The genetic improvement of forage grasses and legumes to reduce greenhouse gas emissions

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Summary for policy makers

Grasslands are one of the most important ecosystems and forms of land use in the world. They are crucial for food production and deliver ecosystem services. However, particularly when used for livestock production, they are also a significant source of pollution including the greenhouse gases methane and nitrous oxide. Energy inputs into grassland agriculture, particularly fertilisers, also contribute to greenhouse gas emissions.

A considerable amount of research has focused on emissions from grasslands: how to measure them and management strategies for mitigation. Similarly, there have been a number of studies on the role of grasslands in terms of carbon sequestration. However, less work has been carried out exploring ways in which genetic improvement of grassland crops can reduce emissions and enhance carbon sequestration.

Plant breeding has been successful at increasing the yield, persistency and stress tolerance of the major grasses and legumes of many grasslands in the world. These same approaches have considerable potential in altering plant traits to enhance the ecological efficiency of grassland agriculture.

This paper focuses on the following areas:

(i) Reducing methane emissions from ruminant animals (cattle, sheep) by modification of plant composition. There are approaches for which the potential is clear but which are not yet fully validated. These include increasing the digestibility and sugar content of grasses and increasing the content of compounds that affect protein breakdown in the rumen.

(ii) Reducing nitrous oxide emissions by making the use of nitrogen more efficient both in terms of going from soil to plant and in the animal. Considerable progress has been made towards increasing plant nitrogen use efficiency in key species of temperate grasslands. This has the potential to allow significantly less nitrogen fertiliser application and reduce nitrous oxide emissions. Changes in plant composition can also influence the efficiency with which protein is used in the rumen and potentially increase meat and milk production whilst reducing nitrogen losses through excreta.

(iii) Plant breeding may also enhance carbon sequestration in grasslands by focusing both on below ground traits of importance (e.g. root morphology and turnover) and on the composition of material returned to the soil as litter.

(iv) The processes involved in fertiliser production, particularly nitrogen fertilisers, result in considerable greenhouse gas emissions. In addition to gains from more efficient utilisation of nitrogen and phosphorus in improved grassland crops, the greater uptake and use of improved varieties of forage legumes, which can ‘fix’ their own nitrogen from the atmosphere, has considerable potential to reduce the carbon footprint of livestock agriculture.

The paper is primarily focused on species of temperate grasslands where most work has been carried out although the potential in tropical areas is discussed briefly.

Plant breeding approaches are cost effective, accessible to farmers through established routes and show high rates of uptake in many parts of the world. Collaboration between plant breeders, animal scientists and soil scientists is critical to future success. Progress is best evaluated within the framework of a comprehensive life cycle analysis approach that guards against emission swapping and develops solutions that consider economic as well as environmental sustainability.

Introduction

Grasslands cover about 70% of the world's agricultural area (Soussana and Luscher, 2007). Their use in a way that is economically, environmentally and socially sustainable is a major challenge facing many countries. Clearly they play an important role in food production. However, there is increasing recognition of the need to approach grasslands from the viewpoint of 'multi-functionality' i.e. they are also important as sources of pollution, particularly from livestock production, whilst at the same time delivering important ecosystems services and underpinning the tourism, amenity and leisure industries in many parts of the world. The changing considerations concerning the use and management of grasslands have been recently reviewed (Kemp and Michalk, 2007).

A considerable number of studies have looked at the effects of climate change on grassland species and on the potential to enhance adaptation by genetic improvement (e.g. drought tolerance) but much less work has been carried out on the ways in which plant breeding can contribute to climate change mitigation through reduced emissions of greenhouse gases and enhanced carbon sequestration.

In a recent review, Povellato *et al* (2007) stated that agriculture (as a whole) and forestry account for 30% of worldwide greenhouse gas (GHG) emissions from human activities and 10% in Europe. They concluded that agriculture and forestry can potentially contribute to GHG emission mitigation and that emission reduction can be achieved cost effectively by reducing methane emissions. Carbon sequestration is an additional way of mitigating GHG emission but more costly than direct abatement. Currently, in terms of carbon mitigation options from livestock agriculture, only a reduction in the number of animals can be registered as a reduction according to the Intergovernmental Panel on Climate Change (IPCC, 1997).

