



Legume-supported cropping systems for Europe

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Environmental implications for legume cropping

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SUMMARY

This report summarises the role of legume cropping in influencing nutrient loss and turnover and shaping biodiversity within agroecosystems.

Nitrogen and phosphorous losses

The loss of nutrients from agricultural systems is recognised as a major environmental problem, contributing to air pollution and nutrient enrichment in rivers and oceans. The use of legumes within agriculture provides an opportunity to reduce some of these impacts in ways which maintain or enhance productivity. Nitrous oxide emissions are particularly important here given that they are the largest contributor to greenhouse gas emissions from many agricultural systems. There are many circumstances in which the use of legume supported cropping systems can reduce overall nitrous oxide emissions and the biological nitrogen fixation process associated with legumes can replace synthetic nitrogen fertiliser use.

In terms of N loss from the soil via N_2O flux and NO_3^- leaching then available evidence would highlight the use of legumes as cover crops/green manure and surface mulches as problematic. Legumes in rotation, forage legumes and legumes as intercrops would be beneficial both in terms of reducing fertiliser inputs and cumulative N_2O emissions, but in the case of nitrification/denitrification, N_2O flux would be dependent on N inputs through mineralisation of the previous crop. Insufficient field data allows a definitive statement on N leaching and in terms of variable results from intercropping may reflect deeper rooting varieties (Pappa *et al.*, 2011). However, of the four cropping systems considered the greatest potential for N loss would be the green manure/cover crop/mulch option. Limited data allows only comment of P loss in terms of soil acidification through rotation of legumes and intercropping with liming of pasture/forage legume systems mitigating the mobilisation of the phosphate pools. Improvement of soil quality through soil structure and carbon sequestration would be pronounced both in long-term legume forage systems and direct application of legume residues to soils as green manures/surface mulches.

Biodiversity

Agriculture is a production process with physical inputs and outputs of resources that are intrinsically linked to the surrounding environment, and by manipulating local and regional ecosystems it plays an important role in shaping the biodiversity of life on earth – biodiversity which encompasses the genetic variability between individuals within a species, the vast range of unique species and the variety of habitats that make up local and regional landscapes. Agriculture relies not only on human endeavour and the physical environment, but also on biological process operating at all levels of biodiversity.

Legume cropping, with its capacity to bolster terrestrial resources through the fixation of atmospheric nitrogen, can impact on such biological interactions throughout the agricultural ecosystem.

The impacts of management, in terms of tillage, fertilisation, pesticide and herbicide application are considered alongside the potential value to be derived from ecosystem services associated with biodiversity. Overall we conclude that management factors remain dominant in legume-supported cropping as in conventional cropping, and that legume biomass tends to increase the carrying potential capacity for associated biodiversity. However, impacts were found to be complex and a clear divide was seen between studies investigating the use of legumes to reduce populations of certain organisms, and those studies investigating legume-treatments for promoting associated biodiversity. Legume-supported cropping can both promote and reduce biodiversity within systems but appears to have a generally positive impact at the widest scales.

Nitrogen and phosphorous losses from legume-supported agriculture

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Introduction

The efficiency of nitrogen (N) fertiliser use in agro-ecosystems is often no better than 50% with 45 to 50% of the N applied being taken up by the crop for growth and the remaining N being lost primarily through the combined processes of denitrification, ammonia volatilization and leaching (Smill, 1999; Crew and Peoples, 2004). The use of legumes in agriculture may reduce reliance on inorganic N fertiliser but in many cases the problem of efficiency of N use remains where legumes are used in rotation or as a cover crop for mulching or green manure.

Grain and forage legume production occupies approximately 180 million hectares or 12 to 15% of the arable land area (Graham and Vance, 2003), accounting for 27% of global primary crop production (Vance *et al.*, 2000). Through their ability to fix N from the atmosphere by symbiosis with rhizobia, legumes play a significant role in N supply in both natural ecosystems and agriculture/agroforestry contributing as much as 500 kg N ha⁻¹ y⁻¹ to agricultural land (Briggs *et al.*, 2005). The potential environmental and agronomic implications of biological fixation have been reviewed recently by Jensen and Hauggaard-Nielsen, (2003), Muñoz *et al.*, (2010) and Jensen *et al.*, (2011) and are summarised in Figure 1. Positive environmental impacts to legume cropping include a reduced reliance on inorganic N fertiliser and improvements in soil structure from residue incorporation. Negative effects are primarily associated with N losses to the atmosphere and groundwater where peaks in available N from mineralisation of N-rich residues occur at periods of low crop growth or high rainfall. Soil acidification may also prove problematic eventually leading to decreases in crop productivity but here liming of soils is an effective treatment although effecting N losses too (Galbally *et al.*, 2010). Positive effects of legume cropping on above and below ground biodiversity are reviewed in the second part of this report. This first part of the report provides a review of recent literature on N losses from legume crops and highlights management options that may reduce N₂O emissions to the atmosphere. In addition enhanced P uptake is considered particularly in respect to legume intercropping.

Nitrous oxide production in agricultural soils

The ability of specific gases in the atmosphere to absorb incoming radiation which is then re-emitted as infra-red radiation is an underlying cause of the warming of the earth's climate. Of these so-called greenhouse gases (GHGs), carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are the most important in terms of their concentration in the atmosphere, the rate at which emissions of these gases are increasing and their potential to force the climate (the global warming potential or GWP).

Evidence highlighting the relationship between anthropogenic emissions of GHG's and the Earth's changing climate patterns is widespread with two sectors being identified globally as critical, agriculture and energy, accounting for 13.5% and 25.9% of anthropogenic emissions respectively (IPCC, 2007b). The agricultural sector is a particular important source of emissions of CH₄ and N₂O globally, these two GHGs being approximately 21 and 300 times more effective at warming the climate than CO₂. In addition atmospheric N₂O plays a significant role in reducing the concentration of the earth's natural filter layer for harmful UVb and UVC radiation, tropospheric ozone.

Measuring the concentration of GHGs in air trapped within ice cores drilled from the Antarctic and Greenland ice caps has enabled an estimation of how the concentration of these gases has changed through several glacial-interglacial cycles dating back as far as 650,000 years. These studies reflect fluctuations in levels of N₂O from peak interglacial values of 270 parts per billion by volume (ppbv) to lower glacial values of 200 ppbv (Sowers, 2001; Fluckiger *et al.*, 2004). Since approximately 1850 though, the concentration of N₂O has increased to over 280 ppbv in 1905, over 300 ppbv by the mid-1970s and currently the atmospheric concentration of N₂O is over 320 ppbv representing approx. 6% of the present day greenhouse effect (IPCC, 2007) and 60% of global agricultural emissions of GHGs (Prather *et al.*, 2001; Smith *et al.*, 2007).

A measure of the present day imbalance between sources and sinks for N₂O is provided in Fowler *et al.*, (2009), and serves to highlight the role of agriculture in N₂O production (Table 1). Here 70 % of the imbalance between sources and sinks can be attributed to increased N₂O production from agriculture, primarily through the addition of inorganic N fertilizer to soils (Kroeze, 1999). N-fertiliser use has increased by over 800% between the years 1960 and 2000 (Fixen and West, 2002) and this trend will likely continue with agricultural N₂O emissions predicted to rise by 30-60% over the next twenty years driven by a steadily increasing population and subsequent stresses on food demand leading to increased N inputs into agricultural systems through synthetic fertilisers, manure, human waste and N₂ fixing crops (Smith 1997; Bruinsma, 2003).

Nitrous oxide production in soils reflects both the oxidation and reduction of inorganic N by a wide range of soil microorganisms (fungi, bacteria and archaea). These have evolved to use inorganic N compounds as essential components of energy-coupled, electron transport systems, either as a source of electrons or, similar to oxygen, as a

terminal electron acceptor. Metabolically these redox transformations form a tight cycling of reactive N in natural undisturbed ecosystems but in agricultural ecosystems this balance has been disturbed by excessive inputs of inorganic N. The collective reaction pathways are illustrated in Figure 2.

The two most important processes in N₂O production in soils are aerobic autotrophic nitrification (oxidation) and anaerobic heterotrophic denitrification (reduction). Nitrification proceeds as a stepwise oxidation of ammonia to nitrate, the initial conversion of ammonia to nitrite being a two stage process involving ammonia-oxidising bacteria in the soil. There is significant evidence to support ammonia oxidation being linked to N₂O production at these two steps where ammonia is initially oxidised to hydroxylamine by an ammonia mono-oxygenase, and then hydroxylamine is further oxidised to nitrite by hydroxylamine oxidoreductase (Hooper *et al.*, 1997). Known bacterial ammonia oxidisers all belong to the two genus *Nitrosomonas* and *Nitrosospira* (Prosser and Nicol, 2008). Nitrite oxidisers, typified by the genus *Nitrobacter*, are responsible for the oxidation of NO₂⁻ to NO₃⁻ and both ammonia and nitrite oxidizers may reduce NO₂⁻ to NO, N₂O or N₂ under low oxygen concentrations giving rise to the phenomena of nitrifier denitrification (Colliver and Stephensen, 2000; Wrage *et al.*, 2001).

As with autotrophic soil bacteria, a number of heterotrophic microorganisms are capable of nitrification although this process maybe more commonly associated with soil fungi than bacteria (Odu and Adeoye, 1970). In general though autotrophic nitrification is the dominant nitrification pathway in arable soils with heterotrophic nitrification being favoured in more acidic soils (Anderson *et al.*, 1993; Bremner, 1997; Robertson and Groffman, 2007).

The highest rates of N₂O production from soils arise from anaerobic denitrification (Williams *et al.*, 1992; Bateman and Baggs, 2005). These collective reactions involve the stepwise reduction of nitrate to nitrogen through NO and N₂O by soil microorganisms capable of using reactive nitrogen as terminal electron acceptors when O₂ is limited (Šimek *et al.*, 2002). Organisms utilizing this energy yielding pathway tend to be facultative heterotrophic bacteria such as *Pseudomonas* and *Alcaligenes* species (Robertson and Groffman, 2007) but fungal denitrification also occurs and this may be the dominant driver of N₂O production in agricultural grasslands (Laughlin and Stevens, 2002). Reduction of nitrate to ammonia at high soil concentrations of nitrate may also involve the production of N₂O (Baggs, 2011; Schmidt *et al.*, 2011).

In terms of the major controlling factors on N₂O production, collectively the rate of nitrification and denitrification will be determined by the microbial capacity of the soil, temperature, pH, substrate supply and the degree of oxygenation of the soil (cf. Flessa *et al.*, 2002; Khalil *et al.*, 2002; Šimek and Cooper, 2002; Smith *et al.*, 2003; Malhi *et al.*, 2006; Ding *et al.*, 2007). In addition, the diffusive properties of the soil will affect the flux rate of N₂O to the atmosphere (Figure 3). Water filled pore space (WFPS) is frequently

highlighted in the literature as the most important controlling variable in agricultural soils as it is directly linked with aeration and oxygen availability (Davidson, 1991; Davidson *et al.*, 2000; Smith *et al.*, 2003). In general, N₂O production is thought to be greatest at intermediate WFPS values in the range of 50 – 80% (Davidson, 1991; Dobbie and Smith, 2003a) with peak denitrification rates being favoured by high WFPS values (80 to 85%) where reduced oxygen availability is also coupled to increased solubility of organic carbon and nitrate (Bowden and Bormann, 1986). Nitrification may also prevail at WFPS values above 50% whilst above 75% denitrification is the major pathway for N₂O production (Well *et al.*, 2006).

Nitrous oxide emissions from legume based systems

Monocrop legumes, legumes in rotation, legumes as intercrops and legumes grown as cover crop/green manures will all influence N₂O emissions from the soil through their input of biological fixed N into the soil. In addition root nodules may directly contribute to N₂O emissions via the inherent capacity of some rhizobial species/strains to reduce nitrite to nitrous oxide. In practise the contribution of legume cropping to soil N₂O emissions maybe divided into three separate processes:-

- (a) *Rhizobial denitrification within the nodules*
- (b) *Nitrification and Denitrification of biologically fixed N, and*
- (c) *Decomposition of N-rich residues to provide inorganic N*

Of the three the addition of N-rich legume residues to soils is the most critical with regard to peak N₂O emissions.

Rhizobial denitrification and N₂O production

Isolated legume nodules and rhizobia bacteroids from a range of plant species have been shown to produce N₂O at limiting concentrations of oxygen and with nitrate as their source of nitrogen (Daniel *et al.*, 1980; O'Hara and Daniel, 1985; Coyne and Focht, 1987; Bedmar *et al.*, 2005; Monza *et al.*, 2006). The nitrogenase enzyme system is a powerful electron donor and reduces a wide range of molecules besides N₂ such as nitrous oxide, ethylene, acetylene and cyanide (Rivera-Ortiz and Burris, 1975), but whilst the reduction of reactive nitrogen in legume nodules was thought originally to be a function of nitrogenase enzyme, both free living and symbiotic rhizobia possess enzymes of the denitrification pathway and hence the ability to produce N₂O. Not all rhizobia share this property, indeed denitrification has been shown in only a few genera of N₂-fixing bacteria and a majority of the species/strains studied lack a full complement of denitrification genes (Monza *et al.*, 2006; Table 2). Possession of Nor activity (nitric oxide reductase) would lead to N₂O production from nitric oxide (NO) but further

reduction by Nos activity (N₂O reductase) would limit the extent of production of this greenhouse gas from the bacteroids.

