



Final Report

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1. Research program

Research in organic farming 2000-2005 (DARCOF II)

2. Project title and number

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

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6. Project period (month, year)

Start of project: 11-2000
End of project: 05-2004

7. Final report

A. Project 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 (N₂O), an important greenhouse gas, and data in the literature suggest that N₂O emissions from e.g. organic dairy farms may be smaller than from conventional systems. The difference in N₂O emissions, however, may depend on the intensity with which dinitrogen (N₂) fixed by the legumes in grass-clover mixtures are recycled in the grazed fields. Particularly urine induces N₂O 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 N₂ fixation. Secondly, hitherto total N₂ fixation estimates usually has not accounted for contributions from plant compartments below grazing-height, which causes a severe bias in estimates on N₂ fixation.

The IPCC guidelines for making inventories of greenhouse gases recommend a N₂O release rate of 1.25% for all N inputs, including N₂ fixed by legumes. Because of the uncertainties in quantifying N₂ fixation, no contribution from N₂ fixation to N₂O emissions from legume pastures has actually been estimated so far. Inventories of N₂O emissions for organic farming systems may therefore be severely biased.

The proposed work will investigate magnitudes and describe characteristics of N₂O emissions, denitrification and N₂ 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.

Table A.1: Work package list (from application)

No.	Work package title	Participants*	Budget (1.000 DKK)	Start	End	Deliverable no(s):
1.1 – 1.2	Process studies of nitrogen exchange between soil and atmosphere	<u>Risø</u> DIAS	1454	11/00	05/04	D1.1-1.7
2.1 – 2.3	Field studies of nitrogen exchange between soil and atmosphere	<u>DIAS</u> Risø	1816	01/01	05/04	D2.1-2.6
3	Modelling of nitrogen exchange between soil and atmosphere	<u>DIAS</u> Risø	390	10/02	05/04	D3.1-3.2

* Responsible participants are underlined

Objectives and expected achievements

The proposed work will investigate magnitudes and describe characteristics of N₂O emissions, denitrification and N₂ 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 were to:

- 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_2 with emphasis on gaseous losses and the accompanying plant uptake
- determine the total N_2 fixation including the contribution from stolons and roots
- estimate N input through N_2 fixation, and gaseous N losses through N_2O emission and denitrification under field conditions
- adapt, parameterise and validate a soil-plant-atmosphere model of nitrogen turn-over for simulation of N_2O 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 were carried out at the organic farming experimental trials of Research Centre Foulum. These activities were supplemented by microcosm experiments under fully controlled conditions at the Risø National Laboratory. Results from the experimental activities were 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_2 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.

C. Progress and results

C.1 Description (summary) of main results and conclusions

Summary of main conclusions and achievements obtained. A more detailed description of individual WP-results is given subsequently.

Main conclusions and achievements from WP 1 (process studies):

- Base-line N_2O fluxes from grass-clover were relatively small and independent of pasture age, and amounted to 0.1 – 0.2% of N input by biological N_2 fixation. The N_2O production was correlated with the soil gross N mineralization and NH_4^+ availability indicating nitrification as dominant source for N_2O .
- The N_2O :nitrification ratio averaged 0.05%, but showed great variability.
- Deposition of urine stimulated mineralization of plant-derived carbon and increased N_2O emissions. A trend in the data suggested that urine with low urea gave rise to less relative N_2O emission (0.26%) than urine with high urea content (0.30%), which could indicate dietary control as a N_2O mitigation option.
- Construction of low-volume growth cabinet for $^{15}\text{N}_2$ -labeling work.
- Nitrous oxide emission from grass-clover pots constituted 0.03-1.1% of the biological N_2 -fixation; newly fixed N_2 only constituted ca. 3 ppm of the emitted N_2O .

Main conclusions and achievements from WP 2.1 and 2.2 (N₂ fixation):

- The amount of stolons and clover roots does not seem to increase further after 1st production year, and P_{stubble+roots} should therefore only be included once during the lifecycle of a grass-clover pasture
- Compared to mowing, frequent cutting ('grazing') has a negative effect (6 - 19%) on the harvested dry matter yield, as well as on below harvest dry matter production (19 - 35%).
- The below harvest biomass is less sensitive to adverse climatic conditions than harvested biomass.
- Soil type does not seem to have direct effects on the N₂ fixation, whereas the growth conditions in the coarse sandy soil seem to be suboptimal for clover growth, resulting in disappearance of the clover after 1st production year, and as such an indirect negative effect on the N₂ fixation.

Main conclusions and achievements from WP 2.3 (N₂O from excreta):

- Tests and improvements of two new methods, image analysis and electrical conductivity, for description of spatial urine distribution in fields.
- Short term (<2 weeks) N₂O emissions from urine patches were caused by nitrification activity.
- It was observed that urea-N levels has qualitative and quantitative effects on soil N transformations in urine patches. However, N₂O emissions were less affected.

Main conclusions from WP3 (modelling):

- A dynamic algorithm to simulate the N₂O production and emission from nitrification and denitrification has been included in the field component of the FASSET whole-farm model.
- The model was linked with the cattle model in FASSET to test the effect of urine and dung patches on nitrogen losses, primarily N₂O and nitrate leaching, from grazed grasslands. The results showed that accounting for patches reduced N₂O emissions by 10-30%, but increased nitrate leaching slightly

Alltogether, the findings in this project has provided useful information to our knowledge on drivers and controls in the interaction between grass-clover systems and the environment and has helped to develop tools for future descriptions of the environmental impacts of grass-clover systems. The experimental as well as model results derived in the DINOG project show that a fixed emission factor for N₂O emissions, as used by IPCC, is a much too simple approach for estimation of the actual emissions. The experimental results suggest generally low, but variable N₂O emissions from N₂-fixation and animal excreta. The model suggests large differences between soil types in the emissions, and that the proportion of fertiliser nitrogen emitted as N₂O is smaller at lower nitrogen rates. This non-linear response of N₂O emissions to nitrogen input may make organic farming an interesting option for reducing greenhouse gas emissions, because the nitrogen input is lower in organic farming.

WP 1: Process studies on relationship between N₂O emissions and gross N turnover, and N₂ fixation and N translocation in ¹⁵N₂-labelled soil-plant systems.

Results from this workpackage has been presented at several seminar and conference meetings (e.g. Ambus 2002, 2004; Thyme and Ambus 2002, 2003) and manuscripts are submitted for or ready for international peer-review (e.g. Ambus, 2005; Thyme and Ambus, 2005). The work has been proceeding also in close co-operation with several European partners within the EU-financed GreenGrass project (EVK2-CT2001-00105) and the preliminary results have been discussed at several project meetings.

Baseline N₂O fluxes and relationship with gross N cycling

The experimental work to study the relationship between base-line (not affected by excreta) N₂O emissions and gross N turnover was completed in 2001/2002 and final analytical work completed 2003.

Evolution of N₂O from soil monoliths did not show significant difference among the 1, 2 and 8 years old grass-clover pasture with cumulated values ranging between 166 g N ha⁻¹ (8 year) to 291 g N ha⁻¹ (1 year). Although these numbers are relatively uncertain due to a limited temporal resolution, the results do indicate the N₂O emissions from grass clover pastures in general are low, corresponding to 0.1 – 0.2 % of the annual input from biological N fixation (*see WP2.1*).