The impact of climate change on European grasslands and their role in climate change mitigation have been recently reviewed (Mannetje, 2007a; Hopkins and Del Prado, 2007; Soussana and Luscher, 2007). Morgan (2005) considered the global picture in terms of the response of grazing lands to increased atmospheric carbon dioxide.

Hopkins and Del Prado (2007) compiled the scenarios for climate change affecting European grasslands. Likely responses include: increased herbage growth, increased use of forage legumes particularly white and red clover and alfalfa (lucerne), reduced opportunities for grazing and harvesting on wetter soils, greater incidence of summer drought and increased leaching from more winter rainfall.

The key species of improved grasslands in substantial parts of Europe, Australasia and to a lesser extent North and South America are forage grasses in the families *Lolium* (ryegrasses) *and Festuca* (fescues) and forage legumes in the families *Trifolium* (clovers) and *Medicago* (particularly *Medicago sativa*, alfalfa (or lucerne)).

These species are the major source of grazed and conserved feed for dairy, beef and sheep production. However, there is an increasing awareness of the important role they play in the delivery of ecosystems services and their value for leisure and amenity.

In Mediterranean climate areas, the use of annual species is common and considerable work has been carried out to assess the suitability of a range of different annual legumes (reviewed in Sulas, 2005). However programmes of genetic improvement of particular species have been more limited.

Jank *et al* (2005) described the potential for breeding to contribute substantially to increased productivity of the 260 million hectares of well drained savannah grassland in South America. Recent decades have seen dramatic increases in the use of the introduced grasses *Brachiaria* species particularly in the Brazilian cerrados region. Over the past 30 yrs more than 70m ha of native vegetation has been replaced by pastures for beef production particularly Brachiaria and *Andropogon.* In seasonally flooded lands *Paspalum atratum* is a grass species native to South America which has attracted research and commercial interest (Jank *et al* 2005). Breeding of tropical grasses, many of which, including important species of *Brachiaria, Papsalum* and *Cenchrus,* are apomictic (i.e. do not reproduce sexually through seed production) is still in its infancy and focused primarily on underpinning more efficient and economic livestock production. Many important forage legume genera have originated in tropical America, e.g. *Stylosanthes, Arachis,* and *Leucaena.* Germplasm collections of these species have been made and new cultivars developed although uptake by farmers appears limited. Mannetje (1997) reviewed the prospects for legume based pastures in the tropics. Tropical legumes fix as much N as temperate ones, e.g. 575 kg/ha/yr for a pure stand of *Leucaena leucocephala,* and there is greater carbon storage in legume based tropical pastures than grass only.

Mosier and Delgado (1997) studied methane and nitrous oxide fluxes in tropical grasslands and Bustamante *et al* (2006) reviewed factors important for nitrogen (N) cycles in tropical and sub tropical savannas. Key factors are the extent of N or phosphorus (P) limitation, role of fire and intensity of grazing. There are still considerable gaps in our knowledge of N cycles in tropical grasslands.

The major challenges of tropical improved grasslands -uptake of improved germplasm, encouragement of on farm diversity, and development of more sustainable production- mean that at the moment adoption of breeding programmes aimed at climate change mitigation is not a priority.

In general, breeding approaches to increasing the efficiency of grassland agriculture can be characterised as:

(i)Accessible: through seed without other inputs

(ii) Lasting and cumulative impacts

(iii)Bringing other benefits e.g. varieties contributing to improved animal performance

(iv) Easy to use and relatively inexpensive to the farmer

(v) Appropriate in the long term, representing sustainable ‘genetic’ rather than ‘input’ based solutions.

Reducing methane emissions from ruminants by modification of plant composition

On a global scale, agriculture and in particular enteric fermentation in ruminants (predominantly cattle and sheep), produces between 21 and 25% of the total anthropogenic emissions of methane. In Australia, for example, ruminant livestock are responsible for 12% of national net GHG emissions (Howden and Reyenga, 1999) and there are high emissions per unit GDP for livestock sector relative to other sectors**.** Kebreab *et al* (2006) found agriculture accounts for 8% of GHG in Canada, mainly methane and nitrous oxide.