The ability of N₂-fixing bacteria to denitrify, albeit antagonistic to N₂-fixation in the N-cycle maybe seen as a means of reducing nitrate and nitrite within the immediate confines of the root nodule. Denitrification may therefore be beneficial to nodule metabolism. Nitrate inhibits nitrogenase activity in legume plant nodules and unsurprisingly nitrate reductase activity has been observed in many symbiotic associations between rhizobia and legumes, 97% of nodular nitrate reductase activity being localised in bacteroids (Polcyn and Lucinski, 2001). Nitrite, the product of this enzyme reaction is itself a marked inhibitor of nitrogenase and may be the main inhibitory reactive N species involved in N₂-fixation (Arresegor *et al.*, 1997). The broader distribution of nitrite reductase amongst N₂-fixing genera maybe understood in this context but again the product of the reaction, nitric oxide (NO) is inhibitory on N₂-fixation (Kato *et al.*, 2010). Nitric oxide reductase gives rise to N₂O, which is a competitive inhibitor of nitrogenase, but both this enzyme and N₂O reductase can reduce N₂O to N₂. Evidence would suggest, at least with symbiotic *B.japonicum*, that rhizobia reduce N₂O primarily by N₂O reductase (Sameshima-Saito *et al.*, 2006).

Denitrification therefore, in particular the reduction of nitrite to nitric oxide may be a mechanism to maintain optimum rates of N₂ fixation within the root nodule. However, this protective mechanism maybe species dependant. Whilst much of the work of rhizobial N cycle metabolism has focussed on a narrow range of species such as slow growing strains of *Bradyrhizobia sp.*, García-Plazaola *et al.*, (1996) working on the faster growing species *Rhizobium meliloti*, found little evidence of nitrification in nitrite detoxification. Another possibility is that denitrification enzymes play a role more in nodule formation than nodule function. Mesa *et al.*, (2004) found the presence of denitrification enzymes in rhizobia was not essential for maintaining N₂ fixation; structural *nir* and *nor* gene mutants of *Bradyrhizobium japonicum* lacking both nitrite and nitric oxide reductase activity still possessed nitrogenase but the extent of nodulation of the infected soybean plants was reduced significantly.

Whatever the distribution and function of denitrification enzymes amongst symbiotic rhizobia, the extent of N₂O production from legume nodules in the field is not clear. Early work on upscaling laboratory rates of denitrification highlighted a considerable potential of N₂-fixing bacteria to remove nitrate from agricultural soils. In the case of *Rhizobium lupina* a measured bacterial density of 10⁴ cells g⁻¹ soil was calculated to give initial rates of denitrification of the order of 20 kg N removed ha⁻¹ (O'Hara *et al.*, 1984), this loss of nitrogen being of a similar magnitude to field rates of N₂-fixation (O'Hara and Daniel, 1985). Despite such concerns, evidence for high rates of denitrification by legume nodules in the field is rare (Zhong *et al.*, 2009). Given the considerable uncertainty in upscaling laboratory rates of N₂O flux by isolated nodules or symbiotic bacteria to the field, useful experiments would be those incorporating suitable controls to

compare N₂O flux from inoculated and non-inoculated plants. In the case of both pea and lentil little difference in N₂O flux has been determined between plants inoculated with strains of *Rhizobium leguminosarum* and control plants, and even between inoculated plants and soils planted with wheat (Zhong *et al.*, 2009). This suggests that N₂O emissions are not directly related to biological N₂ fixation by grain legumes, as further illustrated in soil box experiments incorporating wetting and drying cycles with pea and lentil crops and *R. leguminosarum* (Zhong *et al.*, 2011). Taking the lack of field based data into consideration, N₂ fixation by legumes as a source of N₂O is no longer considered important by the IPCC and has been dropped from their emission calculation guidelines (Rochette and Janzen, 2005; IPCC, 2006).

Nitrification and denitrification of biologically fixed N: grass - clover swards and legume monocrops

A comparison of N₂O emissions from different cropping systems by Muñoz *et al.*, (2010) is summarised in Table 3 and highlights the range of N₂O flux values recorded. Grazed grass/clover pastures have the largest recorded N₂O fluxes with fixed nitrogen being released into the soil through both decay of leaf, stem and root litter and transfer to the soil N pool via faeces and urine from the grazing animals. Leaching of N and acidification of soils is a common problem here (Bouwman *et al.*, 2002), the drop in soil pH due to the acidifying effects of the nitrogenase reaction. This eventually leads to a decline in productivity of the grassland (Williams, 1980) hence liming of grasslands is a common solution (Galbally *et al.*, 2010). As N₂O emissions are reduced when soil pH values fall below pH 5.5, liming may lead directly to increases in N₂O flux although field data on the effect of liming is scarce. Galbally *et al.*, (2010) found no significant effect of liming on N₂O emissions from grazed legume pastures typical of Australia. Laboratory incubations of limed soils with urine added as a source of N also show little effect of raising the soil pH above 5.5 on N₂O flux (Zaman *et al.*, 2007; 2008).

Clover density may also be assumed to effect N₂O flux in such systems through increasing N inputs into the soil, but as with the case of liming very little field data is available. A study of N₂O flux from high and low density clover patches concluded that spatial heterogeneity in clover abundance may have very little impact on field scale N₂O emissions in fertilised grasslands (Katja Klumpp *et al.*, 2011).

Clearly from the data presented legume monocrops show the least emissions (Table 3) but care must be taken in interpretation of short term studies. Nitrification and denitrification of biologically fixed N (BNF) may represent a significant source of N₂O from agricultural systems in the long term where incorporation and mineralisation of legume residues may lead to peaks in available nitrate. The majority of studies on legume monocrops are limited at best to one year and hence focus on the short term. Under these conditions with removal of a high proportion of biologically fixed N to the grain during growth and harvest, short term measurements of N₂O emissions will fail to incorporate the effect of carryover of the remaining plant nitrogen in the soil (Evans *et*

al., 2001; Peoples *et al.*, 2001). Some authors consider the stubble remaining after harvest of grain legumes to be a minor source of N₂O through mineralisation given its low organic N content (Lemke *et al.*, 2007; Peoples *et al.*, 2009). For grass-clover stands or stands of forage legumes long term dynamics of N loss are important. Carter and Ambus, (2006) found only 2% of the total N₂O-N emissions of biologically fixed N lost as N₂O in the short term, highlighting the importance of the long-term mineralisation of plant material for N₂O emissions than recently fixed N. Accepting these limitations **Table 4** illustrates the mean and range of N₂O flux values as summarised by Jensen *et al.*, (2011) for a range of specific legume and non-legume crops. The trend from the literature would be that grain legumes, forage legumes and grass-clover stands receiving minimal inorganic N fertiliser have lower emissions of N₂O than N-fertilised pastures and non-legume crops, but higher emissions than non-fertilized, non-legume crops (Rochette *et al.*, 2005; Jensen *et al.*, 2011). In the case of legume systems showing higher N₂O emissions than non-legume crops grown with no added fertilizer, this would reflect N inputs provided by the legumes. As an example Dick *et al.*, (2006) in a comparison of soils from N-fixing and non-N fixing trees found both a higher N₂O flux and pool of available N (NH₄⁺ and NO₃⁻) in the soil from the those fixing N₂ from the atmosphere.

There are a few exceptions in the literature where very high emissions of N₂O have been recorded from legume monocrops, such as alfalfa (Rochette *et al.*, 2004) and soybean (Parkin and Caspar, 2006), but here the influence of previous land management and sources of N other than biologically fixed N must be considered.

Nitrification and denitrification of biologically fixed N: legumes in rotation, residue incorporation and green manures

Before the widespread availability of inorganic N fertilizer, management of soil fertility in farms was typically by legume rich pastures, cover crops or rotation. These management systems are seen by some as a means of increasing productivity in poorer areas of the globe and also to increase sustainable agricultural production (Crews and Peoples, 2004). For instance cereal legume intercropping is a common crop production system in Africa, incorporation of ground nut into rice-based cropping systems increases productivity and income of small holders in South East Asia (Whitmore *et al.*, 2000), rotation of crops with fast growing tree, shrub and herbaceous N₂ fixing legume species is widely adopted for soil fertility management in humid tropics (Millar *et al.*, 2004) and in southern Brazil the use of legume cover crops is increasingly common in no tillage systems (Mielnickzuk *et al.*, 2003).

Both legume crops in rotation and their use as cover crops involve the incorporation of plant residues into the soil which are high in N. It is this aspect to legume systems, the incorporation of organic N into soils which following mineralisation will provide sufficient substrate for nitrification and denitrification, which represents a significant source of N₂O.

This maybe further compounded by the higher N content and lower C/N ratios of legume tissues compared with other plant material.

In general plant residues with high C:N ratios mineralise to provide high amounts of C, thus stimulating microbial growth and in effect immobilising soil N through incorporation into microbial biomass. In the short term this has the effect of delaying the availability of inorganic nitrate for nitrification/denitrification but also for crop growth. In the long term though plant-available N, yield and N uptake increase following straw addition with mineralisation being extended (Cassman *et al.*, 1996; Eagle *et al.*, 2000). Primarily inorganic N accumulates in soils from plant residues only if it is in excess to C-limited microbial growth. For legume residues this will occur rapidly due to both the high N content and low C:N ratio of the tissue. A threshold C:N value of 20 to 25 has been proposed below which rapid mineralization occurs (Frankenberger and Abdelmagid 1985; Myers *et al.*, 1994).

Figure 4 illustrates typical N content values for a variety of plant residues taken from data presented in Jensen *et al.*, (2011). C:N values vary from approximately 26:1 to 10:1 for legume tissues and from approximately 26:1 to 105:1 for non-leguminous tissues with legume N contents ranging from 29 to 100 kg N 1000 kg C⁻¹ and for non-legumes 10 to 39 kg N 1000 kg C⁻¹. Both the high overall N content and low C:N ratios of legume residues will result in more rapid mineralisation, an excess of N with respect to microbial growth and increased substrate for the combined processes of nitrification and denitrification. In general, therefore greater N₂O emissions are measured after incorporation of high N plant residues (Baggs *et al.*, 2000; Millar *et al.*, 2004; Kaewpradit *et al.*, 2008; Gomes *et al.*, 2009; Frimpong *et al.*, 2011; Frimpong *et al.*, 2012), with the peak in N₂O emissions occurring early after incorporation. Here N₂O production is favoured by increased N inputs, but also from the burst in respiration associated with breakdown of residues in the soil and the increased incidence of anaerobic microsites promoting denitrification (Khalil and Baggs, 2005). Imbalances between the timing, availability and amount of newly mineralised N from legume residues and the onset of plant growth are therefore critical with respect to N₂O emissions, particularly if the legume is a cover crop and ploughed in as a green manure (Baggs *et al.*, 2000) or part of an improved ley ploughed over before cereal planting (Pu *et al.*, 1999).

Table 5 provides a comparison of the percentage effect of legumes in rotation, legume residue management and legumes as cover crops on N₂O emissions using published data from both field and laboratory studies where suitable controls are given. Care must be taken in summarising the data as the bulk of the studies measure N₂O flux over a single growth season or in the case of lab' studies a limited number of days. However the overall trend apparent from the data is that legume crops in rotation with consequent incorporation of high N residues into the soil, lead to an increase in N₂O flux. The few exceptions to this where a decrease in N₂O flux is observed relate to an overall

reduction in N inputs for the legume treatment. Irrespective of the scale of the percentage effect observed, the largest recorded flux values are comparable with those measured from crops fertilised with inorganic N (Table 3). This is to ignore savings in both cost to the farmer in reducing fertiliser usage, environmental costs of reducing fertiliser manufacture and further benefits of N carry over into the following crop.

In percentage terms the highest increase in N₂O emissions are found for legume cover crop and legume residue incorporation studies (Baggs *et al.*, 2003; Millar *et al.*, 2004; Gomes *et al.*, 2009; Frimpong *et al.*, 2011; 2012). Improving the synchrony between N availability and crop growth in these management systems would be critical in reducing N₂O flux and maybe N fertilised systems where top-dressings can match supply of N to demand are better than legume-rotations in this respect (Cassman *et al.*, 2002; Crews and Peoples, 2004). One strategy that may prolong mineralisation of legume residues through the season would be to manipulate the overall C:N ratio of the plant material applied. This may be achieved by mixing high C cereal residues with high N legume residues to allow for some measure of N immobilisation (Vinten *et al.*, 1998; Myers *et al.*, 1994; Schwendener *et al.*, 2005; Kaewpradit *et al.*, 2008; Frimpong *et al.*, 2011).