A ¹⁵N-tracer study was undertaken to study the relationship between N₂O emissions and soil N cycling. In order to handle the high number of injections of ¹⁵N-solutions into the root zone necessary to meet assumptions about homogeneity we developed a semi-automatic injection system based on the use of a Hamilton precision liquid processor. We found that soil concentrations of NH₄⁺ was an important indicator for N₂O emissions, which suggests that nitrification was a key process for the N₂O production in these pastures. The ¹⁵N₂O evolution was positively correlated with the soil ¹⁵NH₄⁺ availability (R²=0.22; P<0.001) showing the relationship $F_{15N2O}=0.19 \times e^{6.92 \times A}$, where *A* denotes the concentration of ¹⁵NH₄⁺. In a contrasting manner, the ¹⁵N₂O evolution was inversely related to the soil ¹⁵NO₃⁻ availability (R²=0.19; P<0.001) with the mathematical relationship $F_{15N2O}=1.60 \times e^{-5.95 \times N}$, where *N* denotes the concentration of ¹⁵NO₃⁻. Generally NH₄⁺ contributed at least 50% to the N₂O production,

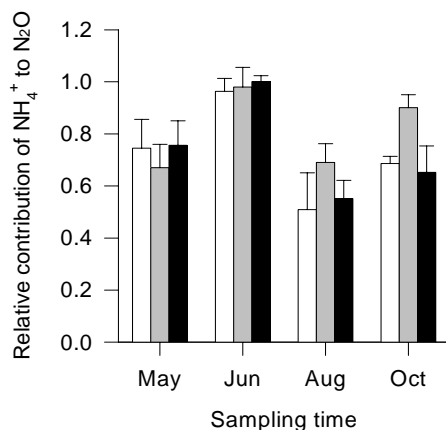


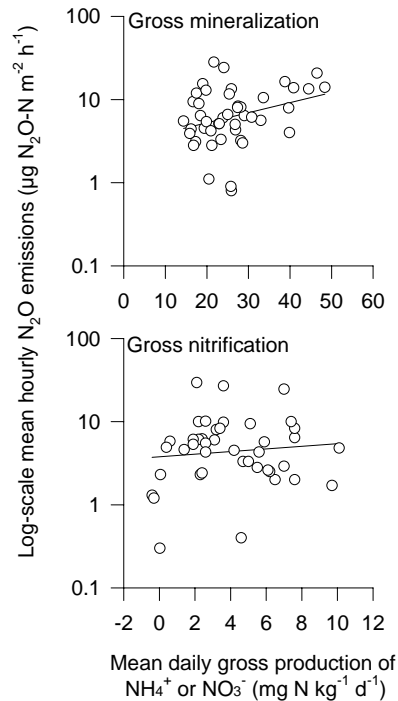
Figure 1.1 Relative contribution of NH₄⁺ to N₂O production in grass-clover root zone.

approaching 100% in June (Fig 1.1).

The N₂O emissions was significantly correlated with soil gross N mineralization (Fig. 1.2). The gross mineralization was independent of pasture age and ranged between 6 and 12 g N m⁻² d⁻¹,

suggesting a seasonal mineralization of 15 ton of N ha⁻¹. The major part of the mineralized N is assimilated by microorganisms and plants and approximately 10 % oxidized to NO₃⁻ via nitrification. An average of 0.05% (range 0.004% to 0.29%) of the oxidized NH₄⁺ was recovered as emitted N₂O within all combinations of pasture age and season, but there was no relationship

Figure 1.2 Relationship between N₂O emissions and gross mineralization (top) and gross nitrification (bottom) in grass-clover. Data points are compiled from measurements in 1 yr, 2 yr and 8 yr old pastures.



between nitrification and n₂o emission intensity (Fig 1.2). The N₂O:nitrification ratio changed significantly with sampling time, was not related to pasture age ($p < 0.05$). Thus, for the sequential sampling times of May, June, August and October the N₂O:nitrification ratio was calculated to 0.01%, 0.13%, 0.03% and 0.04%, respectively.

N₂O fluxes associated with urine deposits

Urine patches are strong sources of N₂O and may provide >20% of the annual N₂O loss from a pasture (see also WP 2.3). First of all, the urine patch has a high availability of inorganic N for nitrification and denitrification. However, urine deposits are also known to induce scorching of vegetation and root death, which may lead to increased accessibility of easily degradable carbon in the soil and thus accelerate the denitrification and N₂O losses further.

In 2003 a six-week pulse labelling study was carried out to assess the carbon- and N-dynamic in grass-clover pastures. Six replicate cores were incubated under an ¹³CO₂ enriched atmosphere for two consecutive days in order to label above- and belowground plant biomass. Immediately following the ¹³C-labeling urine was added to the soil plots and emission of CO₂ and N₂O monitored at regular intervals the following six weeks. Supplemental plots were treated with ¹⁵N-labeled urine to study the source-relationship for N₂O in simulated urine patches.

An increased CO₂ evolution was observed from the urine patches only three hours after application. This increased activity persisted for three days, when urine and non-urine affected plots showed comparable CO₂ evolution. Cumulated CO₂ losses over the course of the experiment, however, indicated a loss of 222 g C m⁻² from urine affected soil, which was 48 g C m⁻² more than from the control soil. This difference is comparable to the amount of organic C

added with the urine (44 g C m⁻²), mainly as urea and hippuric acid, suggesting that this C may have been subject to mineralization.

Taking into account the ¹³C enrichment of the evolved CO₂ it was possible to separate the mineralized C into plant and non-plant (mainly urine) sources by a simple isotope pool dilution approach. These calculations showed that during the first 0-3 days 87% of the evolved CO₂ in response to urine application could be identified as non-plant C. Over the six week course, however, only ~50% of the surplus CO₂ evolved from the urine patches was identified as of non-plant origin, indicating the the urine application stimulated the breakdown of plant-derived carbon compounds and perhaps also root respiration. On the other hand, this also shows that ~50% of the urine C persisted in the soil after the six week period, likely assimilated into the soil microbial biomass.

Parallel measurements of CO₂ and N₂O indicated temporal delay in the N₂O emissions from the urine affected plots suggesting that N₂O production is delinked with C mineralization in urine patches. As discussed later (*WP2.3*) the delayed N₂O may also be caused by inhibition of microorganisms by temporary high levels of ammonia (NH₃).

Application of ¹⁵N-labeled urine and mineral N in variable amounts (26 [low urea urine] and 51 [high urea urine and mineral N] g N m⁻²) showed small relative N₂O emissions ranging between 0.14 – 0.26 % of total N input (Table 1.1). This agrees with the low N₂O emissions from urine also observed in *WP2.3*. The results also indicate a discrepancy in relative emissions of N₂O from urine-N compared with urine-¹⁵N as opposed to similar emission factors for the two pools with mineral N. This suggests that an exchange of N took place between the substrate N-pool and the soil N-pool following application of urine but not mineral N, most likely caused by increased microbial activity in the urine affected soil. The low urea urine gave rise to less N₂O emissions than the high urea urine, suggesting that dietary controls of urine N-composition could offer a N₂O mitigation option.

Table 1.1. Nitrous oxide emission factors from urine with different urea contents and from mineral N.

Treatment	Percentages recovered as N ₂ O emissions over 6 weeks.		
	Total N applied	Applied urea	Applied ¹⁵ N-label
<i>Low urea urine</i>	0.20	0.26	0.15
<i>High urea urine</i>	0.26	0.30	0.22
<i>Mineral N</i>	0.14	-	0.15

The isotopic characteristics of N₂O emitted from the patches with ¹⁵N-labeled urine suggested a temporal shift in the N₂O producing processes. During the first 10-14 days the emitted N₂O was isotopically similar to the soil NH₄⁺ pool indicating that N₂O was formed from nitrification. After the initial two-week period the ¹⁵N enrichment of N₂O rapidly approached the enrichment of the NO₃⁻ pool indicating a shift in N₂O production from nitrification to denitrification. This conclusion supports the observations in the separate experiment in *WP2.3*.

N₂ fixation, N₂O emission and N translocation in ¹⁵N₂-labelled soil-plant systems.

The contribution of biologically fixed N₂ to the N₂O production in grasslands is unknown. To assess the contribution of recently fixed N₂ as a source of N₂O and the transfer of fixed N from clover to companion grass, mixtures of white clover and perennial ryegrass were incubated in a ¹⁵N₂-enriched atmosphere. Due to the high cost of gaseous ¹⁵N, a first task was to construct a minimum-volume gastight growth cabinet suitable for this experiment. Such growth cabinet was

obtained by modifying a chest freezer. Light is introduced from external lamps through a translucent window of Plexiglas mounted in the lid. Temperature, light and CO₂ in the growth cabinet are controlled by computer, an application developed in co-operation with University of Copenhagen. The growth cabinet can host 12 pots of 12 cm × 12 cm size (Fig. 1.3).



