary results indicate that the more diverse All Species Mix (ASM) legume ley can support a larger range of pollinators, both earlier and later in the season, than the less diverse ‘standard’ legume mixtures. This is likely to be due to the different flowering times of the species growing in the mixture. Floral diversity is known to be an important driver for bee community structure, while continuous floral succession between April and July is needed to maintain bumblebee survival; the introduction of diverse sown mixtures has been shown to benefit pollinator communities. The ASM mixture benefits bumblebee and hoverfly species early in the season (May), and hoverfly and solitary bee species late in the season (August). The data suggests that legume leys have the potential to support pollinators throughout the season, outside the major crop flowering times.

Management of the legume leys also affects pollinator community composition, which is likely to be due to floral resource availability under different management types. Sheep grazing significantly reduced floral resources for pollinators throughout the year, in this study. Grazing is likely to affect the species composition, and when grazing pressure was removed from grazed sites, mass flowering of white clover occurred in both ASM and control plots occurred. However, many of the taller flowering species had either been excluded from the ley, or were unable to recover in time to flower, before grazing resumed. Further work is needed to understand how different management practices affect ecosystem services provided by biodiversity, both individually and in combination.

3.5. Conclusions

The **characterisation of legume species** conducted in this study has generated new knowledge on the specific responses of legumes to environmental and management conditions, and the relative merits of various species for use in a range of farming systems. It has shown that the range of currently used species is relatively narrow, and often restricted to just two species (WC and RC); several other species such as BM, BT, CC, LU and SF show great potential to increase the productivity and provision of ecosystem services across the UK. For unlocking this potential however, it is necessary to invest in breeding programmes, to conduct further agronomic research into optimised management and to intensify **knowledge transfer** with regard to best practice.

In addition, the study showed that **mixing different legume** species in the ley has several advantages: it suppresses both early and late weeds; it extends forage availability for key pollinator species; and it increases stability of performance. In addition, mixing species may help to increase the reliability of establishing the ley; further, by producing residues with different residue profiles

(C:N ratios and polyphenol and lignin contents) mixtures also provide opportunities for modifying decomposition rates to improve the synchrony between nitrogen release following incorporation and crop nitrogen demand. Finally, widening the range of legume species increases opportunities to build short term leys into rotations on conventional farms.

This study raises several questions that the conducted experiments were not designed to answer but that are likely to be relevant for drawing further conclusions from the work. One of the points that may limit the conclusions is that only one variety per species was included in the trials. Currently it is unknown whether the differences observed between the different legume and grass species would be similar, larger or smaller than differences within species, based on intraspecific genetic variation. Similarly, differences observed between the species may not only be due to genuine biological properties but may also reflect the breeding effort that has been invested in various species. For example, white clover has received substantial attention from plant breeders and consequently there is currently a wealth of white clover varieties available. In contrast, there were no improved varieties available for white sweet clover and meadow pea. This means that investing **breeding effort** in species other than white and red clover might result in considerable improvement of legume performance and might help to widen the choice of legume species.

A further complex of questions raised by the current study concerns the nutrient dynamics after incorporation of the legumes and grasses into the soil. Critical elements that would be needed for a more comprehensive characterization of legume and grass species in fertility building leys are the composition of below-ground residue and the longer term nitrogen dynamics in the soil.

The LegLINK project, in conjunction with the NFS programme and previous research, has demonstrated the potential for cover crops (and soil amendments in the wider NFS programme) to deliver improvement to both soil systems and yields. However, it should be stressed that **long term rotation experiments** take several seasons to embed the systems. Hence, while the benefits observed in this study are encouraging they should be treated with some caution and further development and refinement of the systems would seem prudent.

Going forward, it is important that long term systems research continues to consider the rotation as a whole and seeks to address the development of increasingly bio-sustainable and resilient rotation systems for conventional cropping. Systems research provides an invaluable facility to showcase, demonstrate and evaluate new ideas and better enable growers to make informed decisions regarding best practice within their farming systems.