Nitrate leaching from legume crops

Leaching of nitrate from agricultural land is a major problem in high intensity systems reflecting both excess N in the soil comparative to crop growth requirements and the amount of water held by the soil immediately following N application (Addiscott and Powlson, 1992; Ledgard, 2001; Jensen and Hauggaard – Nielson, 2003). It is often the most important route of N loss from field soils other than that accounted for by plant uptake. In Europe, nitrate pollution of surface and ground water is a significant environmental problem with the annual nitrate concentration of approximately 30% of ground waters exceeding the EC threshold value of 50 mg L⁻¹ (Al-Kaisi and Licht, 2004; Hooker *et al.*, 2008). In legume systems, particularly legume-rich pastures, leaching may be less of a problem than intensively managed systems (Owens *et al.*, 1994), although field data is lacking. Legume crops in rotation, or as cover crops/green manures may however still leach significant nitrate from the soil due to both the lack of synchrony between N availability and crop growth and the amount of N provided through mineralization of the low C:N plant residues. As almost 75% of legume cover crop biomass is killed and left on the soil surface as a mulch which may be decomposed after 120 days, the potential for N-leaching is high (Quemada *et al.*, 2004;). Comparable field data on the effect of legume cropping on nitrate leaching is scarce in the literature. Beaudoin *et al.*, (2005) observed the highest rates of nitrate leaching in crop rotations including pea for Northern France due to the higher N content of plant biomass and lower N uptake rates from the soil, whilst one recent study on the use of legumes as cover crops in *Capsicum* production showed both high N leaching and a linear correlation between the N accumulated in the legume biomass and the total amount of

nitrate leached (Campiglia *et al.*, 2011). Targeting the reduction of mineral N accumulation in soil, synchronizing N inputs with crop growth and crop N uptake and avoiding the buildup of excess N in soils would contribute towards decreased leaching (Mosier *et al.*, 2002) and one possible way to achieve this would be through intercropping of legumes with cereals, a form of low N input agriculture popular in the tropics and now receiving interest in Europe.

Nitrogen and phosphorus losses from intercropping of legumes

Intercropping of legumes offers an opportunity to increase the input of fixed N into an agro-ecosystem both in the short-term through direct N transfer (Patra *et al.*, 1986; Xiao *et al.*, 2004), and in the long-term through mineralization of residues (Oleson *et al.*, 2002; Thorsted *et al.*, 2006). This may be achieved without compromising N uptake by the cereal crop or crop yield/stability (Hauggaard-Nielsen *et al.*, 2001), and in terms of economic yield may even prove beneficial (Willey, 1979; Hauggaard-Nielsen *et al.*, 2001). As inter-cropping involves both a reduction in applied inorganic N and by virtue of the legume and non-legume plants growing in close proximity, a more efficient use of N, emissions of N₂O maybe expected to be lower than for monocrops. However as with N-leaching a scarcity of information exists whereby direct comparisons between intercrops and monocrops can be made. Dyer *et al.*, (2012) have reported short term N₂O flux data from a temperate maize: soybean system incorporating monocrop data, although in this case cumulative fluxes have not been presented. Here rates of N₂O flux were significantly lower from the intercrop treatments (11.5 to 12 µg N₂O-N m⁻² h⁻¹) than either the soybean or maize crops (13.5 and 14 µg N₂O-N m⁻² h⁻¹ respectively). Only one study however reports cumulative flux values for legume/ cereal intercropping (Pappa *et al.*, 2011). This study concerned both barley /pea and barley clover intercrops and also looked at varietal differences in N₂O flux and N leaching. As the barley mono crop received no added N other than that provided from the previous grass crop, inclusion of the clover and pea (*cv. Nitouche*) crops increased annual N₂O flux by 211 and 267% respectively (Table 6). Of significant interest however is the observation that the second pea variety *cv. Zero* reduced the annual flux by 22 percent and that unlike barley – clover, the barley-pea intercrops reduced nitrate leaching.

Inter-cropping may also have positive effects on plant phosphorous (P) uptake. Phosphorus is an essential plant nutrient but is a relatively immobile element in soils. Following adsorption by soil surfaces and organic matter it forms stable largely insoluble compounds that cannot be removed from soils by leaching or volatilisation. Small amounts of phosphorus are however, released into the soil solution in the form of phosphate ions and it is these that become available for plant uptake and potential loss through drainage.

In many Western countries, fertiliser phosphorus inputs over many years have led to the enrichment of soil with phosphorus in immobile pools. Utilisation of this excess phosphorus can be improved by selecting rotational designs include crops or intercrops that optimise phosphorus uptake (Edwards *et al.*, 2010). Brassicas have been shown to be particularly effective at mobilising phosphorus from the soil possibly as a consequence of their mycorrhizal associations (Walker *et al.* 2012). There is considerable evidence to show that the use of legume based intercropping systems improves the efficiency of soil phosphorus utilisation and it has been suggested that this may be also a consequence of mycorrhizal associations with the roots of legume species (Ren *et al.* 2013). It is considered likely that legume roots are able to alter the pH of the soil and influence phosphorus availability accordingly (Betencourt *et al.* 2012; Li *et al.* 2013). Experiments with Faba beans have shown that the acidity produced by the roots of these plants is more effective at solubilising soil phosphorus than that from other legume species.

Legume-based rotations (including intercrops) are of particular value in soils with lower phosphorus content or in circumstances where phosphorus is applied in insoluble forms. For example organic farming regulations preclude the use of soluble phosphorus fertilisers, preferring instead to use composts or manure or other forms of phosphorus input such as rock phosphate. However extreme phosphorus deficiency (often encountered in low pH soils) can result in reduced growth of legumes in rotation as this becomes the next most limiting nutrient after nitrogen.

Summary

The relative differences between the four legume cropping systems in terms of N and P losses, carbon sequestration, soil quality and biodiversity effects are summarised in Table 7. In terms of N loss from the soil via N₂O flux and NO₃⁻ leaching then available evidence would highlight the use of legumes as cover crops/green manure and surface mulches as problematic. Legumes in rotation, forage legumes and legumes as intercrops would be beneficial both in terms of reducing fertiliser inputs and cumulative N₂O emissions, but in the case of nitrification/denitrification, N₂O flux would be dependent on N inputs through mineralisation of the previous crop. Insufficient field data allows a definitive statement on N leaching and in terms of variable results from intercropping may reflect deeper rooting varieties (Pappa *et al.*, 2011), However, of the four cropping systems considered the greatest potential for N loss would be the green manure/cover crop/mulch option. Limited data allows only comment of P loss in terms of soil acidification through rotation of legumes and intercropping with liming of pasture/forage legume systems mitigating the mobilisation of the phosphate pools. Improvement of soil quality through soil structure and carbon sequestration would be pronounced both in long-term legume forage systems and direct application of legume residues to soils as green manures/surface mulches. The long-term nature of both

forage legume and legume intercropping systems would also be expected to be beneficial on both above and below ground aspects of biodiversity.

N ₂ fixation and reduced N fertilizer use	
Positive	Negative
Decreased GHG emissions	Soil acidification
Decreased fossil energy use	
Increased C and N assimilation	
Increased soil N uptake	

Legume crops: Pre-cropping/cropping	
Positive	Negative
Decreased GHG emissions	N losses from green manure/cover crop/residue incorporation
Decreased ammonia volatilisation	
Decreased N leaching	
Increased soil N uptake	

Legume crops: Post-harvest and long-term	
Positive	Negative
Increased N-benefit to following crop	N losses particularly in intensive systems
Increased soil fertility	
Increased C sequestration	
Increased above/below ground biodiversity	

Figure 1: Environmental effects of legume cropping systems (adapted from Jensen and Hauggard-Nielsen, 2003)

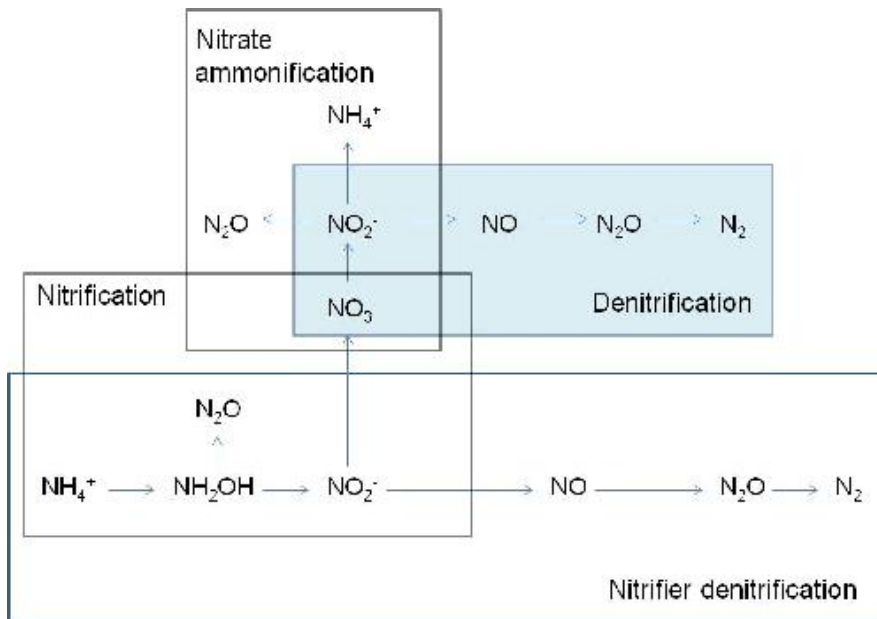


Figure 2: Microbial sources of N_2O in the soil (adapted from Baggs, 2008)

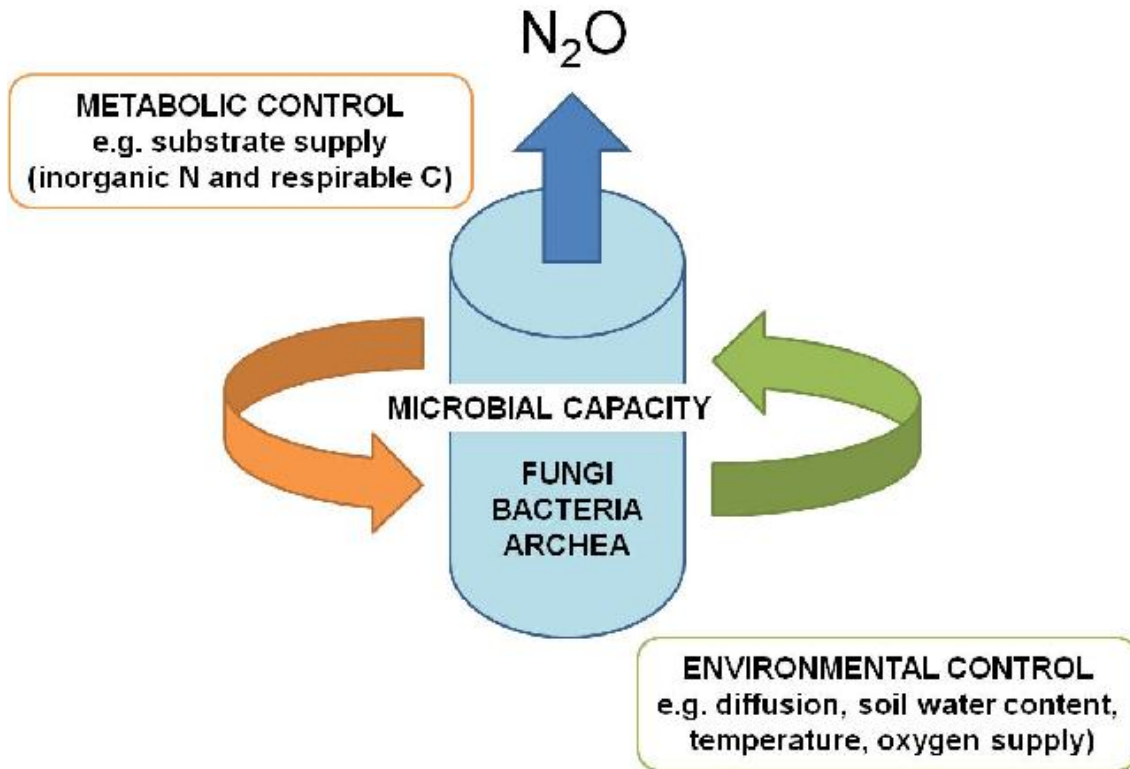


Figure 3: Limiting factors on N₂O production in the soil

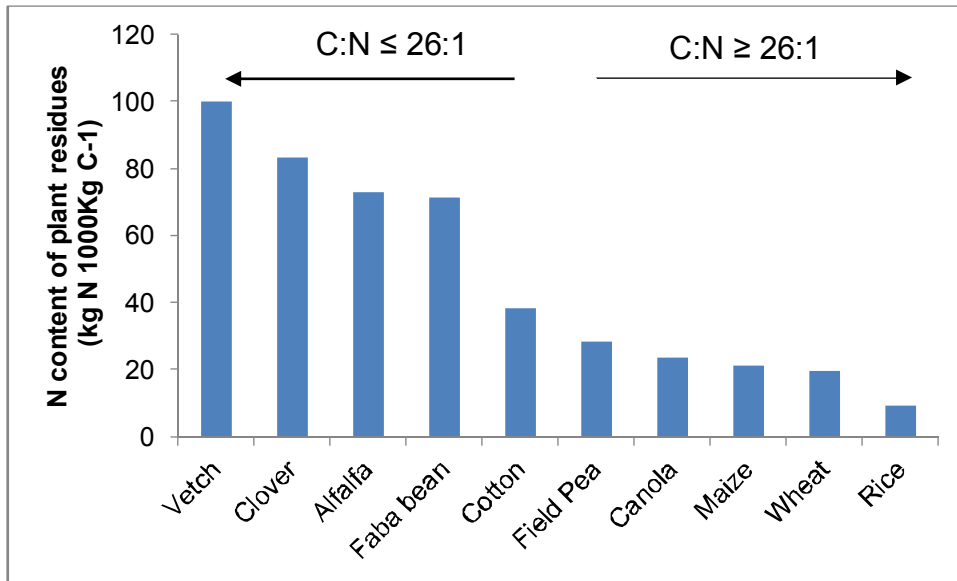


Figure 4: N content and C:N ratios for legume and non-legume plant residues (after Jensen et al., 2013)

Table 1: Sources and sinks of N₂O accumulation in the atmosphere (adapted from Fowler et al., (2009).

