Nitrogen flows in an intercropped arable farming rotation

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Declaration

I declare that this thesis and the papers within it have been composed by myself and that no part of this thesis has been submitted for any other degree or qualification. The work described is my own unless otherwise stated.

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Abstract

Intercropping systems, with legumes as a component, have high potential to provide symbiotically fixed nitrogen (N), increase use of resources, improve crop and soil quality and reduce N losses from agricultural ecosystems. This study aimed to a) understand the agronomic and environmental effects of intercrops in the accompanying and subsequent crop, and b) understand how the choice of species and variety influences N losses in an intercropped low input rotation.

An experiment was established near Edinburgh, SE Scotland, UK, consisting of 12 hydrologically-isolated plots. Treatments were a spring barley (*Hordeum vulgare* cv. *Westminster)* monoculture and intercrops of barley / white clover (*Trifolium repens* cv. *Alice*) and barley/ pea (*Pisum sativum* cv. *Zero 4* or cv. *Nitouche*) in 2006. Spring oats (*Avena sativa* cv. *Firth*) was planted on all plots in 2007. In the third season, all plots were sown with perennial ryegrass. No fertilisers, herbicides or pesticides were used at any stage of the experiment.

At harvest, the total above ground biomass of barley intercropped with clover (4.56 t ha^{-1}) and barley intercropped with pea cv. *Zero 4* (4.49 t ha^{-1}) were significantly different from the barley monocrop (3.05 t ha^{-1}). The grain yield of the barley (2006) intercropped with clover (3.36 t ha^{-1}) was significantly greater than that in the other treatments ($P< 0.01$). The accumulation of N in barley was low in 2006, but significantly higher in the oats grown the following year on the same plots. The intercrops affected the yield and N uptake of the spring oats in the following year.

Nitrate leaching was reduced where legumes were used (Pea cv. *Zero 4*) in comparison with the barley monocrop (cumulative values of 670 g NO_3^-- N ha^{-1} and 3804 g NO_3^--N ha^{-1}, respectively) and gaseous losses were also reduced (cumulative values of 2.14 kg N_2O-N ha^{-1} and 3.20 kg N_2O-N ha^{-1}). Additionally, the leguminous intercrops increased the availability of N during the first growing season and for the following crop. In general, N_2O fluxes were correlated with NH_4^+in soil, DON in soil, water filled pore space (WFPS) and grain yields (cumulative values).

This experiment has highlighted the varied plant growth of barley intercropped with different legumes including different cultivars and species and the additional effects in the following growing year. The two barley/ pea intercrops had similar above ground biomass and grain yield for both component species (barley and peas). However, the accumulation of N in the above ground biomass of barley differed between these two treatments with significantly more N accumulated in barley/ pea cv. *Nitouche* than the barley/ pea cv. *Zero 4*. The two barley/ pea intercrops had significantly different N losses of N_2O and NO_3^-. Barley/ pea cv. *Nitouche* had the highest losses in comparison with barley/ pea cv. *Zero 4* mainly in the year of production, but provided available N deeper in the soil. Barley/ pea cv. *Zero 4* had lower losses than the barley monocrop largely as a consequence of the accumulation of N in the above parts of barley. However, the highest N losses were observed in the barley/ clover treatment but this treatment also provided the most available soil N to the following crop. This study has uniquely shown that different legume species or varieties in an intercropped system result in significant differences in N losses. The need has been demonstrated for long term experiments to help develop sustainable farming systems. The results indicate that legume choice is central to optimising plant productivity and nutrient use efficiency in intercropping designs. The choice of the crop mixture and the initial levels of N in the soil seem to be the most important drivers for N losses.

Keywords: cereals, clover, grain yields, intercropping, N losses, pea

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CONTENTS

1. Introduction

Nitrogen (N) plays a major role in supporting the growth of plants and is essential for high grain yields in arable agricultural systems. The use of synthetic fertilisers has increased in the last decades contributing to the environmental pollution of Earth's atmosphere (Erisman *et al.*, 2008). Synthetic fertilisers contribute both to air pollution by greenhouse gas emissions mainly nitrous oxide (N_2O), and water pollution by leaching into drainage waters. These two pollutants can easily overlap, when attempting to estimate the losses of one or the other (Mosier *et al.*, 1998).

Nitrate leaching is driven by the application of N and related to the surplus of N applied. However, changes in practices, such as timing of application to match crop demand, have reduced the nitrogen excess from a maximum 70 kg ha^{-1} in the early 1980s to about 25 kg ha^{-1} nowadays in conventional farms (Goulding, 2000). Soil texture and structure can influence the amount of leachate, as lighter soils have poor water holding capacity.

Agriculture has an important role in greenhouse gas emissions, given that agricultural soils are the most important source of N_2O, and account for 24% of global N_2O emissions (Smith and O'Mara, 2007) and 83% of total N_2O emissions in Scotland (The Scottish Goverment, 2008). N_2O concentrations grew from 270 ppb in 1850 to 410 ppb in 2000 (Fowler *et al.*, 2008). N_2O has 298 times greater global warming potential than carbon dioxide (CO_2) and can enhance depletion of the ozone layer in the upper atmosphere. Investigating possible fluxes of these gases from soil and their mitigation are high priorities under the current trends of climate change. Soil and crop management can contribute to the reduction of N_2O emissions, but there is a lack of information on how such approaches can help to develop sustainable practices. Nitrate leaching levels are very important for assessing the quality of water resources (Vinten *et al.*, 1992). Poor fertiliser practises can cause nitrate pollution of ground-water. Developing fertiliser practices that maintain the farmers' profitability while minimising the environmental impact on ground-water can help the sustainability of many cropping systems (Arregui and Quemada, 2006).

The use of legumes is well recognised as an alternative practice that can contribute to the reduction of nitrogen fertiliser use. Legumes can provide fixed nitrogen to the soil without the need for the fossil fuel inputs that are necessary to produce and apply fertilisers, either synthetic or organic. However, there is not much evidence regarding the extent to which legumes contribute to N losses and specifically N_2O emissions including the important factors of grain yields and soil N availability (Thorsted *et al.*, 2006; Chen *et al.*, 2008). The use of legumes in mixtures is mainly centred on grasslands (e.g. mixtures of clover and grass) across Europe (Djurhuus and Olsen, 1997; Høgh-Jensen, 2001) and is less common in mixtures of cereal and legumes (Fujita *et al.*, 1992; Thorsted *et al.*, 2002; Hauggaard-Nielsen *et al.*, 2009). The contribution of legumes to N_2O emissions and what the losses are from the intercropped systems within the year of use and the following year are uncertain.

This thesis has used different legume varieties and species in combination (intercropped) with barley in a low input system to both understand and measure the effects of legumes on the current and subsequent crop. Specific aims were:

- To estimate the effect of intercropping different legumes (species and varieties) with barley on grain yield, dry matter production, N uptake in barley and in the subsequent crops in the following two years (Paper 1)

- To assess N use efficiency of intercrops and monocrops (Paper 1 and 3)

- To determine the accumulation of N in shoots and roots of intercrops in the year of growth and following years (Paper 1 and 3)

- To investigate and quantify N losses from intercrops in a three-year low input rotation (Paper 2)

- To investigate factors controlling N_2O fluxes (direct and indirect) from an intercropped low input rotation and the drivers responsible for NO_3-leaching (Paper 2 and 4).

2. Literature Review

2.1 Organic agriculture and Climate Change

The current change in global climate is occurring largely as a consequence of the burning of fossil fuel (coal, oil, natural gas) and the mineralisation of organic matters as a result of land use change (Gruber and Galloway, 2008). These processes have been accelerated by human use of fossil sources, clearing of natural vegetation and use of these soils for cropping. According to the IPCC, the annual amount of greenhouse gases emitted by the agricultural sector was estimated at between 5.1 and 6.0 Gt CO_2 equivalents[1] in 2005 (Barker *et al.*, 2007). This represents approximately 10-12% of total greenhouse gas (GHG) emissions. CH_4 accounts for 3.3 Gt equivalents and N_2O for 2.8 Gt CO_2 equivalents annually, while net emissions of CO_2 are small at 0.04 Gt equivalents per year.

Predictions regarding the future global trends for greenhouse gas emissions from agriculture largely depend how human activities develop in the coming decades, given that there are highly divergent choices ranging from fossil fuel intensive development to options based upon a high degree of environmental protection (Solomon *et al.,* 2007). These pathways or scenarios will determine future emission trajectories and will force societies to make choices that include future consumption of C based energy resources, land use deforestation, and consumer attitudes and diet (Smith *et al.,* 2007). According to current projections, total greenhouse gas emissions from agriculture are expected to reach 8.3 Gt CO_2 equivalents per year in 2030, compared to the current level of approximately 6.0 Gt CO_2 equivalents annually (Smith and O'Mara, 2007). Agriculture can also help to mitigate climate change by reducing emissions of greenhouse gases and sequestering CO_2 from atmosphere in the soil. In 2004, IFOAM published a scoping study on "The Role of Organic Agriculture in Mitigating Climate Change". The study looked at how organic agriculture could contribute to reducing greenhouse gas emissions and mitigate the impacts of climate change (Kotschi and Müller-Sämann, 2004). Organic agriculture minimizes CO_2 emissions from agricultural ecosystems, and can also

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[1] A measure used to compare the emissions from various greenhouse gases based upon their global warming potential (GWP). Carbon dioxide equivalents are commonly expressed as 'million metric tonnes of carbon dioxide equivalents (MMTCDE)'. The carbon dioxide equivalent for a gas is derived by multiplying the tonnes of the gas by the associated GWP. MMTCDE = (million metric tonnes of a gas) * (GWP of the gas). For example, the GWP for methane is 21 and for nitrous oxide 310. This means that emissions of 1 million metric tonnes of methane and nitrous oxide respectively is equivalent to emissions of 21 and 310 million metric tonnes of carbon dioxide.

contribute to carbon sequestration because of the systematic application of manure and compost from animal and crop residues, crop-legume rotations, green manuring with legumes, and agroforestry with multipurpose leguminous trees. Soil is the most important sink for methane where high bacterial activity oxidizes it. Controlled anaerobic digestion of animal manure can contribute significantly to reducing methane emissions. Nitrous oxide emissions are minimized in an organic system.

2.1.1. Organic farming

''Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved.'' (IFOAM, 2008).

The main concept that farmers try to follow is 'organic farming is production without chemicals', but this sentence is incomplete without including some fundamental characteristics. According to IFOAM, the principal characteristics and aims of organic farming are summarised as:

- Producing high quality food in sufficient quantities.
- Encouraging and enhancing the biological cycles within the farming system, involving micro-organisms, soil flora and fauna, plants and animals.
- Maintaining and increasing long term fertility of soils.
- Maintaining the genetic diversity of the production system and its surroundings.
- Using, as far as possible, renewable resources in a locally organized production system.
- Creating a harmonious balance between crop production and animal husbandry.
- Minimising all the forms of pollution.
- Considering the wider social and ecological impact of the farming system.

2.1.2. Organic farming in Europe and in UK

Since the beginning of the 1990s, organic farming has spread rapidly in almost all European countries; however, the rate of increase has slowed in recent years. In Europe almost 6.3 million hectares were managed organically by almost 170,000 farms in 2003. This constitutes 3.4% of the agricultural area and 2% of the farms in the EU. In the

European Union between 1986 and 1996 the land under organic management grew by 30%, annually (Fig. 1).

A major development in the European Union in 2004 was the launch of the European Action Plan for Organic Food and Farming. There are also important differences between individual countries as to the relative importance of organic farming. More than 12% of agricultural land is organic in Austria and 10% in Switzerland. Some other countries have yet to achieve the 1% mark. The strongest growth is established in Scandinavia and the Mediterranean countries with Italy having the highest number of organic farms and the greatest organic land use.

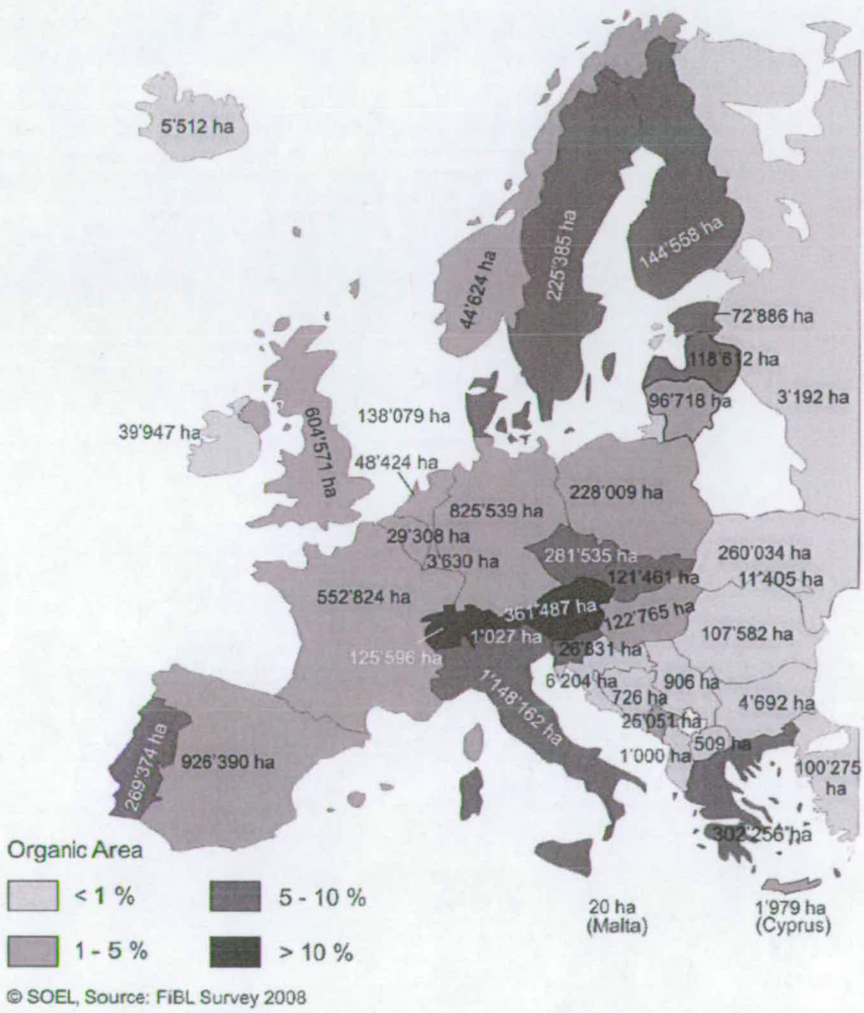


Figure 1: Organic farming in Europe (The World of Organic Agriculture 2008, Statistics and Emerging Trends)

Almost one fifth of the EU's organic land and farms and more than a quarter of the organic farms are located there. In Central and Eastern Europe, there has also been a great increase in the number of organic farms reaching 25% of the total (Willer and Yussefi, 2005).

The total area of organic and in-conversion land in the UK as of January 2007 was 604,571 ha. This is little changed from 2006 and represents 3.5% of the total agricultural area (excluding common grazing). Of this figure, 20% was in conversion and 80% was fully organic (Table 1). Since January 2006 the fully organic area has decreased by 7%, although the area of land in conversion has risen by 41%. The area of fully organic or in-conversion land in England and Wales has increased in 2007 although the area in Scotland and Northern Ireland has decreased by around 5% since January 2006. Most of the reduction in area in Scotland is due to a decrease in organically certified permanent pasture (DEFRA, 2005). In the EU, crop and livestock products sold as organic must be certified as such under EC Regulation 2092/91. The Soil Association is the UK's largest certification body, and was founded in 1946 by a group of farmers, scientists and nutritionists, who observed a direct connection between farming practice and plant,

Table 1: Organic and In-conversion Land in the UK (Jan 2007) Defra

	In conversion	Organic	Total (ha)	Total agricultural area [1] (ha)	% of total agricultural area
North East	6,923	22,618	29,540	589,077	5.0%
North West	1,781	19,438	21,219	935,870	2.3%
Yorkshire & Humberside	3,387	9,032	12,419	1,097,396	1.1%
East Midlands	2,061	12,465	14,526	1,229,436	1.2%
West Midlands	3,974	26,310	30,284	959,623	3.2%
Eastern	3,630	10,785	14,415	1,432,429	1.0%
South West	31,588	93,415	125,003	1,877,866	6.7%
South East (Inc London)	13,181	35,798	48,979	1,206,867	4.1%
England	66,525	229,861	296,386	9,328,564	3.2%
Wales	15,427	63,546	78,973	1,448,683	5.5%
Scotland	35,194	200,103	235,298	5,607,010	4.2%
Northern Ireland	3,991	5,136	9,127	1,028,495	0.9%
UK	121,137	498,646	619,783	17,412,752	3.6%

animal, human and environmental health. The catalyst was the publication of *The Living Soil* by Lady Eve Balfour in 1943. The book presented the case for an alternative, sustainable approach to agriculture that has since become known as organic farming. In the ensuing years the organisation has developed organic standards and now works with consumers, farmers, growers, processors, retailers and policy makers and certifies 80% of all organic products sold in UK (Soil Association, 2008).

Organic farming is a steadily increasing production in European agriculture. However, a further expansion of organic farming is needed to meet increasing consumer worldwide demand for products, which are healthy, safe, and of high quality and produced with consideration for animal welfare and the environment (Marsden *et al.*, 2002). European organic farming and the research within this area are at the forefront internationally and offer alternative food production systems, which could strengthen the competitiveness of EU agriculture.

2.1.3. The potential of Organic Farming to reduce Greenhouse Gas Emissions

Organic farming has considerable potential for reducing emissions of greenhouse gases, as the global warming potential (GWP) of organic farming is smaller than that of conventional or integrated systems when calculated per unit area, but not as low as when calculated per product unit, as conventional yields are generally higher (Badgley, 2007).

Organic farming can be self-sufficient in nitrogen being dependant on the fixed nitrogen and other management practices (Pietsch *et al.*, 2007). Mixed organic farms aim to target practice highly efficient recycling of manures from livestock and crop residues. Leguminous crops can deliver additional nitrogen in sufficient quantities for the rotations. Badgley *et al.* (2007) calculated the potential fixed nitrogen by leguminous plants via intercropping and off-season cropping to be 154 million tonnes per year worldwide, a potential which exceeds by far the nitrogen production from fossil fuel and which is not fully understood within conventional farming techniques.

In organic farming, the prohibition on applied mineral nitrogen and the reduction of livestock units per hectare considerably decreases the concentration of easily available mineral nitrogen in soils and thus N_2O emissions (IPCC, 2007). A balance between the inputs and outputs and the nitrogen use efficiency is essential, as the availability of N is restricted.

2.1.4. Organic farming and the potential to adapt to climate change

Agricultural production in most parts of the world will have to deal with more unpredictable weather conditions. Developing countries such as South Asia and Southern Africa could suffer reductions in yield of several crops if no investment is made into improving the adaptiveness of the production systems. Besides specific technical measures (irrigation, breeding for drought or heat tolerant crops), the resilience of whole production systems should remain a very important focus (Niggli *et al.*, 2008).

Soil fertility-building and soil conserving techniques potentially place organic farming in a position to maintain productivity in the event of drought, irregular rainfall, flooding, and rising temperatures. Such techniques include i) the on-farm flux of manure from livestock production to cropland, ii) the use of composts, iii) the use of leguminous crops and green manure in rotations, iv) diversified crop sequences with permanent soil cover and different rooting depths as well as v) minimum or shallow tillage.

Although organic agriculture is not designed to use water efficiently, different agricultural techniques used in organic agriculture affect water use efficiency of organic arable crops in a positive way (Dalgaard *et al.*, 2001). In addition, organic management practices also decrease pollution in water effluent as the main pollutants, such as mineral nitrogen and pesticides, are prohibited.

An additional strength of organic farming systems is their diversity – including the diversity of crops, fields, rotations, landscapes and farm activities. The high level of diversity of organic farms provides many ecological services that significantly enhance farm resilience. Positive effects of enhanced biodiversity on pests and diseases as well as on better utilization of soil nutrients and water prevention are well documented (Jensen and Hauggaard-Nielsen, 2003; Altieri, 2005).

Genetic diversity of crop plants is generally considered a fundamental resource for adaptation and therefore crucial for maintaining the stability of food supply. As resilience and robustness to environmental stress are multigenic characteristics, *in situ* conservation and on-farm breeding are likely to be more successful than genetic engineering (Altieri, 2005; Bengtsson, 2005; Niggli *et al.*, 2008).

2.2. Nitrogen fixation by legumes

2.2.1. Nitrogen

Nitrogen is one of the 17 chemical elements required for plant growth and reproduction and is an abundant element on and around Earth – about 78 percent of the Earth's atmosphere is nitrogen gas (N_2). As with all plant nutrients nitrogen must be in specific forms to be utilised by plants. Despite nitrogen being one of the most abundant elements on earth, nitrogen deficiency is probably the most common nutritional problem affecting plants worldwide. Healthy plants often contain 3 - 4% nitrogen in their above- ground tissues. Nitrogen is essential for many biological processes; for example, it is included in all amino acids (the stem *amin* derives from ammonia), is incorporated into all proteins and is present in the four bases that make up nucleic acids, such as DNA, and RNA.

2.2.2. The nitrogen cycle in soil

The nitrogen cycle is one of the most important processes in nature for living organisms. According to Henderson's dictionary of biological terms (1999): ''nitrogen cycle is the sum total of processes by which nitrogen circulates between the atmosphere and the biosphere or any subsidiary cycles within this overall process' (Fig. 2).

Soil nitrogen exists in many chemical forms although soils tend to be dominated by organic nitrogen compounds, ammonium (NH_4^+) ions, and nitrate (NO_3^-) ions. At any given time, 95-99% of the potentially available nitrogen in the soil is in organic forms, either in plant and animal residues, in the relatively stable soil organic matter or in living soil organisms, mainly microbes such as bacteria and fungi. This nitrogen is not directly available to plants, but some can be converted to available forms by micro-organisms. The majority of plant-available nitrogen is in the inorganic (known as mineral nitrogen) NH_4^+ and NO_3^- forms.

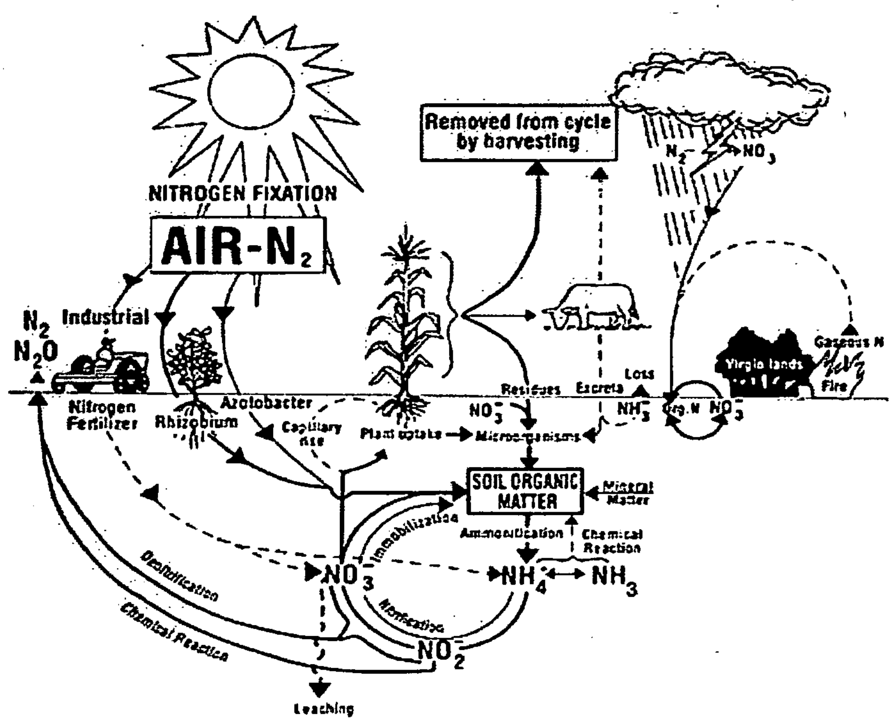


Figure 2: The nitrogen cycle in soils (http://ohioline.osu.edu/aex-fact/images/463_1.jpg).

The conversion of organic N to NH_4^+ and NO_3^- is known as *mineralization*, a process which occurs in soil as micro-organisms convert organic N to plant-available inorganic N. The first step of *mineralization* is called *aminization*, in which micro-organisms (primarily heterotrophs) break down complex proteins to simpler amino acids, amides, and amines. Heterotrophic micro-organisms require preformed organic compounds as sources of carbon and energy. Autotrophic micro-organisms can derive energy from the oxidation of inorganic elements or compounds.

Aminization: Proteins $\rightarrow$ $R\text{-}NH_2 + CO_2$ (Equation 1)

Ammonification is the second step of mineralization in which amino (NH_2) groups are converted to ammonium. Again, micro-organisms (primarily heterotrophic) accomplish this action.

Ammonification: $R\text{-}NH_2 + H_2O \rightarrow NH_3 + R\text{-}OH$ (Equation 2)

Nitrification is the next process in which the previous compound is oxidized to NO_3^-. Microbial activity is responsible for the two steps of nitrification. *Nitrosomonas* (obligate autotrophic bacteria) convert ammonium to nitrite. Nitrification inhibitors, such as nitrapyrin (N-Servea) or dicyandiamide (DCD) interfere with the function of these bacteria, blocking ammonium conversion to leachable nitrate. The second step of nitrification occurs through *Nitrobacter* species, which convert nitrite to nitrate. This step rapidly follows ammonium conversion to nitrite, and consequently nitrite concentrations are normally low in soils (Focht and Verstraete, 1977).

Immobilization, or the temporary tying up of inorganic nitrogen by soil micro-organisms decomposing plant residues, is a recycling process and the reverse of mineralization. Immobilized nitrogen will be unavailable to plants for a time, but will finally become available again as residue decomposition proceeds and populations of micro-organisms decline (Prasd and Power, 1997; Ferguson, 2004). Immobilization of mineral N can occur (often quickly) by integration of fresh organic material into soil, depending on the humification coefficient or effectual organic matter content and the ratio of carbon (C) to nitrogen (C:N ratio) in the incorporated organic material. When utilizing organic material with a low N content, the micro-organisms need supplementary N, reducing the soil mineral N pool with a resulting decrease in plant N availability (Hofman, 2004). There is often no net immobilization when a legume crop, such as alfalfa or white clover, is ploughed under, because the low C:N ratio results in net mineralization (Anderson, 1998).

Ammonium-N is produced in the soil by nitrogen fixing organisms which produce the multicomponent key enzyme complex, referred to as *nitrogenase*. It consists of two oxygen-sensitive, water-soluble proteins. The larger molecule known as dinitrogenase or P1 is a molybdenum- and iron- containing protein, while the smaller molecule is an iron protein known as dinitrogenase reductase or P2. Both enzymes are required for nitrogen fixation in a ratio of one or two P2 for each P1; neither component alone will fix nitrogen. For the nitrogenase reaction, energy is provided by the hydrolysis of ATP.. Estimates of the energy costs of nitrogen fixation in vivo have been based on the stoichiometry of ATP hydrolysis in reaction mixtures which contain a reductant and purified nitrogenase. The stoichiometry between the number of moles of ATP hydrolysed per mole of nitrogen reduced depends on the reaction conditions (pH, temperature, ratio of components protein of the nitrogenase complex), but it is independent of the substrate. Under optimal conditions, at least two molecules of ATP are hydrolysed per electron transferred to substrate and the overall reaction for nitrogen reduction can be written as:

$$N_2 + 8H^+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi_{\text{(inorganic phosphorus)}}$$

(Equation 3)

2.2.3. *Nitrogen-Fixing Systems*

The capability of biological fixation of atmospheric nitrogen (N_2) is restricted to organisms with a prokaryotic cell structure, namely bacteria and blue-green-algae. Three major strategies of N_2 fixation can be differentiated in terrestrial ecosystems, symbiotic, associative, and free living nitrogen fixing organisms, differing in both energy source and fixation capability. On average, symbiotic systems have the highest fixation capability since not only the energy in the form of carbohydrates is provided by the plant, but also other conditions (e.g. export of reduced N) are optimised for efficient N_2 fixation (Cocking, 2003). The plants benefit directly since more than 90% of the fixed nitrogen is rapidly translocated from the bacteria to the plant. Nodulated legumes, such as clover and peas, in symbiosis with Rhizobium and Bradyrhizobium are among the most prominent N_2-fixing systems in agriculture (Zahran, 1999). Our understanding of the *Rhizobium*-legume symbiosis, from the nature of the infection specificity to the biochemistry of the nitrogen-fixing process and the energy economics in the plant, has advanced considerably since the 1970's (Brockwell *et al.*, 1995).

The first phase of the interaction occurs soon after the germination of the legume seed in soil containing Rhizobium species through the nodulation of roots. The bacteria must penetrate the root for the infection to occur, and it is possible in this phase to establish the specificity between recognition and the host-*Rhizobium* (Vincent *et al.*, 1979). Once the bacterial cells are transferred to the root cortex, they are released into the cortex cells. Then, the nodule meristem is formed and the nodule expands. After that, the infected cortex cells are enlarged inside the nodules (Gardener *et al.*, 1985).

One of the most remarkable events during the development of nitrogen fixing legume nodules is the production of haemoglobin, which becomes a major nodule protein. It is now certain that the haem is synthesized by the rhizobia (Beringer *et al.*, 1979) and the globin is produced by the plant (Sedloi-Lumbroso *et al.*, 1978). The chief role of haem is to promote a flux of oxygen in the nodules sufficient to maintain oxidative phosphorylation by the bacteroids in micro-aerobic conditions (Rhodes and Robert, 1982).

2.2.4. *Key factors affecting nitrogen fixation*

Interactions between the micro-symbiont and the plant are influenced by edaphic, climatic and management factors. A legume-*Rhizobium* symbiosis might perform well in a loamy soil but not in a sandy soil, because of the effect that factors listed below have either on the micro-symbiont and/ or the host-plant.

Edaphic Factors

The six main edaphic factors that relate to the soil and that can limit biological nitrogen fixation are: excessive soil moisture, drought, pH, P deficiency, excess mineral N, and deficiency of Ca, Mo,, Co and B.

Excessive moisture and water-logging prevent the development of root hair and sites of nodulation, and therefore interfere with the normal diffusion of O_2 in the plants root system (Yin et al., 2009).

Drought reduces the number of rhizobia in soils, and can inhibit nodulation and N_2 fixation. Prolonged drought will also promote nodule decay. However, deep-rooted legumes exploiting moisture in lower soil layers can continue fixing N_2 when the soil is drying (Athar and Johnson, 1997). Mycorrhizal fungal infection has also been found to improve tolerance of plants to drought.

Soil acidity and related problems of Ca deficiency and aluminium and manganese toxicity adversely affect nodulation, N_2 fixation and plant growth. Most legumes plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N_2 fixation (Bordeleau, 1994). The failure of legumes to nodulate under acid-soil conditions is common, especially in soils of pH less than 5.0. The inability of some rhizobia to persist under such conditions is one cause of nodulation failure, but poor nodulation can occur even where a viable *Rhizobium* population can be demonstrated. It has been observed destructive effects of acidic soils on *Rhizobium*-legume symbiosis and N_2 fixation. Low pH reduced the number of *R. leguminosarum* bv. trifolii cells in soils, which resulted in no or ineffective nodulation by clover plants. The number of nodules, the nitrogenase activity, the nodule ultrastructure, and the fresh and dry weights of nodules were affected to a greater extent at a low medium pH (<4.5) in comparison with a medium high pH (Zahran, 1999).

Phosphorus deficiency reduces nodulation, N_2 fixation and plant growth. Identification of plant species adapted to low-P soils is a good strategy to overcome this soil constraint. The role of mycorrhizal fungi has increased plant P uptake with beneficial effects on N_2 fixation. Dual inoculation with effective rhizobia and mycorrhizal fungi shows synergistic effects on nodulation and N_2 fixation in low P soils. Trees are usually colonised by mycorrhizal fungi in natural ecosystems in the tropics. The significance of this symbiosis in nature should be better recognised (Hogh-Jensen, 1996).

Mineral N inhibits the *Rhizobium* infection process and also N_2 fixation. The former problem probably results from impairment of the recognition mechanisms by nitrates, while the latter is probably due to diversion of photosynthates toward assimilation of nitrates. Application of large quantities of fertilizer N inhibits N_2 fixation, but low doses (<30 kg N ha^{-1}) of fertilizer N can stimulate early growth of legumes and increase their overall N_2 fixation. The amount of this starter N must be defined in relation to available soil N (Zahran, 1999).

Various micro-elements (Cu, Mo, Co, B) are necessary for N_2 fixation. Some of these are principlal components of nitrogenase, for example Mo.

Climatic Factors

The two important climatic determinants affecting biological nitrogen fixation are temperature and light.

Extreme temperatures affect N_2 fixation adversely. This is easy to understand because N_2 fixation is an enzymatic process. However, there are differences between symbiotic systems in their ability to tolerate high (>35°C) and low (<25°C) temperatures.

The availability of light regulates photosynthesis, upon which biological nitrogen fixation depends. An example that has been demonstrated by diurnal variations in nitrogenase activity, where only a very few plants can grow and fix N_2 under shade.

Biotic Factors

Among biotic factors, the absence of the required rhizobia species constitutes the major constraint in the nitrogen fixation process. The other limiting biotic factors could be excessive defoliation of host plant, crop competition and insects and nematodes (Zahran, 1999).

2.2.5. Nitrogen fixation and the global nitrogen cycle

Until relatively recently, the contribution of nitrogen fixation to the global nitrogen cycle probably had not changed for centuries, having been in approximate balance with the denitrification process that converts combined nitrogen back to atmospheric nitrogen.

Fixation did not occur to excess because biological nitrogen fixation is inhibited by the presence of mineral nitrogen. During the past 40 years, the global nitrogen cycle has been affected by the increase in industrial fixation of nitrogen, but the environmental impacts are yet to be measured and assessed. It would be prudent to minimise the further perturbation of the global nitrogen cycle, a major natural cycle (Galloway and Cowling, 2002).

2.2.6. Legumes as a nitrogen source for agriculture

The use of legumes in agriculture may require or encourage completely different methods of farming from those associated with conventional farming. A wide variety of methods can be adopted to use the N fixed by legumes and can be divided into three main functions:

1) Providing protein for human or animal consumption

and/or

2) Providing fixed nitrogen for the benefit of other crops

and/ or

3) Reducing environmental pollution, such as from greenhouse gases

Legumes can also be grown as green manure crops and then ploughed into the soil in order to improve the nitrogen status prior to sowing of a non-fixing crop (Schmidt *et al.* 1999) Forage legumes such as clover can also be undersown together with a taller crop, such as cereals. This provides analogous benefits to mixed cropping and intercropping, and can also be an advantage to follow on crops, control weeds and provide a habitat for beneficial invertebrates (Armstrong and McKinlay, 1997; Brandsaeter, 1998). Cereals can be direct drilled into a permanent clover sward (Schmidt, 2001) or into legume which, in the following year, suppressed weed (White and Scott, 1991).

In a ley-arable crop rotation, grass and a forage legume, usually clover, are grown for several years, grazed by animals and/or cut for silage or hay (Philipps *et al.*, 1996; Elgersma *et al.*, 2000), before being ploughed up so that arable crops can be sown. The last arable crop to be grown before the ley phase is usually undersown with clover. In the ley phase, the grass obtains greater N from the presence of the clover grown to establish the ley and in addition, the grazing animals obtain a high protein diet. Manure from the grazing animals transfers fixed N to grass and can be spread on arable crops, which can also benefit from the accumulated fixed N release when the ley is ploughed (Hogh-Jensen, 1996; Philipps *et al.*, 1996). Before the invention of the Haber-Bosch process, ley-arable crop rotations were the main systems for agriculture in Europe, an example is that of the Norfolk four course rotations (Lampkin, 1990).

2.2.7. Benefits of legume use in agriculture

The ability of legumes to fix atmospheric N_2 and thus add external N to the crop-soil ecosystem is a distinct benefit of legume culture. The amount of N biologically fixed each year by legumes varies greatly from zero to several hundred kg N per ha (Power, 1987; Provorov and Tikhonovich, 2003). Many grain legumes are efficient at N fixation. Variables affecting quantity of N fixed include not only legume species and cultivar, but also factors such as soil type and texture, pH, soil nitrate-N levels, temperature and water regimes, availability of other nutrients, and crop (especially harvest) management, with the latter extremely important. For instance, alfalfa (*Medicago saliva*) may add up to several hundred (300-400) kg N/ha to the soil if a final cutting of hay is not removed, compared to less than 150 kg N if only the roots and stubble remain (Heichel, 1987).

The primary use of a grain legume is to provide protein (obtained as far as possible from biological N fixation) for human or animal consumption, but residues of the crop may provide fixed N for the successive crop. If the grain legumes are fed to animals (or humans) and the animal (or human) faeces are returned to the soil, then much of the fixed N in the grain becomes available to other crops. Grain legumes can be grown in alternate rows with a non-N-fixing non-grain species (intercropping), or sown in a mixture with a non-N fixing grain (mixed cropping).

The economic value of the N fixed by legumes varies widely. One must consider the cost of production of the legumes, the amount of fixed N returned to the soil, and the availability of this N for future crops. Often, these costs are compared directly against the cost of purchasing and applying an equal quantity of N fertiliser plus the net income lost by producing a legume instead of a grain crop (if the legume is grown in rotation). In the past few decades, the cost of production and price of N fertilisers have been such that this type of calculation would generally favour the use of inorganic N fertiliser. This fact is largely responsible for the decreased use of legumes in our crop production systems during the past 40 years (Power, 1987). However, current economics are starting to favour the increased use of legumes, due to the high cost of management of the N fertilisers due to multiple application and increase rates.

There are other benefits from using legumes in a cropping system which also should be included into any comparison with fertiliser-N, but unfortunately, they are often omitted due to the difficulty in quantifying them. Usually, yields of a grain crop grown in rotation are at least 10 to 20% greater than those of continuous grain, regardless of the amount of fertiliser applied. This yield benefit is often referred to as the rotation effect, due to the additional N supply, which will not completely reduce this yield difference of the grain. Cook (1984) and others have shown that rotation of crops can reduce the populations and activity of some pathogenic soil organisms. Similarly, rotations can also break the weed and insect cycles that often prevail with year on year cropping.

Legumes may have additional long-term benefits in some soils but again these benefits are difficult to convert into active monetary values. Usually legume rotations, compared with continuous cropping, result in enhanced soil organic matter content and mineralisable N. As previously described, legumes are high in protein, and therefore, nitrogen rich. The nitrogen supplied by legumes facilitates the decomposition of crop residues in the soil and conversion to soil building organic matter. This provides not only better control of N availability, but also enhanced soil structure and requires less energy for cultivation, as well as less erosion (Hoyt and Hargrove, 1986).

Forage legumes are particularly good for improving soil structure, because they have a high rate of root turnover, providing substrates for bacteria that produce polysaccharides, which are important structural components of soil. Decomposing legume roots and mycorrhizal hyphae bind soil aggregates together, increasing their stability (Miller and Jastrow, 1996).

Decreasing erosion, over a period of decades, can have a major influence on the properties and productivity of some soils (Mielke and Schepers, 1986). The enhanced mineralisable N levels in soils with legume rotations compared to those for continuously cropped soils may greatly aid water quality. With the use of legumes, not only is less fertiliser-N required, but the level of nitrate N in the soil at any one time is usually less, so there is less nitrate to leach below the root zone (Power, 1987).

In addition to the above, there are many more benefits to the use of legumes in the development of soil quality. Soil porosity is increased as several legumes have active taproots reaching 1.5 m to 2.5 m in length that open pathways deep into the soil. Additionally, nitrogen-rich legume residues encourage earthworms and the burrows they create, promoting air movement and water percolation deep into the soil (Schmidt *et al.*, 2001; Eisenhauer and Scheu, 2008). Moreover, earthworms have the ability to recycle crop nutrients that are deep in the soil profile. According to research in both the United States and Canada, the protein, glomalin, produced symbiotically along the roots of the legumes and other plants, serves as 'glue' that helps keep the soil together into stable aggregates by increasing the pore space and tilth and reducing soil erosion and surface cracking (Nyatsanga and Pierre, 1973).

Legumes can also lower the pH and promote increased plant-soil-microbial activity in soils with a pH above the range for optimum crop growth and development. In a greenhouse experiment, alfalfa and soybeans lowered the pH in a Nocollet clay loam soil by one whole pH unit (Nyatsanga and Pierre, 1973). Moreover, legumes contribute to an increased diversity of soil microbiology leading to a greater stability to the total life of the soil. Legumes also provide an excellent break in a crop rotation that reduces the build-up of grassy weed problems, insects and diseases (Wright and Upadhyaya, 1997).

In New Zealand, the use of grass-white clover pastures has demonstrated their great economic continuation. The production from these pastures is among the most economical in the world (Frame *et al.*, 1998) and the favorable weather conditions allow the white clover to grow almost all year round. It has been estimated that UK livestock producers having legume-based systems could provide an economic benefit of approximately £300m a year (Doyle and Bevan, 1996). The environmental benefits of legume systems are of great importance. Losses of N as N_2O (a greenhouse gas) can be greater in fertiliser based systems than in legume based systems (Jensen and Hauggaard-Nielsen, 2003).

Organic farming is widely believed to cause less N pollution of ground-water than conventional agriculture producing similar yields (Drinkwater *et al.*, 1998). Goulding (2000) reviewed data on leaching from conventional and organic leys and found that conventional grass-only first year leys leached around 1.5 times as much N as first year organic grass-clover leys, and second year conventional leys leached around 3 times as much N as second year organic leys. However, leaching from first year organic arable crops was around twice as high as leaching from first year conventional crops, owing to the greater N losses resulting from ploughing of grass-legume leys. N losses from second year conventional arable crops were around 1.5 times higher than N losses from second year organic arable crops. In the third year of arable crops leaching losses were around 1.5 times higher in the conventional system. Over the whole rotation, leaching losses of N were only slightly lower from the organic system.

Mixed farming systems can also use nitrogen more efficiently than purely arable farms or pastoral farms (Granstedt, 1991). There is however, a risk of nitrate leaching, following the ploughing of pastures (Scholefield and Smith, 1996). Phillips and Stopes (1995) note that while leaching from this stage of the rotation is high, the lower losses during the rest of a typical organic rotation compensate for this, so the average nitrogen loss is 10.3-20.8 kg N ha yr^{-1}.

Milk yields from grass-clover pasture with no applied N in New Zealand were found to be 83% of those from pastures receiving 400 kg N ha^{-1}, but the efficiency of N use was much greater when no N fertiliser was applied. At the high rate of N application, only 26% of the fertiliser N and biologically fixed N was recovered in milk and other produce. When the pasture was reliant on biologically fixed N, 52% of the N input was recovered in the farm produce (Ledgard, 1999).

It is difficult to make comparisons between conventional and organic farms because so many conventional farms are not self-contained or self-sufficient units, as organic farms ideally should be, but rather they are often part of a broader national and international food chain. In particular, the separation of arable production and animal production that has resulted from the use of N fertiliser has directly affected the efficiency of N use.

2.2.8. The amount of N fixation

The amount of N fixed by different legumes is determined by the intrinsic ability of the crop/ rhizobium symbiosis to fix N, modified by the crop's growing conditions (e.g. soil, climate, disease), crop management and length of time for which the crop is grown. Thus, the influence of all of these factors means that a variety of values has been reported by different researchers. Nevertheless, there is usually a strong relationship between yield and the quantity of N fixed for a particular legume species (Peoples *et al.,* 1995).

Part of the fixed nitrogen remains in the soil as root residues and nodules or returns to the soil with litter fall. In annual species some of the fixed nitrogen after harvest becomes available for the next crop. In mixed stands of legumes and non legumes (e.g. grasses, cereals), direct transfer of fixed nitrogen from legumes to non-legumes during the growing season is possible, although the extent to which it occurs is small, around 10% or less of the total nitrogen fixed.

The following figure (Fig. 3) shows the ranges of N fixed quantities and remaining after harvest in the UK conditions. There have been many studies of the amount of N fixed by white clover and red clover, which are very important for the pastures in UK.

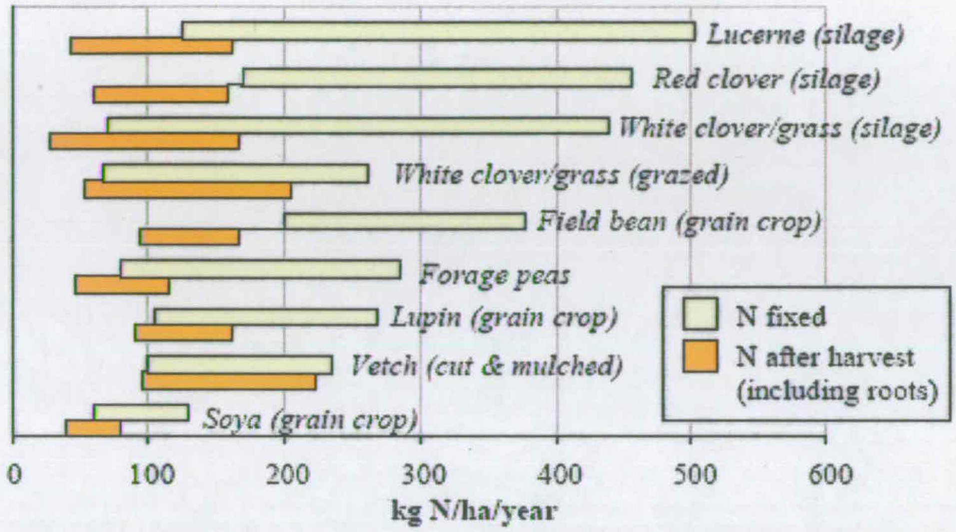


Figure 3: Provisional ranges of N fixed quantities and remaining after harvest in the UK conditions (Defra, 2005)

2.3. The fate of fixed N

To sustain and maximise agricultural production in order to supply the nutritional needs of a continually growing world population, agricultural systems need nitrogen (N) inputs. In its inert form as basic dinitrogen (N_2) gas in the atmosphere (78%), nitrogen does not impact environmental quality. But the widespread use of N in agricultural systems and the associated transformations of that N into various ions or gaseous forms contribute to leaks from the beneficial parts (e.g. farming systems) to the non beneficial (e.g. atmosphere). These N losses may contribute to the degradation of water, air, and soil in many regions of the world. Nitrate (NO_3^-) is one of the most mobile ions in agricultural systems, and NO_3^- leaching is a primary source of the excess nitrate concentrations in drinking water. Soil erosion that transports soil particles and also N contributes to surface water pollution. The gaseous transport of ammonia (NH_3) from manures and the denitrification of NO_3^- and nitrite (NO_2^-) ions and their conversion into gaseous forms of N such as nitrous oxide (N_2O) and nitric oxide (NO) can contribute to air quality and global warming impacts.

2.3.1. Nitrate leaching losses

Leaching is often the most important route of N loss from field soils other than that accounted for in plant uptake. Nitrate (NO_3^-) is the primary form of N that leaches into ground-water. It is totally soluble at the concentrations found in soil and its movement is therefore closely related to subsurface water movement. As described by Jury and Nielson (1989), the movement of an NO_3^- ion through soil is governed by convection, mass-flow, and diffusion within the soil solution. The widespread appearance of NO_3^- in ground- water is a consequence of its high solubility, mobility, and easy displacement by water. Nitrate leached below the root zone in most agronomic crops will eventually leach downward until it reaches a saturated zone, either at an aquifer or aquitard. Nitrate leaching below the saturated level is generally unrecoverable by most crops except the deep-rooted species such as alfalfa. In autumn, cultivations are likely to cause the largest losses of NO_3^-especially if following a fertility-building ley, because of the bare ground and heavier precipitation and less plant growth for N uptake over the winter (ADAS, 2003).

Most soil types and environmental parameters, which influence the transport of dissolved NO_3^- through field soils, vary substantially, even over short distances. Nitrate is not always easily leached in well structured loams and clays. It is mostly formed and held in crumbs (percolating water primarily moves down through cracks and coarse pores between the crumbs, so nitrate only gets into the escaping water by diffusion, which is a slow process).

In organic systems, leaching losses will occur mainly from sudden, rapid mineralisation and nitrification of organic nitrogen, especially following cultivation when mineralisation will be enhanced. It has been postulated that organic farming reduces nitrate leaching, a major environmental concern in Europe (Drinkwater, 1998). The average leaching of nitrate over a crop rotation was low per unit area from organic systems when compared with conventional (Korsaeth, 2008). However, an accurate comparison of leaching between systems requires soil type, climatic conditions, pH and yields to be considered and this is not accomplished due to differences in the sequence and type of crops grown, differences in the input intensity of N and a general lack of yield data. Incorrect generation of leaching data are common, as pointed out by Andrén *et al.* (1999).

Berntsen *et al.* (2006) studied two different types of soil (sand and loamy sand). On each of the farm a three year old grass-clover field was selected. Half of the field was ploughed the first year and the other half was ploughed the next year. Spring barley (*Hordeum vulgare L.*) was sown after the spring ploughing. Measurements showed a low N leaching during the pasture period (9-64 kg N ha^{-1}) but a high leaching the first (63-216 kg N ha^{-1}) and second (61-235 kg N ha^{-1}) year after ploughing. In addition, a high residual positive N effect of the pasture was observed on the barley yields in both years. There was a low response to manure application on the sandy soil both during the first and second year after ploughing in comparison with the greater response of the loamy sand soil.

Davis and Barraclough (1988) monitored different time points in the rotation on an organic farm in the UK. They found that the amount of N lost by leaching was closely dependent on the position in the rotation. Moreover, nitrate leaching was reduced the longer the field had been in the organic rotation. In Denmark, the impact of organic compared with conventional farming practices on N leaching loss has been studied for mixed dairy and arable farms. The results show a lower N leaching loss from organic than conventional mixed dairy farms, primarily due to lower N inputs and the increasing soil N pool on organic arable farms over time (Knudsen, 2005).

2.3.2. Nitrous oxide fluxes from soils

Nitrous oxide (N_2O) is a powerful greenhouse gas and one of its largest global sources in agriculture (Nevison, 2000). It contributes to the depletion of the stratospheric ozone layer and the global greenhouse gas budget (IPCC, 2007). Although quantities in the atmosphere are small compared to CO_2 and water vapour, the contribution to global warming is considerable, accounting for almost 5% of the total greenhouse effect (Bouwman, 1996). This is caused by the long atmospheric lifetime (~150 years) and the strong radiative warming effect of 310 times that of CO_2.

Soils have been identified to be the most important source of N_2O, accounting for 65% of the total global emissions (Kroeze *et al.*, 1999). N_2O production occurs primarily via microbial nitrification and denitrification. Davidson *et al.* (2000) have used the ' hole in pipe' concept (Fig. 5) to describe the N losses via the two procedures. Ammonium enters the first pipe and leaves it as nitrate, whereas holes in this pipe represent the escape of nitric and nitrous oxide. Nitrates enter the second pipe leaves as nitrogen gas (N_2) with the holes representing the escape of nitric and nitrous oxides as well as the entrance of them produced elsewhere. The N_2 flows into the pipe and/ or total N output via crop residues. Blocking one or more holes of this pipe system (without decreasing the total input and/ or output) usually leads to increased fluxes from the rest of the holes. Nitrification and denitrification have contrasting requirements for oxygen and are describe on details below (Bremner, 1997):

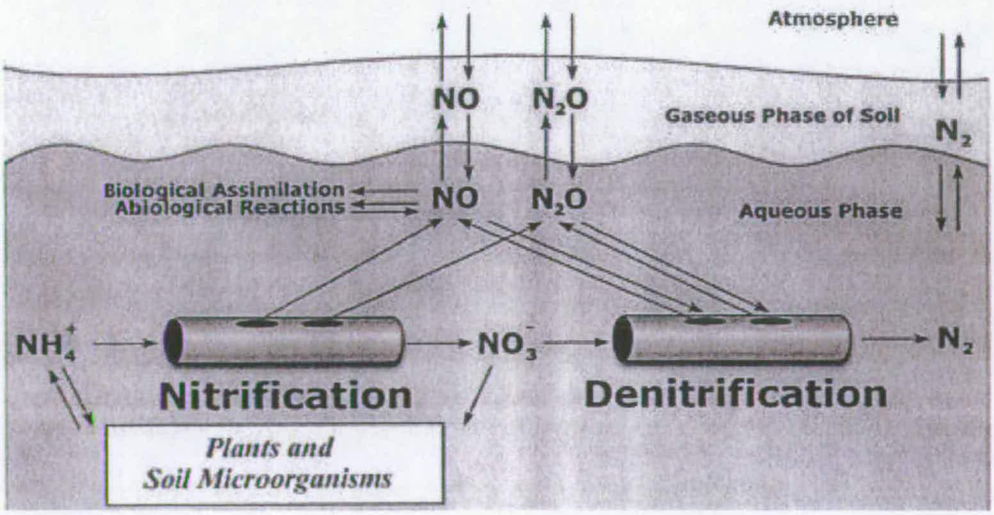


Figure 5: 'Hole in pipe' conceptual model of the two microbial processes (nitrification and denitrification) (Davidson *et al.*, 2000)

2.3.2.1. Nitrification

Nitrification is an aerobic process, performed by both heterotrophic and autotrophic organisms (Fig. 6). Nitrification is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonia or nitrite. In the first step of nitrification, bacteria oxidize ammonia to nitrite according to equation (4).