3.6. Output and dissemination record

3.6.1. Publications

Baddeley JA, Döring T, Hatch D, Marshall A, Pearce B, Roderick S, Stobart R, Storkey J, Watson C, Wolfe M. 2010. Using legume-based mixtures to enhance the nitrogen use efficiency and economic viability of cropping systems. Proceedings of SAC/SEPA Conference, "Climate, Water and Soil: Science, Policy and Practice". Edinburgh, 31 March–1 April 2010, p.178-182.

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3.6.2. Events

Nr.	Date	Audience	Location	Number of Participants	Duration (LegLINK)	Event title	KT Details	Partners responsible
1	30/6/09	Farmers	Berks	~30	~0.5 h	Arable day	LegLINK presentation, first results	ORC
2	5/8/09	Farmers & advisors	Scotland	~20	0.5 h	Organic farm walk	Project demonstrated as part of an Open Day	SAC
3	5/8/09	Farmers	Cornwall	~50	1 day	Managing the future of your soil—the role of green manures	Intro to project and demo of trial plots	Duchy College (with Garden Organic)
4	7/1/10	Farmers	Glos	~50		Organic Producer Conference	LegLINK workshop held at the Organic Producer Conference. Thomas Döring (ORC) described first impressions from preliminary data collected in year one of the project.	ORC
5	19/5/10	Farmers	Glos	~50	3 h	Barrington Park Grassland Day	LegLINK project introduced with visits to trial plots	Duchy, ORC
6	6/10	Advisors	Cambs	~15	1 day	IOTA / Nafferten Ecological Farming Group	Workshops at Bluebell Farm. LegLINK presented as part of a workshop on the latest soil fertility research	IOTA
7	6/10	Farmers	Norfolk			NIAB-TAG open day at Morley	LegLINK introduced with demonstration plots, as part of presentations on latest agricultural research	NIAB-TAG
8	15/6/10	Public	Suffolk	~20	0.5 h	Wakelyns Open Day 2010	LegLINK was presented and the trial plots shown to visitors	ORC
9	19/6/10	Farmers	Cambs	~50	2 days	Cereals 2010	A stand in the HGCA section at Cereals 2010 was staffed by ORC researchers who talked to visitors about LegLINK with the aid of a demonstration tub of ASM sward	ORC, HGCA
10	24/5/10	Public	Berks	~150	[-]	HRH PoW visit to Elm Farm	LegLINK Demonstration bed sown with mixture, with signs to read	ORC
11	6/7/10	Industry	Scotland			Royal Highland Show	LegLINK was introduced by Christine Watson at the SOPA stand	SAC

Nr.	Date	Audience	Location	Number of Participants	Duration (LegLINK)	Event title	KT Details	Partners responsible
12	16/7/10	Public	Berks	~20	2 h	ORC Open Day 2010	LegLINK was introduced by researchers; small beds had been established to demonstrate the ASM and pots to demonstrate the component species individually; later farm visit to participating farmer	ORC
13	29/7/10	Farmers & advisors	Scotland	~20	0.5 h	Organic farm walk	Project demonstrated as part of an Open Day	SAC
14	17/1/11	Industry	Wilts	~50	0.5 h	Organic Producer Conference	Information about LegLINK disseminated at Organic Producer Conference	ORC
15	11/5/11	Farmers	Wales		1 day	Farmer Focus event		IBERS, OCW
16	6/11	Farmers	Norfolk			NIAB-TAG open day at Morley	LegLINK introduced with demonstration plots, as part of presentations on latest agricultural research	NIAB-TAG
17	8/6/11	Farmers	Cornwall	~15	1 day	Farmer focus group	Farmer event hosted by participatory farmer	Duchy, ORC
18	15/6/11	Industry	Lincolns	~40	2 days	Cereals 2011	A plot sown with the LegLINK mixture was exhibited and plants in pots were also shown. See separate report	ORC, HGCA
19	22/6/11	Public	Suffolk	~20	0.5 h	Wakelyns Open Day 2011	LegLINK was presented and the trial plots shown to visitors	ORC
20	23/2/12	Farmers and advisors	Glos	~30	1 day	LegLINK advisor workshop	Selecting and managing legumes to maximise fertility and forage production – new results from research and experience	IOTA, ORC
21	3/4/12	Researchers	Scotland	~250	(2 days)	Agriculture and the Environment IX	SAC and SEPA Biennial Conference; Valuing Ecosystems: Policy, Economic and Management Interactions; 3 Legume LINK posters displayed	ORC, SAC
22	6/12	Farmers	Norfolk			NIAB-TAG open day at Morley	LegLINK introduced with demonstration plots, as part of presentations on latest agricultural research	NIAB-TAG
23	27/8/12	Researchers	Finland	~275	(2 days)	European Agronomy congress	1 Legume LINK poster displayed	ORC
24	10/9/12	Students	Devon	~15	2 h	MSc course Sust. horticulture	Overview of most important LegLINK results, with particular emphasis on the effect of mixing species	ORC