Sources	10 ⁶ tonnes N ₂ O ha ⁻¹ y ⁻¹	Sinks	10 ⁶ tonnes N ₂ O ha ⁻¹ y ⁻¹	Source – Sinks 10 ⁶ tonnes N ₂ O ha ⁻¹ y ⁻¹
Oceans	3.8 (1.8 – 5.8)	Stratosphere	12.5 (1.8 – 5.8)	3.7
Atmosphere	0.6 (0.3 – 1.3)	Soils	1.5 – 3	
Soils	6.6 (3.3 – 9.0)			
Agriculture	2.8 (1.7 – 4.8)			
Biomass Burning	0.7 (0.2 – 1.0)			
Energy and Industry	0.7 (0.2 – 1.8)			
Others	2.5 (0.9 – 4.1)			
Total Sources	17.7 (8.5 – 27.7)	Total Sinks	14.0 (11.5 – 18.0)	

Table 2: Distribution of denitrification genes in N_2 -fixing bacteria (from Monza et al., 2006)

N_2 Fixing Species	Denitrification Genes
<i>Bradyrhizobium japonicum</i>	<i>Nap, Nir, Nor, Nos</i>
<i>Sinorhizobium meliloti</i>	<i>Nap, Nir, Nor, Nos</i>
<i>Rhizobium galegae</i>	<i>Nap, Nir, Nor</i>
<i>Azospirillum brasilense</i>	<i>Nap, Nir</i>
<i>Mesorhizobium loti</i>	<i>Nir</i>
<i>Rhizobium etli</i>	<i>Nir</i>
<i>Rhizobium sllae</i>	<i>Nir</i>

Key: *Nap* – Nitrate Reductase (periplasm)

Nir – Nitrite Reductase

Nor – Nitric Oxide Reductase

Nos – Nitrous Oxide Reductase

Table 3: N₂O fluxes from different soil use and management (from Muñoz et al., 2010)

System	Range N ₂ O flux (kg N ₂ O-N ha ⁻¹ yr ⁻¹)	Country	References
Cropping			
Continuous and rotation crops	0 – 44	Brazil, Canada, Denmark, New Zealand	<i>Wagner-Riddle and Thurtell (1998); Gregorich et al., (2005); Metay et al., (2007); Saggar et al., (2008); Chirinda et al., (2010); Allen et al., (2010)</i>
Leguminous crop	0.3 – 4.7	Canada	<i>Gregorich et al., (2005)</i>
Rice	0 – 36	Australia, USA, Japan, China, Philipines, Indonesia, Taiwan, India	<i>Majumdar (2009)</i>
Shrub land/ Natural Landscape	0 – 21	New Zealand, Finland	<i>Maljanen et al., (2006); Saggar et al., (2008)</i>
Pasture			
Animal waste applied	0 – 156	Canada, New Zealand, England, The Netherlands, Japan, Canada, Denmark, USA	<i>Gregorich et al., (2005); Saggar et al., (2009)</i>
Grazing	0.1 – 183	UK, New Zealand, Australia	<i>Saggar et al., (2008); Matthews et al., (2010); Cardenas et al., (2010); Galbally et al., (2010)</i>

Table 4: Comparison of N₂O emissions from legume and non-legume crops (from Jensen et al., 2011)

Crop	Total N₂O emissions per year or growing season (kg N₂O-N ha⁻¹)
Grassland	
N-fertilised pasture (grass)	4.5 (0.3 - 18.6)
Mixed pasture sward (grass-clover)	0.5 (0.1 - 1.3)
Pure legume stands	
Alfalfa	2.0 (0.7 – 4.6)
White Clover	0.8 (0.5 – 0.9)
Grain legumes	
Faba bean	0.4
Lupin	0.05
Chickpea	0.06 (0.03 – 0.16)
Field pea	0.7 (0.4 – 1.7)
Soybean	1.6 (0.3 – 7.1)
Mean of all legumes	1.3
N-fertilized crops	
Wheat	2.7 (0.09 – 1.6)
Maize	2.7 (0.16 – 12.7)
Canola	2.7 (0.13 – 8.6)
Mean N-fertilized crops	3.2

Soil (no legumes or fertilizer)	1.2 (0.03 – 4.8)
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Table 5: Change in N₂O emissions due to legumes in rotation, legume residue management and legume cover crops

Rotation – Field Experiments	N₂O emission	Effect relative to cereal control/control (%)	Author
<i>Maize-Maize¹</i>	768 g N ha ⁻¹		<i>Halvorson et al., 2008</i>
<i>Maize-Dry bean¹</i>	1637g N ha ⁻¹	+113	
<i>Maize-Maize</i>	183 g N ha ⁻¹		
<i>Maize-Dry bean</i>	200 g N ha ⁻¹	+9	
<i>Maize-Maize-Maize-Maize¹</i>	3.3 kg N ha ⁻¹		<i>McKenzie et al., 1997</i>
<i>Soybean-Soybean-Soybean-Soybean¹</i>	1.7 kg N ha ⁻¹	-48	
<i>Soybean-Maize-Soybean-Maize¹</i>	2.16 kg N ha ⁻¹	-35	
<i>Maize-Soybean-Alfalfa-Maize¹</i>	2.3 kg N ha ⁻¹	-30	
<i>Maize-Maize-Maize-Maize</i>	1.5 kg N ha ⁻¹		
<i>Soybean-Soybean-Soybean-Soybean</i>	1.2 kg N ha ⁻¹	-20	
<i>Soybean-Maize-Soybean-Maize</i>	1.9 kg N ha ⁻¹	+27	
<i>Maize-Soybean-Alfalfa-Maize</i>	2.9 kg N ha ⁻¹	+93	
<i>Maize-Maize</i>	2.6 kg N ha ⁻¹		<i>Drury et al., 2008</i>
<i>Soybean-Maize</i>	1.3 kg N ha ⁻¹	-50	
<i>Wheat-Wheat</i>	130 g N ₂ O-N ha ⁻¹ 2y ⁻¹		<i>Barton et al., 2013</i>
<i>Lupin-Wheat</i>	100 g N ₂ O-N ha ⁻¹ 2y ⁻¹	-23	
<i>Wheat-Wheat²</i>	90 g N ₂ O-N ha ⁻¹ 2y ⁻¹		
<i>Lupin-Wheat²</i>	110 g N ₂ O-N ha ⁻¹ 2y ⁻¹	+22	
<i>Wheat-Maize</i>	289 gN ₂ O-N ha ⁻¹		<i>Guo et al., 2009</i>
<i>Faba bean-Maize</i>	345 g N ₂ O-N ha ⁻¹	+19	

Legume-supported cropping systems for Europe

<i>Millet-Millet</i> ³	1535 g N ha ⁻¹ y ⁻¹		<i>Dick et al., 2008</i>
<i>Bean-Millet</i> ²	635 g N ha ⁻¹ y ⁻¹	-59	
<i>Millet-Millet</i> ⁴	882 g N ha ⁻¹ y ⁻¹		
<i>Bean-Millet</i> ⁴	917 g N ha ⁻¹ y ⁻¹	+4	
Rotation/Cover Crop Field Experiments			
<i>Oat-Maize</i>	-0.074 kg N ha ⁻¹		<i>Gomes et al., 2009</i>
<i>Vetch-Maize</i>	0.8 kg N ha ⁻¹	+1181	
<i>Lablab-Maize</i>	1.12 kg N ha ⁻¹	+1608	
<i>Pigeon Pea-Maize</i>	1.32 kg N ha ⁻¹	+1888	
Residue Incorporation – Field Experiments			
<i>Maize + Rye Straw</i> ¹	590 g N ₂ O-N ha ⁻¹		<i>Baggs et al., 2003</i>
<i>Maize + Bean Residue</i> ¹	1034 g N ₂ O-N ha ⁻¹	+75	
<i>Maize + Rye Straw</i>	158 g N ₂ O-N ha ⁻¹		
<i>Maize and Bean Residue</i>	790g N ₂ O-N ha ⁻¹	+80	
Rice			
<i>Rice</i>	0.66 g N ₂ O-N m ⁻²		<i>Kaewpradit et al., 2008</i>
<i>Rice + Groundnut residue</i>	0.73 g N ₂ O-N m ⁻²	+11	
<i>Rice + Rice Straw</i>	0.71 g N ₂ O-N m ⁻²	+7.8	
Maize + Residue			
<i>Maize + Natural Fallow Residue</i>	30 g N ₂ O-N ha ⁻¹ t residue ⁻¹		<i>Millar et al., 2004</i>
<i>Maize + Sesbania Residue</i>	539 g N ₂ O-N ha ⁻¹ t residue ⁻¹	+1700	
<i>Maize + Sesbania/Macroptilium</i>	557 g N ₂ O-N ha ⁻¹ t residue ⁻¹	+1760	
<i>Maize + Crotalaria</i>	162 g N ₂ O-N ha ⁻¹ t residue ⁻¹	+440	
<i>Maize + Macroptilium</i>	113 g N ₂ O-N ha ⁻¹ t residue ⁻¹	+277	

Legume-supported cropping systems for Europe

Residue Incorporation – Lab¹ Experiments			
Control Soil	4.56 mg N m⁻² d⁻¹		Frimpong et al., 2011
+ Cowpea residue (100)	13.4 mg N m⁻² d⁻¹	+194	
+ Cowpea/Maize residue (75:25)	13.27 mg N m⁻² d⁻¹	+191	
+ Cowpea/Maize residue (25:75)	8.76 mg N m⁻² d⁻¹	+92	

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Table 6: N-losses from spring barley-clover and barley-pea intercrops (after Pappa et al., 2011)

Crop	N ₂ O flux (kg N ₂ O-N ha ⁻¹)	% change compared to control	Nitrate leached (g NO ₃ ⁻ -N ha ⁻¹)	% change compared to control
Barley	0.9		0.3	
Barley – clover	2.8	+ 211	1.3	+ 333
Barley – pea cv. <i>Nitouche</i>	3.3	+ 267	0.2	- 33
Barley – pea cv. <i>Zero</i>	0.7	- 22	0.1	- 66

Table 7: Summary of environmental/agronomic effects of legume-based systems

	Legumes in Rotation	Forage Legumes	Legume Intercrops	Legumes as Green Manures/Mulches
N ₂ O mitigation	++	++	++	---
N leaching	+	+	+/--	+++
Phosphate leaching	++	+	++	na
Carbon sequestration	na	++	na	+++
Soil quality	+	++	+	+++
Biodiversity benefits	+	+	+	na

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Biodiversity and ecosystem services in legume-supported cropping

Susannah Cass, Michael Williams and Jane Stout

Abstract

Agriculture is a production process with physical inputs and outputs of resources that are intrinsically linked to the surrounding environment, and by manipulating local and regional ecosystems it plays an important role in shaping the biodiversity of life on earth – biodiversity which encompasses the genetic variability between individuals within a species, the vast range of unique species and the variety of habitats that make up local and regional landscapes. Agriculture relies not only on human endeavour and the physical environment, but also on biological process operating at all levels of biodiversity. Legume cropping, with its capacity to bolster terrestrial resources through the fixation of atmospheric nitrogen, can impact on such biological interactions throughout the agricultural ecosystem. This chapter summarises the role of legume-supported cropping in shaping biodiversity within agroecosystems. The impacts of management, in terms of tillage, fertilisation, pesticide and herbicide application are considered alongside the potential value to be derived from ecosystem services associated with biodiversity. Overall we conclude that management factors remain dominant in legume-supported cropping as in conventional cropping, and that legume biomass tends to increase the carrying potential capacity for associated biodiversity. However, impacts were found to be complex and a clear divide was seen between studies investigating the use of legumes to reduce populations of certain organisms, and those studies investigating legume-treatments for promoting associated biodiversity. Legume-supported cropping can both promote and reduce biodiversity within systems but appears to have a generally positive impact at the widest scales.