Figure 1.3 Pots with grass-clover for ¹⁵N₂-labelling and close up of growth cabinet. Water is provided through a silicon tube to each pot

Biological N₂ fixation measured in grass-clover shoots and roots as well as in soil constituted 342, 38 and 67 mg N m⁻² d⁻¹ at 4, 6 and 8 months after emergence, respectively (Fig. 1.4). The drop in N₂ fixation was most likely due to a aphid attack on the clover component. Transfer of recently

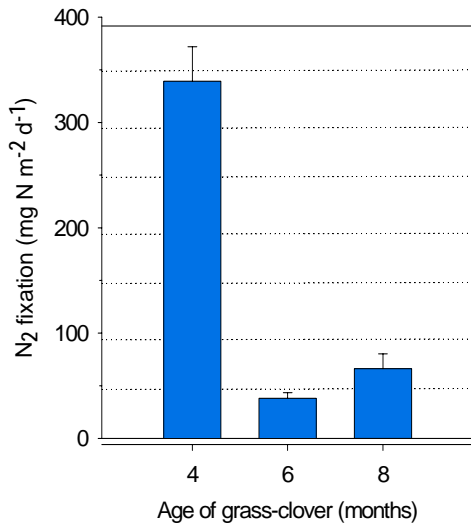


Figure 1.4. Symbiotic N₂ fixation measured in grass-clover shoots and roots; n = 4, means ± SE.

fixed N from clover to companion grass was detected at 6 and 8 months after emergence and amounted to 0.7 ± 0.1 mg N m⁻² d⁻¹, which represented 1.7 ± 0.3 % of the N accumulated in grass shoots during the labelling period. Total N₂O emission was 91, 416 and 259 μg N₂O-N m⁻² d⁻¹ at 4, 6 and 8 months after emergence, respectively. Only 3.2 ± 0.5 ppm of the recently fixed N₂ was emitted as N₂O on a daily basis. Thus, recently fixed N released via easily degradable clover residues appears to be a minor source of N₂O.

The experiment was conducted at relatively high soil water content, which should promote N₂O production via denitrification. In spite of this, <<1 % of the fixed ¹⁵N₂ was emitted as ¹⁵N₂O. This indicates that the N₂O emission factor for recently biologically fixed N₂ in a grass-clover pasture might be lower than the standard emission factor of 1.25 % suggested by IPCC. The aphid

attack on clover led to enhanced translocation of fixed N from clover to companion grass. This was probably because of 1) raised clover rhizodeposition of nitrogenous compounds and/or 2) reduced competition for light - both factors enabling increased growth of grass.

WP 2.1 and 2.2: N₂ fixation

Field estimates of total N₂ fixation based on measurements of the harvested dry matter require knowledge about the contribution from the below harvest components, i.e. stolons and roots. Furthermore, the N₂ fixation is often measured under mowing conditions, i.e. less frequent cuttings and no influence from grazing cattle, and to be able to estimate the N₂ fixation under grazing conditions knowledge about the effects of frequent cutting on clover growth is required. These aspects have been studied in combination in series of field trials and a greenhouse experiment, which are briefly summarised below with reference to previous status reports and to

publications listed in part E.

Effects of pasture age

During the growing season 2001 measurements of dry matter yields and N₂ fixation in above and below harvest biomass were carried out in a 1st, 2nd and 8th year grass-white clover. This experiment was briefly described in the 2002-status report along with preliminary results, and has been published in Eriksen and Vinther (2002, 2003, 2004) and in Eriksen *et al.* (2004a), as well as included in the publications Eriksen *et al.* (2004b) and Vinther (2003a, 2003b, 2003c, 2004b).

Effects of cutting frequency

The effects of cutting management were studied in a greenhouse experiment with red and white clover, and in a 2-year field experiment with a ryegrass-white clover mixture under two cutting regimes termed 'mowing' and 'grazing'. The 'mowing' treatment was cut at monthly intervals (3 times in 2002 and 4 times in 2003) and the 'grazing' treatment at 8-14 day intervals (7 times in 2002 and 12 times in 2003).

- The greenhouse experiment was briefly described in status report 2003 and is published in Vinther (2005a).

- Preliminary results of the field experiment were described in status report 2003, and some results have been published in Vinther (2004a, 2004c, 2005a).

Effects of soil type

The above-mentioned field experiments were carried out on a sandy loam soil, and for comparison the N₂ fixation, including contribution from belowground biomass, has been measured in a coarse sandy soil with grass-white clover. Additionally, estimates of N₂ fixation were made during the growing season 2001 at two dairy farms on coarse sandy soils, which were used as study sites for the DARCOF-project BIOMOD. Some of the results were described in status report 2002 and will appear in Grant *et al.* (2004).

Summary of N₂ fixation studies (WP 2.1 and 2.2).

The field studies of N₂ fixation has primarily focused on the contribution from below harvest, i.e., stubble, stolons and roots, with pasture age and cutting strategy as the major factors of interest. As indicated above, some of the results have been described in various publications. However, a more complete publication is being prepared (Vinther, 2005b), from where main results and conclusions are summarised below.

Table 2.1. Contribution of below-harvest biomass, i.e., stubble, stolons and roots, to the total dry matter production, total N uptake and total N₂ fixation in the various field experiments in WP 2.1 and 2.2.

	Soil type	Below-harvest as percentage of harvested				
		Dry matter		Total N		Fixed N (P _{root+stubble})
		Clover	Grass	Clover	Grass	
Foulumgaard (2002)						
- 'mowing', 1 st year	Sandy	50	283	29	99	24
- 'grazing', 1 st year	loam soil	60	220	36	84	29
Foulumgaard (2003)						
- 'mowing', 2 nd year	Sandy	141	420	105	197	76
- 'grazing', 2 nd year	loam soil	75	341	49	134	41
Jydeved (2002)	Coarse					
- mowing	sandy soil	101	255	52	202	38
Burrehøjvej (2001)						
- 1 st year	Sandy	50	90	36	47	31
- 2 nd year	loam soil	45	168	32	73	26
- 8 th year		59	210	41	105	34

In an empirical model for quantification N_2 fixation in grass-clover mixtures (Høgh-Jensen *et al.*, 2004) we suggested that the contribution from roots and stubble ($P_{\text{root+stubble}}$) to be 25% of fixed shoot N at the end of the growing period. This is apparently slightly lower than what we have found in DINOG, where $P_{\text{root+stubble}}$ was ranging between 24 and 76% (Table 2.1). However, the values of $P_{\text{root+stubble}}$ shown in Table 2.1 represent the total amount of N fixed from establishment of the grass-clover until sampling. For example, the 34% present in roots and stubble in the 8th year grass-clover represent what has been accumulated during eight years, and not only 34% of the harvested N_2 fixation in the actual production year.

Measurements of clover and grass biomass below harvest height at different stages during the lifetime of the pastures indicate that roots and stolons of clover reach their maximum level already during the first production year, whereas biomass of grass stubble and roots continues to increase for a longer period of time (Fig. 2.1). Although there possibly is a high turnover and renewal of root hairs and fine roots, which however could not be measured in our project, the results indicate that the major root system of clovers continues to function throughout the lifetime of the pasture.

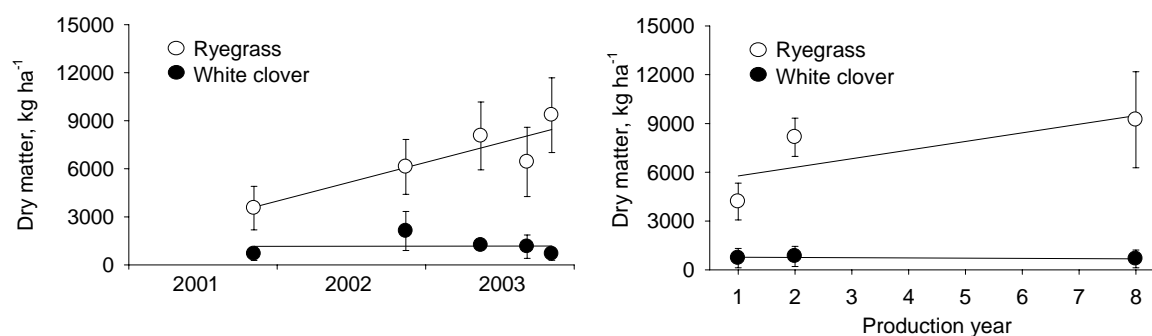


Figure 2.1. Development in below-harvest biomass of ryegrass-white clover during 1st (2002) and 2nd (2003) production year (left) and from 1st to 8th production year (right).

