



HAL
open science

Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review

Erik Jensen, Mark Peoples, Robert Boddey, Peter Gresshoff,
Hauggaard-Nielsen, Alves, Malcolm Morrison

► To cite this version:

Erik Jensen, Mark Peoples, Robert Boddey, Peter Gresshoff, Hauggaard-Nielsen, et al.. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agronomy for Sustainable Development*, 2012, 32 (2), pp.329-364. 10.1007/s13593-011-0056-7. hal-00930531

HAL Id: hal-00930531

<https://hal.science/hal-00930531>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review

Erik Steen Jensen · Mark B. Peoples · Robert M. Boddey · Peter M. Gresshoff · Henrik Hauggaard-Nielsen · Bruno J.R. Alves · Malcolm J. Morrison

Accepted: 8 September 2011 / Published online: 19 October 2011
© INRA and Springer-Verlag, France 2011

Abstract Humans are currently confronted by many global challenges. These include achieving food security for a rapidly expanding population, lowering the risk of climate change by reducing the net release of greenhouse gases into the atmosphere due to human activity, and meeting the increasing demand for energy in the face of dwindling reserves of fossil energy and uncertainties about future reliability of supply. Legumes deliver several important services to societies. They provide important sources of oil, fiber, and protein-rich food and feed while supplying nitrogen

(N) to agro-ecosystems via their unique ability to fix atmospheric N₂ in symbiosis with the soil bacteria rhizobia, increasing soil carbon content, and stimulating the productivity of the crops that follow. However, the role of legumes has rarely been considered in the context of their potential to contribute to the mitigation of climate change by reducing fossil fuel use or by providing feedstock for the emerging biobased economies where fossil sources of energy and industrial raw materials are replaced in part by sustainable and renewable biomass resources. The aim of this review was to collate the current knowledge regarding the capacity of legumes to (1) lower the emissions of the key greenhouse gases carbon dioxide (CO₂) and nitrous oxide (N₂O) compared to N-fertilized systems, (2) reduce the fossil energy used in the production of food and forage, (3) contribute to the sequestration of carbon (C) in soils, and (4) provide a viable source of biomass for the generation of biofuels and other materials in future biorefinery concepts. We estimated that globally between 350 and 500 Tg CO₂ could be emitted as a result of the 33 to 46 Tg N that is biologically fixed by agricultural legumes each year. This compares to around 300 Tg CO₂ released annually from the manufacture of 100 Tg fertilizer N. The main difference is that the CO₂ respired from the nodulated roots of N₂-fixing legumes originated from photosynthesis and will not represent a net contribution to atmospheric concentrations of CO₂, whereas the CO₂ generated during the synthesis of N fertilizer was derived from fossil fuels. Experimental measures of total N₂O fluxes from legumes and N-fertilized systems were found to vary enormously (0.03–7.09 and 0.09–18.16 kg N₂O–N ha⁻¹, respectively). This reflected the data being collated from a diverse range of studies using different rates of N inputs, as well as the large number of climatic, soil, and management variables known to influence denitrification and the portion of

E. S. Jensen (✉)
Department of Agrosystems,
Swedish University of Agricultural Sciences,
P.O. Box 104, 230 53 Alnarp, Sweden
e-mail: Erik.Steen.Jensen@SLU.SE

M. B. Peoples
CSIRO Sustainable Agriculture Flagship, CSIRO Plant Industry,
G.P.O. Box 1600, Canberra, ACT 2601, Australia

R. M. Boddey · B. J.R. Alves
Embrapa Agrobiologia,
Caixa Postal 75.505, 23890-000 Rio de Janeiro, Brazil

P. M. Gresshoff
Center for Integrative Legume Research,
University of Queensland,
St Lucia, QLD 4072, Australia

H. Hauggaard-Nielsen
Biosystems Division,
Risø DTU National Laboratory for Sustainable Energy,
4000 Roskilde, Denmark

M. J. Morrison
Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed
Research Centre, Central Experimental Farm,
Ottawa, Ontario, Canada K2E 6A8

the total N lost as N_2O . Averages across 71 site-years of data, soils under legumes emitted a total of $1.29 \text{ kg } N_2O-N \text{ ha}^{-1}$ during a growing season. This compared to a mean of $3.22 \text{ kg } N_2O-N \text{ ha}^{-1}$ from 67 site-years of N-fertilized crops and pastures, and $1.20 \text{ kg } N_2O-N \text{ ha}^{-1}$ from 33 site-years of data collected from unplanted soils or unfertilized non-legumes. It was concluded that there was little evidence that biological N_2 fixation substantially contributed to total N_2O emissions, and that losses of N_2O from legume soil were generally lower than N-fertilized systems, especially when commercial rates of N fertilizer were applied. Elevated rates of N_2O losses can occur following the termination of legume-based pastures, or where legumes had been green- or brown-manured and there was a rapid build-up of high concentrations of nitrate in soil. Legume crops and legume-based pastures use 35% to 60% less fossil energy than N-fertilized cereals or grasslands, and the inclusion of legumes in cropping sequences reduced the average annual energy usage over a rotation by 12% to 34%. The reduced energy use was primarily due to the removal of the need to apply N fertilizer and the subsequently lower N fertilizer requirements for crops grown following legumes. Life cycle energy balances of legume-based rotations were also assisted by a lower use of agrichemicals for crop protection as diversification of cropping sequences reduce the incidence of cereal pathogens and pests and assisted weed control, although it was noted that differences in fossil energy use between legumes and N-fertilized systems were greatly diminished if energy use was expressed per unit of biomass or grain produced. For a change in land use to result in a net increase C sequestration in soil, the inputs of C remaining in plant residues need to exceed the CO_2 respired by soil microbes during the decomposition of plant residues or soil organic C, and the C lost through wind or water erosion. The net N-balance of the system was a key driver of changes in soil C stocks in many environments, and data collected from pasture, cropping, and agroforestry systems all indicated that legumes played a pivotal role in providing the additional organic N required to encourage the accumulation of soil C at rates greater than can be achieved by cereals or grasses even when they were supplied with N fertilizer. Legumes contain a range of compounds, which could be refined to produce raw industrial materials currently manufactured from petroleum-based sources, pharmaceuticals, surfactants, or food additives as valuable by-products if legume biomass was to be used to generate biodiesel, bioethanol, biojet A1 fuel, or biogas. The attraction of using leguminous material feedstock is that they do not need the inputs of N fertilizer that would otherwise be necessary to support the production of high grain yields or large amounts of plant biomass since it is the high fossil energy use in the synthesis, transport, and application of N fertilizers that often negates much of the net C benefits of many other bioenergy

sources. The use of legume biomass for biorefineries needs careful thought as there will be significant trade-offs with the current role of legumes in contributing to the organic fertility of soils. Agricultural systems will require novel management and plant breeding solutions to provide the range of options that will be required to mitigate climate change. Given their array of ecosystem services and their ability to reduce greenhouse gas emissions, lower the use of fossil energy, accelerate rates of C sequestration in soil, and provide a valuable source of feedstock for biorefineries, legumes should be considered as important components in the development of future agroecosystems.

Keywords Legumes · Biological N_2 fixation · Carbon sequestration · Greenhouse gases · Biorefinery · Biofuels

Contents

1. Introduction	1
2. The potential for legumes to mitigate climate change . 3	
2.1 Mitigation of green-house gas emissions	4
2.1.1 CO_2 emissions arising from N-fertilizer production and symbiotic N_2 fixation.	4
2.1.2 N_2O emissions.	5
2.1.2.1 N_2O emissions from legume and N-fertilized systems.	6
2.1.2.2 N_2O emissions derived from legume residues.	10
2.2 Comparisons of energy use by legume-based and N-fertilized systems.	12
2.3 Soil carbon sequestration and land use change. . . 13	
2.3.1 Legume effects on soil carbon sequestration.	14
2.3.2 Pastures.	15
2.3.3 Cropping sequences.	16
2.3.4 Woody perennial legumes.	18
3. A role for legumes to replace fossil resources?.19	
3.1 Legume biomass yields potentials and constituents	20
3.2 Legumes as biorefinery feedstocks for biofuels, materials, and chemicals.	22
3.2.1 Biofuels.	24
3.2.1.1 Bioethanol.	24
3.2.1.2 Biodiesel.	25
3.2.2 Biogas and digestate for fertilizer.	27
3.2.3 Thermochemical conversion for production of heat, electricity, and biochar.	28
3.2.4 Materials and chemicals.	29
4. Concluding remarks.	29
5. Acknowledgments.	30
6. References.	30

1 Introduction

Biomass from agriculture, forestry, and marine environments is expected to play an important role in replacing scarce energy sources in the transition from a fossil economy to a biobased economy (IEA 2009; Bessou et al. 2010; Fairley 2011). A biobased economy is defined as the replacement of fossil fuels in the production of industrial chemicals, transportation fuels, electricity, heat, and other products by biomass in so-called biorefinery concepts. The political and scientific arguments for this transition are multiple: diversification of energy sources due to declining fossil reserves (energy security), less dependence on fossil raw material exporters (energy security), new uses of biomass to stimulate rural development, and the reduction of greenhouse gases (GHG) to mitigate global climate change (Bessou et al. 2010; Langeveld and Sanders 2010).

Climate change is brought about by the increasing atmospheric content of a range of gases such as carbon dioxide (CO₂), nitrous oxide (N₂O), methane (CH₄), ozone (O₃), and chlorofluorocarbons (CFCs). These greenhouse gases (GHG) are all increasing as a result of human activity (e.g., Table 1; Blasing 2010). As the GHG concentration increases, more sunlight is absorbed and the energy converted to heat. At the current rate of GHG production, the average surface temperatures of the planet are predicted to rise by 2°C or more by 2100 (IPCC 2007). Such a warming could impact terrestrial ecosystems either positively or negatively depending on current regional climatic conditions.

Rising concentrations of CO₂ are the main concern since CO₂ emissions from the combustion of fossil fuels account for >50% of the estimated increased greenhouse effect (IPCC 2007). The agricultural contribution to the global GHG emission has been estimated to be 13.5% of the total GHG CO₂-equivalents (IPCC 2007) and is derived from (1) the use of fossil energy for the manufacture and transport of fertilizer nitrogen (N), other fertilizers and agrichemicals, and the consumption of petroleum-based fuels for on-farm machinery operation; (2) changes in land-use that release GHG due to the net decomposition of soil organic matter, or when the carbon (C) in the wood is converted to CO₂ by burning when land is deforested for cropping or livestock; (3) the release of N₂O from soil as a result of inefficiencies in crop recovery of fertilizer and other sources of N; and (4) CH₄ released from the enteric digestion of forage within the rumen of livestock, on-farm manure management, and rice (*Oryza sativa*) cultivation on wetlands (Jenkinson 2001; Crews and Peoples 2005; Bessou et al. 2010). To mitigate climate change from agriculture, it is important to adopt strategies that reduce these sources of GHG emissions.

Leguminous crops (e.g., field pea, *Pisum sativum*; faba bean, *Vicia faba*; chickpea, *Cicer arietinum*; soybean,

Glycine max), forages [e.g., clovers, *Trifolium* spp.; alfalfa (lucerne), *Medicago sativa*], trees, and shrubs (e.g., species of *Leucaena*, *Callinadra*, *Gliricidia*, *Acacia*, and *Sesbania*) provide a range of agroecosystems services for humans. These include (1) N (protein)-rich foods, feeds, and green-manures; (2) a lowering of the need for fertilizer N to support crop and pasture production as the result of contributions of symbiotically fixed dinitrogen (N₂) to the growth of the legume host, and the subsequent improvement of soil fertility through inputs of legume organic residues (Rochester et al. 2001; Jensen and Hauggaard-Nielsen 2003; Crews and Peoples 2004); (3) improvements in soil structural characteristics (Rochester et al. 2001; McCallum et al. 2004); (4) direct impacts on soil biology by reducing the incidence of cereal root pathogens, and/or encouraging beneficial microorganisms (Kirkegaard et al. 2008; Osborne et al. 2010); (5) diversification of species grown in rotations reducing the requirement for pesticides and other agrichemicals, encouraging systems resilience and biodiversity (Jensen and Hauggaard-Nielsen 2003; Köpke and Nemecek 2010); (6) deep-rooted perennial legumes reducing the risk of groundwater contamination by nitrate (NO₃⁻), or the development of dryland salinity, due to their ability to grow and extract water all year round (Angus et al. 2001; Entz et al. 2001; Lefroy et al. 2001); and (7) the revegetation and reclamation of degraded or cleared lands (Thrall et al. 2005; Chaer et al. 2011; De Faria et al. 2011). Even though legumes obtain N through biological nitrogen fixation (BNF), rather than through fossil energy-derived fertilizer N, they are generally not considered as a mitigation option (Smith et al. 2007). With the exception of soybean, legumes are also usually not regarded as particularly relevant as biomass crops or as crop components as feedstock in biorefinery for biofuel and/or biomaterials production (Venendaal et al. 1997; Brehmer et al. 2008; Bessou et al. 2010).

This paper reviews the potential new roles for the use of legumes in future agriculture to (1) reduce the emissions of the key GHG CO₂ and N₂O; (2) lower fossil energy consumption during the production of food, forage, and fiber; (3) increase the sequestering of organic C in soils; and (4) provide an energy-efficient biomass source for biorefineries to produce biofuels, chemicals, and materials to replace fossil-resource-derived products.

2 The potential for legumes to mitigate climate change

2.1 Mitigation of greenhouse gas emissions

As GHG concentrations rise, it has become increasingly important to account for losses of CO₂ and N₂O arising from agriculture (Table 1). Emissions of these gases may

Table 1 The Global Warming Potential (GWP) of some major greenhouse gases and historic trends in changes in their atmospheric concentrations

Gas	GWP ^a (100 year)	Year and surface air concentration (ppm on a volume basis)				
		1800	1900	1950	1995	2008
CO ₂	1	280	297	311	361	385
CH ₄	21	0.80	0.87	1.15	1.73	1.80
N ₂ O	298	0.28	0.28	0.29	0.31	0.32

Collated from data presented by IPCC (2007); Lægread et al. (1999); Blasing (2010)

^a The GWP provides a simple measure of the radiative effects of emissions of various greenhouse gases integrated over a 100-year time horizon, relative to an equal mass of CO₂ emissions

occur either *directly* as the result of farming activities (e.g., cultivation and harvesting) or *indirectly* during the production and transport of required inputs (e.g., fertilizers, herbicides, and pesticides). The potential role of N₂-fixing legumes in reducing GHG emissions through direct effects on CO₂ and N₂O fluxes in the production of high-protein grain and forage will be compared to the applications of fertilizer N in the following sections.

2.1.1 CO₂ emissions arising from N fertilizer production and symbiotic N₂ fixation

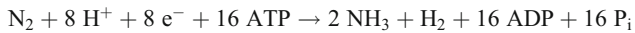
A century after its invention, the Haber–Bosch process of ammonia (NH₃) production essentially remains unchanged. Ammonia is synthesized from a 3:1 volume mixture of H₂ and N₂ at elevated temperature and pressure in the presence of an iron catalyst (Smil 2001). All the N₂ used is obtained from the air and the H₂ can be obtained by either (a) partial oxidation of heavy fuel oil or coal, or (b) steam reforming of natural gas or other light hydrocarbons (natural gas liquids, liquefied petroleum gas, or naphtha; Smil 2001; Crews and Peoples 2004). It has been estimated that the fossil energy requirements associated with providing the high temperature and pressures and the generation of H₂ feedstock required for the synthesis of N fertilizer represents 1–2% of the total world energy consumption (Smil 2001; Jenkinson 2001). It has also been calculated that the varying efficiencies of different processing plants result in the release of between 0.7 and 1.0 kg of CO₂-C (equivalent to 2.6–3.7 kg CO₂ gas) per kilogram of NH₃-N produced (Jenkinson 2001; Jensen and Hauggaard-Nielsen 2003). About half of the CO₂ generated during NH₃ production will be reused if the NH₃ is converted to urea, which is the most widely used form of N fertilizer applied to agroecosystems (67% of total fertilizer N consumed in 2007; IFA 2010). However, once the urea is applied to the soil, it is rapidly hydrolyzed by the enzyme urease to NH₃ and the CO₂ originally captured during urea production will also be released (Jenkinson 2001). Consequently, the annual global fertilizer production of around 100 Tg N (1 Tg=10¹² g; IFA

2010) manufactured with an efficiency of 2.6–3.7 kg CO₂ generated per kilogram of N synthesized represents around 300 Tg of CO₂ being released into the atmosphere each year.

There are nearly 18,000 legume species, many of major agricultural importance. Legumes range from herbaceous annuals plants to gigantic trees (e.g., Moreton Bay chestnut, *Castanospermum australe*). Many legumes possess the ability to form nitrogen-fixing symbioses with soil bacteria broadly called “rhizobia” (see Ferguson et al. 2010 for an up-to-date review). The symbiosis is initiated through an exchange of chemical signals; specifically legume roots secrete not only sugars but also flavones and isoflavones. These exist as “chemical cocktails” of “rhizobial” gene activators and repressors, representing part of host specificity. For example, a bacterium that normally induces nodules in white clover will not nodulate or fix nitrogen with soybean, and vice versa. The flavone signal also works as a chemo-attractant to “rhizobia” which then attach to root hairs in the susceptible zone right behind the growing root tip region [there are some exceptions to root hair nodulation process—for example groundnut (*Arachis hypogae*) where rhizobia rely upon entry through root cracks]. Here, they activate bacterial genes (*nod* and *nol* genes) that cooperate to synthesize and secrete a nodulation (Nod) factor. Nod factor perception leads to two interrelated processes, namely root hair/root cortex infection, and cortical and pericycle cell divisions. The combined meristems form the nascent root nodule, well-plumbed with a bifurcated vascular system, designed to provide photosynthate (usually as sucrose-derived malate; Udvardi et al. 1988) and to transport the products of symbiotic N₂ fixation back to the plant. The young cells inside the emerging nodule become invaded by the “rhizobia”, which now differentiate into N₂-fixing bacteroids. Bacterial N₂ fixation genes express the components of the nitrogenase enzyme complex (NifH, NifD, and NifK), that together with critical genes for regulation, iron and molybdenum supply, electron transport facilitate the conversion of atmospheric N₂ into NH₃ (ammonia) which in turn is assimilated within the nodule cell cytoplasm to glutamine. Glutamine in turn serves as the N donor for the subsequent

synthesis of a complex set of amino acid and or N transport compounds (such as ureides in soybean, or glutamine and asparagine in temperate legumes; Peoples and Herridge 1990). Nodule development is regulated internally by an “autoregulation of nodulation” (AON) circuit (cf., Gresshoff et al. 2009; Reid et al. 2011) and externally by stress as well as nitrate (Carroll et al. 1985; Ferguson et al. 2010).

Overall the nitrogenase reaction associated with N₂ fixation in the nodule is as follows:



One notes the large energy demand in electrons and ATP. This occurs through the action of oxidative phosphorylation under microaerobic conditions, requiring the red heme protein leghemoglobin to act as a limiting oxygen carrier to the N₂-fixing and rapidly respiring bacteroids. As a result, the process of N₂ fixation in legume nodules is generally considered to have much higher energy and C requirements for N assimilation than plants dependent upon NO₃⁻ reduction for growth (Fig. 1; Atkins 1984; Kaschuk et al. 2009). However, conclusions about what this means with respect to CO₂ losses from BNF are not so straightforward. Some experiments comparing below-ground losses of CO₂ from the root systems of N₂-fixing legumes with NO₃⁻-fed legumes have found similar respiratory losses (e.g., 22–23 g CO₂ per gram of N assimilated; Minchin and Pate 1973), while other studies have reported that between 10 g (Pate et al. 1979) and 20 g more CO₂ to be respired per gram of N accumulated by nodulated legumes than by plants dependent upon NO₃⁻ for growth (Minchin et al. 1980; Finke et al. 1982). This range of estimates of additional respiratory losses due to BNF reflects key differences between species in the main site of NO₃⁻ reduction. The smallest differences between the C costs of N₂ fixation and NO₃⁻ uptake and reduction by N-fertilized plants occurred where much of the incoming NO₃⁻ was reduced in roots (field pea and white lupin, *Lupinus albus*) rather than in leaves (soybean and cowpea, *Vigna unguiculata*; Atkins 1984).



Fig. 1 Nodules on alfalfa root fixing atmospheric nitrogen. Photo: T. Råberg, SLU

Current global estimates of annual amounts of N₂ fixed by agricultural legumes range between 33 and 46 Tg N (Herridge et al. 2008). Assuming that the N₂ fixation process respire on average 10 g more CO₂ from a legume’s nodulated root system for every gram of N assimilated than plants utilizing fertilizer or soil N for growth, then between 350 and 500 Tg of additional CO₂ might be respired from the nodulated roots of legumes each year as a direct result of BNF.

Conclusions Globally, the amount of CO₂ respired from the root systems of N₂-fixing legumes could be comparable to, or higher than, the CO₂ generated during N-fertilizer production. However, the CO₂ respired from the nodulated roots of legumes originated from the atmosphere via photosynthesis, so any of the CO₂ that was not subsequently recaptured by the plant and eventually escaped from the legume canopy to the atmosphere would essentially be C neutral. By contrast, all the CO₂ released during the synthesis of fertilizer N would be derived from fossil energy and represents a net contribution to atmospheric concentrations of CO₂.

2.1.2 N₂O emissions

About 5% of the total atmospheric greenhouse effect is attributed to N₂O of which 60% to 70% of the annual global anthropogenic emissions have been calculated to come from animal and crop production (Mosier 2001; IPCC 2007). While N₂O can be generated in the process of nitrification where nitrite is converted to NO₃⁻, N₂O losses as the result of denitrification are generally considered to be the more important source in most cropping and pasture systems (Rochester 2003; Peoples et al. 2004b; Soussana et al. 2010). Denitrification occurs when the soil is very moist and O₂ supply is restricted, a suitable mineralizable organic C is present to be used as an energy source by denitrifying microbes, and there are high concentrations of NO₃⁻ (Peoples et al. 2004b; Stehfest and Bouwman 2006). Many species of soil bacteria are able to survive in anoxic conditions by using the denitrification process. Essentially, NO₃⁻ is substituted for O₂ as a respiratory electron acceptor; the NO₃⁻ is reduced to nitrite and in sequence to N₂O and N₂. The bulk of the gaseous losses will be as N₂, but the small proportion of the total emissions in the form of N₂O (i.e., the ratio of N₂O/N₂) can be affected by many different variables such as N application rate, soil organic C content, soil pH, and texture (Rochester 2003; Stehfest and Bouwman 2006; Peoples et al. 2009b). This illustrates the potential difficulty in reliably measuring or predicting specific losses of N₂O from what is essentially a very complex, transient, and variable process.