Introduction

Leguminous plants, through symbiotic interactions with rhizobial bacteria in root nodule structures, fix atmospheric nitrogen into organic form and when successfully nodulated are a source of biologically available N (Graham and Vance, 2000). Legume root, shoot and leaf biomass provides an enriched N resource for above- and belowground fauna whilst growing, and, as both surface litter and incorporated soil organic material, for the decomposer community and soil microorganisms after senescence and recombination (Mattson, 1980). Below ground, root exudates and living and senescent root biomass provide additional N enriched inputs to the soil (see Williams *et al.* this volume). Through subsequent trophic interactions, these N resources are transferred to all elements of the food web into which the leguminous plant is integrated. From associated vegetation communities to top insectivorous predators or transient herbivorous macrofauna, all organisms within the associated ecosystem are potentially affected by the presence of N-fixing legumes. Legumes have been repeatedly seen as a key functional group in a diverse range of ecological studies. These functions include: including: vegetation diversity-productivity gradients, vegetation community invasibility, natural enemy dynamics, soil structure and functioning, ecology and conservation of avifauna, greenhouse gas emissions from agriculture and carbon sequestration in soils (Drinkwater *et al.* 1998, Bullock *et al.* 2001, Curry and Schmidt, 2007, Fargione *et al.* 2007, Birkhofer *et al.* 2011). In the context of our increasingly urgent global need to tackle both the biodiversity crisis and climate change; these findings indicate that legumes ecology is an important research area.

Studies have shown that legumes can influence biodiversity which is increasingly recognised as providing ecological services of great importance to agriculture (Altieri, 1999). Within agroecosystems it may be useful to consider biodiversity, elsewhere defined as 'diversity within species, between species and of ecosystems' (Article 2 of the UN Convention on Biological Diversity, 1992), in terms of productive, resource and destructive biota (Swift and Anderson, 1993). Productive biota include those crops, trees and animals selected by the farmer to produce harvested resources; resource biota includes those organisms offering 'ecosystem services' to the system such as pollination, biological control and decomposition; and destructive biota include those weeds, insect pest, microbial pathogens etc. which actively reduce the ultimate quantity or quality of productive biota (Swift and Anderson, 1993). Biodiversity may be planned, as in the case of crop diversification, or the associated biodiversity of un-sown plants and wild fauna (Vandermeer and Perfecto, 1997). There is increasing pressure to consider biodiversity as a tool within agricultural design to simultaneously target species conservation and food security (Brussaard *et al.* 2010) which requires an understanding of the underlying mechanisms which generate and maintain diverse systems. Four main factors contribute to biodiversity within agricultural systems: the diversity of vegetation within and around the cultivated area; the permanence of various crops within the

system; the intensity of management of the system and the extent of isolation from areas of natural vegetation. These complementary aspects together contribute to the net effect any particular cropping system will have on biodiversity.

It is widely recognised that the presence of legumes can significantly increase biodiversity in some vegetation communities (Tilman et al. 1997, van der Heijden et al. 1998, Fargione et al. 2007), however to date research into the fundamental processes by which legumes may impact on biodiversity has focussed predominantly on the evolution and maintenance of natural or semi-natural, relatively temporally-stable vegetation communities. Such studies have shown legumes promote growth of neighbouring non-leguminous plants, alter competitive interactions within vegetation communities, increase invasibility and result in the maintenance of greater vegetation diversity (Smith and Gross 2007, Eisenhauer and Scheu 2008b). Methods for calculating an economic value for services such as biodiversity maintenance, pollination and pest regulation are being developed (Mace et al. 2012, Bommarco et al. 2013).

Legume-supported agroecosystems

Anthropogenic pressures of population growth, climate change, land use change and intensification of land management have led to a reduction in traditional legume-supported systems and there is now, in response to environmental and biodiversity crises, a need to better understand the potential for sustainable application of legumes in modern agricultural production (Peoples et al. 1995). This requires application of existing knowledge of fundamental legume ecology to agricultural systems in which management factors play a far greater role. These interactions are often limited to far shorter timescales, plant species compositions are heavily proscribed and manipulated (physical weeding, herbicide application), significant levels of exogenous organic and inorganic nutrients may be applied to the system, there is significant removal of organic material (harvest), soil may suffer regular disturbance (tillage, compaction) and many other non-plant species or groups of organisms may also be artificially manipulated (insecticides, fungicides, nematocides, biocontrol introductions).

Leguminous species are used variously as grain crops, forage, green manure, as 'catch' or 'alternative host' intercrops, in rotations and in agroforestry (Graham and Vance 2003). Each application places the plant at a different position in the agroecosystem and subtly moderates the strengths and directions of its interactions with surrounding organisms. The potential effects of legume-supported agricultural systems and practices can be usefully separated into two broad themes separating management from biological impacts. Management impacts can be thought of as those which are to some extent removed from the identity of the crops as legumes *per se* and result more from the management practices generally applied to the system. In contrast, biological effects result specifically from the functional traits of legumes as nitrogen fixing, dicotyledonous, flowering plants. It is important to understand how these traits have the

potential to impact on energy flow through the agricultural ecosystem and affect the biological communities and processes present by alteration of integral nutrient balances.

Impacts of legume cropping on associated vegetation

Weeds, unsown species, non-crop vegetation – what is vegetation biodiversity?

Non-crop flora are often generically termed ‘weeds’, yet it is important to recognise that whilst some species are detrimental to crop production, many species may be neutral in this regard, and a significant number of species may provide additional benefits as alternative hosts for pest species, attractants to pollinators or simply competitive stimulants to the overall productivity of the vegetation community (Albrecht 2003, Hyvönen and Huusela-Veistola 2008). Agricultural weeds are a source of food for phytophagous consumer fauna– fauna which may, in the absence of these weed species, have fed solely on the crop itself. As such agricultural weeds are seen as important indicators for biodiversity in agricultural systems (Albrecht 2003). In the context of this review we consider any additional biodiversity to be a broadly positive element, however in many cases legume-supported crops such as clover-ryegrass leys are included within rotations specifically for the purpose of limiting weed cover, particularly in organic systems (Hole et al. 2005).

Management effects of legume-supported cropping on vegetation biodiversity

Herbicide use has a direct impact on non-crop vegetation communities and is responsible for significant declines in flowering plant species once common in agricultural habitats (Hole et al. 2005). Therefore legume-supported crops used in systems which also have reduced chemical inputs may see improved vegetation biodiversity compared to conventionally managed crops. Soil disturbance caused by ploughing and other forms of tillage can also have a major impact on vegetation communities by directly uprooting and burying plants and altering the viability and germination of the soil seed bank (Feldman et al. 1997, Tuesca et al. 2001b).

Biological effects on vegetation biodiversity

As dicotyledonous species, legumes compete with non-crop plants for space and resources in a way that contrasts with grass-type crops. Shading by broadleaf legume canopy can reduce light penetration to ground level, and climbing and creeping growth forms add further structural complexity, which is of particular relevance in the case of intercropped and undersown systems with high leaf area index (Bilalis et al. 2010).

Legume crops can increase the availability of N to neighbouring non-legume crop plants by mechanisms of N sparing (Kumar et al. 1999), whereby the biologically fixed N

reduces the legume's requirement for soil N uptake; or by direct or indirect transfer of fixed N to surrounding plants (Hogh-Jensen 2006; Rasmussen et al. 2007). Such niche partitioning leads to more efficient use of resources and can allow a greater abundance of vegetation to inhabit a set area and may result in increased diversity of non-crop vegetation (Tilman et al. 1997)

Legume-supported systems can also have long-term impacts on vegetation communities via changes to soil structure, seed bank and soil chemistry over the course of several cropping cycles. Arable weed communities have been shown to remain influenced by previous land use after four years (Albrecht, 2003, Büchs, 2003) indicating that the annual flora of fields in crop rotations will be influenced by both the current species in cultivation and those used over several previous years. Due to these lasting effects on the non-crop flora, legumes can be used in rotations to make changes to the weed community for example Meiss et al. (2010c) used indicator species analysis to demonstrate that alfalfa (*Medicago sativa*) may suppress many weeds that are common in arable cropping whilst favouring other species. Problematic infestations of recognised 'noxious weeds' such as *Galium aparine* and *Cirsium arvense* were reduced following alfalfa cropping in this study (Meiss et al. 2010b, Meiss et al. 2010c). Crops such as alfalfa may release allelopathic compounds which directly limit the growth of weed flora in later stages of a crop rotation (Xuan and Tsuzuki, 2002).

Legume-supported cropping may also impact vegetation communities indirectly via secondary interactions with other organisms. For example legume crops which are allowed to reach flowering may influence the pollination of other flowering plants. Either by attracting additional pollinators and facilitating pollination in other species, or by competing for pollinators (Rathcke, 1983, Brookes et al. 1994, Ghazoul, 2006). By providing a spatially complex vegetation cover legume-supported cropping systems may stimulate seed predation by vertebrates, arthropods and earthworms (Eisenhauer et al. 2009, Meiss et al. 2010a). This is one reason why a general increase in biodiversity can be beneficial for long term stability, resilience and sustainability of the agroecosystem as a whole (Gurr et al. 2003, Kleijn et al. 2006). Within any particular legume supported system the net impact of legumes on associated vegetation will be the result of the local balance between all such, often conflicting, factors.

Vegetation biodiversity in legume-supported systems

Grass and forage

Impacts in grass systems depend on competitive interactions between grasses and legumes, in some cases by suppressing non-sown species and in others by opening gaps in dense grass swards allowing additional species to colonise (Andersson and Milberg 1998, Picasso et al. 2008, Connolly et al. 2009, Frankow-Lindberg et al. 2009). Legumes tend to favour vegetation species richness and diversity in natural and semi-natural mixed swards on soils with low to moderate mineral nitrogen reserves due to the

release of additional N resources to neighbouring plants and the conservation of the existing soil reserves (Fargione et al. 2007). However in highly fertile managed agricultural grasslands this may not be the case as nitrogen is not a limiting nutrient. Legumes such as red clover may struggle to compete with particularly vigorous high yielding forage grass species (Frankow-Lindberg et al. 2009) and may facilitate greater biodiversity due to patches opened up in these otherwise dense grass swards when the legume plants senesce. However in contrast, fast growing and creeping species such as white clover may prove equally or more effective than grass species in preventing invasion of swards by un-sown species (Connolly et al. 2009, Frankow-Lindberg et al. 2009).

Cover and green manure crops

Cover and green manure cropping systems are designed specifically to simultaneously reduce 'weeds' and enrich the soil with organic material to benefit neighbouring or succeeding crops. It is therefore unsurprising that these systems are seen to reduce abundance and biodiversity of associated vegetation species (Albrecht 2005, Gago et al. 2007, Chikoye et al. 2008, Bilalis et al. 2009, Huqi et al. 2009). Such cropping systems are often used in combination with reduced or no-till soil management in permanent cropping systems such as orchards and vineyards to reduce weed pressure without the nutrient losses that might otherwise be associated with harrowing and other mechanical weed reduction methods. Unlike mechanical weeding and herbicide application, legume based cover and green manure crops may alter the community structure of associated vegetation in favour of broad leaved species rather than competitive grasses which may lead to the maintenance of a low abundance yet none the less diverse community featuring a greater range of rare species with potentially beneficial impact to other organisms within the ecosystem (Meiss et al. 2010c).

Agroforestry and woody mulches

Whilst legume-supported cropping in Europe is predominantly concerned with herbaceous plants there are also a wide range of leguminous tree species utilised in agricultural systems, particularly in tropical areas with particularly nutrient poor soils and predominantly low input subsistence farming practices (Graham and Vance, 2003). Material pruned from leguminous trees and hedgerows can be used in much the same as living green manures and cover crops- to suppress unsown species by physical crowding, shading of the soil surface and promotion of decomposition rates to reduce the viability period of the soil seedbank (Egbe et al. 1998, Ekeleme et al. 2004). Some woody leguminous tree species such as Acacia have also been found to have additional allelopathic properties leading to enhanced suppression of weeds (El-Khawas and Shehata, 2005).

Intercropping and undersowing

Intercropped and undersown systems are designed to fertilise the companion crop and suppress non-crop vegetation. Where the legume component of such systems is not

sufficiently vigorous, often due to climatic conditions, these systems may have negligible effect on weed abundance (Hyvonen and Salonen, 2002, Lundkvist et al. 2008, Deveikyte et al. 2009). However in the majority of reported studies on a wide variety of intercropped and undersown systems (Table 1), non-crop vegetation was significantly reduced in legume supported cropping (Jorgensen and Moller, 2000, Hyvonen and Salonen, 2002, Chikoye et al. 2004, Bilalis et al. 2010, Koepke and Nemecek, 2010). It may in fact be beneficial to legume grain yield to intercrop with cereal to increase competitiveness with weeds in some environments (Salonen et al. 2005).

Grain legume crops

Legumes grown in monoculture for harvest in their own right are the most variable in terms of their impacts on associated vegetation biodiversity. As with any monoculture, occurrence of unsown species is related to the physical structure of the crop, competitiveness, cropping season and soil and pest management practices utilised – all of which vary significantly within the wide range of legume species commonly used in agriculture (Graham and Vance 2003). Grain legume crops are seen to have relatively high associated vegetation biodiversity in conditions where they weak competitors and in systems where high non-crop biomass is not significantly detrimental to production (Shrestha et al. 2002, Albrecht, 2008, Lundkvist et al. 2008). However, in intensively managed systems grain legumes are seen to have particularly low non-crop vegetation biodiversity as herbicide and tillage effects are strong (Mas and Verdu, 2003, Chikoye et al. 2008, Deveikyte et al. 2009).

