

## **I.13 Dinitrogen Fixation and Nitrous Oxide Losses in Organic Grass-Clover Pastures: An Integrated Experimental and Modelling Approach**

**Acronym:** DINOG

**Date:** 15 February 2000

### **1. Summary**

Organic farming practices, and in particular dairy production systems, are becoming increasingly abundant within Danish agriculture. Grazed pastures may be a significant source of nitrous oxide ( $N_2O$ ), an important greenhouse gas, and data in the literature suggest that  $N_2O$  emissions from e.g. organic dairy farms may be smaller than from conventional systems. The difference in  $N_2O$  emissions, however, may depend on the intensity with which dinitrogen ( $N_2$ ) fixed by the legumes in grass-clover mixtures are recycled in the grazed fields. Particularly urine induces  $N_2O$  emissions, which also constitutes a major loss of N.

Dinitrogen fixation in grass-clover pastures is influenced by grazing and excretal deposits, which thus needs to be taken into account when estimating total  $N_2$  fixation. Secondly, hitherto total  $N_2$  fixation estimates usually has not accounted for contributions from plant compartments below grazing-height, which causes a severe bias in estimates on  $N_2$  fixation.

The IPCC guidelines for making inventories of greenhouse gases recommend a  $N_2O$  release rate of 1.25% for all N inputs, including  $N_2$  fixed by legumes. Because of the uncertainties in quantifying  $N_2$  fixation, no contribution from  $N_2$  fixation to  $N_2O$  emissions from legume pastures has actually been estimated so far. Inventories of  $N_2O$  emissions for organic farming systems may therefore be severely biased.

The proposed work will investigate magnitudes and describe characteristics of  $N_2O$  emissions, denitrification and  $N_2$  fixation in organically managed grass-clover pastures under different grazing intensities and variable sandy soil textures. Quantitative results will be implemented in submodules of a whole-farm N flow model. To meet these goals a number of field experiments will be initiated at organic farming experimental trials, supplemented by microcosm experiments under fully controlled conditions.

The information provided by this project will (i) provide information necessary for a holistic evaluation of the environmental impact of organic farming practices, (ii) be a significant support for decision making by local and regional organic farming extension services, and (iii) supply very useful information for the construction of national and regional inventories of greenhouse gas emissions.

### **2. Research group**

#### Risø National Laboratory (Risø)

*Per Ambus, senior research scientist (project co-ordinator)-PAn.  
nn. PhD student*

#### Danish Institute of Agricultural Sciences (DIAS)

*Finn P. Vinther, senior scientist-FPV  
Søren O. Petersen, senior scientist-SOP  
Jørgen E. Olesen, senior scientist-JEO  
Nick Hutchings, senior researcher-NH*

The activities in the proposed project are undertaken in collaboration between three research groups located at two institutes. PA is the project co-ordinator, and is responsible for process studies using  $^{15}\text{N}$  pool-dilution and tracing in growth chambers and field microplots (*WP1*). FPV is responsible for the field-scale studies on  $\text{N}_2$  fixation and  $\text{N}_2\text{O}$  emission (*WP2*). JEO is responsible for the modelling work (*WP3*), and is the principal investigator of the experimental sites. It is hoped to include a Ph.D. student in the project via external funding.

### 3. Introduction

In this project issues on organic dairy production practices are combined with environmental issues through an integrated experimental and modelling approach. Organic farming is gaining increasing support from consumers as well as government authorities. Presently 6% of the Danish agricultural land is managed by organic farming practises, mainly dairy production systems, and the proportion of organic farming continues to increase ([www.lr.dk](http://www.lr.dk)).

Both public health and environmental issues are expected to benefit from the increase in organic farming by e.g. a reduction in the exposure to pesticides and hormones, and by reduced risks of nutrient export to environments surrounding the agroecosystem. However, dairy production typically involves cattle grazing on pastures for a significant part of the year, and excretal nitrogen deposited during this time is at risk of being lost to the atmosphere or via leaching. In addition, the grass-clover pastures is via the dinitrogen ( $\text{N}_2$ ) fixing components expected to have a higher level nitrogen, and both factors could influence emissions of N oxides such as nitric oxide (NO) and nitrous oxide ( $\text{N}_2\text{O}$ ) and leaching of nitrate ( $\text{NO}_3^-$ ) from this cropping system.

Emissions of  $\text{N}_2\text{O}$ , which is a significant greenhouse gas, are mainly derived from agricultural soils. Thus far, however, there have only been a few accurate estimates of total  $\text{N}_2\text{O}$  emissions from grassland livestock production systems, and understanding of the factors controlling  $\text{N}_2\text{O}$  emissions remains unsatisfactory (Oenema *et al.*, 1998). The emissions from intensively managed systems clearly appear to be large. Emissions from less intensively managed systems (e.g. organic dairy farms) may be smaller, and altered practices of grassland management with a higher proportion of grass-legume pastures is suggested as a mitigation option to reduce  $\text{N}_2\text{O}$  (Velthof *et al.*, 1998). This may, however, depend on the intensity with which  $\text{N}_2$  fixed by the legumes in grass-clover mixtures are recycled in the grazed fields.

In organic as well as conventional dairy farming grass-clover pastures is an important component of the cropping system. This because grass-clover is an excellent cattle fodder, and because the clover via symbiotic  $\text{N}_2$  fixation is the major contributor to the nitrogen pool in these cropping systems. Although  $\text{N}_2$  fixation is a key process in organic grass-legume systems, several gaps exist in our current knowledge concerning this mechanism. In particular, this concerns the contribution from clover components below normal grazing or harvest height to the total  $\text{N}_2$  fixation.

The global, as well as national budgets of  $\text{N}_2\text{O}$  emissions are based on the IPCC guidelines for making inventories of greenhouse gases (IPCC, 1997). This methodology assigns emission factors to the inputs of N to the agroecosystem. A fixed  $\text{N}_2\text{O}$  release rate of 1.25% is used for all N inputs as fertiliser, manure, green manure or  $\text{N}_2$  fixed by legume crops. Because of the uncertainties in quantifying  $\text{N}_2$  fixation from legume pastures no contribution from  $\text{N}_2$  fixation to  $\text{N}_2\text{O}$  emissions has been estimated so far (Mosier *et al.*, 1998). This may cause a severe bias in the inventories of  $\text{N}_2\text{O}$  emissions for organic farming systems, where the main input of N is through  $\text{N}_2$  fixation.