The IPCC (2006) suggested that for every 100 kg of fertilizer N added to the soil, on average 1.0 kg of N can be expected to be emitted as N₂O. As a GHG, N₂O absorbs approximately 300 times as much infra-red radiation per kilogram as CO₂ (Table 1), and since an emission of 1.0 kg N₂O–N equates to 1.57 kg N₂O gas, the impact of every kilogram of N₂O–N released would be equivalent to around 470 kg CO₂. In addition to this amount, the IPCC includes further sources of N₂O as 1.0% of the N deposited on the soil surface as residues (IPCC 2006). For many years, the IPCC reference manual (IPCC 1996) and the good practice guide for inventories (IPCC 2000) (erroneously) considered 1.25 kg of N₂O–N to be emitted for every 100 kg of biologically fixed N₂. In other words, if BNF by legumes was responsible for emissions from the soil (rhizosphere) or from the nodules of this magnitude during growth, with subsequent additional

losses when organic N in above-ground and below-ground legumes residues were mineralized, then legumes would be no more favorable than N fertilizer in terms of GHG emissions. Although the recent IPCC publications no longer includes BNF as a source of N₂O (IPCC 2006), some countries around the world continue to utilize the former recommendations. The following sections review N₂O emissions both during a legume's growth cycle, and subsequently from legume residues, and compare the magnitude of these N₂O losses with fertilized systems.

2.1.3 N₂O emissions from legume and N-fertilized systems

The range of field-based measures of N₂O emissions detected in different legume and N-fertilized cropping and pasture systems are exceptionally wide (Table 2; see also

Table 2 Examples of total N₂O emissions from field-grown legumes, N fertilized grass pastures and crops, or un-fertilized soils in North and South America, Europe, South Asia, East Asia, Australia, and New Zealand

Category and species	Number of site-years	Total N ₂ O emission per growing season or year (kg N ₂ O–N ha ⁻¹)	
		Range	Mean
Pure legume stands ^a			
Alfalfa	14	0.67–4.57	1.99
White clover	3	0.50–0.90	0.79
Mixed pasture sward ^a			
Grass–clover	8	0.10–1.30	0.54
Legume crops ^a			
Faba bean	1	–	0.41
Lupin	1	–	0.05
Chickpea	5	0.03–0.16	0.06
Field pea	6	0.38–1.73	0.65
Soybean	33	0.29–7.09	1.58
<i>Mean of all legumes</i>	<i>71</i>		<i>1.29</i>
N-fertilized pasture ^b			
Grass	19	0.3–18.16	4.49
N-fertilized crops ^b			
Wheat	18	0.09–8.57	2.73
Maize	22	0.16–12.67	2.72
Canola	8	0.13–8.60	2.65
<i>Mean of fertilized systems</i>	<i>67</i>		<i>3.22</i>
Soil ^c			
No N fertilizer or legume	33	0.03–4.80	1.20

Collated from the data presented by Ruz-Jerez et al. (1994); Bouwman (1996); Wagner-Riddle et al. (1997); Hénault et al. (1998); Kamp et al. (1998); Mahmood et al. (1998); Teira-Esmatges et al. (1998); Goossens et al. (2001); Aulakh et al. (2001); Rochette and Janzen (2005); Parkin and Kaspar (2006); Jones et al. (2007); Barton et al. (2008); Ciampitti et al. (2008); Chen et al. (2008); Barton et al. (2010); Schwenke et al. (2010); Barton et al. (2011), and includes unpublished data of Morrison et al. (unpublished data)

^a Data come from systems where either no N fertilizer was used, or legumes were supplied with just 5 kg fertilizer-N ha⁻¹ as “starter N” at sowing, except for two experiments with grass–clover pastures and three soybean studies where 35–44 kg fertilizer-N ha⁻¹ had been applied

^b Data have been restricted to trials where only N fertilizer was used. Treatments that included applications of animal manures have been excluded. Emissions from grasslands include both grazed and mown systems

^c Includes data from either unplanted soils or non-legume species where no N fertilizer was applied

reviews by Bouwman 1996; Rochette and Janzen 2005). This is consistent with the large number of climatic, soil, and management variables known to influence the denitrification rate, and the amount of N_2O emitted (Rochester 2003; Jones et al. 2007; Peoples et al. 2009b), and reflects the data having been derived from many unrelated experiments undertaken in a diverse range of farming systems from different environments and soil types around the world receiving vastly different amounts of N. While it is clear from Table 2 that sizeable losses of N_2O can occur from soil from under legume-based pastures and crops, the origin of this N_2O cannot always be conclusively identified since many experiments do not include controls to quantify background soil emissions. Where appropriate controls have been included within the experimental design, the losses of N_2O from soil have commonly not differed significantly from the legume treatment (e.g., Kilian and Werner 1996; Rochette et al. 2004; Ciampitti et al. 2008; Barton et al. 2011). This general observation is also supported when the measures of total N_2O emissions from legume systems are averaged across all experiments and legume species ($1.29 \text{ kg N}_2\text{O-N ha}^{-1}$, calculated from 71 site-years of data; Table 2) are compared to the equivalent mean data for unfertilized non-legume crops or un-planted soil (mean of $1.20 \text{ kg N}_2\text{O-N ha}^{-1}$, calculated from 33 site-years of data; Table 2).

When considered in its entirety, the data of Table 2 suggest that while total N_2O emissions during legume growth (Table 2) can be similar to N-fertilized systems, more often the N_2O losses from soils under legumes tend to be lower than N_2O fluxes from fertilized grasslands and non-legume crops (mean of $3.22 \text{ kg N}_2\text{O-N ha}^{-1}$, calculated from 67 site-years of data; Table 2). This is illustrated in the experimental data presented in Table 3. The Brazilian study in Table 3 showed that the N_2O emissions from a soybean–vetch (*Vicia* spp.) sequence were similar to N_2O

fluxes measured from cereal-only systems receiving relatively low rates of N fertilizer ($45\text{--}60 \text{ kg N ha}^{-1}$) over two consecutive years of experimentation. The USA example provided in Table 3 on the other hand indicated that N_2O emissions were considerably lower from soybean than from soil under the N-fertilized maize crop in both years of the trial where more commercial rates of N fertilizer (215 kg N ha^{-1}) had been supplied. Large peaks in N_2O fluxes also seem to be a feature of N-fertilized pastures, particularly immediately following applications of N fertilizers (Jones et al. 2007; Soussana et al. 2010; Klumpp et al. 2011). As a consequence, total N_2O losses from heavily N-fertilized grasslands can be up to 4-fold higher than measured from unfertilized legume–grass pastures included in the same experiment (Ruz-Jerez et al. 1994).

The original assertion that BNF could be an important source of N_2O emissions during legume growth seems to be based on reviews of the literature by Bouwman (1996) which included the results of studies undertaken in the 1980s where relatively high emissions had been recorded with legumes. There had also been some supporting experimental evidence that the process of BNF could give rise to N_2O since strains of different species of rhizobia had been demonstrated to possess nitrate and nitrite reductase and were capable of denitrifying NO_3^- to N_2O *in vitro* in the free-living form, in legume root nodules, or as isolated bacteroids (e.g., O'Hara and Daniel 1985; van Berkum and Keyser 1985; Smith and Smith 1986). However, subsequent studies examining different strains and species of rhizobia have indicated that the capacity of rhizobia to produce large amounts of N_2O in anaerobic liquid medium amended with NO_3^- is very variable, and many strains produce only trace quantities under the same conditions (e.g., Table 4; Breitenbeck and Bremner 1989; Garcia-Plazaola et al. 1993; Rosen et al. 1996; Zhong et al. 2009).

Table 3 Measurements of N_2O emissions for different cropping sequences over two consecutive years in Brazil (Jantalia et al. 2008) and the USA (Parkin and Kaspar 2006)

Crop(s) in 2003	N fertilizer applied (kg N ha ⁻¹)	N_2O emissions (kg $\text{N}_2\text{O-N ha}^{-1}$)	Crop(s) in 2004	N fertilizer applied (kg N ha ⁻¹)	N_2O emissions (kg $\text{N}_2\text{O-N ha}^{-1}$)
Brazil					
Soybean–wheat	0 (soybean) 45 (wheat)	0.81a	Soybean–wheat	0 (soybean) 45 (wheat)	0.64a
Soybean–vetch	0	0.73a	Sorghum–wheat	60 (sorghum) 45 (wheat)	0.66a
Maize–wheat	0 (maize) 45 (wheat)	0.83a	Soybean–vetch	0	0.68a
USA					
Soybean	44	2.4b	Maize	215	12.7a
Maize	215	8.6a	Soybean	44	7.1b

Data have been averaged over several tillage systems. For each experiment and year, values followed by the same letter are not significantly different ($P < 0.05$)

Table 4 Nitrous oxide fluxes normalized by the optical density (o.d.) of growth medium of several *Bradyrhizobium* spp. strains recommended for various grain and forage legumes (Alves et al., unpublished data)

<i>Bradyrhizobium</i> strains	Legume host species	Optical density	N ₂ O flux (μmol N ₂ O h ⁻¹ o.d. ⁻¹)
BR 446	<i>Stylosanthes</i> sp.	0.87	1.13
BR 2003/2811 (mixture)	<i>Crotalaria</i> sp.	0.72	0.002
BR 2407	<i>Dolichos lablab</i>	0.69	0.001
BR 85 (CPAC 7)	<i>Glycine max</i>	0.86	0.02
BR 86 (CPAC 15)	<i>Glycine max</i>	0.98	0.49
BR 1435	<i>Arachis</i> sp.	0.59	0.42

The results of several controlled-environment studies with soybean have also raised considerable doubt about the relative importance of contributions of N₂O fluxes from the process of N₂ fixation in legume systems. In one study N₂O emissions from N₂-fixing soybean was found to be low and statistically similar to a non-nodulating soybean line and unplanted pots of soil even though large quantities of N were fixed (Table 5). A second key study showed that >90% of the total growing season emissions of N₂O occurred between grain-filling and maturity, and it was proposed that this N₂O was more likely to be the result of N released from the decaying nodules and roots in the rhizosphere than to have arisen from BNF (Yang and Cai 2005).

Very similar results were observed in soybean field experiments in both Canada (Fig. 2) and Argentina (Ciampitti et al. 2008). No significant differences in N₂O emissions were detected in the Canadian investigation between the N₂-fixing and non-fixing soybean isolines during seed-filling when rates of BNF would generally be expected to be highest (Zapata et al. 1987; Bergersen et al. 1989). Differences were only observed in the final stages of seed maturation and following grain harvest (Fig. 2). Almost 70% of the total N₂O lost during soybean's growing season occurred in the period between grain-filling and grain maturity in both studies (Fig. 2; Ciampitti et al. 2008). In the Argentinean experiment, the peak in N₂O emissions from the soybean plots also corresponded with a spike in N₂O fluxes from the unplanted soil control plots implying that background soil factors were largely responsible for the

generation of N₂O, not the presence of the legume (Ciampitti et al. 2008).

Further evidence that N₂O emissions are unlikely to be directly linked to BNF comes from investigations by Jantalia et al. (2008) in Brazil where N₂O fluxes were monitored in different double-cropping systems (i.e., one summer crop and one winter crop grown in each year) over two consecutive years (Table 3). In that study, the soybean in the soybean–wheat sequence fixed between 100 and 200 kg N ha⁻¹ in above-ground biomass, while in neighboring plots, the soybean–vetch sequence, the total amounts of N fixed by both legume crops represented 165 to 280 kg N ha⁻¹ (Jantalia et al. 2008). Yet despite the large inputs of fixed N by the legumes, their measured emissions of N₂O were not significantly different from the N₂O fluxes coming from maize–wheat or sorghum (*Sorghum bicolor*)–wheat sequences receiving between 45 and 60 kg fertilizer N ha⁻¹ (Table 3).

Inexplicably high N₂O fluxes have sometimes been detected in legume studies (e.g., soybean in 2004, Table 3; see also alfalfa data presented by Rochette et al. 2004 and some experiments collated by Helgason et al. 2005). If these are not associated with BNF, then what is the origin of the elevated emissions of N₂O? Some of the measured fluxes of N₂O from legume fields could include the denitrification of NO₃⁻ derived from sources such as unutilized N fertilizer from the preceding crop, and/or from the decomposition of the previous crops residues and other background soil sources. But since denitrification and the proportion of the denitrified N released as N₂O tends to be

Table 5 Soybean shoot dry matter (DM) and N accumulation, and cumulative N₂O emissions from soil over 64 days

Treatment	Shoot DM (g plant ⁻¹)	Shoot N (g N plant ⁻¹)	N ₂ O emission (mg N pot ⁻¹)
Bare soil	NA	NA	0.54a
Soybean cv Conquista	26.1a	0.60a	0.52a
Non-nod soybean cv T-201	2.25b	0.03b	0.67a

Pots containing 10 kg of Rhodic Ferralsol were either sown to a nodulating cultivar of soybean (*Glycine max* cv Conquista) double inoculated with two strains of *Bradyrhizobium japonicum* (SEMIA 5080 and SEMIA 5019), or to a non-nodulating soybean mutant (cv T-201). The amounts of N₂ fixed by Conquista soybean were estimated to be 0.57 g N plant⁻¹ by comparing the amounts of shoot N accumulated to the non-nodulating line. A bare soil treatment was included as a control (Alves et al., unpublished data). Means in the columns followed by the same letter are not statistically different at $P < 0.05$ Tukey's test

NA not applicable

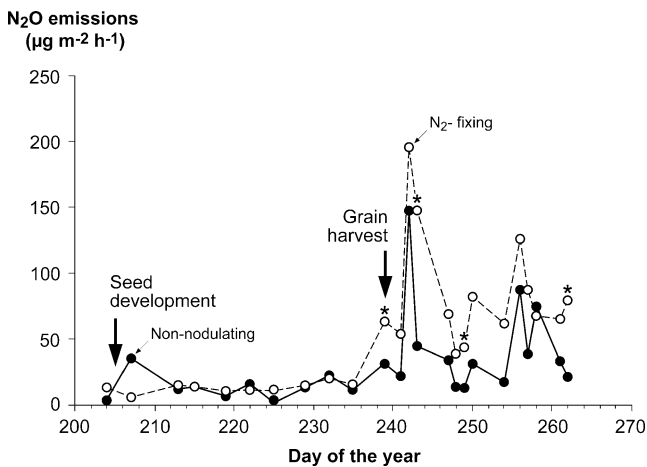


Fig. 2 Field measurements of N_2O emissions from a trial at Ottawa, Ontario, Canada comparing non-nodulating (solid circle and line) and nodulated isolines (open circle and dashed line) of the soybean cultivar Presto during reproductive development and the immediate post-harvest period (Morrison et al., unpublished data). Determinations of N_2O flux were achieved by placing chambers between the soybean rows every 3 or 4 days and sampling N_2O in the gas head space over time. Each point represents the mean of four replicates, and an asterisk (*) indicates samplings where significant ($P < 0.05$) differences in N_2O emissions between nodulating and non-nodulating isolines were recorded. The arrows indicate the commencement of seed development and grain harvest. Cumulative estimates of N_2O losses from the nodulated soybean line were calculated to be $1.2 \text{ kg } N_2O\text{-N ha}^{-1}$ prior to the completion of seed development, $2.5 \text{ kg } N_2O\text{-N ha}^{-1}$ between seed development and grain maturity, and $3.8 \text{ kg } N_2O\text{-N ha}^{-1}$ during the first 30 days after grain harvest

correlated with concentrations of soil NO_3^- (Wagner-Riddle and Thurtell 1998; Peoples et al. 2004b), denitrification of NO_3^- that is often observed to build up beneath N_2 -fixing legumes towards the end of the annual growing season (Peoples et al. 1995a; Chalk 1998) might also be a source of N_2O . Some of the inorganic N accumulating beneath legumes could represent “spared” soil NO_3^- not assimilated during legume growth due to the poor efficiency with which legume roots recover plant-available soil mineral N (Herridge et al. 1995; Chalk 1998; Jensen et al. 2010). Other

Fig. 3 Intercropping of grain legumes and cereals enhances biomass yields, improves the use of resources, due to competitive interactions, and increases the yield stability compared to sole crop grain legumes. In addition, soil mineral N levels may be lower and the C-to-N ratio of crop residues more balanced than in the sole crop grain legume. Left: pea–barley intercrop; right: faba bean–spring wheat intercrop. Photos: H. Hauggaard-Nielsen, Risø DTU



potential sources include the rhizodeposition of N via exudates and secretions from living legume roots, or the mineralization of organic legume N released during the turnover of fine roots and nodules and from senesced leaf litter lost from the legume canopy during growth (Bergersen et al. 1989; Peoples et al. 1995a; Wichern et al. 2008). Intercropping of grain legumes with cereals may reduce soil mineral N levels during autumn and winter as well as the risk of N_2O emission and leaching (Hauggaard-Nielsen et al. 2003, Fig. 3).

Shifts in the species composition of soil microbial populations and a high microbial diversity commonly detected in the legume rhizosphere (Lupwayi and Kennedy 2007; Osborne et al. 2010) could also be contributing factors to the high N_2O flux since the release of readily metabolizable substrates into the legume rhizosphere stimulates microbial growth and activity and promotes oxygen consumption. This could conceivably create temporary anaerobic microsites in soil that would favor denitrification (Bertelsen and Jensen 1992; Lemke et al. 2007). There is also some evidence to suggest that the increased populations of microbes associated with the root systems of N_2 -fixing legumes include denitrifying bacteria (Zhong et al. 2009).

Conclusions Emissions of N_2O tend to be lower under legumes than N-fertilized crops and pastures, particularly when commercially relevant rates of N fertilizer are applied. This undoubtedly reflects differences in both the relative size of the N inputs and the concentrations of soil NO_3^- available to be denitrified. There is little evidence to support a direct association between BNF and N_2O emissions from legume fields. While the source(s) of N responsible for the N_2O emitted during a legume’s growing season have not been identified, it is likely that the N_2O is derived from the denitrification of NO_3^- that often accumulates in soil either as the result of inefficient recovery of NO_3^- by legume roots or the mineralization of labile sources of legume N released from the nodulated

roots and fallen leaf material (Bertelsen and Jensen 1992; Rochette and Janzen 2005).

2.1.4 N_2O emissions derived from legume residues

The decomposition and mineralization of organic N in legume residues into inorganic forms following a legume phase is a microbial-mediated process associated with the breakdown of organic compounds being used to provide the soil microbes with a C source for respiration and growth (Fillery 2001). Much of the simple organic N released from legume residues is rapidly assimilated (immobilized) by the soil microbial population (Bremer and van Kessel 1992; Murphy et al. 1998; Peoples et al. 2009b). Inorganic N (mineral N—ammonium, NH_4^+ , and NO_3^-) only accumulates in soil if the amounts of N released from residues exceed the C-limited microbial requirement for N for growth. Since legume tissues tend to have higher N contents and lower C/N ratios than non-leguminous material, legume residues are more likely to result in net mineralization and a build-up of inorganic N in soil (Peoples and Herridge 1990; Kumar and Goh 2000). Concentrations of inorganic N in field soils are generally observed to be higher when sowing a subsequent crop in a rotation if it follows a legume crop or pasture than a cereal (Chalk 1998; Fillery 2001; Jensen and Hauggaard-Nielsen 2003). This can often be related to the amounts of legume N accumulated during a pasture phase or the amounts of crop legume N remaining in residues following grain harvest (Evans et al. 2003; Peoples et al. 2001, 2004a).

Since legume residues provide a source of easily decomposable C substrate for denitrifying microorganisms, emissions of N_2O could occur either during the process of nitrification of N derived from legume residues or as a result of the denitrification of the NO_3^- pool that subsequently builds up in the soil. In general terms, the susceptibility of N derived from legume residues to loss processes is determined by how well the release (supply) of mineralized N is synchronized with the demand for N by following crops (Crews and Peoples 2005).

The fate of legume or fertilizer N is often measured using ^{15}N -labeled materials. These studies indicate that while a much lower proportion of the N originally present in legume residues is usually taken up by a subsequent wheat, rice, or maize crop (on average 15–20%; Peoples et al. 1995a; Fillery 2001; Peoples et al. 2009b) than from fertilizer (on average 30–40%; Peoples et al. 1995a; Krupnik et al. 2004; Crews and Peoples 2005), considerably more legume N is retained in the soil system than fertilizer N (60% vs. 30%, respectively; Crews and Peoples 2005; Peoples et al. 2009b). While the extent of losses will be influenced by whether the system is rainfed or irrigated, average losses from cereals appear to be in the order of 10–20% for legume N and 30–

40% for fertilizer (Peoples et al. 2004b, 2009b). Meta-analysis of ^{15}N field experiments has shown that the extent of losses is driven by the size of the N inputs regardless of the source and has indicated that total losses of legume N tend to be less than from fertilizer when both are applied at rates of $<125 \text{ kg N ha}^{-1}$ (Gardner and Drinkwater 2009). While quite a lot may be known about total losses of legume or fertilizer N, it is more difficult to generalize about denitrification as the pathway of N loss, or more specifically about how much of the losses from above- and below-ground legume residues might be in the form of N_2O .