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APPENDIX

Additional data tables

Field characterisation of legume and grass species (WP1)

Table A1. List of weed species at Barrington Park, 24 June 2009

Species	**Cover (%)**
Chenopodium album	30
Papaver rhoeas	30
Fumaria officinalis	10
Sinapis arvensis	10
Sonchus arvensis	10
Veronica persicae	5
Capsella bursa pastoris	4
Anagalllis arvensis	3
Cirsium arvense	2
Matricaria matricarioides	2
Stellaria media	2
Thlaspi arvense	<0.5
Galium aparine	<0.5
Viola arvensis	<0.5
Fallopia convolvulus	<0.5
Rumex obtusifolius	<0.5
Polygonum aviculare	<0.5
Euphorbia helioscopia	<0.1
Senecio vulgaris	<0.1
Polygonum persicaria	<0.1
Galium spec.	<0.1
Lamium amplexicaule	<0.1
Silene alba	<0.1
Plantago major	<0.1
Euphorbia exigua	<0.1
Melilotus officinalis	<0.1
Atriplex patula	<0.1

Decision support model for rotation planning (WP5)

Table A2. Proportions of sown species and weeds in Years 1 – 4 of LBM and clover/grass leys as used in the current version of the FBC-Link model (dummy values).

	Year 1	**Year 2**	**Year 3**	**Year 4**
Mix A				
Red clover	**0.39**	**0.34**	**0.29**	**0.17**
White clover	**0.18**	**0.15**	**0.13**	**0.13**
Lucerne	**0.25**	**0.34**	**0.38**	**0.36**
Black medic	**0.15**	**0.12**	**0.10**	**0.09**
Weeds	**0.00**	**0.05**	**0.10**	**0.25**
Mix A + grass				
Red clover	**0.28**	**0.20**	**0.16**	**0.07**
White clover	**0.13**	**0.10**	**0.09**	**0.09**
Lucerne	**0.20**	**0.25**	**0.26**	**0.22**
Black medic	**0.12**	**0.09**	**0.08**	**0.05**
Perennial ryegrass	**0.27**	**0.31**	**0.31**	**0.32**
Weeds	**0.00**	**0.05**	**0.10**	**0.25**
Mix B				
Sainfoin	**0.27**	**0.31**	**0.31**	**0.26**
White clover	**0.27**	**0.19**	**0.16**	**0.13**
Lucerne	**0.31**	**0.35**	**0.35**	**0.29**
Black medic	**0.15**	**0.10**	**0.08**	**0.07**
Weeds	**0.00**	**0.05**	**0.10**	**0.25**
Clover/grass				
White clover	**0.50**	**0.40**	**0.30**	**0.20**
Perennial ryegrass	**0.50**	**0.55**	**0.60**	**0.55**
Weeds	**0.00**	**0.05**	**0.10**	**0.25**

Table A3. Carbon, N, lignin and polyphenolic concentrations in herbage and root + stubble for sown species and weeds as used in the current version of the FBC-Link model (dummy values). Concentrations of N in perennial ryegrass are adjusted according to the proportion of legume in the total herbage, within the ranges shown. Polyphenolic concentrations are expressed as % tannic acid equivalents.

	Herbage			
	C (%)	**N (%)**	**Lignin (%)**	**Polyphenols (%)**
Red clover	40	3.1	5.0	0
White clover	40	3.8	5.0	0
Lucerne	40	3.2	11.5	0
Black medic	40	3.5	11.0	0
Sainfoin	40	3.1	5.5	4
Perennial ryegrass	40	1.4 - 3.0	5.0	0
Weeds	40	2.0	6.0	0
	Root + stubble			
Red clover	40	2.6	7.5	0
White clover	40	3.4	7.5	0
Lucerne	40	2.8	15.0	0
Black medic	40	3.0	14.0	0
Sainfoin	40	2.6	7.5	3
Perennial ryegrass	40	1.0 - 2.2	7.5	0
Weeds	40	1.7	9.0	0

Soil analytical methods

Soils were analysed at NRM Ltd., Bracknell, UK. Soil analytical methods followed DEFRA Reference Book RB427, ISBN 0 11 242762 6 and NRM Ltd SOP JAS-399.

The soil pH was measured potentiometrically in the soil-water suspension (ratio 1:2.5) in a controlled temperature environment. Available phosphorus was extracted from the soil at 20°C by shaking with Olsen’s Reagent (0.5M sodium bicarbonate solution) at pH 8.5.

Inorganic phosphorus was determined spectrophotometrically at 880nm after reacting with acid ammonium molybdate and ascorbic acid. Available potassium was extracted from the soil by shaking with M ammonium nitrate at 20°C for 30 minutes. After filtration, the concentration of potassium in the extract was determined by flame photometry.

Available magnesium was extracted from the soil by shaking with M ammonium nitrate at 20°C for 30 minutes. After filtration, the concentration of magnesium in the extract was determined by atomic absorption spectroscopy. The addition of a releasing agent to the sample before analysis eliminated interference by phosphate.

The soil texture (particle size distribution of the soil) was determined using a Pipette sedimentation method. The organic matter was removed from the soil sample. The sand fraction was removed by sieving at 63µm and the resulting sample was mixed to form a suspension with water. The clay and silt fractions were determined by pipette sampling from suspension at different sedimentation times according to Stokes Law. Textural classes followed the UK Classification (Sand 2.00-0.063mm, Silt 0.063-0.002mm, Clay < 0.002mm).

Mineral nitrogen content was determined from cooled soil samples after chopping the soil and mixing it to obtain a homogenous sample. Stones were removed. A portion was shaken with 2M KCl to extract the mineral-N fractions. The Nitrate-N, Nitrite-N and Ammonium-N were measured colourimetrically.

Abbreviations

Species and mixtures

AC:	Alsike Clover, *Trifolium hybridum*
ASM:	All Species Mix (see Table 1, p. 19)
CC:	Crimson Clover, *Trifolium incarnatum*
BM:	Black medic, *Medicago lupulina*
BT:	Birdsfoot trefoil, *Lotus corniculatus*
IM:	Inoculated All Species Mix
IR:	Italian ryegrass, *Lolium multiflorum*
LBM:	Legume Based Mixture
LT:	Large Birdsfoot trefoil, *Lotus pedunculatus*
LU:	Lucerne, *Medicago sativa*
MF:	Meadow fescue, *Festuca pratensis*
MP:	Meadow Pea, *Lathyrus pratensis*
NM:	Non-inoculated All Species Mix
PR:	Perennial ryegrass, *Lolium perenne*
SC:	White sweet clover, *Melilotus alba*
SF:	Sainfoin, *Onobrychis viciifolia*
RC:	Red clover, *Trifolium pratense*
TY:	Timothy, *Phleum pratense*
WC:	White clover, *Trifolium repens*
WV:	Winter vetch, *Vicia sativa*

Other abbreviations

C:	carbon
DNDC:	DeNitrifiction DeComposition model
FBC:	Fertility Building Crops (Model), see Cuttle (2006)
IPCC:	Intergovernmental Panel on Climate Change
K:	Potassium
L:	lignin (in WP5 model)
Mg:	Magnesium
N:	Nitrogen
P:	Phosphorus
Pp:	polyphenol (in WP5 model)