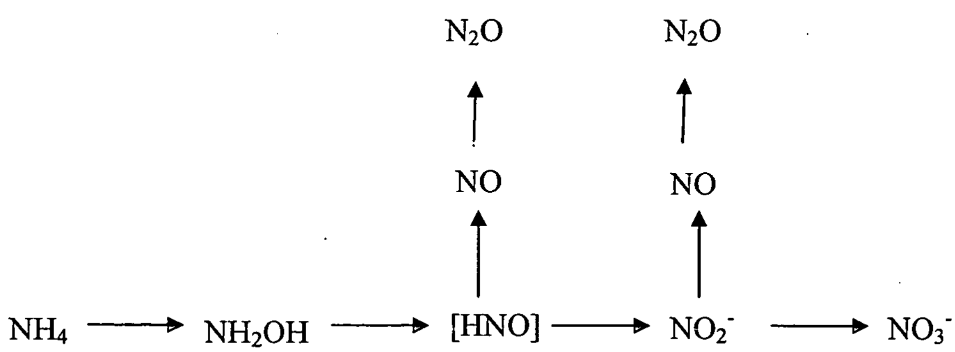
$$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^- \quad (4)$$

Nitrosomonas is the most frequently identified genus associated with this step, including *Nitrosococcus* and *Nitrosospira.* Some subgenera, *Nitrosolobus* and *Nitrosovibrio,* can also autotrophically oxidize ammonia. In the second step of the process, bacteria oxidize nitrite to nitrate according to equation (5).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \quad (5)$$

Nitrobacter is the most frequently identified genus associated with this second step, although other genera, including *Nitrospina*, *Nitrococcus*, and *Nitrospira,* can also autotrophically oxidize nitrite.

Figure 6: Autotrophic nitrification pathway



The factors that influence nitrification in soils are physical, environmental and chemical and the interactions between them have an important role. Soil matrix, moisture status, aeration, pH and temperature play dominant roles in the nitrification of soil or added ammonium to nitrate (Groffman, 1991).

2.3.2.2. *Denitrification*

Denitrification occurs in anaerobic sites in the soil and is carried out by a wide range of mainly heterotrophic but also autotrophic organisms. Denitrification refers to the process in which nitrate is converted to gaseous compounds (nitric oxide, nitrous oxide and N_2) by micro-organisms (Fig. 7). The sequence usually involves the production of nitrite (NO_2^-). Several types of bacteria perform this conversion when growing on organic matter in anaerobic conditions. Because of the lack of oxygen for normal aerobic respiration, they use nitrate in place of oxygen as the terminal electron acceptor. This is termed anaerobic respiration and can be illustrated as follows (equation 6):

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O + \text{energy} \quad (6)$$

Figure 7: **Denitrification pathway**

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

The conditions in which we find denitrifying organisms are characterised by (1) a supply of oxidisable organic matter, and (2) absence of oxygen but availability of reducible nitrogen sources. A mixture of gaseous nitrogen products is often produced because of the stepwise use of nitrate, nitrite, nitric oxide and nitrous oxide as electron acceptors in anaerobic respiration. The ratio of N_2O to N_2 production depends on the species of denitrifer involved (Robertson and Kuenen, 1991), on the degree of anaerobicity in soil, soil carbon and NO_3 content and soil pH. The common denitrifying bacteria include several species of *Pseudomonas*, *Alkaligenes* and *Bacillus*. Their activities result in substantial losses of nitrogen into the atmosphere, roughly balancing the amount of nitrogen fixation that occurs each year. The largest rates of N_2O emission tend to be associated with denitrification.

The factors that affect denitrification are oxygen concentration, nitrate concentration and carbon content. They are in turn affected by various biological and physiological factors, such as temperature and soil water content, which make the regulation of denitrification rather complex.

Soil conditions favourable for nitrification are much more common, so that the contribution of nitrification to the total global N_2O emission may not be trivial; however rates of N_2O production by nitrification tend to be lower than denitrification depending on edaphic and climatic conditions (Williams *et al.*, 1998). The balance between the two processes, nitrification and denitrification, contributing to the N_2O emission will vary with climate, soil conditions and soil management. Generally, high rainfall, poor drainage, fine soil texture and high organic carbon content promote denitrification and associated N_2O production, whereas low rainfall, good drainage, aeration and coarse texture promote nitrification and associated N_2O. However, in most soils the prevalence of nitrification or denitrification as the main source of N_2O is not static and can switch very rapidly, as the soil aeration state within the biologically active sites changes due to e.g. rainfall or increased O_2 demand caused by the presence of easily mineralisable organic matter (Scholes *et al.*, 1997b).

2.3.2.3 Chemodenitrification

Chemodenitrification is a non-biological process, which involves various chemical reactions of NO_2^- ions and yields a number of nitrogenous gases like N_2, N_2O and NO (Bremer and Nelson, 1968). However, chemodenitrification produces mostly NO. NO is found from biological denitrification only in very small amounts as it is not only a product but also a substrate of this process. Thus the presence of large amounts of NO can be an indicator of chemodenitrification (equation 7 and 8).

Under acidic conditions (pH < 4.9) and a redox of 0 to 200 mV, HNO_2 dismutases chemically to form the nitrogenous gases NO_2 and NO which are further reduced to N_2O /N_2 by organic substances.

$$3HNO_2 \rightarrow 2NO + HNO_3 + H_2O \quad (7)$$

or

$$HNO_2 \rightarrow NO + NO_2 + H_2O \quad (8)$$

However, with increasing pH, the HNO_2 levels decline, resulting in a decrease in N_2O production through HNO_2 dismutation. The chemical reaction between NH_2OH, an intermediate of nitrification with NO_2, may also be responsible for N_2O production in well aerated as well as anaerobic soils. Further, decomposition of organic matter oxides with nitrous acid also results in the formation of N_2O.

Agricultural soils represent a very large and growing global source of N_2O. Current estimates for annual emissions from this source range from 2 to about 4 million tonnes of N_2O - N globally (IPCC, 2007). With a rapid increase in population growth and a consequent need for more food production, both the area of agricultural soils and intensity of their use is likely to continue to rise rapidly in the coming decades. In addition, the conversion of forest to agricultural land and increased use of nitrogen fertilisers in agriculture have contribute to high emissions from soils (Matson and Vitousek, 1990).

2.4. Direct sources of Nitrous Oxide

The use of fertilisers (mineral or organic form) is a major source of N_2O from agricultural soils. Mineral nitrogen (ammonium or nitrate) is transformed quicker in comparison with organic N that is decomposed slower into the mineral form. The mineral form of N is integrated into the soil organic matter which is decomposed. Ammonium and nitrate are absorbed by the plants, but nitrate is usually susceptible to leaching.

The demand for greater crop yields and more intensive farming practices have driven farmers to extend the use of mineral N based fertilisers. Large amounts of N_2O production are connected with favourable denitrification soil conditions. Some additional N_2O is thought to arise in agricultural soils through the process of N fixation (Bowman *et al.*, 2002).

2.4.1. Method of calculating direct N_2O emissions from agricultural soils

Until 2004, the emissions from agricultural soils were calculated for most sources as described in the IPCC (2006 and 2007). Total emissions from a country (kg N_2O-N yr^{-1}) are calculated as N_2O = N_2O-direct+N_2O-animal+N_2O-indirect. For direct N_2O emissions from agricultural soils due to N inputs the following sources can be distinguished: synthetic fertilisers (F_{SN}), animal manure (F_{AM}), cultivation of N-fixing crop (F_{BN}), crop residues (F_{CR}), N mineralisation associated with loss of soil organic matter resulting from change of land use or management of mineral soils (F_{SOM}); and drainage/ management of high organic content soils (e.g. histosols) (F_{OS}).

In the most basic approach (Tier 1) described in the latest IPCC Guidelines (IPCC, 2007), direct N_2O emissions from agricultural soils are estimated as follows:

$$N_2O Direct - N = N_2O - N_{N\,inputs} + N_2O - N_{OS} + N_2O - N_{PRP} \quad (9)$$

Where:

$$N_2O - N_{N\,inputs} = F_{SN} + F_{ON} + F_{CR} + F_{SOM}) * EF1) + ((F_{SN} + F_{ON} + F_{CR} + F_{SOM}) FR * EF_{1FR}) \quad (10)$$

$$N_2O - N_{OS} = ((F_{OS,CG,Temp} * EF_{2CG,\,Temp}) + (F_{OS,\,CS,\,Trop} * EF_{2CG,\,Trop}) + (F_{OS,F,TempNR} * EF_{2F,\,TempNR}) + (F_{OS,F,TempNP} * EF_{2F,\,TempNP}) + (F_{OS,\,F,\,Trop} * EF_{2F,\,Trop}) \quad (11)$$

$$N_2O\text{–}N_{PRP} = ((F_{PRP,CPP} \bullet EF_{3PRP,CPP}) + (F_{PRP,SO} \bullet EF_{3PRP,SO})) \quad (12)$$

Where:

N_2ODirect–N = annual direct N_2O–N emissions produced from managed soils

N_2O–NN inputs = annual direct N_2O–N emissions from N inputs to managed soils

N_2O–NOS = annual direct N_2O–N emissions from managed organic soils

N_2O–NPRP = annual direct N_2O–N emissions from urine and dung inputs to grazed soils

F_{SN} = annual amount of synthetic fertiliser N applied to soils

F_{ON} = annual amount of animal manure, compost, sewage sludge and other organic N additions applied to soils

F_{CR} = annual amount of N in crop residues (above-ground and below-ground), including N-fixing crops, and from forage/pasture renewal, returned to soils

F_{SOM} = annual amount of N in mineral soils that is mineralised, in association with loss of soil C from soil organic matter as a result of changes to land use or management

F_{OS} = annual area of managed/drained organic soils, ha

F_{PRP} = annual amount of urine and dung N deposited by grazing animals on pasture, range and paddock

EF_1 = emission factor for N_2O emissions from N inputs

EF_{1FR} is the emission factor for N_2O emissions from N inputs to flooded rice,

EF_2 = emission factor for N_2O emissions from drained/managed organic soils,

EF_{3PRP} = emission factor for N_2O emissions from urine and dung N deposited on pasture, range and paddock by grazing animals,

Emission factor Default value

EF1 for N additions from mineral fertilisers, organic amendments and crop residues, and N mineralised from

mineral soil as a result of loss of soil carbon [kg N_2O –N (kg N)$^{-1}$]= 0.01

EF1FR for flooded rice fields [kg N_2O –N (kg N)$^{-1}$]= 0.003

EF2 CG, Temp for temperate organic crop and grassland soils (kg N_2O –N ha^{-1}) =8

EF2 CG, Trop for tropical organic crop and grassland soils (kg N_2O –N ha^{-1}) =16

EF2F, Temp, Org, R for temperate and boreal organic nutrient rich forest soils (kg N_2O –N ha^{-1}) = 0.6

EF2F, Temp, Org, P for temperate and boreal organic nutrient poor forest soils (kg N_2O –N ha^{-1}) = 0.1

EF2F, Trop for tropical organic forest soils (kg N_2O –N ha^{-1}) = 8

EF3PRP, CPP for cattle (dairy, non-dairy and buffalo), poultry and pigs [kg N_2O –N (kg $N)^{-1}$] = 0.02

EF3PRP, SO for sheep and 'other animals' [kg N_2O –N (kg $N)^{-1}$] = 0.01

In the light of new evidence, the default value[2] for EF_1 has been set at 1% of the N applied to soils or released through activities that result in mineralisation of organic matter in mineral soils. In many cases, this factor will be adequate, however, there are recent data to suggest that this emission factor could be disaggregated based on environmental (climate, soil organic C content, soil texture, drainage and soil pH) and management-related factors (N application rate per fertiliser type, type of crop, with differences between legumes, non-leguminous arable crops, and grass) (Bowman *et al.*, 2002; Stehfest and Bouwman, 2006). Countries that are able to disaggregate their activity data from all or some of these factors may choose to use disaggregated emission factors known as Tier 2 (IPCC, 2007).

2.5. Indirect sources of Nitrous Oxide

The first of indirect sources pathway is the volatilisation of N as NH_3 and oxides of N (NOx), and the deposition of these gases and their products NH_4^+ and NO_3^- onto soils and the surface of lakes and other waters. The sources of N as NH_3 and NOx are not confined to agricultural fertilisers and manures, but also include fossil fuel combustion, biomass burning, and processes in the chemical industry. Thus, these processes cause N_2O emissions in an exactly analogous way to those resulting from decomposition of agriculturally derived NH_3 and NOx, following the application of synthetic and organic N fertilisers and /or urine and dung deposition from grazing animals (IPCC, 2007).

Most indirect agricultural N_2O emissions occur from aquatic environments, since much of the nitrogen lost from agricultural land through leaching, runoff, crop harvest and human consumption ultimately ends up in ground-water, rivers, lakes and estuaries. Measurements of fractional N_2O yields are sparse in aquatic environments, although the yields appear to be governed by many of the same variables described for soils. In rivers and estuaries, N_2O may be produced by nitrifiers and denitrifiers both in bottom sediments and in the water column. Commonly, a uniform yield of 0.5% for both nitrification and denitrification has been assumed for such environments (Mosier *et al.*, 1998).

In the latest IPCC Guidelines (IPCC, 2006), indirect N_2O emissions from runoff/ leaching of agricultural soils are estimated as follows:

$$N_2O_{(L)}\text{–}N = F_{SN} + F_{ON} + F_{PRP} + F_{CR} + F_{SOM} \bullet Frac_{LEACH-(H)} \bullet EF_5 \; (13)$$

Where:

N_2O (L)–N = annual amount of N_2O–N produced from leaching and runoff of N additions to managed soils in regions where leaching/runoff occurs, kg N_2O–N yr^{-1}

FSN = annual amount of synthetic fertiliser N applied to soils in regions where leaching/runoff occurs, kg N yr^{-1}

FON = annual amount of managed animal manure, compost, sewage sludge and other organic N additions applied to soils in regions where leaching/runoff occurs, kg N yr^{-1}

FPRP = annual amount of urine and dung N deposited by grazing animals in regions where leaching/runoff occurs, kg N yr^{-1}

FCR = amount of N in crop residues (above- and below-ground), including N-fixing crops, and from forage/ pasture renewal, returned to soils annually in regions where leaching/runoff occurs, kg N yr^{-1}

FSOM = annual amount of N mineralised in mineral soils associated with loss of soil C from soil organic matter as a result of changes to land use or management in regions where leaching/runoff occurs, kg N yr^{-1}

FracLEACH-(H) = fraction of all N added to/mineralised in managed soils in regions where leaching/runoff occurs that is lost through leaching and runoff, kg N (kg of N additions) $^{-1}$

EF5 = emission factor for N_2O emissions from N leaching and runoff, kg N_2O–N (kg N leached and Runoff).

Uncertainties in estimates of indirect N_2O emissions from managed soils are caused by uncertainties related to natural variability and to the emission, volatilization and leaching factors, agricultural activity data, and lack of measurements. Additional uncertainty will be introduced in an inventory when values for these factors that are not representative of all conditions in a country are used. In general, the reliability of activity data will be higher than that of the emission, volatilisation and leaching factors. As with direct emissions, further uncertainties may be caused by missing information on observance of laws and regulations related to handling and application of fertiliser and manure, and changing management practices in farming. Generally, it is difficult to obtain information on the actual observance of laws and possible emission reductions achieved as well as information on farming practices (IPCC, 2007).

Indirect agricultural sources of N_2O remain poorly defined in most cases. There are many ways that the indirect emissions occur arising from NO_3 leaching and run-off from agricultural soils. The combination of heavy rain and fertiliser application lead to leaching of large amounts of N from the soil to drainage ditches, streams, rivers and finally ground-water and estuaries. N_2O is emitted to the atmosphere as soon as the drainage water is exposed to air. Further indirect N_2O emissions are produced from drainage waters when the leached N fertiliser undergoes the processes of nitrification or denitrification in aquatic and estuarine sediments. In addition, the volatilisation and deposition of NH_3 from fertiliser application and the consumption of crops followed by sewage treatment are important indirect N_2O sources from agricultural soils (Reay *et al.*, 2004b) (Fig. 8).

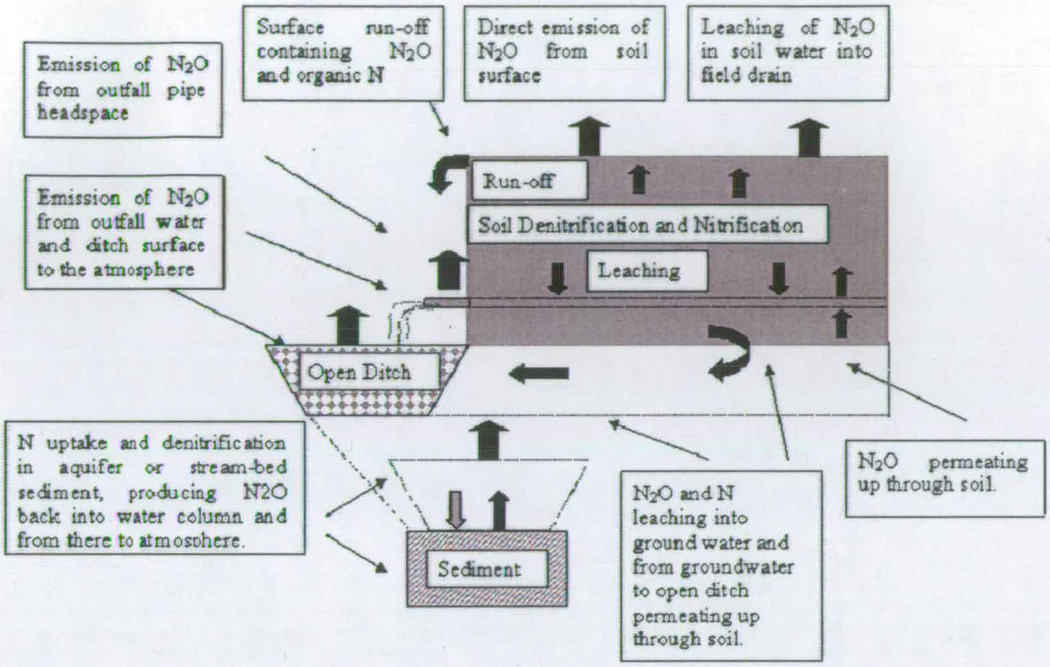


Figure 8: Schematic showing N_2O production and loss from generalised agricultural system. Solid black arrows denote movement of N_2O through system. Note that N_2O consumption via denitrification may at times be important. Arrow sizes do not reflect relative magnitude of fluxes after Reay *et al.*, 2004b.

Future agriculture is likely to embrace a spectrum of production systems. These will range from those that produce commodities based on strict yield and quality criteria (e.g. spring barley for malting) to those that are based on more ecological approaches to the supply of nutrients, and the management of weeds, pests and diseases (e.g. organic farming). Research should aim to develop management practices flexible enough to be used across a wide portion of this spectrum, ideally through understanding the biological and ecological processes that operate within the systems. Sustainable cropping systems of the future will need to be resilient in order to produce acceptable yields under changing environmental conditions. Additionally, they should have minimal adverse environmental impacts and, preferably, positive environmental benefits in terms of, for example, biodiversity and soil quality.

2.6. Factors controlling N_2O emissions

The soil environmental conditions (soil moisture, temperature, pH and oxygen), the organic carbon concentration and the amount of nitrogen cycling in soils control microbial processes and thus N_2O production and consumption.

Soil O_2 concentration and water content

As it has been mentioned earlier nitrification and denitrification processes are controlled by the oxygen level in soils, which is mainly influenced by water content, temperature and microbial activity. Denitrification requires anaerobic conditions which are promoted by soils with high soil moisture content or aerobic soils with anaerobic microsites. Waterlogging promotes complete denitrification with N_2 as a final product, whereas at lower moisture contents N_2O is the main gas produced. In nitrification, the N_2O/ NO_3^- ratio increases with decreasing O_2 levels as nitrifiers use NO_2 as an alternative electron acceptor, producing N_2O. Optimal moisture contents for nitrification range between 30% and 70%, whereas N_2O production occurs mainly at WFPS (Water Filled Pore Space) between 50% and 70% (Fig. 9). At low moisture contents, microbial activity is depressed and mineralisation of organic N will be slow, so limiting NH_4 availability for nitrification. As a result in both processes, N_2O is the favoured product at intermediate moisture contents. Wetting of a dry soil through rain or irrigation has been observed to result in rapid, large, but fairly short duration N_2O and NO emissions, which are often referred to as ' pulses' of N oxides (Scholes *et al.*, 1997a). It has been suggested that these fluxes result from an accumulation of NO_3 and NH_4 in the soil over the dry months.

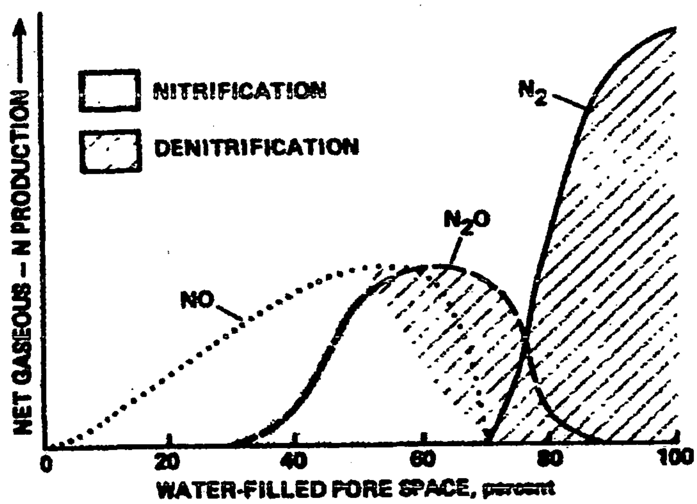


Figure 9: Effect of water-filled pore space on nitrification and denitrification and contributions to emissions of NO and N_2O (Davidson et al., 2000).

Soil pH

There is an optimum pH for both denitrification and nitrification, which ranges between 7.0 and 8.0 (Xie, 1999). However, Hadas *et al.*(1989) observed an optimum pH range between 7 and 9 for nitrification. Since the N_2O reductase is highly sensitive to low pH, the N_2O:N_2 is enhanced with a pH decrease. Notably, N_2O is the major product of denitrification at low pH 4.0. At higher pH values, N_2O is further reduced to N_2 as the end product of denitrification. However, Davidson (1993) did not observe any clear cut effect of pH on N_2O fluxes in field conditions.

Temperature

Nitrification and denitrification are affected by temperature; rates are very low below 5°C but increase rapidly with increasing temperature. Oterr (1999) found that there is an exponential increase of NO and N_2O with increased temperature. The temperature for denitrification and nitrification ranges from 25°C to 37°C and from 25°C to 35°C, respectively. The temperature dependency of denitrification and nitrification results in seasonal and daily variations on N_2O emissions from soils in temperate climates. In order to produce accurate annual estimates of soil N_2O emissions, frequent seasonal flux measurements have to be taken to account for temporal variability. The daily variation can be exaggerated with fertiliser applications (Flechard *et al.*, 2007). A large proportion of the annual N_2O emissions can be produced within only a few days. These typically occur either in winter during freeze-thaw cycles or in summer through rewetting of dry soil, driven by climate and weather parameters. Additionally, emissions occur in situations with elevated mineral nitrogen concentrations in moist soils after fertilisation or during the decomposition of crop residues, controlled by management and the interactions with climate, soil and site parameters and (Freibauer and Kaltschmitt, 2003).

Organic Matter

The availability of organic C is an important factor regulating the denitrification process in the soil. Rates of denitrification are usually correlated positively to the water-soluble C content of soils. Hence, large N_2O fluxes are reported from organic peat-soils (Luo *et al.*, 1999) Addition of organic materials in the form of crop residues, organic manures, waste effluents and sewage sludge, enhances N losses in the form of N_2O from soils. High availability of organic C induces complete reduction of NO_3-N, leading to N_2 emission as the end product instead of N_2O. An increased C supply reduces the N_2O/ N_2 ratio evolved during denitrification. As organic C is a substrate for respiration, high organic C contents can lead to the increase of anaerobic microsites, favouring denitrification. These 'hot spots' describe the high spatial variability of soil denitrification observed experimentally (Schmidt *et al.*, 1988).

Nitrogen sources

While denitrification is limited by availability of NO_3^-, the nitrification process is influenced by NH_4^+ which may be derived from either decomposition of organic matter, the mineralisation of organic compounds, as fertiliser and atmospheric N inputs and plant species residues. Low NO_3^--N concentrations can lead to N_2O consumption as denitrifiers use N_2O instead of NO_3^-. High NO_3^- concentrations in the soil increase N_2O emissions from denitrification and also enhance the N_2O/N_2 ratio as NO_3^- usually inhibits N_2O reduction to N_2 (Mosier *et al.*, 1998). High NH_4^+ concentrations enhance the production of N_2O by nitrification and/or denitrification after an application of NH_4^+ forming fertiliser.

2.7. Intercropping

Intercropping is the growing of two or more crops simultaneously in the same field, which intensifies cropping in both time and space dimensions. There is intercrop competition during all or part of crop growth. Crop spatial arrangement, crop density, maturity dates of the crops and plant architecture will all affect crop and weed growth in intercropping systems and must be always considered. There are at least four basic arrangements used in intercropping:

- *Mixed intercropping.* "Growing two or more crops simultaneously with no distinct row arrangement." This is frequently the form taken in indigenous slash-and-burn or fallow agriculture.
- *Row intercropping.* "Growing two or more crops simultaneously where one or more crops are planted in rows." This is the pattern usually encountered in intensive agriculture, where the plough has replaced the machete and fire as the main tool of land preparation.
- *Strip intercropping.* "Growing two or more crops simultaneously in different strips wide enough to permit independent cultivation but narrow enough for the crops to interact agronomically." This form of intercropping is more common in highly modernized systems, especially where the intensive use of machinery is desired.

- *Relay intercropping.* "Planting a second crop into a standing crop at a time when the standing crop is at its reproductive stage but before harvesting." This form of intercropping may actually include the other three as subsets, since its primary categorization variable is time (Vandermeer, 1989).

To optimise total plant density, the seeding rate of each crop in the mixture is lowered rate. If full rates of each crop were planted, neither would yield well because of the interspecific competition. By reducing the seeding rates of each, the crops have a chance to yield well within the mixture. Planting intercrops that feature staggered maturity dates or development periods takes advantage of variations in peak resource demands for nutrients, water and light. Having one crop mature before its companion crop lessens the competition between the two crops. Plant architecture is a commonly used strategy to allow one member of the mix to capture light that would not otherwise be available to the others.

The use of two or more crops may improve utilisation of local resources, reduce or eliminate use of pesticides, herbicides and fertilisers, which can encourage local flora and fauna, increase the size and diversity of the soil microbial community; remove the dependence on one crop, and increase the diversification of cultivated land. All of these factors tend to make intercropping systems more resilient to environmental perturbations than monocrop systems. Intercropping is widely used in the less intensive agricultural systems typical of Africa and Asia. In Europe, intercrops largely disappeared through the 20^{th} century with an increase in mechanisation and artificial chemical support, except for grass-clover swards. Intercropping ideally allows for improved resource utilisation and beneficial biological interactions between the crops. In other words, light, water and nutrients are used by the crops instead of the weeds, and some plants may enhance the growing environment for their companion crop plant.

Although there is interest in the reintroduction of intercropping, this is hampered by a lack of credible scientific evidence. The main potential advantages are greater and more stable yields, as there is less competition and better contribution of common resources; protection against risk and environmental extremes, and ecological approaches to manage pests, diseases and weeds through natural competitive principles. The approach can contribute to the prevention of nitrogen leaching that is sometimes observed from monocrops such as grain legumes due to changes in incorporated residues involved in nutrient turnover and an improvement in protein content of cereal. Moreover, the inclusion of N_2 fixing crops in an intercrop leads to the utilisation of the renewable resource of atmospheric nitrogen which can increase the sustainability of the agroecosystem (Phool, 1986)

Intercrops are used globally for all the above benefits but lately they are also used to provide biofuel as the intercrop composition can be designed to produce a medium (for microbial fermentation) containing all essential nutrients. Thereby addition of e.g. urea and other fermentation nutrients from fossil fuels can be avoided especially N when including leguminous species, whereby addition of e.g. urea and other fossil based fermentation nutrients can be reduced. (Thomsen and Haugaard-Nielsen, 2008).

2.7.1. Intercropping in Europe and Worldwide

Modern European intercropping systems have been used for either organic agriculture and/ or conventional systems, where the tactical use of fertilisers and pesticides provide powerful additional management options. Recent work on intercropping at SAC has successfully quantified the transfer of N from clover to cereal in the establishment year (i.e. clover undersown into a spring cereal) (Rees *et al.*, 2006). Results show that during this first phase there is little nutritional benefit to the crop, although there is a net benefit to the system in terms of developing ground cover for the winter period. Clover root death and leaf litter decomposition taking place over winter may be an important source of N for a cereal crop in the following year, but as yet such contribution has not been quantified, and, in any case, is expected to vary from situation to situation depending on the degree of leaching and denitrification. Management practices such as cutting and the use of herbicide to kill part of the developed understorey have the potential to create a supply of N in synchrony with crop demand (Drinkwater *et al.*, 1998). The amount of control of mineralisation/ immobilisation processes that can be achieved, and the proportion of this N transferred to the crop compared with that lost through denitrification and leaching all require quantification. Results obtained to date also suggest that the timing of many of these management options will be critical in determining their outcome, making thorough, mechanistic knowledge of the system vital. A model has been developed recently that describes root competition and nutrient flows in intercropping systems (Wu *et al.*, 2007). The model known as SPACSYS is a mixed dimensional, multi-layer, field scale, weather-driven and daily time-step dynamic simulation model. The current version includes a plant growth and development component, a nitrogen cycling component, a carbon cycling component, plus a soil water component that includes representation of water flow to field drains as well as downwards through the soil layers, together with a heat transfer component.

The use of intercrop systems requires both environmental and economic evaluation of their potential contribution to sustainability but also investigation of their use in a spatial dimension for managing the farm landscape. The latter concept applies not only to buffer strips and field margins, but potentially within the cropped areas of the farm for production of fodder crops so as to provide a better use of the resources .

In more detail, intercrops of pea and barley have been shown to use available growth resources more efficiently than their corresponding monocrops (Hauggaard-Nielsen and Jensen, 2001). The increased resource use efficiency may be explained by the fact that the two intercropped species do not compete for exactly the same resource and thereby give rise to some degree of resource complementarity (Hauggaard-Nielsen *et al.*, 2001). Barley has been shown to be much more competitive for soil inorganic N than pea (Jensen, 1996), most likely as a result of faster and deeper root growth of barley compared to pea (Hauggaard-Nielsen *et al.*, 2001), forcing the grain legume to increase reliance on symbiotic N_2-fixation (Jensen, 1996; Karpenstein-Machan and Stuelpnagel, 2000). A better utilization of resources through resource complementarity may also result in reduced weed growth in intercrops compared to sole crops (Liebman and Dyck, 1993), an aspect that is of great importance to low-input farming systems, such as organic farming (Hauggaard-Nielsen *et al.*, 2001). In an intercrop, the degree of resource complementarity attained, the total yield measured and the relative contribution of the individual components is determined by both inter- and intra-specific competition, which again is influenced by the availability of environmental resources and the relative frequency and density at which the component crops are sown (Vandermeer, 1989). Recommended monocrop plant population densities are well established for most crops (Bulson *et al.*, 1997). However, intercrop components may utilize growth resources more efficiently than monocrops. Consequently, the optimum plant density in intercrops could be greater than the optimum density of each of the sole crops. With increased crop density competitive dynamics will inherently be affected and, as noted by Willey (1979), the impact of the dominant will often increase as intercrop density is raised. The proportions at which intercrop components are sown may be of great significance in determining yields and production efficiency of cereal–legume intercrop systems (Ofori *et al.*, 1987) and changes in the relative frequency of intercrop components have been shown to alter the competitive dynamics between component species (Willey, 1979). Intercrop competition studies usually base their conclusion on data from one single, final harvest of crops grown at one density, thereby implying that competitive strength or other measures of performance are constant. However, species interactions are complex, varying with cropping density, the nutrient environment and time (Connolly *et al.*, 1990). Another factor is the rate of development of the crops. Planting intercrops that feature staggered maturity dates or development periods takes advantage of variations in peak resource demand for nutrients, water, and sunlight. Selecting crops or varieties with different maturity dates can also assist staggered harvesting and separation of grain commodities.

Intercropping is of special relevance and importance in future organic farming systems, because it offers a number of significant enhancements of the net productivity of organic farming and the ecosystems in farming regions as a result of the increased diversity of the cropping system.

3. Overview of the thesis

The main study aims were to a) study the potential benefits of the use of intercrops in a low input rotation in Eastern Scotland and b) estimate the nitrogen (N) losses of an intercropped low input rotation and c) understand what controls and drives the fluxes from the different intercropped systems. The main study was based at an experiment on the Bush Estate, near Edinburgh, Scotland, on 12 hydrologically isolated plots previously used for leaching studies.

The thesis is composed of four individual papers. Paper 1 describes the effects of different legumes' (species and/ or varieties) on grain yield and total N uptake of the barley and subsequent crops. Monthly measurements of gas fluxes, soil parameters and water were made across the three growing seasons including a comparison of the data with the final harvests. Land Equivalent Ratios (LER) were calculated to evaluate the intercrop systems.

To compliment the above agronomic work, environmental issues were considered. N losses from the drainage plots were investigated by weekly measurements of nitrous oxide (N_2O) fluxes and nitrate (NO_3^-) leachate in a 30 month period (Paper 2). N_2O fluxes were measured using static chambers across the plots. NO_3^- leachate was calculated by 'spot' sampling and 'integral' sampling. In addition to the above two measurements, N_2O fluxes from the drainage were measured in the second growing season to estimate the indirect losses. To finalise this study, soil cores with intervals of 20 cm were collected monthly during the experimental period and analysed for available NO_3^-, NH_4^+ and dissolved organic nitrogen (DON).

Paper 3 focuses on below ground biomass (roots). Specifically, it examines the effects of the accompanying legume on barley root growth/ development and the influence of the legume' roots on the next cereal crop. This is important since legume roots supply N to the cereal crop, fix N that affects N_2O fluxes and may affect the competition for nutrients in the following year's crop. Root samples were collected at key growth stages, when demand for N from the cereals had reached the highest level. Soil cores were collected from the treatments in two depths (0-20 cm and 20-40 cm).

Finally, Paper 4 examines the factors that may control the N_2O fluxes and the losses from the experiment including data for soil water release properties and water filled pore space. We also compared the possible factors controlling N_2O fluxes under the same soil type, topography and climate without any input. Grain yields were used to test the correlations with cumulative N_2O fluxes, as higher yields related with higher amount of N in the soil related with increase N_2O fluxes. Seasonal variability was considered for the correlations.

Weather data were recorded throughout the experimental period. Average monthly temperature and monthly rainfall are presented in the papers from data provided by the SAC weather station at Bush Estate. Tables and Figures are labelled sequentially throughout the thesis with the exception of the individual papers (Chapters 5, 6, 7 and 8) where Tables and Figures are labelled as they will appear in the final published articles.

The thesis concludes with a discussion of the most important points arrive from the work, suggestions for further work and new ideas coming from the presented results.

4. Materials and Methods

4.1.Study site

The experiment was conducted in a 0.4 ha field under laying by a sandy loam soil (Macmerry series) developed from partially sorted glacial till at Bush Estate, 8 km south of Edinburgh, Scotland (lat. 55° 51'N, long. 3° 12'W) (Fig.1). The soil profile is described on Table 1. The soil is light with some stones in the subsoil horizons and this contributes to a high bulk density.

Twelve hydrologically isolated plots were installed in 1992. The plots were aligned downslope with 1.5 m deep trenches backfilled with gravel and lined with polythene on the downslope edge. The plots were installed as shown in Fig. 1c. A trenchless drainage machine first laid 10 runs of approximately 100 m of 100 mm pipe in approximately 1.5m depth with gravel backfill to the soil surface.. Plastic lined porous drainage matting was laid over each of these pipes at the base of each of the four plots. The trench between adjacent plots was filled with bentonite to prevent lateral movement of water. The closed pipes connected to one of three instrument pits (Fig.1, 2, 3). More information can be found at Vinten et al., 1994.

The field was fallow for the period 2003-2005. Temperature and precipitation for the field site over the course of the study are shown in Figure 4, which was measured in two weather stations, less than 5 miles distance each. Total annual precipitation and mean annual temperature were 927 mm and 8.5 °C for 2006 and 1288 mm and 8.2 °C for 2007.

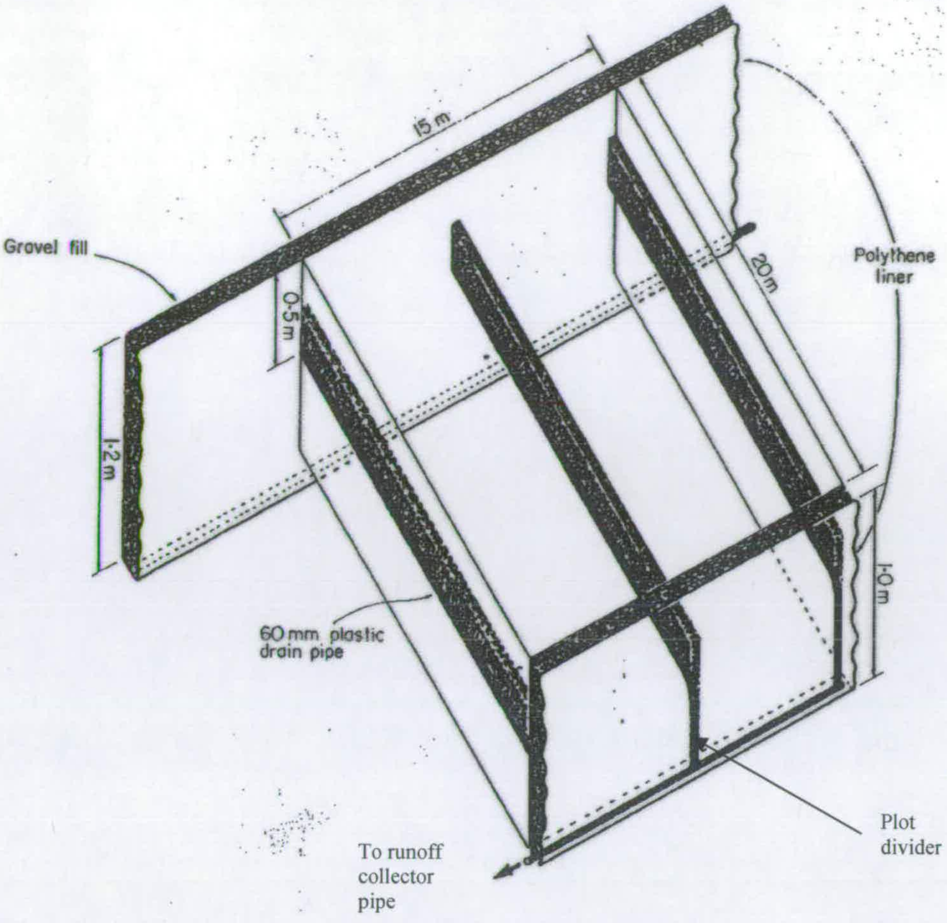


Figure 1: Two plots isolation (Vinten *et al*., 1994)

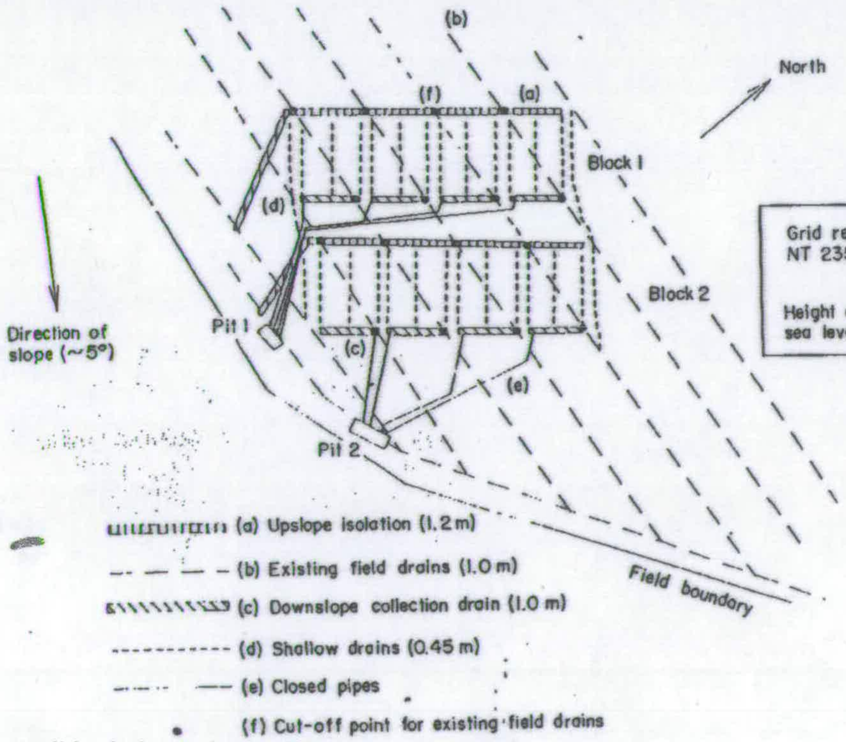


Figure 2: Overall isolation scheme (Vinten *et al*., 1994)

Figure 3: (a) Location of sample site located in Mid Lothian, Scotland, U.K. The field is highlighted with a red circle in (b) (scale 1:25.000). (c) Detailed plan of the Section 3 field at the bottom right photo. Plots 1, 6, 11: Barley/ Clover; Plots 2, 3, 5: Barley/ Pea cv. Zero 4; Plots 8, 10, 12: Barley/ Pea cv. Nitouche and Plots 4, 7, 11: Barley (mon).

Table 1: Typical soil profile description of No. 3 field, Bush Estate (Vinten et al., 1992).

Title:SCAE No.3 Field Association: Winton/ Rowanhill Series: Macmerry Drainage: Imperfect	Nat Grid: NT243640 Land Capability: 3.1 Altitude: 199m	
Parent material: till, with partially watersorted upper horizons, derived mainly from sedimentary rocks of carboniferous age		
Horizon	Depth (cm)	Description
Ap	32	Dark brown (7.5YR4/2); sandy loam, moderate medium subangular blocky to moderate fine subangular block; moist; friable; common very fine fibrous roots; few small subangular stones; clear wavy change to:
Bg	65	Greyish brown (10YR5/2) with common fine prominemt and sharp strong brown (7.5YR5/8) mottles; loamy sand; moist and very friable; common very fine fibrous roots; common medium subrounded stones; clear smooth change to:
Bg	95	Brown (7.5YR5/4) with common fine prominent and sharp strong brown (7.5YR5/8) mottles and common medium light brownish grey (10YR6/2) gley patches; loamy sand locally sandy loam; weak medium subangular; moist; friable to firm; few very fine fibrous roots; common medium and few large subrounded stones; clear smooth change to:
Cg	160+	Brown (7.5YR4/4)with few fine prominent and sharp strong brown (7.5YR5/8) mottles and common medium pinkish grey (5YR6/2) gley patches; sandy clay loam to clay loam; massive; slight moist; firm; no roots; common medium subangular and few large subangular stones mainly sandstones and intermediate igneous

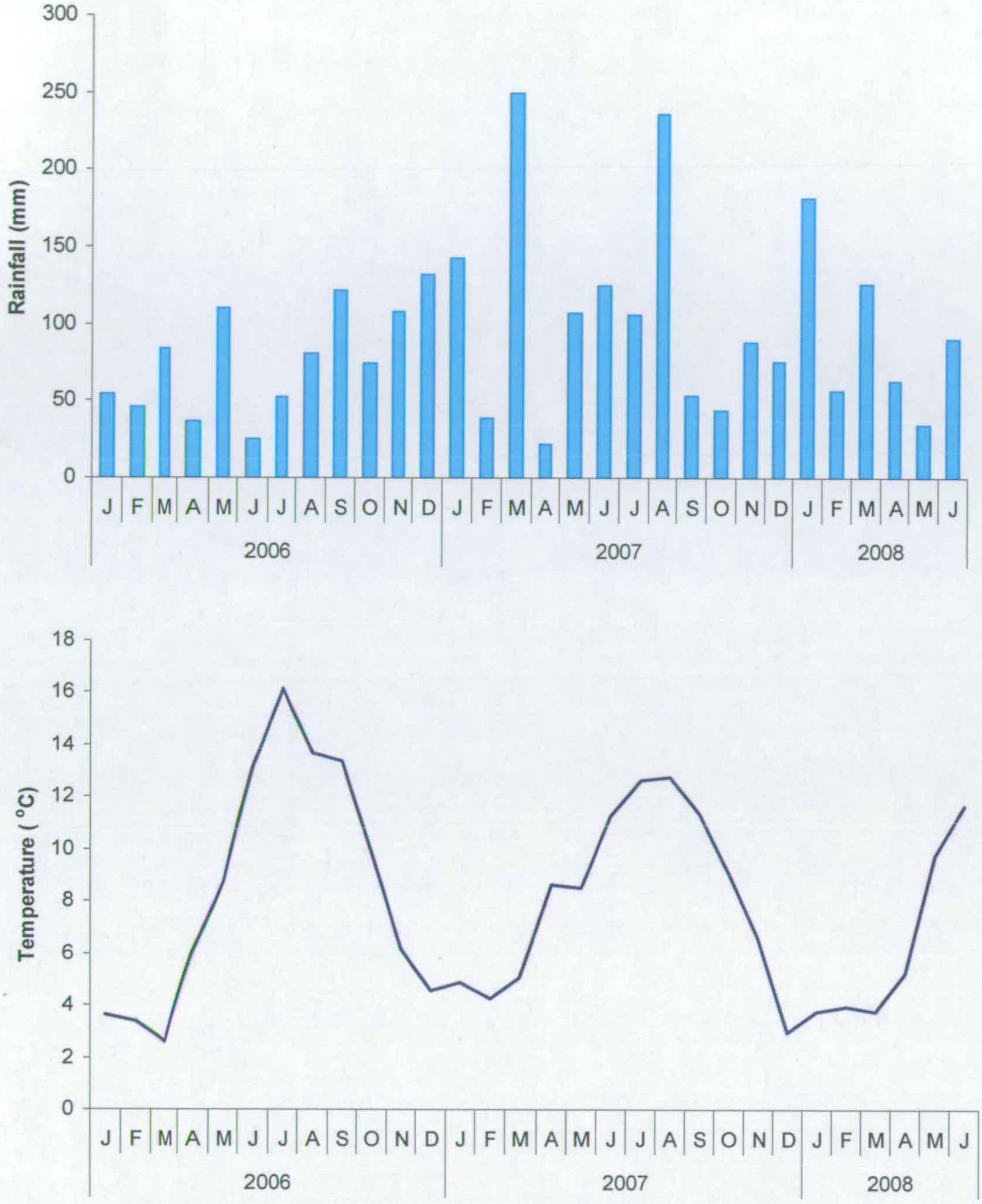


Figure 4: Mean monthly precipitation (a) and air temperature (b) for the experimental period.

4.2. Methods

4.2.1. N_2O fluxes

Fluxes were measured at the experimental site using the static chamber method described by (Clayton, 1994). Chambers consisted of a 20 cm length polypropylene vent pipe (diameter 40 cm) fitted with a 4.5 cm-wide outward-facing polyvinylchloride (PVC) flange at one end to seat a square aluminium lid during flux measurements (Fig. 5). The soil-atmosphere flux is calculated by measuring the change in concentration over time in a known volume of air inside an enclosure. To calculate a valid flux rate the enclosure must be designed and employed in a way that limits interference. There are many variations in chamber design including chamber volume, material, air circulation and pressure equilibration. The time that the chamber will be closed for and the number of samples collected from each enclosure chamber are important issues affecting the results. Moreover, the closure time must be long enough to allow sufficient concentration change for analytical detection and short enough to prevent significant changes in the temperature of the enclosed chamber. Further considerations include the number of chambers per plot, the layout across the experimental area, the frequency of measurements and the time during the day.

One chamber (volume 25120 cm^3; cover area 1256 cm^3) was located in each of the 12 plots. The chambers were sealed for 60 minutes with an aluminium lid having a small open sampling point sealed with grommet to insert the syringe. Gas samples were collected weekly in portable evacuated aluminium vials (Scott *et al.*, 1999). Samples were analysed for N_2O by electron-capture gas chromatography. For consistency, gas sampling was carried out between 10:00 and 12:00 hrs (Clayton *et al.*, 1994). Additionally, three more samples of background air were collected for use as ambient during the calculations.

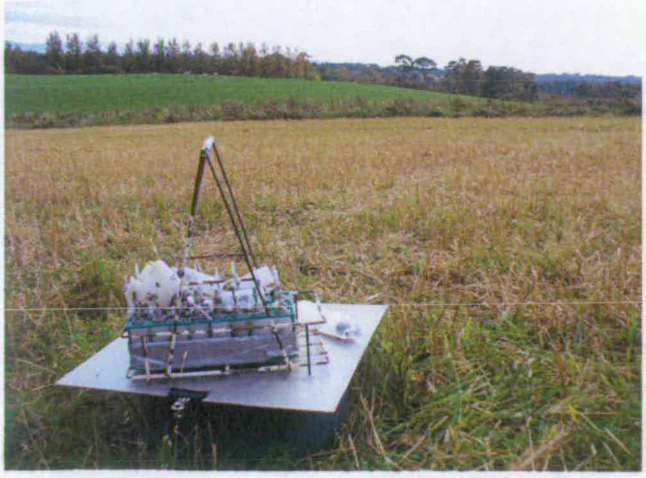


Figure 5: Static chamber in the field.

4.2.4. Dissolved N_2O- headspace method

Concentrations of both dissolved N_2O and inorganic N were measured in water samples collected from the field drainage. All samples were analysed within 48 hr of collection. Nitrous oxide concentrations were assessed in the laboratory by analysis of duplicate 5 mL subsamples of each sample. Each subsample was injected with a syringe into a 22 mL vial sealed with a septum and shaken vigorously for 2 min, followed by a 30 min standing period.

Nitrous oxide concentrations in the headspaces were determined by gas chromatography using an Agilent 6890 GC fitted with a 1.8 m Porapak-N column and electron capture detector. *In situ* dissolved N_2O concentrations were then calculated, based on N_2O solubility at laboratory temperature and pressure versus *in situ* temperature and pressure, and allowing for the atmospheric N_2O concentration.

4.2.3. NO_3- leachate

Two sampling methods were used to sample the drain flow from the pipes. The first was an integrated sampler, which consisted of a closed plastic bucket with a plastic pipe to connect the two sides that carried water from one side of the tipping bucket. At one side it was connected a collection bottle and at the other side it was open for the excess water to run out. A small hole in the pipe allowed some of the water which tipped into the pipe via the bottle (about 10 ml) to flow into the bucket (Fig. 6, 7).

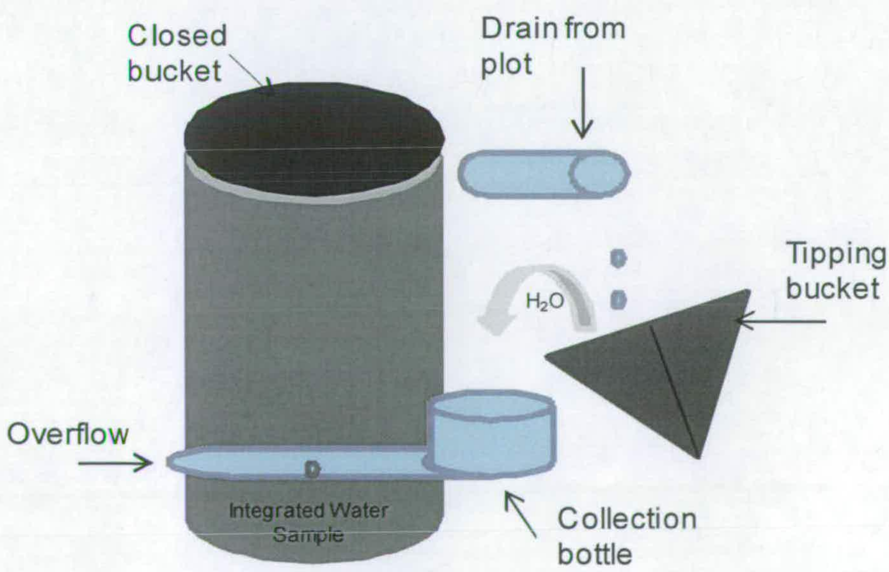


Figure 6: Schematic presentation of the integrated water sampler.

Water samples from the tipping buckets accumulated in the collecting bucket, and a single sample of the water in the bucket was collected at the end of each sampling period. The volume of each individual sample to the bottle varied but this is unimportant because the number of samples collected is so large *(n* = 300 for 10 mm drain flow). This system has a significant benefit over the second sampling method, because only one sample was needed for analysis to obtain the average concentration of the flow between samplings. The second method was a spot sampling comparing collection of the water flowing from the pipe at a specific time/ date which was used for checking the integrated sampler results. The concentrations of the NO_3- from the integrated sampler were compared from a sample collected on the day of sampling collection from the drainage pipe, so as to test if there was any change on the concentrations. All the samples were collected weekly and transferred in the laboratory and analysed for nitrate and ammonium concentrations. If they were not analysed the same day of collection, they were stored in the freezer for analysis at a later stage. The determination was done by continuous flow analysis using the methods of Hendrickson & Selmer Olsen (1970) and Crooke & Simpson (1971), respectively. The samples were transferred to the laboratory and placed according to date and number of plot. 3 ml of the each sample was transferred to a clear plastic disposable capsule and placed on the auto-sampler. Determination of NO_3- and NH_4^+ concentrations were taken place. Water based standards were used. NH_4^+ were insignificant, so they have not presented. For the analyses of the data, only the two blocks of the three were used, as all the plots had not equal runoff. That was a limitation of the experiment, as some of the plots were not properply drained since the establishment of them.