The “window of asynchrony” between N supply and demand, and the period of highest risk for denitrification losses, is likely to be greatest in legume-based systems when a fallow period follows a legume and/or early in the subsequent growing season because either no plants are present to capture and utilize the generated NO_3^- or the demand for N by newly sown crops is small. Emissions of N_2O were observed to rise immediately following soybean grain harvest in Canada and represented an average of $41 \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$ or a total of $3.8 \text{ kg N}_2\text{O-N ha}^{-1}$ over the 30 days of measurement (Fig. 2). Differences in N_2O flux between the nodulated and non-nodulated soybean treatments during the post-harvest period in this particular experiment were attributed to decomposition of nodule N (see also the conclusions of Rochette and Janzen 2005). Similar data collected during the post-harvest summer–autumn fallow period (November–April) between crops in Western Australia in a drier environment on the other hand showed little evidence of elevated N_2O emissions where relatively small amounts of senesced stubble from narrow-leafed lupin (*Lupinus angustifolius*) had been retained [$2.3 \text{ Mg above-ground DM ha}^{-1}$ ($1 \text{ Mg}=10^6 \text{ g}$, or 1 t), containing 26 kg N ha^{-1} , C/N ratio=45] compared to a bare soil control (0.07 and $0.04 \text{ kg N}_2\text{O-N ha}^{-1}$, respectively; Barton et al. 2011). It should be acknowledged that for any mineralization of residues or denitrification to occur in the Mediterranean-type climate (wet winters and dry summers) of Western Australia would greatly depend upon the timing and intensity of summer storms, and there may be years when N_2O emissions will be more likely during the fallow period between crops than others (Barton et al. 2008). The potential for post-harvest N_2O emissions is probably greater in the summer-dominant rainfall region of the northern cropping zone of eastern Australia. Experimentation undertaken in northern New South Wales measured losses of $0.06 \text{ kg N}_2\text{O-N ha}^{-1}$ during the first 2 months of the summer fallow period after a chickpea crop (Schwenke et al. 2010, Fig. 4), while $0.24 \text{ kg N}_2\text{O-N ha}^{-1}$ was emitted over the same period following a N-fertilized canola treatment included in the same trial. Most of the fallow emissions in this particular study occurred during a week of continual rain when the trial plots received $>120 \text{ mm}$ of rainfall (Schwenke et al. 2010).



Fig. 4 Chamber used to quantify N_2O emissions from legume residues. The lid of a chamber in one of the four replicates of each treatment automatically closes on a rotational basis each hour of the day to continually monitor changes in concentrations of N_2O in the chamber headspace. Photo: M. B. Peoples, CSIRO

Another key period of risk for N losses from legume systems in the cool-temperate climates of the northern hemisphere occurs during winter and early spring thaw since high rates of nitrification can occur in cool wet soils (Magid et al. 2001), while any plant roots present will be unlikely to be actively assimilating the NO_3^- mineralized from legume residues (Jensen and Hauggaard-Nielsen 2003). Emissions of N_2O collected from either lentil or field pea residues immediately following spring snow melt were not significantly different from neighboring wheat stubble plots in Saskatchewan ($0.1 \text{ kg N}_2\text{O-N ha}^{-1}$) and Alberta, Canada (0.4 to $0.6 \text{ kg N}_2\text{O-N ha}^{-1}$), suggesting that N_2O emissions by crop legume residues remaining from the previous year can be negligible (Lemke et al. 2007). However, the situation was found to be very different elsewhere in Canada following the autumn termination and plough-down of N-rich alfalfa biomass where significantly higher fluxes of N_2O were measured during winter and early spring ($5.38 \text{ kg N}_2\text{O-N ha}^{-1}$) than detected coming from a bare soil during the same period ($2.84 \text{ kg N}_2\text{O-N ha}^{-1}$; Wagner-Riddle et al. 1997). There was also evidence that these elevated emissions may persist for up to 2 years after removal of the alfalfa stand (Wagner-Riddle and Thurtell 1998). By way of comparison, the initial losses of N_2O following alfalfa plough-down over the winter-early spring period was more than 3-fold greater than the N_2O emissions from barley, canola, or maize crops (1.05 – $1.31 \text{ kg N}_2\text{O-N ha}^{-1}$) that were subsequently grown in different treatment plots at the same experimental sites that were fertilized with between 75 and 100 kg N ha^{-1} (Wagner-Riddle et al. 1997).

Another situation analogous to the alfalfa plough-down example that would be conducive to generating high concentrations of soil NO_3^- susceptible to denitrification losses occurs when fresh legume biomass is either green-manured (i.e., either physically incorporated into soil or used as mulch) or brown-manured (killed prior to maturity

with a knock-down herbicide). Certainly higher N_2O emissions have been observed coming from soil under a maize crop in the UK where 3.9 Mg ha^{-1} of over-wintering faba bean foliage containing 180 kg N ha^{-1} ($\text{C/N ratio}=12$) had been green-manured prior to sowing ($0.79 \text{ kg N}_2\text{O-N ha}^{-1}$ over 65 days) than detected coming from the nil residue control treatment (0.23 – $0.31 \text{ kg N}_2\text{O-N ha}^{-1}$; Baggs et al. 2003). Fluxes of N_2O during the growth of lowland (wetland/flooded) rice in India were also considerably higher from sesbania (*Sesbania aculeate*) green-manured plots ($11.5 \text{ kg N}_2\text{O-N ha}^{-1}$ over 119 days) receiving 40 Mg ha^{-1} of shoot material containing 176 kg N ha^{-1} ($\text{C/N ratio}=18$) than where 6 Mg ha^{-1} of wheat stubble containing 27 kg N ha^{-1} ($\text{C/N ratio}=94$) were either retained ($6.6 \text{ kg N}_2\text{O-N ha}^{-1}$) or removed ($5.0 \text{ kg N}_2\text{O-N ha}^{-1}$; Aulakh et al. 2001). While green-manuring may be a good strategy to economize (financially) on N fertilizer, it is clearly a risky practice with regards to GHG emissions. In the case of the study of Aulakh et al. (2001), losses of N_2O from the green-manured plots were equivalent to where 120 kg N ha^{-1} was supplied to rice as N fertilizer, although a lower proportion of the applied N was calculated to be lost as N_2O from the sesbania mulch (6.5%) than from the N fertilizer (8.8%).

Conclusions There is a real risk of elevated N_2O emissions from legume residues. Low C/N ratio of leguminous material can potentially stimulate N_2O losses as they are a source of N for rapid mineralization and nitrification, and legume residues provide a source of easily decomposable C substrate for microorganisms to support the denitrification of NO_3^- that accumulates in soil. Clearly, there are situations where large amounts of labile legume organic N is returned to soil such as when legume-based pastures have been terminated prior to cropping, or where legumes are used for green-manure. Under these conditions, N_2O emissions can be comparable to, or greater than, where crops receive N fertilizer (Wagner-Riddle et al. 1997; Aulakh et al. 2001). However, the senesced, vegetative stubble that typically remains after grain harvest of legume crops is unlikely to represent a major source of N_2O loss above normal background soil emissions (Lemke et al. 2007) since the quantities of organic N returned to the soil tend to be relatively small and the C/N ratio of the residues are less favorable for rapid mineralization to build up high concentrations of soil NO_3^- (Kumar and Goh 2000; Fillery 2001; Peoples et al. 2009b).

2.2 Comparisons of energy use by legume-based and N-fertilized systems

Fossil fuel is used in both legume and non-legume cropping and forage systems in the production of seed for sowing; by

Table 6 Fossil energy consumed in pea, barley, and forage crops in Denmark and the amount of product dry matter (DM) harvested (Peoples et al. 2009b)

Parameter ^a	Pea	Barley	Grass–clover	Grass
Direct energy in diesel use (MJ ha ⁻¹)	3,320	3,400	3,940	4,880
N fertilizer (kg N ha ⁻¹)	0	130	125	400
N fertilizer (MJ ha ⁻¹)	0	6,500	6,250	20,000
Seeds and non-N fertilizers (MJ ha ⁻¹)	2,770	1,860	Manure ^b	Manure ^b
Pesticides (MJ ha ⁻¹)	900	900	50	50
Total fossil energy use (MJ ha ⁻¹)	6,990 (55%)	12,660	10,240 (41%)	24,930
Harvested product (kg DM ha ⁻¹)	6,000	8,000	7,700	11,700
Energy input (MJ kg DM ⁻¹)	1.16 (73%)	1.58	1.32 (62%)	2.13

Values in parentheses represent energy use in legume systems expressed as a percentage of the energy used by the non-legume comparison

^a Basis of calculations: diesel=41 MJ L⁻¹; N fertilizer=50 MJ kg N⁻¹; pesticides=300 MJ kg⁻¹ active ingredient

^b Energy costs associated the collection and spreading of manures to forage crops are not included in the calculations, but were assumed to be similar for both the grass and the grass–clover mixture

on-farm machinery for tillage, sowing, and harvesting of agricultural produce; and in the manufacture, transport, and application of fertilizers and other agrichemical inputs used to either supplement crop nutritional requirements or for crop protection. A comprehensive comparison of the use of energy by field pea or barley (*Hordeum vulgare*) crops, and in grass–clover mixtures or pure grass systems in Denmark revealed that total energy costs were 55% and 41% lower in the legume cropping and forage systems, respectively (Table 6). The single most energy-expensive input was N fertilizer accounting for 51% of the total on-farm use of fossil energy for barley and 81% of the fossil energy for the grass forage crop (Table 6).

Lower total fossil energy use by legume production systems was also apparent when similar analyses were collated for a larger range of crop comparisons elsewhere in

Europe and in North America (Table 7). The production of field pea and faba bean crops in Switzerland required 25% less energy than canola (oilseed rape, *Brassica napus*), 36% less than wheat (*Triticum aestivum*) and barley, and 60% less energy than maize (*Zea mays*) production (Table 7). In North America, field pea required 35% less energy than flax (*Linum usitatissimum*) and 45–48% less than wheat, while soybean required 39% less energy than maize (Table 7). Since dry matter (DM) production and grain yield by legumes tend to be lower than N-fertilized crops, the differences in energy use between legume and non-legume crops were markedly reduced, and in some instances may be little different, when calculated on the basis of fossil energy use per kilogram of DM of product harvested (Tables 6 and 7). Although it should be noted that if the data were reconsidered on a per kilogram

Table 7 Comparisons of the amounts of N fertilizer used and energy consumed in the production of a range of legume and non-legume grain crops, and the amount of product dry matter (DM) harvested in the cropping systems of Switzerland (Köpke and Nemecek 2010) and North America (Zentner et al. 2004; Rathke et al. 2007)

Parameter	Crop					
	Pea	Faba bean	Wheat	Barley	Canola	Maize
Switzerland						
N fertilizer applied (kg N ha ⁻¹)	5	5	136	100	105	110
Total energy use (MJ ha ⁻¹)	14,100	13,500	22,900	20,100	18,400	34,100
Harvested product (kg DM ha ⁻¹)	3,340	3,290	5,461	5,803	2,926	7,980
Energy input (MJ kg DM ⁻¹)	4.22	4.10	4.19	3.46	6.29	4.27
North America						
N fertilizer applied (kg N ha ⁻¹)	19	0	76	106	59	113
Total energy use (MJ ha ⁻¹)	4,584	5,938	8,400	8,730	7,040	9,713
Harvested product (kg DM ha ⁻¹)	2,504	2,350	2,446	2,519	1,800	6,470
Energy use efficiency (MJ kg DM ⁻¹)	1.83	2.53	3.43	3.46	3.91	1.50

Data have been averaged across different tillage treatments

grain crude protein produced, then the comparisons would be far more favorable for legumes (Köpke and Nemecek 2010).

Since legumes can convey significant benefits for following crops (Kirkegaard et al. 2008; Peoples et al. 2009a,b), calculations of energy demand on an individual crop basis such as depicted in Tables 6 and 7 will underestimate the wider implications of total energy consumption at a rotational level. Life cycle analyses (LCA) of cropping sequences with and without the inclusion of a legume found that fossil fuel energy use with legumes included was on average 12–30% lower per year compared to a rotation where legume crop(s) were not included in all but one of the four locations in Europe (Table 8), while they were an average 13–24% lower for four locations in North America (Table 8). It was only in the low-input cropping system in Spain where the introduction of field pea had no real effect on energy use as little or no N fertilizer was applied to the sunflower (*Helianthus annuus*) crop it replaced, therefore no N fertilizer was saved (Nemecek et al. 2008). Presumably both sunflower and pea would also have had similar effects in reducing cereal root diseases and breaking pest and weed cycles (Kirkegaard et al. 2008).

The largest energy savings occurred where a legume crop was grown every second year (field pea and soybean in Switzerland, lentil, *Lens culinaris*, at Swift Current Saskatchewan, and soybean at Lincoln, Nebraska) rather than just once in the rotation (Table 4). Whether this intensity of legume use is a wise strategy remains to be seen given the increased risk of accelerating the build-up of legume diseases or the development of herbicide resistance by weeds.

Recently, the influence of introducing grain legumes into a cereal-based cropping system in Canada has been evaluated by including the CO₂ equivalent emissions (CO₂e) derived from GHG release associated with farming activities, in addition to direct energy costs to compare the

“C footprint” of different cropping sequences (Gan et al. 2011). Averaged across five site-years of data, the C footprint of durum wheat grain produced in a cereal–cereal–durum system was calculated to represent 0.42 kg CO₂e per kilogram of grain harvested. This compared to estimates of 0.30 kg CO₂e per kilogram of durum grain when the durum was preceded by a grain legume (chickpea, lentil, or pea) in the previous year. In other words, the C footprint was 28% lower than when the durum crop was grown following a cereal (Gan et al. 2011).

Conclusions The reduced energy use and lower C footprint resulting from growing legumes largely reflected the removal of the need to apply N fertilizer and the subsequently lower N fertilizer requirements for the crops grown following the legumes. However, the total energy balance was also assisted by a lower use of agrichemicals since the diversification of the cropping sequence reduced the incidence of cereal pathogens and pests and changed weed populations, although it should be noted that the overall impact of legumes on energy use was greatly diminished if comparisons with N-fertilized systems were calculated on the basis of the amounts of biomass or grain produced.

2.3 Soil carbon sequestration and land use change

Soils contain large amounts of C in both inorganic and organic forms. Inorganic forms of C are derived from geologic or soil parent material sources and are usually present in soils as carbonates and bicarbonates. The amount of soil organic C (SOC) present in soil can represent from <20 to >200 Mg C ha⁻¹ in the top 30 cm of soil (Arrouays et al. 2001; Hoyle et al. 2011). Soil organic C exists in several different pools of varying size. Plant roots, fresh residues, living microorganisms, and macrofauna represent <15% of the total SOC pool, while partially

Table 8 Comparisons of estimates of average annual energy use (MJ ha⁻¹ per year) by rotations with (+) or without (–) the inclusion of a legume in the cropping sequence at four locations in Europe (Nemecek et al. 2008) and North America (Zentner et al. 2001, 2004; Rathke et al. 2007)

Geographic region	Location and rotation with (+) or without (–) a legume crop							
Europe	Saxony-Anhalt, Germany		Barrois, France		Canton Vaud, Switzerland		Castilla y León, Spain	
	+	–	+	–	+	–	+	–
Annual energy use	21,100 (86%)	24,500	19,900 (88%)	22,500	21,900 (69%)	31,500	13,100 (102%)	12,800
North America	Saskatchewan, Canada #1 ^a		Saskatchewan, Canada #2 ^a		Saskatchewan, Canada #3 ^a		Nebraska, USA	
	+	–	+	–	+	–	+	–
Annual energy use	4,305 (75%)	5,699	7,704 (86%)	8,913	7,092 (87%)	8,151	7,773 (80%)	9,713

Values in parentheses represent energy use in legume systems expressed as a percentage of the energy used by the non-legume comparison

^a The Canadian data were derived from three locations in Saskatchewan, Canada: Swift current (site #1; diesel fuel use converted to MJ on the basis of 41 MJ L⁻¹, as described in Table 6), Tisdale (site #2), and Indian Head (site #3). Data represent the mean of several different tillages treatments

decomposed plant residues, humus (the product of the breakdown of plant residues and soil microbes), and very resistant forms of organic C such as charcoal represent the balance (Dalal and Chan 2001; Hoyle et al. 2011).

While the atmosphere contains around 750 Pg (1 Pg = 10^{15} g which is equivalent to 1 Gt) of C as CO₂, globally the top meter of soil stores approximately 1,500 Pg in SOC and 900–1,700 Pg as inorganic C, and exchanges 60 Pg C each year with the atmosphere (Eswaran et al. 1993). The sheer size of the SOC pool and the annual flux of C passing through the soil are two reasons why so much focus has been given to the possible role sequestering C in soil might play in mitigating GHG emissions (Lal 2004; Soussana et al. 2010).

The amount of C accumulated in a soil is dependent upon the balance between C inputs and losses. In the absence of the transport and incorporation of large amounts of offsite organic wastes or biochar, new C can only be introduced to the soil via photosynthesis by plants. Consequently, any farm management practice that enhances total plant production and the retention of plant shoot and root residues, and/or reduces C losses can theoretically contribute to increasing soil C content (Hoyle et al. 2011). There is also an upper limit to the annual C inputs in plant residues, particularly in rainfed agriculture, where the availability of water and nutrient supply constrains photosynthesis and plant productivity.

Losses of C from soil result from leaching of dissolved and particulate C, wind and water erosion, and the microbial decomposition and associated mineralization processes that convert C in fresh plant residues and SOC into CO₂ (Dalal and Chan 2001; Kindler et al. 2011). The rate of microbial decomposition is heavily influenced by climate (Christopher and Lal 2007) and soil texture, factors that provide physical protection for SOC (Soussana et al. 2004, 2010; Hoyle et al. 2011), the source of organic residues (Gregorich et al. 2001; Rochester 2011), and farming practices, such as cultivation that increases soil disturbance and exposes plant residues and SOC to microbial decomposition (Dalal and Chan 2001; Christopher and Lal 2007). Depending upon climatic conditions, between 50% and 75% of the C in plant residues can be expected to be respired as CO₂ by microbes during the first year of decomposition (Hoyle et al. 2011).

Changes in land use could shift the relative balance between C inputs and losses in either direction depending on the nature of the change. All soils will eventually attain a dynamic equilibrium level when soil C gains equal soil C losses (i.e., a steady state when the rate of change in SOC is zero) which represents the upper limit of the amount of C that can be sequestered as defined by the inherent physiochemical properties of the different soil pools, and factors such as silt and clay content, clay mineralogy, and microaggregation (Soussana et al. 2004; Stewart et al.

2008; Chan et al. 2011). The following sections examine the potential impact that legumes and management can have on SOC.

2.3.1 Legume effects on soil carbon sequestration

In terms of mitigating emissions of CO₂, the C sequestered in soil should ideally be stored in stable forms of SOC such as humus (Christopher and Lal 2007). Humus represents 40–60% of the SOC and is the main stable form of C that can be influenced by human activity. It may take decades before increases in humus resulting from changes in soil management or farm inputs can be reliably quantified (Hoyle et al. 2011). With the exception of charcoal/biochar, all the other forms of SOC are more labile than humus and decompose within months or years. Nutrients such as N, phosphorus (P), and sulfur (S) are tied up along with C in humus and SOC cannot be effectively sequestered unless adequate amounts of these nutrients are available (Williams and Donald 1957; Kirkby et al. 2011). There is evidence to suggest that humification is particularly limited by the availability of N (Christopher and Lal 2007). The elemental composition of SOC tends to be very similar in almost all soils (e.g., C/N ratios depicted in Fig. 5; similar relationships for SOC in C/P and C/S ratios can be found in Cleveland and Liptzin 2007 and Kirkby et al. 2011). Around 80 kg N is associated with every megagram of organic C accumulated in soil (Fig. 5; Table 9). Since the C/N ratios and amounts of N provided per megagram of C in legume residues are generally much closer to that of soil

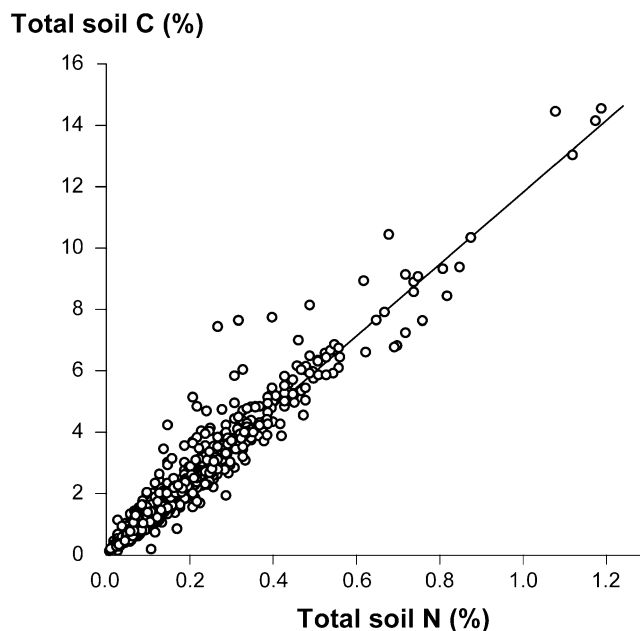


Fig. 5 Relationship between total soil C and N derived from 598 soils from around the world (modified from Kirkby et al. 2011). Linear regression: $C = 12(N) - 0.04$ ($R^2 = 0.91$)

Table 9 Examples of the amounts (kg) of N per 1,000 kg of C and the ratio of C/N expressed on a mass basis of shoot residues of different plant species and selected components of the soil

		Residues of different plant species ^a				
		Wheat	Maize	Rice	Canola	Cotton
Non-legumes	Carbon (C)	1,000	1,000	1,000	1,000	1,000
	Nitrogen (N)	19.6	21.4	9.5	23.8	38.5
	C/N ratio	51:1	47:1	105:1	42:1	26:1
Legumes	Alfalfa		Vetch	Clover	Field pea ^b	Faba bean
	Carbon (C)	1,000	1,000	1,000	1,000	1,000
	Nitrogen (N)	73.2	100	83.3	28.6	71.4
	C/N ratio	14:1	10:1	12:1	35:1	14:1
		Different soil components ^c				
		Microbial biomass	Fungi	Bacteria	Soil organic C	
	Carbon (C)	1,000	1,000	1,000	1,000	
	Nitrogen (N)	135	106	178.5	83.3	
	C/N ratio	7:1	9:1	6:1	12:1	

^aCollated from Peoples et al. (2009b), Rochester (2011), and unpublished data

^bSenesced shoot material from field-grown crops collected after grain harvest

^cCalculated from Fig. 2 and data presented by Cleveland and Liptzin 2007 and Kirkby et al. 2011

microorganisms and soil organic matter than non-legume species (Table 9), and leguminous organic matter tends to have higher P concentrations than other species (e.g., Pinkerton and Randall 1994), the inclusion of legumes in farming systems might be expected to be more conducive to C sequestration and the build-up of SOC over time.