In this work total N<sub>2</sub> fixation inputs and emission losses of N<sub>2</sub>O and N<sub>2</sub> are measured in organic grass-clover systems on different soil types, and grazed by cattle with different grazing intensities. The underlying processes for N<sub>2</sub>O formation are examined. Quantitative results will be used to parameterise and validate a soil-atmosphere flux model. Overall, these data will provide significant information necessary for a holistic evaluation of the environmental impact of organic farming practices.

#### 4. State of the art

##### *N<sub>2</sub>O emission losses.*

It is acknowledged that a substantial part of whole farm emissions of N<sub>2</sub>O in dairy production systems can be attributed to the soil plant compartment. In particular, this has been recognised in intensively managed systems in the U.K. and in the Netherlands (Chadwick *et al.*, 1999; Velthof *et al.*, 1998). Since fertiliser requirements for grass-clover systems are smaller than for pure-grass systems, and because biologically fixed N<sub>2</sub> is released only slowly into the soil, introducing grass-clovers has been suggested as a mitigation option to reduce grassland N<sub>2</sub>O emissions. (Velthof *et al.*, 1998). They suggest the N<sub>2</sub>O losses from grass-clover systems to constitute 0-1% of the symbiotic N<sub>2</sub> fixation (SNF), which is lower than the general emission factor of 1.25% recommended by the IPCC (1997) for N<sub>2</sub>O inventories. However, the knowledge of the releases of biologically fixed N<sub>2</sub> as N<sub>2</sub>O in grass-legume systems is very sparse, partly due to the difficulties of estimating the SNF (Bouwman, 1996; Mosier *et al.*, 1998). Indeed, almost no work has been done on low-input grass-clover pastures within organic rotations.

Animal excreta have a substantial influence on the N<sub>2</sub>O emissions and denitrification losses from grass-clover swards used for grazing. A number of studies in recent years have focused on the N<sub>2</sub>O emissions from urine and dung affected grassland. It has been reported that up to 16 % of urine N is lost as N<sub>2</sub>O during transient periods following applications, equivalent to 50 kg N ha<sup>-1</sup> (de Klein and van Logtestijn, 1994). Recently, Yamulki *et al.* (1998) estimated that up to 22% of the total annual N<sub>2</sub>O loss from U.K. grasslands may originate from urine and dung affected areas. Short-term losses within 24 hours from urine patches may contribute 8 % of total annual N<sub>2</sub>O losses from grazed swards (Williams *et al.*, 1999). Since a major part (approx. 30 %) of SNF in grazed grass-clover systems may be subject to aboveground transfer via animal excreta (Ledgard, 1991), grazing activity may significantly accelerate the loss of SNF as N<sub>2</sub>O and denitrification. Treading by livestock (Clayton *et al.*, 1994) and changes in plant community composition and plant physiology (Ledgard and Steele, 1992) may also affect N<sub>2</sub>O emissions in grazed pastures. Enhanced N<sub>2</sub>O emissions due to dung and urine have been attributed to nitrification (Koops *et al.*, 1997) and denitrification (Monaghan and Barraclough, 1993), but is most likely linked to a complex interaction of nitrification and denitrification activity associated with decomposition processes in the vicinity of urine and dung patches (Lovell and Jarvis, 1996; Yamulki *et al.*, 1998). Differences in soil texture classes (drainage) and climatic conditions are important factors for the soil environment and may explain the contrasting results in the literature on N<sub>2</sub>O formation (Yamulki *et al.*, 1998).

Nitrous oxide emissions from soils are highly stochastic, both in space and time, and estimates accordingly associated with high uncertainties (Ambus and Christensen, 1994). Losses of N<sub>2</sub>O in pastures are particularly influenced by the highly variable spatial distribution of urine and dung patches.

In order to fully understand the casual relationships between N<sub>2</sub>O emissions and livestock grazing, it is therefore necessary to determine the N<sub>2</sub>O emissions at a high spatial resolution

in the field. It is also necessary to combine these measurements with detailed experimental approaches addressing the soil processes of N turnover at similar spatial and temporal resolutions.

### *N<sub>2</sub> fixation*

The importance of symbiotic N<sub>2</sub> fixation by pasture legumes for improving the nitrogen status of soils, and for maintaining a sustainable production level in organic dairy farming, has long been recognised, and numerous attempts have been made to quantify the amount of N<sub>2</sub> fixed. In a recent review, the range in estimates of N<sub>2</sub> fixation was given as 55-295 kg N ha<sup>-1</sup> year<sup>-1</sup> (Ledgard and Steele, 1992). However, these results are based on measurements of SNF in the harvested clover biomass only. Total SNF may be significantly underestimated, if the root, nodule and stolon components are not accounted for, and indeed the general IPCC methodology (IPCC, 1997) prescribes a doubling of harvested legume biomass to come to estimates of total N<sub>2</sub> fixation. This is in accordance with recent research showing that the below harvest-height components of pasture legumes contain at least 40% of the total plant N (Peoples *et al.*, 1998; Vinther and Jensen, 2000), and the absolute amounts of N<sub>2</sub> fixed could be nearly twice of that measured in shoots. In a short-term (3 weeks) field study Jørgensen and Ledgard (1997) found that the N<sub>2</sub> fixation could be quantified by analysing the leaf fraction and then multiply by 1.7 to obtain the total input of nitrogen. This relationship might vary according to changes in the environment, and more work is needed to establish how the N accumulation in clover tissues below harvest-height is influenced by factors such as soil type, defoliation/grazing, and soil moisture and nutrient availability.