Figure 6: Drainage pits for collection of drainage water (taken on March, 2006)

4.2.4. Dissolved Organic Nitrogen (DON) in soil and Inorganic Nitrogen

Dissolved organic nitrogen (DON) is an important component of the soil, and in many cases, it is the main vector for N loss from the soil via leaching. Several methods exist for the analysis of DON and total N, including Kjeldahl digestion. The method that has been used here is the persulfate digestion technique, because it is simpler and non toxic. Persulfate N includes DON, nitrate and ammonium.

Soil samples were collected at two depths (0–20 cm and 20-40 cm) at monthly intervals from each plot. Fresh soil was extracted prior to drying. If that was not possible, samples were frozen at -16 °C until analysis. Prior to extraction, soil was sieved with a 2 mm sieve to obtain representative subsamples and to exclude large particles, and finally mixed. All sample weights were converted to an oven-dry (105 °C) basis, determined by oven drying subsamples (>24 hours) taken at the time of sample analysis. When a sieved fraction was used for analysis, the material larger than mesh diameter was oven-dried (105 °C) in a Qualitemp 300 from Laboratory Process Equipment (LTE) oven and weighed to provide a correction factor between sieved oven-dry weight and unsieved oven-dry weight.

A duplicate sample of 10 gr of sieved soil each was weighed into a 150 ml polyethylene shaker bottle and 50 ml of 0.5 M K_2SO_4 added. The shaker bottle was tightly taped and placed on a Gallenkamp Orbital shaker at 100 rev min^{-1} for 2 hours. After standing for 5 min, the clear excess was filtered through a 150 cm Whatman No 42 fluted filter paper and the extract was collected into a freezer proof airtight polythene bottle. A sub-sample of K_2SO_4 was also filtered for use as a baseline blank determination. The extract was stored in the fridge for up to 24 h before analysis. If the extracts could not be analysed within 24h, they were frozen. The K_2SO_4 extract was analysed for NH_4^+ and NO_3^- on the auto-analyser using a range of standards made up in 0.5 M K_2SO_4 (0.5, 1, 1.5 and 2 ppm).

For the oxidation process, a 5 ml aliquot of the K_2SO_4 extract was accurately pipetted into an autoclaved universal bottle and 1ml of persulphate oxidiser was added. The persulphate oxidiser used was 1.34 g $K_2S_2O_8$ + 0.3 g NaOH dissolved in 100 ml deionised water. The bottle was then tightly capped and placed in the autoclave for 30 mins at 110 °C. This processed extract was only analysed for NO_3^- and NH_4^+ again on the auto-analyser at a dilution of 1.5 ml of extract to 0.09 ml of 0.1 M NaOH. The suggested range of standards used was started at 0.5 ppm up to 5 ppm.

The calculations of the N content in the soil as NH_4^+ and NO_3^- were made by using the following equations:

N-*content* ($\mu g\ g^{-1}$) = (extract concentration * extractant volume) / eqdw (14)

and

eqdw (g)= (dw / fw) * fsw (15)

where *extract concentration* is the nitrate or ammonium value of the analyser in $\mu g*ml^{-1}$; *extractant volume* is 50 ml K_2SO_4; *eqdw* is the equivalent dry weight; *dw* is the dry weight of the soil sample in g; *fw* is the fresh weight of the soil in g and *fsw* is the fresh weight of the sample used for nitrogen analysis in g (i.e. 10 g).

The calculation of DON was calculated by using the following equation:

$$\text{DON} (\mu g\ N/L) = \text{Persulphate N-Inorganic N}$$

4.2.5. *Root measurements*

Root samples were collected from the plots by using 7 cm diameter tubes up to 0.9 m depth once in each growing season. The cores were analysed in 1-2 days or stored below 0 °C for later analyses. The cores were placed in water and washed the next day using different sieves (from 1 cm to 1mm). This can be the most difficult and laborious step in the measurement if plants are grown in a solid medium. Soil or growth media with a high sand content can greatly simplify this step, while high clay or organic matter content can make it extremely tedious. Harvesting of younger plants can also simplify this step. Since it was impractical to scan the whole root system of the plant, a representative sample was scanned after removal of any organic debris and dead roots and analyzed before determining dry mass. The roots were scanned by an A3 Epson, Expression 836 XL dual scanner and the Win RHIZO software program was used to measure the root length, root area and root volume. The following settings were chosen before the start of scanning each root: professional mode, film, 8bit greyscale depth, 600 dpi optical resolution, original size and normal preview. The root samples were then scanned in a transparent, water proof tray filled with water. The colours used for drawing the roots were coded according to their diameter. Root length and diameter were measured with Regent's unique method and with an indirect statistical method (Tennant, 1975). With Regent's method, measurements were made continuously at each point along the root. Root overlap was taken into account to provide accurate measurements of length and area. Image edition was also available to override decisions made by the system (Fig. 7, 8). Analysis results can be sensitive to the threshold parameters used which are automatically set by WinRhizo. Images were also saved in files for later validations, analyses or for visualization in other programs (like word processors). Finally, the fresh weight was measured, after the excess water had been removed and the roots were transferred to dry at 100 °C overnight. The material was stored for analyses of the mineral N.

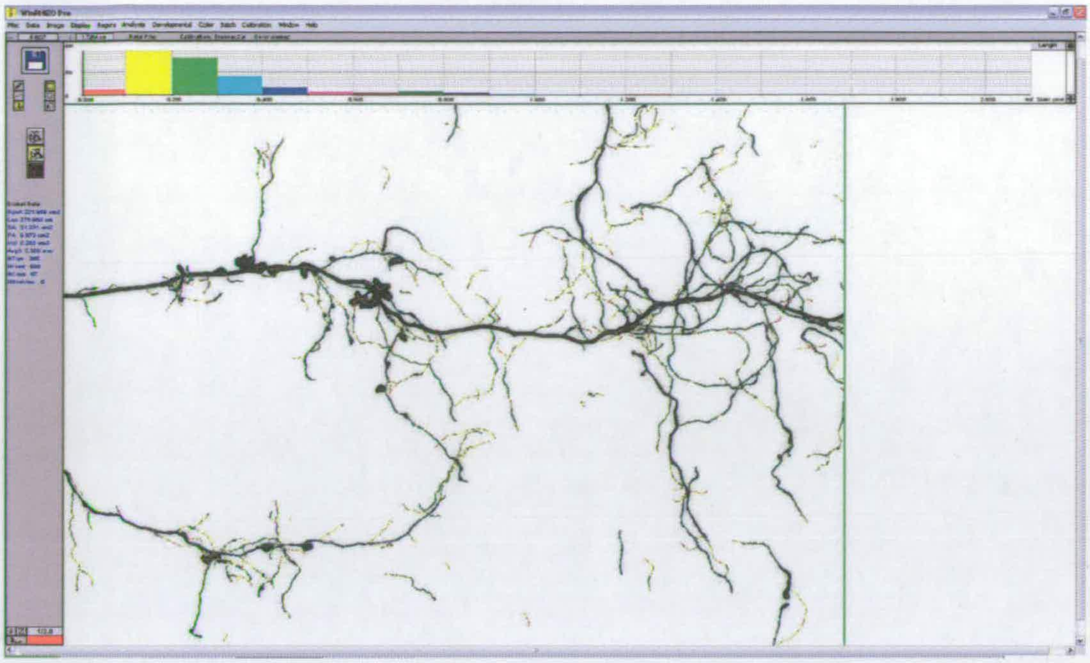


Figure 7: Scanning stage using the Win Rhizo program. Each root is highlighted with a different colour depending on each diameter. Each colour represents the root diameter in increments of 0.1mm at the top of figure a histogram of the total area.

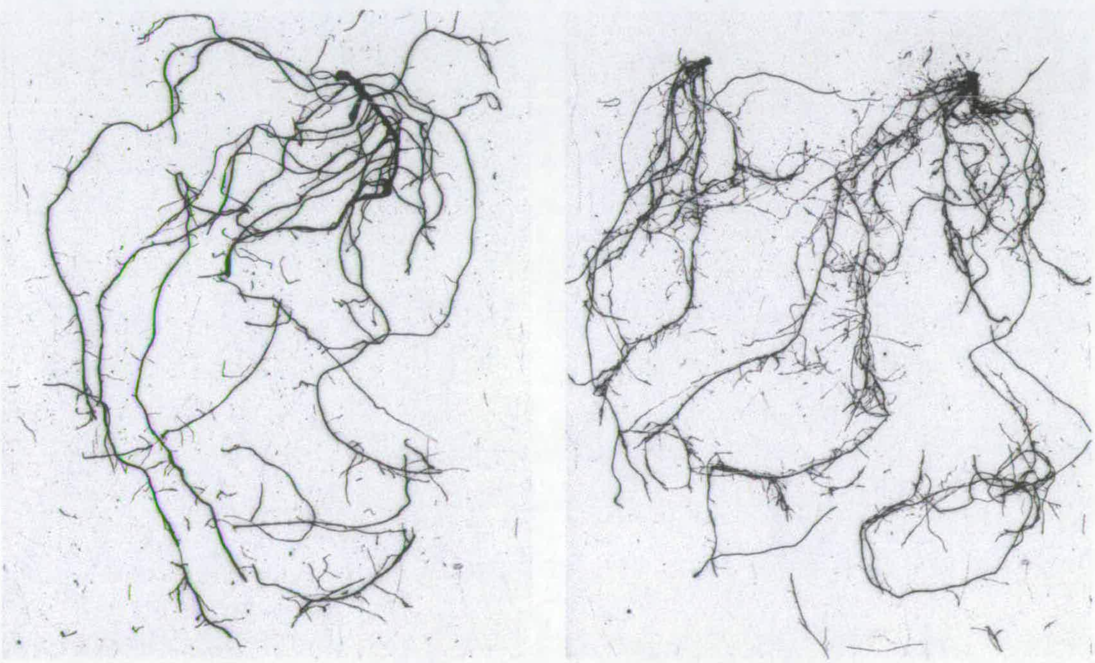


Figure 8: Scanned roots using the Win Rhizo program of barley cv. Westminster (left) and pea cv. Nitouche (right) at the first growing season (2006) at the full maturity stage.

4.2.6. *Bulk density and Soil Water Content*

Bulk density (ρ_{bulk}) is a measure of the weight of the soil per unit volume, usually given on an oven-dry (110 °C) basis. Variation in bulk density is attributable to the relative proportion and specific gravity of solid organic and inorganic particles and to the porosity of the soil. Most soils have bulk densities between 1.0 and 2.0. Samples for bulk density determination were collected in a 9 cm diameter ring. The samples were each placed in a bag and transferred to the lab, where each bag was weighed. The soil from each bag was placed on a metal tray to dry over-night in an oven at 110°C. The soil bulk density was calculated as the dry mass of the soil divided by the volume. It is expressed as g cm^{-3}.

4.2.7. *Volumetric/ Gravimetric water content*

Soil water content was expressed on a gravimetric or volumetric basis. Gravimetric water content (θg) is the mass of water per mass of dry soil. It was measured by weighing a fresh soil sample (*mwet*), oven drying the sample to remove the water, then weighing the dried soil on 105°C (*mdry*). It is expressed as g g^{-1}

$$\theta g = \frac{m\,water}{msoil} = \frac{mwet - mdry}{m\ dry}$$

Volumetric water content (θv) is the volume of liquid water per volume of soil. It is expressed as g cm^{-3}. Volume is the ratio of mass to density (ρ), which gives:

$$\theta v = \frac{volume\ water}{volume\ soil} = \frac{\frac{mwater}{\rho water}}{\frac{msoil}{\rho soil}} = \frac{\theta g \cdot \rho soil}{\rho water}$$

Two soil ring cores from each plot were collected, plastic caps were placed at each end and then they were transferred on the laboratory. Excess soil was trimmed from the core and a piece of gauze of known weight was placed at the end of the core and secure with a rubber band to keep the soil in place. Each core was weighed with the band and gauze and the cores were placed on a large aluminium tray, where they were sprayed with a few ml of 4% formaldehyde solution to kill any living organisms. The tray was filled with water to a depth of 10 mm, left for 4 h, filled to 25 mm, left for 4 h and filled so that the cores were completely immersed and left for 24 h. After that, the cores were weighed and immediately after placed on the tension table. When placing the core in position, firm pressure was applied to ensure a good contact with the silica sand (Ball and Hunter, 1988). When all the cores were set, the water head was lowered to the first standard height, -30 cm. The excess water flowed out the over the tension table at a steady rate. When the flow had decreased to the occasional drip then the apparatus was left for a period until the container needed to be emptied again. The cores were left for 7days until no more water came out of the tension table. They were then removed carefully for weighing after brushing off any silica sand (*mfre*). After weighing, the cores carefully replaced on the tension table after the sand surface had been sprayed with de-aired water giving them a small twist when in position in order to get the best possible hydraulic contact. The drainage beakers were lowered to the next matrix potential of -60 cm. That was repeated for -90 cm and -120 cm. One more measurement was taken at -300 cm. For this purpose, a low pressure plate was used. At the end, the samples were dried at 105 °C overnight and the weight was recorded and at the same time the empty weight of the metal rings was recorded (*mdry*).

4.2.8. Plant Biomass

All plants were harvested above ground every growing season and initially separated into cereal, legumes and weeds. Plant biomass was measured monthly using quadrats (50 cm x 50 cm) by harvesting all the plant material within it. The fresh weights were recorded and then the samples were separated further into straw, and grain (for cereals and peas). The fresh weight of these fractions was recorded and then dried at 60 °C overnight. The dry weight was recorded and the material was ground for storage for further analyses (mineral N).

4.2.9. Total N in plants

Total N and C are major nutrient pools and essential for determining nutrient balances. Total N and C in the plants were determined using dry Micro-Dumas combustion, which is based on a transformation to gas phase by extremely rapid and complete flash combustion of the sample material. Between 10 mg and 15 mg of finally dried in powder form ground sample material was weighed into a tin capsule. The sample was introduced into the combustion tube (which was maintained at about 1000 °C) via an auto-sampler. The sample and tin container melted in a violent reaction, (flash combustion) in the enriched atmosphere of oxygen. All the substances in the sample were completely oxidised.

The combustion products were carried in the carrier gas, helium, through an oxidation catalyst (chromium oxide), at 1000 °C. To ensure complete oxidation a layer of silver coated cobalt oxide was placed at the bottom of the combustion tube. The mixture of combustion products (carbon dioxide, nitrogen, oxides of nitrogen and water) passed through the reduction tube which contained copper kept at 650 °C. The excess oxygen was removed and the oxides of nitrogen are reduced to nitrogen which with the carbon dioxide and water pass through an absorbent filter (magnesium perchlorate), to remove water.

The nitrogen and carbon dioxide are taken by the carrier gas to the chromatographic column and then to a thermal conductivity detector which generates electrical signals proportional to the concentrations of nitrogen and carbon dioxide present. Determination of the percentage composition of the samples is made by comparison of the gas areas of standards with known elemental compositions to gas peak areas of the sample.

4.2.10. Statistics

Descriptive statistics (mean and standard error) were performed as a measure of the variability in N_2O fluxes measurements by crop type and seasons. Scatter plots were used to identify relationships between grain yields, water filled pore space, soil moisture, soil temperature, air temperature and N_2O fluxes. Multiple regression analyses were used to examine the functional relationship(s) between the dependent variables (total N, NO_3^-N in soil and water, NH_4^+-N, N_2O fluxes) and the potential predictor variables (crop, seasons, soil moisture and soil temperature). Site means for each treatment grouping were used for developing the models. Simple regression analyses were also carried out to describe specific relationships between response variables and explanatory variables. Simple and multiple regression analyses were performed using Genstat® Statistical software, 8^{th} edition. One-way analysis of variance (ANOVA) was used to test for differences in mean soil properties and gas flux among treatments and seasons. These were performed using Minitab 15. All charts presented were performed using Microsoft Excel 2007. The raw data for N_2O flux were not normally distributed and were log10 transformed for regression and ANOVA analyses.

5. Paper 1

Intercropping of legumes and cereals: effect on yield and N balances in a three year low input crop rotation

Valentini A. Pappa , Robert M. Rees, Robin L. Walker, John A. Baddeley , and

Christine A. Watson

Submitted to 'Field Crops Research'

Paper 1

Intercropping of legumes and cereals: effect on yield and N balances in a three year low input crop rotation

Valentini A. Pappa, Robert M. Rees, Robin L. Walker, John A. Baddeley and Christine A. Watson

Abstract

Intercropping systems, that include legumes, can provide symbiotically fixed nitrogen (N) and potentially increase yield through improved resource use efficiency. The aims of this study were to: a) establish whether the yield benefit of an intercropping phase in a rotation extended beyond its growth season; b) determine whether the effects depended on choice of legume and c) explore the relative contribution of legume derived N to companion crops in two growing seasons. An experiment was established near Edinburgh, in the UK, consisting of 12 hydrologically-isolated plots. Treatments were a spring barley (*Hordeum vulgare* cv. *Westminster)* monoculture and intercrops of barley / white clover (*Trifolium repens* cv. *Alice*) and barley / pea (*Pisum sativum* cv. *Zero 4* or cv. *Nitouche*) in 2006. Spring oats (*Avena sativa* cv. *Firth*) were planted on all plots in 2007. In the third season, all plots were sown with perennial ryegrass. No fertilisers, herbicides or pesticides were used at any stage of the experiment. Above ground biomass (barley, clover, peas, oats), grain yields (barley, peas and oats) and land equivalent ratio (LER) were measured at key stages during the growing seasons of 2006 and 2007. At harvest, the total above ground biomass of barley intercropped with clover (4.56 t ha^{-1}) and barley intercropped with pea cv. *Zero 4* (4.49 tha^{-1}) were significantly different from the barley monocrop (3.05 t ha^{-1}; $P< 0.05$). The grain yield of the barley (2006) intercropped with clover (3.36 t ha^{-1}) was significantly greater than that in the other treatments ($P< 0.01$). The accumulation of N in barley was low in 2006, but significantly higher in the oats grown the following year on the same plots. The intercrops affected the yield and N uptake of the spring oats in the following year, the effect was related to the different legume species and cultivars present in the previous years intercrop. Legume choice is central to optimising plant productivity in intercropping designs. Cultivars for intercropping purposes must be chosen with care, taking into account the effects upon the growth of the partner crop/s as well as to the following crop, including environmental factors.

Keywords: Intercropping, Grain yield, Rotation, Drainage plots

1. Introduction

Intercropping can be defined as the simultaneous cultivation of two or more crops on the same area of land. The crops can be sown together or at different times, but they are usually grown simultaneously for a considerable proportion of their growing periods. Intercropping has been shown to increase yield compared with monocrops in low input systems (Ofori *et al.*, 1987), has greater yield stability (Willey, 1979) and also reduces the chance of crop failure (Anil *et al.*, 1998). It also has the potential to improve yield stability (Hauggaard-Nielsen *et al.*, 2006) and plant resource utilisation of water, light and nutrients (Willey, 1990; Jensen, 1996a; Whitmore and Schroder, 2007; Xu *et al.*, 2008; Zhang *et al.*, 2008). Where legumes are included as an intercrop, nitrogen transfer from biological nitrogen fixation (BNF) has been reported (Jensen, 1996b; Hauggaard-Nielsen and Jensen, 2001; Corre-Hellou *et al.*, 2007). Intercropping of barley (*Hordeum vulgare L.*) with pea (*Pisum sativum L.*) improves the use of plant growth resources in comparison with the associated monocrop due to different resource use, as they are different species (Willey, 1979; Hauggaard-Nielsen and Jensen, 2001).

There is a particular interest in intercropping in low input and organic systems (Jones, 1993; Pridham and Martin, 2005). Recent rises in fertiliser costs, coupled with concern about the environmental impacts of excess N use and associated legislation are driving farmers to consider alternative nitrogen (N) sources and approaches to N management such as intercropping (Tilman *et al.* 2002; Erisman *et al.* 2008). Minimising external inputs (e.g. fertilisers and pesticides) and more efficient use N (e.g. from biological nitrogen fixation) (BNF) can increase the economic, environmental, ecological and social sustainability of agricultural systems.

In developing more sustainable cropping systems it is important to examine stability of crop yield and N dynamics over time (whole rotations or periods of several years as well as within growing seasons). To date, most studies of intercrops have based their conclusions only on measurements of final yield (Connolly *et al.*, 2001) and data from only one growing season (Hauggaard-Nielsen and Jensen, 2001; Andersen *et al.*, 2005). More recently, Hauggaard-Nielsen *et al.* (2009) found that there was 30-40% more efficient use of N resources by pea-barley intercropped compared with the respective monocrop across Europe by using the same variety. Some studies (Hauggaard-Nielsen *et al.*, 2003; Pappa *et al.*, 2008) have considered the dynamics of intercrops over a longer period and the implications of this in terms of a range of mitigation options of N.

The main objectives of the experiment reported here were to: a) evaluate the effect of different legumes (species and varieties) and barley on grain yield, dry matter production, N uptake of the intercrop treatments compared with the associated cereal monocrop and their effect to the next grain yields; b) to assess the utilisation of N use efficiency of intercrops and monocrops and c) to determine the accumulation of N in shoots of the crops used in a low input rotation.

2. Materials and Methods

2.1. Site description and experimental design

The experiment was sited at the Bush Estate (lat. 55° 51'N, long. 3° 12'W), near Edinburgh, Scotland, UK. This drained-plot experimental facility was established in 1990, and consists of 12 hydrologically-isolated plots (25m x 9m) that have been used previously for nitrate leaching studies (Vinten *et al.*, 1994). Prior to the current experiment, the plots had been fallow for the past three years (2003-2005). The soil is a sandy loam (Eutric Cambisol, Macmerry Series) developed from partially sorted glacial till, and the upper 0.5 to 1.5 m of the soil profile is freely drained. Further details of the experimental facilities are given in Vinten *et al.*(1992) The rainfall and air temperature for the three years of the current study are shown in Table 1. The 25 year annual average precipitation at Bush Estate is 676.2 mm.

Table 1: Monthly total rainfall (mm) and monthly average air temperature (°C) for Bush Estate, Edinburgh, UK.

	2006		2007		2008	
	Rainfall	Air temperature	Rainfall	Air temperature	Rainfall	Air temperature
January	55	3.6	143	4.9	181	3.8
February	46	3.4	39	4.2	57	3.9
March	84	2.6	249	5.0	126	3.8
April	37	6.1	22	8.6	63	5.3
May	111	8.8	107	8.5	35	9.7
June	25	13.2	125	11.3	90	11.6
July	53	16.2	106	12.6	109	13.9
August	80	13.7	236	12.8	136	13.8
September	122	13.4	54	11.4	60	11.8
October	74	9.9	44	9.1	79	7.5
November	108	6.2	88	6.5	76	4.9
December	132	4.6	76	2.9	121	3.6
Total	927		1288		1132.4	
Average	77	8.5	107	8.2	91.9	7.8

In 2006 when the current study commenced, the treatments were: barley (*Hordeum vulgare* cv. Westminster) as a monocrop; Pea (*Pisum sativum* cv. *Zero 4*)/ barley intercrop; Pea (*Pisum sativum* cv. *Nitouche*)/ barley intercrop; White clover (*Trifolium repens* cv. Alice) /barley intercrop. Westminster is a medium-tall variety of barley that is widely grown for malting and livestock feed due to its combination of longer than average straw and good disease resistance characteristics (HGCA, 2008). *Nitouche* is a popular large blue pea variety and has a consistent performance, good agronomic characters and suitability for premium markets. *Nitouche* produces a large, smooth, round pea and retains its colour well. *Nitouche* also has a high level of resistance to downy mildew and long straw with good standing ability making it relatively easy to harvest. *Zero 4* is a small blue combining pea with a unique combination of agronomic characteristics: short straw, excellent standing ability and very early maturity. When sown at its optimum seed rate of 110 seeds/ m^2, *Zero 4* has a similar yield potential to *Nitouche* (optimum seed rate of 70 seeds / m^2) (Nickerson, 2008). Alice is a tall, large leaved white clover developed for exceptional yields of palatable, high quality, high protein forage in pasture mixtures. Its vigorous spring and summer growth makes it a good choice for cutting or grazing management, as well as for N fixation throughout the seasons and good winter- hardiness and cover (Barenbrug, 2006).

Each treatment was replicated three times in a blocked design. In the intercrop treatments, the seed rates for the pea and barley followed a 50:50 replacement design. This means that the target intercrop density was 50% of the monoculture density of each crop. Based on monocropping seed rates of 200 kg ha^{-1} for the barley and 250 kg ha^{-1} for the peas which equated to approximately 350, 75 and 110 germinable seeds per m^{-2} for Westminster, *Nitouche* and *Zero 4* respectively, the intercrop components were half these values. The sowing date for these treatments was the 24th April 2006. In the second growing season (2007), all the plots were sown with spring oats (*Avena sativa* cv. Firth) on the 3rd April at a seed rate of 250 kg ha^{-1} (approximately 450-500 germinable seeds per m^{-2}). Firth is a very popular spring oat variety and exhibits a high kernel content and good resistance to mildew (HGCA, 2008). In the third growing season, perennial ryegrass (PRG) was sown in all plots in a 50:50 mixture of *Aberavon* and *Aberdart* at a total seed rate of 35 kg ha^{-1}. The plots were tilled using a mouldboard plough followed by cultivating (rotary hoe), seeding and rolling. No fertilisers, herbicides or pesticides were used. The cropping pattern is shown in Table 2.

Table 2: Cropping 2003-2008 on the drained plots

2003	*2004*	*2005*	**2006**	**2007**	**2008**
Fallow	*Fallow*	*Fallow*	**Intercropping spring barley/ legumes**	**Spring Oats**	**Perennial Ryegrass**

Treatments (2006)
1. Barley/ Clover
2. Barley/ Pea cv. Zero 4
3. Barley/ Pea cv. Nitouche
4. Barley

2.2 Harvest and analysis

Above ground plant material was collected just prior to crop harvest (11th September 2006, 11th September 2007 and 26th June 2008) by cutting the plants 5 cm above the soil surface from a 1 m^2 area (four 0.25 m^2 quadrats randomly placed in each plot). After the harvest, the residues were removed from the plots. During the winter, the clover plants continued to grow due to mild winter temperatures and incorporated in the soil by ploughing in the spring (3rd April 2007). Biomass samples were separated into barley, pea, clover and weeds, dried at 70 °C for 24 h and weighed. For grain yield, crop plants were hand swathed at the time of crop maturity from the above biomass sample and grain yield was calculated at 85% dry matter. Each part (stems, ears, pods) of each crop was weighed and subsamples taken for determination of the total N concentration (%) of the aboveground material. The oven-dried samples were ground using a Glen Creston hammer mill with a 1 mm mesh. A sub sample of this was then ball milled to a flour-like consistency using a Retch ball mill. These samples were analysed for total N by combustion using a PDZ Europa Mass Spectrometer. Nitrogen accumulation and grain N yield were calculated by multiplying dry matter and grain yield, respectively, by their corresponding N concentration. The same procedure was used for the spring oat crop (2007 season) which was separated into straw, grain and ryegrass (2008).

2.3. Calculations

2.3.1. Land Equivalent Ratio

The nature of the interactions between the yield components and productivity of the intercropping system depends on the morphology, physiology, density and spatial arrangement of the components in relation to the climatic, edaphic and biotic environment in which they are grown, together with the ensuing management regime (Anil *et al.*, 1998). Such complex interactions generally mean that intercrops have both complementary and antagonistic interactions. However, the measurement of yield is usually taken as a primary consideration in the assessment of the potential of intercropping practices and has led to several measurement criteria being put forward (Szumigalski and Van Acker, 2006). An important tool for the study and evaluation of intercropping systems is the Land Equivalent Ratio (LER) (Dhima *et al.*, 2007). LER provides a measure of the yield advantage obtained by growing two or more crops or varieties as an intercrop compared to growing the same crops or varieties as a collection of separate monocrops. The LER for the barley/ pea intercrop is calculated using the following formula:

$$L_B = Y_{Bint} / Y_{Bmon}$$

$$L_L = Y_{Lint} / Y_{Lmon}$$

$$LER = L_B + L_L,$$

where L_B and L_L are the land for barley and legumes, respectively, Y_{Bint} and Y_{Bmon} are the yield of barley in intercrops and monocrop, respectively, and Y_{Lint} and Y_{Lmon} are the yields of the legume in the intercrop and monocrop, respectively. An LER value of 1.0 indicates no difference in yield between the intercrop and monocrop. Any value greater than 1.0 shows a yield advantage for intercropping. An LER of 1.3 for example, indicates that the area planted to monocultures would need to be 30% greater than the area planted to intercrop to produce the same combined yield. The calculation of LER is the most common method adopted in intercropping studies, in particular in tropical regions where intercropping is commonly practised.

For barley plants (2006), the cereal growth stages GS 23 (main shoot and 3 tillers), GS 65 (flowering half-way), GS 77 (late milk), GS 92 (grain hard (not dented by nail) for the growing season 2006 (barley) were 30, 70, 100 and 140 days from sowing, respectively. For oat plants (2007), the growth stages GS 16 (six leaves unfolded), GS 23, GS 65, GS 77 and GS 92 were 40, 65, 100, 135, 161 days from sowing respectively (HGCA, 2005/06).

2.4. Statistics

All measured variables were normally distributed tested by Anderson-Darling test (significance .05) and statistical analyses by ANOVA were performed using either Minitab 15 or GenStat 8 software. In all cases, significant differences were calculated at or below the 5% level.

3. Results

3.1. Growth stages and height

3.1.1. Barley plants

During the 2006 season, barley height was influenced by companion variety choice and agronomic practice. The treatments were significantly different with barley in the barley/ pea cv. *Nitouche* treatment being the tallest followed by barley in the barley/ clover treatment. The height of the barley intercropped with the two pea varieties was also significantly different with the barley grown with pea cv, *Nitouche* resulting in taller plants by 0.29 m ($P<0.05$) in harvest stage (GS 92) in comparison with the rest of the treatments (Fig. 1A).

3.1.2. Oat plants

The intercropped treatments were followed by spring oats the next year (2007). The oats grown on the previous year's barley/ clover treatment proved to be the strongest and tallest plants in comparison with the rest of the treatments. The two treatments that had the pea intercrops were significantly different in height with barley/ pea cv. *Nitouche* by 0.14 m ($P<0.05$), but they had almost the same biomass (see above ground biomass results) (Fig. 1B).

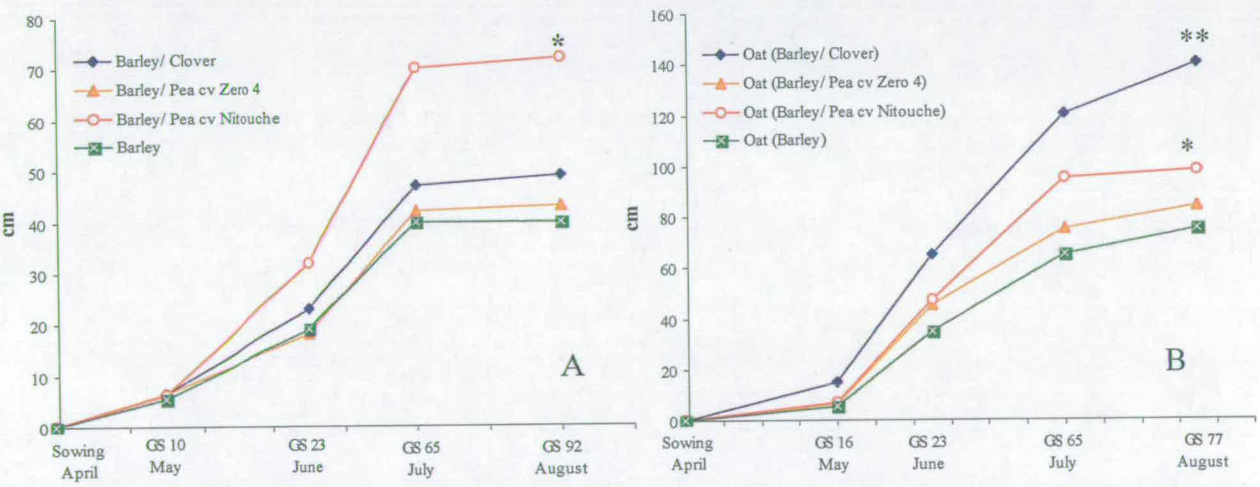


Figure 1: Height of barley in 2006 (A) and oats in 2007 (B) plotted against growth stages. All values are means (n=3). Asterisks indicate whether treatments are significantly different at * P<0.05; **P<0.01

3.2. Above ground biomass

3.2.1. Spring barley and oats

The barley grew rapidly in 2006 between GS 23 and 65 after the canopy had closed generating almost 70% of its total final dry matter. At the end of grain filling (GS 85), when the maximum crop dry weight occurred, the stems and leaves started losing weight due to falling leaf tissue and senescence varying by 7 days between monocrop and intercropped treatments with the monocrop to senescence first. The barley had the maximum biomass in the barley/ clover treatment. Due to observations, the senescence started earlier in the barley monocrop and its dry biomass reduced very quickly (Fig. 2A).

The growth of the 2007 oat plants were significantly affected by the previous crop treatment. Oats grown following barley/ clover grew more rapidly than the other treatments and had the greatest biomass (17.31 t ha^{-1}) at GS 92 (Fig. 2B).

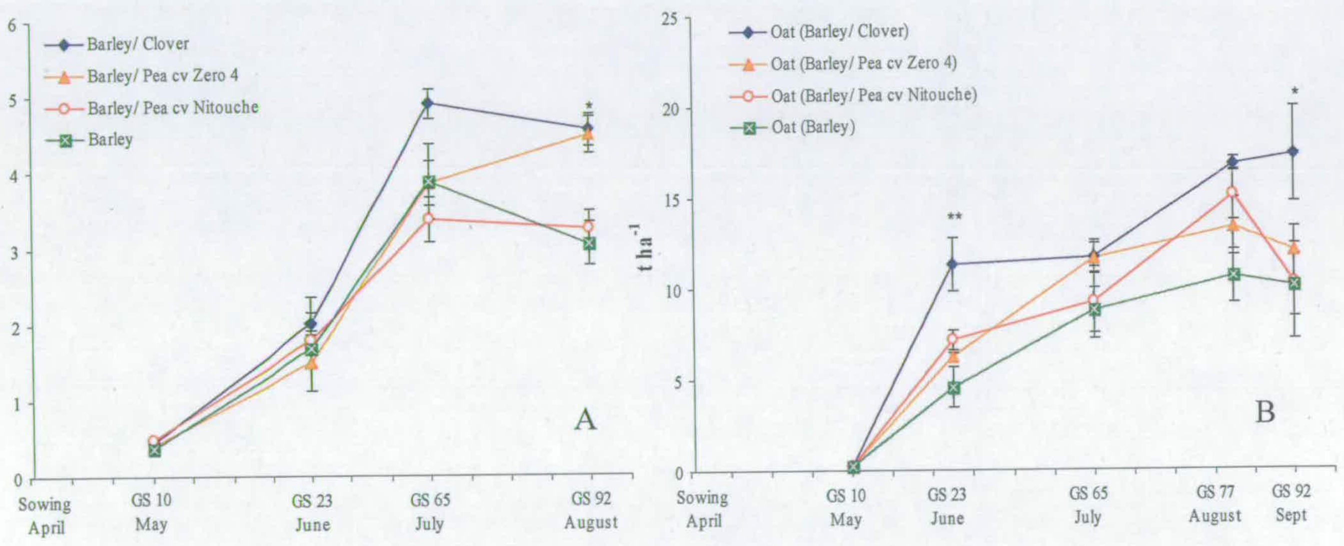


Figure 2: Above ground biomass of barley in 2006 (A) and oats in 2007 (B) plotted against growth stages. All values are means (n=3) ± SE (bars) within the treatment. Asterisks indicate whether treatments are significantly different at * P<0.05; **P<0.01

3.2.2. Legume plants

In 2006, the above ground legume biomass was significantly different at the beginning of the growing season (June 2006; Barley growth stage 23) with pea cv. *Nitouche* greater by 0.7 t ha^{-1} ($P<0.05$); and clover having the lowest biomass (Fig. 3). The biomass of the two pea varieties was not significantly different during the growing season even though the pea cv. *Nitouche* was double the height of pea cv. *Zero 4* (Fig. 1A).

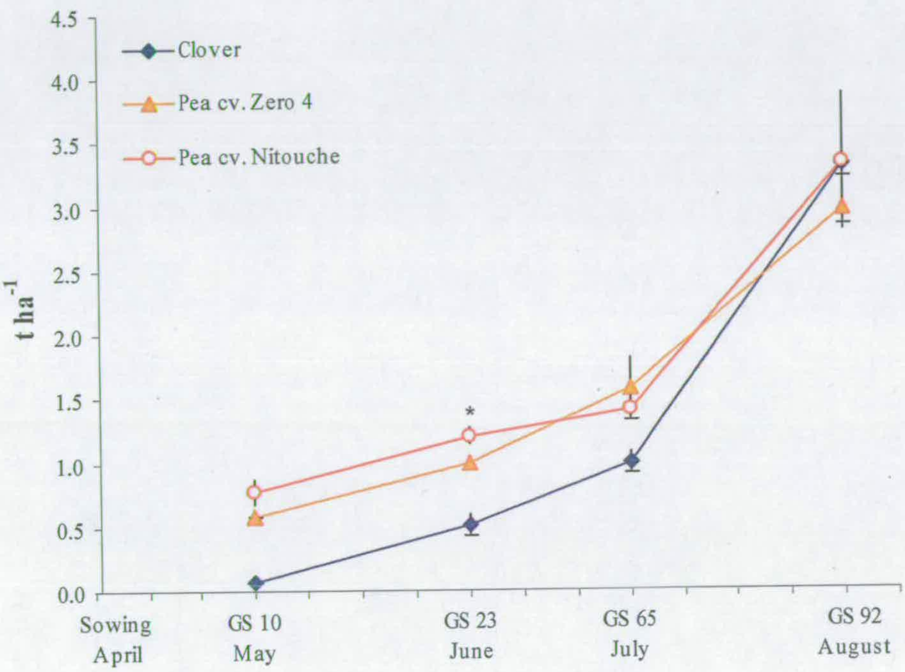


Figure 3: Above ground biomass of legume plants plotted against cereal (barley) growth stages during the growing season (2006). All values are means (n=3) ± SE (bars) within the treatment. Asterisks indicate whether treatments are significantly different at * $P<0.05$.

3.2.3. Perennial ryegrass

In 2008, the above ground biomass was not significantly different within the treatments. All the treatments had close to 1.3 t ha^{-1} dry matter after the summer harvest (Fig.4).

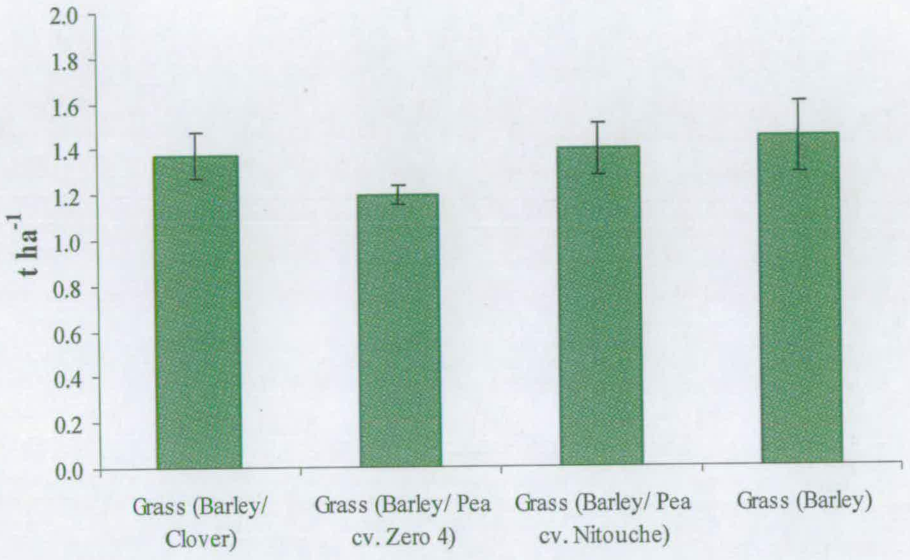


Figure 4: Above ground biomass of perennial ryegrass in 2008. All values are means (n=3) ± SE (bars) within the plots. The initial treatments (2006) are presented in parenthesis.

3.3. Grain yields

3.3.1. Spring barley and peas during the first year of the cropping sequence (2006)

The pea cultivars differed in time of flowering/ maturity and stem length with *Zero 4* being the earliest to mature and shortest (close to 0.40 m tall). The composition of established intercrops was close to the target of 50:50 with 162 barley (intercropped); 47 Pea cv. *Nitouche* (intercropped); 56 Pea cv. *Zero 4* (intercropped) and 261 barley (monocrop) plants m^{-2}. The grain yield of the barley intercropped with clover was significantly different ($P<0.01$) possessing 182% of the average barley monocrop yield. No yield differences were observed between the pea cultivars and the intercropped barley grain yields (Fig. 5).

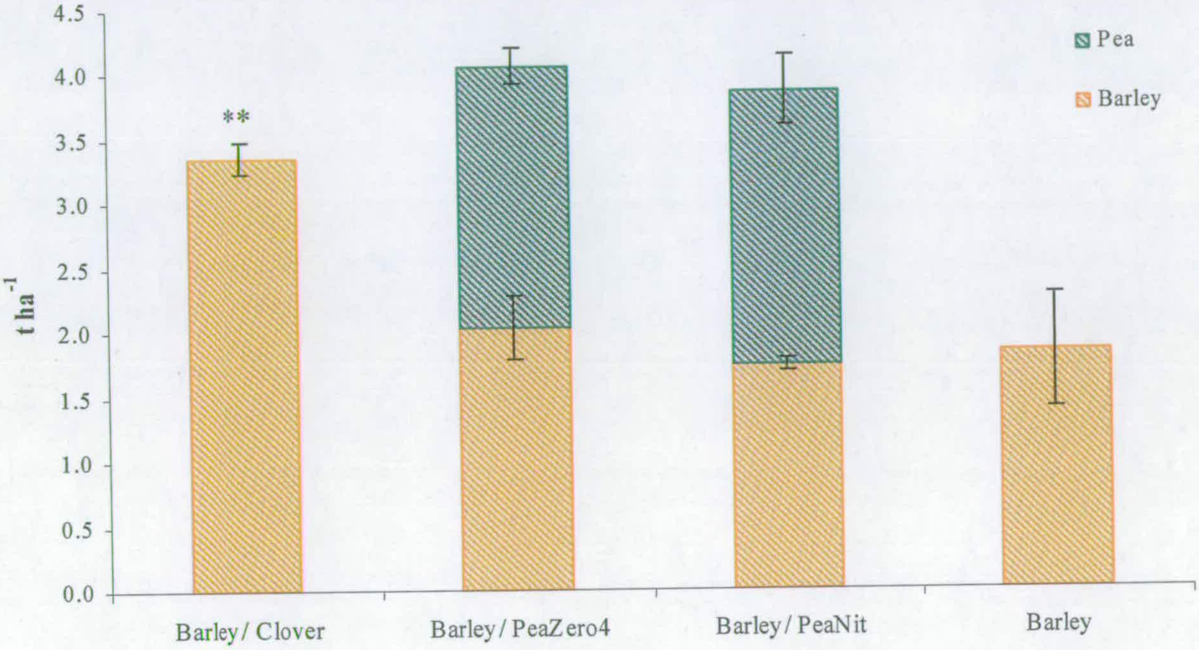


Figure 5: Grain yields of barley and pea in 2006. All values are means (n=3) ± SE (bars) within the treatment. Asterisks indicate whether treatments are significantly different at (** $P<0.01$) from the other treatments.

3.3.2. Spring oats in the second year of the cropping sequence (2007)

Spring oat grain yield was influenced by the previous year's treatments with a significant difference ($P<0.05$) between the oats grown after the clover/ barley (6.68 t ha^{-1}) treatment and the other treatments (Fig. 6). During the winter prior to sowing the oats, the clover continued to grow well after harvest due to mild winter temperatures and was incorporated in the soil by ploughing in the spring (3rd April, 2007).

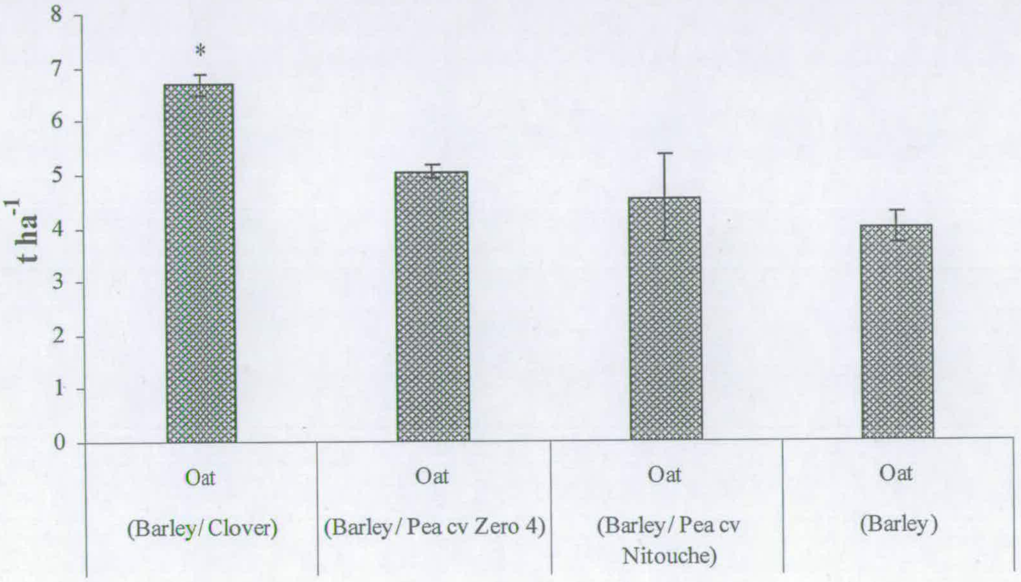


Figure 6: Spring oat grain yields in 2007. The initial treatments (2006) are presented in parenthesis. All values are means (n=3) ± SE (bars) within the treatment. Asterisks indicate whether treatments are significantly different at * $P<0.05$ from the other treatments.

3.4. Production efficiency of intercrops

Land Equivalent Ratio (LER) was estimated in order to determine the efficiency of the intercrops in relation to the component monocrops and the potential advantages and disadvantages of them. The average LER for the clover/ barley treatment was 1.27 compared to 1.48 for barley/ pea cv. *Zero 4* and barley/ pea cv. *Nitouche*. The overall advantages were 27% for barley/ clover and 48% for barley/ pea (Fig. 7).

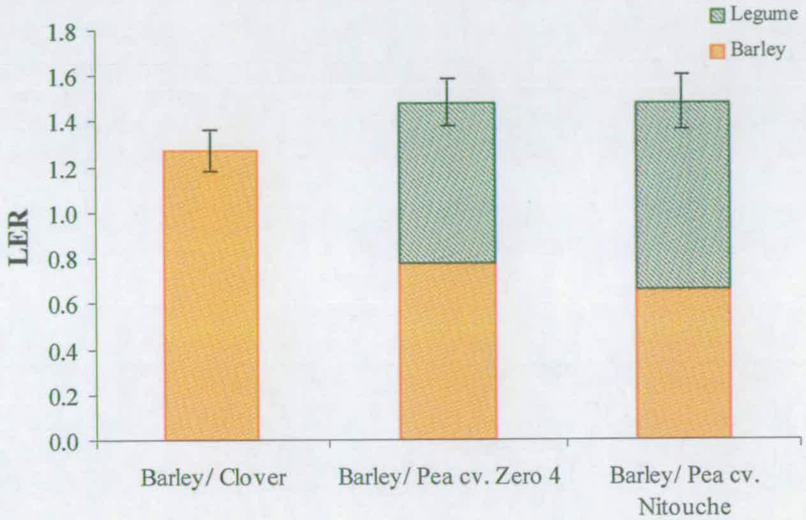


Figure 7: Land Equivalent Ratio (LER) for the intercrops (2006). All values are means (n=3) ± SE (bars).

3.5. N accumulation in crops

3.5.1. Spring barley

Total above ground N accumulation in barley was not significantly different between treatments in 2006 until harvest ($P<0.05$), when barley/ pea cv. *Zero 4* reached 52 kg N ha^{-1} in the above ground biomass (Fig. 8A). For the last harvest analyses of total N were carried out on separate samples of straw and grain. Total N in barley straw did not differ between treatments. There were significant differences ($P<0.05$) in total N in the barley grain yield with barley/ pea cv. *Zero 4* having the highest value (45.6 kg N ha^{-1}) (Fig. 9).

3.5.2. Legumes (Clover and Peas)

The intercropped pea cv. *Nitouche* plants had the highest levels of total N ($P<0.01$) of 97 kg N ha^{-1} at harvest with 78.5 and 18.5 kg N ha^{-1} in grain and straw respectively (Fig. 9). Pea cv. *Zero 4* plants were not significantly different in total N in comparison with the pea cv.

Nitouche plants having 63 kg N ha^{-1} in grain and 20 kg N ha^{-1} in straw followed by the clover plants with 79 kg N ha^{-1} (Fig. 10).

3.5.3. Spring oats

Total above ground N accumulation was not significantly different between treatments at the beginning of the second growing season (2007). However, eight weeks after sowing, the oat plants growing on the previous barley/ clover plots had significantly more total N in the above ground parts on average about 143 kg N ha^{-1} than the other treatments ($P<0.001$). This difference continued until the end of the growing season (Fig. 8B). The total N in the oats growing in the previous two barley/ pea plots was significantly different at GS 23 ($P<0.05$) having 71 kg N ha^{-1} and 53 kg N ha^{-1} on previous barley/ pea cv. *Nitouche* and barley/ pea cv. *Zero 4*, respectively. At the end of the growing season, the total N in straw for the spring oats grown in the previous clover/ barley treatment was significantly different from the rest of the treatments reaching almost 38 kg N ha^{-1}. The total N contained in the grain did not differ between the plots where pea crops and monoculture barley were grown in the previous year (Table 3).

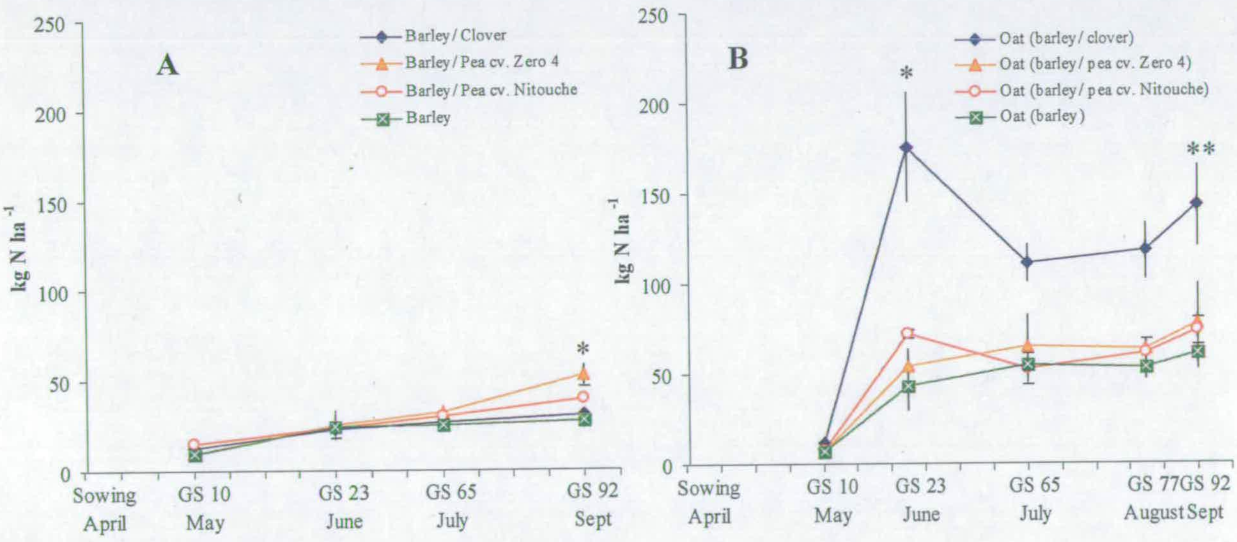


Figure 8: Accumulation of N in above ground biomass at different growth stages for spring barley (2006) (A) and spring oats (2007) (B). All values are means (n=3) ± SE (bars). Asterisks indicate whether treatments are significantly different at * $P<0.05$ and * * $P<0.01$ from the other treatments.

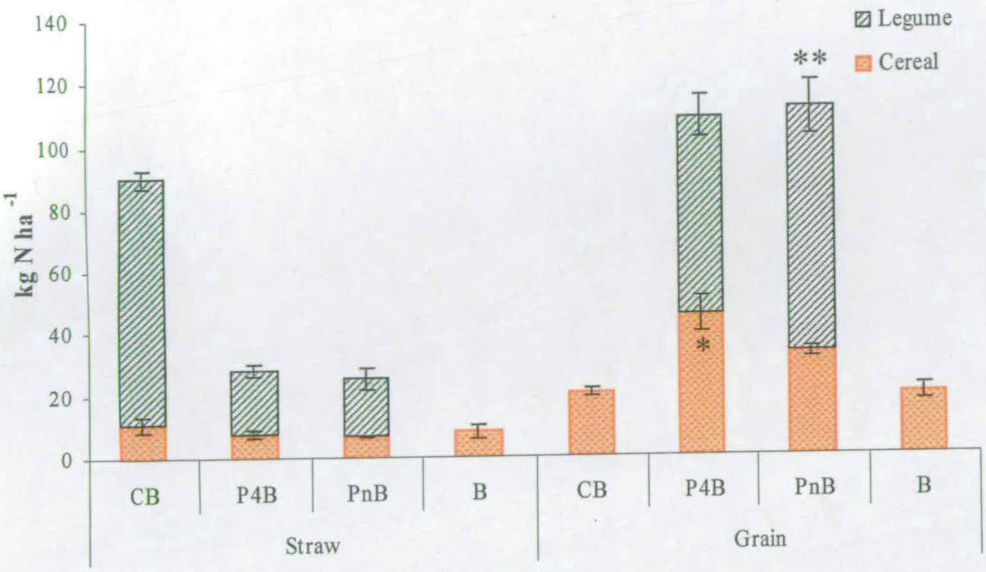


Figure 9: Accumulation of N in above ground parts (straw and grain) of barley and legumes (clover and peas) expressed as kg N ha^{-1} at the final harvest in 2006. All values are means (n=3) ± SE (bars). Treatments represented by CB: Clover/ Barley; P04B: Pea cv. Zero 4/ Barley; PNitB: Pea cv. Nitouche/ Barley and B: Barley. Asterisks indicate whether treatments are significantly different at * P<0.05; **P<0.01 from the other treatments.

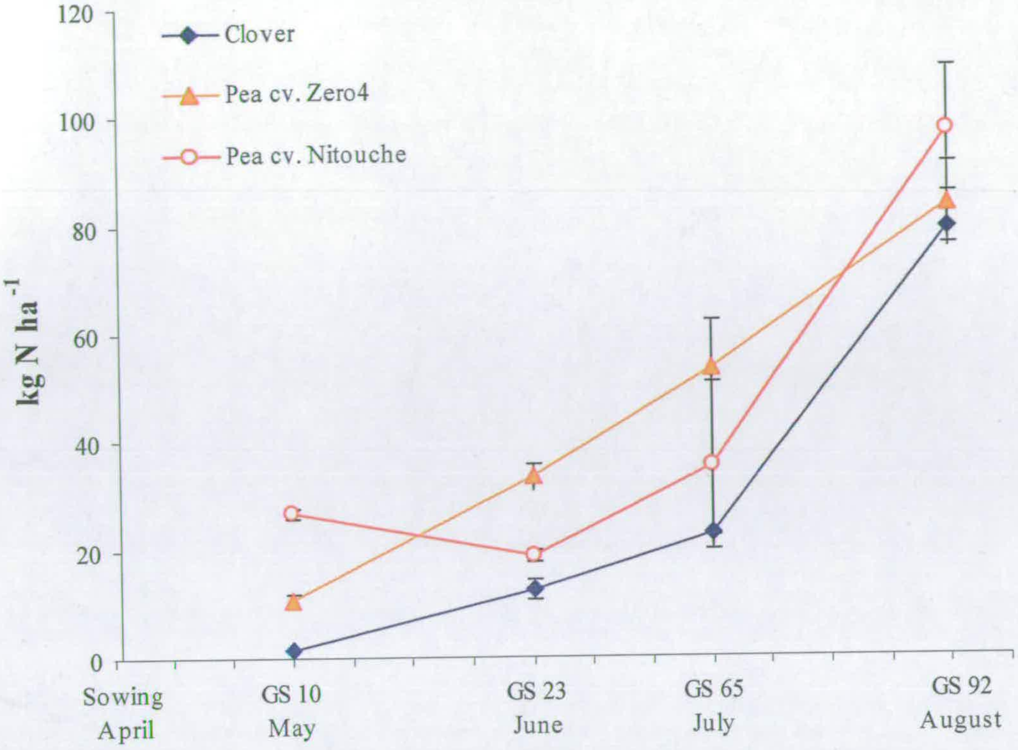


Figure 10: Accumulation of N in above ground biomass of the legumes according to cereal (barley) growth stages in 2006. All values are means (n=3) ± SE (bars).

Table 3: Accumulation of N in above ground parts (straw and grain) of spring oats (2007) expressed as kg N ha^{-1}. All the values are the mean (n=3) ± SE. The values with different letters (a, b) are significant different (P<0.05)

Treatments	Straw (kg N ha^{-1})			Grain (kg N ha^{-1})			Total (kg N ha^{-1})		
Oat (barley/ clover)	37.9	± 4.2	b	105	± 17.1	b	132	± 22.4	b
Oat (barley/ pea cv. Zero 4)	13.1	± 0.7	a	64.6	± 7.12	a	70.2	± 7.30	a
Oat (barley/ pea cv. Nitouche)	11.3	± 2.9	a	62.0	± 15.0	a	59.2	± 21.9	a
Oat (barley)	10.6	± 0.8	a	50.5	± 13.2	b	57.0	± 8.5	a

4. Discussion

The interactions between intercropped cereals and legumes are affected both by differences in the morphology and physiology of the species and through environmental controls. Barley and legume plants respond differently to light and cereal plants can cause shading which leads to growth restrictions in legume plants (Fujita *et al.*, 1992). Barley absorbs nutrients, especially N, rapidly between growth stages 31 (first node detectable) and 39 (flat leaf blade all visible), as the canopy size increases through leaf emergence and tiller survival. It then slows until growth stage 59 (ear completely emerged); after that the N taken up is redistributed within the plant, (HGCA, 2005/06). Barley plants intercropped with clover had the highest biomass and yields of barley (Fig. 2A and 5), and the barley grown in the two barley/ pea treatments was equal in biomass yet both had lower yields than the barley grown in the barley/ clover treatment at GS 92 (Fig 2A.). This was probably a result of the taller peas increasing the interspecific competition for light during vegetative growth compared with the lower growing clover. In addition competition for soil water during grain filling of the barley and peas (Thorsted *et al.*, 2006), as well as possible higher levels of available N in the soil and/ or the effect of shading of the weed understorey during the growing period from the clover (Grashoff and d'Antuono, 1997) may have influenced this outcome.

The nutrient dynamics of both cereals and legumes would be expected to be influenced when grown as intercrops. The legume has the potential to provide nitrogen (N) to the non-legume directly through mycorrhizal links, root exudates, or decay of roots and nodules (Vandermeer, 1999). Another possible mechanism is that legumes can 'bank' large quantities of soil N, which might otherwise have leached out of the system and release it through soil organic matter turnover to the non-legume companion crop later during the growing season, or to the following crops (Vinten *et al.*, 1992). In peas, there is evidence to indicate that the process of BNF begins approximately a month after sowing and lasts for up to two months (Balandreau and Dommergues, 1973). If this were the case in our study then it would suggest that for an early variety such as pea cv. *Zero 4*, BNF would have continued during seed development whereas for a later variety (e.g. *Nitouche*), it would have stopped after flowering (Cousin, 1997). Anil *et al.* (1998) found that the amount of N_2 fixed by legumes generally declines with increasing soil N availability, and if the legumes are continuously shaded their ability to fix N_2 is further impaired (Willey, 1979). The two pea cultivars had different growth rates and biomass even though at the harvest stage they had similar grain yields (Fig. 2A and 5). The competition between the barley and peas reduced the amount of above ground biomass in the barley intercropped with pea cv. *Nitouche*, with the barley being more shaded than when grown with the shorter pea cv. *Zero 4*. The impedance of photosythetically active radiation

(PAR) to the legumes, as a result of competition for this resource by the barley, reduced the photosynthesis rate very probably resulting in decreased BNF. Pea cv. *Zero 4* was the shorter of the two pea cultivars and it is possible that its growth and physiological activity was affected by shading, more so than the pea cv. *Nitouche*. Less shading of the legume component in an intercropped system may increase both the photosynthesis rate of the legume as well as the rate of BNF (Fujita *et al.*, 1992).

The LER values indicate good resource use efficiency. Intercropping can also provide improvements in soil quality and more stable yields (Yildirim and Guvenc, 2005). The LER values were always greater than one for the intercrop treatments in this study which can be considered to represent a high level of biological efficiency (Vandermeer, 1999; Hauggaard-Nielsen and Jensen, 2001). The LER values were also used to compare cultivar performance in the intercrops, relative to the barley/ peas and barley monocrop, with gains of up to 22% (Hauggaard-Nielsen and Jensen, 2001). Results presented in our study indicate comparable values up to 27% and 42% for the barley/ pea and barley/ clover intercrop, respectively, compared to the barley alone, representing a significant yield benefit for these treatments (Fig. 6). Some previous studies have also shown LERs of greater than one from intercropping experiments. For instance, Mazaheri (2006) recorded LERs of 1.19 when working with maize and kale. Research on wheat and beans by Bulson (1997) estimated LERs of significantly greater than 1 in situations where crop densities were sufficiently high, whereas Newman (1986) studying vegetables and fruit found LERs more than 2 under some circumstances. The increased LERs that are observed in intercropping experiments are likely to be a consequence of a number of interacting factors. However, given that the companion species predictably occupy different ecological niches, it is likely that there will be some increased exploitation of resources (light, water and nutrients).

There were significant differences in the accumulation of N in the barley plants between treatments during the first growing season (2006) indicating a possible effect of available N in the soil and N inputs by fixation (Bandyopadhyay and De, 1986). Many studies have been conducted using barley to elucidate the factors controlling N uptake. It has been shown that different yield response in barley is linked to the crop's varying N uptake and its N use efficiency (Perby and and Jensén, 1983; Tillman *et al.*, 1991; Delogu *et al.*, 1998). During plant growth, from germination up to harvest stage, N has a key role in dry matter formation and accumulation. Barley plants grown in intercrops have an opportunity to increase N use efficiency and therefore yield through improved exploitation of soil N resources and N transfer from companion legume species (Delogu *et al.*, 1998).

In most cases, the crop mixture contained more N than the component monocrops, indicating improved N use by the intercrops. Other studies confirm the N uptake and efficiency benefits of growing a legume with a non-legume (Martin and Snaydon, 1982; Szumigalski and Van Acker, 2008). A number of mechanisms exist that enable utilisation of these growth resources more efficiently than the associated monocrop. For example, different spatial arrangements can influence nutrient transfer directly, but also have an effect on weed and disease pressure as well as competition for light and water (Anil *et al.*, 1998; Thorsted *et al.*, 2006).

The most important result emanating from this study is arguably the quantification of the substantial effect the previous legume treatments had on the following oat crop. Oats grown in the barley/ clover plots had the highest biomass, grain yield and accumulation of N in straw and grain in comparison with the other treatments (Fig. 2B, 6, 8B and Table 3). It has been reported in other studies that where clover continues to grow after the harvest there is the potential for reduced N loss from the soil and possible N transfer through legume residue decomposition and turnover (Baggs *et al.*, 2002; Miller *et al.*, 2008). It has also been suggested that there are additional benefits on soil structure during the growing season and into subsequent seasons (Mytton *et al.*, 1993; Papadopoulos *et al.*, 2006).

The management practices employed here may be appropriate for the manipulation of nitrate leaching and N_2O losses from agricultural ecosystems (Thomsen, 2005), as the plant N uptake was different for each treatment. The rate and morphology of barley root development in combination with the available N in the soil might be responsible for the different N uptakes (Eghball and Maranville, 1993; Mengel *et al.*, 2006; Herrera *et al.*, 2007; Paper 4). Root biomass can control the amount of N uptake by plants, as increased root biomass will enable plants to exploit a larger volume of soil (Rees *et al.*, 2005).

Agricultural farming systems have a higher potential to benefit from the use of legumes as part of a rotation in order to improve soil fertility and structure. Subsequent crops can then benefit by using the N stored in the soil, which is released by mineralisation (Watson *et al.*, 2002). This experiment has highlighted the higher potential grain yields from the use of intercrops with additional benefits of improved yields in subsequent crops. This work demonstrates the need for long-term experiments to evaluate the advantages, or disadvantages of intercropping systems. It is likely that in addition to the environmental and agronomic benefits of intercropping systems reported in this paper that there will be positive economic effects (Rao and Willey, 1980; Hauggaard-Nielsen and Jensen, 2001).

5. Conclusions

Agricultural farming can benefit from the use of legume-cereal intercropping. The choice of legume cultivar or species is a key factor influencing the amount of N available to the system in the year of use and/ or the following year, with this impacting significantly on the final grain yield in these years. The two barley/ pea intercrops had similar above ground biomass and grain yield for both component species (barley and peas). However, the accumulation of N in the above ground biomass of barley differed between these two treatments with significantly more N accumulated in barley/ pea cv. *Nitouche* than the barley/ pea cv. *Zero 4*. Undersowing cereals, even at low seed rates, with clover can contribute significantly to accumulation of N in the cereal plants. If the residues remaining after the harvest of a barley/ clover crop are incorporated into the soil by ploughing in the spring, it can provide available N for the following crop.

The magnitude of these effects is highly sensitive to management. For example, the choice of legume is important in optimising plant productivity in intercropping designs. However, more extensive study on the effects that different species/ varietal combinations and ratios have on the productivity of the system as well as their environmental impact is required in order to optimise such systems. Important issues for further investigations will include the effect of intercrops on greenhouse gas balances, the influence root development and compatibility of component species/ varieties, and the impact of soil and climate for agriculture. Gaining a greater understanding of the interactions taking place within a cereal/ legume intercrop its role within a rotation has the potential to be a very useful management tool in the development of more sustainable cropping and agricultural systems.

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References

Andersen, M., Hauggaard-Nielsen, H., Ambus, P., Jensen, E., 2005. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant and Soil 266, 273-287.

Anil, L., Park, R.H., Miller, F.A., 1998. Temperate intercropping of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass & Forage Science 53, 301-317.

Baggs, E.M., Rees, R.M., Castle, K., Scott, A., Smith, K.A., Vinten, A.J.A., 2002. Nitrous oxide release from soils receiving N-rich crop residues and paper mill sludge in eastern Scotland. Agriculture, Ecosystems & Environment 90, 109-123.

Balandreau, J., Dommergues, Y., 1973. Assaying nitrogenase (CH,) activity in the field.In T. Rosswall (ed.), Modem methods in the study of microbial ecology. Bull. Ecol. Res. Commun. (Stockholm) 17:247-254.

Bandyopadhyay, S.K., De, R., 1986. Nitrogen relationships and residual effects of intercropping sorghum with legumes. The Journal of Agricultural Science 107, 629-632.

Barenbrug, 2006. www.barenbrug.com/site/modules/custom/products/getfile.php?siteid=1001&filename=Alice.pdf accessed January, 2006.

Bulson, H.A.J., Snaydon, R.W., and, S., C.E. , 1997. Effects of plant density on intercropped wheat and field beans in an organic farming system. Journal of Agricultural Science 128 59-71.

Connolly, J., Wayne, P., Bazzaz, F.A., 2001. Interspecific Competition in Plants: How Well Do Current Methods Answer Fundamental Questions? The American Naturalist 157, 107-125.

Corre-Hellou, G., Brisson, N., Launay, M., Fustec, J., Crozat, Y., 2007. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. Field Crops Research 103, 76-85.

Cousin, R., 1997. Peas (Pisum sativum L.). Field Crops Research 53, 111-130.

Delogu, G., Cattivelli, L., Pecchioni, N., De Falcis, D., Maggiore, T., Stanca, A.M., 1998. Uptake and agronomic efficiency of nitrogen in winter barley and winter wheat. European Journal of Agronomy 9, 11-20.

Dhima, K.V., Lithourgidis, A.S., Vasilakoglou, I.B., Dordas, C.A., 2007. Competition indices of common vetch and cereal intercrops in two seeding ratio. Field Crops Research 100, 249-256.

Eghball, B., Maranville, J.W., 1993. Root Development and Nitrogen Influx of Corn Genotypes Grown under Combined Drought and Nitrogen Stresses. Agronomy Journal 85, 147-152.

Fujita, K., Ofosu-Budu, K.G., Ogata, S., 1992. Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant and Soil 141, 155-175.

Grashoff, C., d'Antuono, L.F., 1997. Effect of shading and nitrogen application on yield, grain size distribution and concentrations of nitrogen and water soluble carbohydrates in malting spring barley (Hordeum vulgare L.). European Journal of Agronomy 6, 275-293.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2003. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutrient Cycling in Agroecosystems 65, 289-300.

Hauggaard-Nielsen, H., Andersen, M.K., Jørnsgaard, B., Jensen, E.S., 2006. Density and relative frequency effects on competitive interactions and resource use in pea-barley intercrops. Field Crops Research 95, 256-267.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009. Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crops Research 113, 64-71.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. Field Crops Research 72, 185-196.

Herrera, J.M., Stamp, P., Liedgens, M., 2007. Dynamics of Root Development of Spring Wheat Genotypes Varying in Nitrogen Use Efficiency. Wheat Production in Stressed Environments, pp. 197-201.

HGCA, 2005/06. The barley growth guide.

HGCA, 2008. http://www.hgca.com/content.output/512/512/Varieties/Spring%20oats/Firth.mspx.

Jensen, E.S., 1996a. Barley uptake of N deposited in the rhizosphere of associated field pea. Soil Biology and Biochemistry 28, 159-168.

Jensen, E.S., 1996b. Rhizodeposition of N by pea and barley and its effect on soil N dynamics. Soil Biology and Biochemistry 28, 65-71.

Jones, L., and Clements, R.O., 1993. Development of a low input system for growing wheat (Triticum vulgare) in a permanent understory of white clover (Trifolium repens). Annals of applied Biology 123, 109-119.

Martin, M.P.L.D., Snaydon, R.W., 1982. Root and Shoot Interactions Between Barley and Field Beans When Intercropped. The Journal of Applied Ecology 19, 263-272.

Mazaheri, D., Madani, A., and, O., M. , 2006. Assessing the land equivalent ratio (LER) of two corn [Zea mays L.] varieties intercropping at various nitrogen levels in Karaj, Iran. Journal of Central European Agriculture. 7(2): 359-364.

Mengel, K., Hütsch, B., Kane, Y., 2006. Nitrogen fertilizer application rates on cereal crops according to available mineral and organic soil nitrogen. European Journal of Agronomy 24, 343-348.

Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., Trevors, J.T., 2008. Crop residue influence on denitrification, N2O emissions and denitrifier community abundance in soil. Soil Biology and Biochemistry 40, 2553-2562.

Mytton, L.R., Cresswell, A., and Colbourn, P., 1993. Improvement in soil structure associated with white clover. Grass & Forage Science 48, 84-90.

Newman, S.M., 1986. Pear and vegetable interculture systems, land Equivalent Ratios, light use efficiency and productivity. Experimental Agriculture 22, 382-392.

Nickerson, 2008. http://www.nickersonseeds.co.uk/.

Ofori, F., Stern, W.R., Brady, N.C., 1987. Cereal-Legume Intercropping Systems. Advances in Agronomy. Academic Press, pp. 41-90.

Papadopoulos, A., Mooney, S.J., Bird, N.R.A., 2006. Quantification of the effects of contrasting crops in the development of soil structure: an organic conversion. Soil Use and Management 22, 172-179.

Pappa, V.A., Rees, R.M., Baddeley, J.A., Walker, R.L., 2008. Intercrops and N use efficiency in low input agricultural systems. Proceedings QLIF, Soil nitrogen: research and extension. Driebergen, TheNetherlands.

Perby, H., and Jensén, P., 1983. Varietal differences in uptake and utilization of nitrogen and other macro-elements in seedlings of barley, Hordeum vulgare. Physiologia Plantarum 58, 223-230.

Pridham, J.C., Martin, E., 2005. Effect of Intercropping and Cultivar Mixtures on Organic Wheat Production. Presented at the ASA-CSSA-SSSA International Annual Meetings. UT, Salt Lake City.

Rao, M.R., and Willey, R.W., 1980. Evaluation of yield stability in intercropping: studies on sorghum/pigeonpea. Exp. Agric. 16 pp. 105-116.

Rees, R.M., Bingham, I.J., Baddeley, J.A., Watson, C.A., 2005. The role of plants and land management in sequestering soil carbon in temperate arable and grassland ecosystems. Geoderma 128, 130-154.

Szumigalski, A.R., Van Acker, R.C., 2006. Nitrogen Yield and Land Use Efficiency in Annual Sole Crops and Intercrops. Agronomy Journal 98, 1030-1040.

Szumigalski, A.R., Van Acker, R.C., 2008. Land Equivalent Ratios, Light Interception, and Water Use in Annual Intercrops in the Presence or Absence of In-Crop Herbicides. Agronomy Journal 100, 1145-1154.

Thomsen, I.K., 2005. Nitrate leaching under spring barley is influenced by the presence of a ryegrass catch crop: Results from a lysimeter experiment. Agriculture, Ecosystems & Environment 111, 21-29.

Thorsted, M.D., Olesen, J.E., Weiner, J., 2006. Width of clover strips and wheat rows influence grain yield in winter wheat/white clover intercropping. Field Crops Research 95, 280-290.

Tillman, B.A., Pan, W.L., Ullrich, S.E., 1991. Nitrogen Use by Northern-Adapted Barley Genotypes Under No-Till. Agronomy Journal 83, 194-201.

Vandermeer, J.H., 1999. The ecology of Intercropping. Cambridge Univ. Press, New York.

Vinten, A.J.A., Vivian, B.J., and Howard, R.S., 1992. The effect of nitrogen fertilizer on the nitrogen cycle of two upland arable soils of contrasting textures. Proceedings of the Fertilizer Society.

Watson, C.A., Atkinson, D., Gosling, P., Jackson, L.R., and Rayns, F., 2002. Managing soil fertility in organic farming systems. Soil Use and Management 18, 239-247.

Whitmore, A.P., Schroder, J.J., 2007. Intercropping reduces nitrate leaching from under field crops without loss of yield: A modelling study. European Journal of Agronomy 27, 81-88.

Willey, R.W., 1979. Intercropping: Its importance and research needs.Competition and yield advantages. Field Crop Abstr. Part 1., 1-10.

Willey, R.W., 1990. Resource use in intercropping systems. Agricultural Water Management 17, 215-231.

Xu, B.C., Li, F.M., Shan, L., 2008. Switchgrass and milkvetch intercropping under 2:1 row-replacement in semiarid region, northwest China: Aboveground biomass and water use efficiency. European Journal of Agronomy 28, 485-492.

Yildirim, E., Guvenc, I., 2005. Intercropping based on cauliflower: more productive, profitable and highly sustainable. European Journal of Agronomy 22, 11-18.

Zhang, L., van der Werf, W., Bastiaans, L., Zhang, S., Li, B., Spiertz, J.H.J., 2008. Light interception and utilization in relay intercrops of wheat and cotton. Field Crops Research 107, 29-42.

6. Paper 2

Direct and indirect nitrogen losses from an intercropped arable farming rotation

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To be Submitted to 'Agriculture, Ecosystems and Environment'

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Abstract

Intercropping, the cultivation of two or more crops in the same space at the same time, has disappeared from many European farming systems over the last 50 years as these systems have become increasingly dependant on the use of herbicides and mineral fertilisers to maintain productivity. Cereal–legume intercropping offers potential benefits in low-input cropping systems, where nutrient inputs, in particular nitrogen (N), are limited. It can increase the input of leguminous symbiotically-fixed N to the cropping system and reduce negative impacts on the environment. The research aims were to explore the effects of intercropping on post-harvest and the following years' N dynamics, the risks of gaseous and leaching N losses and to estimate the available soil N. The main hypotheses were that: a) the intercrops can reduce system N_2O emissions compared to monocrops; b) the choice of legume species and cultivars can minimise N losses from a system and influence soil available N. A drainage-plot experiment was established on the South-East Scotland, UK, consisting of 12 hydrologically-isolated plots. The treatments were a spring barley (*Hordeum vulgare* cv. *Westminster)* monoculture and intercrops of barley/ white clover (*Trifolium repens* cv. *Alice*) and barley/ pea (*Pisum sativum* cv. *Zero 4* or cv. *Nitouche*). Spring oats (*Avena sativa* cv. *Firth*) were planted on all plots in 2007. In the third season, all plots were sown with perennial ryegrass. No fertilisers, herbicides or pesticides were used at any stage of the experiment. No fertilisers, herbicides or pesticides were used throughout the experiment. Soil mineral N, Dissolved Organic Nitrogen (DON) in soil, gaseous N fluxes (direct and indirect) and N leaching were measured over two year of the experimental period .

Nitrate leaching was reduced where legumes were used (Pea cv. *Zero 4*) in comparison with the barley monocrop (cumulative values of 670 g NO_3^-- N ha^{-1} and 3804 g NO_3^--N ha^{-1}, respectively) and gaseous losses were also reduced (cumulative values of 2.14 kg N_2O-N ha^{-1} and 3.20 kg N_2O-N ha^{-1}). Additionally, the leguminous intercrops increased the soil available N during the first growing season and for the following crop. These results show that intercropping may reduce N losses from low input agricultural systems. However, they also demonstrate that there is a need to choose suitable cultivars for intercropping purposes with care, taking into account the effects upon the growth of the main crop and the influence on the wider enviroment.

Keywords: Barley, drainage plots, intercropping, legumes, nitrogen losses, nitrous oxide, organic farming

1. Introduction

In 2005, agriculture accounted for an estimated emission of 5.1 to 6.1 gigatonnes (Gt) carbon dioxide (CO_2) eq/yr (10-12 % of total global anthropogenic emissions of greenhouse gases) (IPCC, 2006). Despite large annual exchanges of CO_2 between the atmosphere and agricultural lands, the net flux is estimated to be approximately balanced, with CO_2 emissions around 0.04 Gt CO_2/yr. Agriculture is thus an important source of greenhouse gas (GHG) emissions and accounts for approximately one fifth of the annual increase in radiative forcing* and this increases to one third when land use changes, like conversion of forest to cropland, are included (Robertson *et al.*, 2000). Nitrous oxide is released from fixed nitrogen in soils by microbial processes and has a 100-yr average global warming potential (GWP) 296 times larger than an equal mass of CO_2 (Prather *et al.*, 2001; Smith and O'Mara, 2007). It is generated by the microbial processes of nitrification and denitrification in soils and manures and is often enhanced where available nitrogen (N) exceeds plants requirements, especially under wet conditions (Smith and Conen, 2004). Of global anthropogenic emissions in 2005, agriculture accounted for about 60% of N_2O. Nitrous oxide can be produced during biological N fixation, and also when legume residues are returned to the soil (Crews and Peoples, 2004; Lupwayi and Kennedy, 2007). The last route is well-established – when residues rich in N (low C:N ratio) decompose in soil, they can release large amounts of mineral N which is then liable to N_2O loss during nitrification and denitrification (Larsson et al., 1998; Baggs et al., 2000); (Huang et al., 2004); (Rochette and Janzen, 2005). Nitrate leaching can also contribute to significant N loss from the rooting zone (Hansen and Djurhuus, 1997) resulting in water pollution and additional indirect N_2O losses. The amount and intensity of rainfall, evaporation rate, temperature, soil texture and structure, type of land use and cropping practices are all parameters that influence the amount of nitrate in the soil.

It is widely recognised that new agricultural management practices need to be developed to reduce global emissions of N_2O whilst maintaining food production (Raddatz, 2007).

* 'Radiative forcing is a concept used for quantitative comparisons of the strength of different human and natural agents in causing climate change.' Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D.W. Fahey, J. Haywood, J. Lean, D.C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz and R. Van Dorland, , 2007. Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)]. Cambridge University
Press, Cambridge, United Kingdom and New York, NY, USA.

Emissions are most likely to be reduced by increasing N use efficiency (Flessa *et al.*, 2002), and by targeting management practices associated with significant N_2O losses(Ball, Rees, and Sinclair). Emissions of N_2O increase markedly following incorporation of leguminous crops, probably as a result of mineralisation of biologically fixed residue N with enhanced microbial respiration during residue decay (Flessa *et al.*, 2002). The underlying mechanisms behind N_2O emissions are well understood, but we know less about the relative contributions and interactions of environmental and management drivers, including N supply (Bouwman, 1996; Maggiotto, 2000), temperature (Castaldi, 2000; Freibauer and Kaltschmitt, 2003; Flechard *et al.*, 2007), pH (Mogge *et al.*, 1999) and soil moisture (Dobbie, 1999; Zheng *et al.*, 2000; Dobbie and Smith, 2003). More recent research suggests management of the system, e.g. ploughing (Djurhuus and Olsen, 1997; Ball *et al.*, 2002), as well as the crops (Chen *et al.*, 2002) themselves and the cropping systems (Halvorson *et al.*, 2008; Jantalia *et al.*, 2008) can have an impact on N_2O release.

Nitrogen losses in drainage water occur both as a consequence of leaching and through transport of dissolved N_2O. According to Carpenter *et al.* (1998), total export of N from agricultural ecosystems to water, as a percent of fertiliser inputs, ranges from 10-40% from loam and clay soils to 25-80% for sandy soils. There is however much uncertainty regarding the proportion of this which is present as N_2O (Jarvis *et al.*, 2001). Crutzen *et al.* (Crutzen et al. 389-95) suggested that indirect N_2O losses from fixed N application are higher (3-5 times) than previously assessed and have a high impact for climate. Indirect agricultural sources of N_2O remain poorly defined in most cases as they are difficult to quantify and measure because N_2O is emitted to the atmosphere as soon as the drainage water is exposed to the air (Reay *et al.*, 2004a).

Manipulation of agricultural management practices is a possible mechanism to control both loss of nitrogen in drainage water (leaching) and GHG emissions (Smith and Conen 255-63). More efficient land management systems should aim to decrease mineral N accumulation in soil and synchronise N inputs with crop growth and crop N uptake, avoiding the build up of excess N in soils (Mosier et al, 2002). Haugaard-Nielsen *et al.* (2003) found a small reduction in nitrate leaching from lysimeters cropped with pea-barley mixtures compared with monocrops. Currently, this system is attracting increased interest in low-input crop production systems and is being extensively investigated using N inhibitors for reducing the N losses (Hauggaard-Nielsen and Jensen, 2001; Zhang and Li, 2003) and improving soil conditions (Smith *et al.*, 2003).

Intercropping, or the growing of two or more species together at one time, is a widespread practice in tropical agriculture but less widely used in temperate regions. It relies on the two crops having complementary rather than competing traits and thus using resources more efficiently. The inclusion of a leguminous crop has two possible benefits (a) reduced competition for soil nitrogen because the legume fixes its own N, and (b) increased residual nitrogen available to a following crop (Hauggaard-Nielsen and Jensen, 2001; Sangakkara *et al.*, 2003; Thorsted *et al.*, 2006).

In systems that include intercrops, legumes can provide N for the cereals through direct N transfer (Patra *et al.*, 1986; Xiao *et al.*, 2004), while long-term transfer through effects on soil organic N is most important for yield improvements at the crop rotation level (Olesen *et al.*, 2002; Thorsted *et al.*, 2006). Intercropping of legumes and cereals offers an opportunity to increase the input of fixed N into an agro-ecosystem without compromising the use of N from the cereals, yield levels and stability (Hauggaard-Nielsen *et al.*, 2001). Some experiments have shown a clear yield advantage in intercrops, for example beans and maize (Willey, 1979) and pea and barley (Hauggaard-Nielsen *et al.*, 2001), whereas other studies have not, for example intercropping found no benefit from intercropping wheat with clover (Thorsted *et al.*, 2006).

The main objectives of the present experiment were to: a) investigate the N losses from the use of intercrops in a three-year low input rotation; b) quantify the N losses from legumes in comparison with the monocrop and c) to estimate the availability of N in the soil throughout the two year experimental period.

2. Materials and Methods

2.1. Site description and experimental design

A drained-plot experiment at the Bush Estate (lat. 55° 51'N, long. 3° 12'W), near Edinburgh, Scotland, (Fig.2) was used for measuring N_2O emissions from soil, nitrate (NO_3^-) leaching, N_2O from drainage water and mineral N in soil. This facility was established in 1990, and consists of 12 hydrologically-isolated plots (each 25 m x 9 m) that have been used previously for NO_3^- leaching studies (Vinten *et al.*, 1994). The soil was a sandy loam (Eutric Cambisol, Macmerry Series) developed from partially sorted glacial till. The upper 0.5 to 1.5 m of the profile is freely drained. The annual average precipitation in the last 25 years at Bush Estate is 676.2 mm. During the experimental period, the annual precipitation was 927 mm, 1288 mm and 1132 mm for 2006, 2007 and 2008, respectively (Fig 1). The day before sowing in 2006, the soil was sampled and the water content at field capacity was 19 ± 0.5 % (v/v) and the soil bulk density was 1.19 ± 0.01 Mg m^{-3} (mean ± SE, n=5) in 0-20 cm. The top soil was a sandy loam (65% sand, 20% silt and 15% clay)

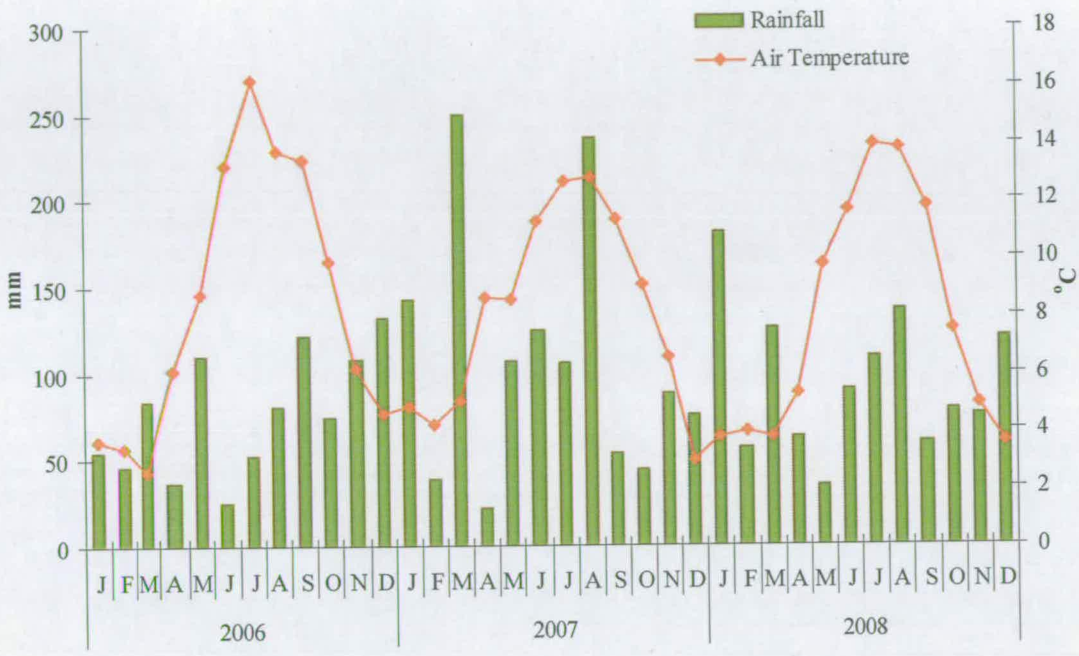


Figure 1: Monthly total rainfall (mm) and monthly average air temperature (°C) at Bush Estate, Scotland during the experimental period.

In the first growing season, the crops were barley (*Hordeum vulgare* cv. Westminster), as a monocrop (mon); a pea (*Pisum sativum* cv. *Zero 4*)/ barley intercrop; a pea (*Pisum sativum* cv. *Nitouche*)/ barley intercrop; and a white clover (*Trifolium repens* cv. *Alice*) /barley intercrop. The barley cv. Westminster is a medium-tall variety that is widely grown for malting and feeding due to its combination of longer than average straw and good disease characteristics (HGCA, 2008). The pea cv. *Nitouche* is a large blue variety with a consistent performance, good agronomic characters and suitability for premium markets. *Nitouche* produces a large, smooth, round pea and retains its colour well. It has long straw with good standing ability making it easy to harvest and high level of resistance to downy mildew. Pea cv. *Zero 4* is a small blue combining pea with a unique combination of agronomic characters: short straw, excellent standing ability and very early maturity.

Figure 2: Sample site located in Midlothian, Scotland, U.K (A). The field is highlighted with a red circle on the top right map (B). Detailed plan of the Section 3 field at the bottom right photo (C). Plots 1, 6, 11: Barley/ Clover; Plots 2, 3, 5: Barley/ Pea cv. *Zero 4*; Plots 8, 10, 12: Barley/ Pea cv. *Nitouche* and plots 4, 7, 9: Barley (mon) in 2006 followed by spring oats (2007) and ryegrass (2008).

When sown at its optimum seed rate of 110 seeds/ m^2, *Zero 4* has a similar yield potential to *Nitouche* (optimum seed rate of 70 seeds / m^2) (Nickerson, 2008). White clover cv. *Alice* is a tall, large leaved clover developed for exception yields of palatable, high quality, high protein forage when included in pastures mixtures. Its vigorous spring and summer growth makes it a good choice for cutting or grazing management and it has good winter hardiness (Barenbrug, 2006).

Each treatment was replicated three times in a blocked design (Fig.2C). In the intercrops the seed rates for the pea and barley followed a 50:50 replacement design. Based on monocrop seed rates of 200 kg ha^{-1} for the barley and 250 kg ha^{-1} for the peas which equated to approximately 375, 75 and 110 germinable seeds m^{-2} for Westminster, *Nitouche* and Zero-4 respectively, the intercrop components were half these values, and the sowing date was on the 24th April 2006. In the second growing season (2007), all the plots were sown with spring oats (*Avena sativa* cv. Firth) on 3rd April at a seed rate of 25 kg ha^{-1} (approximately 450-500 germinable seeds m^{-2}). This is a cultivar that remains a very popular with a high kernel content and good resistance to mildew (HGCA, 2008). In the third growing season, perennial ryegrass (PRG) was sown in all plots in a 50:50 mixture of cv. Aberavon and cv. Aberdart at a total seed rate of 35 kg ha^{-1}(Table 2). In all years, the plots were tilled using a mouldboard plough followed by a rotary hoe, seed drilling and rolling. No fertilisers, herbicides or pesticides were used.

All the data are presented with reference to the cereal growth stages GS 23 (Main shoot and 3 tillers), GS 65 (Flowering half-way), GS 77 (Late milk), GS 92 (Grain hard (not dented by nail). For the 2006 growing season (barley) these stages were at 30, 70, 100 and 140 days, respectively. For oat plants (2007), the growth stages GS 16 (six leaves unfolded), GS 23, GS 65, GS 77 and GS 92 were 40, 65, 100, 135, 161 days from sowing respectively (HGCA, 2005/06). Some of the data presented here have been separated into different periods as follow: Summer '06: 24th April – 11th September; Winter '06: 12th September 06– 2nd April 07; Summer '07: 3rd April – 11th September; Winter '07: 12th September 07- 21st April 08; Summer '08: 22nd April – 30th August.

Table 1: The three-year rotation at the Edinburgh site.

2003	2004	2005	2006	2007	2008
Fallow	*Fallow*	*Fallow*	**Intercropping spring barley/ legumes**	**Spring Oats**	**Grass**

Treatments

1. Barley/ Clover
2. Barley/ Pea cv. Zero 4
3. Barley/ Pea cv.Nitouche
4. Barley

2.2. Sampling and analysis methods

2.2.1. Nitrous oxide fluxes

Fluxes of N_2O were measured using the static chamber technique (Clayton H, 1994). One chamber (volume 25120 cm^3; cover area 1256 cm^3) was located in each of the 12 plots. The chambers were sealed for 60 minutes with an aluminium lid having a small open sampling point sealed with a grommet in which the syringe was inserted.Air and soil temperature were recorded the same time. Gas samples were collected in portable evacuated aluminium vials (Scott *et al.*, 1999) and analysed for N_2O by electron-capture gas chromatography. For consistency, gas sampling was carried out between 10:00 and 12:00 hrs (Clayton *et al.*, 1994).

2.2.2. Nitrate leaching

The drainage flux from each plot was measured using tipping-bucket flow meters mounted in instrument pits. Tips were counted by a hand-counter connected at the side of the tipping bucket. Rainfall was measured at the weather station at Boghall Farm (0.5 km away) and the Centre of Ecology and Hydrology (0.5 km away). The composition of the drainage water was estimated using samples collected with a simple integrated sampler which collected a small volume (ca. 10 ml) of water into a large black plastic bucket, every second tip of the buckets (Vinten *et al.*, 1991). The concentrations of the NO_3- from the integrated sampler were compared from a sample collected on the day of sampling collection from the drainage pipe, so as to test if there was any change on the concentrations. NO_3^- and ammonium (NH_4^+) concentrations in the water samples were determined by continuous flow analysis. Water based standards were used (0, 2, 4, 6, 8 and 10 ppm). NH_4^+ was insignificant, so it is not presented. For the analyses of the data, only the two blocks of the three were used, as all the plots had not equal runoff. That was a limitation of the experiment, as some of the plots were not properly drained since the establishment of them. In Fig. 5, the flow volume is presented for eight out of the twelve plots representing the plots used for the calculation of the leaching.

The leaching loss of nitrogen was calculated, making the following assumptions.

1. Differences in drain flow on different plots were not the result of differences in evapotranspiration or change in storage. It was due to construction, so eight plots with constant drainage were used.

2. The nitrate concentration in deep percolation was the same as that occurring in drainage water.

2.2.3. Dissolved N_2O *concentrations*

Additional samples of drainage water from each plot were collected weekly for almost all the second growing season for measuring the N_2O in drainage water. Containers were filled completely and sealed with a gas tight inner seal, held in place by a screw- top lid. Water samples were stored in a cool box, immediately transferred to the laboratory and stored at 4 °C for up to 48 h until further analysis. N_2O concentration in drainage was assessed in the laboratory by analysis of duplicate 5 ml subsamples from each initial sample. These were injected with a syringe into a 22 ml sealed vial which was shaken vigorously for 1 min. N_2O was then measured using a gas chomatograph (Agilent 6890 GC) fitted with a 1.8m Porapak-N column and electron capture detector (Reay *et al.*, 2004b).

2.2.4. Mineral N

Soil samples from each plot were collected at two depths (0–20 cm and 20-40 cm) at monthly intervals during the growing season and every two months during the winter period. Extractions were made from fresh soil prior to drying. Prior to extraction, the soil was sieved by using a 2 mm sieve to obtain representative subsamples and to exclude large particles and finally mixed. All sample weights were converted to an oven-dry (105 °C) weight. A 10 g sample of sieved soil was weighted into a 150 ml shaker bottle with 50 ml of 0.5M K_2SO_4 and shaken in a Gallenkamp Orbital shaker for 2 hours. After standing for 5 min, the clear excess was filtered through a Whatman No 42 fluted filter paper and the extract collected into a freezer proof airtight polythene bottle. A sub-sample of K_2SO_4 was also filtered for use as a baseline blank determination. The extract was either stored in the fridge for up to 24 h prior to analysis or frozen. The K_2SO_4 extract was analysed for NH_4^+ and NO_3^- on the auto-analyser using a range of standards made up in 0.5M K_2SO_4).

2.2.5. Dissolved Organic Nitrogen (DON) in soil and Inorganic Nitrogen

Dissolved organic nitrogen (DON) is an important component of the soil, and in many cases, it is the main vector for N loss from the soil via leaching. Several methods exist for the analysis of DON and total N, including Kjeldahl digestion. The method that has been used here is the persulfate digestion technique, because it is simpler and non toxic. Persulfate N includes DON, nitrate and ammonium.

The soil extraction for NO_3^- and NH_4^+ were used for this analysis. For the oxidation process, a 5 ml aliquot of the K_2SO_4 extract was accurately pipetted into an autoclaved universal bottle and 1ml of persulphate oxidiser was added. The persulphate oxidiser used was 1.34 g $K_2S_2O_8$ + 0.3 g NaOH dissolved in 100 ml deionised water. The bottle was then tightly capped and placed in the autoclave for 30 mins at 110 °C. This processed extract was only analysed for NO_3^- and NH_4^+ again on the auto-analyser at a dilution of 1.5 ml of extract to 0.09 ml of 0.1 M NaOH. The suggested range of standards used was started at 0.5 ppm up to 5 ppm. DON was measured only two years of the experiment.

2.3. Statistics

All measured variables were normally distributed by Anderson-Darling test (significance .05) and statistical analyses using analysis of variance (ANOVA) were performed with Minitab 15 and GenStat 8.

3. Results

3.1. Leachate

NO_3^- leaching was measured over the whole experimental period. Peaks of NO_3^- mass appeared a few weeks after ploughing and during the autumn months (Fig.3, 4). That can be observed with the flow volume per plot during the winter months presented in Fig. 5. The concentrations had the same pattern having high levels during the whole experimental except the summer '06, which was a very dry period. Barley/ clover had the highest amount of N leachate (1276 g NO_3^- - N ha^{-1}) during the first growing season (2006) which was significantly greater than other treatments ($P<0.001$, Table 2). The barley/ pea treatments showed no differences during the growing season (2006), but after harvest over the 2006 autumn/ winter period, barley/ pea cv. *Nitouche* leached almost 10 times more NO_3^- than the barley/ pea cv. *Zero 4* (cumulative values for this period: barley/ pea cv *Nitouche*: 5373 g NO_3^- - N and barley/ pea cv. *Zero 4*: 573 g NO_3^- - N) ($P<0.005$). During the 2007 growing season, oats growing in the barley/ clover plots (1565 g NO_3^- - N ha^{-1}) had the highest NO_3^- leaching and this continued during the winter (2007-2008). The N leaching from oats growing after the barley/ pea treatments were significantly different from the other treatments during the growing season ($P<0.05$), but not during the winter (2007). The monocrop barley and following oat had a considerable amount of NO_3^- leached usually during at the winter periods, after the harvest and removing of the plant material. The NH_4^+ concentrations in drainage water were negligible (<0.01 mg L^{-1}) in all samples.

Figure 3: Weekly total rainfall and average temperature and NO_3^- : N (g NO_3^- : N ha^{-1} $week^{-1}$) leaching during the experimental period 2006-2008 for the four treatments referring to the initial treatment for the whole experimental period. All the values are the mean (n=2) ± SE (bars).

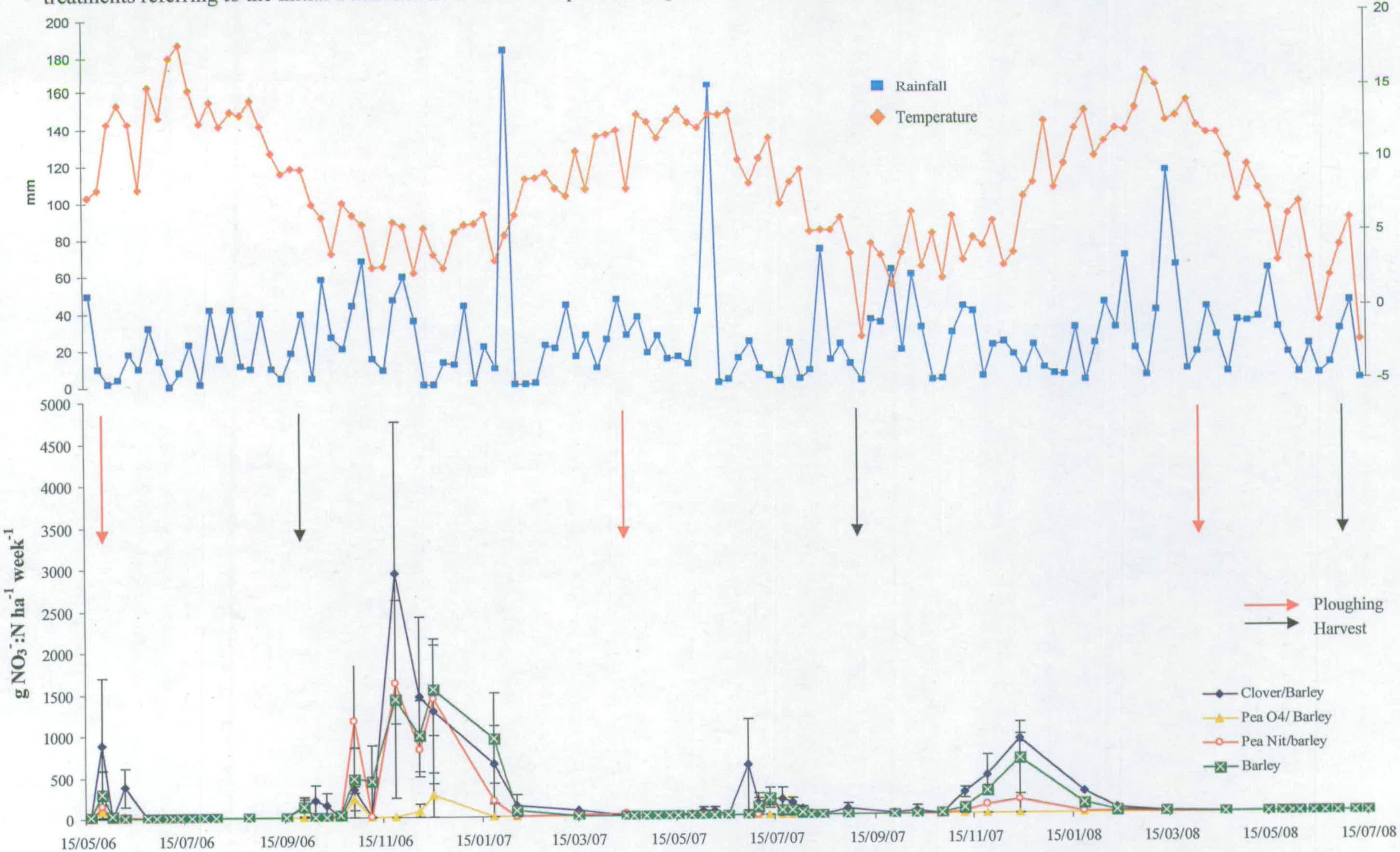
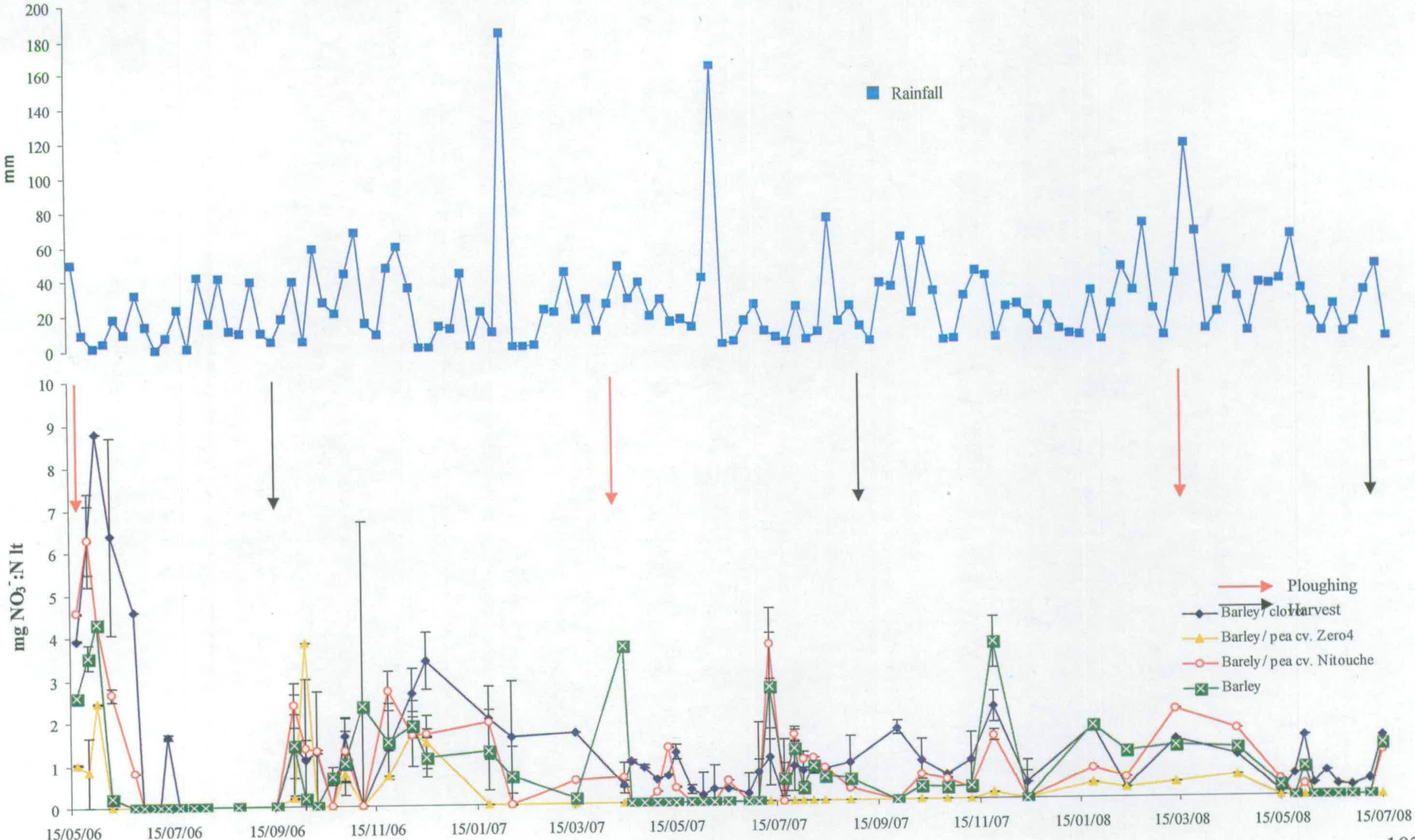


Figure 4: Weekly total rainfall and concentrations of NO_3^- : N (mg NO_3^- : N lt^{-1}) leaching during the experimental period 2006-2008 for the four treatments referring to the initial treatment for the whole experimental period. All the values are the mean (n=2) ± SE (bars).



	Clover/ Barley	Pea Zero 4/ Barley	Pea Nit/ Barley	Barley
Intercropping (2006)	1277 a	95 b	160 b	334 c
Winter (2006-2007)	7240 a	573 b	5374 c	1996 d
Oat (2007)	1566 a	0 b	261 c	458 c
Winter (2007-2008)	1698 a	0 b	167 b	1016 c
Grass (2008)	0 a	2 b	0 a	0 a
Total	11781	671	5963	3804

Table 2: Cumulative NO_3^-: N leaching (g NO_3^- : N ha^{-1}) from plots planted with barley/ legume intercrop during 2006, oats on 2007 and grass on 2008. The same letter within the same row indicates treatments not significantly different from each other (P>5%). Results are presented as nitrate (NO_3^- - N); ammonium (NH_4^+ - N) concentrations were negligible (< 0.01 mg L-1) in all samples. Periods: Summer '06: 24th April – 11th September; Winter '06: 12th September – 2nd April; Summer '07: 3rd April – 11th September; Winter '07: 12th September- 21st April; Summer '08: 22nd April – 30th August.

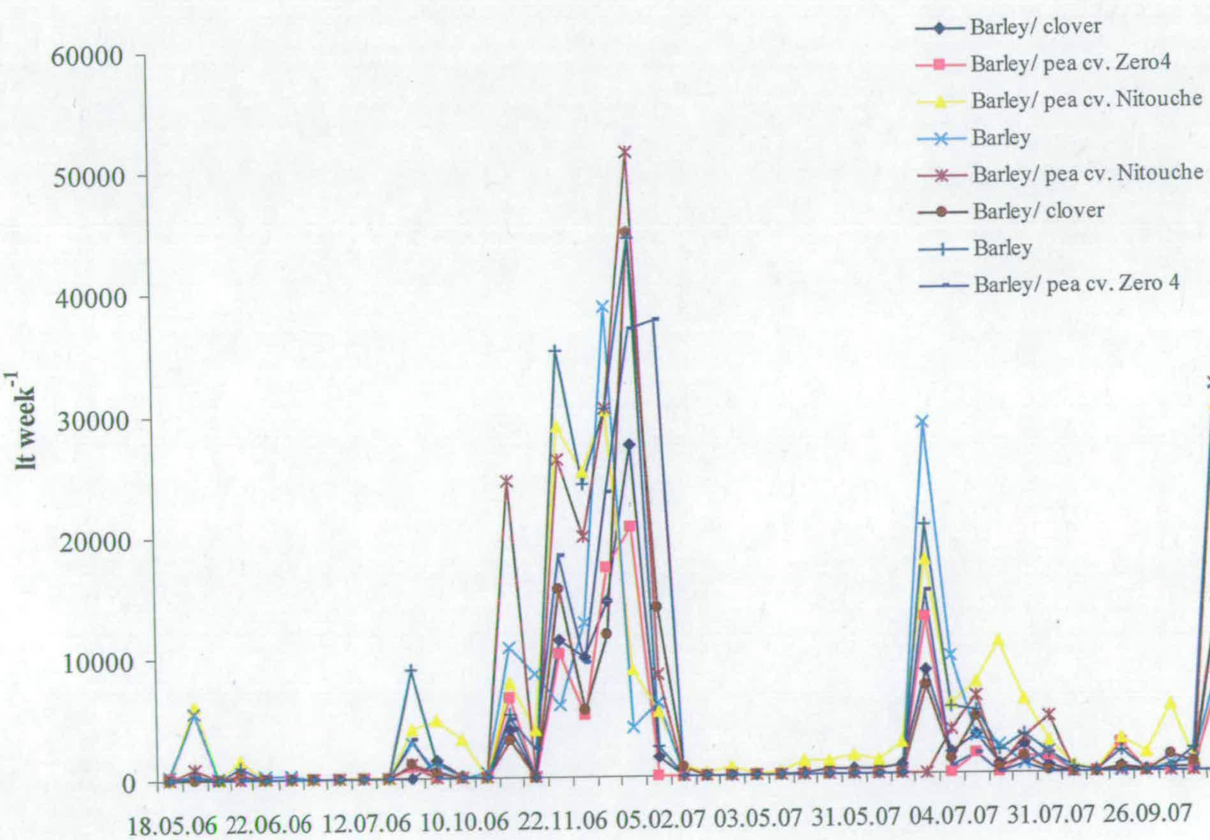
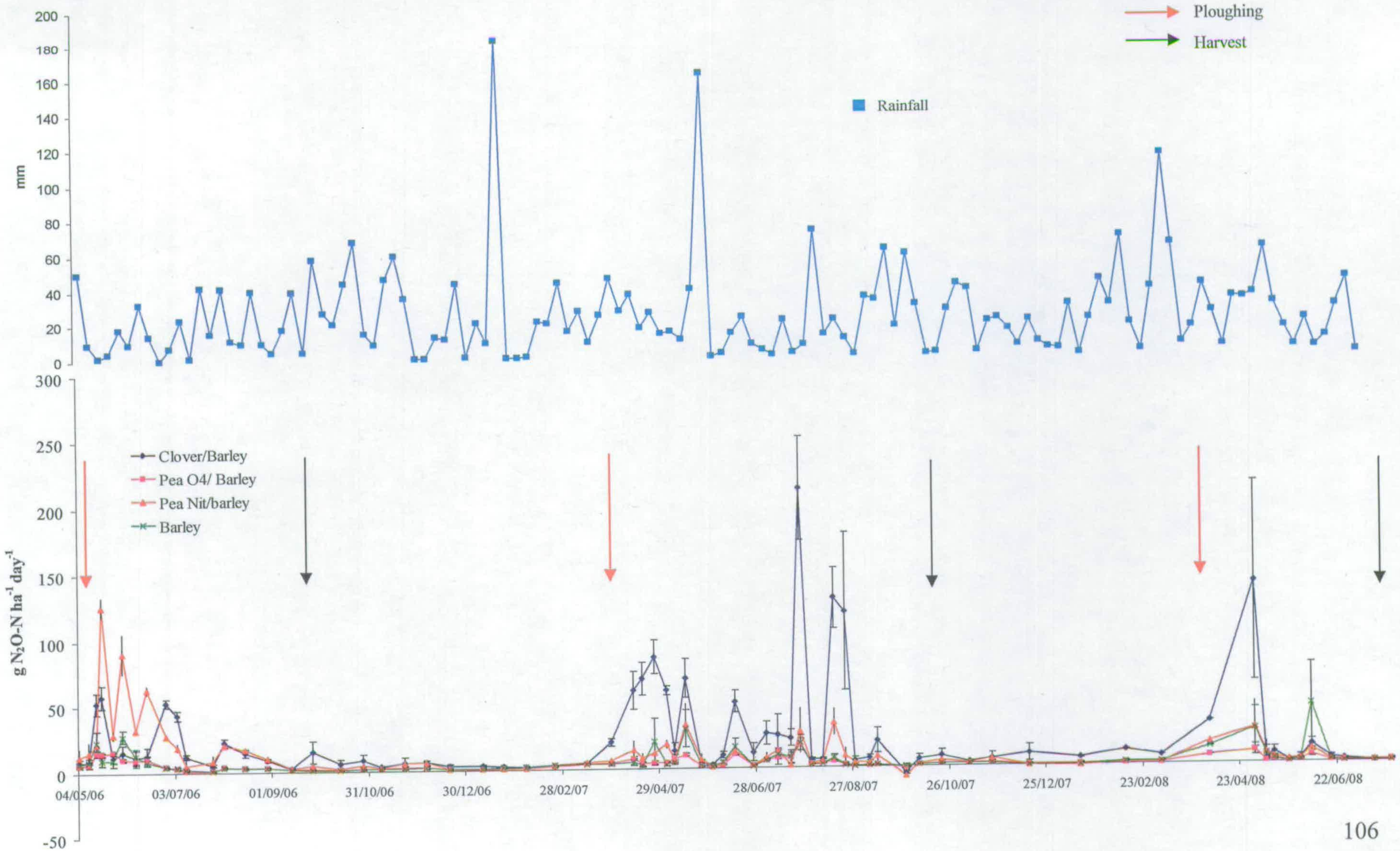


Figure 5: Weekly flow volume of leaching during the experimental period 2006-2008 for the four treatments referring to the initial treatment for the whole experimental period. Only presented the plots considered for the leaching calculations.

3.2. Nitrous oxide

At the end of the first growing season the cumulative N_2O fluxes differed significantly between treatments ($P<0.05$), with the largest emissions (3.35 kg N ha^{-1}) observed from the Pea cv. *Nitouche*. This was significantly greater than emissions the barley/ pea cv. *Zero 4* (0.74 kg N ha^{-1}) ($P<0.05$). Clover/ barley treatment had the second highest fluxes reaching 2.78 kg N ha^{-1}(Table 3). The treatment effect continued during the winter period when the clover/ barley had highest fluxes as the clover kept growing after the harvest. During the second growing season, a carry-over from the previous year's treatments was observed (Fig. 6). In this year cumulative emissions from the clover/ barley were 8.57 kg N ha^{-1}, 1.31 kg N ha^{-1} barley monocrop, 2.30 kg N ha^{-1} from barley/ pea cv. *Nitouche* and 0.98 kg N ha^{-1} from barley/ cv. *Zero 4* ($P<0.005$) (Table 3).

Figure 6: Weekly Rainfall and N_2O fluxes (g N_2O-N ha^{-1} day^{-1}) for the drainage plots, Bush, Edinburgh from April 2006 to September 2008. Initial treatments (2006) are used for describing the treatments for the whole duration of the experiment. All the values are the mean (n=6) ± SE (bars).



Treatments	Summer '06		Winter '07		Summer '06		Winter '07		Summer '08		Total	
A	2778	a	508	a	8566	a	141	a	1091	a	13083	a
B	744	b	188	b	976	b	67	a	163	b	2137	c
C	3345	a	321	a	2294	b	-278	a	397	a	6081	b
D	876	b	201	b	1308	b	138	a	669	a	3193	c

Table 3: Cumulative N_2O fluxes (g N_2O-N ha^{-1}) for different season. Treatments: (2006) A: Barley/ clover; B: barley/ pea cv. *Zero 4*; C: barley/ pea cv. *Nitouche*; D: barley (monocrop); (2007) A, B, C, D: Oat; (2008) A, B, C, D: Grass. The values with different letters (a, b) are significant different (P<0.05)

Periods: Summer '06: 24th April – 11th September; Winter '06: 12th September – 2nd April; Summer '07: 3rd April – 11th September; Winter '07: 12th September- 21st April; Summer '08: 22nd April – 30th August. All the values are the mean (n=3) ± SE (bars).

3.3. Dissolved N_2O *concentrations*

The N_2O fluxes and concentrations on the drainage were measured only in the second growing season. N_2O fluxes in drainage were greatest for the oats growing in the previous barley/ pea cv. *Nitouche* (Fig. 7). However, the concentrations were higher for the previous barley/ clover treatment (Fig. 8). The oats growing after the barley/ pea cv. *Zero 4* treatment had minimal leaching during the growing season and the dissolved N_2O was difficult to be measured, as there was no flow of drainage.

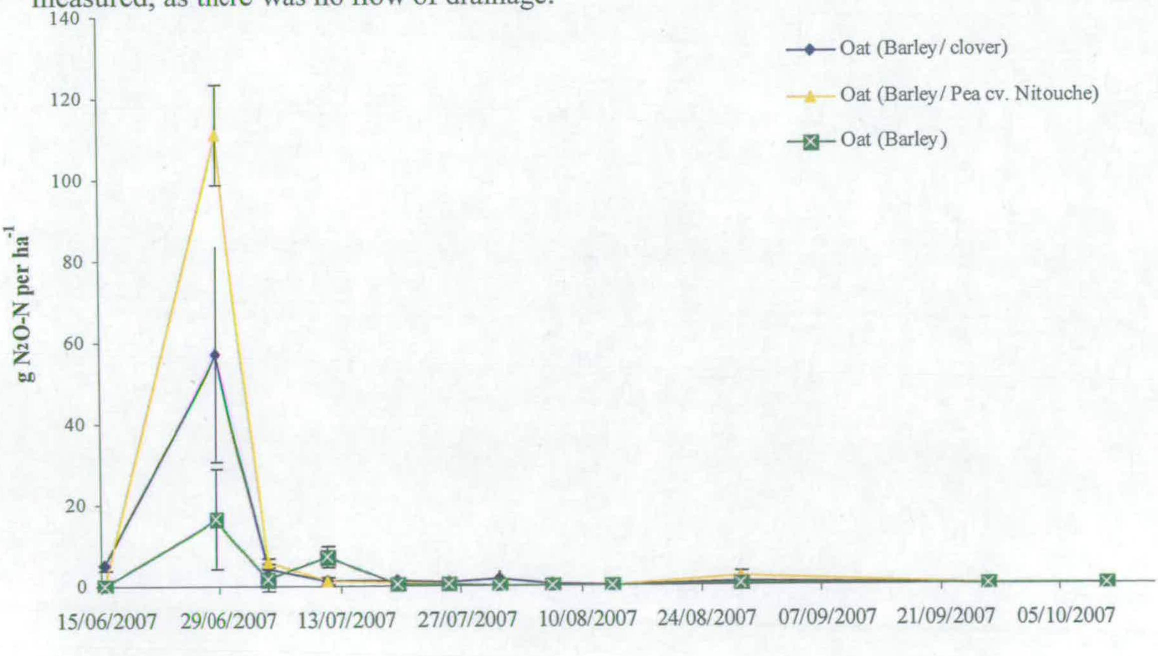


Figure 7: N_2O emissions (g N_2O – N per ha^{-1}) from drainage water (indirect losses) for the oat crop distinguished by the previous (initial) treatment. All the values are the mean (n=3) ± SE (bars).

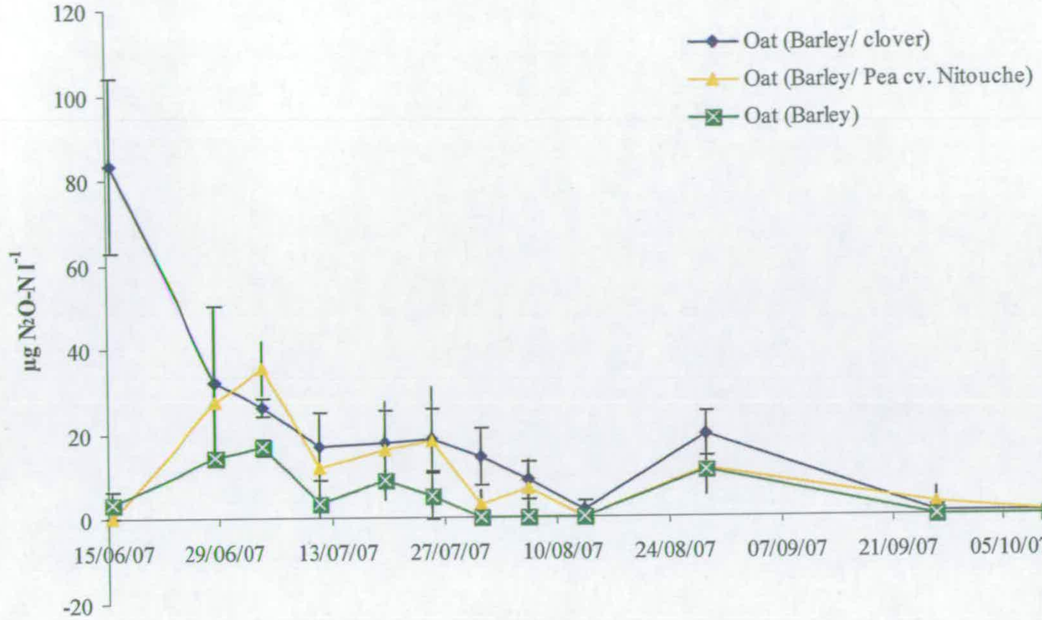


Figure 8: N_2O emissions (μg N_2O – N l^{-1}) from drainage water (indirect losses) for the oat crop distinguished by the previous (initial) treatment. All the values are the mean (n=3) ± SE (bars).

3.4. Soil mineral N

3.4.1. Ammonium

Soil NH_4^+ concentrations in the 0-20 cm soil layer from all the treatments were measured monthly during the growing seasons and every two months during winter periods. During the 2006 growing season, the levels of NH_4^+- N for the two pea varieties were significantly different ($P<0.05$) with barley/ pea cv. *Zero 4* having the highest value of 29.44 mg NH_4^+- N kg^{-1}. Barley monocrop had the second highest value followed by barley/ clover and barley/ cv. *Nitouche*. After the harvest (12th September 2006), the amount of NH_4^+-N in the soil was significantly ($P<0.05$) higher for the barley/ clover treatment until the spring, 2007, ploughing. After that management, the values of ammonium were low and not significantly different between treatments (Fig. 9A).

Ammonium concentrations in the 20-40 cm layer, showed patterns that mirrored those of the surface (0-20 cm) in the first growing season. Barley/ pea cv. *Zero 4* had the highest concentration of 18.82 mg NH_4^+- N kg^{-1}. The levels of ammonium remained low (below 5 mg NH_4^+- N kg^{-1}) for the remainder of the experiment after the ploughing in the second year (Fig. 9B).

3.4.2. Nitrate

Surface NO_3^- concentrations (0-20 cm) increased up until growth stage 65 in June, 2006. Barley/ pea cv. *Nitouche* had the highest concentration (11.50 mg NO_3^- -N kg $^{-1}$) followed by barley/ pea cv. *Zero 4* (9.85 mg NO_3^- N kg $^{-1}$). Both treatments were significantly different ($P<0.05$) from barley/ clover and barley monocrop at this stage (Fig 9C). The concentrations of NO_3^- fell during the ear filling (GS 23-65) and the maturity stages of the peas, but they increased again after the harvest of the crops. In the barley/clover treatments nitrate concentrations remained high during the winter months. After ploughing in 2007, there was an increase in nitrate concentrations with highest values in the barley/clover (10.3 mg NO_3^-N kg $^{-1}$) followed by barley/ pea cv. *Zero 4* (6.42 mg NO_3^-N kg^{-1}), which were significantly different ($P<0.001$) from the barley/pea cv. *Nitouche*. After ploughing at the beginning of the third year, there were high concentrations of nitrates (above 13 mg NO_3^--N kg^{-1}) in all the treatments, which then declined as soon as the new crop was established (Fig. 9C).

In the 20-40 cm soil layer, there were significant differences between the treatments ($P<0.05$) during the establishment period in the first growing season. Barley/ pea cv. *Nitouche* had the highest concentrations of NO_3^--N (6.87 mg NO_3-N kg^{-1}) which was significantly higher than that from barley/pea cv. *Zero 4* (3.02 mg NO_3^--N kg soil^{-1}; P<0.05). After harvest, concentrations remained between 3 mg NO_3^- - N kg soil^{-1}and 5 mg NO_3^- -N kg^{-1} until the spring ploughing. The NO_3^- concentration under barley/ pea cv. *Zero 4* was higher than that of the barley/ pea cv. *Nitouche*, (11.64 and 7.09 mg NO_3^- -N kg^{-1}, respectively) during the first and second growing season. After the establishment of the oat crop, the concentration of nitrate in the soil was higher where barley/ pea cv. *Nitouche* had been grown previously and significantly higher (P<0.05) than the previous barley/ pea cv. *Zero 4*. This difference remained until the following year's ploughing (Fig. 9D).

3.4.3. Dissolved Organic Nitrogen (DON)

In the 0-20 cm soil layer, there was a reduction in DON during the first year of the experiment particularly in the barley/pea cv. *Nitouche* treatment, where concentrations decreased significantly (P<0.001) by 11 mg kg^{-1}. During the winter period, the concentrations of DON were constant for all the treatments around 12 mg kg^{-1}. After ploughing in spring 2007, there was an increase in the concentration of DON in plots previously cropped with barley/legume intercrops, when concentrations in plots previously cropped with barley/clover reached 31 mg kg^{-1} (P<0.05). However, during the establishment of the oat crop those values reduced by 11 mg kg^{-1} (on average). During the experimental period, the DON values for the barley monocrop remained relatively stable around 15 mg kg^{-1} (Fig. 10A).

In the 20- 40 cm soil layer, the DON concentration remained constant (at around 15 mg kg^{-1}) except in the barley/pea cv. In the *Nitouche* and barley monocrops, concentrations reduced by 4 mg and 6 mg DON kg $^{-1}$, respectively. During the winter period there was no significant change until the spring ploughing (2007), when the concentrations increased under all the treatments, but especially where barley/ legumes treatments had occured. Similarly, in the top layer (0-20 cm), the earlier barley/ clover crop had a significantly higher concentration of DON (35 mg DON kg ¯1; P<0.001) followed by barley/ pea cv. *Nitouche* (25 mg DON kg $^{-1}$). During the establishment period of the oat crop, an average reduction of 11 mg DON kg $^{-1}$ was measured in all treatments (Fig. 10B).

Figure 9: Ammonium and nitrate concentrations in soil during the experiment in two soil layers (0-20 and 20-40 cm). All the values are means (n=6) ± SE (bars). A) Ammonium: 0-20 cm; B) Ammonium 20-40 cm; C) Nitrate: 0-20 cm; D) Nitrate: 20-40 cm. Arrows symbolise the ploughing time.

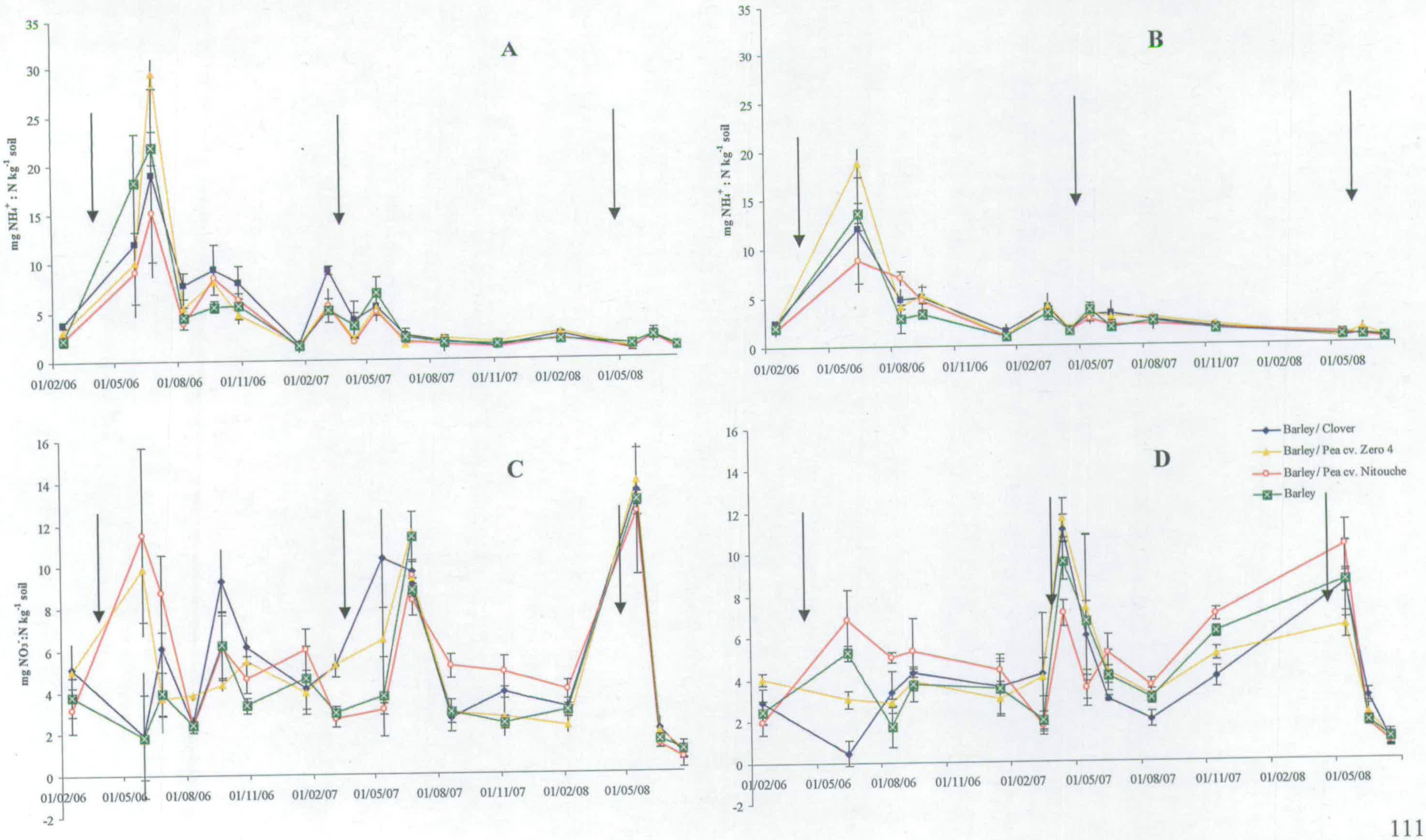
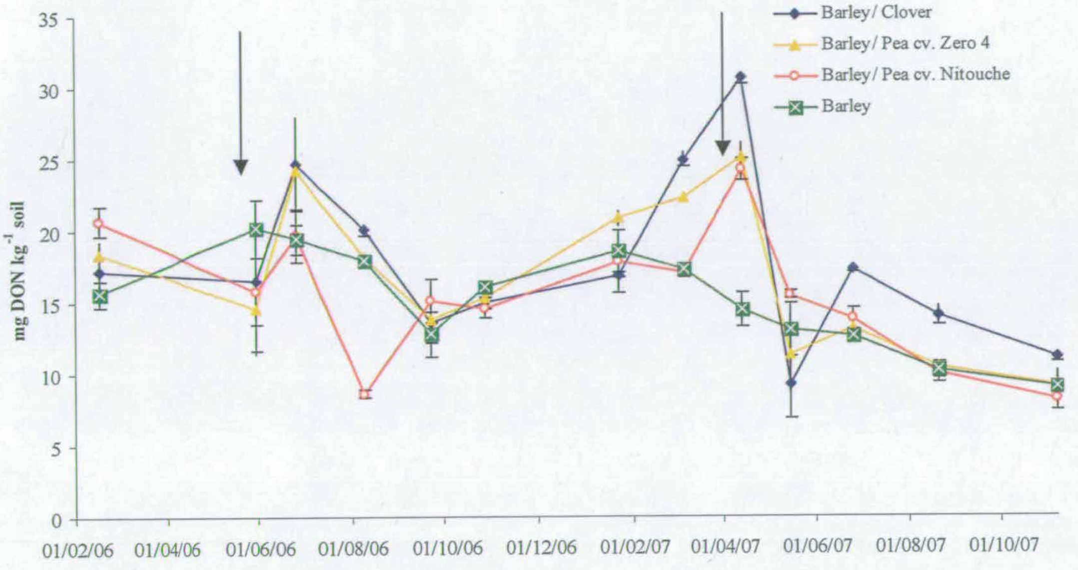
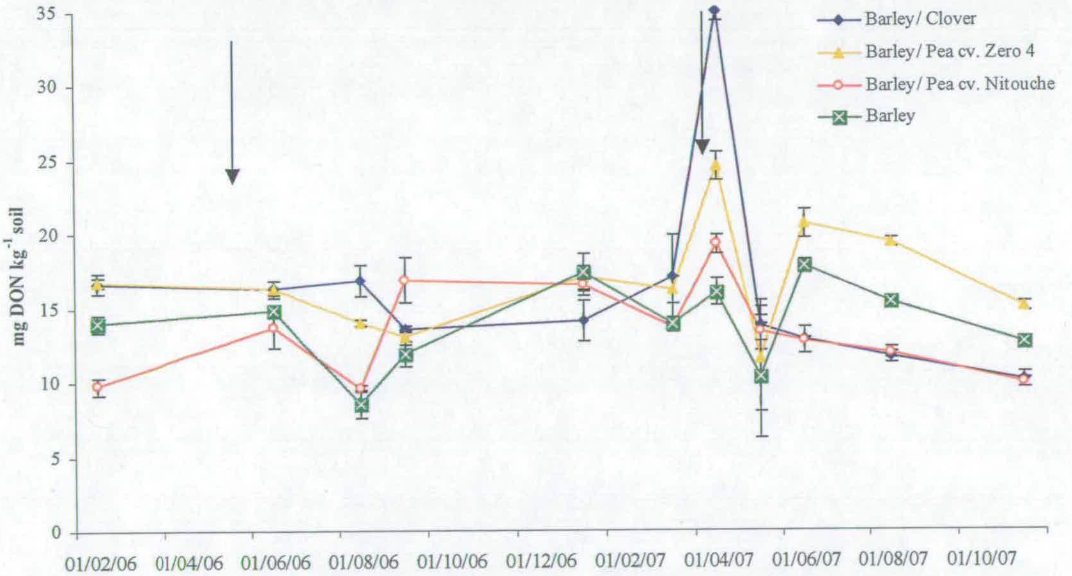


Figure 10: Dissolved Organic Nitrogen (DON) concentrations in soil during the experiment in two soil layers (0-20 cm (A) and 20-40 cm (B)). All the values are means (n=6) ± SE (bars).

A)



B)



4. Discussion

This study has shown that large differences in N_2O emissions can result from intercropping treatments that use different legume species and cultivars. The highest of N_2O fluxes were observed during spring, summer and autumn 2006, probably due to nitrification being enhanced by the higher temperatures, drier conditions and fixed N from the legume plants (Skiba *et al.*, 1993; Skiba *et al.*, 2006; Jones *et al.*, 2007). The annual rainfall at this site is usually around 700 mm. In 2006, the total rainfall was 927 mm, whilst in 2007 it was almost double the average, reaching 1288 mm. Also the distribution of rainfall between the two years differed considerably. For example, total rainfall in the period between 1 June and 30 September was 280 mm in 2006 and 521 mm in 2007. In 2006, the average temperature during this period was 14.2°C whilst 2007 was cooler with an average temperature of 12°C. These meteorological differences might influence the differences in N_2O fluxes between the two years. Emissions were generally lower in 2006 than in 2007. An important observation from the first year was the different flux rates from the two barley/ pea treatments. Barley/ pea cv. *Nitouche* had cumulative fluxes of 3.35 kg N ha^{-1} in contrast with barley pea cv. *Zero 4* of 0.74 kg N ha^{-1}. Such a difference in N_2O fluxes between cultivars of the same species has not been reported in any previous experimental study.

Additionally, similar results regarding the carry-over of N_2O fluxes from the treatments for the second growing season have not been previously observed in another study. The fluxes continued to be high especially from the plots that followed the barley/ clover treatment, with the barley/ pea cv. *Nitouche* treatment only slightly lower. These results might be due in part to different water filled pore spaces attributed to the treatments' different root systems and/ or mineral N (Dobbie and Smith, 2003). Another factor that might have contributed to the high fluxes might have been the disruption of soil by ploughing (Ball *et al.*, 2007) during spring 2007, as re-growth of clover plants occurred and pea plants germinated from the seedbed left after harvest continuing the mineralisation of N from organic N stored over the winter months (Vinten *et al.*, 2002).

The barley/ clover treatment had the highest overall N losses in the first year (2006) the highest N_2O fluxes and NO_3^- leaching rates. Studies have shown that nitrogen fixation in clover can vary widely with values between 11 and 373 kg N ha^{-1} year $^{-1}$ under different conditions reported worldwide (Lampkin, 1990; Wood, 1996; Vinther and Jensen, 2000; Ledgard, 2001). These different rates of N fixation can indirectly affect the amount of N loss via leaching (Pastor and Binkley, 1998). In addition to this, the amount of mineral N in the soil and organic N can contribute to higher N_2O losses by nitrification and denitrification (Jones *et al.*, 2005), possibly related with the results of the barley/ pea cv. *Nitouche* treatments. Nitrous oxide emissions in this study showed seasonal patterns with low emissions during the winter months, which then increase with rising temperature and cultivation, as has been observed in other studies (Jones *et al.*, 2007; McTaggart *et al.*, 1994).

Soil mineral N concentrations increased during the first growing season especially in the barlye/ pea cv. *Nitouche* and barley/ clover treatments. Soil NH_4^+ concentrations in the 0-20 cm soil layer increased after the establishment of the legumes, but declined rapidly at the ear filling stage, when the cereal demands N, indicating uptake by the cereal plants or microbial consumption. The concentrations of NO_3^- increased after disturbance of the soil (e.g. ploughing). Different soil management options such as reduced or min-tillage might provide an opportunity to reduce the losses of available N (Fischer *et al.*, 2002; Hansen, 2002). Although this approaches can be generally used under specific climatic and soil conditions, factors closely related with the cumulative values of leaching and/ or gaseous losses.

In general, nitrate leaching tended to be higher from crop rotations with legumes than from those without legumes. It has been confirmed by experiments Beaudoin (2005) observed the highest rates of nitrate leaching in crop rotations including pea for Northern France. This was because legumes fix N, which results in higher N contents in the biomass and a lower N uptake from the soil. To some extent this is also related to other properties of the crop or its management, such as the peas' relatively shallow root system, which support only the higher soil layers or the sowing and harvest dates of the crop in relation to the period until the subsequent crop (Carrouée *et al.*, 2006). However, legume-based systems may have lower N losses than conventional and farmyard manure based systems (Drinkwater *et al.*, 1998). Hauggaard-Nielsen *et al.* (2003) found a decrease in nitrate-leaching from lysimeter plots intercropped with pea-barley in comparison with monocrops, due to N-content and rate of decomposition of roots and residues. The current study has shown that the highest rate of NO_3^- leaching was from the barley/ clover treatments, which continued during the whole of the experiment. This was enhanced by the continued growth of clover during the winter (2006) due to higher than average temperatures (Murray, 2000; Goulas, 2003), which allowed the growth of nodules during winter months (Bergensen *et al.*, 1963; Marriott, 1988). The two barley/ pea intercrops were significantly different in NO_3^- leaching throughout the experiment with barley/ pea cv. *Zero 4* having the lowest NO_3^- leaching losses; less than the barley monocrop. This strong varietal effect on nitrate leaching, like that on N_2O fluxes, has not been previously reported for such a long experimental period.

In 1979, it was the first time when the N_2O transport from soil to water drainage was measured (Dowdell *et al.*, 1979), which considered to be an indirect measurement on N loss.

Nitrous oxide is fairly soluble in water (1.0 ml gas per ml water at 5°C), so it can be lost from the soil by leaching in amounts comparable with those lost in the gas phase from the soil surface. The N_2O appearing in outfall water is likely to derive at least in part from both nitrification and denitrification at the soil surface, in deeper soil and even in the field drain (Reay *et al.*, 2004a). We found that the N_2O emissions from the drainage from the previous barley/ clover were much higher by comparison with the oats grown in barley/ pea cv. *Nitouche*. However, the actual drainage output (g N ha^{-1}) showed the reverse effect, as the oats growing in the previous barley/ pea cv. *Nitouche* had greater runoff reaching a peak of 111g N_2O-N ha^{-1}. This result might be due to differences in the soil water filled pore space (WFPS), bulk density, root density and amount of mineral N (Jensen, 2003; Nemecek and Erzinger, 2005). At the field scale, relatively low emissions from the soil surface (direct flux) have been attributed to the low rainfall experienced for the study period. However, indirect losses of N_2O have appeared less sensitive to low rainfall conditions, perhaps due to the greater soil depth at which such leached N_2O was generated. However, the diverse grain yields during the two growing seasons (Paper 1) in relation with environmental effects is a important step for the choice of the appropriate treatment. Pappa et al. (Paper 1) has shown that the grain yields between the treatments were similar with the barley/ clover to have the highest yield in both growing seasons and showing the highest cumulative N_2O fluxes for the experimental period. However, the two barley/ pea treatments had similar yields (both barley and peas), but the cumulative N_2O fluxes were three times higher and the NO_3^- ten times higher with barley/ pea cv. Nitouche to have the lowest values showing the great environmental effect that a treatment can have.

This experiment has highlighted the diverse N losses from the use of intercrops including different cultivars and species and the additional effects in the following growing years. It has also demonstrated the need for longer term experiments to develop these systems and quantify their benefits or not in the subsequent crop and in a rotation. However, it is important not only to concentrate in the environmental effects of such systems, but also on the economic effects considering global inventories for mitigating N losses.

5. Conclusions

Low input and organic systems can benefit from the use of legume-cereal intercropping. The study showed that the use of legume intercropped with cereals can be an alternative way to provide nitrogen and minimise the losses from agricultural systems. Mixtures of cereals with legumes can have fewer losses than those from monocrops. However, the choice of legume cultivar and species is a key factor influencing the amount of N losses in the year of use and/ or the following year, with this impacting significantly on the cumulative N losses.

The magnitude of these effects is highly sensitive to soil management. For example, the choice of legume is important in optimising N fixation in intercropping designs. However, a more extensive study of the environmental impacts that different species/ varietal combinations and ratios is needed in order to optimise such systems. Important factors to investigate further will include the influence of root development and compatibility of component species / varieties. Having a better understanding of the interactions taking place within a cereal/ legume intercrop and how this can fit in to a rotation has the potential to be a very useful management tool in the development of more sustainable cropping and agricultural systems.

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References

Baggs, E.M., Rees, R.M., Smith, K.A., Vinten, A.J.A., 2000. Nitrous oxide emission from soils after incorporating crop residues. Soil Use and Management 16, 82-87.

Ball, B.C., McTaggart, I.P., Watson, C.A., 2002. Influence of organic ley-arable management and afforestation in sandy loam to clay loam soils on fluxes of N_2O and CH4 in Scotland. Agriculture, Ecosystems & Environment 90, 305-317.

Ball, B.C., Watson, C.A., Crichton, I., 2007. Nitrous oxide emissions, cereal growth, N recovery and soil nitrogen status after ploughing organically managed grass/clover swards. Soil Use and Management 23, 145-155.

Ball, B. C., Rees R. M., and Sinclair A. H.. "Mitigation of Nitrous Oxide and Methane Emissions From Agricultural Soils – a Summary of Scottish Experience". 2008.

Barenbrug,2006. www.barenbrug.com/site/modules/custom/products/getfile.php?siteid=1001 &filename=Alice.pdf accessed January, 2006.

Beaudoin, N., Saad, J.K., Van Laethem, C., Machet, J.M., Maucorps, J., Mary, B., 2005. Nitrate leaching in intensive agriculture in Northern France: Effect of farming practices, soils and crop rotations. Agriculture, Ecosystems & Environment 111, 292-310.

Bergensen, F.J., Hely, F.W., and Costin, A.B., 1963. Overwintering of clover nodules in alpine conditions. Australian Journal of Biological Science 16, 920-921.

Bouwman, A.F., 1996. Direct emission of nitrous oxide from agricultural soils. Nutrient Cycling in Agroecosystems 46, 53-70.

Carrouée, B., Bourgeais, E., and Aveline, A., 2006. Nitrate leaching related to dry pea in arable crop rotations. Grain legumes and the environment: how to assess benefits and impacts, Zurich, November 18-19, 2004. , 117-124.

Castaldi, S., 2000. Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment. Biology and Fertility of Soils 32, 67-72.

Chen, X., Cabrera, M.L., Zhang, L., Wu, J., Shi, Y., Yu, W.T., Shen, S.M., 2002. Nitrous oxide emission from upland crops and crop-soil systems in northeastern China. Nutrient Cycling in Agroecosystems 62, 241-247.

Clayton H, A.J., Smith KA, 1994. Measurement of nitrous oxide emissions from fertilised grassland using closed chambers. Journal of. Geophysical Research 99, 16599-16607.

Clayton, H., Arah, J.R.M., Smith, K.A., 1994. Measurement of nitrous oxide emissions from fertilised grassland using closed chambers. Journal of. Geophysical Research 99, 16599-16607.

Crews, T.E., Peoples, M.B., 2004. Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agriculture, Ecosystems & Environment 102, 279-297.

Crutzen, P. J., et al. "N_2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels." Atmospheric Chemistry and Physics 8.2 (2008): 389-95.

Djurhuus, J., Olsen, P., 1997. Nitrate leaching after cut grass/clover leys as affected by time of ploughing. Soil Use and Management 13, 61-67.

Dobbie, K.E., and Smith, K.A., 2003. Nitrous oxide emission factors for agricultural soils in Great Britain: the impact of soil water-filled pore space and other controlling variables. Global Change Biology 9, 204-218.

Dobbie, K.E., McTaggart, I.P. , Smith, K.A., 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research D: Atmospheres 104.

Dobbie, K.E., Smith, K.A., 2003. Nitrous oxide emission factors for agricultural soils in Great Britain: the impact of soil water-filled pore space and other controlling variables. Global Change Biology 9, 204-218.

Dowdell, R.J., Burford, J.R., Crees, R., 1979. Losses of nitrous oxide dissolved in drainage water from agricultural land. Nature 278, 342-343.

Drinkwater, L.E., Wagoner, P., and Sarrantino, M., 1998. Legume based cropping systems have reduced carbon and nitrogen losses Nature 396 262-265.

Fischer, R.A., Santiveri, F., Vidal, I.R., 2002. Crop rotation, tillage and crop residue management for wheat and maize in the sub-humid tropical highlands: I. Wheat and legume performance. Field Crops Research 79, 107-122.

Flechard, C.R., Ambus, P., Skiba, U., Rees, R.M., Hensen, A., van Amstel, A., Dasselaar, A.v.d.P.-v., Soussana, J.F., Jones, M., Clifton-Brown, J., Raschi, A., Horvath, L., Neftel, A., Jocher, M., Ammann, C., Leifeld, J., Fuhrer, J., Calanca, P., Thalman, E., Pilegaard, K., Di Marco, C., Campbell, C., Nemitz, E., Hargreaves, K.J., Levy, P.E., Ball, B.C., Jones, S.K., van de Bulk, W.C.M., Groot, T., Blom, M., Domingues, R., Kasper, G., Allard, V., Ceschia, E., Cellier, P., Laville, P., Henault, C., Bizouard, F., Abdalla, M., Williams, M., Baronti, S., Berretti, F., Grosz, B., 2007. Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. Agriculture, Ecosystems & Environment 121, 135-152.

Flessa, H., Ruser, R., Dörsch, P., Kamp, T., Jimenez, M.A., Munch, J.C., Beese, F., 2002. Integrated evaluation of greenhouse gas emissions (CO2, CH4, N_2O) from two farming systems in southern Germany. Agriculture, Ecosystems & Environment 91, 175-189.

Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D.W. Fahey, J. Haywood, J. Lean, D.C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz and R. Van Dorland, , 2007. Changes in Atmospheric Constituents and in Radiative Forcing. In:

Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Freibauer, A., Kaltschmitt, M., 2003. Controls and Models for Estimating Direct Nitrous Oxide Emissions from Temperate and Sub-Boreal Agricultural Mineral Soils in Europe. Biogeochemistry 63, 93-115.

Goulas, E., Le-Dily, F., Ozouf, J., and Ourry, A., 2003. Effects of a cold treatement of the root system on white clover (Trifolium repens L.) morphogenesis and nitrogen reserve accumulation. Journal of Plant Physiology 160, 893-902.

Halvorson, A.D., Del Grosso, S.J., Reule, C.A., 2008. Nitrogen, Tillage, and Crop Rotation Effects on Nitrous Oxide Emissions from Irrigated Cropping Systems. Journal of Environmental Quality 37, 1337-1344.

Hansen, E.M., Djurhuus, J., 1997. Nitrate leaching as influenced by soil tillage and catch crop. Soil and Tillage Research 41, 203-219.

Hansen, E.M., Hogh-Jensen, H., and Djurhuus, J. , 2002. Biological nitrogen fixation in a grazed perennial grass/clover ley and correlation with herbage and soil variables. European Journal of Agronomy 16, 309-320.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. Plant and Soil 236, 63-74.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2003. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutrient Cycling in Agroecosystems 65, 289-300.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. Field Crops Research 72, 185-196.

HGCA, 2005/06. The barley growth guide.

HGCA, 2008. http://www.hgca.com/content.output/512/512/Varieties/Spring%20oats/Firth.mspx.

Huang, Y., Zou, J., Zheng, X., Wang, Y., Xu, X., 2004. Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. Soil Biology and Biochemistry 36, 973-981.

IPCC, 2006. Guidelines for National Greenhouse Gas Inventories., N_2O emissions from managed soils, and CO2 emissions from lime and urea application. Published by the

Institute for Global Environmental Strategies (IGES) for the IPCC. ISBN 4-88788-032-4

Jantalia, C., dos Santos, H., Urquiaga, S., Boddey, R., Alves, B., 2008. Fluxes of nitrous oxide from soil under different crop rotations and tillage systems in the South of Brazil. Nutrient Cycling in Agroecosystems.

Jarvis, S.C., Yamulki, S., and Brown, L., 2001. Sources of nitrogen oxide emissions in intensive grassland management. Phyton, 41, 107-118.

Jensen, E.S.a.H.-N., H. , 2003. How can increased use of biological N2 fixation in agriculture benefit the environment? . Plant and Soil 252, 177-186.

Jones, S.K., Rees, R.M., Skiba, U.M., Ball, B.C., 2005. Greenhouse gas emissions from a managed grassland. Global and Planetary Change 47, 201-211.

Jones, S.K., Rees, R.M., Skiba, U.M., Ball, B.C., 2007. Influence of organic and mineral N fertiliser on N_2O fluxes from a temperate grassland. Agriculture, Ecosystems & Environment 121, 74-83.

Lampkin, N., 1990. Organic Farming. p.715.

Larsson, L., Ferm, M., Kasimir-Klemedtsson, A., Klemedtsson, L., 1998. Ammonia and nitrous oxide emissions from grass and alfalfa mulches. Nutrient Cycling in Agroecosystems 51, 41-46.

Ledgard, S.F., 2001. Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. Plant and Soil 228, 43-49.

Lupwayi, N.Z., Kennedy, A.C., 2007. Grain Legumes in Northern Great Plains: Impacts on Selected Biological Soil Processes. Agronomy Journal 99, 1700-1709.

Maggiotto, S.R., Webb, J.A., Wagner-Riddle, C., Thurtell, G.W. , 2000. Nitrous and nitrogen oxide emissions from turfgrass receiving different forms of nitrogen fertilizer. Journal of Environmental Quality 29.

Marriott, C.A., Thomas, R.J., Smith, M.A., Logan, K.A.B., Baird, M.A., and Ironside, A.D., 1988. The effects of temperature and nitrogen interactions on growth and nitrogen assimilation of white clover. Plant and Soil 111, 43-51.

Mogge, B., Kaiser, E.-A., Munch, J.-C., 1999. Nitrous oxide emissions and denitrification N-losses from agricultural soils in the Bornhöved Lake region: influence of organic fertilizers and land-use. Soil Biology and Biochemistry 31, 1245-1252.

Murray, P.J., and Jorgensen, M., and Gill, E., 2000. Effects of temperature on growth and morphology of two varieties of white clover (Trifolium repens L.)and their impact on soil microbial acivity. Annals of Applied Biology 137, 305-309.

Nemecek, T., Erzinger, S., 2005. Modelling Representative Life Cycle Inventories for Swiss Arable Crops (9 pp). The International Journal of Life Cycle Assessment 10, 68-76.

Nickerson, 2008. http://www.nickersonseeds.co.uk/.

Olesen, J.E., Rasmussen, I.A., Askegaard, M., and Kristensen, K., 2002. Whole-rotation dry matter and nitrogen grain yields from the first course of an organic farming crop rotation experiment. Journal of. Agricultural Science 139 361-370.

Pastor, J., Binkley, D., 1998. Nitrogen fixation and the mass balances of carbon and nitrogen in ecosystems. Biogeochemistry 43, 63-78.

Patra, D.D., Sachdev, M.S., Subbiah, B.V., 1986. 15N studies on the transfer of legume-fixed nitrogen to associated cereals in intercropping systems. Biology and Fertility of Soils 2, 165-171.

Prather, M., Ehhalt, D., al.:, e., 2001. Atmospheric chemistry and greenhouse gases, in: Climate Change. The Scientific Basis, edited by: Houghton, J. T., Ding, Y., Griggs, D. J., et al., 239- 287, Cambridge University Press, Cambridge, UK, 2001.

Raddatz, R.L., 2007. Evidence for the influence of agriculture on weather and climate through the transformation and management of vegetation: Illustrated by examples from the Canadian Prairies. Agricultural and Forest Meteorology 142, 186-202.

Reay, D.S., Edwards, A.C., Smith, K.A., 2004a. Determinants of nitrous oxide emission from agricultural drainage waters. Water, Air, & Soil Pollution: Focus 4, 107-115.

Reay, D.S., Smith, K.A., Edwards, A.C., 2004b. Nitrous Oxide in Agricultural Drainage Waters Following Field Fertilisation. Water, Air and Soil Pollution: Focus 4, 437-451.

Robertson, G.P., Paul, E.A., Harwood, R.R., 2000. Greenhouse Gases in Intensive Agriculture: Contributions of Individual Gases to the Radiative Forcing of the Atmosphere. Science 289, 1922-1925.

Rochette, P., Janzen, H., 2005. Towards a Revised Coefficient for Estimating N_2O Emissions from Legumes. Nutrient Cycling in Agroecosystems 73, 171-179.

Sangakkara, U.R., Richner, W., Steinebrunner, F., Stamp, P., 2003. Impact of the Cropping Systems of a Minor Dry Season on the Growth, Yields and Nitrogen Uptake of Maize Grown in the Humid Tropics during the Major Rainy Season. Journal of Agronomy and Crop Science 189, 361-366.

Skiba, U.,. DiMarco, C., Hargreaves, K., Sneath, R., McCartney, L., 2006. Nitrous oxide emissions from a dung heap measured by chambers and plume methods. Agriculture, Ecosystems & Environment 112, 135-139.

Skiba, U., Smith, K.A., and Fowler, D., 1993. Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. Soil Biology and Biochemistry 25 pp. 152-156.

Smith, K.A., Conen, F., 2004. Impacts of land management on fluxes of trace greenhouse gases. Soil Use and Management 20, 255-263.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F., O'Mara, C.R., B. Scholes, O. Sirotenko 2007. Agriculture. In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change In: [B. Metz, O.R.D., P.R. Bosch, R. Dave, L.A. Meyer (eds)], (Ed.). Cambridge University Press,Cambridge, United Kingdom and New York, NY, USA.

Smith, K. A. and F. Conen. "Impacts of land management on fluxes of trace greenhouse gases." Soil Use and Management 20 (2004): 255-63.

Thorsted, M.D., Olesen, J.E., Weiner, J., 2006. Width of clover strips and wheat rows influence grain yield in winter wheat/white clover intercropping. Field Crops Research 95, 280-290.

Vinten, A.J.A., Ball, B.C., O'Sullivan, M.F., Henshall, J.K., Howard, R., Wright, F., Ritchie, R., 2002. The effects of cultivation method and timing, previous sward and fertilizer level on subsequent crop yields and nitrate leaching following cultivation of long-term grazed grass and grass-clover swards. The Journal of Agricultural Science 139, 245-256.

Vinten, A.J.A., Howard, R.S., and Redman, M.H., 1991. Measurement of nitrate leaching losses from arable plots under different nitrogen input regimes. Soil Use and Management 7, 3-13.

Vinten, A.J.A., Vivian, B.J., Wright, F., Howard, R.S., 1994. A comparative study of nitrate leaching from soils of differing textures under similar climatic and cropping conditions. Journal of Hydrology 159, 197-213.

Vinther, F.P., Jensen, E.S., 2000. Estimating legume N2 fixation in grass-clover mixtures of a grazed organic cropping system using two 15N methods. Agriculture, Ecosystems & Environment 78, 139-147.

Willey, R.W., 1979. Intercropping: Its importance and research needs.Competition and yield advantages. Field Crop Abstr. Part 1., 1-10.

Wood, M., 1996. Nitrogen fixation: How much and at what cost? Legumes in Sustainable Farming systems Systems, 26-35.

Xiao, Y., Li, L., Zhang, F., 2004. Effect of root contact on interspecific competition and N transfer between wheat and fababean using direct and indirect 15N techniques. Plant and Soil 262, 45-54.

Zhang, F., Li, L., 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. Plant and Soil 248, 305-312.

Zheng, X., Wang, M., Wang, Y., Shen, R., Gou, J., Li, J., Jin, J., Li, L., 2000. Impacts of soil moisture on nitrous oxide emission from croplands: a case study on the rice-based agro-ecosystem in Southeast China. Chemosphere - Global Change Science 2, 207-224.

7. Paper 3

Root development effects during and after the use of intercropping.

Valentini A. Pappa, Robert M. Rees, John A. Baddeley, Robin L. Walker,

Christine A. Watson

To be Submitted to 'Plant and Soil'

Paper 3

Root development effects during and after the use of intercropping.

Valentini A. Pappa, Robert M. Rees, John A. Baddeley, Robin L. Walker,

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Abstract

Intercropping, the growth of two or more crops on the same area and the same time has been used to maximise use of resources, improve crop and soil quality and reduce the N losses of agricultural ecosystems. The hypotheses of this study were that the cereal (barley) roots are affected by the accompanying legume variety/ species and that there is an effect of the following crop in an organically-managed rotation. An experiment was established near Edinburgh, SE Scotland, UK, on 12 hydrologically-isolated plots. Treatments were a spring barley (*Hordeum vulgare* cv. *Westminster)* monoculture and intercrops of barley / white clover (*Trifolium repens* cv. *Alice*) and barley/ pea (*Pisum sativum* cv. *Zero4* or cv. *Nitouche*) in 2006. Spring oats (*Avena sativa* cv. *Firth*) were planted on all plots in 2007. No fertilisers, herbicides or pesticides were used at any stage of the experiment. Root length density, average diameter, volume, total N content and root:shoot area were measured at key stages in 2006 and 2007 for barley and oats, respectively. The two barley/ pea intercrops had different below-ground characteristics. These had different effects on the following crop, with barley/ pea cv. *Zero 4* benefiting in the year of growth and the barley/ pea cv. *Nitouche* benefiting in the following year. Barley/ pea cv. *Zero 4* had the highest above-ground plant density and widest root diameter (in 30-60 cm depth) in comparison with the barley plants growing with pea cv. *Nitouche*. Barley roots intercropped with clover had the highest root length density and widest root diameter (in 0-30 cm depth) of all the treatments. Barley monocrop roots had the highest root:shoot ratio. The following year, the oat plants where growing barley/ pea cv. *Nitouche* had been grown previously, the highest root length density and volume including the highest root:shoot ratio (0.33). Oats grown where there had previously been a barley monocrop had the smallest root diameter (0.59 mm).

Keywords: barley, intercropping, nitrogen, oat, roots

1. Introduction

There is an urgent need to develop agricultural systems that have a high level of productivity, but are at the same time, sustainable and have a reduced impact on the environment (Crews and Peoples, 2004). Intercropping, the growing of two or more crops on the same piece of land in the same year, may help achieve this goal. Intercropping has been practised in many parts of the world (Fujita *et al.*, 1992), but almost 80% of published data are from warmer climates such as Southern Asia and Africa (Connolly *et al.*, 2001).

The main objective of intercropping has been to maximise the use of resources such as space, light and nutrients (Willey, 1990; Li *et al.*, 2001), as well as to improve crop quality and quantity (Ofori *et al.*, 1987; Baumann *et al.*, 2002) and reduce accumulation of NO_3–N in the soil profile (Li *et al.*, 2005). Other benefits include improvements in the quality of drainage water where intercropping reduces the need for synthetic fertilisers (Horwith, 1985; Jensen and Hauggaard-Nielsen, 2003).

When cereals are growing with legumes, physiological and morphological differences affect their mutual relationship. While this has been studied extensively above-ground, there is very little data on belowground processes in species mixtures. Many processes that occur in the rhizosphere of mixtures need further research, as they are not clearly understood and several factors affect the microbial activity and root structure (Connolly *et al.*, 2001; Zhang and Li, 2003). The rhizosphere is the zone around the roots where soil, micro-organisms and roots mutually contribute to the ecosystem. Compared with the bulk soil or non-rhizosphere soil, the rhizosphere has different biological, physical and chemical soil properties. The rhizosphere is rich in root exudates, and can, play a major role in nutrient mobilisation and microbial activities (Dakora and Phillips, 2002; Dakora, 2003).

Most annual crop mixtures such as cereals and legumes are grown at the same time and in the same space, and develop root systems that explore the same soil zone for resources (Horwith, 1985; Jensen, 2003; Ndakidemi, 2006). Under such conditions, below-ground competition for resources such as nutrients and water is likely to occur. For example, research has shown that activities in mixed cropping systems involving maize and cowpea occur between the top 30 – 45 cm of soil, and their root density decreased with depth (Kumar and Goh, 2000). Because of these interactions, cowpea yields can be reduced significantly relative to that of maize (Watiki *et al.*, 1993). Root systems in mixtures can provide some advantages in soil such as carbon enrichment through carbon turnover (Vanlauwe *et al.*, 1997), and release of phenolics, phytosiderophores and carboxylic acids as root exudates by component plants (Dakora and Phillips, 2002; Dakora, 2003).

The most important benefit, though, is the potential for improved nitrogen supply by biological nitrogen fixation from the legume crops (Giller and Cadisch, 1995). However, studies on N_2 fixation in complex cereal/legume mixtures are few (Stern, 1993a; Hauggaard-Nielsen *et al.*, 2001). In intercropping, the legume usually fixes N_2 that benefits the cereal component, since it depends on nitrogen for maximum yield (Ofori *et al.*, 1987). Controlled environment studies have shown a significant direct transfer of fixed-N to the associated non-legume (Stern, 1993a; McNeill *et al.*, 1997; Høgh-Jensen, 2000; Dahlin and Mårtensson, 2008). In mixed stands, where row arrangements and the distance between legume and cereal are large, nitrogen transfer could decrease (Thorsted *et al.*, 2006). However, Jensen (1996) found that barley plants are more competitive than pea due to faster growth and deeper root systems (Hauggaard-Nielsen *et al.*, 2001; Hauggaard-Nielsen *et al.*, 2009).

The hypotheses of this study were that the cereal (barley) root growth is affected by the accompanying legume variety/ species and there is an effect on the following crop in an organically-managed rotation.

2. Materials and Methods

2.1. Site description and soil

The experiment was carried out 8 km south of Edinburgh, Scotland (lat. 55° 51'N, long. 3° 12'W). The overall field size is 0.4ha and is divided in 12 plots (each 9 m x 25 m). Further details of the full experimental facilities are given in (Vinten *et al.*, 1992).The plots are hydrologicaly isolated in 1992. The 25 year mean annual rainfall is 676 mm. Monthly average air temperature and rainfall during the experimental period are shown in Figure 1. The plots were fallow for the period 2003-2005. The day before sowing (23rd April 2006), the soil was sampled at 0-20. In the top soil (0-20 cm), the water content at field capacity was 19 ± 0.5 % (v/v) and the soil bulk density was 1.19 ± 0.01 Mg m^{-3} (mean ± SE, n=5). The top soil was a sandy loam (Eutric Cambisol, Macmerry Series) with 65% sand, 20% silt and 15% clay developed from partially sorted glacial till, the upper 0.5 to 1.5 m of the soil profile is freely drained.

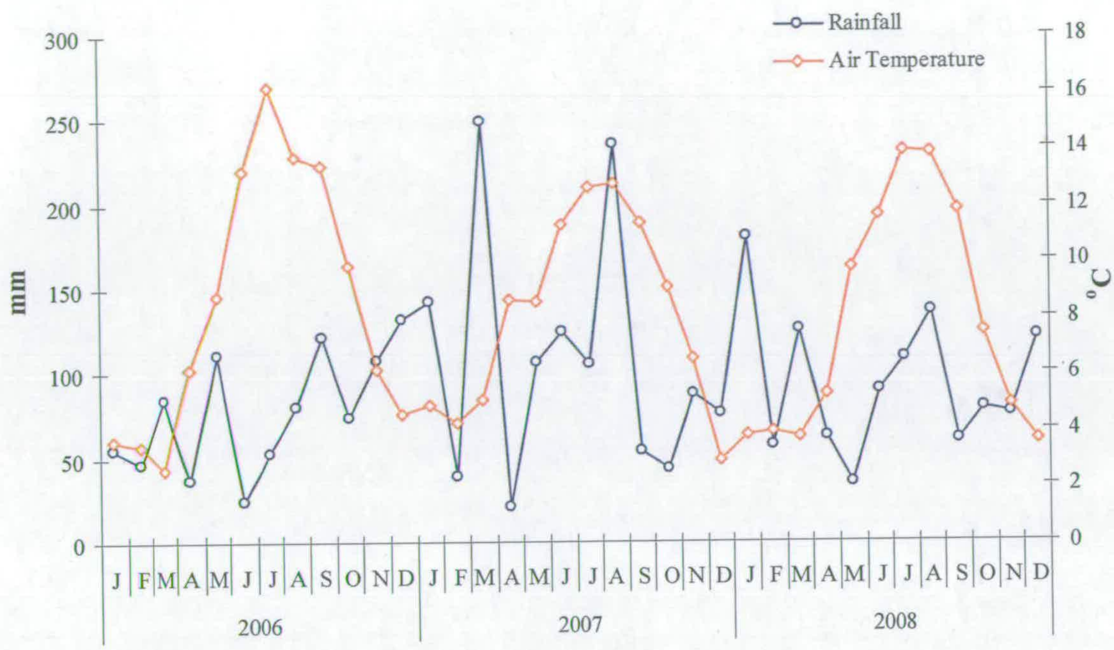


Figure 1: Monthly total rainfall (mm) and monthly average air temperature (°C).

In 2006 when the current study commenced, the treatments were: barley (*Hordeum vulgare* cv. Westminster) as a monocrop; Pea (*Pisum sativum* cv. *Zero 4*)/ barley intercrop; Pea (*Pisum sativum* cv. *Nitouche*)/ barley intercrop; White clover (*Trifolium repens* cv. Alice) /barley intercrop. Westminster is a medium-tall variety of barley that is widely grown for malting and livestock feed due to its combination of longer than average straw and good disease resistance characteristics (Nickerson, 2008). *Nitouche* is a popular large blue pea variety and has a consistent performance, good agronomic characters and suitability for premium markets. *Nitouche* produces a large, smooth, round pea and retains its colour well. *Nitouche* also has a high level of resistance to downy mildew and long straw with good standing ability making it relatively easy to harvest. *Zero 4* is a small blue combining pea with a unique combination of agronomic characteristics: short straw, excellent standing ability and very early maturity. When sown at its optimum seed rate of 110 seeds/ m^2, *Zero 4* has a similar yield potential to *Nitouche* (optimum seed rate of 70 seeds/ m^2) (Barenbrug, 2006). *Alice* is a tall, large leaved white clover developed for exceptional yields of palatable, high quality, high protein forage included in pastures mixtures. Its vigorous spring and summer growth makes it a good choice for cutting or grazing management, as well as for N fixation through out season and good winter- hardiness and cover (HGCA, 2008).

2.2. Experimental design

Each treatment was replicated three times in a blocked design. In the intercrop treatments, the seed rates for the pea and barley followed a 50:50 replacement design. This means that the target intercrop density was 50% of the monoculture density of each crop. Based on mon-cropping seed rates of 200 kg ha^{-1} for the barley and 250 kg ha^{-1} for the peas which equated to approximately 350, 75 and 110 germinable seeds m^{-2} for Westminster, *Nitouche* and Zero-4 respectively, the intercrop components were half these values. The sowing date for these treatments was the 24th April 2006. In the second growing season (2007), all the plots were sown with spring oats (*Avena sativa* cv. Firth) on the 3rd April at a seed rate of 250 kg ha^{-1} (approximately 450-500 germinable seeds m^{-2}). Firth is a very popular spring oat variety and exhibits a high kernel content and good resistance to mildew (Szumigalski and Van Acker, 2006). In the third growing season, perennial ryegrass (PRG) was sown in all plots in a 50:50 mixture of *Aberavon* and *Aberdart* at a total seed rate of 35 kg ha^{-1}. The plots were tilled using a mouldboard plough followed by cultivating (rotary hoe), seeding and rolling. No fertilisers, herbicides or pesticides were used. The cropping pattern is shown in Table 1.

Table 1: Cropping 2003-2008 on the drainage plots.

Year	Crops	Treatments	*Varieties*
2003	Fallow		
2004	Fallow		
2005	Fallow		
2006	Intercropping cereal/ legumes	Spring Barley/ White clover Spring Barley/ Spring Pea 1 Spring Barley/ Spring Pea 2 Spring Barley	*Westminster/ Alice* *Westminster/ Zero 4* *Westminster/ Nitouche* *Westminster*
2007	Cereal	Spring Oat	*Firth*
2008	Grass	Perennial ryegrass	*AberAvon & Aberstar*

2.3. Harvest and analyses

2.3.1. Root biomass

Below-ground samples were collected prior to crop harvest (11th September 2006 and 11th September 2007) by collecting soil cores to 100 cm depth (10 cm in diameter) in 2006 and 40 cm depth in 2007 (7 cm in diameter). The cores were analysed within 1-2 days or stored below -5 °C for later analysis. The cores from 2006 were split into intervals of 30 cm to allow comparison between different soil depths. In 2007, the cores were analysed as one sample (0-40 cm) for each of the treatments. At the time of analysis the cores were placed in water and washed the next day using three different sieves (0.5 mm to 0.2 mm to 0.05 mm). The separation of the two root systems (barley and legume) was difficult, but was achieved by including one plant of each intercropped species in the soil core and recognising the difference in root architecture/ structure between the cereals and legumes. Before measurement, any organic debris or dead root material was removed. The root samples were then arranged in a transparent, water-proof tray filled with water. The roots were scanned with an A3 Epson, Expression 836XL scanner operating at 600 dpi and fitted with a transparency adaptor to produce a uniform, shadow-free white background. Images were 8-bit greyscale and saved in uncompressed TIFF format. Scans were analysed with Win RHIZO software (2003b) (Régent Instruments, Québec, Canada), which measured the root length, root volume and root diameter for the barley and oat crops. Images were saved in files for later validations, analyses or for visualization in other programs. Then, the root fresh weight was recorded, after the excess water had been removed and the roots were dried at 60 °C for 24 h. The oven-dried samples were ground using a Glen Creston hammer mill with a 1 mm mesh. A sub sample of this was then ball milled to a flour-like consistency using a Retch ball mill. These samples were analyzed for total N by combustion using a PDZ Europa Mass Spectrometer.

Length density of the roots is the root scanned length per unit scanning area and was calculated by divide the root length with the column of soil-root that were collected. Root: shoot surface area was calculated by divide the roof surface area and the shoot area (including shoots and leaves) after scanning the samples with the A3 scanner.

2.3.2. Plant biomass

All plants were harvested above-ground every growing season and initially separated into cereal, legumes and weeds. Plant biomass was measured monthly using quadrats (50 cm x 50 cm) by harvesting all the plant material within it and then dried at 60°C for 24 h and weighed. Each part (stems, ears, pods) of each crop was weighed separately and subsamples were taken for determination of the total N concentration (%) of the above-ground crop. The same procedure was followed the next year (2007) for the spring oat crop which was separated into straw and grain.

For barley plants (2006), the cereal growth stage GS 69 (flowering completed) was at 80 days from sowing. For oat plants (2007), the growth stage GS 77, was at 100 days from sowing respectively (HGCA, 2005/06).

2.4. Statistical analyses

All measured variables were normally distributed tested by Anderson-Darling test (significance .05) and statistical analyses by ANOVA were performed using either Minitab 15 or GenStat 8 software. In all cases, significant differences were calculated at the 5% level.

3. Results

3.1. Root lengths and diameters

3.1.1. Intercropping, 2006

Root length density is one of the most functionally important and frequently-measured traits in agricultural studies. The intercrop treatments resulted in changes in total root length in the surface soil horizons (0-30 cm). Barley growing in the barley/ clover intercrop had a root length density of 1.54 cm cm^{-3}, which was significantly greater ($P<0.001$) than the barley monocrop, at 1.19 cm cm^{-3}. The two pea varieties intercropped with barley were significantly different; barley/ pea cv. *Zero 4* had a significant higher value ($P<0.05$) of 1 cm cm^{-3} in comparison with barley/ pea cv. *Nitouche* at 0.79 cm cm^{-3}. In the lower soil layers (30-60 cm and 60-90 cm), there were no significant differences in root length (Fig 2).

The average diameter of barley roots growing at 0-30 cm depth in the barley/ clover treatment was 0.37 mm which was also significantly greater ($P< 0.05$) than that in the two pea treatments (0.32 mm and 0.34 mm for barley/ pea cv. *Zero 4* and barley/ pea cv. *Nitouche*, respectively). The average root diameter of barley monocrop was 0.37 mm. At a depth of 30-60, barley intercropped with pea cv. *Zero 4* and clover had the widest diameter (0.37 mm and 0.35 mm, respectively) and this was significantly different from the other barley/ pea cv. *Nitouche* (0.32 mm) and barley monocrop (0.30mm) ($P<0.05$). At a depth of 60-90 cm, the barley intercropped with clover had the highest value of 0.40 mm and this was significantly different from the other treatments ($P<0.005$). There was no significant difference between he two pea treatments (Fig.3).

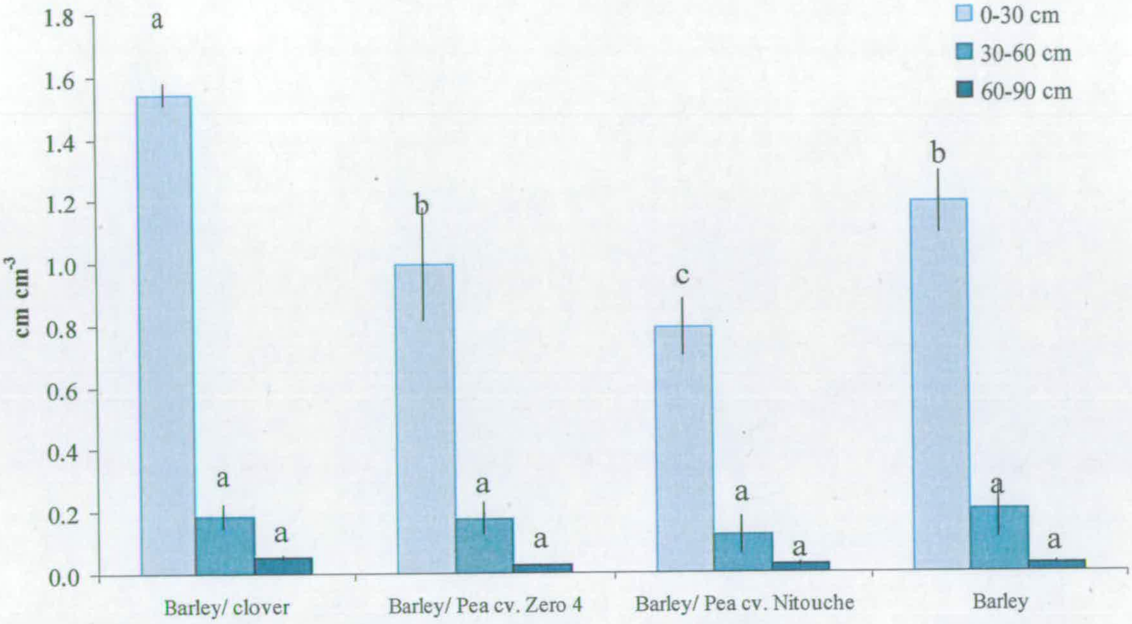


Figure 2: Mean **barley roots length density for three depths (0-30 cm; 30-60 cm; 60-90 cm) in July, 2006 at growth stage 69 (flowering completed) (n=3) ± SE (bars).** The values with different letters (a, b) are significant different (P<0.05) between each depth.

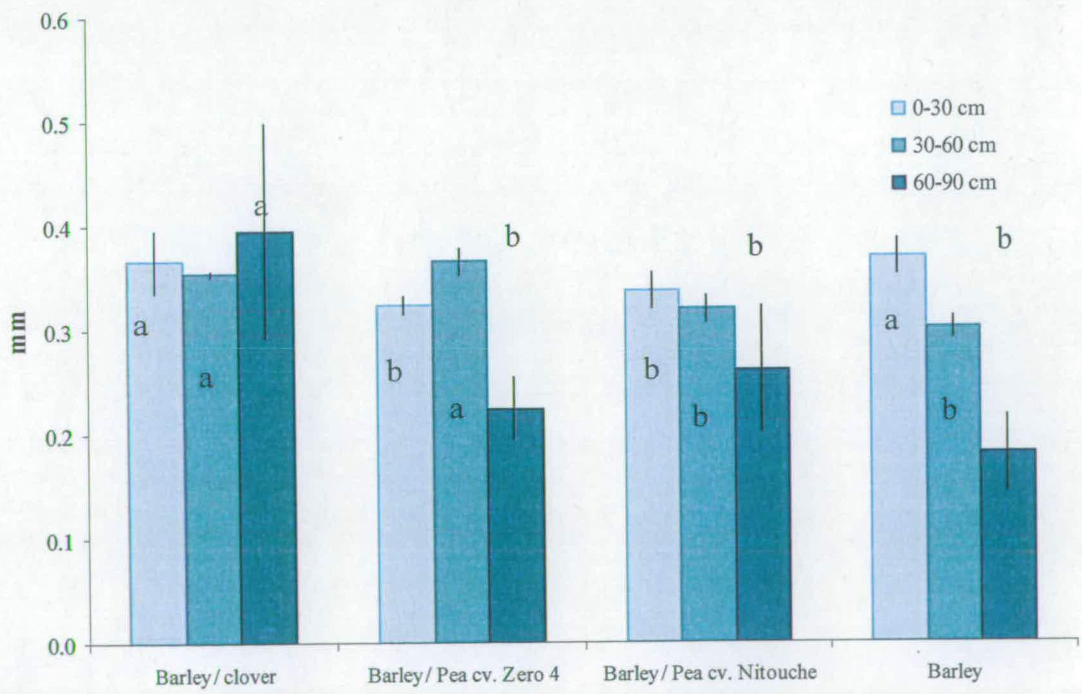


Figure 3: Average diameter of barley roots for three depths (0-30 cm; 30-60 cm; 60-90 cm) in July, 2006 at growth stage 69 (flowering completed) (n=3) ± SE (bars). The values with different letters (a, b) are significant different (P<0.05) between each depth.

3.1.2. Oats, 2007

Oats were grown across the experiment in 2007 on plots that had in the previous year been used for the intercrops. In these plots the root length density of the oat crop sampled previously under barley/ pea cv. *Nitouche* had the highest values (5.98 cm cm^{-3} at 0-40 cm depth) being significantly different from the other treatments ($P<0.001$). The other treatments were not significantly different (Fig. 4).

The average diameter for the roots of oats grown in the previous treatments with legumes was significantly different from those grown in the barley monocrop treatment (0.59 mm) ($P<0.05$). The root diameters of oats from the other three legume treatments were not significantly different; however oat roots from the barley/ pea cv. *Nitouche* had the largest diameter of 1.24 mm (Fig. 5).

The volume of oat roots where barley/ pea cv. *Nitouche* had previously been grown was 8.50 cm^3 and was significantly greater than that in other treatments ($P<0.001$) (Fig. 6). The roots of oats in which barley/ pea had previously been grown were significantly different with the oats grown in the barley/ pea cv. *Zero 4* having the largest root volume of 8.5 cm^3 ($P<0.005$).

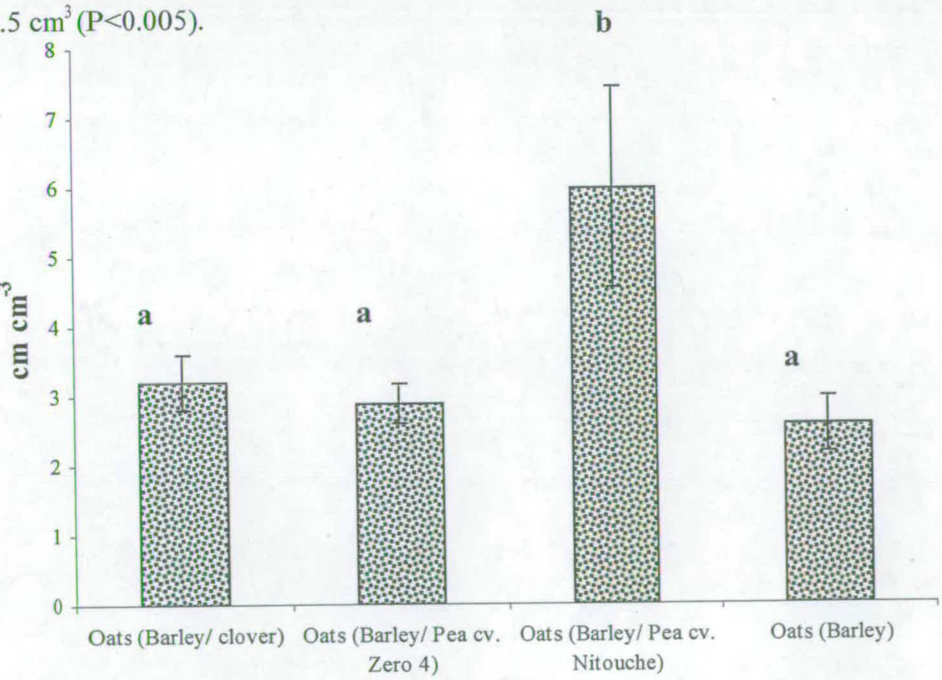


Figure 4: Root length density of oat roots for 0-40 cm soil depth in July, 2007 at the growth stage of 77 (late milking). The label on the x axis refers to the previous season's crop treatments. Values are means ± SE (bars). The values with different letters (a, b) are significant different ($P<0.05$) .

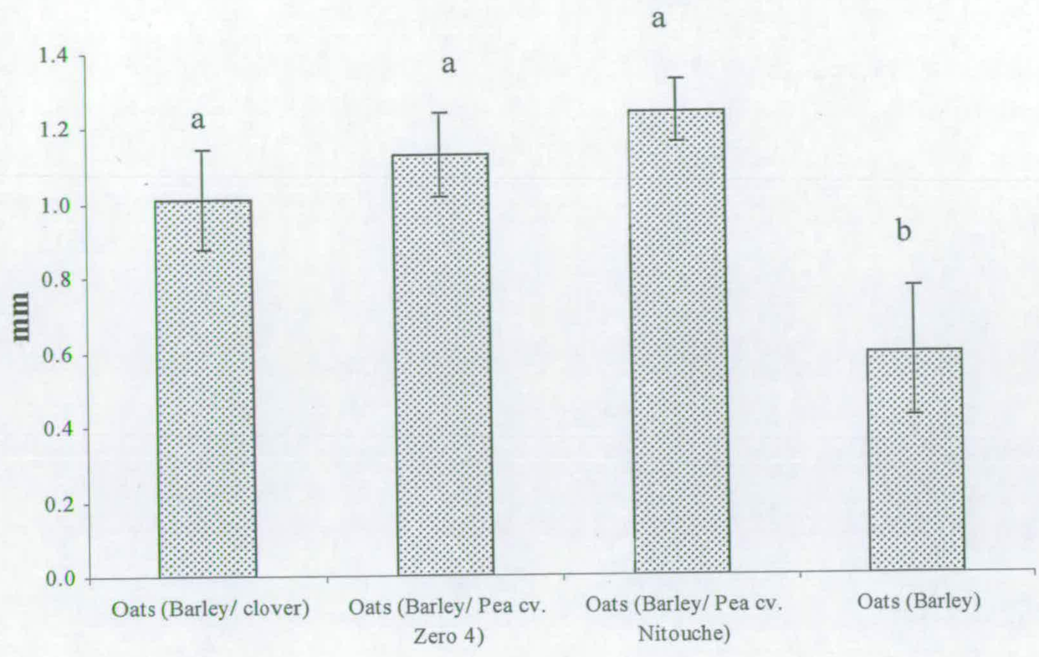


Figure 5: Average diameter of oat roots for 0-40 cm soil depth in July, 2007 at the growth stage of 77 (late milking). The label on the x axis refers to the previous season's crop treatments ± SE (bars). The values with different letters (a, b) are significant different (P<0.05).

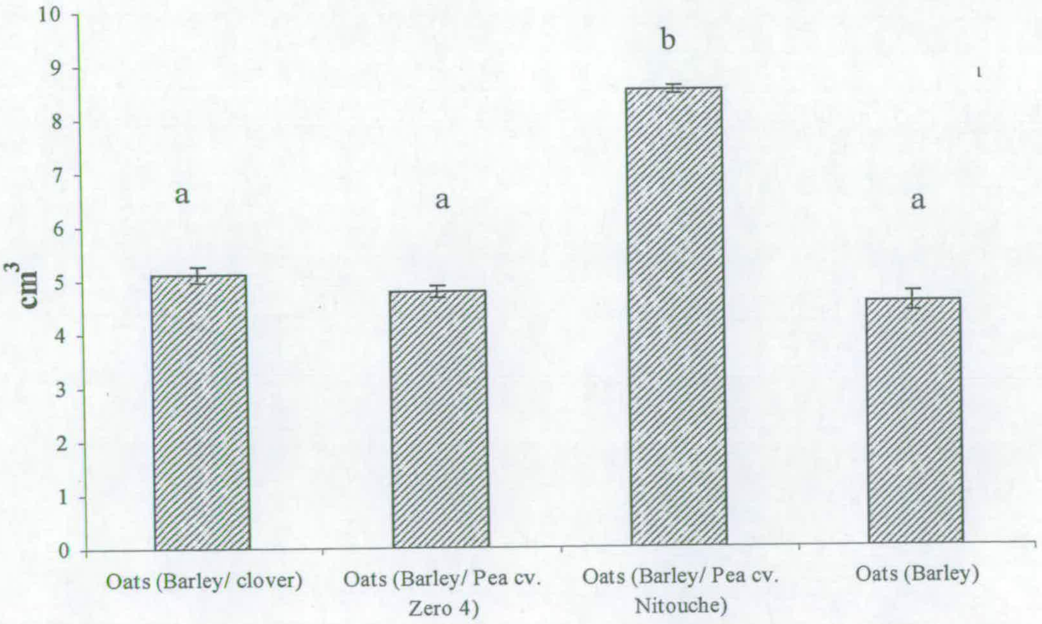


Figure 6: The root volume of oat roots for 0-40 cm soil depth in July, 2007 at the growth stage of 77 (late milking). The label on the x axis refers to the previous season's crop treatments ± SE (bars). The values with different letters (a, b) are significant different (P<0.05).

3.2. Root: shoot surface area ratio

3.2.1. Barley

The root: shoot surface area ratio of barley plants during the intercropping season was significantly different ($P<0.01$) with the barley monocrop having the highest ratio (26.16) for 0-90 cm soil depth. The barley/ clover treatment had the second highest ratio (24.47), but this was not significantly different from the barley monocrop. The barley/ pea cv. *Nitouche* (14.95) and barley/ cv. *Zero 4* (13.93) treatments were significantly lower from each of the other two treatments ($P<0.05$) (Fig. 7). The same pattern for the root: shoot area was found using the data for the root area in the 0-30 cm soil layer (data not presented).

3.2.2. Oats

The root: shoot ratio of oats in the second growing season was calculated by using the root area for the 0-40 cm soil layer. The ratio in the oat plants growing in plots previous cropped with barley/ pea cv. *Nitouche* (0.33) was significantly higher ($P<0.01$) than the other treatments having double the ratio of the barley/ pea cv. *Zero 4* (0.16) (Fig. 8).

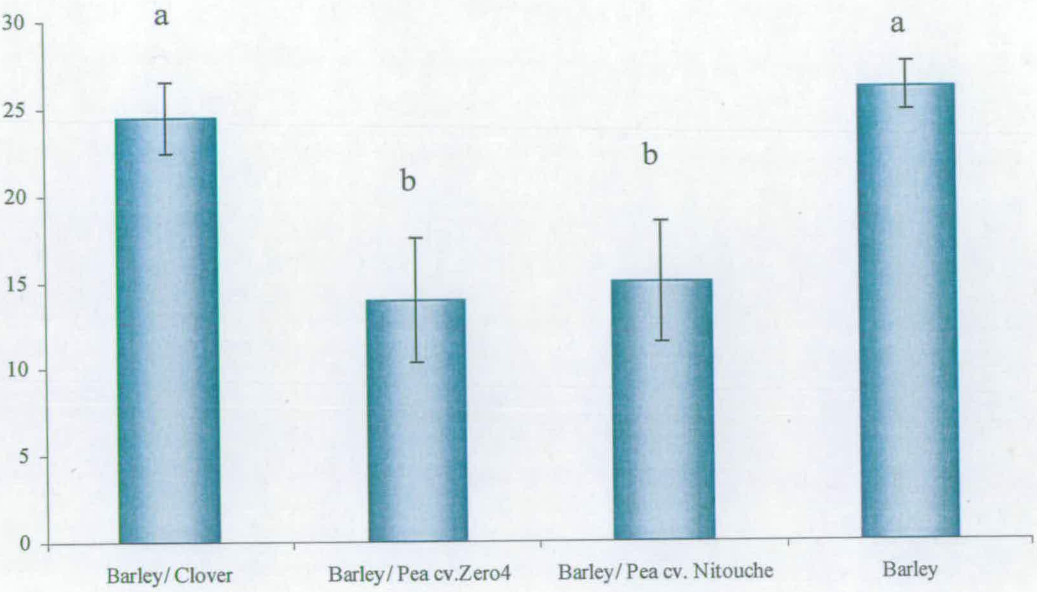


Figure 7: Root: shoot area ratios of barley plants at growth stage 69 (flowering completed) (July, 2006) in the four different treatments. The root area used here is in 0-90 cm soil depth ± SE (bars). The values with different letters (a, b) are significant different (P<0.05).

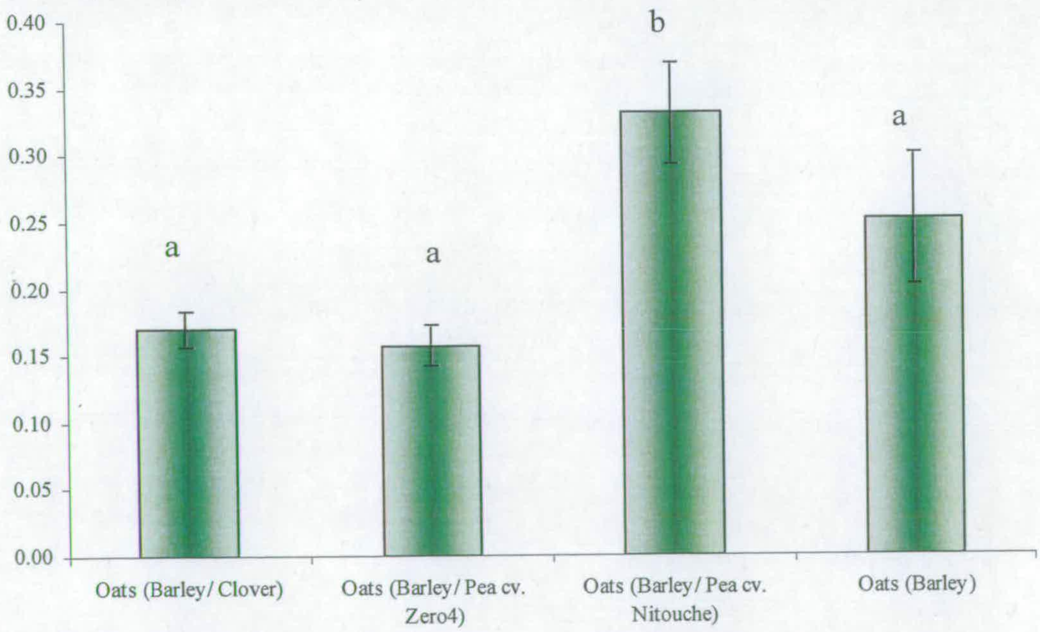


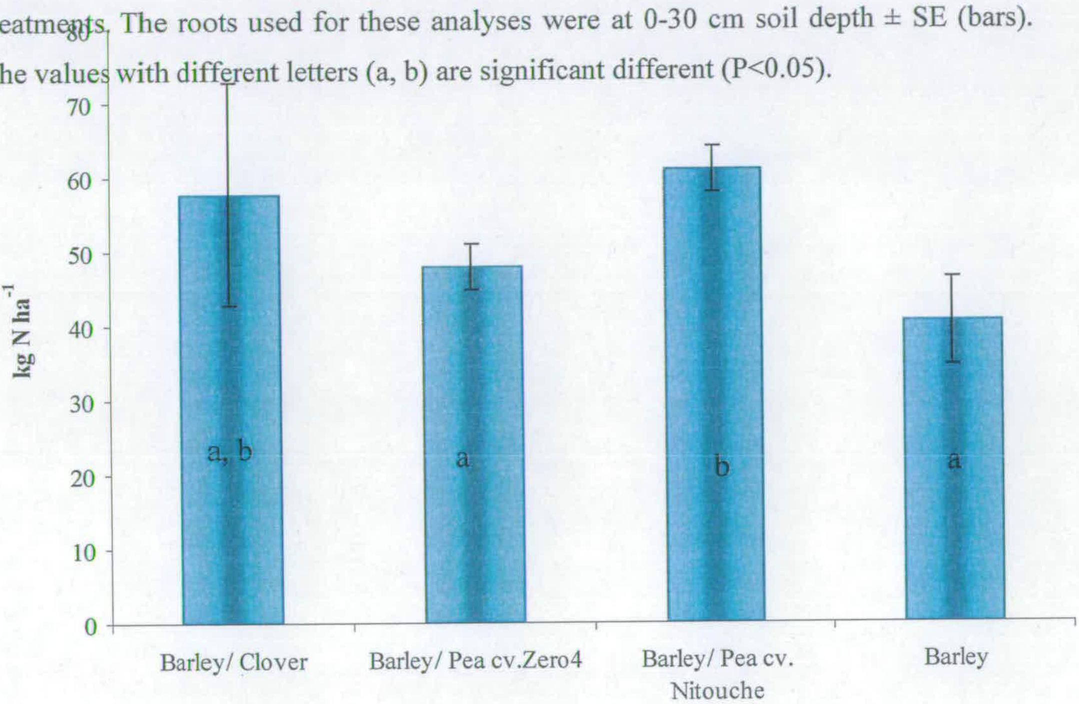
Figure 8: Root: shoot area ratios of oat plants at growth stage 77 (late milking) (July, 2007) in the four different treatments. The root area used here is in 0-40 cm soil depth. The labels on the x axis refer to the previous season's crop treatments ± SE (bars). The values with different letters (a, b) are significant different (P<0.05).

3.3. *Total N in roots*

3.3.1. *Barley plants*

The total N content of barley roots in soil depth of 0-30 cm in the four different treatments was significantly higher for barley/ pea cv. *Nitouche* (61 kg N ha^{-1}) when compared with the barley/ pea cv. *Zero 4* (48 kg N ha^{-1}) and barley monocrop (40 kg N ha^{-1}).

Figure 9: Total N in barley roots at the growth stage 69 (July, 2006) at the four different treatments. The roots used for these analyses were at 0-30 cm soil depth ± SE (bars). The values with different letters (a, b) are significant different (P<0.05).



3.3.2. Oat plants

In the second year of the experiment, the N content of the oat roots was significantly greater ($P<0.05$) where the previous crop had been barley/ clover, with an N content of 86 kg ha^{-1}. There were no differences in the N contents of oat roots growing in the areas that had previously been used for the two barley/ pea treatments (Fig.10).

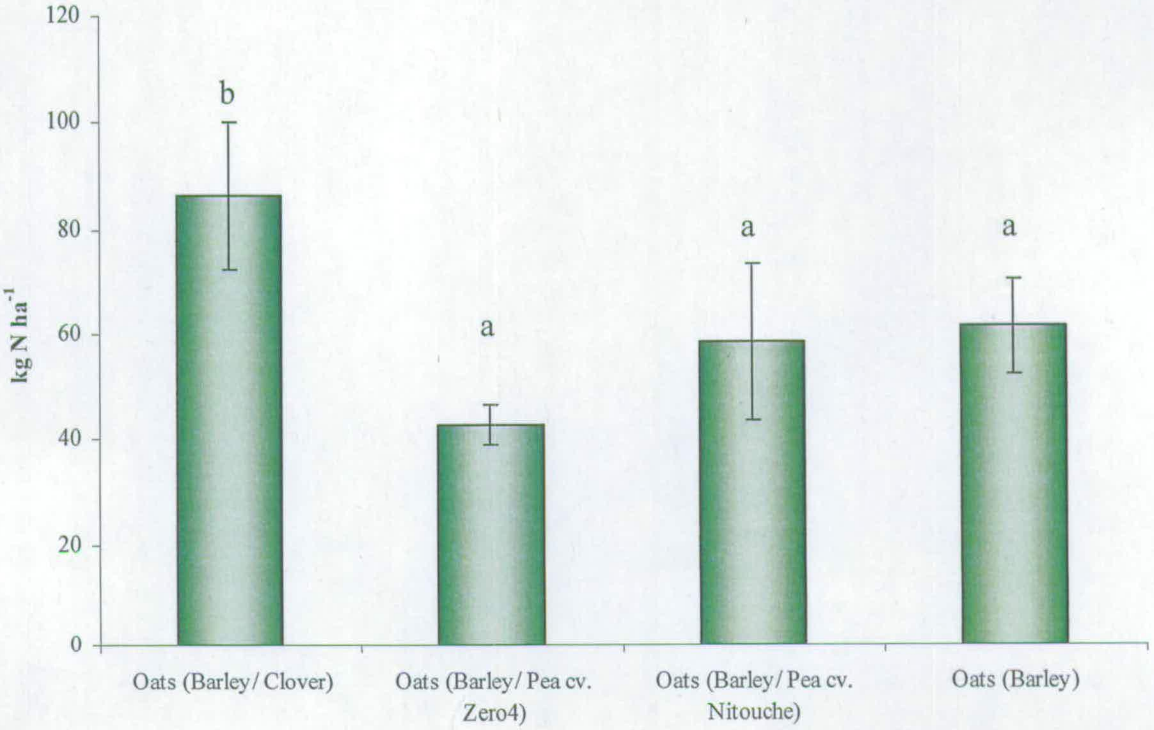


Figure 10: Total N in oat plants at the GS 77 (2006) at the four different treatments. The roots used were in 0-30 cm soil depth ± SE (bars).

4. Discussion

Cereal-legume intercrops exhibit large changes in above and below-ground biomass dynamics as a consequence of intra-species competition leading to a complex pattern of resource partitioning. Barley intercropped with clover during the first year of the experiment had higher root length density than other treatments. Root length density is the attribute most related to the rate of nutrient uptake as it has a clear functional significance and is closely correlated with the volume of soil explored (Hoad *et al.*, 2001). Additionally, we found that the barley plants intercropped with clover had the largest diameter in most of the depths, which can be attributed to thicker roots caused by higher water filled pore space (WFPS) (Rosolem *et al.*, 2002). The accumulation of root mass at 0-30 cm was highly correlated with the availability of nutrients as presented by Hauggaard *et al.* (2001) that has shown intercropped barley had a deeper growing root system and a faster side root development than mono-crops. Ryser (1998) found that root length increased with decreasing nutrient availability, as the roots search for available nutrient in the soil.

We found that the two barley/ pea intercrops had similar above-ground biomass and grain yield (Pappa *et al.*, Paper 1). However, when barley was intercropped with peas there were significantly longer roots in the barley/ pea cv. *Zero 4* compared to barley/ pea cv. *Nitouche*. Thus, there is a clear varietal difference affecting the development of the root system of barley. The two pea varieties used for the study have different root systems with pea cv. *Zero* having thinner roots. It has also been reported that different legume species have different microbial populations and activity attributed to the production of root exudates (Grayston *et al.*, 1998; Ryan *et al.*, 2001).

The observation that oat roots grown in plots previously cropped with barley/ pea cv *Nitouche* were longer and had a larger volume indicated a response to soil nutrient availability with higher NO_3^- concentration in both soil depths (0-20 cm and 20-40 cm). In general, nutrients seems to become available when the roots die either because through tissue death and turnover (Bingham and Rees, 2008) or as a consequence of accelerated damage from below-ground herbivores/ pathogens or because of frost and drought, or management practices such as ploughing (Hoad *et al.*, 2001). At the experimental it, during the winter of 2006-2007, there were few days of frost (Fig. 1), resulting in minimum damage of roots and plant material. Clover plants were re-grew and pea plants germinated from the seed after the harvest resulting in a turn-over during spring cultivation. The longer root system of the intercropped pea might have also increased the aeration and the infiltration of water, as well as loosening the soil structure, which might have increased both the accessibility of organic matter to microorganisms and the mineralization rate (Stirzaker *et al.*, 1996).

The ratio of root:shoot area had shown that the barley monocrop had the highest value possibly due to low supply of nutrients that affect the root development more than that of the shoots. However, the barley intercropped with the different legume species and varieties had significantly higher shoot: root area ratios with barley/ clover having the highest value out of the legume intercrops, indicating a nutrient response. The availability of N in the plants can maintain the leaf area of the crop. As a result, a crop with an adequate N supply has lower root: shoot ratio than an N-deficient crop. If the soil N availability is lower, the rate of shoot growth usually decreases while the root growth can remain constant (Basra, 1994). The nitrogen accumulation in barley roots was greater in barley/clover, and significantly different between the two barley/ pea treatments; this was the opposite pattern observed in root length. This pattern also continued into the second year where the highest N accumulation in oat roots was observed where the previous crop had been barley/ clover perhaps due to the growth of clover plants during the mild winter months.

The use of legumes in combination with cereals can be advantageous as part of a rotation in low input and organic farming systems. There are benefits for both the current and the following crop by using the fixed N and the stored N, respectively (Watson *et al.*, 2002). Additionally, crop mixtures and rotations mimic the diversity of natural agro-ecosystems more closely than intensive mono-cropping systems. Alvey *et al.* (2003) found that a crop rotation can cause significant shifts in rhizosphere bacterial communities partially influenced by different species. Varying the type of crops grown can increase the level of soil organic matter, enhance the carbon capture and benefit agriculture and climate (Paul *et al.*, 1996; Paustian *et al.*, 1997; Adiku *et al.*, 2008).

Intercropping has been proven to enhance nutrient acquisition by the crops. The transfer of N can take place by movement of exudates, turnover and breakdown of legume roots and direct root to root transfer (Swinnen, 1994; Rogers *et al.*, 2001; Paynel and Cliquet, 2003; Yao *et al.*, 2003). It has been reported that a non-legume (e.g. cereal) intercropped with a legume has greater N acquisition than a non-legume monocrop (Francis and Brady, 1989; Stern, 1993b; Vandermeer, 1999; Li *et al.*, 2001) including different species. Hogh-Jensen and Schjoerring (2000) have found that N transfer was significantly higher from white clover to ryegrass than from red clover .

However, the root differences between the two pea varieties and legume species intercropped with spring barley in this study may have been caused by other factors, such as water availability may affect root distribution (Morris and Garrity, 1993) and solar radiation affects the growth of the two crops differently (Keating and Carberry, 1993), because in our study pea cv. *Nitouche* was a bigger variety than pea cv. Zero 4. In the future, the use of models might help to clarify some of these interacting effects by separating out component mechanisms responsible for observed differences in intercrop performance (Corre-Hellou *et al.*, 2007; Launay *et al.*, in press).

This experiment has highlighted the diverse root growth of barley intercropped with different legumes including different cultivars and species and additional effects in the following growing year. It has also demonstrated the need for longer term experiments to develop these systems and quantify their benefits in subsequent crops and in a rotation.

5. Conclusions

Low input and organic farming systems can benefit from legume-cereal intercropping. The study shows that the choice of legume cultivar or species is a key factor for the establishment of the cereal crop in the year of use and/ or the following year. Under-sowing clover even at low seed rates with cereals can contribute to a supply of N in cereal plants both years and more efficient retention of N by the system. The two barley/ pea intercrops had different below-ground characteristics. These had different effects on the following crop, with barley/ pea cv. *Zero 4* benefiting in the year of growth and the barley/ pea cv. *Nitouche* benefiting the following year.

The magnitude of these effects is highly sensitive to soil available N. For example, the choice of legume is important in optimising N fixation in intercropping designs. However, a more extensive study of the environmental impacts that different species/ varietal combinations and ratios have in order to optimise such systems is required. Having a better understanding of the interactions taking place within a cereal/ legume intercrop and how this can fit into a rotation has the potential to be a very useful management tool in the development of more sustainable cropping and agricultural systems. Using mathematical models to describe plant interactions in terms of functional attributes including root structures and development is useful for projecting scenarios for different climatic, environmental and management conditions.

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References

Adiku, S., Narh, S., Jones, J., Laryea, K., Dowuona, G., 2008. Short-term effects of crop rotation, residue management, and soil water on carbon mineralization in a tropical cropping system. Plant and Soil 311, 29-38.

Alvey, S., Yang, C.H., Buerkert, A., Crowley, D.E., 2003. Cereal/legume rotation effects on rhizosphere bacterial community structure in west african soils. Biology and Fertility of Soils 37, 73-82.

Barenbrug, 2006. www.barenbrug.com/site/modules/custom/products/getfile.php?siteid=1001&filename=Alice.pdf accessed January, 2006.

Basra, A.S., 1994. Mechanisms of plant growth and improved productivity: modern approaches Published by CRC Press, ISBN 0824791924, 9780824791926, 476 pages.

Baumann, D.T., Bastiaans, L., Kropff, M.J., 2002. Intercropping System Optimization for Yield, Quality, and Weed Suppression Combining Mechanistic and Descriptive Models. Agronomy Journal 94, 734-742.

Bingham I J and Rees R M 2008 Senescene and N release from clover roots following permanent excision of the shoot. Plant Soil 303, 229-240.

Connolly, J., Wayne, P., Bazzaz, F.A., 2001. Interspecific Competition in Plants: How Well Do Current Methods Answer Fundamental Questions? The American Naturalist 157, 107-125.

Corre-Hellou, G., Brisson, N., Launay, M., Fustec, J., Crozat, Y., 2007. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. Field Crops Research 103, 76-85.

Crews, T.E., Peoples, M.B., 2004. Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agriculture, Ecosystems & Environment 102, 279-297.

Dahlin, A., Mårtensson, A., 2008. Cutting regime determines allocation of fixed nitrogen in white clover. Biology and Fertility of Soils 45, 199-204.

Dakora, F.D., 2003. Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. New Phytologist 158, 39-49.

Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil 245, 35-47.

Francis, C.A., Brady, N.C., 1989. Biological Efficiencies in Multiple-Cropping Systems. Advances in Agronomy. Academic Press, pp. 1-42.

Fujita, K., Ofosu-Budu, K.G., Ogata, S., 1992. Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant and Soil 141, 155-175.

Giller, K.E., and Cadisch, G., 1995. Future benefits from biological nitrogen fixation: An ecological approach to agriculture. Plant and Soil 174, 255-277.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369-378.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. Plant and Soil 236, 63-74.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009. Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crops Research 113, 64-71.

HGCA, 2005/06. The barley growth guide.

HGCA, 2008. http://www.hgca.com/content.output/512/512/Varieties/Spring%20oats/Firth.mspx.

Høgh-Jensen, H., and Schjoerring, J.K., 2000. Below-ground nitrogen transfer between different grassland species: Direct quantification by 15N leaf feeding compared with indirect dilution of soil 15N. Plant and Soil 227, 171-183.

Høgh-Jensen, H.a.S., J.K. , 2000. Below-ground nitrogen transfer between different grassland species: Direct quantification by 15N leaf feeding compared with indirect dilution of soil 15N. Plant and Soil 227, 171-183.

Horwith, B., 1985. A role for intercropping in modern agriculture. BioScience 35, 5.

Jensen, E.S., 1996. Rhizodeposition of N by pea and barley and its effect on soil N dynamics. Soil Biology and Biochemistry 28, 65-71.

Jensen, E.S., and Hauggaard-Nielsen, H., 2003. How can increased use of biological N2 fixation in agriculture benefit the environment? . Plant and Soil 252, 177-186.

Jensen, E.S.a.H.-N., H. , 2003. How can increased use of biological N2 fixation in agriculture benefit the environment? . Plant and Soil 252, 177-186.

Keating, B.A., Carberry, P.S., 1993. Resource capture and use in intercropping: solar radiation. Field Crops Research 34, 273-301.

Kumar, K., Goh, K.M., 2000. Crop residues and management practices: Effects on soil quality, soil nitrogen dynamics, crop yield and nitrogen recovery. Advance in Agronomy 68, 197.

Launay, M., Brisson, N., Satger, S., Hauggaard-Nielsen, H., Corre-Hellou, G., Kasynova, E., Ruske, R., Jensen, E.S., Gooding, M.J., in press. Exploring options for managing strategies for pea-barley intercropping using a modeling approach. European Journal of Agronomy In Press, Corrected Proof.

Li, L., Sun, J., Zhang, F., Li, X., Rengel, Z., Yang, S., 2001. Wheat/maize or wheat/soybean strip intercropping: II. Recovery or compensation of maize and soybean after wheat harvesting. Field Crops Research 71, 173-181.

Li, W., Li, L., Sun, J., Guo, T., Zhang, F., Bao, X., Peng, A., Tang, C., 2005. Effects of intercropping and nitrogen application on nitrate present in the profile of an Orthic Anthrosol in Northwest China. Agriculture, Ecosystems & Environment 105, 483-491.

McNeill, A.M., Zhu, C., and Fillery, I.R.P., 1997. Use of in situ 15N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil/plant systems. Australian Journal of Agricultural Research 48, 295-304.

Morris, R.A., Garrity, D.P., 1993. Resource capture and utilization in intercropping: water. Field Crops Research 34, 303-317.

Ndakidemi, P.A., 2006. Manipulating legume/cereal mixtures to optimize the above and below-ground interactions in the traditional African cropping systems. African Journal of Biotechnology 5, 2526-2533.

Nickerson, 2008. http://www.nickersonseeds.co.uk/.

Ofori, F., Stern, W.R., Brady, N.C., 1987. Cereal-Legume Intercropping Systems. Advances in Agronomy. Academic Press, pp. 41-90.

Paul, E.A., Elliott, E.T., Cole, C.V., 1996. Soil organic matter in temperate agroecosystems: long-term experiments in North America. Published by CRC Press, 414 pages.

Paustian, K., Andrén, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., Noordwijk, M., Woomer, P.L., 1997. Agricultural soils as a sink to mitigate CO2 emissions. Soil Use and Management 13, 230-244.

Paynel, F., and Cliquet, J.B., 2003. N transfer from white clover to perennial ryegrass, via exudation of nitrogenous compounds Agronomie 23, 503-510.

Rogers, J.B., Laidlaw, A.S., and Christie, P., 2001. The role of arbuscular mycorrhizal fungi in the transfer of nutrients between white clover and perennial ryegrass. Chemosphere 42, 153-159.

Rosolem, C.A., Foloni, J.S.S., Tiritan, C.S., 2002. Root growth and nutrient accumulation in cover crops as affected by soil compaction. Soil and Tillage Research 65, 109-115.

Ryan, P.R., Delhaize, E., Jones, D.L., 2001. FUNCTION AND MECHANISM OF ORGANIC ANION EXUDATION FROM PLANT ROOTS. Annual Review of Plant Physiology and Plant Molecular Biology 52, 527-560.

Ryser, P., 1998. Intra- and interspecific variation in root length, root turnover and the underlying parameters., Inherent variation in plant growth: physiological mechanisms and ecological consequences. Lambers H, Poorter H, Van Vuuren MMI, , pp. 441-465.

Stern, W.R., 1993a. Nitrogen fixation and transfer in intercrop systems. Field Crops Research 34, 335-356.

Stern, W.R., 1993b. Nitrogen fixation and transfer in intercrop systems. Field Crops Research 34, 335-356.

Stirzaker, R., Passioura, J., Wilms, Y., 1996. Soil structure and plant growth: Impact of bulk density and biopores. Plant and Soil 185, 151-162.

Swinnen, J., 1994. Rhizodeposition and turnover of root-derived organic material in barley and wheat under conventional and integrated management. Agriculture Ecosystems & Environment 51, 115-128.

Szumigalski, A.R., Van Acker, R.C., 2006. Nitrogen Yield and Land Use Efficiency in Annual Sole Crops and Intercrops. Agronomy Journal 98, 1030-1040.

Thorsted, M.D., Olesen, J.E., Weiner, J., 2006. Width of clover strips and wheat rows influence grain yield in winter wheat/white clover intercropping. Field Crops Research 95, 280-290.