Legume crops in rotations

Increasing the diversity of crops within an agricultural system has clear potential biodiversity benefits in the form of a greater range of habitats and niches for species to exploit. However the overall impacts on non-crop vegetation of legumes in rotation are again seen to vary widely between studies, demonstrating that exact system design and management impacts have an overriding influence in specific cases. Weed diversity and abundance of certain species were reported to benefit from legume crops in rotation in some cases (Murphy et al. 2006, Graziani et al. 2012) whilst reductions in weed abundance and weed seedbanks are seen in other cases (Murphy et al. 2006, Koepke and Nemecek 2010). These results suggest that inclusion of legume crops in rotations may promote diversity whilst reducing the abundance of the non-crop vegetation community.

Table 1 Reported impacts of legume-supported cropping on elements of non-crop vegetation biodiversity.

Cropping system	Directional impact on biodiversity	Elements of biodiversity measured	Systems studied	References
Pasture, grass leys and forage	+	Weed abundance	Grass/clover ley, red clover, clover,	(Andersson and Milberg, 1998), (Connolly et al. 2009), (Frankow-Lindberg et al. 2009)
	-	Weed abundance	Alfalfa, white clover	(Picasso et al. 2008)
Green manure and cover-crops	-	Weed seedbank	Grass/clover leys	(Albrecht, 2005)
	-	Weed abundance	<i>Pueraria phaseoloides</i> , <i>Aeschynomene histrix</i> ; vetch; vineyard with grass/clover cover crop; olive orchard with pea/rye cover crop	(Chikoye et al. 2008), (Bilalis et al. 2009), (Gago et al. 2007), (Smith and Gross, 2007, Huqi et al. 2009)
Agroforestry and woody mulches	-	Weed abundance	<i>Pterocarpus</i> and <i>Millettia</i> prunings as mulch, woody legume prunings, <i>Acacia auriculiformis</i> prunings	(Egbe et al. 1998), (Ekeleme et al. 2004)
Intercropping and undersowing	=	Weed abundance	pea/spring cereal	(Deveikyte et al. 2009), (Hyvonen and Salonen, 2002), (Lundkvist et al. 2008)
	-	Weed abundance	Spring maize/cowpea, maize/bean (<i>Phaseolus vulgaris</i>), maize/bean (<i>Vicia faba</i>), Maize/Phacelia, maize/velvet (<i>Mucuna cochinchinensis</i>), bean	(Hyvonen and Salonen, 2002), (Bilalis et al. 2010), (Jorgensen and Moller, 2000), (Chikoye et al. 2004), (Koepke and Nemecek, 2010)

Grain legume monocrops	+	Weed abundance	White lupine, pea, (kidney bean, white bean, soybean combined)	(Albrecht, 2008), (Lundkvist et al. 2008), (Shrestha et al. 2002), (Tuesca et al. 2001a)
	=	Weed abundance	pea	(Andreasen and Skovgaard, 2009)
	-	Weed abundance	Pea, soybean, cowpea	(Deveikyte et al. 2009), (Chikoye et al. 2008), (Mas and Verdu, 2003)
Legumes in rotation	+	Weed seedbank diversity	Soybean in rotation	(Murphy et al. 2006)
	+	Amaranthus retroflexus and Chenopodium album seedbanks	Legumes in Mediterranean rotations	(Graziani et al. 2012)
	+	Weed abundance and diversity	Alfalfa/ryegrass hay in rotation	(Sosnoskie et al. 2009)
	=	Weed abundance and diversity	Soybean in rotation	(Sosnoskie et al. 2009)
	-	Weed abundance	Faba bean in rotation	(Koepke and Nemecek, 2010)
	-	Weed seedbank density	Soybean in rotation	(Murphy et al. 2006)

Impacts on above-ground invertebrate biodiversity

Pests, predators, pollinators – what is invertebrate biodiversity?

Key above ground invertebrates in agroecosystems can be divided into herbivores, granivores and flower-feeding primary consumers; secondary consumers and higher predators; and parasitoides. Each group has the potential to significantly impact on the flow of nutrients through the ecosystem and thus potentially affect crop production. Intensive agricultural techniques have proved that it is possible to produce crops without needing to maintain a diverse ecosystem of invertebrates. However, it is becoming increasingly apparent that this is not a sustainable approach, and with significant declines in populations of pollinators (Biesmeijer et al. 2006) and other farmland wildlife,

it is increasingly likely that even the most intensive agricultural systems may not be able to continue profitable production without concessions to biodiversity. Invertebrates are some of the key biota affecting the productivity, stability and resilience of ecosystems in the face of perturbation and it is important that any long-term sustainable agricultural system is supported by a diverse invertebrate fauna (Duelli, 1997, Hawksworth, 1991) that is able to provide ecosystem services such as pollination and natural enemy control of pest populations (Perrings et al. 2006).

Effects of crop management on invertebrate biodiversity

The application of chemical pesticides is clearly the predominant management factor affecting invertebrate biodiversity in agricultural systems (Geiger et al. 2010, Goulson, 2013). Numerous studies have clearly demonstrated the biodiversity increases associated with organic or similarly termed pesticide free systems (Rundlöf et al. 2008, Gabriel et al. 2010, Power and Stout 2011, Gabriel et al. 2013) and biodiversity of invertebrates in any legume-supported system will be heavily dependent on the type of and degree to which pesticides are used. As legume-supported cropping is often used as a tool in organic agriculture it is likely that legume cropping on average has a higher associated invertebrate biodiversity than 'conventional' systems. However, in comparisons between conventional and organic production, it is often hard to distinguish the individual impacts on invertebrate biodiversity of reduced inorganic pest control, wider landscape context and legume crops.

Biological effects of legume crops on invertebrate biodiversity

As with vegetation biodiversity, invertebrate biodiversity in legume-supported cropping is heavily influenced by the spatial structure of the crop habitat. Increases in structural complexity of vegetation lead to increased habitat for invertebrate species and thus cover-crops, undersowing, inter-cropping and mulches may all be expected to have positive impacts on invertebrate biodiversity. Beneficial arthropods are known to benefit from ecotones, the presence of which is increased in diversified agricultural landscapes (Duelli, 1997) resulting from the introduction of legume crops into a farming system.

As primary producers legumes have a significant impact at higher trophic levels due to the low C:N ratio of their N enriched biomass providing high quality, accessible nutrients to consumers (Sileshi and Mafongoya, 2007). This rich nutrient resources is available indiscriminately to invertebrate herbivores and therefore benefits pest species as much as it does those with neutral or positive impacts on crop productivity. Without a diverse and well-structured community of invertebrates and other organisms the attractiveness of legume vegetation could have a detrimental effect on production in cropping systems, however in a healthy ecosystem increased pest populations can lead to increased

predator and parasitoid populations with a resulting equilibrium between pests and natural enemies (Price et al. 1980).

As flowering plants legumes also provide a significant additional resource to invertebrate pollinator species including Hymenoptera, Diptera and Coleoptera which may be particularly important in landscapes dominated by wind-pollinated cereals (Brookes et al. 1994, Potts et al. 2009). The section below covering pollination services provides further details on this subject.

Legume-supported cropping may support invertebrate communities by providing habitat and food resources at times in the season when such resources are scarce. This is particularly relevant in the case of cover crops where conventional systems would have bare fallow. Legume-based seed mixtures in crop margins, as opposed to margin-free fields, may support predator species whilst within the field the soil is left bare and thus maintain a standing population of natural enemy control agents able to respond rapidly to seasonal increases in pest populations (Pywell et al. 2011).

Impacts of legume-supported cropping on invertebrate herbivores may have further indirect impacts on neighbouring plants and soil food webs as herbivory can alter nutrient transfer. An increase in biologically fixed N was found by Ayres et al. (2007) in perennial ryegrass (*Lolium perenne*) when neighbouring white clover (*Trifolium repens*) was defoliated, soil microbial biomass was also significantly increased in the same system.

Aboveground invertebrate biodiversity

Grass and forage

Studies demonstrate flower-feeding flying invertebrates such as Hymenoptera and Lepidoptera benefit from legume-rich grass and forage systems. Grass-clover leys have been shown to support significantly higher abundances of non-pest butterflies in organic rotations (Feber et al. 1997) and both butterfly and bumblebee abundance was increased in intensive forage swards with sown legumes (Potts et al. 2009). Similar results for butterfly abundance were also seen in biofuel swards rich in legume species (Myers et al. 2012).

Cover and green manure crops

The habitat provided by sowing legumes as ground cover in orchards and vineyards has been seen to promote arthropod richness and diversity (Osler et al. 2000, Fernandez et al. 2008). Hymenoptera, Diptera and Aranaea were also seen to benefit from alfalfa (*Medicago sativa*) used as green manure in some situations, however the effects on Diptera, Aranaea and Coleoptera were not consistent (Curry 1986). One study reported a reduction in Lepidoptera larvae, a commercial pest, in broccoli using yellow sweetclover (*Melilotus officinalis*) as a cover crop (Hooks and Johnson 2001).

Agroforestry and woody mulches

Adding leguminous shade trees (*Erythrina poeppigiana*) to coffee plantations diversifies the habitat and resulted in higher species richness and diversity of hopper (Homoptera, suborder Auchenorrhyncha) species in one study (Rojas et al. 2001). The authors note that although hoppers can be significant pests in some coffee plantations they were not seen as a detrimental species in the studied system which indicates that there may be influence of predatory and parasitoid species preventing damaging outbreaks. In contrast a study of leguminous hedgerows and woody mulch in maize cropping found no discernible effect on maize stem-borers and stem-borer parasitoid species (Midega et al. 2004).

Intercrops

Predatory and parasitoid species are the focus of several legume intercropping studies with positive effects on parasitoid wasps and predatory invertebrates reported in three papers (Midega et al. 2008, Midega et al. 2009, Caballero-Lopez et al. 2012) and, as in the agroforestry element of the same study, no effect reported in one (Midega et al. 2004). These increases are likely to be due to concurrent increases in herbivore communities, for aphids in the case of Caballero-Lopez et al. (2012). However the resulting population balance between herbivores and natural enemies can reduce pest population as seen in a maize/bean intercropping system (Belay et al. 2009).

Grain legume crops

The number of nests of the bumblebee *Bombus pascuorum* was found to be positively influenced by field bean crops (Knight et al. 2009) indicating that the floral abundance of flowering legume crops can be beneficial to pollinator species. High spider species richness in soybean compared to most non-legume grain crops was suggested by Uetz et al. (1999). However research where the specific effects of grain legumes on invertebrate diversity can be extracted from other management factors appears to be lacking.

Legumes in field margins

The use of flowering legume species in field margins is specifically recommended for the promotion of farmland biodiversity and it is no surprise that the literature supports this. The abundance and diversity of many insect groups and species including bumblebees, cuckoo bees, pollinators and beneficial arthropods are all reported (Lagerlof et al. 1992, Carvell et al. 2007, Pywell et al. 2011). Field margins provide stable and diverse habitats which support invertebrate fauna in general, including herbivorous species (Pywell et al. 2011), but studies suggest that herbivore numbers in neighbouring fields are kept in check in such systems by increased abundance of predators (Denys and Tschardtke, 2002).

Table 2 Reported impacts of legume-supported cropping systems on elements of above-ground invertebrate biodiversity.

Cropping system	Directional impact on biodiversity	Elements of biodiversity measured	Systems studied	References
Pasture, grass leys and forage	+	Butterfly abundance	Legume-rich biofuel swards; sown legume-rich forage swards	(Myers et al. 2012), (Potts et al. 2009, Feber et al. 1997)
	+	Bumblebee abundance	Sown legume-rich forage swards	(Potts et al. 2009)
	+	Ant abundance	<i>Arachis pintoii</i> in pastures	(Velasquez et al. 2012)
Green manure and cover-crops	+	Arthropod richness and diversity	Apple orchard/fescue+alfalfa mulch; apple orchard/strawberry clover mulch; lupine; walnut orchard/alfalfa; <i>Pueraria phasioloides</i>	(Fernandez et al. 2008), (Osler et al. 2000), (Stamps et al. 2002), (Vohland and Schroth, 1999)
	+	Hymenoptera, Diptera, Aranaea	Alfalfa	(Curry, 1986), (Uetz et al. 1999)
	=	Diptera, Aranaea, Coleoptera	Alfalfa	(Curry, 1986)
	-	Lepidoptera larvae	Broccoli/Yellow sweetclover (<i>Melilotus officinalis</i>) cover	(Hooks and Johnson, 2001)
Agroforestry and woody mulches	+	Hopper species diversity	<i>Erythrina poeppigiana</i> shaded coffee plantations	(Rojas et al. 2001)

Legume-supported cropping systems for Europe

	=	Stem-borer parasitoids	Gliricidia hedgerows, leucocephala hedgerows	sepium Leucaena	(Midega et al. 2004)
Intercropping and undersowing	+	Activity of stem-borer specialist parasitoid wasps	Maize/Desmodium		(Midega et al. 2009)
	+	Forb aphids and predatory invertebrates	Winter cover	wheat-legume	(Caballero-Lopez et al. 2012)
	+	Spider abundance and diversity	Maize/desmodium		(Midega et al. 2008)
	=	Stem-borer parasitoid activity	Maize/Cowpea,		(Midega et al. 2004)
	-	Stem-borer abundance	Maize/bean		(Belay et al. 2009)
Grain legume monocrops	+	Bumblebee nests (<i>Bombus pascuorum</i>)	Field beans		(Knight et al. 2009)
	+	Spider species richness	Soy bean		(Uetz et al. 1999)
Legumes in field margins	+	Abundance and diversity of bumble and cuckoo bees	Legume-based and nectar seed mixture	pollen	(Carvell et al. 2007), (Lagerlof et al. 1992)
	+	Insect abundance	Legume-based field margin	sown	(Lagerlof et al. 1992)
	+	Pollinator herbivore species, beneficial arthropod predators	Legume-based field margin	sown	(Pywell et al. 2011)

Impacts on below-ground biodiversity

What is belowground biodiversity?