2.3.2 Pastures

Despite the widespread utilization of mixed pastures in Europe based on ryegrass (*Lolium multiflorum*) and clovers (*Trifolium* spp.) there are relatively few quantitative studies of the impact of the legume introduction on soil C accumulation. Soussana et al. (2004) used models and data from a large survey of SOC under different land uses and soil types in France (Arrouays et al. 2001) to show that the conversion of short-term N-fertilized grass leys to grass–legume mixtures could result in the accumulation of 10 Mg C ha⁻¹ in the soil over a period of 20 years. Conant et al. (2001) in a review of soil C changes beneath temperate and tropical pastures also identified the inclusion of legumes as one of the many variables that can contribute to increased soil C stocks. Other factors found to influence the accumulation of SOC in pastures and rangelands include (1) climate and whether the pastures are rainfed or irrigated through effects on the net primary productivity of plants and C loss processes; (2) stocking rate and grazing management through defoliation and trampling effects on leaf area, photosynthesis, root biomass and soil microbial communities, and the impact of animal excreta on C and nutrient cycling; (3) the botanical composition of the pasture (i.e., the percentage of total pasture biomass present as grass or legumes); and (4) the age of the pasture and the initial state of the soil system since the rate of change in SOC tends to be greater where the initial soil C stocks are low (e.g., where SOC had been depleted by cropping) than where the soil is closer to

its C equilibrium (Conant et al. 2001; Soussana et al. 2004; Klumpp et al. 2009; Soussana et al. 2010; Chan et al. 2011).

Conceptually, whether the forage legume is a perennial or annual could also be important. Although a long-term Australian study failed to detect major differences in the rate of SOC increase in the top 30 cm of soil between rainfed pastures containing the perennial legume alfalfa or annual clovers (Chan et al. 2011), alfalfa would be expected to have a higher potential for C allocation below 30 cm than clovers as a direct result of alfalfa's much deeper rooting systems (Angus et al. 2001; Peoples and Baldock 2001). Certainly, other investigations have reported significantly greater gains in SOC where alfalfa or other perennial species such as siratro (*Macroptilium atropurpureum*) or desmanthus (*Desmanthus virgatus*) were grown compared to where annual pasture or crop legumes had been used (Dalal et al. 1995; Armstrong et al. 1999; Whitbread et al. 2000; Young et al. 2009). Soil C stocks have also been found to be substantially higher (130–134 Mg ha⁻¹, 0–70 cm) when maize was grown in rotation with alfalfa [undersown beneath oats (*Avena sativa*) and grown for 2.5 years in every 4 years] than under maize monoculture (109–115 Mg ha⁻¹; Gregorich et al. 2001). By employing a solid-state ¹³C nuclear magnetic resonance analytical technique in this experiment, they demonstrated that <15% of the C in maize residues was retained in soil compared to >50% of the residue C contributed by the alfalfa and oats (Gregorich et al. 2001).

Rotations based on alternating periods of legume-based pastures and cropping are common in the dryland farming systems of Australia (Peoples and Baldock 2001; Kirkegaard et al. 2011). Even though SOC might accumulate under legume-based pastures, total C stocks will inevitably decline when the land is returned to cropping (Dalal et al. 1995; Chan 1997; Persson et al. 2008). In the long term, whether rotating pastures with crops results in net C sequestration,

helps maintain SOC stocks, or simply slows the rate of loss of SOC compared to continuously cropped soils will be influenced by the prevailing climatic effects on C inputs and C loss processes, and the frequency or duration of the pasture phase (Grace et al. 1995; Dalal and Chan 2001; Young et al. 2009; Chan et al. 2011).

The potential for soil C sequestration is likely to be greatest in intensively managed permanent pastures and grasslands (Soussana et al. 2004). In the USA, Wright et al. (2004) reported that at low-grazing intensity, the SOC concentration (0–15 cm) under a long-term N-fertilized (350 kg N ha⁻¹ per year) Bermuda grass (*Cynodon dactylon*)/rye grass pasture in Texas increased by 39% over a 19-year period, whereas SOC was increased by 67% under a Bermuda grass/clover (*Trifolium* sp.) mixture receiving no N fertilizer. Not only was there a more rapid increase in SOC by changing from a heavily N-fertilized pure grass pasture to a mixed grass/clover sward but this would also have reduced N₂O emissions (Ruz-Jerez et al. 1994). Similar legume effects on SOC were observed when yellow-flowering alfalfa (*M. sativa* ssp. *falcata*) was interseeded into temperate grassland in the range lands of North Dakota (Mortensen et al. 2004) where average annual rates of soil C accumulation (0–100 cm) were increased by 1.56, 0.65 and 0.33 Mg C ha⁻¹ per year 4, 14, and 36 years after alfalfa had been introduced, respectively. These data illustrate the fact that as time passes, C sequestration rates will decrease as a new equilibrium between C inputs and losses is attained.

In South America, grass-only pastures based on *Brachiaria* (*Brachiaria decumbens*, *Brachiaria humidicola*, and *Brachiaria brizantha*) have been shown to accumulate more SOC than was originally present under the native savanna vegetation (Fisher et al. 2007). The potential to further increase the rates of soil C sequestration with forage legumes has been demonstrated by Fisher et al. (1994) who found that in the eastern savanna of Colombia, soil C accumulation (0–100 cm) was increased by 7.8 Mg ha⁻¹ per year where *Arachis pintoii* had been introduced into the sward, above that achieved by pure grass alone, despite the legume contributing only 20% of the total root biomass. Another study undertaken in the south of Bahia in Brazil showed that the introduction of *Desmodium ovalifolium* into a *Brachiaria* sward approximately doubled the rate of soil C accumulation (0–100 cm) from 0.66 to 1.17 Mg C ha⁻¹ per year over a 9-year period (Tarré et al. 2001).

In some cases, sub-optimal nutrition can severely limit the impact of legumes. A good example of this was in the acidic soils of southeastern Australia where the accumulation of SOC under permanent subterranean clover (*Trifolium subterraneum*) pastures was found to vary directly with the amount of superphosphate fertilizer applied (Williams and Donald 1957). It was proposed that the rate of build-up of organic matter was constrained by P and S deficiencies

in the soils. Alleviating these constraints has also been shown to stimulate the productivity of subterranean clover and greatly increase the amounts of N₂ fixed (Peoples et al. 1995b).

Conclusions There is evidence from many different regions and environments that SOC concentrations can be increased when legumes are included in pastures (e.g., Conant et al. 2001; Wright et al. 2004; Boddey et al. 2009; Chan et al. 2011). The impact of forage legumes appear to be greatest in permanent pastures and with perennial legume species. The effects of perennial legumes on SOC are associated with (1) lower losses of C from their organic residues than from annual legumes as a result of a lower soil water content maintained under perennials reducing microbial activity and respiratory losses of the organic C (Angus et al. 2001; Young et al. 2009), and (2) higher potential inputs of C due to the capacity of perennials to respond to rainfall and grow outside an annual's normal growing season (Peoples and Baldock 2001). Regardless of whether the legume is an annual or a perennial, a key factor contributing to the rate of SOC build-up will be the nutritional management of pastures (Williams and Donald 1957; Conant et al. 2001; Chan et al. 2011).

2.3.3 Cropping sequences

Cultivation and cropping leads to substantial losses of SOC principally via the decomposition of humus (Dalal and Chan 2001; Christopher and Lal 2007). The conversion of grassland to cropping has been reported to result in a decline in soil C stocks of between 25% and 43% at rates of up to 0.95 Mg C ha⁻¹ per year (Soussana et al. 2004). Consequently, arable soils inevitably have lower levels of SOC than pastures (Arrouays et al. 2001). Until recently, there was a general consensus that a change from conventional tillage (CT) to reduced or zero tillage (ZT) systems that maintain at least 30% of the soil surface cover would lead to positive impacts on SOC in almost any cropping system as the tillage-induced losses of C would be avoided (Christopher and Lal 2007). This was challenged by Baker et al. (2007) who pointed out that in almost all the earlier studies of the effects of tillage the soil was sampled to <30 cm depth only, often <20 cm. Evidence was presented that where soils had been sampled to greater depths (e.g., 80 or 100 cm) more C was found at depth under CT and there was little or no difference between ZT and CT in total SOC down the soil profile (e.g., VandenBygaart et al. 2003). This led Baker et al. (2007) to conclude that the apparent accumulation of soil C observed under ZT compared to CT was largely an artifact of the sampling depth.

Many of the early studies undertaken in both temperate and sub-tropical cropping systems that compared the interactions of tillage and the inclusion of legumes such as lupin, vetch, or cowpea in rotations also sampled the soil to <30 cm depth and found that soil C concentrations were often considerably higher under ZT than under CT and that legume–cereal rotations tended to lose smaller amounts of SOC than continuous cereal cropping (e.g., Heenan et al. 1995; Bayer et al. 2000). Freixo et al. (2002) working in Brazil showed that even after 13 years of continuous wheat (winter)–soybean (summer) double-cropping systems (i.e., two crops every year), there were no significant differences in stocks of SOC between ZT and CT management when assessed to 40 cm. Sisti et al. (2004) also found no significant difference between SOC stocks in continuous wheat–soybean under the different tillage systems (167 cf. 168 Mg Cha⁻¹ 0–100 cm under CT and ZT, respectively; Fig. 6), although SOC stocks were approximately 10 Mg C ha⁻¹ higher after 13 years of cropping where vetch had been included along with soybean in two different rotations as a winter green-manure crop before maize (178 and 179 Mg Cha⁻¹), but only under ZT management. Where vetch was grown under CT, SOC was considerably lower (161 and 163 Mg Cha⁻¹). Between 46% and 68% of the difference between SOC in the ZT and CT treatments that included vetch occurred below 30 cm (Sisti et al. 2004). The impact of vetch green-manure on SOC under ZT was confirmed in Brazil by Boddey et al. (2010) who found that SOC stocks under ZT (0–100 cm) were increased above that achieved with CT representing an average rate of change of between 0.48 and 1.53 Mg Cha⁻¹ per year. Over 60% of this additional C was also found to be located below 30 cm depth. A positive impact of mucuna (*Mucuna pruriens*) cover-crops on SOC in the top 40 cm of soil was reported in cultivated maize systems in Benin, Africa (Barthès et al. 2004). In this case, the inclusion of mucuna cover-cropping resulted in an additional annual return of 11.9 Mg DM ha⁻¹ in shoot residues over and above that occurring in the more traditional unfertilized, cultivated maize monoculture. This represented increased inputs of



Fig. 6 Soybean cultivated on zero-till land. Photo: B. Alves, Embrapa

organic C of 6.5 Mg Cha⁻¹ per year, which contributed to an average change in SOC over 12 years equivalent to 1.3 Mg Cha⁻¹ per year (Barthès et al. 2004). Analyses of the ¹³C signatures of soil and litter indicated that only 2% of the organic C was derived from maize residues compared to 57% from mucuna.

Rochester (2011), working in irrigated, minimum-tilled cotton (*Gossypium hirsutum*) cropping systems in Australia, found that after 10 years the SOC was 7.3 Mg Cha⁻¹ higher to 90 cm (representing a 7% increase) under cropping sequences that included either vetch green-manures or legume crops with relatively low N harvest indices (i.e., low ratio of grain N/total plant N), such as faba bean, compared to non-legume alternatives (wheat–cotton, or continuous cotton). The rotations that included legumes returned 49% more stubble-C and 133% more stubble-N, and around 60% of the additional SOC was located below 30 cm (Rochester 2011).

The results of Diekow et al. (2005) indicate that the desired effects of legumes on SOC can also be achieved when legumes are intercropped with maize as cover-crops under ZT. After 17 years of ZT management, SOC (0–108 cm) ranged between 124 and 128 Mg Cha⁻¹ under a continuous oat–maize sequence in either the absence or presence of N fertilizer (120–180 kg Nha⁻¹), but reached 141 to 149 Mg C ha⁻¹ where lablab (*Lablab purpureum*) or pigeon pea (*Cajanus cajan*) were present as inter-crops without N fertilizer. This was not significantly different ($P < 0.05$) to measures of SOC under the native grassland 31 years earlier (152 Mg Cha⁻¹).

Conclusions The cropping sequence studies described in this section demonstrated the potential contributions of N₂-fixing legumes to SOC stocks under ZT or minimum tillage systems, although it was not always possible to discern from these studies whether the observed beneficial effects of legumes on SOC compared to continuous cereals resulted from a net increase in SOC stocks or simply reflected a slower decline in soil C reserves due to lower losses of organic C. An important discovery common to almost all the studies was that the impact of legumes on the accumulation of soil C under ZT was greatest in the subsoil below 30 cm depth. These observations differ from the original conclusions of Baker et al. (2007). Long-term experiments indicate that it can be difficult for legumes to influence SOC in CT systems (Freixo et al. 2002; Sisti et al. 2004; Boddey et al. 2010), but the data of Barthès et al. (2004) suggested that it might be possible to increase SOC under cultivation provided the inputs of legume organic C and N were greater than the increased losses of SOC stimulated by tillage.

It appears that the net N-balance of the system is a key driver of C stock changes in the soil. When a high N

harvest index legume crop like soybean is the only legume present in a rotation, SOC stocks are not maintained because large quantities of N are exported from the field in the grain. By contrast, large amounts of organic C and N are returned to the soil where legume green-manures and cover-crops are utilized, or when legume crops with high vegetative residues are grown regularly.

2.3.4 Woody perennial legumes

Of all the land management options available, afforestation of land is often considered to result in the greatest C sequestration (Christopher and Lal 2007). Given the potential for fast-growing multipurpose legume trees and shrubs to accumulate biomass (e.g., Peoples et al. 1996), and to contribute substantial amounts of organic C and nutrients to soil through either leaf litter, the periodic harvest of foliage prunings for use as green-manure, or when grazed by ruminants, it is perhaps not surprising that woody perennial legumes have been considered a promising option to enhance SOC stocks while revegetating cleared land, degraded soils, or mining wastes (Thrall et al. 2005; Chaer et al. 2011; Radrizzani et al. 2011; Fig. 7).

It has been estimated that 27% of the total land area in South America is degraded. In Brazil alone, degraded land represents 236 million ha, approximately four times larger than the area dedicated to arable crops (Chaer et al. 2011). At least 50 million ha of the degraded areas in Brazil are former agricultural areas in the central savanna (Cerrado) region, which could potentially be reclaimed for food production. However, much of the remaining areas are located in the deforested hillsides in the Atlantic coastal region where there are few reclamation options. The land was first cleared for sugarcane (*Saccharum* spp.), then on a



Fig. 7 Revegetation of land used for disposal of mine waste (bauxite) by legumes trees. *Small picture* inserted: land before planting. *Main picture*: land after 3 years of legume growth. *Photo*: R. M. Boddey, Embrapa

much wider scale for coffee (*Coffea robusta*), and in the last century for charcoal for iron founding (Dean 1995; Boddey et al. 2003). Some of the land has become so degraded that it has been completely abandoned, while in other areas the land is utilized by small landholders for only extremely low productivity crop production or for extensive cattle grazing (Szott et al. 1999). In both cases, it is common practice to burn off the vegetation at least once a year either to facilitate planting or to exploit grass regrowth for forage. Burning exacerbates the loss of nutrients and soil organic matter; erosion becomes a problem and the degradative processes are accelerated. Few attempts have been made to revegetate seriously degraded areas, but over the last 20 years a team at Embrapa Agrobiologia has developed a technology based on the use of fast-growing legume trees from the genus *Acacia* (*Acacia mangium*, *Acacia holosericea*, *Acacia angustissima*, and *Acacia auriculiformis*), *Albizia lebeck*, *Mimosa caesalpinifolia*, *Pseudosamanea guachapele*, *Enterolobium contortisiliquum*, *Sclerolobium paniculatum*, and *Styryphnodendrum purpureum* (Chaer et al. 2011; De Faria et al. 2011).

In cleared land and severely degraded soils, or where new tree legume species are being grown for the first time, populations of rhizobia capable of nodulating tree legumes and forming an effective symbioses tend to be extremely low and tree legumes commonly fail to fix N_2 or prosper if planted without appropriate rhizobial inoculation (Galiana et al. 1998; Thrall et al. 2005). Consequently, considerable effort has been placed on isolating and selecting effective rhizobia for a number of woody legume species suitable for use in temperate or tropical environments (Franco and de Faria 1997; Galiana et al. 1998; Thrall et al. 2005).

Recently, three Brazilian studies have highlighted the potential effects of tree legume species on the soil C stocks where they have been used in degraded areas. The first study was at a sloping site where, in 1989, a large amount of the top 40 cm of soil was removed to construct an irrigation dam reducing the SOC stock to $44.5 \text{ Mg C ha}^{-1}$. Originally, the slope was covered by Guinea grass (*Panicum maximum*). The SOC stocks (0–60 cm) were found to have been increased by 21 Mg C ha^{-1} (average rate of 1.4 Mg C ha^{-1} per year) over a period of 15 years by growing *M. caesalpinifolia* and by 55 Mg C ha^{-1} (average rate of 3.7 Mg C ha^{-1} per year) with *A. auriculiformis* and *P. guachapele* (Boddey et al. 2009; Chaer et al. 2011).

The second study, near Angra dos Reis on the coast of Rio de Janeiro, was a steep slope ($\sim 50^\circ$) which had been deforested and the top soil removed (Macedo et al. 2008). A recovery operation began in 1991 by planting *A. mangium*, *A. holosericea*, and *M. caesalpinifolia*. Part of the deforested hillside was left unplanted while 1,000 m

further east along the hillside was an area of the original forest. All three sites (unplanted, tree legumes, and original forest) were sampled to a depth of 60 cm. The SOC under the undisturbed Atlantic forest was 108 Mg C ha⁻¹ while the SOC under the unplanted hill and tree legume hill was 65 and 88 Mg C ha⁻¹, respectively.

A further study was undertaken on an experimental area established in degraded secondary forest near the town of Valença (Rio de Janeiro state; Torres et al. 2007). Replicated plots (25×50 m) of mixtures of different N₂-fixing and non-N₂-fixing legume tree species and non-legume trees were planted in different proportions: 0%, 25%, 50%, and 75% N₂-fixing legumes. In 6 years, the C in tree biomass (including roots) and litter was estimated to represent 16 Mg C ha⁻¹ where non-N₂-fixing trees were planted, and 47 Mg C ha⁻¹ in the treatment with 75% N₂-fixing legume trees. Soil C stocks (0–60 cm) under the plots with 50% legumes (84 Mg C ha⁻¹) was significantly greater than where no legume was present (71 Mg C ha⁻¹) representing an annual rate of SOC change of 2.17 Mg C ha⁻¹ per year (Torres et al. 2007).

The limited data on tree legume effects on SOC from elsewhere in the world suggest that the Brazilian case studies described above may not be unique. The average annual rates of SOC accumulation in the topsoil (0–15 cm) from leucaena (*Leucaena leucocephala*) compared to grass pastures or cropping soils in tropical Australia were reported to range from 0.08 to 0.26 and 0.76 Mg C ha⁻¹ per year following 38, 20, and 14 years of leucaena, respectively (Radrizzani et al. 2011). The applications of leucaena and *Senna siamea* residues (C/N=13:1 and 18:1, respectively) to an *Imperata cylindrical* grass fallow in West Africa were also found to be more effective at increasing SOC (0–20 cm) than applications of similar amounts of residue C as maize stover (C/N=58:1; Gaiser et al. 2011).

Conclusions The case studies described here confirm the perceived benefits of using N₂-fixing woody perennials to accumulate SOC in soil in addition to their capacity to provide a strong sink to sequester CO₂ in their biomass. Interestingly, the increases in SOC stocks (1.4–2.2 Mg C ha⁻¹ per year, 0–60 cm) observed in the Brazilian studies were achieved solely through litter fall from the canopy. This may have represented between 5 and 11 Mg DM ha⁻¹ per year (Chaer et al. 2011). There is potential to more intensively manage some legume shrub and tree species by regularly harvesting foliage which could allow for up to 20 to 30 Mg DM ha⁻¹ (Peoples et al. 1996) to be applied as green-manure to accelerate C accumulation in soil. However, the data of Radrizzani et al. (2011) remind us that the rates of change in SOC beneath woody perennial legume systems will inevitably decline over time.

3 A role for legumes to replace fossil resources?

Biomass can potentially be used to replace fossil hydrocarbons for heat, power, solid and liquid fuels, materials, or chemicals (Bessou et al. 2010). The global energy demand is expected to increase by about 45% by 2030 with the main increase occurring in non-OECD countries (IEA 2009). While fossil fuels are expected to still account for 80% of the world energy requirement in 2030 with oil remaining as the dominant energy source, biomass is projected to be the most important primary source of renewable energy. Biomass is predicted to provide about 9% of the total energy requirement and around 5% of the world road transport fuels (IEA 2009). This represents a 40% increase compared to 2006 in terms of million tons of oil equivalents (Mtoe).

In a biobased economy, fossil resources are replaced by biomass for the production of industrial chemicals, transportation fuels, electricity, heat, and other products. The world demand for biomass in renewable energy production is predicted to increase from 1,186 Mtoe to 1,660 Mtoe in 2030 (IEA 2009) with the EU and USA targeting a 25–30% replacement (Mabee et al. 2006). Second-generation biofuel technologies based on lignocellulosic feedstock are expected only to be deployed to a minor degree in 2030 due to the major improvements that will be required in conversion technologies involving the enzymatic hydrolysis of sugars (Mabee et al. 2006; IEA 2009). A major part of the biomass is projected to come from agricultural crops and residues as well as forest residues, with a growing share coming from purpose-grown energy crops (IEA 2009; Fig. 8).

Concerns about dwindling petroleum reserves and needs to supply sources of energy with lower GHG emissions are not the only drivers for these changes. The re-emergence in interest in biofuels and biomass feedstocks have also been encouraged by insecurities about on-going petroleum



Fig. 8 Straw and stover from annual crops are valuable resources for soil carbon sequestration, but sustainable use for biorefineries may be possible from fertile land with high soil C contents. Photo: E. S. Jensen, SLU

supplies in light of the recent instabilities in the oil-rich Arab world, and Japan's nuclear crisis following the 2011 tsunami which has caused many countries to re-assess their reliance upon nuclear power as a source of low-C electricity (Fairley 2011).

There are also a number of concerns about the environmental credentials and socio-economic effects of present bioethanol and biodiesel production from crops (Pimentel 2003). Foremost of these concerns are (1) the implications for food availability and security where energy crops displace food production, (2) GHG emissions if the increased demand for cropping land for biomass crops either directly or indirectly results in the clearing of forested areas, and (3) supplying fertilizer inputs to support the growth of high yielding and high biomass crops. This final issue is one of the key factors contributing to the reduction of the C neutrality of biomass systems because the fossil fuels involved in fertilizer production and transport can effectively negate the whole of life-cycle energy benefits. The attraction of legumes is their ability to satisfy their own N requirements from symbiotic N₂ fixation (Herridge et al. 2008). Although it should be noted that legume species differ enormously in their reliance upon N₂ fixation for growth in the field, with dry bean (also known as common bean or French bean; *Phaseolus vulgaris*) often fixing the least, and soybean and faba bean fixing the most (Peoples et al. 2009a).