Vandermeer, J.H., 1999. The ecology of Intercropping. Cambridge Univ. Press, New York.

Vanlauwe, B., Diels, J., Sanginga, N., and Merckx, R., 1997 Residue quality and decomposition: An unsteady relationship? Driven By Nature: Plant Litter Quality and Decomposition, 157-166.

Vinten, A.J.A., Vivian, B.J., and Howard, R.S., 1992. The effect of nitrogen fertilizer on the nitrogen cycle of two upland arable soils of contrasting textures. Proceedings of the Fertilizer Society.

Watiki, J.M., Fukai, S., Banda, J.A., Keating, B.A., 1993. Radiation interception and growth of maize/cowpea intercrop as affected by maize plant density and cowpea cultivar. Field Crops Research 35, 123-133.

Watson, C.A., Atkinson, D., Gosling, P., Jackson, L.R., and Rayns, F., 2002. Managing soil fertility in organic farming systems. Soil Use and Management 18, 239-247.

Willey, R.W., 1990. Resource use in intercropping systems. Agricultural Water Management 17, 215-231.

Yao, Q., Li, X.L., Ai, W.D., and, C., P., 2003. Bi-directional transfer of phosphorus between red clover and perennial ryegrass via arbuscular mycorrhizal hyphal links. European Journal of Soil Biology 39, 47-54.

Zhang, F. and Li, L., 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. Plant and Soil 248, 305-312.

8. Paper 4

Factors controlling N losses from an intercropped low input rotation.

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To be submitted to Soil Use and Management

Paper 4

Factors controlling N losses from an intercropped low input rotation.

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Abstract

Nitrous oxide and nitrate are environmentally significant nutrient losses from agricultural systems. Low input farming systems have limited sources of inputs and depend on biologically fixed N. Intercropping offers an alternative supply of crop N, and can influence N retention and loss within a crop rotation. The process of nutrient loss is controlled by an interaction between environmental conditions, such as climate and soil type, and management. We report here on an experiment studying N loss and turnover from intercrops and monocrops for 12 hydrologically-isolated plots near Edinburgh in the UK. Treatments were a spring barley (*Hordeum vulgare* cv. *Westminster)* monoculture and intercrops of barley / white clover (*Trifolium repens* cv. *Alice*) and barley/ pea (*Pisum sativum* cv. *Zero 4* or cv. *Nitouche*) in 2006. Spring oats (*Avena sativa* cv. *Firth*) were planted on all plots in 2007. In the third growing season, all plots were sown with perennial ryegrass. No fertilisers, herbicides or pesticides were used at any stage of the experiment. During the experimental period, N_2O (direct and indirect) fluxes, NO_3^--N leachate, available N, crop yields, water filled pore space (WFPS) and climatic conditions were recorded. In general, N_2O fluxes were controlled by available NH_4^+-N, DON in soil, WFPS and grain yields (cumulative values). Specifically, N_2O fluxes from barley/ clover were related into soil available NH_4^+-N ($P<0.05$; $R=0.83$), barley/ pea cv. *Zero 4* yields to WFPS ($P<0.05$; $R=-0.62$); barley/ pea cv. *Nitouche* yields to DON ($P<0.05$; $R=0.97$) and WFPS ($P<0.05$; $R=-0.60$) and barley yields to DON ($P<0.05$; $R=0.38$). Grain yields were related to cumulative N_2O fluxes for the whole growing season ($P<0.05$; $R=0.78$) and initial levels of soil available NH_4^+-N ($P<0.05$; $R=0.31$).

Introduction

Nitrogen (N) losses from agricultural systems are mainly through gaseous losses, as nitrous oxide (N_2O), and leachate, as nitrate (NO_3^--N). N_2O is a "greenhouse gas" with a 100-yr average global warming potential (GWP) 296 times larger than an equal mass of CO_2 (Prather et al., 2001; Smith and O'Mara, 2007). Direct emissions of N_2O from soils result mainly from microbially driven nitrification and denitrification processes, as well as chemodenitrification (Bremner, 1997) and there are also small contributions by dissimilatory reduction of NO_3^--N to NH_4^+-N (DNRA) (Casella et al., 1984; Stevens and Laughlin, 1998). Farming systems include a complex mix of tillage, timings and frequencies in combination with fertilisation rates, residues and crop, all interacting with local climate, topography and soil type. The processes are controlled by several factors which affect the amount of N losses from the systems, such as soil water content regulates oxygen supply (Scholes *et al.*, 1997); temperature affects most organisms (Freibauer and Kaltschmitt, 2003; Flechard et al., 2007); available N (NO_3^--N and NH_4^+-N), as it regulates the reaction rates; available organic carbon, as the denitrifiers require carbon for respiration and it also regulates oxygen levels and pH, that controls both nitrification and denitrification (Davidson, 1993; Singh et al., 2007).

Leaching is often quantitatively the most important channel of N loss from field soils other than that accounted for in plant uptake (Vinten 988-96). NO_3^--N is the primary form of N leached into groundwater, is totally soluble at the concentrations found in soil, and its movement is closely related to water movement. Most soil and environmental parameters, which influence the transport of dissolved NO_3^--N through natural field soils, vary substantially at different locations, even at short separation distances. In organic systems, leaching losses will occur mainly from sudden, rapid mineralisation and nitrification of organic nitrogen, especially following cultivation when mineralisation will be enhanced. It has been postulated that organic farming reduces nitrate leaching, a major environmental concern in Europe (Drinkwater *et al.*, 1998). However, Trewavas (2001) states ploughing in of legume crops on organic farms to improve soil fertility and continued manure breakdown leads to nitrate leaching into aquifers and waterways at identical rates to conventional farms. Nevertheless, the average leaching of nitrate over a crop rotation was reported to be rather lower per unit area from organic systems than conventional (Korsaeth, 2008). On the other hand, an accurate comparison of leaching between systems requires yields to be considered. This is difficult to achieve as there are differences in the sequence and type of crops grown and in the input intensity of N. Inaccurate interpretations of leaching data are common, as pointed out by Andrén *et al* (1999). When looking at cumulative nitrate leaching, there was a 50% higher rate from the fertilizer-based system, although not statistically significant (P = 0.06). But the whole difference for the 15-

year period is based on the observation that only one year out of the five measured had a higher leaching rate. This was interesting, but there was no attempt to discuss this difference.

Low-input farming systems, such as arable organic farming, often have limited sources of nitrogen (N), which can minimise the productivity of these systems by limiting the amount of available N during crop growth (Berry *et al.*, 2002). This may be linked to the observation that organic yields are, on average, 50-95% of the conventional yield, depending on species and position in the organic rotation (Watson *et al.*, 2002). Most organic systems depend on biological N fixation (BNF) to supply N for intercropped cereals and/or for the following crops (Elgersma *et al.*, 2000; Thorsted *et al.*, 2006). BNF in legumes is a fundamental process for maintaining soil fertility and the continued productivity of organic cropping systems. The amount of N_2 fixed and the N contribution from leguminous crops are influenced by a number of environmental factors, including soil type, soil nutritional status, species and varieties, water availability and temperature, as well as soil and crop management (Vinther and Jensen, 2000).

Stern (1993) found that the direct transfer of N from a N_2-fixing legume to an intercropped cereal is usually insufficient for cereal production. However, the transfer of N from legumes to non-legumes is promoted by defoliation or maturing of the legume resulting in the death of the roots and nodules. Mechanical control of legumes and in particular white clover can decrease the interspecific competition and increase N transfer from the legume (clover) to cereals (Thorsted *et al.*, 2006). Pappa et al (Paper 1) have found that intercrops of legumes with barley have a positive effect on the partner and the following grain yields and biomass of cereal.

The concentration of mineral N in the soil is affected by soil moisture. In field conditions, soil moisture affects N mineralisation in both direct and indirect ways. The direct effect is related to the water availability for microbial activity, and in this case it is suitable to express soil moisture in terms of water potential (Orchard and and Cook, 1983). Water also affects N mineralisation by controlling oxygen (O_2) diffusion within the soil and the volume of soil supporting aerobic microbial activity. To study this, soil water content is normally expressed as water-filled pore space (WFPS) (Skopp *et al.*, 1990).

Because of all these factors controlling the N losses and more specifically N_2O losses from agricultural systems we compared the most common factors controlling them under the same soil type (sandy loam), topography and climate without any addition of fertiliser and residues. The N_2O emissions from a low input rotation were used to investigate the seasonal variation and the treatment effects; and to determine the soil, plant production and climatic factors controlling N_2O release from soil to atmosphere. The main aims were to investigate whether the

N_2O fluxes are principally controlled by the intercropped type and influenced by the soil structure. Additionally, seasonal cumulative values of N_2O will be related to the final yield.

2. Materials and methods

2.1. Site description and soil

The experiment was carried out 8 km south of Edinburgh, Scotland (lat. 55° 51'N, long. 3° 12'W). The overall field size was 0.4 ha and 12 hydrologicaly isolated plots were installed in 1990. Further details of the full experimental facilities are given in (Vinten et al., 1992).The plots were fallow for the period 2003-2005. The day before sowing (23rd April 2006), the soil was sampled in 0-20cm (n=8 per plot). The water content at field capacity was 19 ± 0.5 % (v/v) and the soil bulk density was 1.19 ± 0.01 Mg m^{-3} (mean ± SE, n=5). The top soil was a sandy loam (Eutric Cambisol, Macmerry Series) with 65% sand, 20% silt and 15% clay developed from partially sorted glacial till, the upper 0.5 to 1.5 m of the soil profile is freely drained.

In 2006 when the current study started, the treatments were: barley (*Hordeum vulgare* cv. Westminster) as a monocrop; pea (*Pisum sativum* cv. *Zero 4*)/ barley intercrop; pea (*Pisum sativum* cv. *Nitouche*)/ barley intercrop; White clover (*Trifolium repens* cv. Alice) /barley intercrop established on the 24th April 2006. Westminster is a very high yielding spring malting variety with long, stiff straw. It has excellent all-round disease resistance. Westminster has all of the necessary qualities for brewing and export markets including feed barley growers (HGCA, 2008). *Nitouche* is a popular large blue pea variety and has a consistent performance, good agronomic characters and suitability for premium markets. *Nitouche* produces a large, smooth, round pea and retains its colour well. *Nitouche* also has a high level of resistance to downy mildew and long straw with good standing ability making it relatively easy to harvest. *Zero 4* is a small blue combining pea with a unique combination of agronomic characteristics: short straw, excellent standing ability and very early maturity (Nickerson, 2008). When sown at its optimum seed rate of 110 seeds/ m^2, *Zero 4* has a similar yield potential to *Nitouche* (optimum seed rate of 70 seeds/ m^2). Alice is a tall, large leaved white clover developed for exceptional yields of palatable, high quality, high protein forage included in pastures mixtures (Barenbrug, 2006). Its vigorous spring and summer growth make it a suitable choice for cutting or grazing management, as well as for N fixation throughout the season and good winter- hardiness and cover (HGCA, 2008). Each treatment was replicated three times in a blocked design. In the intercrop treatments, the seed rates for the pea and barley followed a 50:50 replacement design. This means that the target intercrop density was 50% of the monoculture density of each crop. Based on monocropping seed rates of 200 kg ha^{-1} for the barley and 250 kg ha^{-1} for the peas which equated to approximately 350, 75 and 110 germinable seeds m^{-2} for Westminster, *Nitouche* and *Zero-4* respectively, the intercrop components were half these values.

In the second growing season (2007), all the plots were sown with spring oats (*Avena sativa* cv. Firth) on 3rd April at a seed rate 250 kg ha^{-1}. Firth is a very popular spring oat variety and exhibits a high kernel content and good resistance to mildew (Szumigalski and Van Acker, 2006). In the third growing season, perennial ryegrass (PRG) was sown in all plots in a 50:50 mixture of Aberavon and Aberdart at a seed rate of 35 kg ha^{-1}(Table 1). The plots were tilled using a mouldboard plough followed by cultivating (rotary hoe), seeding and rolling. No fertilisers, herbicides or pesticides were used.

Table 1: Cropping 2003-2008 on the drainage plots.

Year	Crops	Treatments	*Varieties*
2003	Fallow		
2004	Fallow		
2005	Fallow		
2006	Intercropping cereal/ legumes	Spring Barley/ White clover	*Westminster/ Alice*
		Spring Barley/ Spring Pea 1	*Westminster/ Zero 4*
		Spring Barley/ Spring Pea 2	*Westminster/ Nitouche*
		Spring Barley	*Westminster*
2007	Cereal	Spring Oat	*Firth*
2008	Grass	Perennial ryegrass	*AberAvon & Aberstar*

All the data are presented with reference to the cereal growth stages GS 23 (main shoot and 3 tillers), GS 65 (flowering half-way), GS 77 (late milk), GS 92 (grain hard (not dented by nail). For the 2006 growing season (barley) these stages were at 30, 70, 100 and 140 days, respectively. For oat plants (2007), the growth stages GS 16 (six leaves unfolded), GS 23, GS 65, GS 77 and GS 92 were 40, 65, 100, 135, 161 days from sowing respectively. Some of the data presented here have been separated in to different periods as follow: Summer '06: 24th April – 11th September; Winter '06: 12th September '06 – 2nd April '07; Summer '07: 3rd April '07 – 11th September '07; Winter '07: 12th September '07- 21st April '08; Summer '08: 22nd April '08– 30th August '08.

2.2. Sampling and analyses

Nitrous oxide fluxes from soil were measured using static chambers located randomly within each of the plots using the method described by Ball *et al.* (1997). One chamber (volume 25120 cm^3; cover area 1256 cm^3) was located in each of the 12 plots. The chambers were sealed for 60 minutes with an aluminium lid having a small open sampling point sealed with a grommet in which the syringe was inserted. Air and soil temperature were recorded the same time. Gas samples were collected in portable evacuated aluminium vials (Scott *et al.*, 1999) and analysed for N_2O by electron-capture gas chromatography. For consistency, gas sampling was carried out between 10:00 and 12:00 hrs

Soil samples were collected monthly and randomly from within the plots to a depth of 40 cm and separated into 0-20 cm and 20-40 cm layers. Soil mineral N (NH_4^+-N and NO_3^--N) and dissolved organic N concentrations were determined following extraction in 0.5 M K_2SO_4 (Pappa *et al.*, paper 2). N_2O fluxes and mineral N data are presented in Pappa *et al.* (Chapter 6: paper 2).

The drainage flux was measured using tipping bucket flow meters mounted in the instrument pits. Tips were measured with a hand-counter connected at the side of the tipping bucket. Rainfall was obtained from the weather station at Boghall Farm (0.5 km away) and the Center of Ecology and Hydrology (0.5 km away). The composition of the drainage water was estimated using samples collected with a simple integral sampler which collected a small volume (ca. l0 ml) of water into a large black plastic bucket, every second tip of the buckets (Vinten *et al.*, 1991) (Pappa *et al.*, Chapter 6: paper 2).

Additional drainage water samples were collected weekly by hand for almost all of the second growing season for N_2O measurements in drainage water. Containers were filled completely and sealed with a gas tight inner seal, held in place by a screw-top lid. Water samples were stored in a cool box and immediately transferred to the laboratory and stored at 4 °C until analysis. All samples were analysed within 48 h of collection. Nitrous oxide concentrations in drainage were assessed in the laboratory by analysis using a gas chomatograpgy (Agilent 6890 GC) fitted with a 1.8 m Porapak-N column and electron capture detector of duplicate 5 ml subsamples from each sample injected with a syringe in a 22 ml sealed vial which was shaken vigorously for 1 min. (Reay *et al.*, 2004b).

Water release properties were assessed on undisturbed soil samples taken monthly with the same type of rings as used for bulk density. The samples were saturated with water at atmospheric pressure and equilibrated on tension tables and pressure plates as described by Ball and Hunter (1988). Total porosity was taken as an estimate of water content at saturation. Field capacity was defined as the moisture content at -6 kPa (Duncan, 1979). The dry bulk density of the sample, was calculated from its dry weight and volume, and gravimetric water content was measured by using a separate sample of oven-dry soil (Ball and Hunter, 1988).

2.3. Statistics

The measured variables were tested with Anderson-Darling test (significance .05) for normality distributed and statistical analyses by ANOVA were performed using Minitab 15 (2006) and GenStat 8 (2005). The N_2O data were transformed by using $\log_e$ transformation. Relationships between fluxes and environmental parameters were investigated using correlation analysis.

3. Results

3.1. Weather data

The last 25 year mean annual rainfall is 676 mm, mean annual air temperature is with maximum and minimum daily air temperature of 19 °C (July) and 0.7 °C (January). During the experiment the rainfall was higher that the average, in 2006 the total annual rainfall was 927 mm, in 2007 it was 1288 mm and in 2008 it was 1132 mm. Monthly average air temperature and total rainfall during the experimental period are shown on the Figure 1.

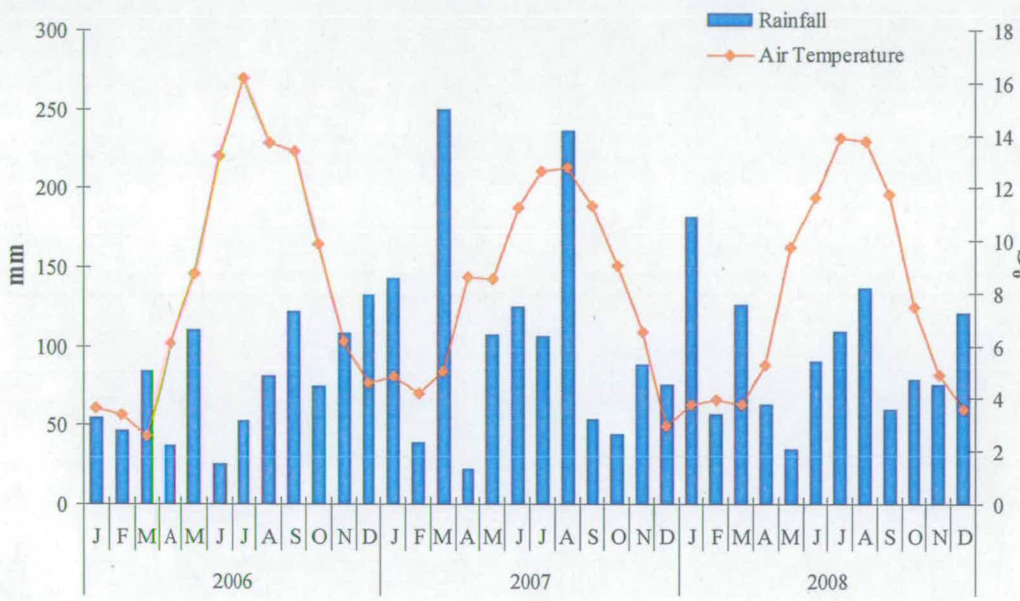


Figure 1: Monthly total rainfall (mm) and monthly average air temperature (°C).

Total rainfall in the period between 1 May and 30 September (when soil temperatures and therefore microbial activity are highest) was 390 mm in 2006 and 627 mm in 2007.

	2006	2007	2008
January	3.5	4.8	4.1
February	3.4	4.2	4.2
March	2.9	5.1	4.4
April	5.8	8.1	5.9
May	9.2	10.0	10.2
June	12.3	12.9	12.9
July	14.6	14.4	14.9
August	14.2	14.6	14.9
September	13.2	13.2	13.1
October	10.7	10.6	7.5
November	7.3	8.2	8.4
December	5.6	4.8	2.7
Average	8.6	9	8.6

Table 1: Average soil temperature (°C) for 2006-2008 in 0-10cm depth.

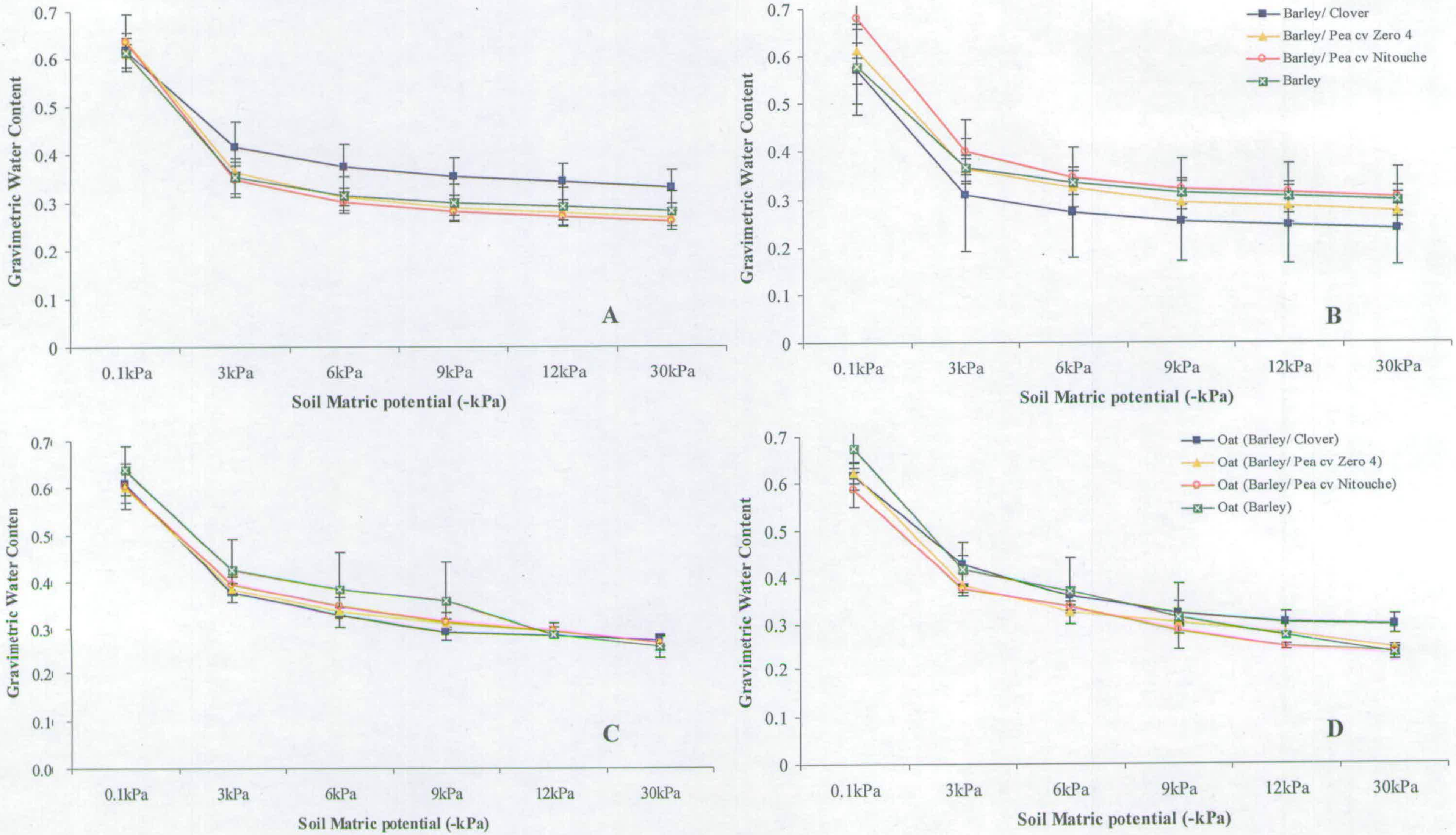
Soil temperature remained relatively constant between years (Table 1). At the sowing periods, a higher soil temperature by 2 °C was observed during April, compared to previous winter months during 2006-2007.

3.2. Water release properties

At GS 23, in 2006, the barley/ clover had the highest gravimetric water at all the different tensions following the intercrops of barley with the two pea cultivars (Fig. 2A). At GS 92, the gravimetric water content of the barley/ pea intercrops were significantly different each other. Barley/ pea cv. *Nitouche* had a higher value under all the different tensions with a difference of almost 0.05 from barley/ pea cv. *Zero 4* ($P<0.05$) (Fig. 2B).

In the second growing season (2007), the water contents of different treatments were not significantly different. The previous barley monocrop had the highest gravimetric water content until the -9 kPa tension. However, the previous barley/ pea cv. *Nitouche* had higher values than other intercrops (Fig. 2C). At GS 92, oats grown in the presenting barley/ clover treatment had a significantly higher gravimetric water from the rest of the treatments ($P<0.05$) (Fig. 2D).

Figure 2: Soil gravimetric water content at four different times during the experimental period under different tensions. All values are means (n=3) ± SE (bars) within the treatment. A) 26th June 2006 – GS 23; B) 11th September 2006 – GS 92; C) 24th June 2007 – GS 23; D) 30th August 2007 – GS 92.



3.3. Water Filled Pore Space (WFPS)

In 2006, the WFPS was decreased as soon as the plants were established. During summer 2006, rainfall was very low and the WFPS decreased rapidly during July to 30%. At the same time, the average daily soil temperature ranged from 10 °C to 18 °C in June and July. The intercrops of barley and pea had similar WFPS patterns at 0-20 cm depth without any significant differences during the first year of the experiment (Fig. 3).

In 2007, the WFPS remained around 50% during the growing season of oats. The previous barley/ pea intercrops were different with barley/ pea cv. *Nitouche* having a higher WFPS in comparison with barley/ pea cv. *Zero 4*. However, the previous barley monocrop had the highest WFPS reaching 60%at the end of the growing season. During the winter, the WFPS remained high for all the treatments until the spring 2008. The previous barley monocrop had the highest WFPS ($P<0.01$), but when the grass was established and it decreased to 30% for all treatments. The same pattern as 0-20 cm is seen for depth 20-40 cm during 2006. However, the barley/ pea cv. *Nitouche* had the lowest WFPS during the total experimental period ($P<0.05$). Throughout winter 2006 and second growing season (2007), the previous barley/ pea cv. *Zero 4* had the highest WFPS and was significantly different from barley/ pea cv. *Nitouche* ($P<0.05$).

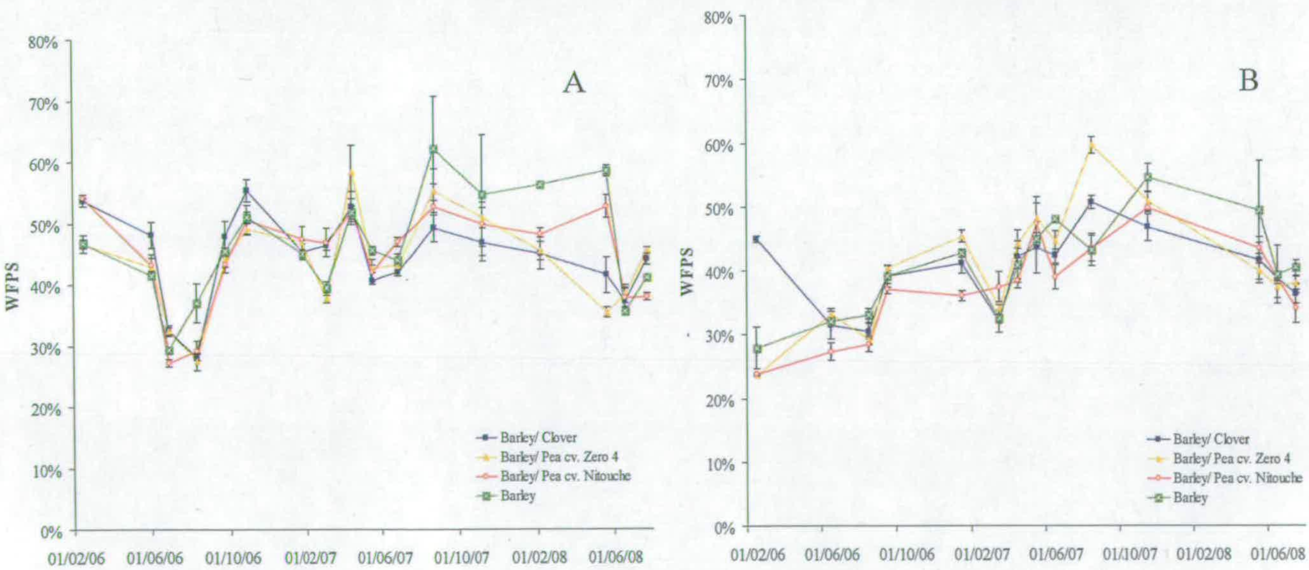


Figure 3: Water filled pore space (WFPS) during the experiment period in the soil layers at 0-20 cm (A) and 20-40 cm (B). All values are means (n=3) ± SE (bars) within the treatment.

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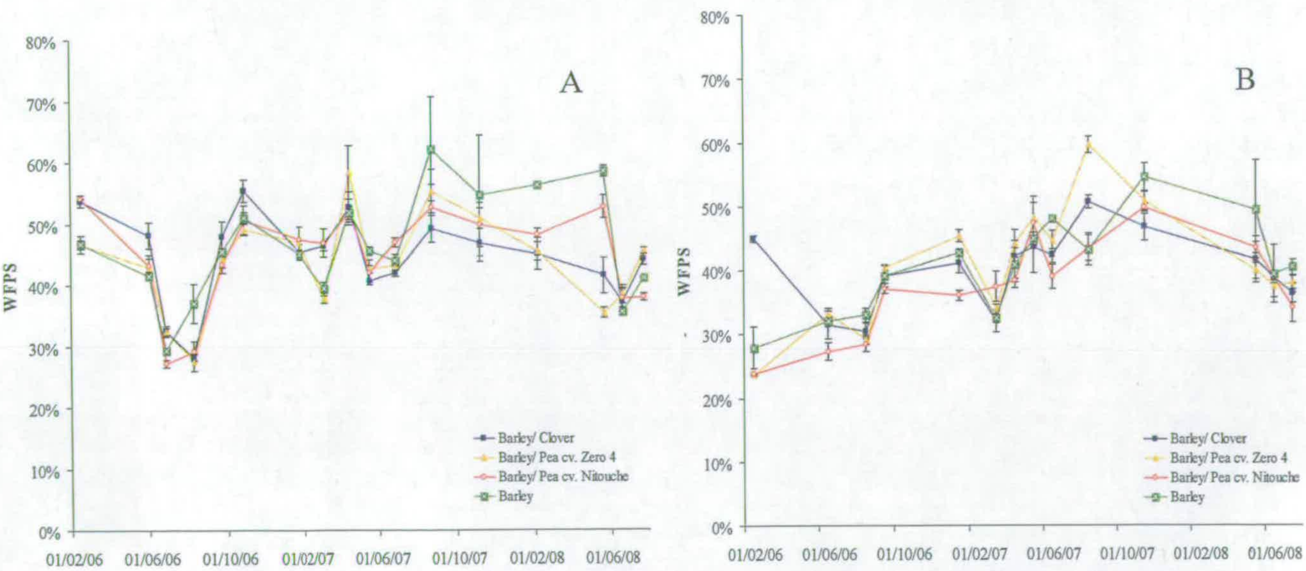

Figure 3: Water filled pore space (WFPS) during the experiment period in the soil layers at 0-20 cm (A) and 20-40 cm (B). All values are means (n=3) ± SE (bars) within the treatment.

3.4. Correlations of N_2O *losses*

3.4.1. Ammonium (NH_4^+-N*) in soil*

Ammonium has an important role in the production of terrestrial N_2O, as N_2O is generated during the nitrification process. There was a low correlation between terrestrial N_2O losses and NH_4^+-N in the soil at the same date of sampling for the total duration of the experiment ($P<0.05$; $R=0.31$). However, when the N_2O fluxes data were separated for each of the treatments, there was no correlation between treatment and NH_4^+-N. When the experimental period was divided into seasons, in the barley/ clover treatment during the first growing season (2006) ($P<0.05$; $R=0.91$) $\log_{10}$ N_2O fluxes were highly correlated with NH_4^+-N. Additionally, there was a high correlation of log N_2O losses and NH_4^+-N in the soil for the previous barley/ pea cv. *Nitouche* ($P<0.05$; $R=0.92$) and barley monocrop ($P<0.05$; $R=0.98$) during the second growing season (2007), when oat plants were growing. Data for NH_4^+-N and N_2O are not presented here (see Chapter 6 - paper 2).

3.4.2. Nitrate (NO_3^--N*) in soil*

N_2O losses from soil occur during the denitrification process, when soil nitrate is converted to soil N_2O and N_2 gas by several micro-organisms. There was not significant correlation between N_2O on the day of sampling and NO_3^--N in the soil ($P>0.05$; $R\leq0.5$) during the whole experimental period and also within individual periods (spring-summer 2006; winter 2006-2007; spring summer 2007; winter 2007-2008). Data for NO_3^--N are not presented (see paper 2).

3.4.3. Dissolved Organic Nitrogen (DON) in soil

Terrestrial N_2O losses were positively correlated with DON for the whole duration of the experiment ($p<0.05$; $R=0.44$) (Fig.4). Separating the N_2O fluxes by treatment, they were only correlated for the barley monocrop for the whole experimental period ($P<0.05$; $R=0.38$). Separating the treatment into seasons, there was only one significant correlation for barley/ pea cv. *Nitouche* for the first growing season ($P<0.05$; $R=0.97$) between N_2O and DON. Data for DON are not presented here (see paper 2).

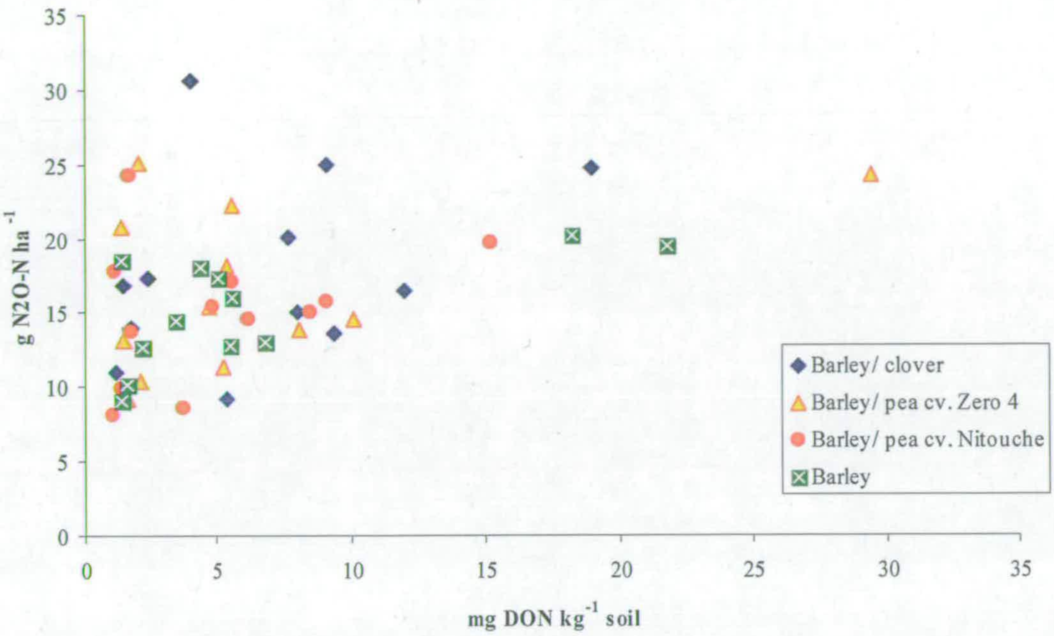

Figure 4: Scatter plot of the N_2O and DON for the two years of the duration of the experiment. Each point represents the mean of three (n=3) replicates for measurements every two months. Treatments refer to the initial experimental layout (2006).

3.4.4. Temperature and Rainfall

Soil and air temperature and rainfall had no significant effect on the terrestrial N_2O losses during the whole experimental period either when analysed as the whole data set or for individual treatments and seasons.

3.4.5. Water Filled Pore Space (WFPS)

Water filled pore space had a negative correlation with the log N_2O losses ($P<0.05$; $R\leq -0.28$). Separating the fluxes by treatments, we found that only the two barley/ pea treatments had a significant correlation of N_2O with WFPS for the whole duration of the experiment (barley/ pea cv. *Nitouche*: $P<0.05$; $R=-0.60$ and barley/ pea cv *Zero 4*: $P<0.05$; $R=-0.62$). However, there was no seasonal correlation between each of the treatments.

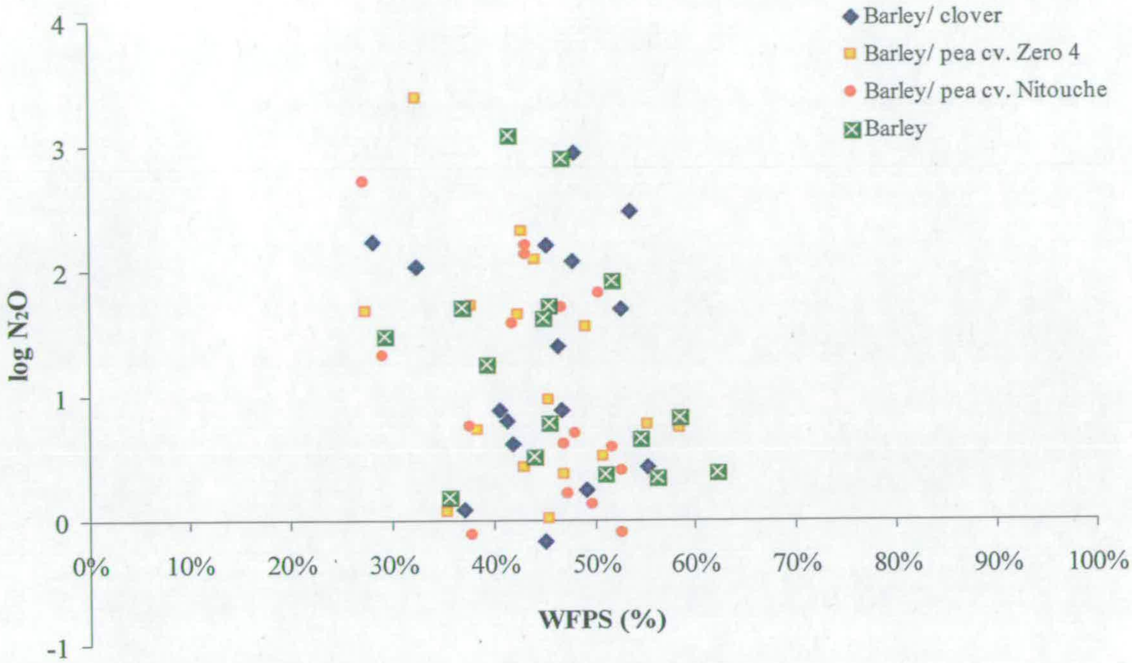

Figure 5: Scatter plot of the log N_2O and WFPS for the whole duration of the experiment. Each point represents the mean of three (n=3) replicates for monthly measurements. Treatments refer to the initial experimental layout (2006).

3.4.6. N_2O from the drainage and NO_3^--N from the soil

There was no correlation between the NO_3^--N in the soil and the indirect N_2O losses from the drainage for the second growing season (2007).

3.4.7. $N_2O$from the soil and N_2O from the drainage

There was no correlation between the soil $\mathbf{N_2O}$ and the indirect $\mathbf{N_2O}$ losses from the drainage for the second growing season (2007) for all of the treatments.

3.5 Correlations of grain yield and N losses

3.5.1. Barley grain yield and initial levels of NH_4^+-N in soil

There was a significant correlation between the initial available NH_4^+-N in 2006 in the soil and the final grain yield of barley ($P<0.05$; $R=0.68$). However, when the different treatments were tested a significant correlation was only found for barley/ pea cv. *Zero 4* ($P=0.05$; $R=0.99$). No other correlation was found with NO_3^--N in soil and DON.

3.5.2. Barley grain yield and cumulative N_2O fluxes from the soil

There was a significant correlation between the cumulative N_2O fluxes and the final grain yield for all the treatments ($P<0.01$; $R=0.78$). Separating the fluxes into treatments there was a found high correlation in the first growing season (April-September, 2006) and the final grain yield of barley for the barley/ clover ($P<0.05$; $R=0.99$). However, the barley monocrop and barley/ pea cv. *Zero 4* cumulative N_2O fluxes were less correlated with barley yield.

3.5.3. Oat grain yield and cumulative N_2O fluxes

There was a high correlation between the cumulative N_2O fluxes in the second growing season (April-September, 2007) and the final grain yield of oat crop grain in the previous barley/ clover treatment ($P=0.001$; $R=1$), but not for oats grain in the previous barley/ pea cv. *Nitouche* and barley/ pea cv. *Zero 4* treatments ($P>0.05$).

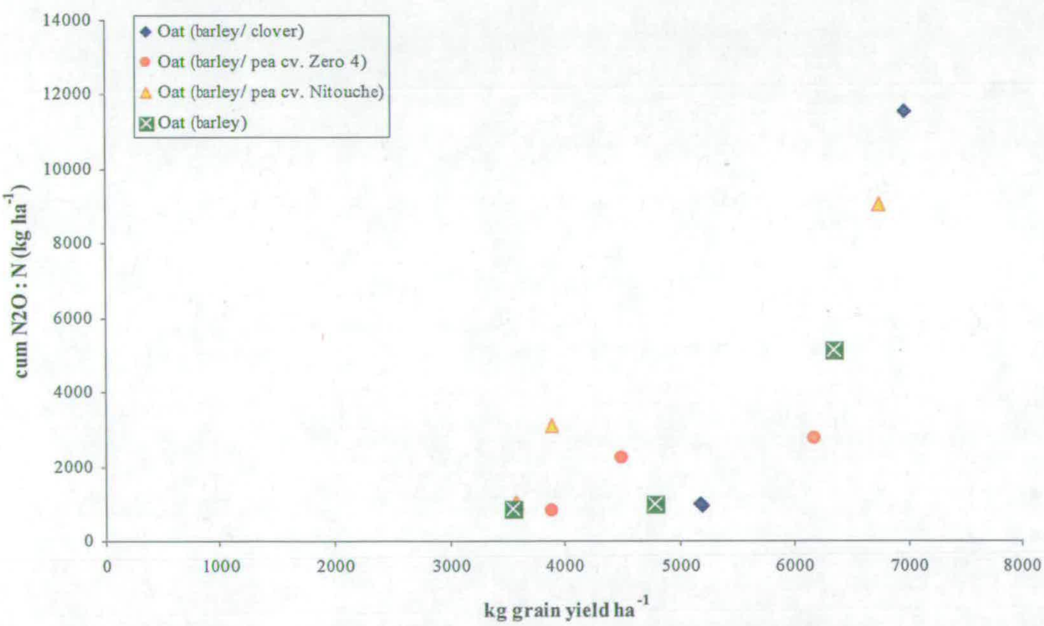

Figure 6: Scatter plot of the cumulative N_2O and final oat grain yield. Each point represents the mean of three (n=3). Treatments refer to the initial experimental layout (2006).

4. Discussion

4.1. Local Conditions

4.1.1. Temperature

As for any biological process, the rates of nitrification and denitrification are affected by changes in temperature. Nitrous oxide emissions from the rotation had no correlation with the temperature (soil and air) during the experimental period. Dobbie *et al.* (1999) found that if soil WFPS or available N content are limiting, there may not be a clear relationship with temperature. However, when only those data points where the other factors are non-limiting are considered, there is evidence of very steep responses of temperature in agricultural soils in Scotland (Dobbie and Smith, 2001).

4.1.2. Rainfall

Positive correlations have been observed in several studies between rainfall and N_2O fluxes. The production of N_2O via either nitrification or denitrification may be altered by changes in wetness (Davidson, 1992; Zheng et al., 2000). In Scotland, high fluxes of N_2O have been reported from a temperate grassland thought to be largely as a consequence of denitrification, which was particularly prevalent in the wetter of two different years (Jones *et al.*, 2007). However, in our study, there was no correlation between the N_2O fluxes and rainfall maybe because the time of sampling wasn not that frequent to detect response to rainfall. Skiba *et al.* (2000) reported that higher N_2O emissions occured during summer months when the rainfall was high. However, a heavy rain event after a dry period can produce peaks of N_2O fluxes, as the inorganic N is accumulated and the reactivation of the microbial activity contribute to metabolism of inorganic N (Davidson, 1993).

4.1.3. WFPS

Soil water content (through its effect on aeration), together with N supply, has been shown to be the dominant variable controlling the N_2O emission rate. In our study there were high correlations with WFPS for the N_2O fluxes produced during the experimental period. Dobbie *et al.* (1999) suggest that maximum N_2O fluxes occur at a WFPS of 80-85%, values that were rarely observed through out the experiment. Below 60% WFPS nitrification, an aerobic process, is often the dominant process producing N_2O (Linn and and Doran, 1984).

4.2. Management

4.2.1. Land management

Agricultural practices, such as irrigation, can alter the oxygen status of the soil and at the same time increase N_2O emissions (Halvorson *et al.*, 2008). Under conventional tillage cultivation of agricultural soils can cause the loss of SOC or maintain relatively constant levels of SOC which results in higher fluxes of N_2O. In contrast, reduced tillage can increase SOC in soils (Lal, 2004) resulting in reduced erosion and improved water quality and drainage (D'Haene *et al.*, 2008).

Parkin and Kaspar (2006) compared N_2O fluxes from soybean and corn crops in a soybean-corn rotation, recording higher N_2O emissions from the corn when compare with the soybeans. They found that the controlling factor was the available N from the applied fertiliser. The material remaining after harvest decomposed promoting mineralization and NH_4^+-N-N, production which then led to nitrification and denitrification with consequent N_2O emissions. Agricultural practices can also increase ammonia volatilisation and NO_3^--N leaching. N_2O in drainage is another indirect loss (Reay *et al.*, 2004a), but there were very low correlations in our study for all treatments in 2007. A portion of the NO_3^--N that is leached or discharged in drainage can also be denitrified and result in N_2O emissions (Del Grosso *et al.*, 2001). In our study there was no strong relationship between the N_2O and NO_3^--N released from the drainage water (data not presented) which may have been a consequence of the long residence time of water in the soil profile (Vinten et al., 1992).

Davis and Barraclough (1988) monitored different points in time in a rotation on an organic farm in the UK. They found that the magnitude of leaching was closely dependent on the position of a field in the rotation. Nitrate leaching was reduced the longer the field had been in the organic rotation. In Denmark, the impact of organic compared with conventional farming practices on N leaching loss has been studied for mixed dairy and arable farms. The results show a lower N leaching loss from organic than conventional mixed dairy farms, primarily due to lower N inputs and increase the soil in N pool was increasing on organic arable farms over time, but the N leaching loss was comparable to conventional arable farms (Knudsen, 2005).

4.2.2. Crop type

Crop type is a very important factor in determining the N_2O fluxes. Several studies have shown differences in fluxes between crops, for example potatoes and broccoli have higher emissions than barley or wheat (Smith et al., 1998; Dobbie, 1999; Petersen et al., 2006). Nitrous oxide emissions from grasslands can be higher than for many arable crops (Skiba *et al.*, 1993). Pappa *et al.* (paper 2) found that the N_2O fluxes differ between species and the cultivars. N_2O fluxes and the final grain yield had a high correlation ($P<0.05$) for most of the treatments, except the treatment that had the highest fluxes that growing season. Cultivation of nitrogen fixing plants contributes to N_2O emission in a number of ways. Atmospheric N fixed by legumes can be nitrified and denitrified in the same way as fertilizer nitrogen. thus providing a source of N_2O. Symbiotically Rhizobia in root nodules are able to denitrify and produce N_2O (Freney, 1997).

Rochette *et al.* (2005) found that legume crops have higher fluxes than the non-legume crops grown with no added fertiliser, an observation that may be linked to the N inputs provided by legume based systems. Comparing soil collected from N-fixing trees and non N-fixing trees, Dick *et al.*(2006) found that more N_2O was emitted from soil associated with the N-fixing trees and correlated with a larger pool of available N (NH_4^+-N and NO_3^-) in the soil. A correlation between N_2O and NH_4^+-N in soil for the barley/ clover (2006) and previous barley/ pea cv. *Nitouche* (2007) treatment was reported in this study. DON had a high correlation with N_2O for the barley/ pea cv. *Nitouche* (2006). Additionally, Cui et al. (2006) have found that all the treatments with the N_2O fluxes for the whole duration of the experiment between the initial NH_4^+-N in the soil and the final grain yield of barley (2006) were highly correlated.

Additionally, root architecture of different species can affect the amount of NO_3^--N leachate and subsequent conversion to N_2O. Root depth and density affects the water content in the soil and the availability of N. The undesirable leaching of nitrogen to groundwater occurs in soil profiles with a coarse sand texture and a shallower rootable depth (Bowman *et al.*, 1998) and also the effect of leaching on bulk density (Smit and Groenwold, 2005).

Winter cover crops (e.g. wheat (*Triticum aestivum* L. and rye (*Secale cereal* L.)) can effectively prevent NO_3^--N leaching in the winter months on permeable soils, and reduce drainage losses of NO_3^--NN (Feyereisen *et al.*, 2006). Reductions in NO_3^--NN leaching/drainage losses are likely to reduce overall N_2O emissions from water (Snyder *et al.*, in press) and reduce the N_2O indirect losses from low input rotations (Paper 2).

Finally, the use of models to estimate the N_2O emissions from arable soils is essential to devise strategies to mitigate the impact of agriculture on global warming, but we have to consider the complexities of the processes involved, which include influences of soil texture, water input, fertilisers, crops and local conditions (Del Grosso *et al.*, 2006; David *et al.*, 2009).

5. Conclusions

N_2O fluxes (direct and indirect) and NO_3^--N (leachate and in the soil) were the main losses examined in this study trying to discover the factors controlling these losses in that intercropped low input rotation. N_2O fluxes seemed not to be directly affected by the local conditions (temperature, rainfall and WFPS). However, the management factors, such as different crops, had a significant influence on N_2O and NO_3^--N loss. Available soil NH_4^+-N, DON and NO_3^--N were significantly related to N_2O fluxes (direct, indirect) depending on the treatment. Final grain yields were highly positively correlated with the cumulative N_2O fluxes for the season that the crops were growing, as well as with the initial NH_4^+-N levels in the soil. The choice of the crop mixture and the initial levels of N in the soil seem to be the most important drivers for N losses. The use of several models for prediction and mitigation options of N_2O is essential for future estimation of N losses.

References

Andrén O, Kirchmann H and Pettersson O (1999). Reaping the benefits of cropping experiments. Nature, 399, 14.

Ball, B.C., Horgan, G.W., Clayton, H., Parker, J.P., 1997. Spatial Variability of Nitrous Oxide Fluxes and Controlling Soil and Topographic Properties. Journal of Environmental Quality 26, 1399-1409.

Ball, B.C., Hunter, R., 1988. The determination of water release characteristics of soil cores at low suctions. Geoderma 43, 195-212.

Barenbrug,2006. www.barenbrug.com/site/modules/custom/products/getfile.php?siteid=1001&filename=Alice.pdf accessed January, 2006.

Berry, P.M., Sylvester-Bradley, R., Philipps, L., Hatch, D.J., Cuttle, S.P., Rayns, F.W., and Gosling, P., 2002. Is the productivity of organic farms restricted by the supply of available nitrogen? Soil Use and Management 18, 248-255.

Bowman, D.C., Devitt, D.A., Engelke, M.C., Rufty, T.W., Jr., 1998. Root Archutecture Affects Nitrate Leaching from Bentgrass Turf. Crop Science 38, 1633-1639.

Bremner, J.M., 1997. Sources of nitrous oxide in soils. Nutrient Cycling in Agroecosystems 49, 7-16.

Casella, S., Leporini, C., Nuti, M.P., 1984. Nitrous oxide production by nitrogen-fixing, fast-growing Rhizobia. Microbial Ecology 10, 107-114.

D'Haene, K., Van den Bossche, A., Vandenbruwane, J., De Neve, S., Gabriels, D., Hofman, G., 2008. The effect of reduced tillage on nitrous oxide emissions of silt loam soils. Biology and Fertility of Soils 45, 213-217.

David, M., Del Grosso, S., Hu, X., Marshall, E., McIsaac, G., Parton, W., Tonitto, C., Youssef, M., 2009. Modeling denitrification in a tile-drained, corn and soybean agroecosystem of Illinois, USA. Biogeochemistry 93, 7-30.

Davidson, E.A., 1992. Sources of Nitric Oxide and Nitrous Oxide following Wetting of Dry Soil. Soil Science Society of American Journal 56, 95-102.

Davidson, E.A., Matson,P. A., Vitousek,P. M., Riley, R., Dunkin, K., Garciamendez, G., and Maass,J. M. , 1993. Processes regulating soil emissions of NO and N2O in a seasonally dry tropical forest. Ecology 74, 130-139.

Del Grosso, S.J., Parton, W.J., Mosier, A.R., Hartman, M.D., Keough, C.A., Peterson, G.A., Ojima, D.S., Schimel, D.S., Follett, R.F., Hatfield, J.L.,

2001. Simulated effects of land use, soil texture, and precipitation on N gas emissions using DAYCENT. Nitrogen in the Environment: Sources, Problems and Management. Elsevier Science, Amsterdam, pp. 413-431.

Del Grosso, S.J., Parton, W.J., Mosier, A.R., Walsh, M.K., Ojima, D.S., Thornton, P.E., 2006. DAYCENT National-Scale Simulations of Nitrous Oxide Emissions from Cropped Soils in the United States. Journal of Environtal Quality 35, 1451-1460.

Dobbie, K.E., McTaggart, I.P. , Smith, K.A., 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research: Atmospheres 104.

Dobbie, K.E., Smith, K.A., 2001. The effects of temperature, water-filled pore space and land use on N₂O emissions from an imperfectly drained gleysol. European Journal of Soil Science 52, 667-673.

Feyereisen, G.W., Wilson, B.N., Sands, G.R., Strock, J.S., Porter, P.M., 2006. Potential for a Rye Cover Crop to Reduce Nitrate Loss in Southwestern Minnesota. Agronomy Journal 98, 1416-1426.

Flechard, C.R., Ambus, P., Skiba, U., Rees, R.M., Hensen, A., van Amstel, A., Dasselaar, A.v.d.P.-v., Soussana, J.F., Jones, M., Clifton-Brown, J., Raschi, A., Horvath, L., Neftel, A., Jocher, M., Ammann, C., Leifeld, J., Fuhrer, J., Calanca, P., Thalman, E., Pilegaard, K., Di Marco, C., Campbell, C., Nemitz, E., Hargreaves, K.J., Levy, P.E., Ball, B.C., Jones, S.K., van de Bulk, W.C.M., Groot, T., Blom, M., Domingues, R., Kasper, G., Allard, V., Ceschia, E., Cellier, P., Laville, P., Henault, C., Bizouard, F., Abdalla, M., Williams, M., Baronti, S., Berretti, F., Grosz, B., 2007. Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. Agriculture, Ecosystems & Environment 121, 135-152.

Freibauer, A., Kaltschmitt, M., 2003. Controls and Models for Estimating Direct Nitrous Oxide Emissions from Temperate and Sub-Boreal Agricultural Mineral Soils in Europe. Biogeochemistry 63, 93-115.

Freney, J.R., 1997. Emission of nitrous oxide from soils used for agriculture. Nutrient Cycling in Agroecosystems 49, 1-6.

Halvorson, A.D., Del Grosso, S.J., Reule, C.A., 2008. Nitrogen, Tillage, and Crop Rotation Effects on Nitrous Oxide Emissions from Irrigated Cropping Systems. Journal of Environmental Quality 37, 1337-1344.

HGCA, 2008. http://www.hgca.com/content.output/512/512/Varieties/Spring%20oats/Firth.mspx.

Jones, S.K., Rees, R.M., Skiba, U.M., Ball, B.C., 2007. Influence of organic and mineral N fertiliser on N2O fluxes from a temperate grassland. Agriculture, Ecosystems & Environment 121, 74-83.

Korsaeth, A., 2008. Relations between nitrogen leaching and food productivity in organic and conventional cropping systems in a long-term field study. Agriculture, Ecosystems & Environment 127, 177-188.

Lal, R., 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science 304, 1623-1627.

Linn, D.M., and Doran, J.W., 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. . Soil Science Society of America Journal 48, 1267-1272.

Nickerson, 2008. http://www.nickersonseeds.co.uk/.

Orchard, V.A., and Cook, F.J., 1983. Relationship between soil respiration and soil moisture. Soil Biology and Biochemistry 15 447-453.

Parkin, T.B., Kaspar, T.C., 2006. Nitrous Oxide Emissions from Corn-Soybean Systems in the Midwest. Journal of Environmental Quality 35, 1496-1506.

Petersen, S.O., Regina, K., Pöllinger, A., Rigler, E., Valli, L., Yamulki, S., Esala, M., Fabbri, C., Syväsalo, E., Vinther, F.P., 2006. Nitrous oxide emissions from organic and conventional crop rotations in five European countries. Agriculture, Ecosystems & Environment 112, 200-206.

Prather, M., Ehhalt, D., al.:, e., 2001. Atmospheric chemistry and greenhouse gases, in: Climate Change. The Scientific Basis, edited by: Houghton, J. T., Ding, Y., Griggs, D. J., et al., 239- 287, Cambridge University Press, Cambridge, UK, 2001.

Reay, D.S., Edwards, A.C., Smith, K.A., 2004a. Determinants of nitrous oxide emission from agricultural drainage waters. Water, Air, & Soil Pollution: Focus 4, 107-115.

Reay, D.S., Smith, K.A., Edwards, A.C., 2004b. Nitrous Oxide in Agricultural Drainage Waters Following Field Fertilisation. Water, Air and Soil Pollution: Focus 4, 437-451.

Rochette, P., Janzen, H., 2005. Towards a Revised Coefficient for Estimating N2O Emissions from Legumes. Nutrient Cycling in Agroecosystems 73, 171-179.

Scholes, M.C., Martin, R., Scholes, R.J., Parsons, D., Winstead, E., 1997. NO and N2O emissions from savanna soils following the first simulated rains of the season. Nutrient Cycling in Agroecosystems 48, 115-122.

Singh, S., Ghoshal, N., Sight, K.P., 2007. Influence of resource quality of organic inputs on rice-barley dryland agro-ecosystem: Variations in biological productivity, grain yield and efficiency on nitrogen use. Experimental Agriculture 43, 149-161.

Skiba, U., Smith, K.A., 2000. The control of nitrous oxide emissions from agricultural and natural soils. Chemosphere - Global Change Science 2, 379-386.

Skiba, U., Smith, K.A., and Fowler, D., 1993. Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. Soil Biology and. Biochemistry. 25 pp. 152-156.

Skopp, J., Jawson, M.D., and Doran, J.W., 1990. Steady-state aerobic microbial activity as a function of soil water content. Soil Science Society of America Journal 54 1619-1625.

Smit, A.L., Groenwold, J., 2005. Root characteristics of selected field crops: Data from the Wageningen Rhizolab (1990–2002). Plant and Soil 272, 365-384.

Smith, K.A., Thomson, P.E., Clayton, H., McTaggart, I.P., Conen, F., 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmospheric Environment 32, 3301-3309.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F., O'Mara, C.R., B. Scholes, O. Sirotenko 2007. Agriculture. In Climate Change 2007:

Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change In: [B. Metz, O.R.D., P.R. Bosch, R. Dave, L.A. Meyer (eds)], (Ed.). Cambridge University Press,Cambridge, United Kingdom and New York, NY, USA.

Snyder, C.S., Bruulsema, T.W., Jensen, T.L., Fixen, P.E., in press. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agriculture, Ecosystems & Environment In Press, Corrected Proof.

Stern, W.R., 1993. Nitrogen fixation and transfer in intercrop systems. Field Crops Research 34, 335-356.

Stevens, R.J., Laughlin, R.J., 1998. Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. Nutrient Cycling in Agroecosystems 52, 131-139.

Szumigalski, A.R., Van Acker, R.C., 2006. Nitrogen Yield and Land Use Efficiency in Annual Sole Crops and Intercrops. Agronomy Journal 98, 1030-1040.

Thorsted, M.D., Olesen, J.E., Weiner, J., 2006. Mechanical control of clover improves nitrogen supply and growth of wheat in winter wheat/white clover intercropping. European Journal of Agronomy 24, 149-155.

Trewavas, A., 2001. Urban myths of organic farming. Nature 410, 409-410.

Vinten, A.J.A., Howard, R.S., and Redman, M.H., 1991. Measurement of nitrate leaching losses from arable plots under different nitrogen input regimes. Soil Use and Management 7, 3-13.

Vinten, A.J.A., Vivian, B.J., and Howard, R.S., 1992. The effect of nitrogen fertilizer on the nitrogen cycle of two upland arable soils of contrasting textures. Proceedings of the Fertilizer Society.

Vinther, F.P., Jensen, E.S., 2000. Estimating legume N2 fixation in grass-clover mixtures of a grazed organic cropping system using two 15N methods. Agriculture, Ecosystems & Environment 78, 139-147.

Watson, C.A., Atkinson, D., Gosling, P., Jackson, L.R., and Rayns, F., 2002. Managing soil fertility in organic farming systems. Soil Use and Management 18, 239-247.

Vinten, A. J. A. "Predicting nitrate leaching from drained arable soils derived from glacial till." Journal of Environmental Quality 28.3 (1999): 988-96.

Zheng, X., Wang, M., Wang, Y., Shen, R., Gou, J., Li, J., Jin, J., Li, L., 2000. Impacts of soil moisture on nitrous oxide emission from croplands: a case study on the rice-based agro-ecosystem in Southeast China. Chemosphere - Global Change Science 2, 207-224.

9. General discussion

Nitrogen (N) production has more than doubled globally in the last century because of human activities and population growth. The increased demand for global food production including high amounts of dairy and meat consumption in human diets has had to be supported by increased use of N fertiliser. Nitrogen is one of the most important nutrients for the development and growth of plants in agricultural systems. However, the consequences of excessive use of N results in several effects on air, water and soil quality, and the wider ecology of agricultural systems. Global greenhouse gas budgets have been modified in addition to the formation of N oxides and tropospheric ozone resulting in the loss of stratospheric ozone and changes in radiative balance. Water quality has also been affected, resulting in the pollution of drinking water, groundwater and oceans, and soil quality has been negatively modified through soil acidification and nutrient excesses resulting in higher levels of carbon in soil.

Nitrogen losses from agro-ecosystems have steadily increased due to low efficiency of N use, leading to greater environmental pollution. Nitrous oxide (N_2O) from agriculture contributes a high proportion of the total greenhouse gas emissions and consequently it is considered one of the main environmental problems. However, EU targets for reduced fluxes by 2020 and 2050 drive the search to find alternative ways of supplying N to crops and to mitigate the N losses. Nitrate (NO_3^-) leaching from agricultural systems is the primary form of N leached into groundwater; this is totally soluble and its movement is related to water movement.

An increased use of legume crops has also enhanced biological N-fixation, due to symbiosis with nitrogen-fixing bacteria in nodules. They also do not require N fertilisers, so the use of fossil energy use and gaseous losses are consequently less. However, the grain legumes are not produced in high amounts in Europe, as the production of them has decreased the last 10 years and the import of legumes from South America is essential for feeding.

In this thesis the effects of intercrops on cereal production were studied in order to examine whether such an approach as growing cereals with legumes could lead in a more efficient N utilisation and reduce N losses. Possible carry-over effects to the following year's crops were also measured focusing on N losses. In order to understand the processes regulating N losses, the influence of the variety/ genotype as well as the influence of the different species were investigated without any input of inorganic or organic fertiliser.

9.1. N_2O losses

Influence of varieties and species on gaseous N losses

Different varieties have different yields and N uptake (Willey, 1979; Szumigalski and Van Acker, 2006; Kihara *et al.*, 2007), but what was not known was the influence of varieties on the environment, specifically N_2O emissions. N_2O fluxes varied between treatments, but also showed seasonal patterns and varied between years in the same treatments. This indicates as has been shown in previous studies that the principal variable controlling the rate of N_2O fluxes were not only the soil N but also the treatments and the climatic conditions, such as soil moisture (Flechard et al. 135-52).

The field experiment on the drainage plots in 2006/2007 investigated the different patterns of N_2O emissions from different varieties and species of legumes intercropped with spring barley. The highest fluxes were observed from barley/ clover for the whole duration of the experiment increasing throughout the growing seasons. Barley/ pea cv. *Nitouche* had the second highest cumulative emissions of N_2O, but in the growing year 2006, it was larger in magnitude. On the other hand, comparing the same species but different variety, it was found that barley/ pea cv. *Zero 4* had the lowest fluxes of all treatments, even in comparison to barley monocrop. High N_2O fluxes from low input systems can partly be explained by the N input from N_2 fixation, providing additional N. Indeed, available N soil content increased during the growing stages of legumes especially for the barley/ clover and barley/ pea cv. *Nitouche*. Such differences in N_2O fluxes between similar species have not been reported in a previous study. The main soil N storage from fixed N was confined to the upper soil profile, where it most vulnerable to loss and most of the N_2O production processes occur. It is important to focus then at the upper soil profile and to manage it appropriately. N_2O emissions during the winter months followed typical seasonal patterns with low emissions during winter months, which then increased with rising temperature and cultivation. Additionally the carry-over effect, has not been observed in any other study. In the following years, the cumulative N_2O fluxes from barley/ clover was almost three times higher than the first growing season with the highest peaks occurring after ploughing.

Calculation of the global warming potential (GWP) for each of treatment, which is a measure of how much a given mass of greenhouse gas is estimated to contribute to global warming, indicated that barley/ clover had the highest GWP of 2.5 tCO_{2e} in comparison with the control barley monocrop (0.6 tCO_{2e}). However, the barley/ pea cv. Nitouche had a GWP value of 1.2 tCO_{2e}, but the lowest GWP was from the intercrop treatment of barley/ pea cv. Zero 4 (0.4 tCO_{2e}) showing the high potential and benefit of some cereal/ legume intercrops.

Intercrops therefore can contribute to lower significant mitigation of N_2O emissions from agriculture by careful choice of appropriate species and/ or varieties. It is an alternative management option that, in combination with other mitigation options, such as improved nutrient use, tillage and residue management can reduce the fluxes (Mosier *et al.*, 1998; Ball *et al.*, 1999; Miller *et al.*, 2008). However, although legume systems can sometimes reduce the external inputs, at the same time fixed N_2 can be a source of N_2O (Rochette and Janzen, 2005) and therefore providing winter cover after the harvest may influence/ mitigate winter losses (Ritter *et al.*, 1998; Dobbie, 1999; Vinten *et al.*, 2002). Although catch crops are commonly used, such an approach may reduce the amount of N left in the soil from the previous crop that can be lost either as NO_3^- leaching or N_2O, and alter the timing of availability to the subsequent crop (Thorup-Kristensen, 1993; 1994). N mineralization of the catch crop residues depends on the time of cut and incorporation, as well as the C:N ratio of the residues (Herrera and Liedgens, 2009).

In conventional farming systems, surplus soil N is highly positively correlated with N_2O emissions. Precise application of fertiliser, using slow release fertilisers or nitrification inhibitors, adjusting the timing of application with the plant N uptake and avoiding unnecessary N applications can reduce the N_2O losses (Robertson *et al.*, 2000; Smith and O'Mara, 2007). Using newer technologies, such as precision farming, which involves spatially explicit information on soils and allows a farmer to target inputs of nutrients to optimise nutrient supplies, can minimise potential losses. Combining that kind of technology with intercropping may be an efficient mitigation option for the future. The different varieties might contribute in addition to the management practices, because of the different use of the available resources.

There is no one solution leading to reduced N_2O emissions from agricultural soils, what is required is a combination of approaches that increase nitrogen use efficiency within the agricultural systems and at the same time improved understanding the different levels of N_2O emissions from varied soil types and climatic conditions. Using different varieties or actually breeding varieties of legumes for intercropping purposes to reduce

N_2O losses, may offer the opportunity to reduce the emissions in parallel with increasing nitrogen use efficiency in the agricultural systems.

Influence of varieties and species on N_2O *losses from the drainage*

N_2O losses from drainage water are generated in the deeper soil layers and comprise a relatively small component of the total agriculturally derived N_2O emissions (Höll *et al.*, 2005), Conversion of NO_3^- leaching or run off from soil via aquatic denitrification and redeposition of volatilised non-N_2O N-oxides and ammonia on soils to N_2O, can be of the order of 200 g N_2O ha^{-1} per year compared with around 8500 g N_2O ha^{-1} total, for the same period of measurements. The highest fluxes were from barley/ pea cv. *Nitouche* , which were significantly different from barley/ clover. Barley/ pea cv. *Zero 4* had minimal drainage, so the fluxes were difficult to measure. At present, these indirect losses are denoted by the IPCC and represent an area of continuing uncertainty in global N_2O budgets. Further research into fluxes from these environments may serve to bridge the gap between the combination of the IPCC default direct emission factor for N_2O (~ 1% of reactive N input) and the indirect emission from leaching (designated by IPCC as "EF_{5-g}"), and the global average figure of 3–5% for total N_2O emissions from inputs of reactive N recently suggested by Crutzen *et al.* (2008). The use of models to estimate the indirect losses from agricultural soils is very important for future estimations of N losses and mitigation strategies. Such process-based models should clarify potential to reduce uncertainties for both direct and indirect N_2O emissions, as such models could account for how climate, soil type, and N inputs affect both total N losses and the proportion of losses that are in the form of N_2O, NO_x, NH_3, and N_2 gases, and NO_3 leaching.

9.2. NO_3^- leaching

Influence of varieties and species on NO_3^- *leachate*

Leaching is a complex function of land use, cropping system, soil type, climate, topography, hydrology and nutrient management with only the biological components being easily controlled. The environmental impact of agricultural practices has received much focus over recent decades. Nitrogen leaching has been one of the issues of concern, due to the negative influence on ground water quality and the eutrophication of coastal waters. In low input systems, such as organic systems, the input of N is provided by legumes or manure application and may be a viable alternative agricultural practice to conventional systems as it may enhance the environmental quality. However, in some studies, the use of manure has been shown to result in equal amounts of N leached as the application of inorganic fertilisers (Thomsen, 1993; Jones et al., 2005). Unfortunately, heavy rainfall, less plant cover and less mivrobial activity for N uptake during the autumn and winter months normally leach appreciable amounts of N, as happened in autumn 2006 and 2007, when the highest NO_3^- leaching losses in this study occurred. However, several studies (Beaudoin *et al.*, 2005; Geijersstam and Mayrtensson, 2006) have confirmed that the NO_3^- leaching is higher in crop rotations that include legume plants due to the fact that legumes fix nitrogen. Undersowning barley with clover during the first growing season provided the benefit of covering the cultivated area during the winter months (Vinten *et al.*, 2002) and therefore resulting possibly in less runoff and leaching. However, ploughing of clover/ grass leys in autumn can contribute highly to NO_3^- leaching as the nitrogen in organic materials can be processed when N uptake from plants is not taking place. There is a lack of knowledge about the interactions between mineralisation and immobilisation; processes that are essential for incorporating plant material and residues into soil. Using intercrops to supply N in the cereal crop, can reduce the addition of synthetic fertilisers. Using alternatives, such as a cover crop during winter or catch crop can reduce the N losses significantly. Intercropping legumes with cereals can reduce the environmental risks of NO_3^- leaching due to higher uptake of soil available N and the higher N utilisation effects if the above ground residues remain on the field. Low input farming systems, including organic systems, have high possibilities to reduce nitrate leaching, as the use of fertilisers is not allowed and the inputs are in general lower than in the conventional systems. However, if leaching is calculated per unit of yield rather than per ha, then it may be as high as in conventional crops (Kirchmann and and Bergström, 2001).

Groundwater pollution by nitrate is a serious problem in the European Union and in many other developed countries. The European Union has implemented the Water Framework Directive, which aims for all water bodies to reach good ecological status by 2015 (Directive and 2000/60/EC, 2000; Letcher and Giupponi, 2005). Use of nitrification inhibitors may be a possible solution as they control the formation of nitrate and in general regulate biological processes (Kirchmann *et al.*, 2002). However, there is still a lack of evidence on the 'controls' of the breakdown of mineralization and further research is needed.

In general, the practices that reduce N_2O emissions often reduce NO_3^- leaching and thereby contribute to a reduction in the pollution of ground-water (Olesen *et al.*, 2006). Reducing the fertiliser application below the expected economic rate, using nitrification inhibitors and sensible use of manures in low input systems can contribute to minimising

NO_3^- leaching (Kirchmann *et al.*, 2002). Nutrient management can result in efficient use of fertilisers avoiding harmful effects on water and air quality of N losses (Olesen *et al.*, 2006)

9.3. Role of N in Grain Yield Production

Crop mixtures contain more N than comparable monocrops confirming the benefits of growing a legume with a non-legume (Szumigalski and Van Acker, 2008). Grain yield from the barley crop was significantly higher between barley/ clover and the other treatments. Clover plants possibly supplied N in the mid boot stage or earlier, as N application at such stages can influence yield whereas later applications usually do not affect yield, but can raise grain N concentrations (Thorsted *et al.*, 2002). The two barley/ pea treatments had no significant effects on grain yield for both crops in comparison with the other treatments and within the barley/ pea treatments, although they did cause significant differences in the above ground biomass of almost 2 t ha^{-1} with barley/ pea cv. Nitouche to have the highest.

The most important result was the quantification of the substantial effect of the previous legume on the oat crop. Oats following the barley/ clover treatment had the highest grain yield, possibly due to the continuous growth during the winter months (2006-2007) of the clover plants regrown after the harvest, providing a cover crop.

Low input and organic farming systems can benefit from the use of legumes in their rotations resulting in a higher potential cereal grain yield derived from the use of intercrops and also benefits for the following crop. The choice of species and/ or varieties must be considered carefully and will be influenced by factors such as maturity dates, potential yields, physiological characteristics and use of the harvested crop (e.g. animal food, human consumption). Undersowning cereals with clover even at low rates can contribute to potentially high supplies of N to the accompanying crop, reduce leaching during the winter months, and benefit soil structure and the subsequent crop (Løes *et al.*, 2006). The use of other legumes, such as peas, intercropped with cereals can have higher combining yields and have a residual effect on the following crop (Paper 1).

9.4. Economic implications of low input intercropped systems

The present research did not address social and economic aspects, but focussed only on agronomic and environmental aspects of the intercrops. That does not imply that such factors are less important. The economic value of the N fixed by legumes varies widely. On the one hand, the cost of production of the legumes must be considered, the amount of fixed N returned to the soil, and the availability of this N for future crops. Often, these costs are compared directly against the cost of purchasing and applying an equal quantity of N fertiliser plus the net income lost by producing a legume instead of a grain crop (if the legume is grown in rotation). In the past several decades, the cost of production and price of N fertilisers have been such that this type of calculation would generally favour the use of N fertiliser. This fact is largely responsible for the decreased use of legumes in our crop production systems over the past 40 years (Power, 1987). Although, current economics are uncertain the increased use of legumes maybe favoured once again.

Intercropping in low input systems has similar effects to use in organic systems. In several studies, intercrops have been shown to be productive than the monocrops (Ofori *et al.*, 1987; Fujita *et al.*, 1992; Hauggaard-Nielsen and Jensen, 2001). The intercrops used in the present study make different use of the resources. The intercrops with LER values higher than one, showed an economic benefit in comparison with their associate cereal monocrop. However, in low input systems, the main problem is weeds and the difficulty of control them. However, the growth of two or more species has better competitive ability towards weeds than monocrops. The differential use of resources by intercrop components may minimise the availability of light, water and nutrients for weeds and thereby reduce weed growth (Brandsaeter *et al.*, 1998; Hauggaard-Nielsen *et al.*, 2006; Szumigalski and Van Acker, 2006; Thorsted *et al.*, 2006).

The choice of an intercropping system is important, as it may influence the amount of N leached and can be an important management tool. However, the choice of cultivar has also been shown to be critical to the economic evaluation which may be added to the environmental benefits, such as the reduction in air (N_2O) and water (leaching) losses. The importance of variety needs to be investigated further to ascertain the full significance of genotypes in relation to minimising losses whilst ensuring economic viability.

9. 5 Models of intercropped systems

Intercropping is a rather complex agricultural system in comparison with monocrops and involves an 'unpredictable' balance between factors influencing environmental and agro-ecological effects of the two species, which make future predictions complicated.

Modelling intercropping systems for the development of more sustainable production systems and involving limited inputs or low soil fertility, such as organic farming systems (Watson *et al.*, 2002) can be very valuable in understanding interspecific interactions and to help manage intercropping. In reality, it is very difficult to control all the different factors related with intercropping, such as available resources (water, light, nutrients). The intercrops optimise differently nitrogen use, as barley compete for soil mineral N and legume depends on BFF (Jensen, 1996; Corre-Hellou *et al.*, 2007; Hauggaard-Nielsen *et al.*, 2009).

Nevertheless, several models have previously simulated different intercrop systems, mainly grass/ clover leys or cereal legume mixtures and include factors such as competition for light, water and nutrients. Corre-Hellou *et al.* (2009) tried to simulate the dry weight production, N accumulation, N_2 fixation and soil inorganic N by adapting a sole crop model for an intercropped situation for predicting the composition of the final mixture according to soil N supply. Modelling two different species, such as barley and peas, which have different sensitivities, growth rate and plant height is complicated (Berntsen *et al.*, 2004). Height should be disconnected from leaf area and should be mainly driven by thermal time (Corre-Hellou *et al.*, 2009). However, the number of plants (plant density) and nitrogen fixation rates of the legumes also need to be considered.

Models can help us to understand intercropping systems in rotations. Creating different scenarios, such as comparing monocrops with different legumes (species), can help to estimate grain yields and how much they depend on nitrogen inputs. Additionally, including edaphic and climatic factors, such as soil moisture, temperature and organic matter, related with N_2O and NO_3^- leaching, can lead to estimation of these N losses.

Long term simulations might improve our understanding of how to increase crop yields and reduce NO_3^- leaching and N_2O emissions by exploring options such as incorporation of crop residues, minimum tillage and use of winter cover crops or catch crops. Furthermore, testing different scenarios of N applications including rate, type and timing can provide information of environmental and agronomic advantage.

Considering the complex and dynamic interactions between the species for nitrogen is essential and should include a wide range of data from different situations and limiting factors occurring in low input systems, such as weed, yields, and water. Additionally, there is a need to include other essential factors influencing grain yields in future models, such as diseases, pests and stress conditions (water, temperature).

Research of this kind may be translated directly into management guidelines which could be incorporated into policy at a later date. Experts from different research backgrounds and policy makers are needed to consolidate this in order to have a holistic approach for the future. Farmers will have to be adaptable in the future in order to respond to environmental change and reduce the Impacts of agriculture on the environment. The general public must also be kept informed of future and current policies within a simple understandable format. The transfer of knowledge between researchers and farmers should be constructive to enable the exchange of new practical and scientific ideas in order to provide economically and environmentally sustainable agricultural systems.

Reference List

Flechard, C. R., Ambus, P., Skiba, U. M., Rees, R. M., Hensen, A., van Amstel, A., van den Pol-van Dasselaar, Soussana, J. F., Jones, M., Clifton-Brown, J., Raschi, A., Horvath, L., Neftel, A., Jocher, M., Ammann, C., Leifeld, J., Fuhrer, J., Calanca, P., Thalman, E., Pilegaard, K., Di Marco, C., Campbell, C., Nemitz, E., Hargreaves, K. J., Levy, P. E., Ball, B. C., Jones, S. K., van de Bulk, W. C. M., Groot, T., Blom, M., Domingues, R., Kasper, G., Allard, V., Ceschia, E., Cellier, P., Laville, P., Henault, C., Bizouard, F., Abdalla, M., Williams, M., Baronti, S., Berretti, F., and Grosz, B. Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. Agriculture, Ecosystems and Environment 121, 135-52. 2007.

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10. Conclusions

This research project has shown that intercrops may have agronomic and environmental advantages over mono-crops related to the selected variety or species. The reduction in gaseous emissions and water pollution from agriculture has increased the need to develop more sustainable agricultural systems, and has provided renewed opportunities for evaluating the use of intercrops in low input rotations aimed at maintaining productivity and providing environmental protection.

This research project demonstrates that low input systems can benefit from legume-cereal intercropping and contribute to achieving the UK government's target of reducing greenhouse gas emissions by 80% by the year 2050. The barley/ pea cv. Zero 4 had the lowest GWP. Simirarly, the N_2O losses in terms of grain yield were similar to GWP with barley/ pea cv. Zero 4 to have 0.18 kg of N2O-N per ton of grain yield. However, there are some uncertainties; the carbon sequestration rate that can additionally contribute to the GWP has not been measured, and the approach to express these values can vary, either as CO_{2e} per area of land or CO_{2e} per product.

The choice of legume species and/ or cultivar and crop rotation, influence gaseous losses and nitrate leaching as well as yields and are therefore critically important to farmers/ growers. The choice of legume variety for intercropping purposes has been shown through this research to have the potential to contribute to reducing the environmental impacts of agriculture. Legume choice is central to optimising plant productivity in intercropping designs. Cultivars for intercropping purposes must be chosen with care, taking into account the effects upon the growth of the partner crop/s as well as to the following crop and include environmental factors. Including intercropping in a rotation may maintain higher yields, reduce N losses and enhance the soil fertility for the subsequent crop. Additionally, longer term experiments can help us to understand the utilisation of the resources and the interactions between intercropping systems in the development of sustainable agricultural systems.

11. Recommended Future Work

After the completion of the experiment, we realised that in hindsight other data could have been collected on a more frequent basis. This data would have helped us gain a better understand of some of the many interactions that are occurring in complex systems such as intercropping. During the beginning of the growing season, daily collection of data might be important, as the range of soil management and plant growth activities become more intensive. Further analyses of biological processes, such as measuring N_2 fixation of the legumes and measuring other nutrients, such as carbon and phosphorus, are closely related with the nitrogen cycle and could help in the understanding and explanation of some of these results. Finally, establishment of the experiment under varied climatic and soil conditions could provide stronger evidence for the effects of legumes.

The differences found in the two pea varieties intercropped with barley requires further study. A more detailed understanding of the root systems focused on microbial activity in the rhizosphere, number of nodules and root formation, would be important. The use of DNA-based techniques could allow the fate of particular genes or organisms to be monitored directly in environmental samples. Such DNA would be extracted directly from the soil. Ammonia-oxidising proteobacteria, nitrite-oxidising microbes and free-living N_2-fixation microbes can be identified using the Polymerase Chain Reaction (PCR) technique (Compton et al., 2004; Wakelin et al., 2006).

In addition, field experiment involving a range of cultivars should be undertaken in order to investigate mitigation of GHG emissions and leaching losses in the year of growth and the subsequent year. Using cultivars of peas and beans, as mono-crops and intercrops with cereals (barley, wheat and oat) should be considered. Cereal crops would be appropriate following a legume in a rotation especially in organic farming. Repetition of the same experiment under different climatic conditions would provide a clearer conclusion about the inter-relationships between N_2O emissions, soil properties and climate. Such an experiment would examine below ground aspects of system performance, such as nodulation, root formation, WFPS, denitrification and soil N fractions as well as above ground characteristics, such as yields, N accumulation and biomass. Farmers would have more information on the benefit of intercropping in comparison with the associated monocrop regarding yield and N supply and additional evidence could be provided of the environmental benefits.

Existing models such as DNDC (DeNitrificationDeComposition) and SPACSYS would be used to test scenarios under different climates and provide up to date information on the contribution of different cropping systems to environmental change. Such models can be further developed to make predictions for GHG emissions under specific climatic and soil conditions. There is a need to provide continuous data for validating models, so as to have reliable predictions. The DNDC model is a process based agro-ecosystem model that runs on a daily time-step and is driven by meteorological data, soil and environmental data and information on crop management. The outputs include estimates of C and N exchange with the environment including CO_2, N_2O and CH_4 emission, and nitrate leaching. The model has been applied extensively to agro ecosystems around the world and is widely acknowledged as a state-of-the-art model for use in assessing nutrient fluxes in arable farming systems (Li *et al.*, 1992; Saggar *et al.*, 2004; Li *et al,*. 2006). The SPACSYS model is an alternative option, which is a multi-dimensional, field scale, weather-driven dynamic simulation model of C and N cycling between plants, soils and microbes. It operates with a daily time-step and also integrates interactions between below- and above- ground plant growth, N and C cycling and water in the plant-atmosphere-soil continuum. Finally, it incorporates a highly detailed module describing root system nutrient cycling processes and is applicable to both monocrop and intercrop systems.

A GHG mitigation route map for agriculture has been set out following recent research by SAC for the Committee on Climate Change and Defra, using marginal abatement cost curves (MACCs). MACCs show how different crop, soil and livestock measures can be used to mitigate (i.e. prevent the release of) greenhouse gases. A MACC ranks measures according to the cost per tonne of CO_{2e} abated. In other words, some appropriate farm measures can be identified as preventing the release of a tonne/ CO_{2e} more cheaply than others. To determine an efficient mitigation budget in agriculture or on a specific farm, it is important for all technologies available to be assessed and to be identified in terms of this approach. Such an approach could be used to understand the potential economic consequences of intercropped low input farming as a mitigation tool. The MACC approach would also allow the benefits of such systems to be compared with other mitigation technologies.

Alternative approaches to soil management are important both to reduce N losses and at the same time to allow enough food to be produced. The advice provided to the farmers must be rapid and the sharing of new knowledge must be constant, so as to be able to develop more sustainable farming practices.

The research challenges of the future will be determined by national and international strategies and will need to balance the needs of sustainable productions against climate change and food security. Reappraisal of production systems, such as the value of intercropping and the role of legumes will be part of meeting these future targets.

References

ADAS, 2003. http://www.adas.co.uk/media_files/Publications/science_review_2003-2004.pdf.

Altieri, M.A., Ponti, L., and Nicholls, C. , 2005. Enhanced pest management through soil health: toward a belowground habitat management strategy. Biodynamics 33-40.

Anderson, G.C., Fillery, I.R.P., Dolling, P.J., and Asseng, S., 1998. Nitrogen and water flows under pasture-wheat and lupin-wheat rotations in deep sands in Western Australia. 1. Nitrogen fixation in legumes, net N mineralisation, and utilisation of soil-derived nitrogen. Australian Journal of Agricultural Research 49, 329-343.

Armstrong, G., and McKinlay, R.G., 1997. The effect of undersowing cabbages with clover on the activity of carabid beetles. Biological Agriculture and Horticulture 15 269-277.

Arregui, L., Quemada, M., 2006. Drainage and nitrate leaching in a crop rotation under different N-fertilizer strategies: application of capacitance probes. Plant and Soil 288, 57-69.

Athar, M., Johnson, D.A., 1997. Effect of drought on the growth and survival ofRhizobium melilotistrains from Pakistan and Nepal. Journal of Arid Environments 35, 335-340.

Badgley, C., Moghtader, J., Quintero, E., Zakem, E., Jahi Chappell, M., Avilés-Vázquez, K., Samulon, A. and Perfecto, I. , 2007. Organic agriculture and the global food supply. . Renewable Agriculture and Food Systems 22, 86-108.

Ball, B.C., Scott, A., Parker, J.P., 1999. Field N_2O, CO_2 and CH_4 fluxes in relation to tillage, compaction and soil quality in Scotland. Soil and Tillage Research 53, 29-39.

Barker, T., Bashmakov, I., Alharthi, A., Amann, M., Cifuentes, L., Drexhage, J., Duan, M., Edenhofer, O., Flannery, B., Grubb, M., Hoogwijk, M., Ibitoye, F. I., Jepma, C. J. and Pizer, W. A. (2007). Mitigation from a cross-sectoral perspective, in Climate Change 2007: Mitigation, Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, eds. B. Metz, O. R. Davidson, P. R. Bosch, R. Dave and L. A. Meyer. Cambridge: Cambridge University Press.

Beaudoin, N., Saad, J.K., Van Laethem, C., Machet, J.M., Maucorps, J., Mary, B., 2005. Nitrate leaching in intensive agriculture in Northern France: Effect of farming practices, soils and crop rotations. Agriculture, Ecosystems & Environment 111, 292-310.

Bengtsson, J., Ahnström, J., and Weibull, A.-C., 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. Journal of Applied Ecology 42, 261-269.

Beringer, J.E., Brewin, N., Johnston, A.W.B., Schulman, H.M., Hopwood, D.A., 1979. The Rhizobium-Legume Symbiosis. Proceedings of the Royal Society of London. Series B. Biological Sciences 204, 219-233.

Berntsen, J., Grant, R., Olesen, J.E., Kristensen, I.S., Vinther, F.P., Mølgaard, J.P., Petersen, B.M., 2006. Nitrate leaching from organic farming systems with rotational grass-clover and arable crops. Soil Use and Management 22, 197-208.

Berntsen, J., Hauggard-Nielsen, H., Olesen, J.E., Petersen, B.M., Jensen, E.S., Thomsen, A., 2004. Modelling dry matter production and resource use in intercrops of pea and barley. Field Crops Research 88, 69-83.

Bordeleau, L.M., and Prevost, D., 1994. Nodulation and nitrogen fixation in extreme environments. Plant and Soil 161, 115-125.

Bouwman, A.F., 1996. Direct emission of nitrous oxide from agricultural soils. Nutrient Cycling in Agroecosystems 46, 53-70.

Bowman, A.M., Peoples, M.B., Smith, W., and Brockwell, J., 2002. Factors affecting nitrogen fixation by dryland lucerne in central-western New South Wales. Australian Journal of Experimental Agriculture 42, 439-451.

Brandsaeter, L.O., Netland, J., and Meadow, R., 1998. Yields, weeds, pests and soil nitrogen in a white cabbage living mulch system. Biological Agriculture and Horticulture 16, 291-309.

Brandsaeter, L.O., Netland, J. and Meadow, R., 1998. Yields, weeds, pests and soil nitrogen in a white cabbage living mulch system. Biological Agriculture and Horticulture 16, 291-309.

Bremner, J.M., 1997. Sources of nitrous oxide in soils. Nutrient Cycling in Agroecosystems 49, 7-16.

Brockwell, J., Bottomley, P.J., Thies, J.E., 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil. 174:143-180.

Bulson HAJ Snaydon RW and Stopes CE 1997. Effects of plant density on intercropped 20 wheat and field beans in an organic farming system Journal of Agricultural 21 Science, Cambridge 128, 59-71.

Chen, S., Huang, Y., Zou, J., 2008. Relationship between nitrous oxide emission and winter wheat production. Biology and Fertility of Soils 44, 985-989.

Cocking, E.C., 2003. Endophytic colonization of plant roots by nitrogen-fixing bacteria. Plant and Soil 252, 169-175.

Connolly, J., Wayne, P., Murray, R., 1990. Time course of plant-plant interactions in experimental mixtures of annuals: density, frequency, and nutrient effects. Oecologia 82, 513-526.

Cook, R.J., 1984. Root health: Importance and relationship to farming practices. Organic farming, 111-127.

Corre-Hellou, G., Brisson, N., Launay, M., Fustec, J., Crozat, Y., 2007. Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. Field Crops Research 103, 76-85.

Corre-Hellou, G., Faure, M., Launay, M., Brisson, N., Crozat, Y., 2009. Adaptation of the STICS intercrop model to simulate crop growth and N accumulation in pea-barley intercrops. Field Crops Research 113, 72-81.

Crutzen, P.J., Mosier, A.R., Smith, K.A., and Winiwarter, W., 2008. N_2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. Atmos. Chem. Phys. 8 pp. 389-395.

Dalgaard, T., Halberg, N., Porter, J.R., 2001. A model for fossil energy use in Danish agriculture used to compare organic and conventional farming. Agriculture, Ecosystems & Environment 87, 51-65.

Davidson, E.A., Keller, M., Erickson, H.E., Verchot, L.V., Veldkamp, E., 2000 Testing a conceptual model of soil emissions of nitrous and nitric oxides bioscience 50, 667-680(614).

Davidson, E.A., Matson,P. A., Vitousek,P. M., Riley, R., Dunkin, K., Garciamendez, G., and Maass,J. M. , 1993. Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest. Ecology 74, 130-139.

DEFRA, 2005. Organic Statistics England. http://statistics.defra.gov.uk/esg/statnot/orgeng.pdf.

Directive, 2000/60/EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

Djurhuus, J., Olsen, P., 1997. Nitrate leaching after cut grass/clover leys as affected by time of ploughing. Soil Use and Management 13, 61-67.

Dobbie, K.E., McTaggart, I.P., Smith, K.A., 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research D: Atmospheres 104, pp. 26891-26899.

Doyle, C.J., and Bevan, K., 1996. Economic effects of legume-based grassland systems. Legumes in Sustainable Farming Systems.

Drinkwater, L.E., Wagoner, P. and Sarrantino, M., 1998. Legume based cropping systems have reduced carbon and nitrogen losses Nature 396 262-265.

Eisenhauer, N., Scheu, S., 2008. Earthworms as drivers of the competition between grasses and legumes. Soil Biology and Biochemistry 40, 2650-2659.

Elgersma, A., Schlepers, H., Nassiri, M., 2000. Interactions between perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.) under contrasting nitrogen availability: productivity, seasonal patterns of species composition, N2 fixation, N transfer and N recovery. Plant and Soil 221, 281-299.

Erisman, J.W., A. Bleeker, A. Hensen, and A. Vermeulen. 2008. Atmospheric Environment, 42:3209- 3217.

Flechard, C.R., Ambus, P., Skiba, U., Rees, R.M., Hensen, A., van Amstel, A., Dasselaar, A.v.d.P.-v., Soussana, J.F., Jones, M., Clifton-Brown, J., Raschi, A., Horvath, L., Neftel, A., Jocher, M., Ammann, C., Leifeld, J., Fuhrer, J., Calanca, P., Thalman, E., Pilegaard, K., Di Marco, C., Campbell, C., Nemitz, E., Hargreaves, K.J., Levy, P.E., Ball, B.C., Jones, S.K., van de Bulk, W.C.M., Groot, T., Blom, M., Domingues, R., Kasper, G., Allard, V., Ceschia, E., Cellier, P., Laville, P., Henault, C., Bizouard, F., Abdalla, M., Williams, M., Baronti, S., Berretti, F., Grosz, B., 2007. Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. Agriculture, Ecosystems & Environment 121, 135-152.

Focht, D.D., and Verstraete, W., 1977. Biochemical ecology of nitrification and denitrification. Advances in Microbial Ecology 1 pp. 135-214.

Fowler, D., Skiba, U., Moncrieff, J.B., Polson, D., 2008. Greenhouse gas emissions, inventories and validation. SEPA/ SAC conference, Land Management in a Changing Environment.

Frame J., Charlton J.F.L., Laidlaw A.S. (1998): Temperate forage legumes. CAB International, Wallingford. 327 p.

Freibauer, A., Kaltschmitt, M., 2003. Controls and models for estimating direct nitrous oxide emissions from temperate and sub-boreal agricultural mineral soils in Europe. Biogeochemistry 63, 93-115.

Fujita, K., Ofosu-Budu, K.G., Ogata, S., 1992. Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant and Soil 141, 155-175.

Galloway, J.N., 1998. The global nitrogen-cycle: Changes and consequences. Environmental Pollution 102, 15-24.

Galloway, J.N., and Cowling, E.B., 2002. Reactive Nitrogen and The World: 200 Years of Change. AMBIO: A Journal of the Human Environment 31, 64-71.

Geijersstam, L.A., Mayrtensson, A., 2006. Nitrogen fixation and residual effects of field pea intercropped with oats. Acta Agriculturae Scandinavica, Section B - Plant Soil Science 56, 186 - 196.

Goulding, 2000. Nitrate leaching from arable and horticultural land. Soil Use and Management 16, 145-151.

Granstedt, A., 1991. The potential for Swedish farms to eliminate the use of artificial fertilisers. American Journal of Alternative Agriculture 6, 122-131.

Groffman, P.M., 1991. Ecology of nitrification and denitrification in soil evaluated at scales relevant to atmospheric chemistry. I: Rogers, J.E., Whitman, W.B. (red.) Microbial production and consumption of greenhouse gases: Methane, nitrogen oxides and halomethanes. American Society of Microbiology, Washington, D.C., pp 201-217.

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451, 293-296.

Hadas, A., Feigin, A., Feigenbaum, S., Portnoy, R., 1989. Nitrogen mineralization in the field at various soil depths. European Journal of Soil Science 40, 131-137.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. Plant and Soil 236, 63-74.

Hauggaard-Nielsen, H., Andersen, M.K., Jørnsgaard, B., Jensen, E.S., 2006. Density and relative frequency effects on competitive interactions and resource use in pea-barley intercrops. Field Crops Research 95, 256-267.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009. Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crops Research 113, 64-71.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. Field Crops Research 72, 185-196.

Heichel, G. H. 1987. Legumes as a source of nitrogen in conservation tillage. In J. F. Power (ed.) Role of legumes in conservation tillage. Soil Cons. Soc. Amer., Ankeny, lowa

Herrera, J.M., Liedgens, M., 2009. Leaching and Utilization of Nitrogen During a Spring Wheat Catch Crop Succession. Journal of Environmental Quality 38, 1410-1419.

Hofman, G., 2004. Soil and Plant Nitrogen. International Fertilizer Industry Association.

Högh-Jensen, H., 1996. Symbiotic N2 fixation in grass-clover mixtures and nitrogen transfer from clovers to the accompanying grass. In Symbiotic Nitrogen Fixation in Crop Rotations with Manure Fertilization, 7-31.

Høgh-Jensen, H.a.S., J.K., 2001. Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys Soil Biology and Biochemistry 33, 439-448.

Höll, B.S., Jungkunst, H.F., Fiedler, S., Stahr, K., 2005. Indirect nitrous oxide emission from a nitrogen saturated spruce forest and general accuracy of the IPCC methodology. Atmospheric Environment 39, 5959-5970.

Hoyt, G.D., and Hargrove, W.H., 1986. Legume cover crops for improving crop and soil management in the southern Hort. Sci. 21, 397-402.

IFOAM, 2008. http://www.ifoam.org/growing_organic/definitions/doa/index.html.

IPCC, 2006. Guidelines for National Greenhouse Gas Inventories., N_2O emissions from managed soils, and CO_2 emissions from lime and urea application. Published by the Institute for Global Environmental Strategies (IGES) for the IPCC. ISBN 4-88788-032-4

IPCC, 2007. Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko. Agriculture: In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jensen, E.S., 1996. Barley uptake of N deposited in the rhizosphere of associated field pea. Soil Biology and Biochemistry 28, 159-168.

Jensen, E.S., and Hauggaard-Nielsen, H., 2003. How can increased use of biological N2 fixation in agriculture benefit the environment? . Plant and Soil 252, 177-186.

Jones, S.K., Rees, R.M., Skiba, U.M., Ball, B.C., 2005. Greenhouse gas emissions from a managed grassland. Global and Planetary Change 47, 201-211.

Jury, W.A., and Nelson, D.R., 1989. Nitrate transport and leaching mechanisms. Nitrogen Management and Ground Water Protection, 139-157.

IPCC, 2007. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007 B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds) Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Karpenstein-Machan, M., Stuelpnagel, R., 2000. Biomass yield and nitrogen fixation of legumes monocropped and intercropped with rye and rotation effects on a subsequent maize crop. Plant and Soil 218, 215-232.

Kihara, J., Kimetu, J.M., Vanlauwe, B., Bationo, A., Waswa, B., Mukalama, J., 2007. Optimising crop productivity in legume-cereal rotations through nitrogen and phosphorus management in western Kenya. Advances in Integrated Soil Fertility Management in sub-Saharan Africa: Challenges and Opportunities, pp. 493-502.

Kirchmann, H., and Bergström, L.F., 2001 Do organic farming practices reduce nitrate leaching? . Communications in Soil Science and Plant Analysis 32 (7&8).

Kirchmann, H., Johnston, A.E.J., Bergstram, L.F., 2002. Possibilities for reducing nitrate leaching from agricultural land. AMBIO 31, 404-408.

Knudsen, M.T., Kristensen, I. S., Berntsen, J., Petersen, B. M., Kristensen, E. S., 2005. The effect of organic farming on N leaching loss. unpublished.

Korsaeth, A., 2008. Relations between nitrogen leaching and food productivity in organic and conventional cropping systems in a long-term field study. Agriculture, Ecosystems & Environment 127, 177-188.

Kotschi, J., Müller-Sämann, K., 2004. The Role of Organic Agriculture in Mitigating Climate Change: A Scoping Study. Bonn, Germany: International Federation of Organic Agriculture Movements.

Kroeze, C., Mosier, A., Bouwman, L., 1999. Closing the global N_2O budget: a retrospective analysis 1500 - 1994. Global Biogeochem. Cycles 13.

Lampkin, N., 1990. Organic Farming. p.715.

Ledgard, S.F., Penno, J.W. and Sprosen, M.S., 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. Journal of Agricultural Science 132, 215-225.

Letcher, R.A., Giupponi, C., 2005. Policies and tools for sustainable water management in the European Union. Environmental Modelling & Software 20, 93-98.

Liebman, M., Dyck, E., 1993. Crop rotation and intercropping strategies for weed management. Ecological Applications 3, 92-122.

Løes, A.K., Henriksen, T.M., Eltun, R., 2006. Repeated undersowing of clover in stockless organic grain production. Paper presented at Joint Organic Congress, Odense, Denmark, May 30-31, 2006.

Luo J, Tillman R W and Ball P R 1999 Factors regulating denitrification in a soil under pasture. Soil Biology and. Biochemistry. 31, 913–917.

Marsden, T., Banks, J., Bristow, G., 2002. The social management of rural nature: understanding agrarian-based rural development. Environment and Planning A 34, 809-825.

Matson, P.A. and Vitousek, P.M., 1990. Ecosystem Approach to a Global Nitrous Oxide Budget. BioScience 40, 667-672.

Mielke, L.N., and Schepers, J.S., 1986. Plant response to topsoil thickness on an eroded loess soil. Journal of. Soil and Water Conservation. 41, 59-63.

Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., Trevors, J.T., 2008. Crop residue influence on denitrification, N_2O emissions and denitrifier community abundance in soil. Soil Biology and Biochemistry 40, 2553-2562.

Miller, R.M., and Jastrow, J.D., 1996. Contributions of legumes to the formation and maintenance of soil structure. Legumes in Sustainable Farming Systems, 105-112.

Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., van Cleemput, O., 1998. Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. Nutrient Cycling in Agroecosystems 52, 225-248.

Mosier, A.R., 1994. Nitrous oxide emissions from agricultural soils. Nutrient Cycling in Agroecosystems 37, 191-200.

Nevison, C., 2000. Review of the IPCC methodology for estimating nitrous oxide emissions associated with agricultural leaching and runoff. Chemosphere - Global Change Science 2, 493-500.

Niggli, U., Fliessbach, A., and, M., P., 2008. Does Organic Farming have Greater Potential to Adapt to Climate Change? 16th IFOAM Organic World Congress, Modena, Italy.

Nyatsanga, T., and Pierre, W.H., 1973. Effect of nitrogen fixation by legumes on soil acidity. Agronomy Journal 65, 936-940.

Ofori, F., Stern, W.R., Brady, N.C., 1987. Cereal-Legume Intercropping Systems. Advances in Agronomy. Academic Press, pp. 41-90.

Olesen, J.E., Schelde, K., Weiske, A., Weisbjerg, M.R., Asman, W.A.H., Djurhuus, J., 2006. Modelling greenhouse gas emissions from European conventional and organic dairy farms. Agriculture, Ecosystems & Environment 112, 207-220.

Paustian, K., Andrén, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., Noordwijk, M., Woomer, P.L., 1997. Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use and Management 13, 230-244.

Peoples, M. B., J. K. Ladha, and D. F. Herridge. 1995. Enhancing legume N2 fixation through plant and soil management. Plant Soil 174:83-101.

Philipps, L., and Stopes, C.E., 1995. The impact of rational practice on nitrate leaching losses in organic farming systems. Nitrogen leaching in Ecological Agriculture, 123-134.

Philipps, L., Stopes, C.E., and Woodward, L., 1996. The role of the grass/clover ley for nitrogen accumulation within organic farming systems Legumes in sustainable farming systems, 124-125.

Phool Chand Gupta, J.C.O.T., 1986. Upland Rice: A Global Perspective International Rice Research Institute.

Pidwirny, M., 2006. "The Nitrogen Cycle". Fundamentals of Physical Geography. 2nd Edition

Pietsch, G., Friedel, J.K., Freyer, B., 2007. Lucerne management in an organic farming system under dry site conditions. Field Crops Research 102, 104-118.

Power, J.F., 1987. Legumes: Their potential role in agricultural production. American Journal of Alternative Agriculture 2, 69-73.

Provorov, N.A., Tikhonovich, I.A., 2003. Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. Genetic Resources and Crop Evolution 50, 89-99.

Reay, D.S., Edwards, A.C., Smith, K.A., 2004a. Determinants of nitrous oxide emission from agricultural drainage waters. Water, Air, & Soil Pollution: Focus 4, 107-115.

Reay, D.S., Smith, K.A., Edwards, A.C., 2004b. Nitrous oxide in agricultural drainage waters following field fertilisation. Water, Air and Soil Pollution: Focus 4, 437-451.

Rees, R.M., Baddeley, J.A., Bingham, I.J., Watson, C.A., 2006. Nitrogen uptake by spring barley grown with red and white clover. Organic Farming and European Rural Development, 300-301.

Ritter, W.F., Scarborough, R.W., Chirnside, A.E.M., 1998. Winter cover crops as a best management practice for reducing nitrogen leaching. Journal of Contaminant Hydrology 34, 1-15.

Robertson, G.P., Paul, E.A., Harwood, R.R., 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the Atmosphere. Science 289, 1922-1925.

Robertson, L.A., and Kuenen, J.G., 1991. Physiology of nitrifying and denitrifying bacteria. In: Microbial Production and consumption of Greenhouse Gases." (JE Rogers and WB Whitman (eds.)) American Society for Microbiology, Washington, DC, 1991, pp. 189-199.

Rochette, P., Janzen, H., 2005. Towards a revised coefficient for estimating N_2O emissions from legumes. Nutrient Cycling in Agroecosystems 73, 171-179.

Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F., Munch, J.C., 2006. Emission of N_2O, N2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. Soil Biology and Biochemistry 38, 263-274.

Willer, H., Yussefi, M., 2005. The World of Organic Agriculture: Statistics and Emerging Trends

IFOAM, 2008. http://www.ifoam.org/growing_organic/definitions/doa/index.html

Willey, R.W., 1979. Intercropping: Its importance and research needs.Competition and yield advantages. Field Crop Abstr. Part 1., 1-10.

Williams, P.H., Jarvis, S.C., Dixon, E., 1998. Emission of nitric oxide and nitrous oxide from soil under field and laboratory conditions. Soil Biology and Biochemistry 30, 1885-1893.

Wright, S.E., and Upadhyaya, A., 1997. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 161, 575-586.

Wu, L., McGechan, M.B., McRoberts, N., Baddeley, J.A., Watson, C.A., 2007. SPACSYS: Integration of a 3D root architecture component to carbon, nitrogen and water cycling--Model description. Ecological Modelling 200, 343-359.

Yin, D., Chen, S., Chen, F., Guan, Z., Fang, W., Morpho-anatomical and physiological responses of two Dendranthema species to waterlogging. Environmental and Experimental Botany 68, 122-130.

Zahran, H.H., 1999. Rhizobium-Legume symbiosis and nitrogen fixation under severe conditions and in an Arid climate. Microbiol. Mol. Biol. Rev. 63, 968-989.

Appendix: Images of the field experiment and study site

Photographs from the experimental area

Photo 1: Drainage plots from air. Photo was downloaded by live maps (taken 2004).

Photo 2: The experimental area after ploughing and sowing in 2006 (taken 21st April 2006).

Photo 5: Intercropping of barley with clover (left) and pea cv. Zero 4 (right) (taken in August and July, 2006, respectively)

Photo 6: A static chamber on the field after the harvest of the crop with the 'bombs' at the top (taken 15th Septemebr 2006).