The amount of N<sub>2</sub> fixed in pastures is primarily regulated by total legume yield and the proportion of clover N derived from the atmosphere (Peoples *et al.*, 1998). Thus, factors limiting white clover growth, or suppressing the N<sub>2</sub> fixation process, will reduce the amount of N<sub>2</sub> fixed in pastures. Major limiting factors in grazed grass-clover sward in contrast to mowed swards are the high cutting frequency caused by the grazing, and excretal deposits from animals (Riffkin *et al.*, 1999). Comparing different white clover varieties under cutting and grazing conditions, Swift *et al.* (1992) found a considerable variation between varieties, but with the general trend that average dry matter production from cutting was about twice of that under grazing. Excretal deposits and, in particular, urine have a significant negative effect on the N<sub>2</sub> fixation in grass-white clover. Vinther (1998) showed that the N<sub>2</sub> fixation process in urine patches was significantly depressed, resulting in a total reduction of 45% in the amount of N<sub>2</sub> fixed over a period of four months after application. The dung only affected (10% reduction) the N<sub>2</sub> fixation for a distance of up to 10 cm from the edge of dung pats. Therefore, when estimating the amount of N<sub>2</sub> fixed in grass-clover pastures the combined effect of intensive grazing and excretal deposits has to be taken into account.

### *Modelling*

The uncertainty in N<sub>2</sub>O emission estimates may be reduced through the application of simulation modelling. Such an approach requires that the models incorporate the main causes of variability in emissions, *i.e.* spatial variability in inorganic nitrogen and carbon concentrations in soils and temporal variability in soil water content. A comparison of models for N<sub>2</sub>O emission has shown a large variation in simulated N<sub>2</sub>O emissions, mainly associated with differences in the simulation of soil water content (Frolking *et al.*, 1998). The main factor influencing N<sub>2</sub>O emission on grazed pastures will, however, be the multiple deposition of urine and dung in patches. It is therefore very important to include this variability in nitrogen concentration explicitly in the model as also suggested by Oenema *et al.* (1998). It is

also important that the model includes all processes of nitrogen turnover and losses, as both direct and indirect interactions may occur between these processes. The FASSET model that will be applied in this project fulfils these requirements, but requires additional modelling with respect to N<sub>2</sub>O emission from nitrification and reparameterisation of the existing model for denitrification.

## 5. Objectives and expected achievements

The proposed work will investigate magnitudes and describe characteristics of N<sub>2</sub>O emissions, denitrification and N<sub>2</sub> fixation in organically managed grass-clover pastures under different grazing intensities and variable soil textures. The results will be implemented in submodules of a whole-farm N flow model. In particular the objectives of the work are to:

- investigate and elucidate relationships between gross rates of mineralization and nitrification and losses of N<sub>2</sub>O and N<sub>2</sub>
- investigate the translocation and fate of biologically fixed N<sub>2</sub> with emphasis on gaseous losses and the accompanying plant uptake
- determine the total N<sub>2</sub> fixation including the contribution from stolons and roots
- estimate N input through N<sub>2</sub> fixation, and gaseous N losses through N<sub>2</sub>O emission and denitrification under field conditions
- adapt, parameterise and validate a soil-plant-atmosphere model of nitrogen turn-over for simulation of N<sub>2</sub>O emission, including simulation of spatial variability caused by urine and dung patches on grazed pastures

In order to meet these goals, a number of field experiments will be initiated at the organic farming experimental trials of Research Centre Foulum. These activities will be supplemented by microcosm experiments under fully controlled conditions at the Risø National Laboratory. Results from the experimental activities will be made available for incorporation into the FASSET whole-farm model.

Organic farming practices, and in particular dairy production systems, are becoming increasingly abundant within Danish agriculture. In Denmark, grass-clover pastures are predominantly located on sandy soils, and data on N<sub>2</sub> fixation from these soils are very sparse. Therefore, the information provided by this project will be a significant support for decision making by local and regional organic farming extension services. Data from this work also provides information necessary for a holistic evaluation of the environmental impact of organic farming practices, and it will supply very useful information for the construction of national and regional inventories of greenhouse gas emissions.

## 6. Workpackages including methods

### *Experimental outline*

The experiments will be carried out at different sites and with different experimental approaches. An overview of the sites and approaches is shown below indicating the soil type and if the grass-clover is grazed or mowed.

Experimental site	Experimental characteristics	Experimental approach
1. Organic Crop Rotation at Jyndevad Experimental Station.	Coarse sandy soil. The crop rotation consists of mowed grass-white clover undersown in barley, 1 <sup>st</sup> to 3 <sup>rd</sup> year grass-clover, oat, rye and potatoes. Two levels of organic manure corresponding to 0.7 and 1.4 LU ha <sup>-1</sup> . Grass-clover mowed.	Determination of total N <sub>2</sub> fixation, incl. contribution from stolons and roots, using <sup>15</sup> N isotopes ( <i>WP2.1</i> ). Field estimates of N <sub>2</sub> fixation in harvested clover biomass ( <i>WP2.2</i> ).
2. Organic Crop Rotation at Foulum Research Centre	Loamy sand soil. The cropping system consists of grass-white clover undersown in barley, 1 <sup>st</sup> and 2 <sup>nd</sup> year grass-clover, barley-pea whole-crop, winter wheat and fodder beets. Two levels of organic manure corresponding to 0.7 and 1.4 LU ha <sup>-1</sup> . Grass-clover grazed by heifers.	Microplots for gross N-turnover and gaseous losses ( <i>WP1.1</i> ). Determination of total N <sub>2</sub> fixation, incl. contribution from stolons and roots, using <sup>15</sup> N isotopes ( <i>WP2.1</i> ). Field estimates of N <sub>2</sub> fixation in grazed clover biomass ( <i>WP2.2</i> ). Spatial variation and field estimates of N <sub>2</sub> O emission ( <i>WP2.3</i> ).
3. Commercial organic "study-farm".	Sandy soil. The cropping system contains grass-white clover undersown in barley, 1 <sup>st</sup> and 2 <sup>nd</sup> year grass-white clover. Grass-clover grazed by dairy cows.	Microplots for gross N-turnover and gaseous losses ( <i>WP1.1</i> ). Field estimates of N <sub>2</sub> fixation in grazed clover biomass ( <i>WP2.2</i> ). Spatial variation and field estimates of N <sub>2</sub> O emission ( <i>WP2.3</i> ).
4. Risø National Laboratory	Soil monoliths and lysimeters incubated in fully controlled growth chambers. Soils from experimental sites.	Detailed study on N <sub>2</sub> fixation, N <sub>2</sub> O emission and N partitioning in the plant-soil system ( <i>WP1.1 and 1.2</i> )