There exists a wealth of diversity among species of macro, meso- and microfauna which spend parts or the whole of their life cycles below the soil surface. Belowground biodiversity in legume crops is heavily dependent on local management, climatic, edaphic and biological factors (Wallwork, 1976) and may provide stability against perturbation. Redundancy among species of microbes and soil fauna can increase the resilience of ecosystem service provision to disturbance (Garbisu et al. 2011).

Among belowground fauna, earthworms are considered ecosystem engineers (Römbke et al. 2005) for their key roles in decomposition, nutrient cycling and soil structuring processes. Due to their key role in belowground ecosystem services, earthworm species richness has been proposed as a priority indicator of soil biodiversity (Bartlett et al. 2010).

Management effects of legume-supported cropping on belowground biodiversity

In comparison to natural ecosystems, agriculturally managed soils can have highly simplified faunal communities and lower activity and abundance of organisms (Curry, 1986). This is predominantly a result of the degradation of soil structure and fertility resulting from low replacement rates of organic material in harvested systems where biomass is not re-incorporated into the soil, soil tillage and soil compaction due to machinery use or livestock trampling (Bartlett et al. 2010, Curry et al. 2008).

Earthworm populations are strongly affected by soil disturbance (Curry et al. 2002) meaning any impacts of legume cropping will be significantly moderated by the methods of cultivation used. However, it has been shown that at least two years of permanent grass/clover cover are required for the full development of earthworm populations even in highly favourable temperate soils (Schmidt and Curry, 2001) indicating that maximal populations may not be reached in annual cropping systems or short term grass leys. Population dynamics within rotations are likely to be highly fluctuating depending on the crop types used, order of rotation and duration of un-tilled recovery periods.

Biological effects on belowground biodiversity

The physical structure of above-ground vegetation and litter in legume-supported systems influences belowground biota. Surface litter cover has been shown to increase soil faunal feeding activity (Römbke et al. 2006) and decomposition by soil microbes has

been shown to be positively correlated to biomass of non-crop flora (Wardle et al. 1999). The presence of soil surface litter reduces earthworm dispersal (Mathieu et al. 2010) and may result in high populations particularly in green manure and inter/under-sown crops where legume litter is maintained as a mulch layer.

Surface litter subsequently becomes a nutrient input to the belowground food web as it decomposes and the C and N inputs of legume-supported cropping significantly impact soil biota, often to a greater extent than conventional fertilisers (Altieri 1999).

Legume cropping is also used to diversify crop systems over time, crops such as alfalfa are used as a 'biological break' in a rotation to reduce pest populations which build up over successive seasons of other arable crops (Altieri 1999) by removing the host plants and potentially by the alteration of soil chemistry with allelopathic compounds.

By promoting soil biodiversity legume-supported cropping may also have additional effects on crop productivity and non-crop vegetation. Grassland diversity experiments have shown earthworm presence to increase total plant community productivity, increase legume shoot biomass, increase the invisibility of plant communities and alter competitive interactions between plant species (Eisenhauer and Scheu 2008a, Eisenhauer et al. 2009). The impacts of clover on N transfer to wheat in an intercropping system were shown to be significantly related to the earthworm population, with earthworms altering inter and intra-crop N allocation and increasing biomass production of wheat in the system (Schmidt and Curry 1999).

The effects of legumes on above-ground invertebrates may also be indirectly influenced by belowground processes, for example the enrichment in N concentration of grass biomass in the presence of earthworms lead additionally to a 95% increase in aphid infestation in a greenhouse mesocosm experiment (Eisenhauer and Scheu 2008a).

Belowground biodiversity in different legume-supported cropping systems

Grass and forage

In a two year study of 92 grassland sites across Germany, feeding activity of soil fauna was found to be positively related to legume and grass species richness whilst no additional impacts of agricultural management were observed indicating that floral biodiversity had a common effect on soil activity above and beyond the influences of local and regional differences in management and environmental conditions (Birkhofer et al. 2011).

Cover and green manure crops

Legume cover-cropping has been shown to have long-term effects on multiple groups of belowground fauna. Maize cultivated with a cover crop of *Mucuna pruriens* var. *utilis* in Benin was characterised by higher densities of termites, earthworms, isopods,

Coleoptera, centipedes, millipedes, and a number of nematode functional groups, in comparison to traditionally grown maize (unfertilised monoculture) and maize fertilised with mineral NPK (Blanchart et al. 2006). The observed increase in facultatively phytophagous, bacterial-feeding, and predatory nematode species under this legume cover crop, combined with a simultaneous decrease in obligatory phytophagous species indicate that legume covers may potentially suppress root damage by pathogenic nematodes and enhance inter-specific biocontrol within the nematofauna.

Cover cropping with legumes can result in both higher densities and higher biomass of earthworms (Blanchart et al. 2006). The relative average densities of earthworms reported in this study: 121 individuals/m² under traditionally cultivated (unfertilised) maize, 360 individuals/m² under NPK fertilised maize and 579 individuals/m² under maize intercropped with *Mucuna pruriens*, indicate a higher carrying capacity of the system resulting from increased soil nutrient levels in the form of accessible organic material. Earthworms have been seen to increase soil aggregate stability and the storage of C and N in a soybean (*Vicia faba*) cropping system (Ketterings et al. 1997).

Agroforestry and woody mulches

Soil impacts were reported from maize cropping systems utilizing woody legume material as mulches. Soil invertebrates including earthworms and centipedes were found to benefit in these systems (Sileshi and Mafongoya 2006, Sileshi and Mafongoya, 2007, Sileshi et al. 2008) suggesting that added nutrients may benefit decomposer groups and the soil food web.

Intercropping and undersowing

Large earthworm populations of more than 1000 individuals m⁻², 250g m⁻² and 7-10 species, have been recorded under wheat under-sown with clover in comparison to conventional wheat crops (Schmidt and Curry, 1999). In some undersown systems the positive effects on earthworm communities of legumes may be sufficiently strong to outweigh the negative impacts of soil disturbance, for example wheat-clover intercropping was shown to have a greater impact on earthworm populations than tillage intensity in a comparison of conventional tillage and direct drilling (Schmidt et al. 2003).

Grain crops

Isoptera were found to benefit from soybean cropping (de Aquino et al. 2008), suggesting that decomposer organisms may benefit from grain legume residues in the same way as in intercropping. A study by Briar et al. (2007) found no significant impacts of soybean monoculture on soil nematode populations. This may be due to the contradictory responses of different nematode groups to the biological and management impacts of legume cropping with shifts in population dominance rather than overall changes in abundance (Neher and Lee Campbell, 1994).

Legumes in rotation

The use of legumes in crop rotations is likely to have complex effects on belowground biodiversity. In some cases legume crops are used for their allelopathic properties to reduce populations of potential pests such as plant feeding nematodes (Stirling et al. 2002, Wang et al. 2002). However legume rotations have also been observed to benefit some groups of soil fauna such as earthworms as seen in rotations incorporating red clover and soybean (Jordan et al. 2004).

Landscape biodiversity and macrofauna

Legume-supported cropping may benefit diversity at the landscape scale in terms of spatial and temporal habitat diversity as additional crop species in areas of monoculture and as additional species in rotations over seasons or years (Altieri 1999). Landscape effects have the greatest impact on larger and more motile organisms such as farmland birds, bats, vertebrates and flying insects through provision of increased foraging and nesting habitats, and range of food and other resources (Wilson et al. 1997, Andersson et al. 2013).

The rapid decline in farmland bird populations associated with the intensification of European agriculture (Wilson et al. 1997, Donald et al. 2001) has for some time been a topic of significant public and scientific concern. Declines have been most severe in those species which had adapted specifically to the environmental niches offered by agricultural landscapes that developed over centuries and before the development of intensive cultivation practices. As a result of intensive production, the carrying capacity of agricultural landscapes for bird populations has been dramatically reduced. The major contributing factors being reduction of hedgerow nesting habitats, reduction in ground cover and increased disturbance for ground nesting birds, reduced retention of stubble leading to a reduction in over-wintering seed availability, increased efficiency of harvest leading to reduced seed spillage, reduced abundance of flowering weeds to attract insects as chickfood and reduction in seed-set by non-crop flora (Newton, 2004). Changes to the non-crop vegetation community resulting from legume cropping may benefit farmland bird populations by favouring species upon which their diets rely (Moorcroft et al. 2002). Dandelions (*Taraxacum officinale*), while considered a weed species in many contexts, is also a key element of the diet of the linnet (*Carduelis cannabina*), an endangered farmland bird species (Moorcroft et al. 2006). Populations of dandelion in wheat crops may be increased by a preceding crop of alfalfa (Meiss et al. 2010b) with subsequent potential benefits to linnet populations.

Increased diversity of non-crop flora associated with some legume-supported systems has been shown to be beneficial for some farmland bird species in Europe. Legume forage crops within a mixed steppe and extensive agriculture landscape matrix have been observed to support high densities of Little Bustard (*Tetrax tetrax*) males in France (Wolff et al. 2001). Santangeli and Dolman (2011) found that bustards selected

landscape areas with higher amounts of legume cropping which may have been related to food requirements and suitable habitat structure for displaying males. Mammals such as the Eastern cottontail rabbit (*Sylvilagus floridanus*) also benefit from legume-cropping habitats (Vidus-Rosin et al. 2009).

Given the reported effects of legume cropping on vegetation communities and ground invertebrates, elements key to the diets of many farmland mammals, it is highly likely that the effects of legumes are passed on to higher trophic levels and mammalian consumers. In a study of cover cropping in organic orchards, Wiman et al. (2009) observed numbers of meadow voles (*Microtus pennsylvanicus*), a species considered a pest in these systems as it is destructive to fruit trees, to be highest in a mixed legume cover treatment, and land area cultivated with peas was positively correlated with proportion of rat remains in the pellets of Mackinder's eagle owls (*Bubo capensis mackinderi*) in central Kenya (Ogada and Kibuthu, 2009).

In landscapes often dominated by wind-pollinated cereal crops, mass flowering species including legumes and others such as oilseed rape (*Brassica rapa*), significantly increase the pollen and nectar resources available to a range of organisms including commercially valuable pollinator species (Stanley and Stout, 2013). Yet little information is currently available on the relative volume and quality of the resources provided by the full range of legume crop species.

Ecosystem service provision

Biodiversity in agricultural systems is now recognised to be vital to the supply of numerous ecological services, including nutrient cycling, soil structure and functioning, hydrological processes and crop protection, as reviewed by (Altieri 1999, Tscharntke et al. 2005, Altieri and Rogé 2010). Legume-supported cropping has the potential to deliver many such ecosystem services and particularly to promote pollination, pest control, soil health and conservation of biological diversity.

Pollination services

It has been estimated that with 84% of crop species cultivated in Europe depending on insect pollinators. Global pollination services have been valued at least €153,000 million annually, and given that legumes in the livestock and dairy industries were not factored into this analysis, it is likely to be a conservative estimate (Gallai et al. 2009). With such a reliance on insect pollinators, the recent declines seen in many of these species are of immediate concern. Many measures are being applied in an attempt to halt the decline and further boost pollinator populations (Carvell et al. 2007, Scheper et al. 2013). Flowering crops which are attractive to pollinator species and provide nectar and pollen rewards aid conservation efforts and improve crop yields and sustainability (Palmer et al. 2009). Legume pollinators include a wide range of social, eusocial and solitary bees, both wild populations and commercially managed (Free 1970, Delaplane and Mayer 2000) and thus legume-supported cropping has the potential to play a significant role in attempts to stabilise bee populations. However, mass flowering crops such as legumes, whilst providing a significant resource for large numbers of pollinators, only do so for a short period of time. Pollinator responses to mass flowering crops differ depending on the wider landscape context (Hanley et al. 2011, Stanley et al. 2013). The effects of legumes on pollinators are also subject to the same complex ecosystem interactions as any other element of biodiversity, for example competitive interactions with grasses were shown to reduce legume flower head production in mixed mesocosms (Eisenhauer and Scheu 2008a) potentially reducing the resource available for pollinators.