The above ground biomass of grass-white clover seems to be more affected by the temperature and precipitation than the roots. For example, in 2002 the total dry matter yield was 7.0 and 5.7 tons ha⁻¹ under ‘mowing’ and ‘grazing’, respectively, whereas in 2003, which was dry and warm during the growing period, the corresponding yields were 3.2 and 3.0 tons ha⁻¹, respectively. In 2002 the total below-harvest dry matter was 9.2 and 7.4 tons ha⁻¹ under ‘mowing’ and ‘grazing’, respectively, and in 2003 12.2 and 7.9 tons ha⁻¹, respectively. So, in spite of a considerably lower harvested yield in 2003 than in 2002, the below-cutting level dry matter, i.e., stubble, stolons and roots, remained at the same level in the two years, or even tended to increase from 2002 to 2003. This has implications for calculating the ratio between yield and below-cutting level dry matter, nitrogen or N_2 fixation, where e.g. N_2 fixation in the below-harvested material in the ‘normal’ production year 2002 amounted to 24-29% of N_2 fixation in the harvested herbage. In the dry low-productive year 2003 the corresponding values were 76% in the ‘mowing’ treatment and 41% in the ‘grazing’ treatment (Table 2.1).

WP 2.3. Nitrous oxide emissions from grazed pastures

This part of the project has addressed the regulation of N_2O emissions derived from N excreted during grazing. Nitrous oxide is a product both of nitrification, an aerobic process, and of denitrification which is basically restricted to anaerobic environments. The regulation of N_2O

emissions is thus extremely complex; emissions appear to be related to stressful or unbalanced conditions for the two processes.

Nitrous oxide emissions from pastures are extremely heterogeneous in space and time. Initial efforts to obtain estimates of N₂O emissions at the field scale were compromised by instrumental problems, as previously described. The main focus of this work package has therefore been on targeted studies describing sources of variability with respect to soil conditions and urine composition, which were hypothesized to influence N₂O emissions.

Nitrous oxide emissions are a function of soil conditions (such as aeration status, N availability and pH). Grazing cattle can influence these properties via compaction due to treading and via N deposition, and soil properties can be influenced by management. A possible approach towards estimating N₂O emission patterns and potential mitigation options would therefore be to describe field-scale variability with respect to soil properties and the associated N₂O emissions for use in future models of N₂O emission. Unfortunately, non-destructive methods to be used on a field scale are not available at this time. As a first step in this direction, some new experimental approaches have been explored, including measurements of soil density and salinity, and image analysis of pasture N status. Some preliminary results have been presented at meetings (Petersen et al., 2004a) and electronically (Petersen et al., 2004b).

Excess N is mainly deposited as urea in the urine, and urine patches are therefore hotspots of N turnover and N₂O emissions. Urea is degraded to ammonium which is a potential stress factor for soil organisms. It was therefore hypothesized that high N surpluses in the diet of cattle would accelerate N₂O emissions, whereas low N surpluses, conversely, would have a relatively low potential for emissions. This question was explored in controlled field plot experiments, which were carried out as a joint effort between DINOg and a European research project (EKV2-CT-2000-00096 “Greenhouse gas mitigation for organic and conventional dairy production” Results from this work has been reported in peer-reviewed journals (Bol et al., 2004; Petersen et al., 2004c, d), and the main conclusions are summarized below.

New methods for describing spatial heterogeneity in pastures

Grazing cattle excrete surplus nitrogen mainly in the urine, and resulting concentrations in urine patches can range from 20 to 80 g N m⁻² or even more. In this extreme environment, the risk for leaching or atmospheric losses of nitrogen is particularly high, and precise estimates of nitrogen balances for grazed pastures must therefore be able to properly account for transformations and losses associated with urine patches.

Urine distribution described by image analysis

Within DINOg, a new approach was tested for mapping the distribution of N deposited during grazing. An instrument designated as the Foulum Image Capture Facility was mounted on a lift and taken to 13 m height, where pictures were taken along the edge of a grazed pasture. A campaign in June 2002 revealed a very patchy distribution in the absorption of photosynthetic light which is related to the nitrogen content of the plant material. We therefore believe that this method offers a possibility for describing N deposition by grazing cattle. The concentration of patches was elevated around the drinking trough, showing that there is macro-scale heterogeneity in grazed pastures that is influenced by management.

Urine distribution described by electrical conductivity

Experimental studies of N₂O emissions from pastures need to ensure that urine patches are properly represented in a measurement program. However, because of the patchy distribution of urine, it can be very difficult to make a representative selection of sampling points. For this purpose the information derived from image analysis can not be used, because it represents nitrogen that has already been removed from the soil solution.

An alternative approach was introduced that is based on measurement of soil ionic strength which in agricultural soils is dominated by inorganic N. We used impedance measurements based on time domain reflectometry (TDR), a method that is routinely used to determine volumetric soil water content, but gives also information about the impedance which is inversely related to electrical conductivity (EC) in the soil water. TDR measurements are largely non-destructive and take <1 min, and the method is therefore suitable for fast and detailed mapping of soil conditions. The data were processed and sorted within an hour, and the information used for selection of sampling points which reflected the distribution of nitrogen more precisely than a random selection of sampling points. Ionic strength includes both ammonium and nitrate and therefore will not reveal whether a urine patch is new or several days old. However, with a suitable number of sampling points, urine patches of different age should be represented.

A third method was introduced, i.e., for non-destructive characterization of soil bulk density based on narrow-probe gamma ray transmission measurements. This method was combined with TDR probe measurements of EC in a campaign describing the time course of N₂O emissions in a pasture. Forty sampling points along four transects were characterized with respect to both EC and density. In Fig. 2.2 (right), the data have been sorted in ascending order. The sampling points selected for subsequent measurements of N₂O over time (blue/red) covered the variability with respect to both variables. Nitrous oxide measurement results, however, showed that the relationship between soil properties and the potential for N₂O emissions is complex. One sampling point (red color) showed high and temporally stable emissions of N₂O, which another sampling point with similar EC and higher density showed little activity. The difference was due to aeration status as revealed by moisture determinations and ammonium/nitrate composition by the end of the study.

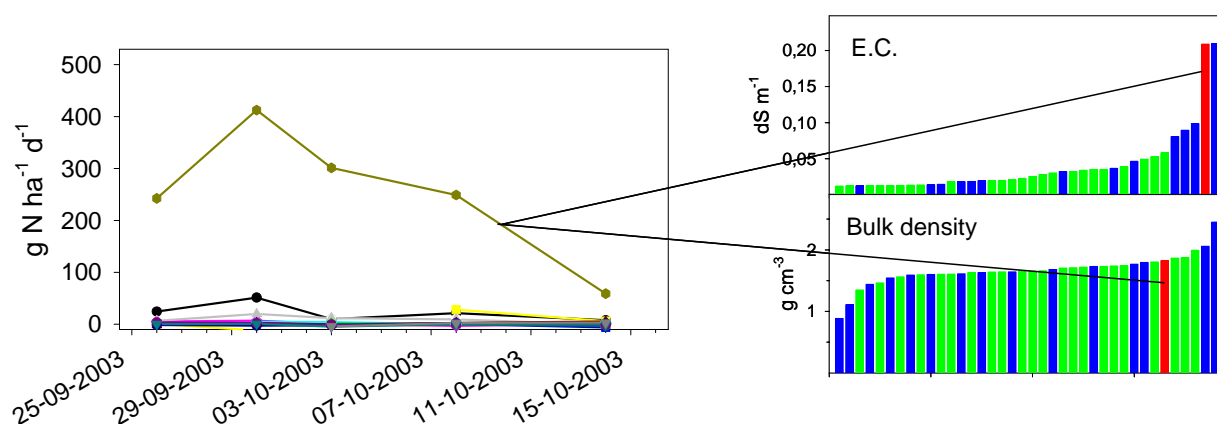


Figure 2.2. Results from a campaign describing N₂O emission over time in 16 sampling points selected on the basis of non-destructive soil bulk density and electrical conductivity measurements.