Clearly, it will be necessary to justify the sustainability of biomass production systems if they are to be seen as a viable alternative to fossil resources and before real progress can be made towards meeting the predicted demand. Biomass systems for energy production will also be required to be multifunctional contributing several components or aspects to society (IAASTD 2009). The following sections analyze the potential role of legumes in contributing to future biobased economies.

3.1 Legume biomass yield potential and constituents

Biomass production for bioenergy feedstock will ideally require a high net biomass per unit area with a low amount of fossil energy input resulting in low fossil energy requirement per kilogram of DM produced. Although legume grain yields have increased in most regions during the last 30 years, yield enhancements are small compared to those observed in wheat and maize. As a result, legume grain yields tend to be lower than cereals in many countries of the world (e.g., Tables 6 and 7; FAOStat 2010). Areas sown to crop legumes have also declined globally for almost all species except soybean in the last few decades (Jensen et al. 2010; FAOStat 2010). Globally, cereals were grown on almost 700 million ha of land in 2009 compared to a total of 193 million ha sown to pulses and legume

oilseed crops such as soybean and groundnut (*Arachia hypogea*).

Second-generation biofuels, power and heat generated by combustion and production of industrial raw materials could be based on legume biomass and residues. However, the amount of legume stubble remaining after grain harvest is often lower than residual cereal straw biomass, which clearly would impact on the relative economics of using legume sources rather than cereals as feedstock.

Part of the reason that legume dry matter yields tend to be lower than of cereals may be explained by the fact the legumes are high in proteins and/or oil and that the energetic cost of producing proteins and oil are higher than producing cell walls, starch, and soluble sugars (Munier-Jolain and Salon 2005). The energetic cost of producing 1 g of pea grain was determined to be 1.8 g glucose equivalents compared to 1.6 g glucose equivalents for 1 g wheat grain. In this calculation, it was assumed that the cost of producing 1 g of starch is 1.21 g glucose g starch⁻¹, whereas it required 1.79 g glucose g protein⁻¹ (Munier-Jolain and Salon 2005). Based on the different constituents of legumes and non-legumes, the relation between average yields and the constituents of a spectrum of arable crop are shown in Fig. 9. Species with similar theoretical C costs of seed production can have different yields (e.g., faba bean and chickpea; Fig. 9). The authors suggested that this could be due to different investments in breeding and development of cultivation techniques of the different species (Munier-Jolain and Salon 2005). However, the greater C cost associated with legume grain synthesis compared with

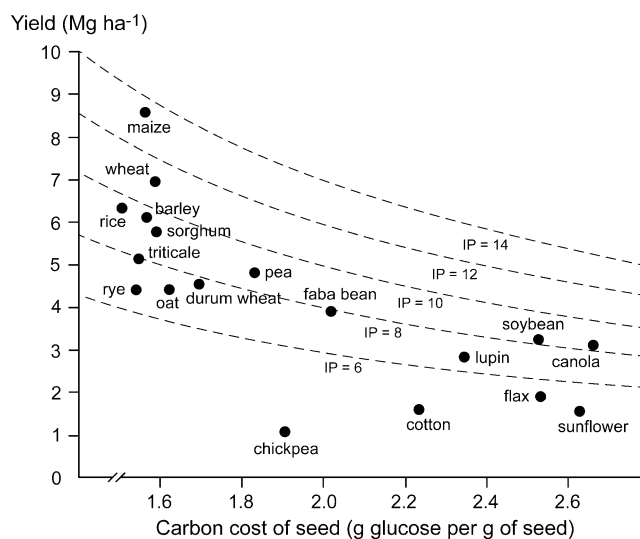


Fig. 9 Relationship between the C cost of seed production for selected legume and non-legume crops. In order to compare crop production performance, various isoproduction (IP) curves expressing the product of the energy cost of 1 g of seed by the yield have been indicated (after Munier-Jolain and Salon 2005)

cereals may be compensated by higher rates of photosynthesis and delayed leaf senescence (Kaschuk et al. 2010).

Less focus has been placed on the use of legumes for biomass feedstock for energy and industrial raw materials. A comprehensive European inventory of crops for bioenergy did not include any legumes (Venendaal et al. 1997). Legumes have high contents of constituents other than carbohydrates, which may be relevant in biorefinery concepts (see Section 3.2), in which the different components could be used for a variety of biobased products. For example, legume biomass might be used to generate biogas (CH₄) and N-rich biofertilizer via anaerobic digestion, and the grain utilized for biodiesel and/or protein feed.

Table 10 and Fig. 10 give an overview of the main constituents of some common legumes as compared to wheat and maize. Since the grain of crop legumes and soybean have high concentrations of protein and oil, the levels of starch tend to be lower than cereals (Table 10). Only pea has a starch content which approaches the levels observed in wheat and maize. Due to their high starch content, pea is often used as an energy component in compound feed. Other carbohydrates in Table 10 include simple sugars, cellulose, hemicelluloses, and lignin. Mature wheat straw and maize stover contain around 40% cellulose (C6) and 25% hemicelluloses (C5), 10–12% lignin, and 9% protein (Duke 1983). By comparison, the vegetative residues of soybean contain 44–83% cellulose and 5–14% lignin, although it should be acknowledged that the determinations of hemicellulose, cellulose, and lignin depend strongly on the analytical methodology used (Reddy and Yang 2009). The protein concentration in the crop legume residues is greater than in cereals and can reach values as high as 10% (Jensen 1989). Similarly, forage legume biomass has high concentrations of protein, which could warrant the use of technology to extract the

protein as a potentially valuable by-product when the biomass is used for biofuels. In addition, legumes contain many interesting compounds, such as alkaloids and isoflavonoids, which may be useful for human and animal health, as well as having applications for industrial uses (Duranti et al. 2008; Dixon and Sumner 2003; Pregelj et al. 2011).

Legume breeding efforts to date have only considered enhancing the concentration of grain protein or oil (in the case of soybean) for livestock or human consumption. The focus of future breeding programs could shift to greater dry matter yields with reduced protein concentrations for biofuels. Legumes can also be grown as part of mixed crop communities with greater diversity to exploit better available resources and deliver several ecosystems services (Jensen and Hauggaard-Nielsen 2003; Figs. 3 and 11).

Conclusions Legumes are lower yielding and have higher protein concentrations than cereals, which have resulted in less interest in their use as sole crops for biofuels. Legumes can be valuable components in mixtures with other species that might be suitable for biorefinery concepts. This is especially the case if the biorefinery is designed to exploit both carbohydrates and protein.

3.2 Legumes as biorefinery feedstocks for biofuels, materials, and chemicals

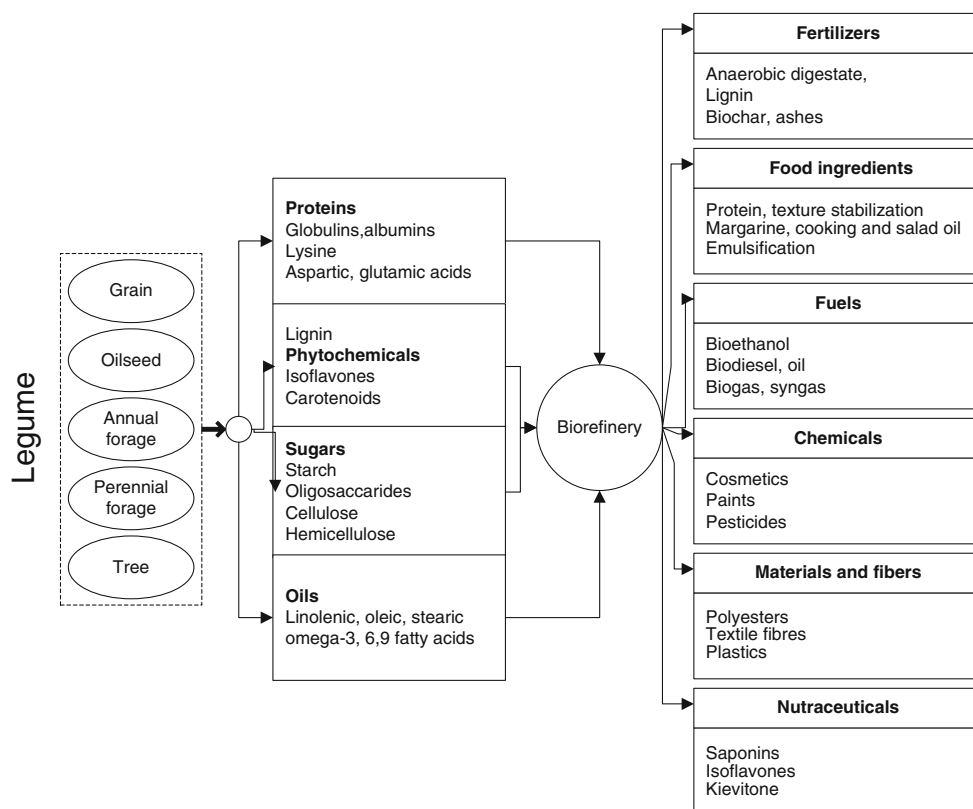
A biorefinery is defined as the sustainable processing of biomass into a spectrum of marketable products and energy (Cherubini et al. 2009) by the use of physical (fractionation, pressing), chemical (acid hydrolysis, synthesis, esterification), thermochemical (pyrolysis, gasification, combustion), and biochemical (enzymatic and fermentation) methods (De

Table 10 Constituents of selected cereal and legume species expressed as percentage of dry matter

Crop and component	Starch (%)	Other carbohydrates (C5, C6, a.o.) (%)	Protein (%)	Fat (%)	Lignin (%)
Grain					
Maize	71	14	9	5	2
Wheat	66	17	13	3	2
Soybean	15	14	41	21	6
Pea	55	18	25	2	6
Lupin	22	23	45	5	16
Faba bean	42	21	31	1	9
Above-ground biomass					
Wheat stubble	0	92	3	2	45
Pea residues	0	81	7	2	41
Grass-clover (30–50% clover)	2	62	22	4	20
Alfalfa (after flowering)	2	72	20	3	31

Collated from data presented by the Danish Feed Analysis (2005), Duke (1981, 1983), and Samac et al. (2006)

Fig. 10 Legume constituents and potential products from legume biorefineries



Jong et al. 2010). The aim is to optimize the sustainable use of specific biomass resources available in a given region to ensure both resource use efficiency and economic/environmental sustainability.

A biorefinery may be simple with only a single or few products such as bioethanol and heat or refined sugar and feed. Alternatively, the biorefinery could produce a spectrum of different biobased products in a way analogous to a petrochemical refinery. Different concepts for biorefineries have been described: well-known simple biorefineries produce sugar, potato starch, wheat starch, soybean oil, and protein. Dry milling refineries use cereals grains for



Fig. 11 Perennial mixtures of forage legumes and grasses, e.g., alfalfa and cocksfoot (*Dactylis glomerata*), can produce abundant amounts of biomass suited for anaerobic fermentation to biogas and biofertilizer. Photo: E. S. Jensen, SLU

bioethanol production and dried distillers grains with solubles (DDGS) for feed. Oleochemical biorefinery produce oils, lubricants, platform chemicals, and biodiesel from canola and soybean (De Jong et al. 2010). Lignocellulosic biorefineries have been used for many years with forestry biomass for the production of paper pulp, chemicals, and energy. During the past decade, there has been a rapid development of sugar platform biorefineries using different types of lignocellulosic biomass (straw, short rotation coppice, perennial energy crops) as feedstock for production of bioethanol, feed, and power (De Jong et al. 2010). A green biorefinery is another concept developed for green biomasses such as grass-clover, alfalfa, and sugar beet (*Beta vulgaris*) leaves to produce amino acids, feed, fibers, and residues for biogas production (Novalin and Zweckmair 2008; De Jong et al. 2010). Even though there is an increasing interest in biorefineries, examples of successful advanced biorefinery concepts which have developed further than the pilot scale are limited (De Jong et al. 2010).

As indicated above, legumes are already used as biorefinery for feedstock. However, for a biorefinery which is either completely or partly based on legume feedstock, to be considered as economically viable, it will be necessary to consider how value can be derived from the high concentration of protein and/or oil, perhaps along with other natural compounds present. An outline of a simplified theoretical biorefinery concept for a legume feedstock is provided in Fig. 12. In the following section, we present

evidence related to the production of biofuels, electricity/heat, materials, and chemicals based on legume feedstock, without emphasis on combining the different technologies in specific legume biorefineries.

Conclusions A biorefinery is a key component in future biobased economies, which will contribute to the replacement of fossil-resource-based economies. Concepts are currently developing quickly worldwide, but soybean is the only legume used to a certain extent for protein feed and biodiesel. There is scope for utilizing other legumes species if their protein and other potentially valuable constituents can be extracted and converted into marketable products.

3.2.1 Biofuels

Renewable sources of energy derived from technologies such as solar panels or wind turbines will be able to supply electricity, but in reality the vast majority of the world's transport systems are based on motor vehicles and aircraft which require liquid fuels and will do so for the foreseeable future. The recognition that sustainable sources of biofuels will need to be a key part of our global energy future is reflected in the trends in the annual output of bioethanol and biodiesel which has expanded more than 6-fold between 2000 and 2010 (IEA 2009; Fairley 2011).

3.2.2 Bioethanol

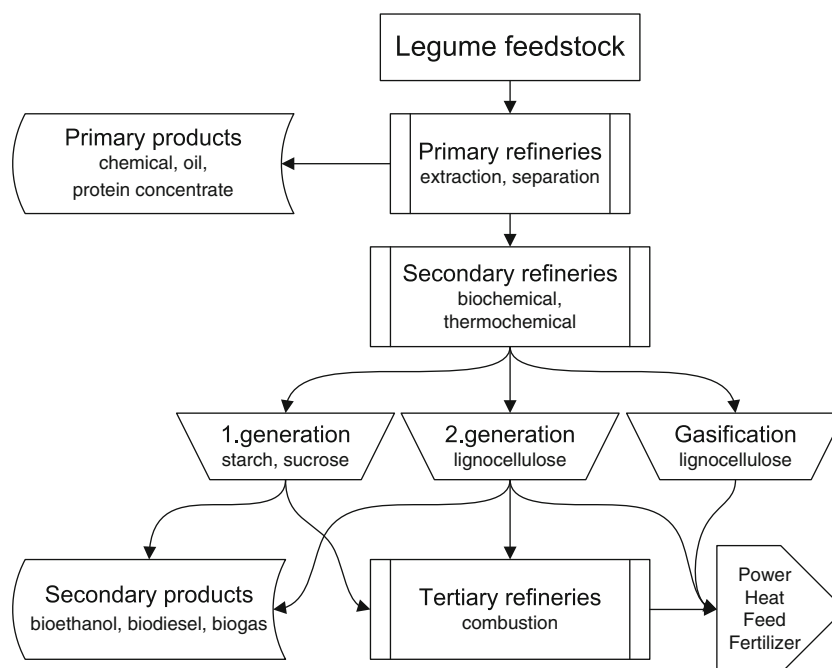
Bioethanol production is based on the microbial fermentation of sugars into ethanol. Bioethanol produced from

simple sugars (e.g., from sugar cane and sugar beet) and starch are termed first-generation bioethanol, whereas bioethanol derived from lignocellulose in straw, stover, perennial biomass crops [e.g., *Miscanthus*; willow (*Salix* spp.); reed canary grass (*Phalaris arundinacea*); mixed grass–clover swards; alfalfa] are termed second-generation bioethanol (Mabee et al. 2006).

Field pea grain has been studied as a potential feedstock either alone or as a supplement with maize grain for first-generation bioethanol production because of its high starch content (Table 10; Nichols et al. 2005; Pryor et al. 2008). Fermentation of whole peas and a dry-separated (starch and protein fraction separated by air classification of milled pea grain) pea starch fraction gave satisfactory ethanol yields (Nichols et al. 2005). The enriched starch fraction in combination with maize starch gave similar or greater ethanol yield than maize starch alone (Pryor et al. 2008). Improved or similar ethanol production occurred with pea starch, despite its less favorable amylase/amylopectin ratio since it has been shown that it is more difficult to convert amylose than amylopectin starch to fermentable sugars, and pea contains 30% to 50% amylose compared to 20% to 30% in maize starch (Pryor et al. 2008).

The consequences of reallocating land from food production to bioenergy purposes, and the overall sustainability of the first-generation technology for bioethanol production, remains controversial (Pimentel 2003; Pimentel and Patzek 2005; Fairley 2011). Hammerschlag (2006) found that the ratio of energy in a liter of ethanol to the non-renewable energy required to produce it with first-generation technologies varied across six different pub-

Fig. 12 Principles of a legume biorefinery



lished studies. In one study, more non-renewable energy was required to produce ethanol from maize grain than was present in the final bioethanol product with an energy balance of 0.84. By contrast, another study with maize estimated an energy balance of 1.65 times more renewable energy generated than non-renewable energy used, when the energy content of the by-products were included in the calculations of the energy return on investment (Hammerschlag 2006). The use of non-renewable energy for fertilizers, especially N, represented from 10% to 20% of the total energy inputs. The lower starch yield per unit area of land by crop legumes compared to cereals will probably prevent their increased use for first-generation bioethanol. However, intercropping grain legumes with high starch-yielding non-legumes may be an alternative option (Hauggaard-Nielsen et al. 2009).

The second-generation conversion technology for lignocellulosic materials into bioethanol appears more promising in terms of the potential of using feedstock and land which is not in direct competition with food production. Lignocellulose conversion technology is more complex than first-generation bioethanol production, primarily due to the presence of both C6 and C5 sugars, which are imbedded in lignin and consequently not easily accessible by the cellulases and hemicellulases that are required to convert cellulose and hemicellulose into fermentable sugars. Consequently, a biomass pre-treatment process is required to open the lignocellulosic structure (Mabee et al. 2006). In recent years interest has increased in second-generation bioethanol from feedstock that is not in direct competition with food production since this concept could be more sustainable in terms of GHG emissions, fossil energy use, energy balance, and resource use. Hammerschlag (2006) reported energy of investment (bioethanol energy per unit non-renewable invested energy) to be up to 4.4 and 6.6:1 for lignocellulose-derived bioethanol from maize stover and poplar.

Vegetative biomass from grain and oilseed legumes is a possible source of feedstock for second-generation bioethanol, but DM yields tend to be lower per unit area and the stubble contains more protein and a lower content of cellulose than cereal alternatives (Table 10). Consequently, the ethanol yield will be lower.

Perennials are a promising option because of their efficient use of resources and lower requirements for farming operations than annual crops (Bessou et al. 2010). Alfalfa is an interesting candidate as a perennial legume feedstock for biofuel and bioproducts since it can yield between 4 and 18 Mg DM ha⁻¹ per year (average of 8 Mg DM ha⁻¹ from three to four annual harvests) for up to 4 years of growth (Samac et al. 2006). Alfalfa requires fertile, deep, and well-drained soils and adequate supply of water, although its deep rooting system makes alfalfa more

resistant to dry periods than many other crops. Alfalfa stems containing 10–12% protein can be used for bioethanol production while the leaves with 26–30% protein can be used as a high-protein feed (Dale 1983) or further processed to a high-value protein product. New germplasm and cultivation methods (plant density and cutting regime) have been developed for alfalfa to provide modified stem/leaf ratios that are more suitable for bioethanol production (Lamb et al. 2003). Research has been done to determine how the polysaccharide composition of alfalfa stems can be modified by cultivation and harvest frequency in order to produce the most optimal biomass for conversion to bioethanol (Samac et al. 2006). Besides being a potential feedstock for bioethanol, stems are also evaluated as feedstock for the production of lactic acid, which can be used for bioplastic, as a replacement for petroleum-based plastics (Koegel et al. 1999).

Other perennial legumes, such as clovers, could be used as feedstock for second-generation bioethanol either grown as sole crops or in mixtures with grasses. Thomsen and Hauggaard-Nielsen (2008) found that the theoretical bioethanol potential (based on carbohydrate composition) of wheat straw and grass–clover crops were similar at 270 and 240 L per megagram of DM. With biomass yields of 5 Mg of wheat straw DM and 10 Mg grass–clover DM ha⁻¹ in Denmark, the production would be 1.35 and 2.4 Mg bioethanol ha⁻¹, respectively. If a grass–clover cover crop was undersown to wheat, a further 0.96 Mg bioethanol ha⁻¹ could be produced from the autumn biomass growth of the cover crop (Thomsen and Hauggaard-Nielsen 2008).

Intensive agroforestry systems also have a potential role in producing large amounts of biomass. For example, densely planted tree legumes such as *Calliandra calothyrsus* and *Gliricidia sepium* in the Australian tropics yielded up to 20 to 30 Mg DM ha⁻¹ as foliage and stem re-growth over an annual cutting cycle when periodically cut as hedgerows (Peoples et al. 1996). Some woody perennial legume species are also suitable for use on marginal or degraded lands (see Section 2.3.4).

The deep-rooted nature of both herbaceous and woody perennial legumes also offer an effective, low-cost method for (1) remediating excess soil N and lowering the risk of groundwater contamination by nitrate (Randall et al. 1997; Entz et al. 2001), and (2) reducing the risk of rising water tables and the development of dryland salinity (Angus et al. 2001; Lefroy et al. 2001).

Conclusions Annual crop legumes do not seem particularly attractive for bioethanol production due to their low starch yield per unit area. Perennial legumes such as alfalfa, on the other hand, offer an interesting resource for future second-generation bioethanol production either as sole crop or in mixed cropping with high-yielding non-legume species.

3.2.3 Biodiesel

Biodiesel is produced by the transesterification of the glyceride molecules in plant oils by methanol to produce glycerine and methyl esters (Mabee et al. 2006), which can readily be used in diesel engines. The production of glycerine and the need for methanol detract somewhat from the attractiveness of biodiesel. New chemical procedures are now in place allowing the production of liquid biofuels (such as aviation fuel) out of plant oils without esterification. The nature of the fatty acid composition controls critical physical properties, such as the cloud point (the temperature at which the diesel will turn cloudy and thus clog injection systems). A low concentration of palmitic (C16:0) and stearic (C18:0) acids and a high concentration of oleic (C18:1) acid is optimal (Kazakoff et al. 2011).

Brazil produced 58 million Mg of soybean in 2007, and extracted 5.7 million Mg of oil, part of which was used for biodiesel production (Elbersen et al. 2010). One megagram of soybean yields about 170 L of biodiesel; consequently, the potential of Brazilian biodiesel production from soybean is about 10 billion liters if all the oil was used for this purpose. In comparison, the global biodiesel production level in 2006 was 2.7 million liters, with Germany being the largest producer of about 1.2 million liters derived from canola (Mabee et al. 2006).

Soybean in Brazil and Argentina is estimated to obtain approximately 80% of its N from BNF (Herridge et al. 2008). In the USA (the world's largest producer), soybean reliance upon BNF for growth is somewhat less (~60%) due to the more fertile soils in the midwest and the residual N fertilizer from maize crops in the rotation. China may have even lower inputs of fixed N by soybean as yields are lower and N fertilizer use is higher (Herridge et al. 2008).

Embrapa Agrobiologia has developed an energy balance for soybean production under typical mechanized farming practice in the Cerrado region of Brazil based on the national average grain yield of 2.82 Mg ha⁻¹ with a total oil yield of 18% (Table 11; Boddey et al. 2009; IBGE 2011; Soares et al. 2007). Since soybean must be re-planted each year, agricultural and transport energy costs proportionately make an important contribution to total fossil energy use (Table 11). Fossil energy inputs for fertilizer are low as little N fertilizer is applied. While fuel inputs under no-till systems were much lower than under conventional tillage, much of the economy in fossil fuel was compensated for by the energy required for herbicide manufacture and application (assumed to be glyphosate in this case). When the fossil energy costs for transesterification is included, the energy balance of biodiesel energy/non-renewable energy is almost unity (1.06:1), suggesting no overall GHG mitigation effect (Table 11). At the same time, >2.3 Mg ha⁻¹ of

high-protein soybean meal was co-produced with an energy content of 38.7 GJ ha⁻¹. If the energy in this high-protein by-product is added to the biodiesel produced, the energy balance becomes much more favorable at 3.2:1 (Table 11), but still compares poorly to estimates of 9.07:1 determined for sugarcane using the same methodologies (Boddey et al. 2008). Unfortunately, and in contrast to the Pimentel and Patzek (2005) study, the energy required to build and maintain the factory infrastructure for processing was not included in the Brazilian calculations for soybean, nor were they considered in similar energy balance study undertaken for soybean biodiesel in the USA which cited a similar energy balance (3.2:1; Sheehan et al. 1998).

The fast-growing legume tree *Pongamia pinnata* (also called *Millettia pinnata*) may be a significant future source of oil for production of biodiesel since the seeds contain around 40% oil, with the predominant fatty acids being oleic, palmitic, stearic, and linoleic (Scott et al. 2008; Kazakoff et al. 2011). *Pongamia* oil is non-edible, but is not toxic to humans. *Pongamia* oil contains about 50–55% oleic acid, with about 7–10% palmitic and stearic acid.

While *Pongamia* is tropical in origin, it will grow and yield well in the coastal Brisbane area of Queensland, Australia (Fig. 13). In winter, it goes dormant allowing it to survive temperatures as low as -5°C (Wilkinson et al. 2012). An initial life cycle and growth analysis of a *Pongamia* plantation at Gatton, on the tablelands of southern Queensland, found that up to 500 flowers developed per tree within 2 years of planting. At a tree density of about 350–450 trees ha⁻¹, 10-year-old trees are capable of producing an oil yield of around 5 Mg ha⁻¹ per year (Scott et al. 2008; Fig. 14). Seeds are mechanically harvested and can be stored before solvent or cold press oil extraction.

The *Pongamia* trees are extremely drought tolerant, owing to their deep root system, waxy leaf, and favorable stress physiology. They are also salt tolerant, so they could be grown on margin lands and in soils unsuited for food production (Wilkinson et al. 2012). In addition to high oil content seeds, the *Pongamia* tree may supply biomass for other biobased applications. The seed pod (casing) is of equal mass to the single seed contained in it and has application in co-firing in electricity plants. The seed cake, after oil extraction, can yield protein concentrate for low quality animal feed supplement (especially ruminants), second generation bioethanol, biogas, or thermochemical conversion and production of biochar (see Section 3.2.4).

Conclusions If soybean is planted solely for the purpose of biodiesel manufacture, there is unlikely to be significant GHG mitigation benefits. If the objective is to produce high-protein feed, and the oil is a by-product, then energy balance may be as high as 3.5:1 and could represent an important GHG mitigation benefit. The legume tree

Table 11 Fossil energy inputs, total energy yield, and energy balance for soybean diesel produced on 1 ha of land under standard Brazilian zero-till management systems

	Units per hectare	Amount	Energy (GJ)
Operation			
1. Field preparation			
Herbicides	L	3.8	1.80
Mineral oil adjuvant	L	0.5	0.02
Seeds for pasture	kg	20	0.03
Lime	kg	1000	1.17
2. Crop establishment			
Soybean seeds	kg	50	1.65
Fungicides	L	0.3	0.12
Inoculant	Dose	2.0	0.01
Fertilizer 00–20–20	kg	500	0.62
3. Crop management			
Ant baits	kg	1.0	0.36
Herbicides	kg	0.65	0.29
Insecticides	L	1.65	0.60
Fungicides	L	1.5	0.45
4. Machinery			
Field operations and transport	h	2.8	2.60
5. Farm labor			
Total	h	8	1.34
6. Fuel			
Diesel oil	L	58	2.76
Total			13.82
Factor			
Soybean bioenergy produced			
Soybean oil	kg	509	19.2
Soybean meal	kg	2,316	38.7
Total bioenergy yield			57.9
Fossil energy use			
Agricultural energy costs			(13.8)
Transesterification (biodiesel production)			(4.3)
Total fossil energy use			(18.1)
Final energy balance for soybean biodiesel ^a			1.06:1.0
Total energy balance including soy meal ^b			3.20:1.0

Grain yield was assumed to be the 2007 national average grain yield (2,824 kg ha⁻¹) with a total oil content of 18% resulting in 480 L of biodiesel per hectare

^aRatio of energy yield of soybean biodiesel to fossil energy used in crop production and transesterification

^bRatio of total bioenergy yield of both biodiesel and soy meal by-product to fossil energy used in crop production and transesterification

Pongamia pinnata offers an interesting possibility for future biodiesel production with potential applications for legume-based biorefineries.

3.2.4 Biogas and digestate for fertilizer from anaerobic digestion

Anaerobic digestion is a key technology for the sustainable use of organic biomasses from industrial and urban organic wastes, animal manures, crop residues, and whole energy crops (Amon et al. 2007). Anaerobic digestion is particularly well suited for heterogeneous feedstock. It sanitizes the feedstock and can be applied at scales from the farm to

big industrial plants. The biogas (mixture of CH₄ and CO₂) produced can be converted to electricity, heat, or upgraded to liquid biofuel for vehicles (Amon et al. 2007; Lehtomäki et al. 2008). Within the European Union, biogas production increased 6-fold from 1995 to 2005 (Eurostat 2007). Simultaneously, the digest residues consisting of nutrients and recalcitrant C enables almost complete nutrient recycling in the system, including N. The digestate can be used as a fertilizer and enhance the synchrony of plant-available N and crop N demand since a major part of the organic N is mineralized to ammonium (Möller et al. 2008).

Legume biomass has as slightly lower CH₄ production potential, as determined in batch fermentation assays, than maize and grass biomasses (Table 12). The CH₄ production

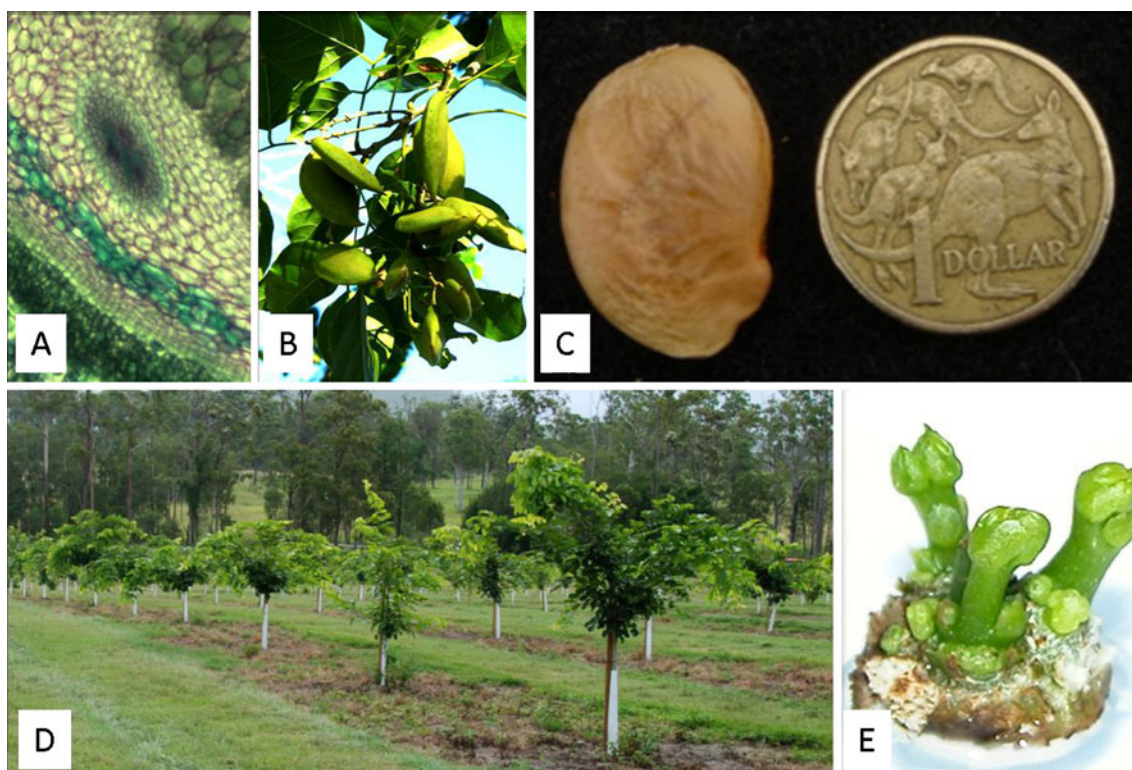


Fig. 13 *Pongamia pinnata*. **A** *Pongamia* nodule section showing infected, cortical, and vascular tissue. **B** Bunch of near-maturity *Pongamia* (*Milletia*) *pinnata* seed pods; up to 25 tons of total seed plus pod wall biomass coming from about 20,000 seeds per tree can be obtained per hectare on an annual basis with elite germplasm. Normally 400–500 trees are planted per hectare. **C** Seed of *Pongamia* (1.8 g average dry weight; 40–45% solvent extractable oil). **D** Rainfed *Pongamia* plantation (28 months old in southeastern Queensland, Australia). **E** *Pongamia* vegetative regeneration in cell culture. Photos: P. M. Gresshoff, CILR

depends on the biochemical composition of the biomass, mainly crude protein, crude fat, crude fiber, and N-free extract, and these four parameters can be used to estimate the CH_4 yield from any biomass (Amon et al. 2007). Crude protein and fat are frequently the most important factors and optimal CH_4 production occurs at C/N ratios of 10 to 30 in the digester (Amon et al. 2007). Thus, high C/N biomass feedstock low in crude protein and fat, such as maize and grasses, could benefit by being enriched with crude protein by mixing legume foliage from species like clover or



Fig. 14 Mature *Pongamia* (*Milletia*) *pinnata* tree in South East Queensland. Photo: P. Scott, CILR

alfalfa to improve efficiencies. But sole crop legume biomass with a high N content would not be optimal either because the concentration of NH_4 in the reactor may become too high and stop the digestion process.

Currently, biogas is produced from monoculture maize feedstock in many European countries, but legume-based energy crops for biogas should be integrated in sustainable crop rotations (Amon et al. 2007), eventually as double cropping systems within the same growing season (Karpenstein-Machan 2001; Fig. 15). Anaerobic digestion is an optimal conversion technology containing legume biomass since the valuable content of N, P, and other nutrients is exploited efficiently via the digestate biofertilizer.

Conclusion Legume biomass is well suited for the production of biogas when mixed with other species since the N and other nutrients in the digestate can be used as a valuable biofertilizer.

3.2.5 Thermochemical conversion for production of heat, syngas, biooil, and biochar

Combustion of a biomass with a high content of N, such as legumes biomass, is not likely to be a sustainable conversion

Table 12 Comparisons of the methane (CH₄) potential of grass or cereal sources to selected legumes and legume containing biomasses

Species	CH ₄ potential (m ³ kg ⁻¹ volatile solids) ^a	Reference
Maize	0.38	Banks (2007)
Ryegrass	0.37	
Alfalfa	0.34	
Clover spp.	0.35	
Lupin spp.	0.33	
Faba bean	0.36	
Pea	0.39	
Vetch	0.28	
Fresh maize	0.43	Oleskowicz-Popiel (2010)
Fresh clover	0.38	
Clover silage	0.40	
Grasses	0.34	Lehtomäki et al. (2008)
Fresh Timothy-red clover ^b mix (10% legume)	0.37	
Red clover	0.29	
Vetch–oat ^c (50% legume)	0.41	
Lupin ^d	0.34	
Maize (silage)	0.39	Amon et al. (2007)
Fresh grass–clover (% legume not determined)	0.34	
Alfalfa	0.34	

^a Volatile solids: dry biomass—ashes (550°C)

^b *Phleum pratense*—*Trifolium pretense*

^c *Vicia sativa*—*Avena sativa*

^d *Lupinus polyphyllus*

technology due to the potential losses of limited nutrients such as P and K along with the ash and the formation of nitrogen oxides (NO and NO₂), which have GHG potential similar to N₂O (Peoples et al. 2004b; Ceotto 2008). Tree legumes such as *L. leucocephala*, *G. sepium*, and *Acacia* spp. are already used as fuel wood and can be important energy sources for households in developing countries (Ryan 1994; Kazakoff et al. 2010; Fig. 16). Key fuel wood characteristics are moisture content and energy density, but consideration should also be made for flame retardants in some species (e.g., *Sesbania grandiflora*) and if possible also the nutrient cycling aspects



Fig. 15 Grass–legume biomass stored as silage to be used continuously as a feedstock for an on-farm biogas plant. Photo: E. S. Jensen, SLU

mentioned above since large amounts of nutrients may be removed from the system with the fuel wood and ash if these are not recycled (Ryan 1994).

Pyrolysis of biomass involves the combustion of the biomass without oxygen and results in syngas, biooil, and biochar. The production ratio of these components depends on the biomass characteristics and the pyrolysis temperature (Bruun et al. 2011). The syngas and biooil can be combusted with oxygen or upgraded to biofuels for vehicles. The biochar, which contain the majority of nutrients except N and



Fig. 16 Multifunctional and multipurpose *Acacia abyssinica* in an Ethiopian highland (c. 2,000 m) village. The tree is grown in field margins and pruned for goat feed (due to its thorny nature) and fuel wood. Photo: E. S. Jensen, SLU

S, can be used to recycle nutrients and provide long-term C sequestration in soil since the C in the biochar is rather recalcitrant to microbial decomposition (Lehmann et al. 2006). Pyrolyzing straw from high biomass soybeans at 500°C resulted in around 70% biooil, 20% biochar, and 10% syngas (Boateng et al. 2010). Boateng et al. (2008) found that the pyrolysis of alfalfa stems produced a lower output of biooil, but slightly higher amounts of biochar than soybean, although the oil was found to have a higher energy content.

Conclusions Legume tree or residue biomasses could be used in thermochemical conversion processes, but it is important to consider that the N and S may be lost from the system. However, in biorefinery concepts, the final conversion process may be a thermochemical or versatile biochemical process such as anaerobic digestion to efficiently recycle the remaining carbohydrates and nutrients in the digestate.

3.2.6 Materials and chemicals

In a biobased economy, there is a requirement to replace products other than energy carriers currently derived from fossil hydrocarbons. Biomass may contribute building blocks for chemicals, biomaterials, and biopharmaceuticals. For example, 56 Tg of textiles were produced worldwide in 1999 of which 54% was synthetic chemical fibers based on fossil hydrocarbons (Lorek and Lucas 2003). Traditionally, legumes have been considered as a source of dietary oil, protein, and fiber for humans and livestock, but legumes contain many constituents that are essentially similar to other sources of biomass—sugars, amino acids, phytochemicals, lignin, tannins, etc. (Fig. 4)—which can be used as building block chemicals to produce surfactants, biopolymers, glues, and a variety of industrial chemicals which are now produced in petrochemical refineries (De Jong et al. 2010). Some natural products are either found only in legumes or in high concentrations in legumes that are of potential use as nutri- or pharmaceuticals, or biopesticides in addition to industrial purposes, and there could be significant benefits in extracting some of the higher value compounds in a biorefinery (Dixon and Sumner 2003; Duranti et al. 2008). For example, there are several reports of a possible role for the use of legume seed proteins to control metabolic disorders. These include the cholesterol-lowering effect of soybean 7S globulin α' subunit and the immobilization of insulin by lupin conglutin γ to control glycemia (Magni et al. 2004; Duranti et al. 2008). Legumes also produce isoflavones which reputedly have estrogenic, anti-angiogenic, antioxidant, and anti-cancer activities, and an ability to prevent osteoporosis and cardiovascular diseases (Dixon and Sumner 2003). Condensed tannins and polyphenols present in legume seed coats are antioxidants with

potential health beneficial effects for cardiac health and immunity, and it has recently been reported that phenolic compounds from faba bean can inhibit human cancer cells (Dixon and Sumner 2003; Siah et al. 2011). Triterpene saponin, which is present in alfalfa, deters herbivore grazing, but these saponins also display allelopathic, antimicrobial, and anti-insect activity, which can be used in other contexts such as surfactants and foaming agents (Dixon and Sumner 2003). The variety of food and non-food products that have been successfully developed and marketed from soybean illustrates what may be possible for other legumes.

Conclusion Legumes are known to contain proteins and bioactive substances, which could be extracted in future biorefineries and used as industrial chemicals, food ingredients or pharmaceuticals, surfactants and bioplastics.

4 Concluding remarks

Legumes are unique plants. They contribute many different functions and ecosystems services that are of great value for agriculture and society (Jensen and Hauggaard-Nielsen 2003; Crews and Peoples 2004; Peoples et al. 2009b; Köpke and Nemecek 2010; De Faria et al. 2011). Including legume food, forage, and tree crops in farming systems is one approach that can contribute to mitigating climate change. Our review of the literature indicates that the ability of the legumes to fix their own N via a symbiosis with rhizobia bacteria reduces emissions of fossil energy-derived CO₂ and results in lower N₂O fluxes compared to cropping and pasture systems that are fertilized with industrial N. Less quantitative data are available concerning N₂O losses from legume residues following a legume phase in a cropping sequence. It was concluded that while the potential losses of N₂O can be large from leguminous residues containing high concentrations N such as nodules, or fresh foliage, the contribution of N₂O emissions from senesced vegetative residues remaining after grain harvest of a crop legume can be small. Further work is needed to better understand how the management and quality of legume residues affects N₂O emissions in subsequent crops.

In addition to legumes resulting in lower GHG emissions, they also appear to play a key role in soil C sequestration. The inclusion of herbaceous legumes in pastures, and either as sole crops, green- or brown-manures, cover-crops, or intercrops in reduced tillage cropping systems, has been shown to enhance soil C accumulation. Woody perennial legume species have also been demonstrated to be extremely useful for revegetating cleared and degraded land to replenish soil organic C stocks.

In the short term, it is unlikely that sole crop annual legumes will be used as biorefinery feedstock due to their relatively low DM yield. Legumes are important compo-

nents of future diversified and sustainable cropping systems, which are not in direct competition for land with food production. Many legumes can be produced on marginal/surplus lands and on degraded or drastically disturbed soils. Perennial legumes (alfalfa, clover, and various tree and shrub species) could have unique roles in generating biomass for biorefineries, without the requirement for N fertilization either as sole crops or in mixtures with grasses.

Advances in conversion/biorefinery technologies will be required which can add value to the by-products of energy generation by extracting and exploiting the high protein content of legume biomass. Examples of potential technologies and products suited for legume biomass include protein extraction for feed (e.g., soybean cake) or pharmaceuticals, renewable materials production, and anaerobic digestion for CH₄ production with the simultaneous production of a biofertilizer containing nutrients for recycling.

Residues from arable crops provide an essential function in maintaining soil fertility, preventing soil erosion and structure in arable soil (Lal and Pimentel 2007). Consequently, the use of legume biomass for bioenergy, materials, and chemicals represents a significant trade-off since the contribution of legume residues to soil organic fertility and C sequestration would be significantly reduced. National strategies for using straw and other residues for biofuels will need to identify the regions that have soils with sufficient organic matter levels to allow the temporary utilization of the straw/stover for bioenergy that are also at low risk of erosion (Nelson 2002). The huge world acreage of soybean potentially could generate massive amounts of organic residues. In the USA, McMurtrey et al. (2005) showed that no soybean residues could be sustainably removed after growing conventional soybean types with conventional tillage practice, whereas between 7% and 30% of the residues could be removed with reduced tillage and zero-tillage scenarios, respectively.

Future sustainable agricultural systems require novel management and plant breeding solutions to assist society with climate change mitigation options for producing biofuels, materials, and chemicals. One of the key paradigms for future sustainable agriculture is multifunctionality of system and crops. Agriculture will need to supply several services from the use of the same piece of land, and the key principle to obtain this is diversity in time and space, involving cropping systems as well as crop species. Thus, legume species, with their multiple arrays of potential ecosystems services combined with their ability to reduce GHG emissions and encourage soil C sequestration, should be given careful consideration as important components of future sustainable food, fiber, and energy production systems for human prosperity.

Acknowledgments Erik S. Jensen wishes to acknowledge that participation in the Fifth International Food Legumes Research Conference helped facilitate the preparation of this review. Mark B.

Peoples is indebted for the support of the Grains Research and Development Corporation (GRDC) to continue research into the role of legumes in Australian farming systems. Peter M. Gresshoff thanks the Australian Research Council (ARC) for a Centre of Excellence grant, the University of Queensland Strategic Fund for continued support, Dr. Paul Scott for academic input, and the Bioenergy Research Pty Ltd for commercial partnership in Pongamia-related research efforts. Malcolm J. Morrison wishes to thank Agriculture and Agri-Food Canada for financial support, Dr. Ed Gregorich and Pat St George from Agriculture and Agri-Food Canada who assisted in the collection of the N₂O data depicted in Fig. 2 along with Brian Couture and Randy Hodgins for field work and technical support.

References

- Amon T, Amon B, Kryvoruchko V, Machmüller A, Hopfner-Sixt K, Bodiroza V, Hrbek R, Fridel J, Pötsch E, Wagentristl H, Schreiner M, Zollitsch W (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Biores Technol* 98:3204–3212
- Angus JF, Gaul RR, Peoples MB, Stapper M, van Hwarden AF (2001) Soil water extraction by dryland crops, annual pastures and lucerne in south-eastern Australia. *Aust J Agric Res* 52:183–192
- Armstrong RD, Kickoff BJ, Millar G, Whitbread AM, Standley J (1999) Changes in soil chemical and physical properties following legumes and opportunity cropping on a cracking clay soil. *Aust J Exp Agric* 39:445–456
- Arrouays D, Deslais W, Bateau V (2001) The carbon content of topsoil and its geographical distribution in France. *Soil Use Manag* 17:7–11
- Atkins CA (1984) Efficiencies and inefficiencies in the legume/Rhizobia symbiosis—a review. *Plant Soil* 82:273–284
- Aulakh MS, Khara TS, Doran JW, Bronson KF (2001) Denitrification, N₂O and CO₂ fluxes in rice–wheat cropping system as affected by crop residues, fertilizer N and legume green manure. *Biol Fertil Soils* 34:375–389
- Baggs EM, Stevenson M, Pihlatie M, Regar A, Cook H, Cadisch G (2003) Nitrous oxide emissions following application of residues and fertilizer under zero and conventional tillage. *Plant Soil* 254:361–370
- Baker JM, Ochsner TE, Venterea RT, Griffis TJ (2007) Tillage and carbon sequestration—what do we really know? *Agric Ecosyst Environ* 118:1–5
- Banks C (2007) Renewable energy from crops and agrowastes (CROPGEN). Final report. 32 p. http://www.cropgen.soton.ac.uk/deliverables/CROPGEN_P FAR2007.pdf (accessed 24 February 2011)
- Barthès B, Azontonde A, Blanchart E, Girardin C, Villenave C, Lesaint S, Oliver R, Feller C (2004) Effect of a legume cover crop (*Mucuna pruriens* var. *utilis*) on soil carbon in an Ultisol under maize cultivation in southern Benin. *Soil Use Manag* 20:231–239
- Barton L, Kiese R, Gatter D, Butterbach-Bahl K, Buck R, Hinz C, Murphy DV (2008) Nitrous oxide emissions from a cropped soil in a semi-arid climate. *Global Change Biol* 14:177–192
- Barton L, Murphy DV, Kiese R, Butterbach-Bahl K (2010) Soil nitrous oxide and methane fluxes are low from a bioenergy crop (canola) grown in a semi-arid climate. *Global Change Biol Bioenergy* 2:1–15
- Barton L, Butterbach-Bahl K, Kiese R, Murphy DV (2011) Nitrous oxide fluxes from a grain–legume crop (narrow-leafed lupin) grown in a semiarid climate. *Global Change Biol* 17:1153–1166

- Bayer C, Mielniczuck J, Amado TJC, Martin-Neto L, Fernandes SBV (2000) Organic matter storage in a sandy clay loam Acrisol affected by tillage and cropping systems in southern Brazil. *Soil Tillage Res* 54:101–109
- Bergersen FJ, Brockwell J, Gault RR, Morthorpe L, Peoples MB, Turner GL (1989) Effects of available soil nitrogen and rates of inoculation on nitrogen fixation by irrigated soybeans and evaluation of $\delta^{15}\text{N}$ methods for measurement. *Aust J Agric Res* 40:761–780
- Bertelsen F, Jensen ES (1992) Gaseous nitrogen losses from field plots grown with pea (*Pisum sativum* L.) or spring barley (*Hordeum vulgare* L.) estimated by ^{15}N mass balance and acetylene inhibition techniques. *Plant Soil* 142:287–295
- Bessou C, Ferchaud F, Gabrielle B, Mary B (2010) Biofuels, greenhouse gases and climate change. A review. *Agron Sustain Dev*. doi:10.1051/agro/2009039
- Blasing TJ (2010) Recent greenhouse gas concentrations. Carbon Dioxide Information Analysis Center Oak Ridge National Laboratory. doi:10.3334/CDIAC/atg.032; http://cdiac.ornl.gov/pns/current_ghg.html (accessed 1 July 2010).
- Boateng AA, Mullen CA, Goldberg NM, Hicks K, Devine TE, Lima IM, McMurtry JE (2010) Sustainable production of bioenergy and biochar from the straw of high-biomass soybeans lines via fast-pyrolysis. *Environ Prog Sustain Energ* 29:175–183
- Boateng AA, Mullen CA, Goldberg NM, Hicks KB, Jung HG, Lamb JF (2008) Production of bio-oil from alfalfa stems by fluidized-bed fast pyrolysis. *Ind Eng Chem Res* 47:4115–4122
- Boddey RM, Xavier DF, Alves BJR, Urquiaga S (2003) Brazilian agriculture: the transition to sustainability. *J Crop Prod* 9:593–621
- Boddey RM, de Soares B, Alves BJR, Urquiaga S (2008) Bio-ethanol production in Brazil. In: Pimentel D (ed) *Biofuels, solar and wind as renewable energy systems: benefits and risks*. Springer, New York, pp 321–356
- Boddey RM, Alves BJR, Soares LHB, Jantalia CP, Urquiaga S (2009) Biological nitrogen fixation and the mitigation of greenhouse gas emissions. In: Emerich DW, Krishnan HB (eds) *Agronomy Monograph 52. Nitrogen Fixation in Crop Production*, Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc Am. Madison, Wisconsin, USA, pp. 387–413
- Boddey RM, Jantalia CP, Zanatta JA, Conceição PC, Bayer C, Mielniczuck J, Dieckow J, dos Santos HP, Denardin JE, Aita C, Alves BJR, Urquiaga S (2010) Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture in southern Brazil. *Global Change Biol* 16:784–795
- Bouwman AF (1996) Direct emissions of nitrous oxide from agricultural soils. *Nutr Cycl Agroecosyst* 46:53–70
- Brehmer B, Struik PC, Sanders J (2008) Using an energetic and exergetic life cycle analysis to assess the best application of legumes within a biobased economy. *Biomass Bioenerg* 32:1175–1186
- Breitenbeck GA, Bremner JM (1989) Ability of free-living *Bradyrhizobium japonicum* to denitrify nitrate in soils. *Biol Fertil Soils* 7:219–224
- Bremer E, van Kessel C (1992) Seasonal microbial biomass dynamics after addition of lentil and wheat residues. *Soil Sci Soc Am J* 56:1141–1146
- Bruun EW, Hauggaard-Nielsen H, Ibrahim N, Egsgaard H, Ambus P, Jensen PA, Dam-Johansen K (2011) Influence of fast pyrolysis temperature on biochar labile fraction and short-term carbon loss in a loamy soil. *Biomass Bioenerg* 35:1182–1189
- Carroll BJ, McNeil DL, Gresshoff PM (1985) Isolation and properties of soybean mutants which nodulate in the presence of high nitrate concentrations. *Proc Natl Acad Sci USA* 82:4162–4166
- Ceotto E (2008) Grasslands for bionenergy production. A review. *Agron Sustain Dev* 28:47–55
- Chaer GM, Resende AS, de Balieiro FC, Boddey RM (2011) Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. *Tree Physiol* 31:139–149
- Chalk PM (1998) Dynamics of biologically fixed N in legume–cereal rotations: a review. *Aust J Agric Res* 49:303–316
- Chan KY (1997) Consequences of changes in particulate organic carbon in vertisols under pasture and cropping. *Soil Sci Soc Am J* 61:1376–1382
- Chan KY, Conyers MK, Li GD, Helyar KR, Poile G, Oates A, Barchia IM (2011) Soil carbon dynamics under different cropping and pasture management in temperate Australia: results of three long-term experiments. *Soil Res* 49:320–328
- Chen S, Huang Y, Zou J (2008) Relationship between nitrous oxide emission and winter wheat production. *Biol Fertil Soils* 44:985–989
- Cherubini F, Jungmeir G, Wellisch M, Wilke M, Skiadas I, Van Ree R, de Jong E (2009) Towards a common classification approach for biorefinery systems. *Biofuels Bioprod Bioref* 3:534–546
- Christopher SF, Lal R (2007) Nitrogen management affects carbon sequestration in North American cropland soils. *Crit Rev Plant Sci* 26:45–64
- Ciampitti IA, Ciarlo EA, Conti ME (2008) Nitrous oxide emissions from soil during soybean [*Glycine max* (L.) Merrill] crop phenological stages and stubbles decomposition period. *Biol Fertil Soils* 44:581–588
- Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochem* 85:235–252
- Conant RT, Paustian K, Elliot ET (2001) Grassland management and conversion into grassland: effects on soil carbon. *Ecol Appl* 11:343–355
- Crews TE, Peoples MB (2004) Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. *Agric Ecosyst Environ* 102:279–297
- Crews TE, Peoples MB (2005) Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. *Nutr Cycl Agroecosyst* 72:101–120
- Dalal RC, Chan KY (2001) Soil organic matter in rainfed cropping systems of the Australian cereal belt. *Aust J Soil Res* 39:435–464
- Dalal RC, Strong WM, Weston EJ, Cooper JE, Lehane KJ, King AJ, Chikien CJ (1995) Sustaining productivity of a vertisol at Warra, Queensland, with fertilizers, no-tillage, or legumes. 1. Organic matter status. *Aust J Exp Agric* 35:903–913
- Dale BE (1983) Biomass refining: protein and ethanol from alfalfa. *Ind Eng Chem Prod Res Dev* 22:466–472
- Danish Feed Analysis (2005) Feed tables 2005. Eds. J. Møller. 49 p. (in Danish)
- De Faria SM, Resende AS, Saggin Júnior OJ, Boddey RM (2011) Exploiting mycorrhizae and rhizobium symbioses to recover seriously degraded soils. In: Polacco JC, Todd CD (eds) *Ecological aspects of plant nitrogen metabolism*. Wiley-Blackwell, New York, pp 195–214
- De Jong E, van Ree R, Sanders JPM, Langeveld JWA (2010) Biorefineries: giving value to sustainable biomass use. In: Langeveld H, Sanders J, Meeusen M (eds) *The biobased economy—biofuels, materials and chemicals in the post-oil era*. Earthscan, London
- Dean W (1995) *With Broadax and Firebrand: the destruction of the Brazilian Atlantic Forest*. University of California Press, Berkeley
- Diekow J, Mielniczuck J, Knicker H, Bayer C, Dick DP, Kogel-Knabner I (2005) Soil C and N stocks as affected by cropping systems and nitrogen fertilization in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil Tillage Res* 81:87–95
- Dixon RA, Sumner LW (2003) Legume natural products: understanding and manipulating complex pathways for human and animal health. *Plant Physiol* 131:878–885
- Duke JA (1981) *Handbook of legumes of world economic importance*. Plenum, New York

- Duke JA (1983) Handbook of Energy Crops. http://www.hort.purdue.edu/newcrop/duke_energy/dukeindex.html (Accessed 23 February 2011).
- Duranti M, Consonni A, Magni C, Sessa F, Scarafoni A (2008) The major proteins of lupin seed: characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends Food Sci Technol* 19:624–633
- Elbersen HW, Bindraban PS, Blaauw R, Jongman R (2010) Biodiesel from Brazil. In: Langeveld H, Sanders J, Meeusen M (eds) *The biobased economy—biofuels, materials and chemicals in the post-oil era*. Earthscan, London, pp 283–301
- Entz MH, Bullied WJ, Forster DA, Gulden R, Vessey JK (2001) Extraction of subsoil nitrogen by alfalfa, alfalfa–wheat and perennial grass systems. *Agron J* 93:495–503
- Eswaran H, Vandenberg E, Reich P (1993) Organic carbon in soils of the world. *Soil Sci Soc Am J* 57:192–194
- Eurostat (2007) Gas and electric market statistics. European Communities, Office for Official Publications of the European Communities, Luxembourg
- Evans J, Scott G, Lemerle D, Kaiser A, Orchard B, Murray GM, Armstrong EL (2003) Impact of legume ‘break’ crops on the residual amount and distribution of soil mineral nitrogen. *Aust J Agric Res* 54:763–776
- Fairley P (2011) Next generation biofuels. *Nature* 474:S2–S5
- FAOstat (2010) <http://faostat.fao.org/> (Accessed 20 September 2010)
- Ferguson BJ, Indrasumunar A, Hayashi S, Lin M-H, Lin Y-H, Reid DE, Gresshoff PM (2010) Molecular analysis of legume nodule development and autoregulation. *J Integr Plant Biol* 52:61–76
- Fillery IRP (2001) The fate of biologically fixed nitrogen in legume-based dryland farming systems: a review. *Aust J Exp Agric* 41:361–381
- Finke RL, Harper JE, Hageman RH (1982) Efficiency of nitrogen assimilation by N₂-fixing and nitrate-grown soybean plants (*Glycine max* [L.] Merr.). *Plant Physiol* 70:1178–1184
- Fisher MJ, Rao IM, Ayarza MA, Lascono CE, Sanz JI, Thomas RJ, Vera RR (1994) Carbon storage by introduced deep-rooted grasses in South American savannas. *Nature* 371:236–238
- Fisher MJ, Braz SP, dos Santos RSM, Urquiaga S, Alve BJR, Boddey RM (2007) Another dimension to grazing systems: soil carbon. *Trop Grassl* 41:65–83
- Franco AA, de Faria SM (1997) The contribution of N₂-fixing tree legumes to land reclamation and sustainability in the tropics. *Soil Biol Biochem* 29:897–903
- Freixo AA, de Machado PLOA, dos Santos HP, Silva CA, de Fadigas FS (2002) Soil organic carbon and fractions of a Rhodic Ferrasol under the influence of tillage and crop rotation systems in southern Brazil. *Soil Tillage Res* 64:221–230
- Gaiser T, Stahr K, Bernard M, Kang BT (2011) Changes in soil carbon fractions in a tropical Acrisol as influenced by the addition of different residue materials. *Agroforest Syst*. doi:10.1007/s10457-011-9417-0
- Galiana A, Gnahoua GM, Chaumont J, Lesueur D, Prin Y, Mallet B (1998) Improvement of nitrogen fixation in *Acacia mangium* through inoculation with rhizobium. *Agroforest Syst* 40:297–307
- Gan Y, Liang C, Wang X, McConkey B (2011) Lowering carbon footprint of durum wheat by diversifying cropping systems. *Field Crops Res* 122:199–206
- García-Plazaola JI, Becerril JM, Arrese-Igor C, Hernandez A, Gonzalez-Murua C, Aparicio-Tejo PM (1993) The contribution of *Rhizobium meliloti* to soil denitrification. *Plant Soil* 157:207–213
- Gardner JB, Drinkwater LE (2009) The fate of nitrogen in grain cropping systems: a meta-analysis of ¹⁵N field experiments. *Ecol Appl* 19:2167–2184
- Goossens A, De Visscher A, Boeckx P, Van Cleemput O (2001) Two-year field study on the emission of N₂O from coarse and middle-textured Belgian soils with different land use. *Nutr Cycl Agroecosyst* 60:23–34
- Grace PR, Oades JM, Keith H, Hancock TW (1995) Trends in wheat yields and soil organic carbon in the permanent rotation trial at the Waite Agricultural Research Institute, South Australia. *Aust J Exp Agric* 35:857–864
- Gregorich EG, Drury CF, Baldock JA (2001) Changes in soil carbon under long-term maize in monoculture and legume-based rotation. *Can J Soil Sci* 81:21–31
- Gresshoff PM, Lohar D, Chan PK, Biswas B, Jiang Q, Reid D, Ferguson B, Stacey G (2009) Genetic analysis of ethylene regulation of legume nodulation. *Plant Signal Behav* 4:9
- Hammerschlag R (2006) Ethanol energy return on investment: a survey of the literature 1990–present. *Environ Sci Technol* 40:1744–1750
- Hauggaard-Nielsen H, Ambus P, Jensen ES (2003) The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. *Nutr Cycl Agroecosyst* 65:289–300
- Hauggaard-Nielsen H, Gooding M, Ambus P, Corre-Hellou G, Crozat Y, Dahlmann C, Dibet A, von Fragstein P, Pristeri A, Monti M, Jensen ES (2009) Pea–barley intercropping for efficient symbiotic N₂-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. *Field Crop Res* 113:64–71
- Heenan DP, McGhie WJ, Thompson F, Chan KY (1995) Decline in soil organic carbon and total nitrogen in relation to tillage, stubble management and rotation. *Aust J Exp Agric* 35:877–884
- Helgason BL, Janzen HH, Chantigny MH, Drury CF, Ellert BH, Gregorich EG, Lemke RL, Pattey E, Rochette P, Wagner-Riddle C (2005) Toward improved coefficients for predicting direct N₂O emissions from soil in Canadian agroecosystems. *Nutr Cycl Agroecosyst* 72:87–99
- Hénault C, Devis X, Lucas JL, Germon JC (1998) Influence of different agricultural practices (type of crop, form of N-fertilizer) on soil nitrous oxide emissions. *Biol Fertil Soils* 27:299–306
- Herridge DF, Marcellos H, Felton WL, Turner GL, Peoples MB (1995) Chickpea increases soil-N fertility in cereal systems through nitrate sparing and N₂ fixation. *Soil Biol Biochem* 27:545–551
- Herridge DF, Peoples MB, Boddey RM (2008) Marschner review: global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 311:1–18
- Hoyle FC, Baldock JA, Murphy DV (2011) Soil organic carbon—role in Australian farming systems. In: Tow P, Cooper I, Partridge I, Birch C (eds) *Rainfed farming systems*. Springer, Heidelberg, in press
- IAASTD (2009) Executive summary of the synthesis report. International Assessment of Agricultural Knowledge, Science and Technology for Development. Intergovernmental plenary Johannesburg, South Africa (7–11 April 2008). 34 p
- IBGE (2011) Instituto Brasileiro de Geografia e Estatística. Retrieved August 2011, from [http://www.sidra.ibge.gov.br/bda/default.asp? t=5&z=t&o=1&u1=1&u2=1&u3=1&u4=1&u5=1&u6=1&u7=1&u8=1&u9=3&u10=1&u11=26674&u12=1&u13=1&u14=1](http://www.sidra.ibge.gov.br/bda/default.asp?t=5&z=t&o=1&u1=1&u2=1&u3=1&u4=1&u5=1&u6=1&u7=1&u8=1&u9=3&u10=1&u11=26674&u12=1&u13=1&u14=1) (Accessed 14 August 2011)
- IEA (2009) World Energy outlook 2008. International Energy Agency. 568 p. IEA, Paris
- IFA (2010) International Fertilizer Industry Association, Paris France. <http://www.fertilizer.org/ifa/Home-Page/STATISTICS> (Accessed 20 August 2010)
- IPCC (1996) Guidelines for National Greenhouse Gas Inventories? Intergovernmental Panel on Climate Change (IPCC)
- IPCC (2000) The science bases. <http://www.ipcc.ch> (Accessed 22 August 2010)
- IPCC (2006) IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, Inst. for Global Strategies (IGES), Hayama