The characteristic floral morphology of legumes is widely attributed to the rapid divergence of the angiosperm line via pollination syndromes and co-evolution with specific pollinator groups. The long corolla and curved nectar tube and colouring of many legume flowers are evidence of their relationships with specific insect pollinator groups whose proboscis and feeding strategy have evolved in tandem with the pollination requirements of the flower in a mutually beneficial manner. Breeding to improve desired traits in crop species has traditionally focused on crop yield increases and improved environmental tolerance, however recent calls have been made for a shift in emphasis towards improving additional environmental functions and better integrating crops into healthy agroecosystems (Klein et al. 2007, Palmer et al. 2009). Improving floral attractiveness through selection for traits including colour, morphology, phenology

and the quantity and quality of nectar and pollen rewards is an area in which crop breeding strategies may achieve these aims whilst simultaneously improving crop productivity through cross-pollination and hybridisation (Palmer et al. 2009). Different categories of legume crops differ in their reliance on insect pollination. Shifts from out-crossing to selfing are prevalent in the recent history of many grain legumes such as soybean, and provide evidence of their intensive domestication, whilst out-breeding remains the dominant mode for the majority of forage legumes and other species not predominantly bred for seed production (Carbonero et al. 2011).

Changes in agricultural practices over time have had significant impacts on pollinator groups. The loss of legumes from cropping systems, including changes from legume-rich hay meadows to silage cutting, are known to be linked to declines in declines in bumblebee numbers for example (Goulson et al. 2005, Fitzpatrick et al. 2007).

Natural enemy pest control

Invertebrate populations may increase in response to the availability of high C:N ratio legume biomass and in turn support larger populations of predatory and parasitoid species. These alternative prey species may be sufficient to maintain predator populations where successful reduction of a specific crop pest may otherwise have led to local extinction of the predator and exposed the system to re-infestation. Increased cover of legumes in organic crops of winter wheat has been seen to significantly increase the richness of aphid parasitoids (Caballero-Lopez et al. 2012). Mass flowering of legume crops may also provide a significant alternative food sources for some predatory species in the form of floral rewards of nectar and pollen, particularly for pollen feeding predator species of the Carabidae and other groups (Hodek et al. 1996, Norris and Kogan 2000).

Noxious Weed Suppression

A number of mechanisms may be responsible for these increases in seed predation. Increased N content, and therefore food quality, of legume crops may supplement the diet of species which consume both vegetation and seed and subsequently support larger populations leading to an ultimate increase in seed predation rates. The reported tendency for legume cropping to support large earthworm populations may increase seed predation by surface foraging anecic species such as *Lumbricus terrestris* which have been observed to consume significant amounts of surface seeds (Eisenhauer et al. 2009). Gallandt et al. (2005) define three pre-dispersal mechanisms by which cover crops may influence the growth and reproduction of non-crop flora and thus affect important classes of seed predators such as ground beetles (Carabidae). They highlight the potential for ground disturbance, associated with establishment of crops and cover crops, to pre-empt weed growth; that cover crops are generally selected for

their capacity for vigorous growth to compete with and suppress weed biomass and reduce seed production; and also suggest that there may be long term residue effects resulting from the recombination of cover crop biomass. Post-dispersal effects on the weed seed bank may also result from legume cropping through improvements in soil quality leading to increase microbial activity, faster rates of decay and subsequently a reduction in the time seeds remain viable in what have been termed 'weed-suppressive' soil conditions (Kremer and Kennedy, 1996).

Non-crop plant communities may also experience natural enemy control in the form of seed predators. Invertebrates are considered to be responsible for levels of seed predation between 56-58% with vertebrates contributing an additional 13-18% (Gallandt et al. 2005). Weed seed predation has been shown to increase with the complexity of vegetation cover and is therefore likely to be a significant influence in legume-supported intercropping, under-sowing and cover-crop systems as demonstrated by findings of high seed predation in continuous alfalfa forage in comparison to both cocksfoot grass forage and bare soil (Meiss et al. 2010a). In this study 16-64% of variation could be explained by vegetation cover, however it must be noted that cutting regime had a stronger influence than crop species, once again indicating that the impacts of legumes must only be considered within the context of additional management practices.

In a wheat intercropping system the influence of red clover ground cover was again shown to double rates of seed predation in comparison to wheat monocropping (Davis and Liebman 2003), and reports from a number of authors (Anderson 1998, Gallandt et al. 2005, Heggenstaller et al. 2006) suggest that legumes may have additional effects beyond those attributed to vegetation structure. Seed predation assays have demonstrated 200% increases in the rate of giant foxtail (*Setaria faberi*) seed removal in wheat-red clover cropping systems compared to a wheat monoculture control, with the difference attributed to a simultaneous eight-fold increase in the activity-density of crickets in the same system (Davis and Liebman, 2003).

Soil quality

Maintaining soil quality is vital to the sustainable use of agricultural land. Soil quality is a measure of a soil's effectiveness in performing such critical functions as nutrient cycling, water management and supporting plant growth and development (Karlen et al. 2003). Biodiversity of a system, particularly but not limited to biodiversity of soil organisms, is integral to efficient soil functioning and biomass, activity and biodiversity of numerous groups including microbes (Garbisu et al. 2011) and earthworms (Bartlett et al. 2010) have been used as indicators for soil quality.

Conservation

Conventional modern agriculture does not always recognise the potential benefits of associated non-crop vegetation, with soil management and herbicide applications generally designed to reduce non-crop plants as far as possible. This has led to many plant species which have specifically adapted to the niches provided by extensive agricultural landscapes over many centuries prior to the industrialisation of modern agriculture, becoming rare and endangered. By example, arable weeds in Germany belong to vegetation types with the highest percentage of endangered species (Albrecht, 2003) and the decline in non-crop vegetation diversity in the 20th Century has been linked to significant knock-on declines in the populations of small mammals and farmland birds (Robinson and Sutherland 2002).

Conclusions

From the literature reviewed here it is clear that legume-supported cropping has significant impacts on biodiversity in agroecosystems both above and below ground, locally and at a landscape scale. However, it is also clear that the relationships between legume crops and non-crop flora and fauna are highly complex and there is no single overriding direction of these impacts. Biodiversity in legume-supported systems appears to be affected in the first instance by the same management factors, in terms of soil disturbance, chemical pest control, nutrient inputs and duration of cropping, as in any other non-legume agricultural system. The precise nature of the response of specific elements of the agroecosystem is then seen to be influenced in more subtle, but none the less active, ways by the legume crops themselves.

Again however, there is no clear directionality to the effects of legumes on biodiversity in agricultural systems reported in the literature. This is immediately obvious from the very nature of the research that covers this area. Research which is quite clearly divided between studies looking at the agronomic value of utilising legumes for increased cropping efficiency, and studies looking at the agricultural practices and conservation potential for increasing beneficial biodiversity using legumes. Research of the first kind is designed to answer questions about the potential of legume cropping to reduce pest and improve productivity and therefore reported results demonstrate systems in which legume-cropping reduces the abundance and diversity of certain organisms. Whereas studies belonging to the latter group are designed to quantify the effectiveness of legume-supported practices which have been adopted specifically in the hope of increasing biodiversity and thus the results tend to show an overall positive effect. Those studies which simply catalogue the flora and fauna of certain cropping systems perhaps provide the best idea of how legume-supported systems compare to conventional systems however the lack of an experimental element to these means that results are often hard to interpret in a statistically significant manner.

We observe that legume-supported cropping does for the most part influence biodiversity in ways that are to be expected based on underlying ecological theories, and note that in some cases the extreme complexity of these interactions has been clearly demonstrated. It is clear however that a novel approach to comparing the biodiversity of legume-supported and conventional cropping over regional and global scales is required before these results can be accurately quantified.

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Table 3 Reported impacts of legume-supported cropping systems on elements of belowground biodiversity.

Cropping system	Biodiversity Impacts	Studies reported in	References
Pasture, grass leys and forage	Soil fauna feeding activity	Temperate grasslands	(Birkhofer et al. 2011)

	+	Bacteria-feeding nematodes	Red clover litter	(Ilieva-Makulec et al. 2006)
	+	Earthworms	<i>Arachis pintoi</i> in pastures	(Velasquez et al. 2012), (Schmidt and Curry, 2001)
	=	Acari	Red clover litter; grass fallow with alfalfa	(Ilieva-Makulec et al. 2006), (Wissuwa et al. 2012)
	=	Collembola	Red clover litter	(Ilieva-Makulec et al. 2006)
	-	Plant parasitic nematodes	Red clover/Timothy	(Briar et al. 2007)
Green manure and cover-crops	+	Soil arthropods	Cotton/ <i>Crotalaria retusa</i> mulch; <i>cotton/Mucuna pruriens</i> mulch; grape vines/subterranean clover mulch	(Brevault et al. 2007), (Favretto et al. 1992), (Sohlenius, 1990)
	+	Collembola	Vetch; alfalfa	(Axelsen and Kristensen, 2000), (Berg and Pawluk, 1984), (Curry, 1986)
	+	Acari	Vetch	(Axelsen and Kristensen, 2000)
	+	Enrichment specialist nematodes	Pea/vetch cover	(DuPont et al. 2009)
	+	Earthworms	Alfalfa	(Sohlenius, 1990)

	=	Collembola	Vetch	(Axelsen and Kristensen, 2000), (Curry, 1986)
	=	Acari	Vetch	(Axelsen and Kristensen, 2000)
	=	Enchytraeidae	Alfalfa	(Lagerlof et al. 1989)
	-	Collembola	Vetch	(Axelsen and Kristensen, 2000)
	-	Acari	Alfalfa	(Berg and Pawluk, 1984)
	-	Thysanoptera	Alfalfa	(Curry, 1986)
	-	Plant feeding nematodes	Legume hay in banana plantations; Alfalfa	(Pattison et al. 2011),
Agroforestry and woody mulches	+	Soil invertebrates, earthworms	Maize/Sesbania, maize/Gliricidia sepium	(Sileshi and Mafongoya, 2006, Sileshi et al. 2008)
	+	Centipedes	Maize/Sesbania	(Sileshi et al. 2008)
Intercropping and undersowing	=	Collembola	Maize/Desmodium	(Midega et al. 2009)
	=	Nematodes	Cucumber/alfalfa; cucumber/ <i>Indigofera hirsute</i> ; squash/alfalfa; squash/ <i>Indigofera hirsute</i>	(Powers et al. 1993)
Grain legume monocrops	+	Isoptera	Soybean	(de Aquino et al. 2008)

	=	Nematodes	Soybean	(Briar et al. 2007)
Legumes in rotation	+	Earthworms	Red clover and soybean	(Jordan et al. 2004)

Table 3 Reported impacts of legume-supported cropping systems on elements of belowground biodiversity.

Cropping system		Biodiversity Impacts	Studies reported in	References
Pasture, grass leys and forage	+	Soil fauna feeding activity	Temperate grasslands	(Birkhofer et al. 2011)
	+	Bacteria-feeding nematodes	Red clover litter	(Ilieva-Makulec et al. 2006)
	=	Acari	Red clover litter; grass fallow with alfalfa	(Ilieva-Makulec et al. 2006), (Wissuwa et al. 2012)
	=	Collembola	Red clover litter	(Ilieva-Makulec et al. 2006)
	-	Plant feeding nematodes	Red clover/Timothy; alfalfa	(Briar et al. 2007), (Sohlenius et al. 1987)
Green manure and cover-crops	+	Soil arthropods	Cotton/ <i>Crotalaria retusa</i> mulch; cotton/ <i>Mucuna pruriens</i> mulch; vines/subterranean clover mulch	(Brevault et al. 2007), (Favretto et al. 1992)

	+	Collembola	Vetch; alfalfa	(Axelsen and Kristensen, 2000), (Berg and Pawluk, 1984), (Curry, 1986)
	+	Acari	Vetch	(Axelsen and Kristensen, 2000)
	+	Enrichment specialist nematodes	Pea/vetch cover	(DuPont et al. 2009)
	=	Collembola	Vetch	(Axelsen and Kristensen, 2000), (Curry, 1986)
	=	Acari	Vetch	(Axelsen and Kristensen, 2000)
	=	enchytraeidae	Alfalfa	(Lagerlof et al. 1989)
	-	Collembola	Vetch	(Axelsen and Kristensen, 2000)
	-	Acari	Alfalfa	(Berg and Pawluk, 1984)
	-	Thysanoptera	Alfalfa	(Curry, 1986)
	-	Plant parasitic nematodes	Legume hay in banana plantations	(Pattison et al. 2011)
Agroforestry and woody mulches	+	Soil invertebrates, earthworms	Maize/Sesbania, maize/Gliricidia sepium	(Sileshi and Mafongoya, 2006, Sileshi et al. 2008)

	+	Centipedes	Maize/Sesbania	(Sileshi et al. 2008)
Intercropping and undersowing	=	Collembola	Maize/Desmodium	(Midega et al. 2009)
	=	Nematodes	Cucumber/alfalfa; cucumber/ <i>Indigofera hirsute</i> ; squash/alfalfa; squash/ <i>Indigofera hirsute</i>	(Powers et al. 1993)
Grain legume monocrops	+	Isoptera	Soybean	(de Aquino et al. 2008)
	=	Nematodes	Soybean	(Briar et al. 2007)
Legumes rotation	in +	Earthworms	Red clover and soybean	(Jordan et al. 2004)