Nitrous oxide emissions from urine patches as influenced by urea level

Urine patches are significant hot-spots of C and N transformations. To investigate the effects of urine composition on C and N turnover and gaseous emissions from a pasture soil, a field plot study was carried out in September 2001. Cattle urine amended with two levels of ¹³C- and ¹⁵N-labelled urea was added to a sandy loam pasture soil, resulting in 23.3 or 39.8 g urea-N m⁻². Pools and isotopic labelling of N₂O and CO₂ emissions, extractable urea, NH₄⁺ and NO₃⁻, and plant uptake were monitored during a 14-d period, while NH₃ losses were estimated in separate plots amended with unlabelled urine. Ammonia volatilization was estimated at 14 and 12% of the urea-N applied in the low (UL) and high (UH) urea treatment, respectively. Nitrous oxide emission rates were low, but generally increased during the 14-d period, as did the proportion derived from

urea-N. On day 14, the contribution from urea was 23 (UL) and 13% (UH treatment), respectively. Cumulative total losses of N₂O during the 14-d period corresponded to 0.021 (UL) and 0.015% (UH) of applied urea-N. Nitrification was probably the only source of N₂O in this study. Urine composition did not influence the potential for N₂O emissions from urine patches under the experimental conditions of this study, but the importance of site conditions and season should be investigated further.

Microbial dynamics may reveal stress effects of urine deposition. The field-plot experiment also investigated ¹³C uptake in microbial lipids. The confined plots without or with cattle urine amendment were sampled after 4 and 14 days, and soil from 0-5 and 10-20 cm depth was analyzed for content and composition of phospholipid fatty acids (PLFAs), and for the distribution of urea-derived ¹³C among individual PLFAs. At the higher urea level, osmotic stress was indicated by the dynamics of cyclopropane fatty acids and branched-chain fatty acids. Incorporation of ¹³C from [¹³C]urea was low, but significant, and the highest amounts of urea-derived C were found in fatty acids that would be consistent with growth of typical NH₄⁺ oxidizing (*Nitrosomonas*) and NO₂⁻ oxidizing bacteria (*Nitrobacter*). PLFA dynamics and the incorporation of urea-derived ¹³C in PLFAs indicated a response of nitrifiers which differed between the two moderate urea concentrations.

Short-term nitrous oxide emissions from pasture soil as influenced by urea level and soil nitrate

A laboratory study was undertaken to describe short-term effects on N₂O emissions and soil conditions, including microbial dynamics, of urea amendment at two different rates (22 and 43 g N m⁻²). The lower urea concentration was also combined with an elevated soil NO₃⁻ concentration. Urea solutions labelled with 25 atom% ¹⁵N were added to the surface of repacked pasture soil cores and incubated for 1, 3, 6 or 9 days under constant conditions (60% WFPS, 14°C). Soil inorganic N (NH₄⁺, NO₂⁻ and NO₃⁻), pH, electrical conductivity and dissolved organic C were quantified. Microbial dynamics were followed by measurements of CO₂ evolution, by analyses of membrane lipid (PLFA) composition, and by measurement of potential ammonium oxidation and denitrifying enzyme activity. The total recovery of ¹⁵N averaged 84%. Conversion of urea-N to NO₃⁻ was evident, but nitrification was delayed at the highest urea concentration and was accompanied by an accumulation of NO₂⁻. Nitrous oxide emissions were also delayed at the highest urea amendment level, but accelerated towards the end of the study. The pH interacted with NH₄⁺ to produce inhibitory concentrations of NH₃(aq) at the highest urea concentration, and there was evidence for transient negative effects of urea amendment on both nitrifying and denitrifying bacteria in this treatment. However, PLFA dynamics indicated that initial inhibitory effects were replaced by increased microbial activity and net growth. It is concluded that urea-N level has qualitative, as well as quantitative effects on soil N transformations in urine patches.

WP 3: Modelling of nitrogen exchange between soil and atmosphere

A dynamic algorithm to simulate the N₂O production and emission from nitrification and denitrification has been included in the field component of the FASSET whole-farm model. Both nitrification and denitrification are included as important sources for N₂O emission in the algorithm. The denitrification model is based on soil organic matter turnover, mineral nitrogen and soil water responses. The model simulates potential production of N₂ and N₂O and calculates the ratio of N₂ to (N₂+N₂O) based on functions of soil texture, soil water content and soil depth.

The model was tested on experimental data of N₂O emissions from typical North European grasslands with mineral soils (Denmark, Finland, UK), differing in climatic conditions, soil properties and management. The model simulated the general time course of N₂O emissions and

captured the observed effects of fertiliser and manure management on emissions. However, emissions from a soil with high clay content were overestimated with the model. The model explained up to 89% of the variation in measured annual nitrous oxide emissions.

The model was tested under different climatic and management scenarios applicable for Denmark. The simulations were done for a 4-year rotation with one year of spring barley undersown with grass, two years of grass and a fourth year of barley undersown with grass. The grasslands were simulated as either grazed with heifers or treated as a cut based system (4 cuts per year), with removal of the grass production. The level of applied mineral N (ammonium nitrate, 50:50) ranged from 0 to 500 kg ha⁻¹. For estimation of average results, 30 independent calculations with the original climatic and soil datasets were done for 3 sites in Denmark.

The simulated emissions of nitrous oxide increase after each fertilisation event (Fig. 3.1). The increase is greatest in mid-summer, where the soil temperature is high and the grass growth may be limited by water shortages. The large recycling of nitrogen in the grazed system leads to a considerably higher level of nitrous oxide emissions. This higher level of nitrous oxide emissions is evident even during spring before the grazing starts, which is a residual effect from the grazing during the previous year.

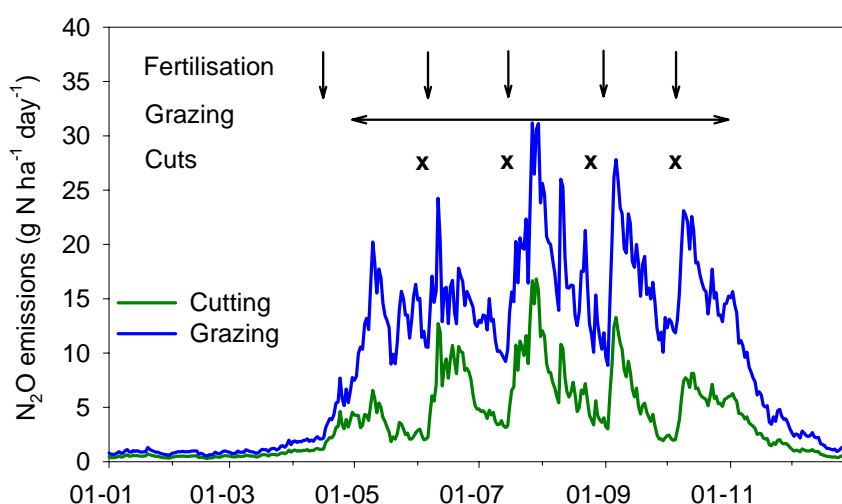


Figure 3.1. Simulated mean daily nitrous oxide emissions from ryegrass on a loamy sand soil fertilised with 200 kg N ha⁻¹ yr⁻¹ and used for either cutting or grazing. The dates of fertilisation are shown with arrows. The line shows the grazing period, and the crosses show dates of cuts.

The model estimates showed considerably higher nitrous oxide emissions from loamy soils compared with a sandy soil (Fig. 3.2). The soil aeration is generally better on the sandy soils and this reduces the risk of nitrous oxide emissions, because conditions for denitrification are more rarely present. The nitrous oxide emissions increased with increasing nitrogen fertilisation. The rate of increase was greater at higher fertilisation rates. This was primarily an effect of a larger addition of mineral nitrogen to the soil at higher nitrogen fertilisation. The increase in nitrous oxide emissions at higher fertiliser rates was particularly large for the loamy soils and under grazing.