- IPCC (2007) Climate change 2007: Synthesis report. Summary for Policymakers. Intergovernmental Panel on Climate Change (IPCC)
- Jantalia CP, dos Santos HP, Urquiaga S, Boddey RM, Alves BJR (2008) Fluxes of nitrous oxide from soil under different crop rotations and tillage systems in the south of Brazil. *Nutr Cycl Agroecosyst* 82:161–173
- Jenkinson DS (2001) The impact of humans on the nitrogen cycle, with focus on temperate agriculture. *Plant Soil* 228:3–15
- Jensen ES (1989) The role of pea cultivation in the nitrogen economy of soils and succeeding crops. In: Planquaert P, Haggard R (eds) *Legumes in farming systems*. Kluwer, Dordrecht, pp 3–15
- Jensen ES, Hauggaard-Nielsen H (2003) How can increased use of biological N₂ fixation in agriculture benefit the environment? *Plant Soil* 252:177–186
- Jensen ES, Peoples MB, Hauggaard-Nielsen H (2010) Faba bean in cropping systems. *Field Crops Res* 115:203–216
- Jones SK, Rees RM, Skiba UM, Ball BC (2007) Influence of organic and mineral N fertiliser on N₂O fluxes from a temperate grassland. *Agric Ecosyst Environ* 121:74–83
- Kamp T, Steindl H, Hantschel RE, Beese F, Munch J-C (1998) Nitrous oxide emissions from a fallow and wheat field as affected by increased soil temperatures. *Biol Fertil Soils* 27:307–314
- Karpenstein-Machan M (2001) Sustainable cultivation concepts for domestic energy production from biomass. *Crit Rev Plant Sci* 20:1–14
- Kaschuk G, Kuyper TW, Leffelaar PA, Hungria M, Giller KE (2009) Are the rates of photosynthesis stimulated by the carbon sink strength or rhizobial and arbuscular mycorrhizal symbioses? *Soil Biol Biochem* 41:1233–1244
- Kaschuk G, Hungria M, Leffelaar PA, Giller KE, Kuyper TW (2010) Differences in photosynthetic behaviour and leaf senescence of soybean (*Glycine max* [L.] Merrill) dependent on N₂ fixation or nitrate supply. *Plant Biol* 12:60–69
- Kazakoff SH, Gresshoff PM, Scott PT (2011) *Pongamia pinnata*, a sustainable feedstock for biodiesel production. In: Halford N, Karp A (eds) *Energy crops*. Royal Society of Chemistry, London, pp. 233–254
- Kilian S, Werner D (1996) Enhanced denitrification in plots of N₂-fixing faba beans compared to plots of a non-fixing legume and non-legumes. *Biol Fert Soils* 21:77–83
- Kindler R, Siemens J, Kaiser K, Walmsley DC, Bernhofer C, Buchmann N, Cellier P, Eugster W, Gleixner G, Grünswald T, Heim A, Ibrom A, Jones SK, Jones M, Klumpp K, Kutsch W, Larsen KS, Lehuger S, Loubet B, McKenzie R, Moors E, Osborne B, Pilegaard K, Rebmann C, Saunders M, Schmidt I, Schrupf M, Seyfferth J, Skib U, Soussana JF, Sutton MA, Tefs C, Vowinkel B, Zeeman M, Kaupenjohann M (2011) Dissolved carbon leaching from soil is a crucial component of the net ecosystem carbon balance. *Glob Change Biol* 17:1167–1185
- Kirkby CA, Kirkegaard JA, Richardson AE, Wade LJ, Blanchard C, Batten G (2011) Stable soil organic matter: a comparison of CNPS ratios in Australian and international soils. *Geoderma* 163:197–208
- Kirkegaard J, Christen O, Krupinsky J, Layzell D (2008) Break crop benefits in temperate wheat production. *Field Crops Res* 107:185–195
- Kirkegaard JA, Peoples MB, Angus JE, Unkovich MJ (2011) Diversity and evolution of rainfed farming systems in Southern Australia. In: Tow P, Cooper I, Partridge I, Birch C (eds) *Rainfed farming systems*. Springer, Dordrecht, The Netherlands, pp 715–754
- Klumpp K, Fontaine S, Arrard E, Le Roux X, Gleixner G, Soussana JF (2009) Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. *J Ecol* 97:876–885
- Klumpp K, Bloor JMG, Ambus P, Soussana JF (2011) Effect of clover density on N₂O emissions and plant–soil N transfers in a fertilised upland pasture. *Plant Soil* 343:97–107
- Koegel RG, Sreenath HK, Straub RJ (1999) Alfalfa fibre as a feedstock for ethanol and organic acids. *App Biochem Biotech* 77-79:110–115
- Köpke U, Nemecek T (2010) Ecological services of faba bean. *Field Crops Res* 115:217–233
- Krupnik TJ, Six J, Ladha JK, Paine MJ, van Kessel C (2004) An assessment of fertilizer nitrogen recovery efficiency by grain crops. In: Mosier AR, Syers KJ, Freney JR (eds) *Agriculture and the nitrogen cycle, the Scientific Committee on Problems of the Environment (SCOPE)*. Island, Covelo, pp 193–207
- Kumar K, Goh KM (2000) Crop residues and management practices: effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. *Adv Agron* 68:197–319
- Lal R (2004) Soil carbon sequestration to mitigate climate change. *Geoderma* 123:1–22
- Lal R, Pimentel D (2007) Biofuels from crop residues. *Soil Tillage Res* 93:237–238
- Lamb JFS, Sheaffer CC, Samac DA (2003) Population density and harvest maturity effects on leaf and stem yield in alfalfa. *Agron J* 295:635–641
- Langeveld JWA, Sanders JPM (2010) General introduction. Chapter 1. In: Langeveld H, Sanders J, Meeusen M (eds) *The biobased economy—biofuels, materials and chemicals in the post-oil era*. Earthscan, London, pp 3–18
- Lefroy EC, Stirzaker RJ, Pate JS (2001) The influence of tagasaste (*Chamaecytisus profliferus* Link.) trees on the water balance of an alley cropping system on deep sand in south-western Australia. *Aust J Agric Res* 52:235–246
- Lehmann J, Gaunt J, Rondon M (2006) Bio-char sequestration in terrestrial ecosystems—a review. *Mitig Adapt Strat Glob Change* 11:403–427
- Lehtomäki A, Viinikainen TA, Rintala JA (2008) Screening boreal energy crops and crop residues for methane biofuels production. *Biomass Bioenerg* 32:541–550
- Lemke RL, Zhong Z, Campbell CA, Zentner R (2007) Can pulse crops play a role in mitigating greenhouse gases from North American agriculture? *Agron J* 99:1719–1725
- Lorek S, Lucas R (2003) Towards sustainable market strategies. A case study on eco-textiles and green power. Wuppertal Institute Report no. 130, 68 p
- Lupwayi NZ, Kennedy AC (2007) Grain legumes in Northern Great Plains: impacts on selected biological soil processes. *Agron J* 99:1700–1709
- Lægread M, Bockman OC, Kaarstad O (1999) *Agriculture, fertilizers and the environment*. CABI, Wallingford
- Mabee W, Saddler J, Nielsen C, Nielsen LH, Jensen ES (2006) Renewable-based fuels for transport. In: Larsen H, Petersen LS (eds) *Risø Energy Report 5. Renewable Energy for Power and transport*. pp 47–50. ISBN 87-550-3515-9, Risø National Laboratory, Denmark
- Macedo MO, Resende AS, Garcia PC, Boddey RM, Jantalia CP, Urquiaga S, Campello EFC, Franco AA (2008) Changes in soil C and N stocks and nutrient dynamics 13 years after recovery of degraded land using leguminous nitrogen-fixing trees. *Forest Ecol Manag* 255:1516–1524
- Magid J, Henriksen O, Thorup-Kristensen K, Mueller T (2001) Disproportionately high N-mineralisation rates from green manures at low temperatures—implications for modelling and management in cool temperate agro-ecosystems. *Plant Soil* 228:73–82
- Magni C, Sessa F, Accardo E, Vanoni M, Morazzoni P, Scarafoni A, Duranti M (2004) Conglutin γ , a lupin seed protein, binds insulin in vitro and reduces plasma glucose levels of hyperglycaemic rats. *J Nutr Biochem* 15:646–650
- Mahmood T, Ali R, Malik KA, Shamsi SRA (1998) Nitrous oxide emissions from an irrigated sandy-clay loam cropped to maize and wheat. *Biol Fertil Soils* 27:189–196

- McCallum MH, Kirkegaard JA, Green T, Cresswell HP, Davies SL, Angus JF, Peoples MB (2004) Improved subsoil macroporosity following perennial pastures. *Aust J Exp Agric* 44:299–307
- McMurtrey JE, Daughtry CST, Devine TE, Corp LA (2005) Spectral detection of crop residues for soil conservation from conventional and large biomass soybeans. *Agron Sustain Dev* 25:25–33
- Minchin FR, Pate JS (1973) The carbon balance of a legume and the functional economy of its root nodules. *J Expl Bot* 24:259–271
- Minchin FR, Summerfield RJ, Neves MCP (1980) Carbon metabolism, nitrogen assimilation, and seed yield of cowpea (*Vigna unguiculata* L. Walp.) grown in an adverse temperature regime. *J Expl Bot* 31:1327–1345
- Mortensen MC, Schuman GE, Ingram LJ (2004) Carbon sequestration in rangelands interseeded with yellow-flowering alfalfa (*Medicago sativa* ssp. *Falcate*). *Environ Manage* 33:S475–S481
- Mosier A (2001) Exchange of gaseous nitrogen compounds between agricultural systems and the atmosphere. *Plant Soil* 228:17–27
- Munier-Jolain NG, Salon C (2005) Are the carbon costs of seed production related to the quantitative and qualitative performance? An appraisal for legumes and other crops. *Plant Cell Environ* 28:1388–1395
- Murphy DV, Fillery IRP, Sparling GP (1998) Seasonal fluctuations of gross N mineralisation, ammonium consumption, and microbial biomass in a Western Australian soil under different land uses. *Aust J Agric Res* 49:523–535
- Möller K, Stinner W, Deuker A, Leitholf G (2008) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic farming systems. *Nutr Cyc Agroecosyst* 82:209–232
- Nelson RG (2002) Resource assessment and removal analysis for corn stover and wheat straw in the Eastern and Midwestern United States. Rainfall and wind-induced soil erosion methodology. *Biomass Bioenerg* 22:349–363
- Nemecek T, von Richthofen J-S, Dubois G, Casta P, Charles R, Pahl H (2008) Environmental impacts of introducing grain legumes into European crop rotations. *Eur J Agron* 28:380–393
- Nichols NN, Dien BS, Wu YV, Cotta MA (2005) Ethanol fermentation of starch from field peas. *Cereal Chem* 82:554–558
- Novalin S, Zweckmair T (2008) Renewable resources—green biorefinery: separation of valuable substances from fluid-fractions by means of membrane technology. *Biofuels Bioprod Bioref* 3:20–27
- O'Hara GW, Daniel RM (1985) Rhizobial denitrification: a review. *Soil Biol Biochem* 17:1–9
- Oleskowicz-Popiel P (2010) Biogas and bioethanol production in organic farming. PhD thesis, Risø National Laboratory for Sustainable Energy, Risø-PhD-64(EN), ISBN 978-87-550-3839-4. 47 p. Risø DTU
- Osborne CS, Peoples MB, Janssen PH (2010) Exposure of soil to a low concentration of hydrogen elicits a reproducible, single member shift in the bacterial community. *Appl Environ Microbiol* 76:1471–1479
- Parkin TB, Kaspar TC (2006) Nitrous oxide emissions from corn-soybean systems in the Midwest. *J Environ Qual* 35:1496–1506
- Pate JS, Layzell DB, Atkins CA (1979) Economy of carbon and nitrogen in a nodulated and nonnodulated (NO₃-grown) legume. *Plant Physiol* 64:1083–1088
- Peoples MB, Baldock JA (2001) Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. *Aust J Exp Agric* 41:327–346
- Peoples MB, Herridge DF (1990) Nitrogen fixation by legumes in tropical and subtropical agriculture. *Adv Agron* 44:155–223
- Peoples MB, Herridge DF, Ladha JK (1995a) Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant Soil* 174:3–28
- Peoples MB, Lilley DM, Burnett VF, Ridley AM, Garden DL (1995b) Effects of lime and superphosphate to acid soils on growth and N₂ fixation by subterranean clover in mixed pasture swards. *Soil Biol Biochem* 27:663–671
- Peoples MB, Palmer B, Lilley DM, Duc LM, Herridge DF (1996) Application of ¹⁵N and xylem ureide methods for assessing N₂ fixation of three shrub legumes periodically pruned for forage. *Plant Soil* 182:125–137
- Peoples MB, Bowman AM, Gault RR, Herridge DF, McCallum MH, McCormick KM, Norton RM, Rochester IJ, Scammell GJ, Schwenke GD (2001) Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. *Plant Soil* 228:29–41
- Peoples MB, Angus JF, Swan AD, Dear BS, Hauggaard-Nielsen H, Jensen ES, Ryan MH, Virgona JM (2004a) Nitrogen dynamics in legume-based pasture systems. In: Mosier AR, Syers KJ, Freney JR (eds) *Agriculture and the nitrogen cycle, the Scientific Committee on Problems of the Environment (SCOPE)*. Island, Covelo, pp 103–114
- Peoples MB, Boyer EW, Goulding KWT, Heffer P, Ochwoh VA, Vanlauwe B, Wood S, Yagi K, Van Cleemput O (2004b) Pathways of nitrogen loss and their impacts on human health and the environment. In: Mosier AR, Syers KJ, Freney JR (eds) *Agriculture and the nitrogen cycle, the Scientific Committee on Problems of the Environment (SCOPE)*. Island, Covelo, pp 53–69
- Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khan DF, Hauggaard-Nielsen H, Jensen ES (2009a) Review article. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis* 48:1–17
- Peoples MB, Hauggaard-Nielsen H, Jensen ES (2009b) The potential environmental benefits and risks derived from legumes in rotations. In: Emerich DW, Krishnan HB (eds) *Agronomy Monograph 52. Nitrogen Fixation in Crop Production*, Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am. Madison, Wisconsin, USA, pp 349–385
- Persson T, Bergkvist G, Katterer T (2008) Long-term effects of crop rotations with and without perennial leys on soil carbon stocks and grain yields of winter wheat. *Nutr Cycl Agroecosyst* 81:193–202
- Pimentel D (2003) Ethanol fuels: energy balance, economics, and environmental impacts are negative. *Nat Resour Res* 12:127–134
- Pimentel D, Patzek TW (2005) Ethanol production using corn, switchgrass, and wood; biodiesel production using soybean and sunflower. *Nat Resour Res* 14:65–76
- Pinkerton A, Randall PJ (1994) Internal phosphorus requirements of six legumes and two grasses. *Aust J Exp Agric* 34:373–379
- Pregelj L, McLanders JR, Gresshoff PM, Schenk PM (2011) Transcription profiling of the isoflavone phenylpropanoid pathway in soybean in response to *Bradyrhizobium japonicum* inoculation. *Funct Plant Biol* 38:13–24
- Pryor SW, Lenling M, Wiesenborn DP (2008) Integrated use of field pea starch and corn for ethanol production. ASABE Paper No. 083999. ASABE, St. Joseph, MI
- Radrizzani A, Shelton HM, Dalzell SA, Kirchoff G (2011) Soil organic carbon and total nitrogen under *Leucaena leucocephala* pastures in Queensland. *Crop Pasture Sci* 62:337–345
- Randall GW, Huggins DR, Russelle MP, Fuchs DJ, Nelson WW, Anderson JL (1997) Nitrate losses through subsurface tile drainage in conservation reserve program alfalfa, and row crop systems. *J Environ Qual* 26:1240–1247

- Rathke G-W, Wienhold BJ, Wilhelm WW, Diepenbrock W (2007) Tillage and rotation effect on corn-soybean energy balances in eastern Nebraska. *Soil Tillage Res* 97:60–70
- Reddy N, Yang Y (2009) Natural cellulose fibers from soybean straw. *Biores Technol* 100:3593–3598
- Reid DE, Ferguson BJ, Gresshoff PM (2011) Inoculation- and nitrate-induced CLE peptides of soybean control NARK-dependent nodule formation. *Mol Plant Microbe Interact* 24:606–618
- Rochester IJ (2003) Estimating nitrous oxide emissions from flood-irrigated alkaline grey clays. *Aust J Soil Res* 41:197–206
- Rochester IJ (2011) Sequestering carbon in minimum-tilled clay soils used for irrigated cotton and grain production. *Soil Tillage Res* 112:1–7
- Rochester IJ, Peoples MB, Hulugalle NR, Gault RR, Constable GA (2001) Using legumes to enhance nitrogen fertility and improve soil condition in cotton cropping systems. *Field Crops Res* 70:27–41
- Rochette P, Janzen HH (2005) Towards a revised coefficient for estimating N₂O emissions from legumes. *Nutr Cycl Agroecosyst* 73:171–179
- Rochette P, Angers DA, Bélanger G, Chantigny MH, Prévost D, Lévesque G (2004) Emissions of N₂O from alfalfa and soybean crops in eastern Canada. *Soil Sci Soc Am J* 68:493–506
- Rosen A, Lindgren P-E, Ljunggren H (1996) Denitrification by *Rhizobium meliloti*. 1. Studies of free-living cells and nodulated plants. *Swed J Agric Res* 26:105–113
- Ruz-Jerez BE, White RE, Ball PR (1994) Long-term measurement of denitrification in three contrasting pastures grazed by sheep. *Soil Biol Biochem* 26:29–39
- Ryan PA (1994) The use of tree legumes for fuelwood production. In: Gutteridge RC, Shelton HM (eds) Forage tree legumes in tropical agriculture. Chapter 5.1. CABI, Wallingford
- Samac DA, Jung H-J, Lamb JFS (2006) Development of alfalfa (*M. sativa* L.) as a feedstock for ethanol and other bioproducts. In: Minter S (ed) Alcoholic fuels. CRC, Boca Raton, pp 79–98
- Schwenke G, Haigh B, McMullen G, Herridge D (2010) Soil nitrous oxide emissions under dryland N-fertilised canola and N₂-fixing chickpea in the northern grains region, Australia. Proc. 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia, pp 228–231
- Scott PT, Pregelj L, Chen N, Hadler JS, Djordjevic MA, Gresshoff PM (2008) *Pongamia pinnata*: an untapped resource for the biofuels industry of the future. *BioEnergy Res* 1:2–11
- Sheehan J, Camobreco V, Duffield D, Graboski M, Shapouri H (1998) Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus. 1-285. U.S. Department of Energy's Office of Fuels Development and U.S. Department of Agriculture's Office of Energy
- Siah S, Konczak I, Agboola S, Wood J, Blanchard C (2011) Phenolic content and potential health benefits of Australian grown faba beans. *British J Nutr* (in press)
- Sisti CPJ, dos Santos HP, Kochhann RA, Alves BJR, Urquiaga S, Boddey RM (2004) Change in carbon and nitrogen stocks in soil under 13 years of conventional or zero tillage in southern Brazil. *Soil Tillage Res* 76:39–58
- Smil V (2001) *Enriching the earth*. MIT Press, Cambridge
- Smith GB, Smith MS (1986) Symbiotic and free-living denitrification by *Bradyrhizobium japonicum*. *Soil Sci Soc Am J* 50:349–354
- Smith P, Martino D, Cai C, Gwary D, Janzen H, Kumar P, McCarl B, Ogle S, O'Mara F, Rice C, Scholes B, Sirotenko O (2007) Agriculture. In: Metz B, Davidson OR, Bosch PR, Dave R, Meyer LA (eds) *Climate Change 2007, Mitigation*. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge
- Soares LH de B, Muniz LC, Figueiredo RS, Alves BJR, Boddey RM, Urquiaga S, Madari BE, Machado PLO de A (2007) Balanço energético de um sistema integrado lavoura-pecuária no Cerrado. *Bol. Pesq.Desenvolv.* No. 26, Embrapa Agrobiologia, Seropédica (available online at: www.cnpab.embrapa.br/publicacoes/download/bot026.pdf)
- Soussana JF, Loiseau P, Vuichard N, Ceschia E, Balesdent J, Chevallier T, Arrouays D (2004) Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use Manag* 20:219–230
- Soussana JF, Tallec T, Blanfort V (2010) Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. *Animal* 4:334–350
- Stehfest E, Bouwman L (2006) N₂O and NO emissions from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. *Nutr Cycl Agroecosyst* 74:207–228
- Stewart CE, Plante AF, Paustian K, Conant RT (2008) Soil carbon saturation: linking concept and measurable carbon pools. *Soil Sci Soc Am J* 72:379–813
- Szot LT, Palm CA, Buresh RJ (1999) Ecosystem fertility and fallow function in the humid and subhumid tropics. *Agrofor Syst* 47:163–196
- Tarré R, Macedo R, Cantarutti RB, de Rezende PC, Pereira JM, Ferreira E, Alves BJR, Urquiaga S, Boddey RM (2001) The effect of the presence of a forage legume on nitrogen and carbon levels in soils under *Brachiaria* pastures in the Atlantic forest region of the South of Bahia, Brazil. *Plant Soil* 234:15–26
- Teira-Esmatges MR, Van Cleemput O, Porta-Casellas J (1998) Fluxes of nitrous oxide and molecular nitrogen from irrigated soils of Catalonia (Spain). *J Environ Qual* 27:687–697
- Thomsen MH, Hauggaard-Nielsen H (2008) Sustainable bioethanol production combining biorefinery principles using combined raw materials from straw and clover-grass. *J Ind Microbiol Biotech* 35:303–311
- Thrall PH, Millsom DA, Jeavons AC, Waayers M, Harvey GR, Bagnall DJ, Brockwell J (2005) Seed inoculation with effective root-nodule bacteria enhances revegetation success. *J Appl Ecol* 42:740–751
- Torres AQA, Jantalia CP, Franco AA, Campello EFC, Resende AS, Urquiaga S, Macedo MO, Moreira JF, Alves TG (2007) Influência de espécies de leguminosas fixadoras de nitrogênio no estoque de C no solo em plantio consorciado no bioma Mata Atlântica (in Portuguese, with English abstract). Congresso Brasileiro de Ciência do Solo 5 to 10 August, Gramada, RS. 3 p. CD-ROM
- Udvardi MK, Price GD, Gresshoff PM, Day DA (1988) A dicarboxylate transporter on the peribacteroid membrane of soybean nodules. *FEBS Lett* 231:36–40
- van Berkum P, Keyser HH (1985) Anaerobic growth and denitrification among different serogroups of soybean rhizobia. *Appl Environ Microbiol* 49:772–777
- VandenBygaert AJ, Gregorich EG, Angers DA (2003) Influence of agricultural management on soil organic carbon: a compendium and assessment of Canadian studies. *Can J Soil Sci* 83:363–380
- Venendaal R, Jørgensen U, Foster CA (1997) European energy crops. *Biomass Bioenerg* 13:147–185
- Wagner-Riddle C, Thurtell GW (1998) Nitrous oxide emissions from agricultural fields during winter and spring thaw as affected by management practices. *Nutr Cycl Agroecosyst* 52:151–163
- Wagner-Riddle C, Thurtell GW, Kidd GK, Beauchamp EG, Sweetman R (1997) Estimates of nitrous oxide emissions from agricultural fields over 28 months. *Can J Soil Sci* 77:135–144
- Wichern F, Eberhardt E, Mayer J, Joergensen RG, Müller T (2008) Nitrogen rhizodeposition in agricultural crops: methods, estimates and future prospects. *Soil Biol Biochem* 40:30–48
- Whitbread AM, Blair GJ, Lefroy RDB (2000) Managing legume leys, residues and fertilisers to enhance the sustainability of wheat cropping systems in Australia. 2. Soil physical fertility and carbon. *Soil Tillage Res* 54:77–89

- Wilkinson CS, Fuskhah E, Indrasumunar A, Gresshoff PM, Scott PT (2012) Growth, nodulation and nitrogen gain of *Pongamia pinnata* and *Glycine max* in response to salinity. *BioEnergy Res* (in review)
- Williams CH, Donald CM (1957) Changes in organic matter and pH in a podzolic soil as influenced by subterranean clover and superphosphate. *Aust J Agric Res* 8:179–189
- Wright AL, Hons FM, Rouquette FM Jr (2004) Long-term management impacts on soil carbon and nitrogen dynamics of grazed bermudagrass pastures. *Soil Biol Biochem* 36:1806–1816
- Yang L, Cai Z (2005) The effect of growing soybean (*Glycine max.* L.) on N₂O emission from soil. *Soil Biol Biochem* 37:1205–1209
- Young RR, Wilson B, Harden S, Bernardi A (2009) Accumulation of soil carbon under zero tillage cropping and perennial vegetation on the Liverpool Plains, eastern Australia. *Aust J Soil Res* 47:273–285
- Zapata F, Danso SKA, Hardarson G, Fried M (1987) Time course of nitrogen fixation in field-grown soybean using Nitrogen-15 methodology. *Agron J* 79:172–176
- Zentner RP, Campbell CA, Biederbeck VO, Miller PR, Selles F, Fernandez MR (2001) In search of a sustainable cropping system for the semiarid Canadian prairies. *J Sustain Agric* 18:117–136
- Zentner RP, Lafond GP, Derksen DA, Nagy CN, Wall DD, May WE (2004) Effects of tillage method and crop rotation on non-renewable energy use efficiency for a thin Black Cherozem in the Canadian prairies. *Soil Tillage Res* 77:125–136
- Zhong Z, Lemke RL, Nelson LM (2009) Nitrous oxide emissions associated with nitrogen fixation by grain legumes. *Soil Biol Biochem* 41:2283–2291