The two major sources of agricultural methane emissions are enteric fermentation in livestock and livestock manures. We will focus on genetic improvement strategies to reduce the former since this is the most important source and the most amenable to improvement through breeding. However it should be noted that approaches to alter the composition of livestock diets will also have an effect on manure composition for example carbon: nitrogen ratio will affect the decomposition rate. Ruminant livestock can produce 250-500L methane/day (Johnson and Johnson, 1995) and these authors described the major factors influencing methane emissions:

- Level of feed intake
- Type of carbohydrate in the diet
- Addition of lipids or ionophores to the diet
- Alteration of the ruminal microflora

Bhatta et al (2007) and Lassey *et al* (2007) reviewed methods of determining methane emissions and the range of strategies to reduce methane emission from enteric fermentation was reviewed by Hopkins and Del Prado (2007). They categorise them as: dietary changes, direct rumen manipulation and systematic changes. The latter include considerations of breed, livestock numbers and intensiveness of production. More intensive production may result in lower methane emission but may be less desirable in terms of other environmental impacts, highlighting the importance of a rigorous life cycle analysis in which trade-offs between different outcomes can be explicitly considered.

Direct rumen manipulation includes reducing protozoa numbers in the rumen (since protozoa parasitise methanogenic bacteria) and the addition of ionophores to enhance propionate levels. However, these approaches have drawbacks: reducing protozoa may lead to metabolic disease and the main ionophores used are antibiotics such as monensin where issues of resistance may limit utility.

Tamminga (1996) reviewed nutritional strategies for methane reduction. Dietary manipulations include the addition of organic acids (aspartate, malate and fumarate) and yeast culture. These compounds encourage the production of propionate and butyrate in the rumen which compete for hydrogen and reduce the ability of methanogenic microbes to produce methane. Research into the efficacy of these approaches and the optimum method of delivery of organic acids is on going. Animal selection approaches based on increased feed efficiency as a result of lower residual feed intake is an objective that may lead to 'substantial and lasting methane abatement' Alford *et al* (2006), and Hegarty *et al* (2007) also demonstrated the potential for animal selection based on residual feed intake. This could reduce the methane costs of growth i.e. increase efficiency.

Plant secondary metabolites such as tannins and saponins have also been employed in attempts to reduce methane emissions from enteric fermentation, although the efficacy of these compounds remains disputed. In the next section we discuss how genetic improvement of tannin containing species offers an approach to studying the impacts of tannins on methane production as well as a potential route to reduced emissions.

In the long term and for many parts of the world where livestock production is important, plant breeding strategies to reduce methane emissions are likely to become of increasing utility. Such strategies may be involved in the development of production systems that result in lower animal numbers without sacrificing economic viability: this is a clear route to reduce methane emissions. That is, emissions per animal would be constant but emissions per unit of production (milk, meat) would decrease. However, another important approach is to build on our (limited) understanding of rumen function and to modify plant composition in such a way as to result in reduced emission per animal without detriment to productivity or health.

It can be seen that a significant factor affecting methane emissions is the animal's diet and this is open to modification through breeding strategies particularly where the animal is fed a diet with a significant forage component (grazed or ensiled). Such approaches build on the considerable success that has been achieved in improving quality traits for animal production e.g. ryegrasses with higher water soluble carbohydrate (WSC) content and increased digestibility. Indeed, in many cases it is likely that improvements in quality for animal production will also lead to reduced emissions. This may be the case for the high WSC grasses where more N is partitioned into meat and milk and less is available for nitrogenous emissions through excreta. At the same time, of course, other diet based strategies are possible including increasing the amount of fibrous concentrate (Lovett *et al* 2005). There is also evidence that using clovers and grasses with high WSC in animal diets can directly reduce methane emissions (Lovett *et a*l 2004). It has been demonstrated that increasing the WSC content in perennial ryegrass by 33g/kg reduces methane production *in vitro* by 9%.