The model was linked with the cattle model in FASSET to test the effect of urine and dung patches on nitrogen losses, primarily nitrous oxide and nitrate leaching, from grazed grasslands. A comparison was made between a situation with uniform distribution of urine and faeces across the grassland and a situation with patches. It was also assumed that the patches will affect growth of the grass and thus have feedback effects on the energy and nitrogen intake by the cattle. The

results showed that accounting for patches reduced N₂O emissions by 10-30%, but increased nitrate leaching slightly. The response for N₂O emissions is due to a saturation of the denitrification in the model at high nitrate levels, which leads to a higher proportion of the losses occurring at nitrate leaching. There is a need to verify this response experimentally.

A test of the nitrogen fixation model in FASSET has been conducted for available datasets in collaboration with the DARCOF II BIOMOD project. The simulated nitrogen fixation of grass-clover pastures is generally in agreement with measured values.

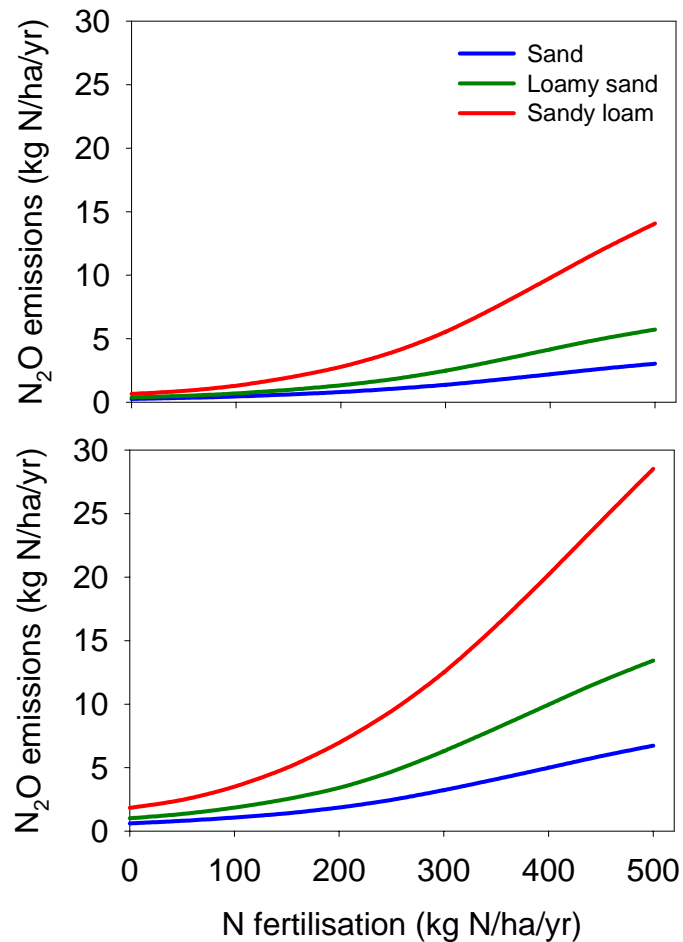


Figure 3.2. Simulated mean annual nitrous oxide emissions with varying nitrogen fertilisation from either cuts or grazing. The simulations were performed for three different soil types.

C.2 Fulfilment of deliverables and milestones

No. and title	Time schedule according to application	Deviations if any*	Full filled
D1.1 Development of method for $^{15}\text{N}_2$ fixation study in lysimeters	05/01	05/02	yes
D1.2 Development of easy, simple method for simultaneous gross N turnover and gas flux measurements in the field	06/01		yes
D1.3 Publication on $^{15}\text{N}_2$ fixation method	12/01	03/05	yes ^a
D1.4 Publication on $^{15}\text{N}_2$ fixation, translocation and gas losses.	09/03	03/05	yes ^a
D1.5 Publication on relationship between field gross N turnover in excreta affected pasture and gas fluxes	05/04	01/05	yes ^b
D1.6 Publication on relationship between gross N turnover and gas fluxes in controlled environment	05/04	01/05	yes ^b
D1.7 First year data available for WP3	03/02		yes
M1.1 Method for $^{15}\text{N}_2$ fixation developed	05/01	05/02	yes
M1.2 Method for field gross N-turnover and gas measurements developed	06/01		yes
M1.3 $^{15}\text{N}_2$ fixation measurements completed	08/02	11/02	yes
M1.4 Gross N turnover and gas flux measurements in the field and controlled environment completed	10/03		yes
D2.1 Report on the relationship between N_2 fixation and leguminous dry matter production	11/01	10/03	yes ^c
D2.2 Publication on the contribution from stolons and clover roots to the total N_2 fixation	05/04	03/05	yes ^d
D2.3 Publication on the combined effects of grazing and urine deposits on N_2 fixation in grass-clover	07/02	03/05	yes ^d
D2.4 Publication on field estimates of N_2 fixation in grass-clover pastures on sandy soil types	05/04	03/05	yes ^e
D2.5 Publication on field estimates of N_2O emission from grass/clover pastures on sandy soil types	05/04	12/04	yes ^f
D2.6 First year field data made available for WP3	03/02		yes
M2.1 Experiments on the combined effects of grazing and urine deposits on N_2 fixation in grass/clover completed.	12/01	10/03	yes
M2.2 Experiments on contribution of stolons and clover roots to N_2 fixation completed.	10/03	03/04	yes
M2.3 Field measurements of N_2O emission and N_2 fixation completed.	10/03	11/03	yes
D3.1 Revised N_2O and N_2 fixation submodels	02/03	04/04	yes
D3.2 Publication on model work	05/04		yes
M3.1 A new N_2O emission sub-model implemented in the whole-farm model FASSET.	02/03	04/03	yes
M3.2 An improved N_2 fixation sub-model implemented in the whole-farm model FASSET.	02/03	04/03	yes
M3.3 Revised FASSET sub-models verified and validated.	11/03	04/04	yes
P1 1 st annual report on DINOG	10/01		yes
P2 2 nd annual report on DINOG	10/02		yes
P3 3 rd annual report on DINOG	10/03		yes

* Deviations are to be further discussed in D

D. *Description of deviations and subsequent adjustments of plans*

There have been no major deviations in the project giving rise to significant changes in research plans or anticipated outputs. The minor deviations were explained in the status report 2001, which caused additional work in the initial phase and delays in some of the subsequent deliveries. The deviations and delays caused by including pasture age studies have primarily affected WP 2.1

and 2.2. These, and some additional deviations in deliverables include:

- a) D1.3 and D1.4 have been merged and is pending review following maternity leave
- b) D1.5 and D1.6 have been merged and is still pending review
- c) D2.1 is included in Høgh-Jensen et al. (2004)
- d) D2.2 and D2.3 have, as mentioned earlier, partly been fulfilled, and will be completed in Vinther (2005b)
- e) D2.4 and D2.5 have been merged and are published in Grant et al. (2005)
- f) the original focus on quantifying field estimates of N₂O were replaced by studies on new methods for describing pasture variability, and on detailed investigations on N₂O emissions and microbial dynamics in urine patches.

E. Project publications and other products

1. Products from Organic Eprints archive

Peer-reviewed and accepted

English

Bol, Roland; Petersen, Søren O.; Christofides, Calliopi; Dittert, Klaus and Hansen, Martin Nørregaard (2004) [Short term N₂O, CO₂, NH₃ fluxes and N/C transfers in a Danish grass-clover pasture after simulated urine deposition in autumn](#) [Udledninger af lattergas, kuldioxid og ammoniak samt N/C-omsætning i en dansk kløvergræsmark efter simuleret afsætning af urin om efteråret]. *Journal of Plant Nutrition and Soil Science* 167:pp. 568-576.*

Eriksen, J and Vinther, FP (2002) [Nitrate leaching in grazed grasslands of different composition and age](#). *Grassland Science in Europe* 7:pp. 682-683.

Eriksen, J.; Vinther, F.P. and Søgaard, K. (2004) [Nitrate leaching and N₂-fixation in grasslands of different composition, age and management](#). *Journal of Agricultural Science* 142:pp. 141-151.