**Table 1: Workpackage list**

Work-package No	Work package title	Responsible participant	Budget 1000DKK	Start	End	Deliv. No
1.1 – 1.2	Process studies of nitrogen exchange between soil and atmosphere	PA	1454	11/00	05/04	D1.1-1.7 Project1-3
2.1 – 2.3	Field studies of nitrogen exchange between soil and atmosphere	FPV	1816	01/01	05/04	D2.1-2.6 Project1-3
3	Modelling of nitrogen exchange between soil and atmosphere	JEO	390	10/02	05/04	D3.1-3.2 Project2-3

**Table 2: Description of workpackages****WP1: Process studies of nitrogen exchange between soil and atmosphere**

Workpackage number: 1  
 Responsible person: PA  
 Contributing persons: PA, nn PhD  
 Person-months: 26

**Objectives WP1**

- Investigate and elucidate relationships between gross rates of mineralization and nitrification and losses of  $N_2O$  and  $N_2$
- Investigate the translocation and fate of biologically fixed N with emphasis on gaseous losses and companion plant uptake

**Description of work***WP1.1 Gross N turnover and losses of  $N_2O$  and  $N_2$ .*

A combination of microcosms kept under controlled conditions, and microplots established in the field by inserting 10-cm diam. PVC cylinders into the soil will be used for a series of  $^{15}N$ -labelling experiments. Sets of plots will be cross-labelled with  $(^{15}NH_4)_2SO_4$  and  $K^{15}NO_3$  and gross mineralization and gross nitrification will be measured over short term incubations (<2 days) by the isotope pool dilution approach (Powlson and Barraclough, 1993). During the short-term incubations, the PVC cylinders will be sealed transiently by gas-tight lids and headspace gas samples collected for  $^{15/14}N_2O$  and  $^{15/14}N_2$  determinations. This approach will provide information on the direct relationship between gross N turnover and the gaseous N losses through nitrification and denitrification (Stevens and Laughlin, 1999). The microplot experiments will include treatments with animal excreta in different amounts to assess the processes for  $N_2O$  production in excreta affected areas and after various times of degradation. Controlled environment microcosms will be used to investigate effects of soil moisture and temperature on the gross N turnover and  $N_2O$  and  $N_2$  fluxes. The experiments in the field will be performed in campaigns co-ordinated with the activities in *WP2.1* and *WP2.3*. In parallel with the  $^{15}N$ -gas measurements, DIAS will measure  $N_2O$  emissions and denitrification by the acetylene-inhibition technique in incubated soil cores.

*WP1.2  $N_2$  fixation,  $N_2O$  emission and N translocation in  $^{15}N_2$ -labelled soil-plant systems.*

Direct measurements of  $N_2$  fixation by determination of  $^{15}N_2$  incorporation into the plant is impeded for practical and economical reasons and has thus far been applied to a limited extent. We will use a sealed lysimeter approach (Fig. 2) in which the soil compartment, or the entire soil-plant system, is sealed under controlled conditions with respect to moisture, temperature,  $CO_2$  and  $O_2$ . Labelled  $^{15}N_2$  is introduced into the soil gas atmosphere in a closed gas loop which is sampled at regular intervals for monitoring the gaseous composition. Nitrous oxide in the gas

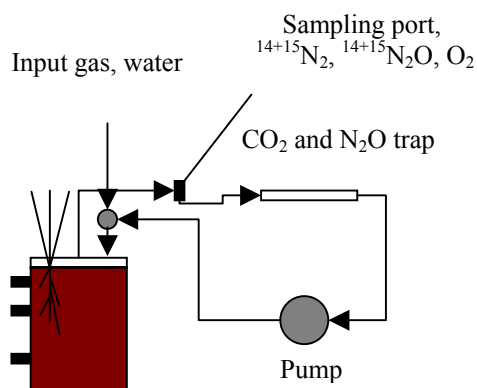


Figure 2. Sealed lysimeter. Simplified after Sims et al. (1983).

loop will be trapped and concentrations and  $^{15}\text{N}$  enrichment measured periodically. This will provide information on the fraction of biologically fixed  $\text{N}_2$ , which is released as  $\text{N}_2\text{O}$ . Secondly, the belowground transfer of N from clover to companion grasses is also assessed. Following the  $^{15}\text{N}_2$ -labelling, the lysimeters will be dissected and soil and plant analysis (total N and  $^{15}\text{N}$ ) performed to trace the translocations and fate of fixed N. In separate lysimeters without labelled  $\text{N}_2$ -gas, the SNF will be estimated by the isotope dilution approach for method validation.

#### **Deliverables WP1**

D1.1 - D1.2 Development/description of methods  
 D1.3 - D1.6 International publications  
 D1.7 Transfer of data for WP3  
 Project1-3 Annual reports

#### **Milestones WP1**

M1.1 Method for  $^{15}\text{N}_2$  fixation developed  
 M1.2 Method for field gross N-turnover and gas measurements developed  
 M1.3  $^{15}\text{N}_2$  fixation measurements completed  
 M1.4 Gross N turnover and gas flux measurements in the field and controlled environment completed

### **WP2: Field studies of nitrogen exchange between soil and atmosphere**

Workpackage number: 2  
 Responsible person: FPV  
 Contributing persons: FPV, SOP  
 Person-months: 35

#### **Objectives WP2**

- Determine the contribution to  $\text{N}_2$  fixation from stolons and roots
- Estimate total  $\text{N}_2$  fixation in grazed grass-clover pastures on two sandy soil types
- Estimate N -emissions in grazed grass-clover pastures on two sandy soil types

#### **Description of work**

##### *WP2.1 $\text{N}_2$ fixation: Contribution from stolons and roots*

Several methods for measuring SNF are available, of which the  $^{15}\text{N}$  isotope techniques (isotope dilution or natural abundance) are the most reliable and currently most used techniques to estimate  $\text{N}_2$  fixation in grass-clover pastures.

Measurements of  $\text{N}_2$  fixation in the various clover components will be carried out using the  $^{15}\text{N}$  isotope dilution method with addition of an immobilising carbon source, and with ryegrass as reference plant (Vinther and Jensen, 2000). This method has proved to give the best results when measurements are to be made over more than one growing season.

In the spring of 2001,  $^{15}\text{N}$ -labelled  $(\text{NH}_4)_2\text{SO}_4$  and a carbon source (glucose) will be applied to plots in the undersown grass-clover and monoculture ryegrass. Samples of aboveground grass-clover will be taken 5-6 times during each production year, and whole-plant samples, i.e. soil blocks of 20 x 20 x 20 cm, will be taken 2-3 times each year. The

samples will be separated into the following components: Clover leaves and petioles, stolons, clover roots, grass leaves, grass roots and soil. Dry matter, concentration of total N and  $^{15}\text{N}$  of each of the components will be determined, and subsequently the total  $\text{N}_2$  fixation, incl. contribution from stolons and roots, and N-transfer from clover to grass will be calculated. The accumulation of fixed N in the various clover components, such as leaves, petioles, stolons and roots is further studied in more details using  $^{15}\text{N}$ -labelled  $\text{N}_2$  gas as described in WP1.2.

### WP2.2 Field estimates of total $\text{N}_2$ fixation in grass-clover

A more simple and less resource demanding technique than  $^{15}\text{N}$  isotopic work will be adopted for estimating total  $\text{N}_2$  fixation in the pastures. Reviewing a large number of data sets available in the literature has revealed a highly significant correlation between SNF and clover dry matter (Fig. 1). Field estimates of  $\text{N}_2$  fixation will be based on this correlation, and subsequently adjusted to total  $\text{N}_2$  fixation according to the findings, described in WP2.1.

In short, the estimation of  $\text{N}_2$  fixation in grass-clover pastures will involve monthly harvests from 1-m<sup>2</sup> plots (4 - 6 replicates) that have not been exposed to grazing between samplings. The harvested plant material will then be separated into clover and grass, and the dry matter production in each of the two components determined by weighing. Finally, the  $\text{N}_2$  fixation in the harvested clover biomass will be calculated using the relationship shown in Fig. 1. Subsamples of the harvested plant material is analysed for total N and  $^{15}\text{N}$  natural abundance, and the natural  $^{15}\text{N}$  abundance method (*e.g.* Shearer and Kohl, 1986) will be applied for validation of field estimates.

The majority of data presented in Fig. 1 are derived from mowed grass/clover trials. In order to estimate effects of grazing and urine deposition on  $\text{N}_2$  fixation, an additional field experiment is planned. The experiment will include the treatments (i) monthly cutting (mowing), (ii) weekly cutting ("grazing"), and (iii) urine addition combined with weekly cutting. The  $^{15}\text{N}$ -isotope dilution method will be used.

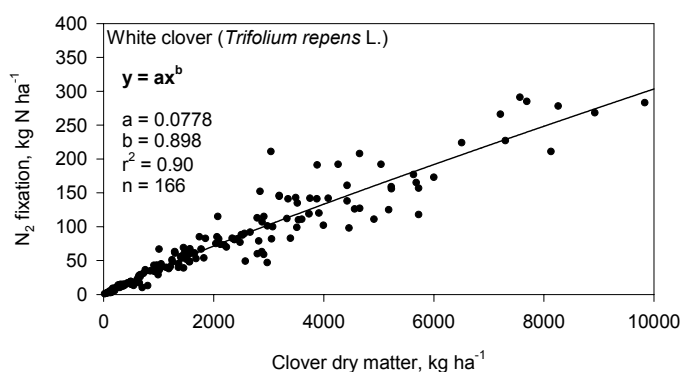


Figure 1. Relationship between  $\text{N}_2$  fixation and clover dry matter (Vinther, 2000).

### WP2.3 Spatial variation and field estimates of $\text{N}_2\text{O}$ emission.

Rates of  $\text{N}_2\text{O}$  emission from the grass-clover pastures will be determined 3-4 times a year in campaigns with frequent measurements using closed static chambers (Vinther and Hoffmann, 2000). Approximately 20 cylindrical metal containers (16 cm diam., 15 cm height) with sharpened edges is inserted into the ground with as little disturbance as possible. The containers are closed with a septum for gas sampling, and 5-mL gas samples are taken from

each chamber headspace at four time points of approximately 30 minutes intervals points; a preliminary experiment on possible disturbance effects due to C or N release from cut-off roots (Livingston and Hutchinson, 1995) will be conducted prior to a decision on sampling intervals. Gas samples are stored in vacutainers and analysed for N<sub>2</sub>O as described by Maag and Vinther (1996). Rates of N<sub>2</sub>O emission are calculated using a non-linear model (Pedersen *et al.*, submitted). After gas sampling the containers will be removed, and soil samples taken for determination of soil moisture and inorganic nitrogen. The spatial distribution of urine deposits will be estimated from measurements of electrical conductivity and visual examination.

#### **Deliverables WP2**

D2.1 International/national report.  
 D2.2 - D2.5 International publications.  
 D2.6 Transfer of data to WP3  
 Project1-3 Annual reports

#### **Milestones WP2**

M2.1 Experiments on the combined effects of grazing and urine deposits on N<sub>2</sub> fixation in grass/clover completed.  
 M2.2 Experiments on contribution of stolons and clover roots to N<sub>2</sub> fixation completed.  
 M2.3 Field measurements of N<sub>2</sub>O emission and N<sub>2</sub> fixation completed.

### **WP3: Modelling of nitrogen exchange between soil and atmosphere**

Workpackage number: 3  
 Responsible person: JEO  
 Contributing persons: JEO, NH  
 Person-months: 7

#### **Objectives WP3**

Adapt, parameterise and validate a soil-plant-atmosphere model of nitrogen turnover for simulation of N<sub>2</sub>O emission, including simulation of spatial variability caused by urine and dung patches on grazed pastures

#### **Description of work**

The modelling study will apply the FASSET whole farm model (Jacobsen *et al.*, 1998). This model simulates the flow of nitrogen at the farm level, including the flows between housing, stores, animals and fields (soil and crop). The model includes a soil-plant-atmosphere model that simulates nitrogen turnover and crop production as affected by availability of water and nitrogen (Olesen *et al.*, 1996). The FASSET model includes models for a range of crops that operate at the stand level and use daily time steps. The simulation of nitrogen turnover in the soil, including mineralisation and denitrification, is currently simulated using the concepts and parameters of the DAISY model (Hansen *et al.*, 1991).

The grass/clover model in FASSET includes a model of competition between a legume and a grass species. The extent of N<sub>2</sub> fixation in the model is thus dependent on both the availability of nitrogen in the soil and on the aboveground competition between the two crop

species. The effect of this on N<sub>2</sub> fixation will be verified against the measurements carried out in the project, which includes measurements of N<sub>2</sub> fixation in both above- and belowground dry matter.

The grazing model in FASSET includes the dynamic creation of spatial heterogeneity in soil nutrient status that arises from spatial and temporal variation in urine and dung depositions (Hutchings and Kristensen, 1995). This is simulated on a daily basis by dynamically creating new instances of soil/crop columns describing the situation with urine/dung patches, including multiple patches. Each instance of the soil/crop columns represents a fraction of the entire area and includes its own separate description of the soil-plant-atmosphere interactions. The sum of nitrogen turnover and production on a field is obtained as the area-weighted sum of the individual soil/crop columns.

The current version of FASSET does not include N<sub>2</sub>O emissions, but only N losses from denitrification. A revised model for N<sub>2</sub>O emission from both nitrification and denitrification will be implemented based on literature data and experimental results from the project. The model selected will also be based on reported experience of modelling approaches used in other models of similar integration level (Li *et al.*, 1992; Grant *et al.*, 1993; Parton *et al.*, 1996; Müller *et al.*, 1997) and on results of model inter-comparisons (e.g. Frolking *et al.*, 1998).

#### **Deliverables WP3**

D3.1 Revised sub-models for N<sub>2</sub>O emission and N<sub>2</sub> fixation in FASSET

D3.2 Report/publication on model work

Project2-3 Annual reports

#### **Milestones WP3**

M3.1 A new N<sub>2</sub>O emission sub-model implemented in the whole-farm model FASSET

M3.2 An improved N<sub>2</sub> fixation sub-model implemented in the whole-farm model FASSET

M3.3 Revised FASSET sub-models verified and validated

## **7. Implementation and time schedule**

**Table 3: Deliverables list**

<b>Deliv.No</b>	<b>Deliverable title</b>	<b>Deliv. date</b>	<b>Meeting<sup>1</sup></b>	<b>Nature<sup>2</sup></b>
D1.1	Development of method for <sup>15</sup> N <sub>2</sub> fixation study in lysimeters	05/01		O
D1.2	Development of easy, simple method for simultaneous gross N turnover and gas flux measurements in the field	06/01		O
D1.3	Publication on <sup>15</sup> N <sub>2</sub> fixation method	12/01		Pu
D1.4	Publication on <sup>15</sup> N <sub>2</sub> fixation, translocation and gas losses.	09/03		Pu
D1.5	Publication on relationship between field gross N turnover in excreta affected pasture and gas fluxes	05/04		Pu
D1.6	Publication on relationship between gross N turnover and gas fluxes in controlled	05/04		Pu

	environment			
D1.7	First year data available for WP3	03/02		O
D2.1	Report on the relationship between N <sub>2</sub> fixation and leguminous dry matter production	11/01		Re
D2.2	Publication on the contribution from stolons and clover roots to the total N <sub>2</sub> fixation	05/04		Pu
D2.3	Publication on the combined effects of grazing and urine deposits on N <sub>2</sub> fixation in grass-clover	07/02		Pu
D2.4	Publication on field estimates of N <sub>2</sub> fixation in grass-clover pastures on sandy soil types	05/04		Pu
D2.5	Publication on field estimates of N <sub>2</sub> O emission from grass/clover pastures on sandy soil types	05/04		Pu
D2.6	First year field data made available for WP3	03/02		O
D3.1	Revised N <sub>2</sub> O and N <sub>2</sub> fixation submodels	02/03		O
D3.2	Publication on model work	05/04		Pu
Project1	1 <sup>st</sup> annual report on DINOG	10/01	G1	Re
Project2	2 <sup>nd</sup> annual report on DINOG	10/02	G2	Re
Project3	3 <sup>rd</sup> annual report on DINOG	10/03	G3	Re

<sup>1</sup>General meetings

<sup>2</sup>Pu= International publications in books and journals; Re= Reports; O= Others

The experimental work will be initiated in late the autumn of year 2000 and will run over 3 years. Implementation of modelling will take place in the final part of the experimental work period. Results from the project will be published in national reports and international journals during 2003 and 2004.



## 8. Collaborative partners

There will be collaboration with several other projects on a national basis. Within the FØJO-programme there will be interactions with project I.3 (Interactions between nitrogen dynamics, crop production and biodiversity in organic crop rotations analysed by simulation models) and I.5 (Grain legumes and cereals - new production methods for increased protein supply). The current project and project I.3 will co-operate with respect to establishing a monitoring programme and with respect to model validation and scenario analyses. Both projects will conduct measurements on the same grazed pasture on a commercial organic farm. Project I.3 will perform a general characterisation of the soil, vegetation and management of crops and animals, and the project will also conduct measurements of nitrate leaching and content of mineral nitrogen in the soil during autumn and winter. The FASSET model will be applied jointly by the two projects for model validation, especially with respect to simulation of spatial variability in both nitrate leaching and N<sub>2</sub>O emission. Project I.5 and the current will interact at the methodological level making available achieved knowledge and competence in the application of <sup>15</sup>N techniques for estimating SNF.

At the international level, the current project has links to several partners. Risø has a continued co-operation with Dr. G.P. Robertson (Michigan State University) with the focus on N<sub>2</sub>O emissions from forests and arable soils. Further, part of the activities of a PhD student expected to be included in the proposed project will take place in the laboratory of Prof. Dr. O. Oenema (Wageningen AB) who is highly competent with the scientific area of greenhouse gas emissions from grasslands. The model work has links to a proposal for EU funding (MIDAIR). The MIDAIR proposal will look at greenhouse gas emissions from conventional and organic dairy farms, and also apply an integrated experimental and modelling approach. It is envisaged that the adapted version of the FASSET model developed here can be used to derive emission factors for N<sub>2</sub>O emission for use in the MIDAIR project.

## 9. Budget

Total budget for the project is 3.66 mill. DKK. A 3 % share of the total budget is allocated Risø for project co-ordination and management.

Specified budget in 1000 DKK.

<b>Risø (WP1)</b>	<b>2000</b>	<b>2001</b>	<b>2002</b>	<b>2003</b>	<b>2004</b>	<b>Σ</b>
Academic costs	81.21	168.6	174.0	180.2	93.1	697.1
Technician costs	0.0	73.0	75.5	78.2	27.0	253.6
Consumables	5.0	60.0	40.0	37.0	5.0	147.0
Durable equipment	70.0	0.0	0.0	0.0	0.0	70.0
Travel	0.0	8.0	8.0	14.0	14.0	44.0
OH (20%)	31.2	61.9	59.5	61.9	27.8	242.3
<b>Σ Risø (WP1)</b>	<b>187.4</b>	<b>371.5</b>	<b>357.0</b>	<b>371.2</b>	<b>166.9</b>	<b>1454.0</b>
<b>DIAS (WP2)</b>						
Academic costs	0.0	242.3	254.2	266.9	93.4	856.8
Technician costs	0.0	126.3	132.7	139.3	4.1	402.4
Consumables	0.0	73.1	76.4	67.8	2.1	219.4
Travel	0.0	6.0	6.0	6.0	16.7	34.7
OH (20%)	0.0	89.5	93.9	96.0	23.3	302.7
<b>Σ DIAS (WP2)</b>	<b>0.0</b>	<b>537.2</b>	<b>563.2</b>	<b>576.0</b>	<b>139.6</b>	<b>1816.0</b>
<b>DIAS (WP3)</b>						
Academic costs	0.0	0.0	84.0	220.0	0.0	304.0
Consumables	0.0	0.0	7.0	14.0	0.0	21.0
OH (20%)	0.0	0.0	18.2	46.8	0.0	65.0
<b>Σ DIAS (WP3)</b>	<b>0.0</b>	<b>0.0</b>	<b>109.2</b>	<b>280.8</b>	<b>0.0</b>	<b>390.0</b>
<b>Σ project</b>	<b>187.4</b>	<b>908.7</b>	<b>1029.3</b>	<b>1228.0</b>	<b>306.4</b>	<b>3660.0</b>

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1988-1990 (18 mo) Graduate student. Departm. Population Biol., Cphgn Univ.  
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Member of Inter-Nordic Workshop on Biomonitoring of Soil Biological Processes and Epiphytic Lichens; Co-ordinator: Joint Field Experiment on N<sub>2</sub>O flux measurement methods within EC STEP project; Member of US Trace Gas Network Group; Reviewer on several international journals, reports and bookchapters. Teaching and supervising experience at graduate and post-graduate levels. Has received four major grants as principal investigator during 1991-1997.

**Five recent relevant peer reviewed publications**

Ambus, P. and G.P. Robertson. 1999. Fluxes of CH<sub>4</sub> and N<sub>2</sub>O from Aspen stands grown under ambient and twice-ambient CO<sub>2</sub>. *Plant and Soil* 209: 1-8.  
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FPV is a senior scientist and heads the research unit on Microbial Ecology, DIAS. He has worked with aspects of microbial ecology in agricultural soils, including soil layers below the root zone. The research has primarily involved studies on the exchange of gases between soil and atmosphere, with emphasis symbiotic N<sub>2</sub> fixation in grass-clover pastures, the influence of biotic and abiotic factors on nitrification and denitrification, and emission of the greenhouse gases methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). FPV has published 24 papers in international journals; 28 contributions in proceedings, and 29 publications as reports, book chapters or in Danish journals.

#### ***Latest five relevant peer reviewed publications***

- Maag, M. and Vinther, F. P. (1996). Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology* **4**, 5-14.
- Vinther, F.P. (1998) Biological nitrogen fixation in grass-clover affected by animal excreta. *Plant and Soil* **203**, 207-215.
- Maag, M. and Vinther, F. P. (1999) Carbon dioxide and nitrous oxide production in animal slurry amended soils under varying temperature and water contents. *Soil Science Society of America Journal* **63**, 858-865.
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Søren O. Petersen works as a senior scientist within the research unit on Microbial Ecology. He has been the national representative of FAIR3-CT96-1877 'Biogenic Emissions of Greenhouse Gases from Arable and Animal Agriculture (1997-1999), and initiated the EU proposal 'Greenhouse Gas Mitigation for Organic and Conventional Dairy Production' (*MIDAIR*) submitted on 15 February. SOP has previously worked with gaseous emissions of N from grazed pastures and arable soil, including NH<sub>3</sub>, N<sub>2</sub>O and N<sub>2</sub>. SOP currently leads projects on effects of application techniques for slurry (BÆR98-5) and on effects of organic wastes on soil fertility, and has published 19 papers in peer-reviewed journals (15 as senior author).

### *Five latest relevant publications:*

Pedersen, A.R., Petersen, S.O. and Vinther, F.P. A stochastic diffusion model for estimating trace gas emissions with static chambers (Submitted to *Soil Science Society of America Journal*).

Sommer, S.G., Petersen, S.O. and Søgaard, H.T. (2000) Greenhouse gas emission from stored livestock slurry. *Journal of Environmental Quality* 29 (In press).

Petersen, S.O. (1999) Nitrous oxide emissions from manure and inorganic fertilizers applied to spring barley. *Journal of Environmental Quality* 28, 1610-1618.

Petersen, S.O., Sommer, S.G., Aes, O. and Søgaard, K. (1998) Ammonia losses from urine and dung of grazing cattle: Effect of N intake. *Atmospheric Environment* 32, 295-300.

Petersen S.O., Nielsen T.H., Frostegaard, A. and Olesen T. (1996) Oxygen uptake, carbon metabolism, and denitrification associated with manure hot-spots. *Soil Biology and Biochemistry* 28: 341-349.

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Born on 28 October 1958. M.Sc. in agriculture from the Royal Veterinary and Agricultural University in Copenhagen, 1983. Senior scientist and head of research unit on Cropping Systems at Department of Crop Physiology and Soil Science.

### Work

My research effort has concentrated on developing and applying models, especially in relation to the influence of weather on processes in the soil plant atmosphere continuum. This has included use of simulation models for assessing effects of climate change on crop production, development of a computer system for irrigation scheduling (MARKVAND), relation between weather factors and volatilisation of ammonia, methods for estimating evapotranspiration and modelling water balance from basins, and simulation of interaction between crop management and weeds, pests and diseases in winter wheat. I was project leader for a large interdisciplinary project on integrated crop protection in winter wheat and I have participated in three EU projects on the effect of climate change on agriculture (EPOCH, CLAIRE and CLIVARA). In the CLIVARA project, I developed methods for upscaling model results on effects of climate change to region and country levels. I have also been in charge of projects on the use of satellite imagery and GIS for land use assessments. I was the leader of the team developing the soil-plant-atmosphere model in the FASSET whole farm simulation model, including adaptation of the model for organic farming. I am currently involved in an experiment on organic crop rotations for grain production, and I have recently (May 1999) organised an international workshop on Designing and Testing Crop Rotations for Organic Farming.

### Other duties

Reviewer for a number of scientific journals, including J. Agric. Sci., Eur. J. Agron., Clim. Res. and Acta Agric. Scand.

External reviewer at the Royal Agricultural and Veterinary University, Copenhagen.

Assistant supervisor for 3 Ph.D. students and 1 M.Sc. student.

### Recent relevant publications

Holst, N., Axelsen, J.A., Olesen, J.E. & Ruggle, P. (1997). Object oriented implementation of the metabolic pool model. *Ecological Modelling* **104**, 175-187.

Olesen, J.E., Eltun, R., Gooding, M.J., Jensen, E.S. & Köpke, U. (Eds) (1999). Designing and testing crop rotations for organic farming. DARCOF Report no. 1.

Olesen, J.E. & Grevsen, K. (2000). A simulation model of climate effects on plant productivity and variability in cauliflower (*Brassica oleracea* L. *botrytis*). *Scientia Horticulturae* **83**, 83-107.

Olesen, J.E., Jørgensen, L.N. & Mortensen, J.V. (in press). Nitrogen application, irrigation strategy and fungicide control in winter wheat on a sandy soil. 2. Radiation interception and conversion. *Journal of Agricultural Science, Cambridge*.

Sommer, S.G. & Olesen, J.E. (in press). Modelling ammonia volatilization from animal slurry applied to cereals. *Atmospheric Environment*.

## Curriculum Vitae, Nicholas John Hutchings

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*Position:* Senior researcher (appointed August 1995)

*Qualifications:*

1976 BSc Plymouth (CNAAB) (Biological Sciences) Class IIA

1982 PhD Keele (Biological Sciences)

*Previous appointments:*

1983-84 Postdoctoral Assistant, University of Strathclyde, Glasgow, UK

1984-95 Modeller, Macaulay Land Use Research Institute, Aberdeen, UK

*Publications:*

Armstrong, H. M., Gordon, I. J., Grant, S. A., Hutchings, N. J., Milne, J. A., and Sibbald, A. R.

(1997) A model of the grazing of hill vegetation by sheep in the UK. I. The prediction of vegetation biomass. *Journal of Applied Ecology* 34: 166-185.

Hutchings, N. J. In the bin - modelling a grazed sward in time and space. *Aspects of Applied Biology* 26, 263-267. 1991.

Hutchings, N. J. (1991) A model of a grass sward continuously grazed to a constant mean height. *Ecological Modelling* 59: 73-91.

Hutchings, N. J. and Kristensen, I. S. (1995) Modelling mineral nitrogen accumulation in grazed pasture: will more nitrogen leach from fertilized grass than unfertilized grass/clover? *Grass and Forage Science* 50: 300-313.

Hutchings, N. J., Sommer, S. G., and Jarvis, S. C. (1996) A model of ammonia volatilization from a grazing livestock farm. *Atmospheric Environment* 30: 589-599.

## **Institute descriptions**

### *Risø*

The Risø group has significant experience in carrying out experiments on the biogeochemical cycling of N and C in the soil-plant-atmosphere system at different scales, spanning from the controlled environment over semi-field to field experiments. We use routinely gas chromatography and stable isotope techniques. The laboratory is hosting CONFIRM, an instrument centre for multidisciplinary work on stable isotope ratio mass spectrometry. The instrumentation includes "state of the art" automated applications for trace gas isotopic analysis in gas, soil and biological materials. Further instrumentation in the laboratory includes GC, HPLC, ion- and carbon analysers and chemoluminescence detector. The proposed project forms a close link to current Risø activities on resource competition in organic intercropping systems, recycling of organic wastes in plant production and impacts of global change on terrestrial soil-plant systems.

### *DIAS*

The DIAS group has considerable experience in research concerning nitrogen transformation processes, and the necessary technical facilities, such as automatized gas chromatographs, ion- and carbon analysers, for carrying out the proposed activities are available at the department. Research ranges from studies of the basic processes in the soil-water-plant-atmosphere system to practical developments in cropping systems, which can meet actual and future cultivation conditions and demands for specific qualities. Further, the DIAS group has considerable experience in modelling nitrogen transformations and flows at both the field and farm levels. A group of 4 to 5 scientists are actively involved in developing and applying the FASSET whole farm simulation model, which will be used in this study. The proposed project builds on and complements the ongoing efforts in the FASSET modelling group to simulate the effects of spatial heterogeneity of urine and dung deposition on nitrogen turnover in grazed grass/clover pastures. The DIAS group also has extensive experience on organic cropping systems, and one of principal investigators in this project recently (1999) organised an international workshop on "Designing and Testing Crop Rotations for Organic Farming".