Another such approach of current interest and supported by some initial evidence is the use of tannin containing forages and breeding of forage species with enhanced tannin content. Forage legumes such as *Lotus corniculatus* (birdsfoot trefoil) and *L. uliginosus* (greater trefoil) possess secondary metabolites known as condensed tannins (CTs) in their leaves. CTs are flavonoid polymers which complex with soluble proteins and render then insoluble in the rumen; yet release them under the acidic conditions found in the small intestine, reducing bloat and increasing amino acid absorption. They are not present on the leaves of white or red clover but are present in the inflorescences. Methane production values were lower in housed fed sheep fed on red clover and birdsfoot trefoil than on a ryegrass/white clover pasture (Ramirez-Restrepo and Barry, 2005). A recent study has shown the extent of variation between and within varieties of *Lotus corniculatus* and *L. uliginosus* (Marley *et al* 2006). Diverse germplasm is now available with CT content ranging from 20mg/g DM to >100mg/g DM for experiments to quantify effect of CT content on methane in combination with other forage species. This will be more feasible using a high throughput CT assay recently developed at IGER which will enable rapid analysis of CT content in the numbers of genotypes required for a breeding programme. Rhizomatous lines of *L. corniculatus* with considerably improved persistence and contribution to mixed swards have been developed at IGER.

Methane can also be released from soils and is produced in layers under the top soil under anaerobic conditions particularly during and after rainfall. Kammann *et al*

(2001) highlighted the importance of the top soil aerobic layer in oxidising methane and therefore reducing the amount released. This shows the value of reducing soil compaction, poaching etc and enhancing soil quality as measured by aeration, and oxygen diffusivity. There is evidence that plant species differ in their visible effects on soil structure (Drury *et al* 1991) and anecdotal reports have long supported a positive role for legumes in this respect. More detailed investigations of the process of soil structuring have been carried out on white clover (Mytton *et al* 1993; Holtham *et al*., 2007), and red clover (Papadopoulos *et al* 2006). It has been reported that the changes in soil structuring brought about by white clover resulted in improvements in water percolation rate (*i.e.* the soil became more freely-drained), and in the extraction by plants of nutrients from the soil. Holtham *et al* (2007) also reported evidence of local structuring of soil around white clover roots and greater drainage of water through soil cores under white clover than under perennial ryegrass monocultures. Similar benefits in terms of soil structure were noted for soil cores under red clover monocultures by Papadopoulos *et al* (2006), although the effects were transient, and were reversed when a cereal crop was sown the following year. Improved soil structure reduces the risk of soil compaction and water run-off, increases the soil's biological activity, and facilitates seedling establishment and root penetration. However, it appears likely that legume-driven improvements in soil structure and drainage also directly result in increased leaching of both fixed and applied nitrate in legume monocultures (Holtham *et al* 2007).

Reducing nitrogenous emissions from grassland agriculture

(i) Increasing the efficiency of plant uptake and use of nitrogen

Nitrous oxide emissions can arise directly from N inputs to soil e.g. animal excreta, fertiliser, manure , crop residues, fixed nitrogen and also indirectly from nitrates. Developments to reduce nitrate leaching or ammonia volatilisation are also likely to reduce nitrous oxide emissions**.** Mannetje (2007b) stated that extensively managed grasslands have negligible emissions of nitrous oxide and ammonia but this is not necessarily the case where inputs are more than minimal. Nitrous oxide emission from temperate grasslands are poorly quantified but Mummey and Smith, (2000) reported estimates from US grasslands of approx 67Gg nitrous oxide N /yr (based on simulated emissions x area). Gregorich *et al* (2005) found that emissions of nitrous oxide from soils increased linearly with the amount of mineral nitrogen fertiliser applied and because systems containing legumes produce lower annual nitrous oxide emissions, alfalfa and other legume crops should be considered differently when deriving national inventories of GHG from agriculture. Rochette *et al (*2004) measured nitrous oxide emissions from soils with alfalfa and soybean cropping looking at soil surface emissions in comparison with perennial grass. Low nitrous oxide emissions were seen under grass and soil mineral N was up to 10 x greater under legumes but soil mineral N pools were not closely related to nitrous oxide emissions. Comparable emissions were seen under Timothy (*Phleum pratense*) as under legumes.

Soil processes controlling nitrous oxide production, i.e. nitrification and denitrification, are affected by a range of abiotic factors (e.g. temperature, pH) but also by fertiliser addition and organic matter content (Hopkins and Del Prado, 2007). Nitrous oxide emissions can be reduced by enhancing the competitiveness of plant uptake against that of competing soil microbial processes. The efficiency by which grassland crops remove mineral soil N has been the focus of much research focusing on optimising fertiliser application timing and rates, increasing fertiliser use efficiency and using chemical nitrification inhibitors .

A key area where genetic approaches can have an impact is in improving the nitrogen use efficiency (NUE) of crops to allow lower fertiliser application and hence reduce nitrogenous emissions through the soil-plant-animal-soil cycle. NUEs from soil to crop are generally lower for grass-based livestock production compared with arable crop production, ranging from 10-40% for whole dairy systems compared with 40-80% for arable systems, on a whole-farm basis (Neeteson *et al* 2004). More efficient use of N brings benefits to farmers both with respect to meeting regulatory requirements and in terms of cost savings from reduced fertiliser use. The case for increasing the efficiency of fertiliser nitrogen use on economic grounds is compelling and global demand for fertilizers continues to rise. 85-90 million tonnes (Mt) of N fertilisers are currently applied annually worldwide (Frink *et al*1999) and this is expected to rise to 240 Mt by 2050 (Tilman *et al* 1999). Breeding forage crops capable of using fertiliser inputs more efficiently offers a clean technology route to increased sustainability of livestock production, via lowering recommended fertilizer rates, reducing the agricultural footprint with respect to pollution and reducing the wider consumption of non-renewable resources. This is particularly so with respect to N, frequently the main determinant of both yield and environmental quality in agricultural systems.

Formal definition of NUE depends on the scope of the system, choice of mass-balance or N flux approaches, and on whether N uptake, utilization and retention within the plant are considered (Garnier and Aronson, 1998; Good *et al.,* 2004). For example, in agronomic terms, NUE = NUpE x NUtE, where NUpE is the N uptake efficiency: the ratio between the amount of N absorbed by the plant and that supplied/available in the soil. NUtE is the utilization efficiency (the unit dry matter (DM) produced per unit N in the dry weight, or the DM flux per unit N flux in a whole stand in units of g biomass/mol of N). This approach has informed previous genetic improvement and mapping studies of N (e.g. Loudet *et al.,* 2003) and can be employed in parallel with a flux-based approach to NUE, developed for ecosystem analysis (Berendse and Aerts, 1987).This offers significant advantages for trait dissection of the NUtE component, given by the product of aNP x MRT, where aNP is mean annual N productivity and MRT is mean residence time of N in the plant. Genetic variation for acquisition (Gorny and Sodkiewicz, 2001), utilization (Witt *et al.,* 1999*),* nutrient retention (Granstedt, 2000) and tissue concentrations (Smith *et al.,* 1999) has been demonstrated in a wide range of species. Quantitative trait loci (QTL) for traits associated with NUE have been identified in *Arabidopsis* (Rauh *et al,* 2002; Loudet *et al,* 2003), maize (Gallais and Hirel, 2004), barley (Mickelson *et al* 2003) and ryegrass (Van Loo *et al.,* 1997, Wilkins *et al* 1999, 2000).

Clearly N leaching can occur under grass/clover swards and in some circumstances this is comparable to swards fertilised at rates commonly used in agricultural practice. The evidence that the use of forage legumes in place of N fertiliser will reduce nitrate leaching is equivocal (reviewed by Frame and Laidlaw, 2005). This is not only as a result of the ploughing of legume rich sward but also leaching taking place under a standing crop, for example following cutting of red clover. There is a need therefore to consider the role of germplasm improvement in reducing such losses. Initial studies at IGER suggest that the processes of root and nodule senescence that contribute to losses of N are under genetic control and amenable to selection. In the long term, enhancement of plant processes that suppress or inhibit nitrification may give a further option. The likely importance of below ground processes including symbioses especially mycorrhizae (Johnson and Wedin, 1997) is largely unexplored but represents another area where genetic understanding leading to breeding approaches may prove valuable.

(ii) Increasing the efficiency of N use in the ruminant animal

The rapid breakdown of herbage proteins in the rumen and inefficient incorporation of herbage nitrogen by the rumen microbial population are major causes of N loss and gaseous emissions. Scarcity of readily available energy during the time of maximal protein degradation restricts microbial protein synthesis. Ammonia accumulates as a waste product and is absorbed from the rumen and excreted as waste nitrogen in urine. When sheep (MacRae and Ulyatt, 1974) and cattle (Ulyatt *et al* 1988) are given fresh forages they can waste 25-40% of forage protein.

Genetic improvement of the forage grasses and legumes that constitute important components of the ruminant diet has the potential to reduce emissions to air. Two possible strategies of increasing the efficiency of conversion of forage-N to microbial-N have been suggested; (i) increase the amount of readily available energy accessible during the early part of the fermentation and (ii) provide a level of protection to the forage proteins, thereby reducing the rate at which their breakdown products are made available to the colonising microbial population. One approach is to develop forage species with a better balance between water soluble carbohydrate (WSC) and crude protein (CP) by increasing the WSC content of the grass or the clover component or reducing the protein content of the legume.

The most advanced of these approaches is the development at IGER of high WSC ryegrasses which are already showing considerable commercial success, particularly in the UK, and for which there is some evidence that increased production is accompanied by reduced emissions as predicted (Miller *et al* 2001). There is also significant variation within white clover and associated material including lower protein content and higher WSC. Unique non fixing inbred genotypes of white clover were used at IGER to demonstrate the principle that material of lower leaf protein content shows much slower protein degradation in the silo (Kingston Smith *et al* 2006). Following this we established that genotypic variation within elite gene pools of white clover is much greater than was previously thought. Interspecific hybrids between white clover and *Trifolium ambiguum* (Kura or Caucasian clover), have a crude protein content 14.2g/kg DM lower than white clover.

Opportunities also exist within forages to select for other specific traits that can reduce protein loss. A good example of this approach is the emerging research on the enzyme polyphenol oxidase (PPO), which is at a particularly high level of activity in red clover in comparison with other species and has a role in protein protection (Owens *et al* 2002). This enzyme converts phenols to quinones which subsequently bind to protein and slow the rate of protein degradation. Thus, in silo, the protein made available for diffuse pollution of nitrogen, e.g. as ammonia, is reduced. Ensiling alfalfa (lucerne) leads to the degradation of 44% to 87% of forage protein to non forage protein (NPN). In comparison, red clover has up to 90% less protein breakdown (Sullivan and Hatfield, 2006). Increasing the level of PPO is a target for genetic improvement in red clover as a route to reduced nitrogenous pollution. Significant variation for PPO activity in red clover germplasm and differences in activity through the year have been shown in recent work at IGER.

Potential of genetic improvement to enhance carbon sequestration in grasslands

The substantial stocks of carbon sequestered in temperate grassland ecosystems are located largely underground in the roots and soil. The roots, senescent leaves, and stems differ in their rate and process of breakdown in the soil (Joffre and Ågren, 2001). However, in a survey of temperate grassland Jobbágy and Jackson (2000) found that only 64% of soil organic carbon existed in the top 40 cm of soil which contained 87% of all roots, the remainder of the carbon is found at greater soil depths probably due to a decreased C turnover at depth in the soil (Jones and Donnely, 2004).

Well managed, improved tropical grasslands store high rates of carbon (230-260 T/ha) with grass-legume mixtures storing more than grass only swards and grazed grasslands more than cut ones (Mannetje, 2007). Estimates of well managed and fertilised grasslands are much lower for carbon (C) deposition than tropical: 65-70T/ha in 100 years. As in temperate systems the depth of deposition of C is important: the deeper the deposit, the longer the turn over time e.g. *Brachiaria* deposits C to a depth of 1m due to deep roots (Mannetje, 2007b).

Ogle *et al* (2004) reviewed management factors affecting soil organic C for temperate and tropical grasslands. Gregorich *et al (*2005) in Eastern Canada showed that management practices that enhance C storage in soils include fertilisation and 'legume and forage based rotations'. Gill *et al* (2000) reviewed factors influencing root turnover e.g. for fine roots of grassland the turnover` rates increased exponentially with mean annual temp. Global patterns were seen but could not be used for specific predictions.

In summary, the principal factors that determine the amount of C sequestration in the soil are:

(i) The rate of input of organic matter,
(ii) The rate of its decomposition,
(iii) Soil depth,
(iv)The physical protection of aggregates and organomineral complexes
(Jones and Donnely, 2004).

Productive forage grasses such as *Festuca. arundinacea* have been shown to increase the soil C pool by 17.2% (equivalent to C sequestration of circa 3 mg C $ha^{-1}yr^{-1}$ over a 6 yr period, Lal *et al* 1998). The amount of C retained by soils is influenced greatly by management practices with those that lead towards reduced soil disturbance and for increased crop persistency having the greatest benefits on C sequestration. It follows that change from arable to perennial grasslands lead to significant improvements in accumulation of soil C (Guo and Gifford, 2002).

Elevated CO_2 levels have been demonstrated previously to increase photosynthesis of perennial ryegrass. Such an effect is likely to increase soil C inputs and microbial biomass through increased root exudates and turnover. However, the benefits of an increased production through elevated CO_2 may well be only short-term and to be sustained will require complex interactions involving the continued availability of N in the soil, the NUE of the plants, and the uptake and loss of nutrients to maintain an effective balance of soil C and N necessary to deliver an effective decomposition process. To some extent, the negative impacts of increases in temperatures could be mitigated for by using multi-species communities that provide for complementary growth patterns and productivity.

The key plant traits likely to influence C sequestration (root depth, structure and architecture; litter composition and amount) are reasonably well established and genetic variation is beginning to be characterized for many of them. Some early progress has been made at IGER with regard to mapping of genes in perennial ryegrass for C sequestration, with effective C return in litter associated with loci on chromosomes 1 and 5

Broader considerations of grassland systems

Productive temperate grasslands typically require significant inputs in the form of fertiliser, particular nitrogen, phosphorus and potassium. Wood and Cowie (2004) carried out a review of studies of greenhouse gas emissions from fertiliser production. Nitrogen fertiliser manufacture brings with it significant greenhouse gas emissions from the Haber-Bosch process of synthesising ammonia and from nitric acid production Synthesis of ammonia, the primary input for most nitrogen fertilisers, is very energy demanding with natural gas the primary energy source. Nitric acid is used in the manufacturing of ammonium nitrate, calcium nitrate and potassium nitrate. The oxidation of ammonia to give nitric oxide also produces a tail gas of nitrous oxide, nitric oxide and nitrogen dioxide. Nitric acid production is the largest industrial source of nitrous oxide although clearly this is also used for purposes other than fertiliser manufacture. Estimates of nitrous oxide emissions from nitric acid manufacture are very variable: 550-5890 CO_2 equivalents /kg nitric acid
Urea accounts for almost 50% of world nitrogen fertiliser production and is synthesised from ammonia and carbon dioxide at high pressure to produce ammonium carbonate which is then dehydrated by heating to give urea and water.

The synthesis of phosphate fertilisers also results in greenhouse gas emissions (reviewed by Wood and Cowie, 2004). Single superphosphate is produced from phosphate rock and sulphuric acid, triple superphosphate from phosphate rock and phosphoric acid. The majority is derived from phosphoric acid which itself is synthesised form phosphate rock and sulphuric acid. Wood and Cowie (2004) state that ‘more sulphuric acid is produced than any other chemical in the world and the largest single user is the fertiliser industry’. Considerable variation in ‘net emissions’ is seen according to method used and efficiency of plant-in some cases the heat generated in production of sulphuric acid is captured.

These figures must be interpreted cautiously as there is a large amount of variation in reports. However they point to the greenhouse gas savings that result from an increased use of legume fixed nitrogen. They also demonstrate the value of approaches to reduce fertiliser inputs by increasing the efficiency with which plants can utilise nitrogen (detailed above) and phosphorus.

Jarvis *et al* (1996) in a systems synthesis study of dairy farms found that use of white clover, especially at relatively low clover contents, was an effective approach to reducing nitrogenous losses. 66% of the support energy for grassland management on a dairy farm came from fertiliser production and that this could be more than halved by the use of white clover. However, there was a cost to production and losses per livestock unit did not differ markedly from those under some alternative management systems. This points to the need for maintaining white clover (or other forage legumes) productivity and persistence in mixed swards and this has been a long term objective of many breeding programmes (reviewed in Abberton and Marshall, 2005)

It is important that the improvements brought about to individual forage species are seen within the context of the whole system at farm and catchment level and in terms of the balance between different outcomes e.g. production, reduced pollution to water and lower emissions to air. To this end the use of modelling approaches is likely to be extremely valuable. Modelling studies can consider the impact of dietary strategies and take into account the full range of economic and environmental attributes important for sustainability. There is a need to consider the whole life cycle e.g. although pelleting of alfalfa may reduce methane emissions it may not be beneficial overall because of the energy costs associated with pelleting (Hironaka *et al* 1996). Life cycle analysis is an emerging and increasingly important tool in the development of sustainable solutions to the delivery of multifunctional agriculture. It is likely that breeding approaches will prove both carbon and cost effective but this needs to be rigorously established and compared with other potential approaches. However, many ‘alternative’ strategies based on management change or animal selection may well prove to be complementary to plant genetic improvement.

Future Possibilities and Needs

The potential for climate change mitigation through the genetic improvement of grassland species remains largely unexplored. However, there are strong *a priori* grounds for believing that this may be a valuable approach, based on success with agronomic and quality traits and some experimental support for this, as detailed above.

Over the last decade, considerable progress has been made in the genomics of model species i.e. those that are, due to small genome size, rapid generation times and relatively simple genome organisation, particularly tractable to molecular genetics approaches including genetic and genomic analysis and sequencing. The original model was *Arabidopsis thaliana* a small dicotyledonous weed, which has now been fully sequenced and which has contributed greatly to our understanding of the genetic control of fundamental processes in plants. More recently, models species have been developed in the major families relevant to grassland agriculture. In the grasses, rice has become a model species as well as a major crop and in the legume family two species have been developed as models, *Lotus japonicus* and *Medicago truncatula*. The latter is in the same genus as alfalfa (*Medicago sativa*) and closely related to the clovers, white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense*). The potential for translating and exploiting the understanding, tools and resources developed in the models to crop species is currently being explored but is likely to be considerable. At the same time significant progress is being made in developing molecular approaches in some of the crop species themselves (Pollock *et al* 2005). This is particularly the case for the temperate grasses in the *Lolium* (ryegrasses) and *Festuca* (fescues).genera, alfalfa and the clovers. Some of the key resources including genetic maps, bacterial artificial chromosome libraries and databases of expressed sequence tags are in place for many of these species. Conservation of gene order or synteny is a powerful tool in transferring information from models to crops and this has been shown to be extensive both within grasses and legumes (e.g. Armstead *et al* 2004). The ability to use genomic insights and to apply molecular approaches to assist plant breeding programmes increases the likelihood of successfully developing new varieties of important forages so as to reduce greenhouse gas emissions and enhance carbon sequestration.

Approaches based on plant genetic improvement have the potential to underpin options for reduction together with other approaches e.g. management, animal selection. They can also bring increased understanding: for instance the use of genetic variation to ‘perturb’ systems and bring greater understanding of processes e.g. in the rumen. Breeding approaches also have the potential to address multi-functionality and trade offs e.g. maintaining productivity and quality whilst reducing inputs.

The key needs for the future are translation from model species, integration of molecular approaches into breeding programmes and collaborations between soil scientists, animal scientists, modellers and plant breeders to seek integrated solutions to the challenge of maintaining economically viable livestock production with a reduced environmental footprint.

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