Høgh-Jensen, Henning; Loges, Ralf; Jensen, Erik Steen; Jørgensen, Finn V. and Vinther, Finn P. (2004) [Empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures](#). *Agricultural Systems* 82:pp. 181-194.*

Petersen, Søren O.; Roslev, Peter and Bol, Roland (2004) [Dynamics of a pasture soil microbial community after deposition of cattle urine amended with \[13C\]urea](#) [Dynamik af det mikrobielle samfund i en afgræsningsmark efter depositin af kvægurin beriget med [13C]urea]. *Applied and Environmental Microbiology* 70(11):pp. 6363-6369.*

Petersen, Søren O.; Simek, Miloslav; Stamatiadis, Stamatis and Yamulki, Sirwan (2004) [Nitrous oxide emissions from grazed grassland: effects of cattle management and soil conditions](#). Paper presented at Greenhouse gas emissions from agriculture. Mitigation options and strategies, Leipzig, Germany, 10-12 February 2004; Published in Weiske, Achim, Eds. *Greenhouse gas emissions from agriculture. Mitigation options and strategies*, page pp. 75-78. Institute for Energy and Environment. Leipzig, Germany.*

Petersen, Søren O.; Stamatiadis, Stamatis and Christofides, Calliopi (2004) [Short-term nitrous oxide emissions from pasture soil as influenced by urea level and soil nitrate](#) [Lattergas-emissions fra græsmarksjord som funktion af urea-niveau og jordens nitratindhold]. *Plant and Soil* 267(1):pp. 117-127.**

Petersen, Dr. Søren O.; Stamatiadis, Dr. Stamatis; Christofides, Dr. C.; Yamulki, Dr. Sirwan and Bol, Dr. Roland (2003) [Urea concentration affects short-term N turnover and N₂O production in grassland soil](#) [Koncentrationen af urea i urin påvirker kvælstofomsætning og

lattergasproduktion i afgræsningsmarker]. Poster presented at 12th Nitrogen Workshop, North Wyke, UK, 22-24 Sept 2003.*

Vinther, F.P. (2005) [Below-harvest biomass and N₂ fixation in grass-clover at different cutting frequencies](#), in Frankow-Lindenberg, B., Eds. *Adaption and Management of Forage Legumes - Strategies for improved Reliability in mixed Swards*. Agricultural University of Sweden, Uppsala.

Vinther, F.P. (2004) [Effect of age and cutting frequency on below-ground biomass in grass-clover](#). Paper presented at Workshop on Adaption and Management of Forage Legumes - Strategies for improved Reliability in Mixed Swards (COST Action 852), Ystad, Sweden, September 19-23 2004; Published in *Abstracts of the Workshop on Adaption and Management of Forage Legumes - Strategies for improved Reliability in Mixed Swards*, page pp. 23-24.

Vinther, F.P. and Dahlmann-Hansen, L. (2004) [Effects of ridging on crop performance and symbiotic N₂ fixation of fababeam \(*Vicia faba* L.\)](#). *Soil Use and Management*.

Vinther, F.P.; Hansen, E.M. and Olesen, J.E. (2004) [Effects of plant residues on crop performance, N mineralisation and microbial activity including field CO₂ and N₂O fluxes in unfertilised crop rotations](#). *Nutrient Cycling in Agroecosystems* 70. Online at <http://www.kluweronline.com/issn/1385-1314/contents>

Submitted for peer-review but not yet accepted

English

Ambus, Dr P (2002) [Sources of N₂O in organic grass-clover pastures](#).. Poster presented at NJF Seminar no. 342 Agricultural soils and greenhouse gases in cool-temperate climate, Reykholt, Iceland, 31 July - 3 August 2002.

Ambus, Dr. P. (2003) [Relationship between nitrogen cycling and nitrous oxide emission in an organic grass-clover pasture](#). [preprint]

Chatskikh, D.; Olesen, J.E.; Berntsen, J.; Regina, K. and Yamulki, S. (2003) [Simulation of effect of climate, soils and management on N₂O emission from grassland](#). [preprint]

Not peer-reviewed

English

Ambus, P. (2004) [Short term N₂O losses in urine patches: a ¹⁵N labelling study](#). Poster presented at International Conference. Greenhouse Gas Emissions from Agriculture - Mitigation Options and Strategies, Leipzig, Germany, 10-12 February, 2004.*

Chatskikh, Dmitri; Olesen, Jørgen E. and Berntsen, Jørgen (2004) [Modelling of N₂O emissions from grasslands in Denmark](#). [oral] Presentation at *Joint meeting of COST Action 627*, Ghent, Belgium, 3-4 May 2004.

Chatskikh, Dmitri; Olesen, Jørgen E.; Berntsen, Jørgen; Regina, Kristiina and Yamulki, Sirwan (2003) [A study of the N₂O emission from grassland with the FASSET farm model](#).

Poster presented at 12th N Workshop, Exeter, Devon, UK, 21-24 September 2003.

Eriksen, J. and Vinther, F.P. (2003) [Nitrate leaching and N₂-fixation in grasslands of different composition, age and management](#). Poster presented at 12th N Workshop, Exeter University, 21-24 September 2003; Published in *Abstracts for the 12th N Workshop. Exeter 21-24 September*.

Hutchings, Nicholas J.; Petersen, Bjørn M.; Olesen, Jørgen E. and Berntsen, Jørgen (2004) [Does modelling of spatial heterogeneity matter?](#). [oral] Presentation at *Joint meeting of COST Action 627*, Ghent, Belgium, 3-4 May 2004.

Petersen, Søren O. (2001) [Nitrous oxide emissions and grassland management](#).*

Petersen, Dr. Søren O.; Bol, Dr. Roland; Dittert, Dr. Klaus; Christofides, Dr. Calliopi; Roslev, Dr. Peter and Hansen, Dr. Martin N. (2004) [Short-term C and N dynamics in urine patches](#):

[Response of nitrifiers to different urinary urea levels](#) . [oral] Presentation at *COST 627 Carbon Storage in Grasslands*, Ghent, BE, 3-5 June 2004.*

Thyme, M. and Ambus, P. (2004) [N₂O emission from grass-clover swards is largely unaffected by recently fixed N₂](#). *DARCOFenews*(1). Online at <http://www.darcof.dk/enews/april04/index.html>.*

Thyme, Mette and Ambus, Per (2004) [From N₂ fixation to N₂O emission in a grass-clover mixture](#). Paper presented at 12th N Workshop 'Controlling N Flows and Losses', Exeter, UK, 21-24 September 2003; Published in Hatch, D.J.; Chadwick, D.R.; Jarvis, S.C. and Roker, J.A., Eds. *Controlling nitrogen flows and losses*, page pp. 365-366. Wageningen Academic Publishers, The Netherlands.*

Thyme, Mette and Ambus, Per (2003) [From N₂ fixation to N₂O emission in a grass-clover pasture](#). Poster presented at 12th N workshop "Controlling N flows and losses", Exeter, UK, 21st - 24th September 2003.*

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Dansk - Danish

Ambus, P. (2004) [Kilovis af N omsættes dagligt i kløvergræs](#) [Kilograms of N is transformed daily in grass-clover]. In *Økologisk Jordbrug*, 20. August, No 318, page 6. Økologisk Landsforening.

Ambus, P. (2002) [Kommer der lattergas fra kløvergræsset?](#) [Is grass-clover a source for nitrous oxide?]. In *Landsbladet Mark*.

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Vinther, F.P. (2004) [N-fiksering - hvor meget og hvordan?](#) [N fixation - how much and how?]. [oral] Presentation at *Plantekongres 2004*, Messecenter Herning, Danmark, 13.-14. januar 2004.

Vinther, F.P. (2004) [N-fiksering - hvor meget og hvordan?](#) [N fixation - how much and how?]. In *Månedsmagasinet Kvæg*, 15. January, page 19.

Vinther, F.P. (2003) [Hvor meget kvælstof henter kløvergræs fra luften?](#) [How much nitrogen is fixed by grass-clover?]. In *Aktuel Økologi fra Familielandbrugets Økologikonsulenter*, No 6, page pp. 4-5.

Vinther, F.P. (2003) [Kvælstof fra luften](#) [Nitrogen from the air]. In *Økologisk Jordbrug*, No 288, page 6.

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Vinther, F.P. and Laier, A.Aa. (2001) [Ærter som kvælstofsamler i vintersæd](#) [Peas collecting nitrogen in winter cereals]. In *Landsbladet*, 7. December.**

2. Other products (oral presentations, public meetings, field days, etc.)

- Ambus, P. 2002. Greenhouse gas emission from agricultural and forest soils. Symposium on climate change and plant-ecosystem interactions, 21 March, Risø, DK. Unpublished.*
- Ambus, P. 2003. Short term C-dynamic in urine affected grass-clover pasture: a ^{13}C pulse labelling study. Invited presentation at the COST-627 action Carbon sequestration opportunities in European grasslands: mitigation scenarios at plot, farm and regional scales, 7-8 September, Clermont-Ferrand, France.*
- Foulum 2001. Presentation of experimental site in connection to workshop within EU Concerted Action 627 'Carbon Storage in Grasslands', 29 Sep.
- Petersen, S.O. 2003. Greenhouse gas emissions from animal manure. Invited presentation at joint Carboeurope-Greengrass concerted action: Synthesis of the European Greenhouse Gas Budget, 4-5 September, Clermont-Ferrand, France.
- Thyme, M. 2001. Nitrous oxide emissions in grass-clover fields. Oral presentation at the Ph.D. summer school on "*Linking Ecology and Organic Farming*", organised by SOAR (Research School for Organic Agriculture and Food Systems), Kongskilde Friluftsgaard, 24-28 September 2001.*
- Thyme, M. 2001. Produktion af lattergas (N_2O) i kløvergræs. Forskerskolen for økologisk jordbrug og fødevarerproduktion - SOAR: Halvårsseminar, Kgl. Veterinær- og Landbohøjskole, 16 November, Tåstrup, DK.*
- Thyme, M. 2001. Production of nitrous oxide in grass-clover pastures. Workshop on clover in Northern areas, Swedish University of Agricultural Sciences, 19-21 November, Umeå, SE.*
- Thyme, M. 2002. Production of nitrous oxide in grass-clover pastures. Ph.D. course: Dynamics of Organic Matter in Soil, 26 May – 1 June, Brorfelde Holbæk, DK.*
- Thyme, M. 2002. Production of nitrous oxide in grass-clover pastures. Plant Research Department, 6 September, Risø National Laboratory, DK.*
- Thyme, M. 2002. Produktion af lattergas (N_2O) i kløvergræs. Half-yearly seminar in SOAR (Research School for Organic Agriculture and Food Systems), 29 November, Risø, DK.*

* 25-75% financed by DARCOF

** 5-25% financed by DARCOF

F. Scientific education

M.Sc. Mette Thyme joined the project as Ph.D.-student beginning 15 September 2001. The Ph.D.-project receives funding by DARCOF II, The Danish Research Councils and Risø National Laboratory.

Martin Nørgaard Hansen, Dept. Agricultural Engineering, DIAS (Ph.D. student) participated in a microplot study with measurements of ammonia volatilisation.

PhD student Daan Beheydt from ISOFYS Laboratory, Gent University, Belgium has visited Department of Agroecology for about 2 weeks in both 2003 and 2004. This has been partly funded by COST action 627. There has been a collaboration on comparing the nitrous oxide emissions modelled with the FASSET model and with the DNDC model, and this is expected to result in an international publication in 2005.

G. National and international cooperation

The activities in DINOG have been closely linked to the EU-project GreenGrass on greenhouse gas emissions from European grasslands. GreenGrass included participants from 7 countries and was joined by Per Ambus and Jørgen E. Olesen.

Data for validating the model was obtained in collaboration with two EU-funded projects,

MIDAIR and GreenGrass. The model estimation of spatial variation due to urine and dung patches in grasslands was also performed in collaboration with the GreenGrass project.

Furthermore, the project has provided a platform for co-operation with numerous other scientists:

Dr. Peter Roslev, Aalborg University

Dr. Klaus Dittert, University of Kiel

Dr. Roland Bol, Institute of Grassland and Environmental Research, Exeter, UK

Dr. Sirwan Yamulki, Institute of Grassland and Environmental Research, Exeter, UK

Dr. Stamatis Stamatiadis, Soil Ecology & Biotechnology Lab, Gaia Center - Goulandris Natural History Museum, Kifissia, Greece

Dr. Martin R. Weisbjerg, Department of Animal Nutrition, Danish Institute of Agricultural Sciences, Denmark

Dr. René Larsen, Department of Agricultural Systems, Danish Institute of Agricultural Sciences, Denmark

Dr. Jeans-Francois Sussana, National Institute for Agricultural Research (INRA), Clermont-Ferrand, France

Dr. Ute Skiba, Centre of Ecology and Hydrology, Bush Estate, Penicuik, Midlothian, UK

Dr. Henning Høgh Jensen, Department of Agricultural Sciences, Organic Farming Unit, Royal Veterinary and Agricultural University, Denmark.

Dr. Anders Michelsen, Botanical Institute, University of Copenhagen, Denmark.

Dr. Kristiina Regina, MTT, Finland

Critical reflection on the project

As discussed in mid-term report 2002, it was decided to put more effort into a generalization of the experimentation in the project, e.g. by including comparisons with conventional systems. The reason for this was partly due to issues raised during the DARCOF-project meetings in 2002 and the international mid-term evaluation, but also to achieve a more intimate co-operation with ongoing EU-funded activities within this research field. For this reason it is thus evident that some activities are diverging from the initial project objectives, exemplified by the inclusion of C-dynamic studies (WP 1) and conventional systems (WP 2).

The experiments in this work have included much detailed studies at the process levels. This approach was emphasized by the international mid-term evaluation panel as being important for the understanding of key-processes in grass-clover systems specifically and to improve understanding of soil-plant-atmosphere interactions in general. The evaluation also emphasized the inclusion of long-term study sites for comparison of grass-clover pastures of different ages, which underlines the importance of maintaining long-term field trials that can be made available for specific works.

Denmark has ratified the Kyoto Protocol, which commits to a 21% reduction in the national emissions of greenhouse gases during the period 1990 to 2010. The focus on emission reduction measures in the agriculture has so far been on the reduction of nitrogen fertiliser use. In the national emissions inventory, N₂O emissions from fertiliser use are estimated as 1.25% of the applied amount of nitrogen. The model results derived in the DINOG project show that this is a much too simple approach for estimation of the actual emissions. There are large differences between soil types in the

emissions, and the proportion of fertiliser nitrogen emitted as nitrous oxide is smaller at lower nitrogen rates. This non-linear response of nitrous oxide emissions to nitrogen input may make organic farming an interesting option for reducing greenhouse gas emissions, because the nitrogen input is lower in organic farming. There is, however, need for further studies to document this. An approach like the DINOG project, which links experimental work with modelling may be very effective to do this. However, unfortunately some technical problems meant that the field data collected in the DINOG project was not suitable for the model validation. Additional data were therefore collected from other studies in Northern Europe.

8. Budget

A. Account for any change in budgets

No changes in overall budget.

B. Budget for the whole project (1,000 DKK)

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	43	24	14	6	44
Technical personnel	25	16	8	1	25

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	1858	1005	623	274	1902
Technical personnel	656	408	217	27	652
Other operational costs	387	261	75	11	347
Equipment	70	70			70
Others (travel)	79	28	20	31	79
Direct costs	3050	1772	935	343	3050
Indirect costs (20% of direct costs)	610	355	187	69	610
Total	3660	2126	1122	412	3660

Comments:

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project			

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: **Danish Institute of Agricultural Sciences**

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	27	14	10	4	28
Technical personnel	15	10	5		15

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	1161	581	443	181	1205
Technical personnel	402	259	139		398
Other operational costs	240	156	38	6	200
Equipment					
Others (travel)	35	12	6	17	35
Direct costs	1838	1008	626	204	1838
Indirect costs (20% of direct costs)	368	202	125	41	368
Total	2206	1210	751	245	2206

Comments:

Name of Institute: **Risø National Laboratory**

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	16	10	4	2	16
Technical personnel	10	6	3	1	10

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	697	424	180	93	697
Technical personnel	254	149	78	27	254
Other operational costs	147	105	37	5	147
Equipment	70	70			70
Others (travel)	44	16	14	14	44
Direct costs	1212	764	309	139	1212
Indirect costs (20% of direct costs)	242	152	62	28	242
Total	1454	916	371	167	1454

Comments: