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Impact of Fermented Organic Fertilizers on *in-situ* Trace Gas Fluxes and on Soil Bacterial Denitrifying Communities in Organic Agriculture

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For my Family

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List of Abbreviations

AMO	ammonia monooxygenase
amoA	gene encoding the alpha-subunit of the ammonia monooxygenase
ANOVA	analysis of variance
AOB	ammonia-oxidizing bacteria
APS	ammonium persulfate
BOD	biological oxygen demand
bp	base pair
BSA	bovine serum albumine
Cd-Nir	cytochrome *cd* nitrite reductase
CEC	cation exchange capacity
CoM	coenzyme M
C_T	threshold cycle
C_t	total carbon
Cu-Nir	copper-containing nitrite reductase
CV	coefficient of variation
DGGE	denaturing gradient gel electrophoresis
dw	dry weight
EB	elution buffer
ECD	electron capture detector
FADase	coenyzme flavine-adenine-dinucleotide
FC	fermented crop material
FID	flame ionization detector
FS	fermented (cattle) slurry
FYM	farmyard manure
HAO	hydroxylamine oxidoreductase
Hin6I	restriction enzyme
HpaII	restriction enzyme
INT	iodnitrotetrazoliumchloride
K_m	apparent half saturation constant
LB-medium	Luria-Bertani medium
MBC	microbial biomass carbon
MDE	mutation detection enhancement solution
MMO	enzyme methane monooxygenase
MPN	most probable number
Nap	periplasmic nitrate reductase
napA	gene encoding the large subunit of the periplasmic nitrate reductase
napB	gene encoding the cytochrome c-subunit of the periplasmic nitrate reductase
Nar	membrane-bound nitrate reductase
narG	gene encoding the alpha-subunit of the membrane-bound nitrate reductase
narH	gene encoding the beta-subunit of the membrane-bound nitrate reductase
narI	gene encoding the gamma-subunit of the membrane-bound nitrate reductase
nirK	gene encoding the copper-containing nitrite reductase
nirS	gene encoding the cytochrome *cd* nitrite reductase
N_{min}	mineral nitrogen (nitrate, ammonium, (nitrite))
NOB	nitrite-oxidizing bacteria

Nor	nitric oxide reductase
NOR	nitrite oxidoreductase
norB	gene encoding the cytochrome *b*-subunit of the nitric oxide reductase
norC	gene encoding the cytochrome *c*-subunit of the nitric oxide reductase
norZ	gene encoding the quinol dehydrogenase-subunit nitric oxide reductase
Nos	nitrous oxide reductase
nosZ	gene encoding the nitrous oxide reductase
NPK	mineral nitrogen-phosphate-potassium fertilizer
N_t	total nitrogen
PCR	polymerase chain reaction
PETG	polyethylenterephthalat
pMMO	particulate form of the methane monooxygenase
precip.	precipitation
qNor	quinol dehydrogenase-subunit nitric oxide reductase
RFLP	restriction fragment length polymorphism
RS	raw (cattle) slurry
SDS	sodium dodecyl sulfate
SIR	substrate-induced respiration
sMMO	soluble form of the methane monooxygenase
SNK	Student-Newman-Keuls test
SSCP	single strand conformation polymorphism
TB	trockener Boden (dry soil)
TBE	Tris-borate-ethylendiamin-tetraacetate buffer
TCD	temperature conductivity detector
TEMED	N, N, N', N' – tetramethylethylenediamine
temp.	temperature
TGGE	temperature gradient gel electrophoresis
T-RFLP	terminal restriction fragment length polymorphism
Tris-EDTA	Tris-ethylendiamin-tetraacetate buffer
UPGMA	unweighted pair group method using arithmetic averages
WFPS	water-filled pore space
WHC	water-holding capacity

Manuring treatments in the cropping system *without livestock*:

w/o L-FC	fermented crops
w/o L-FC+FE	fermented crops + fermented external substrates
w/o L-M	mulching practice

Manuring treatments in the cropping system *with livestock*:

wL-FS	fermented slurry
wL-FS+FC	fermented slurry + fermented crops
wL-FS+FC+FE	fermented slurry + fermented crops + fermented external substrates
wL-FYM	farmyard manure
wL-RS	raw slurry

1 Introduction

1.1 Organic Agriculture

The interest in organic farming and the demand for organically produced food has grown considerably over the last years. In Germany, the organically managed arable land area and the number of organic farms increased continuously up to 811,724 ha corresponding to 4.78% of the total agricultural area and up to 16,791 organic farms corresponding to 3.99% of German farms in 2005, respectively (www.organic-europe.net/europe_eu/statistics). The transaction volume of organic food in Germany steadily rose up to 3.5 billion Euro in 2004 (www.soel.de/oekolandbau/deutschland_ueber.html). In 1991 the European Community released a directive on organic farming (EU regulation 2092/91) to assure comparable production standards in the member states, whereas in Germany already in 1984 common basic standards ("Rahmenrichtlinien") had been developed. The common German seal "Ökoprüfzeichen" for organic products was launched in 1999 which was replaced two years later by the state organic seal "Biosiegel".

Guiding principals of organic agriculture are sustainable cultivation and animal husbandry preserving and enhancing soil fertility, achieving, if possible, a closed nutrient cycle on the farm, and keeping animals in a manner conducive to their welfare. Limited and strictly land-related stocking densities, feeding of farm-grown fodder, and avoidance of antibiotics are features of organic livestock husbandry. Organic farming disallows the use of synthetic fertilizers, pesticides, and growth regulators and instead relies on organic fertilizers, green manuring, biological pest and mechanical weed control in crop cultivation. Wide crop rotations and cultivation of N_2-fixing legumes, intercrops, and green manures characterize organic cropping systems indicating the dependence and importance of biological N_2 fixation, soil management, and cultivation techniques.

Key concerns in organic agriculture such as "sustainability", "soil quality", and "soil fertility" are frequently used terms, however, the finding of appropriate definitions and even more the determination of measurable parameters describing those key words is difficult and problematic. Maintenance of high productivity, sufficient food and fibre production, soil conservation, economic viability, and environmental responsibility seem to be important issues of sustainability (Lal 1994; Kirchmann and Thorvaldsson 2000). Soil quality has been described according to its function of biomass production, capacity to filter, buffer, and transform organic matter, genetic reserve and biological habitat for plants, as well as physical medium for technical and industrial structures, source of raw materials, and cultural heritage (Blum and Santelises 1994). Soil biota are recognized to play an important role in the maintenance of soil fertility and productivity-driving processes like mineralization of organic material, nutrient cycling, availability, and retention, and stabilization of soil aggregates (Insam and Rangger 1997; Wardle *et al.* 1999; Coleman *et al.* 2004). Watson *et al.* (2002b) emphasized crop rotation and management of manures and crop residues as central tools

for maintaining and devolping soil fertility in organic farming systems. Stockdale *et al.* (2002) concluded that the underlying processes supporting soil fertility are not different in organically compared to conventionally managed soils, although the nutrient management differs fundamentally.

Diverse biological soil characteristics like general enzyme activities (FADase, alkaline phosphatase, urease, dehydrogenase, catalase), soil respiration, substrate-induced respiration, microbial biomass C and N content, fungal abundance, arbuscular mycorrhiza, microbial metabolic potential, earthworm population and biomass, and bacterial and archaeal diversity have been investigated in organic farming sytems, partly in comparison to conventional agriculture, entailing wide ranges of results (Fließbach and Mäder 1997; Lundquist *et al.* 1999; Carpenter-Boggs *et al.* 2000; Fließbach and Mäder 2000; Mäder *et al.* 2002; Cardelli *et al.* 2004; Elmholt and Labouriau 2005; Gosling *et al.* 2006; Tu *et al.* 2006b; van Diepeningen *et al.* 2006). Higher biological activities and higher diversities were partly but not necessarily observed in organically managed soils. Shepherd *et al.* (2002) argued that it is not the organic farming system *per se* which is important in promoting better soil structure and higher soil organic matter fraction, but the amount and quality of organic matter returned to a soil. Friedel and Gabel (2001) found significantly elevated soil microbial biomass C and N contents after 41 but not after nine years of organic farming practice in comparison to three years of organic cultivation. Assuming 5 - 6 year crop rotations in organic agriculture, the authors supposed that at least two rotation cycles might be necessary to detect increased amounts of microbial biomass C and N.

The input of mineral fertilizers is not allowed in organic agriculture to meet the plant nitrogen requirement which is a challenge and a crucial role for organic crop production. Nitrogen is frequently considered to be one of the key limiting factors responsible for the limited productivity of organic systems (Eltun 1996; Berensten *et al.* 1998; Thorstensson 1998). Crops under organic management are almost exclusively dependent on soil biological processes which provide nutrients by mineralization of applied organic matter like animal manure, crop residues, or green manuring. Notably in organic farming systems without livestock in which no animal excreta emerge for manuring, cultivation of legumes play a key role due to atmospheric N_2 fixation and hence to import nitrogen into the production system (Watson *et al.* 2002a). However, the nutrient management in those cropping systems is difficult because no additional mobile fertilizer pool like farmyard manure is available. Leguminous intercrops and green manures like lucerne and clover are not necessarily mulched and incorporated to the soil and subsequently decomposed when the nutrient demand of the following crops occurs, resulting in temporal discrepancies between N demand and N supply. Berry *et al.* (2002) questioned whether the productivity in organic systems is restricted by the supply of available nitrogen. They reviewed published results and provided evidence that organic farming systems do have the potential to supply adequate amounts of available N to meet the crop demand. Moreover, even positive N balances for organic farms were often

observed (Watson *et al.* 2002b). However, the level of available N is seldom achieved in practice mainly due to a non-sychronized timing of mineralization with the crop demand as mentioned before.

The asynchronous dynamics of N availability and plant demand in organic crop production in general as well as the lack of a mobile fertilizer pool in organic farming systems without livestock may be compensated through the use of a biogas plant within the production process. Biomass of intercrops and green manures, but also growth of fallow land and crops residues could be taken off the field, fermented in the biogas plant, stored, and then applied as fertilizer when the nutrient demand of the crops occurs. Consequently, a demand-compatible temporal shift of nutrient input to the soil is achieved that enhances the plant nitrogen supply and contemporaneously reduces nitrogen losses from the system. Principles and operation modes of anaerobic fermentation in biogas plants as well as properties of fermented organic fertilizers are summarized in section 1.2.

1.2 Anaerobic Fermentation in Biogas Plants

The use of biogas plants for anaerobic fermentation of agricultural residues is mainly implemented to produce energy entailing an improved manure quality only as "by-product". In Europe, the produced biogas, a mixture of primarily methane (CH_4) and carbon dioxide (CO_2), a renewable energy source, is used for electricity generation in combined heat and power plants (Amon *et al.* 2006; Clemens *et al.* 2006). In Germany, animal slurries and especially cultivated energy crops such as silage maize and cereals are mainly used for biogas production (Eder and Schulz 2006). However, the advantageous use of agricultural organic residues, which do not entail up to now a direct benefit, might be possible. Crop biomass derived from green manures, intercrops, fallow land, crop residues, and forage left-overs might be suitable additional substrates for fermentation. Notably in organic cropping systems without livestock in which no mobile fertilizer is available, the utilization of biogas plants could be interesting. The fermentation of legume-grass mixes, a fundamental component in those crop rotations due to N_2 fixation but without direct use, would create a mobile fertilizer and might reduce partly high volatile nitrogen losses caused by the common mulching practice (Heuwinkel *et al.* 2005).

Various types of biogas plants are in use which can be grouped 1. according to the way of processing (i.e. fully mixed or plug flow digesters which is linked with the fermenter form), 2. according to the operation arrangement (i.e. one, two, or multiple stage digesters with separated processes of fermentation and methanogenesis), 3. according to the mode of feeding (i.e. continuous or batch (mostly applied in "dry fermentation systems" with dry matter contents of ~20 - 40%) operation), or 4. according to the temperature level (mesophilically (~30 - 35°C) or thermophilically (~50 - 55°C) operated) (Eder and Schulz 2006).

During fermentation, complex organic compounds are degraded in a series of microbial metabolic pathways by various *bacteria* (hydrolytic, acidogenic, and acetogenic bacteria) and methanogenic *archaea* mainly to CH_4 and CO_2. The initial step of hydrolyzing the organic polymers (polysaccharides, lipids, and proteins) entails monomers like pentoses and hexoses, free organic acids and other simple compounds such as glycerol, and various amino acids. Those monomers are subsequently metabolized directly or in part via organic acids, volatile fatty acid, or alcohols to acetate (also partly in formate and methanol), hydrogen (H_2), and carbon dioxide (CO_2). During methanogenesis, CH_4 is generated by methanogenic *archaea* either via the acetoclastic (using acetate) or the hydrogenotrophic pathway (using CO_2 and H_2).

Anaerobic digestion yields fermented materials with altered characteristics compared to the respective "raw material". Fermentation of both, slurry and crop biomass results in digested products exhibiting an increased NH_4^+-N content, a smaller ratio of NH_4^+-N/total N, a decreased organic dry matter and total carbon content, a reduced biological oxygen demand (BOD), and a smaller C/N ratio. No alterations arise with respect to the total nitrogen, potassium, and phosphate content. Fermented slurries reveal additionally an elevated pH value, a decreased dry matter content, and a reduced viscosity in comparison to raw slurries (Field *et al.* 1984; Asmus *et al.* 1988; Kirchmann and Witter 1992; Wulf *et al.* 2001). Notably the elevated NH_4^+ concentration in the fermented material indicates its suitability as a plant-available N fertilizer. Due to the decomposition mainly of the easily degradable C compounds during anaerobic fermentation, the digested products are more recalcitrant and therefore might reduce the rate of microbial degradation and oxygen consumption in the soil. Consequently, less anoxic microsites, favorable for denitrifying activities entailing N_2O and N_2 losses, might emerge.

1.3 Trace Gas Fluxes in Agriculture

Nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) are so-called "greenhouse gases", which are present in trace amounts in the atmosphere ("trace gases"). They are important to atmospheric chemistry and earth´s radiative balance. They absorb infrared radiation in the troposphere, thus contributing to the greenhouse effect and global warming. The trace gases are responsible for the "natural greenhouse effect" which causes the global average temperature of 15°C. Without the natural greenhouse effect the temperature on earth would be ~30°C lower, and the earth would be uninhabitable. While the trace gas concentrations in the atmosphere remained relatively constant in pre-industrial times over hundreds of years, concentrations increased between 1750 and 2000 from 270 to 316 ppb N_2O, from 700 to 1750 ppb CH_4, and from 280 to 368 ppm CO_2 corresponding to increments of 17%, 151%, and 31%, respectively (IPCC 2001). The atmospheric lifetimes of N_2O and CH_4 are ~114 and ~12 years, whereas no single lifetime of CO_2 can be defined because of the

different uptake rates by different removal processes resulting in a range of 5 - 200 years. On a per-weight basis and a 100-year time frame, N_2O is about 296 and CH_4 about 23 times more effective at trapping infrared radiation than CO_2 (global warming potential) (IPCC 2001). N_2O is also involved in the depletion of the ozone (O_3) layer in the stratosphere, which protects the biosphere from the harmful effects of ultraviolett radiation (Crutzen 1981).

In 1997, the first step towards a stabilization of the world´s climate was taken with the meeting of more than 160 nations in Kyoto, Japan, to negotiate policies related to greenhouse gas emissions. The nations consented to limit their greenhouse gas emissions, relative to the 1990 emissions. The EU-15 agreed to reduce total greenhouse gas emissions by 8% until 2008 - 2012. With the agreement of the EU-15 member states in 2002 on different emission reduction or limitation targets within the EU, the reduction target for Germany was forced up to 21%. By 2003 the reduction obligation was already mostly fulfilled with a decrease of 18.5% (Nationaler Inventarbericht Deutschland 2005, http://www.bmu.de/klimaschutz/klimaschutzberichterstattung/doc/35575.php).

The agricultural sector is the largest source for N_2O and CH_4 emissions in Germany. Although the agricultural N_2O and CH_4 emissions decreased between 1990 and 2003 by 20%, the contribution of agriculture to the total German greenhouse gas emissions (measured in CO_2 equivalents) remained at 8 - 9%. The percentage of the agriculture-derived N_2O and CH_4 emissions amounted in 2003 to 62.3% and 63.3% of the total German N_2O and CH_4 emissions, respectively. Agriculture´s main emission sources are N_2O from soils and manure management and CH_4 from enteric fermentation and manure management (Umweltdaten Deutschland Online, www.env-it.de/umweltdaten).

Since nitrogen input in organic agriculture is generally restricted and particularly in organic cropping systems without livestock, the N supply is often difficult, nutrient losses from the agricultural production systems, including trace gas emissions, should be minimized. Arable soils as site for N_2O emissions and CH_4 fluxes as well as the respective microbiologically driven processes are introduced in sections 1.4 and 1.5.

1.4 Nitrous Oxide Emissions from Arable Soils

The soil nitrogen cycle (figure 1.1) is a very complex system consisting of various simultaneously ocurring processes like N_2 fixation, N mineralization, N immobilization, nitrification, and denitrification, which import nitrogen, transform nitrogen components or lead to nitrogen losses from soil. Several metabolic pathways like denitrification, nitrification, nitrifier denitrification, chemodenitrification, and dissimilatory nitrate reduction to ammonium are able to generate N_2O either as end product, intermediate product, or side product (overview given e.g. by Bremner (1997) and Stevens and Laughlin (1998)). Depending on many factors such as available nitrogen and carbon, soil texture, pH value, temperature, water-filled pore space, and oxygen concentration, the different metabolic pathways

contribute variously to *in-situ* N_2O effluxes. Microbial denitrification and nitrification are considered to be the two major N_2O-producing processes in soil.

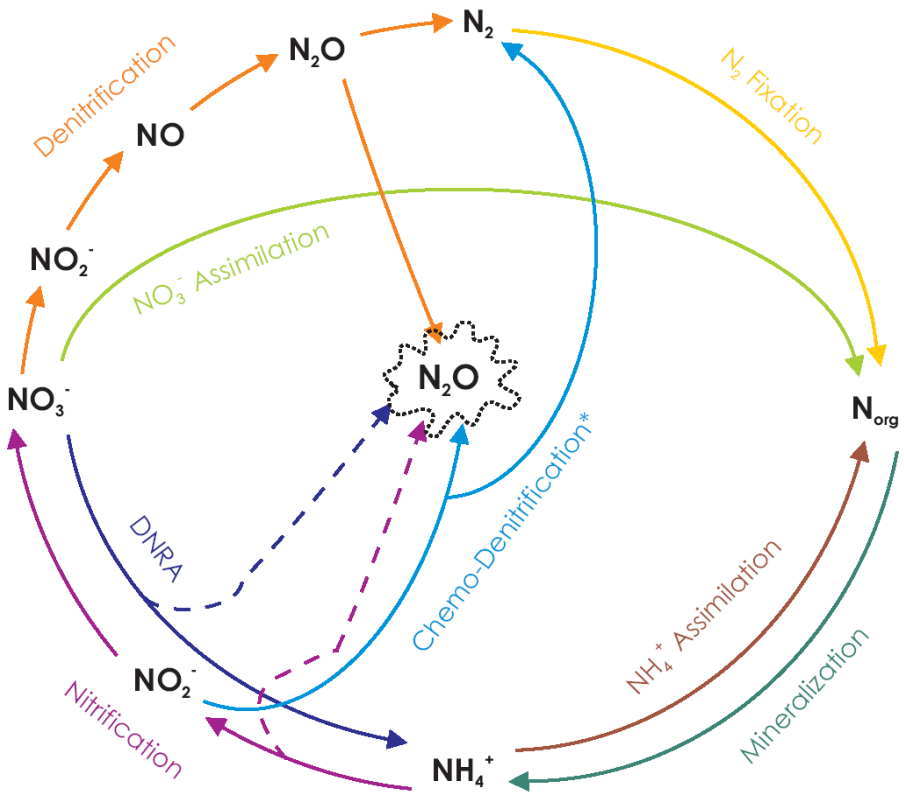


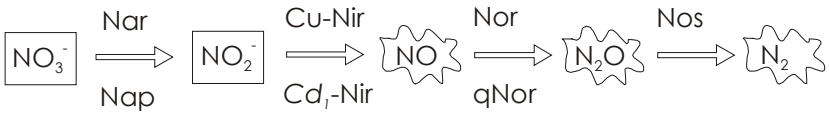
Figure 1.1: Nitrogen transformation processes within the nitrogen cycle. Chemo-Denitrification*: collective term for several non-biological decomposition processes.

1.4.1 Denitrification

Denitrification is a major process contributing to N_2O emissions (Firestone and Davidson 1989). Nitrate reduction to nitrogen gas in prokaryotes is mediated mostly by *Bacteria*, but also a few *Archaea* and some fungi are capable to denitrify. With very few exceptions, denitrifiers can also use oxygen as terminal electron acceptor, which is typically preferred if both terminal oxidants are available. Denitrifiers are ubiquitous in soils but are also present in environments such as sediments, aquatic and marine habitats, and wastewater treatment systems. Generally, denitrification activities increase with elevated organic carbon and nitrate supply, water-filled pore space (WFPS), pH value, and temperature (Tate III 1995). The mole fraction of N_2O in comparison to N_2 as denitrification end product increases with elevated nitrate concentration but decreases with elevated organic carbon content, WFPS, pH value, and temperature (see also reviews by Ferguson (1994), Granli and Bøckmann (1994), Stevens and Laughlin (1998), and Barton *et al.* (1999)).

Complete denitrification is a multi-step process requiring separate enzymes to transform nitrate via nitrite, nitric oxide, and nitrous oxide to dinitrogen. Each redox couple of the

reaction sequence has a more positive redox potential so that every step in denitrification is coupled to energy conservation. The conversion of nitrite to nitric oxide is considered to be the crucial step in the reaction sequence since it causes on the one hand the first gaseous product, and on the other hand it distinguishes denitrifiers from nitrate respirers.



after Philippot and Hallin (2005)

In the following, the different denitrifying enzymes in *Bacteria* responsible for the stepwise nitrogen reduction are introduced. Overviews about the denitrification enzymology are given by Knowles (1982), Ferguson (1994), Zumft (1997), Ferguson (1998), and Shapleigh (2000). Currently, it is assumed that all denitrification enzymes are encoded by single copy genes except *narG* whose copy number varies between one and three per genome (Philippot and Hallin 2005). Most of the denitrifying steps are catalyzed by two different, but functionally equivalent enzymes. The respiratory reduction of NO_3^- to NO_2^- is carried out by two types of dissimilatory nitrate reductases which differ in their location: a membrane-bound (Nar) and a periplasmic (Nap) nitrate reductase. The membrane-bound nitrate reductase is composed of three subunits (alpha, beta, and gamma) which are encoded by the *narG*, *narH*, and *narI* genes, whereas the periplasmic reductase is a heterodimer encoded by the *napA* and *napB* genes. Many microorganisms contain both nitrate reductases (Philippot and Højberg 1999; Richardson *et al.* 2001).

The reduction of NO_2^- to NO is catalyzed by evolutionary unrelated enzymes that are entirely different in terms of structure and the prosthetic metal: the copper- and the cytochrome cd_1-nitrite reductase (Cu-Nir and Cd_1-Nir), which are both located in the periplasm and are encoded by the *nirK* and *nirS* genes, respectively. The two genes seem to occur mutually exclusively in a given strain, but both types have been found in different strains of the same species. Presumably, *nirS* is more widely distributed, while *nirK* might emerge in a greater variety of physiological groups from different habitats (Coyne *et al.* 1989). Previously, *nirS* denitrifiers were supposed to be predominant in marine ecosystems, whereas *nirK* denitrifiers were preferentially found in soils (Braker *et al.* 2000; Avrahami *et al.* 2002). However, Throbäck *et al.* (2004) demonstrated that *nirS* denitrifiers are also common in many different soils harboring a substantial diversity of *nirS* denitrifying bacteria.

The nitric oxide reductase mediates the reduction of NO to N_2O, which represents an unusual reaction in biology, the formation of an N-N bond. The enzyme (Nor) has been described in denitrifying bacteria as complex of a cytochrome *b* and cytochrome *c* subunit being located in the membrane encoded by the *norB* and *norC* genes. Another nitric oxide reductase lacking the cytochrome *c* subunit was purified from the denitrifier *Ralstonia*

eutropha that exhibited a quinol dehydrogenase (qNor) instead. The gene encoding for it was termed *norZ* (Cramm *et al.* 1997). The majority of known denitrifying bacteria seemingly harbors *norB* genes, but also denitrifiers carrying the *norZ* gene exist (Braker and Tiedje 2003). Moreover, a variety of non-denitrifying strains were also found possessing qNor, suggesting an NO-detoxifying function.

The last step of the denitrifying process, the reduction of N_2O to N_2, is catalyzed by the nitrous oxide reductase. In contrast to the other denitrification steps, nitrous oxide reduction is mediated by only one enzyme (Nos) encoded by the *nosZ* gene. It is a periplasmic multi-copper enzyme composed of two identical subuntis. Just as nitrate respiration is not coupled obligatorily to denitrification, bacteria are known that respire N_2O without being denitrifiers, e.g. those, which reduce nitrate via nitrite to ammonia and nitrous oxide to dinitrogen (Yoshinari 1980; McEwan *et al.* 1985; Zumft 1997). Nitrous oxide accumulates during denitrification when nitrate reductase, nitrite reductase, and nitric oxide reductase are more active than nitrous oxide reductase (Betlach and Tiedje 1981). The latter seems to be more sensitive to oxygen than the other three enzymes, low carbon/nitrate ratio, and low pH value – the primary environmental regulators of these enzymes´ synthesis and activity (Firestone and Davidson 1989; Bouwman 1990).

1.4.2 Nitrification

Nitrification is also an important N_2O-producing process in soil. Chemolithoautotrophic nitrification, the aerobic oxidation of ammonia to nitrate via nitrite, involves two different groups of bacteria. Ammonia-oxidizing bacteria (AOB) oxidize ammonia via hydroxylamine to nitrite (nitritation) catalyzed by the enzymes ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO), respectively. The produced nitrite is further oxidized in a one-step reaction to nitrate (nitratation) by nitrite-oxidizing bacteria (NOB) using the nitrite oxidoreductase (NOR).

$$NH_3 + O_2 + 2\,H^+ + 2\,e^- \xrightarrow{AMO} NH_2OH + H_2O \xrightarrow{HAO} NO_2^- + 5\,H^+ + 4\,e^-$$

$$NO_2^- + 0.5\,O_2 \xrightarrow{NOR} NO_3^-$$

The sole energy source of nitrifiers for CO_2 fixation originates from NH_3 and NO_2^- oxidation. However, the substrates are not very effective in energy yield, hence explaining the slow growth of nitrifying organisms. Beside ammonia, a variety of other substrates such as methane, carbon monoxide, and methanol can be oxidized by the ammonia monooxygenase (AMO) (summarized by Bédard and Knowles (1989)). The multiplicity of oxidations carried out by ammonia oxidizers reflects the low substrate specificity of AMO

comparable to the low substrate specificity of the methane monooxygenase (MMO) in methanotrophic bacteria (see section 1.5.1).

N_2O might be formed as side product during NH_3 oxidation through chemical decomposition of NH_2OH, an intermediate between NH_4^+ and NO_2^-, or of NO_2^- itself (Chalk and Smith 1983). Likewise, incomplete oxidation of NH_2OH can also lead to N_2O emissions (Hooper and Terry 1979). Nitrifying bacteria may contribute substantially to N_2O emissions from soils, and many investigators have identified nitrification as an important source of N_2O in soil systems (Breitenbeck and Bremner 1986; Klemedtsson *et al.* 1988b; a; Davidson 1992).

Ammonia-oxidizing bacteria (AOB) are widely distributed in soils, but also in fresh water, seawater, wastewater treatment systems, rocks, and masonry, and require only CO_2, O_2, and NH_4^+ to proliferate. The limiting factor for nitrification in most soils is the availability of NH_4^+ (Hutchinson and Davidson 1993). In addition, low pH value, low water potential, low soil temperature, low organic matter content, and NO_2^- toxicity can limit nitrifying activities (Bremner and Blackmer 1980; 1981; Sahrawat and Keeney 1986).

Known ammonia oxidizers that exist in pure culture comprise two monophyletic groups based on 16S rRNA sequence analysis belonging to the *gamma-Proteobacteria* with *Nitrosococcus oceani* and *Nitrosococcus halophilus* as known species and to the *beta-Proteobacteria* including the genera *Nitrosomonas*, *Nitrosovibrio*, *Nitrosolobus*, and *Nitrosospira* (Purkhold *et al.* 2000; Bock and Wagner 2001). Nitrifiers in soils have been studied by targeting beside the 16S rRNA genes the gene encoding for the ammonia monooxygenase, mainly *amoA*, a subunit that carries the active site of the enzyme (Rotthauwe *et al.* 1995). For environmental investigations *amoA* serves as useful target because it reflects the phylogeny of AOB (Rotthauwe *et al.* 1997; Purkhold *et al.* 2000). According to 16S rRNA sequence analysis, nitrite-oxidizing bacteria (NOB) form the four genera *Nitrobacter*, *Nitrococcus*, and *Nitrospina,* which are assigned to the alpha, gamma, and delta subclass of the *Proteobacteria*, respectively, and *Nitrospira*, the name-giving genus of an independent bacterial phylum (Bock and Wagner 2001).

Recently, Könneke *et al.* (2005) succeeded in the isolation of a marine archaeon that grows chemolithoautotrophically by aerobically oxidizing ammonia to nitrite, hence the first observation of nitrification in the domain of *Archaea*. The presence of putative archaeal AMO encoding genes in marine and soil *Archaea* implies a wide distribution of nitrifying physiology in these organisms. Leininger *et al.* (2006) evidenced in 12 pristine and arable soils of three climatic zones higher abundances of archaeal than bacterial ammonium oxidizers, indicating that (cren-)archaeota may be the most abundant ammonia oxidizing organisms in soil ecosystems on earth.

In addition to autotrophs, heterotrophically living fungi and bacteria can oxidize ammonia or organic N compounds to hydroxylamine, nitrite, and nitrate. In contrast to N oxidation as only energy yielding process in autotrophic nitrifiers, nitrification in heterotrophic organisms is not coupled to energy conservation. Heterotrophic nitrification is considered to be more

common among fungi than bacteria playing an important role in low-pH soils, e.g. in acid forest soils, but not in agricultural and grassland soils (Killham 1990; Landi *et al.* 1993). However, Müller *et al.* (2004a) revealed heterotrophic nitrification activities also in a temperate grassland soil with pH 6.2. Features of autotrophic and heterotrophic nitrification are summarized and reviewed e.g. by Granli and Bøckmann (1994), Bremner (1997), Bothe *et al.* (2000), Bock and Wagner (2001), Kowalchuk and Stephen (2001), and Wrage *et al.* (2001).

1.5 Methane Fluxes in Arable Soils

Net CH_4 fluxes in soil are determined by the relative activites of CH_4 production and CH_4 consumption within the profile. Soils contain both, methane-producing and methane-consuming soil microorganisms that may be active simultaneously in arable terrestrial ecosystems. Harriss *et al.* (1982) performed methane measurements in a swamp soil, which acted as CH_4 source during waterlogged conditions and as CH_4 sink during drought conditions. Singh *et al.* (1996) observed in a rice/wheat agroecosystem during cultivation of rice CH_4 emissions and in the subsequent wheat and fallow period CH_4 consumption. Generally, agricultural soils are recognized as important sinks for atmospheric CH_4. Based on published data, Le Mer and Roger (2001) calculated median CH_4 oxidation rates of 5.5 g CH_4-C ha^{-1} d^{-1} for cultivated soils, 6.5 g CH_4-C ha^{-1} d^{-1} for grassland soils, 8.3 g CH_4-C ha^{-1} d^{-1} for non-cultivated upland soils, and 9.9 g CH_4-C ha^{-1} d^{-1} for forest soils. Hütsch (2001a) reviewed CH_4 consumption rates mainly under temperate climatic conditions and determined mean uptake rates of 0.28, 0.52, and 1.51 mg CH_4 m^{-2} d^{-1} from arable, grassland, and forest soils, respectively. Various land uses and agricultural practices such as tillage and fertilization are the main factors that influence the soil CH_4 oxidation activity. The large differences between minimum and maximum values of CH_4 consumption in both studies indicate a high temporal and spatial variability of CH_4 fluxes.

1.5.1 Methane Oxidation

Methanotrophic bacteria are responsible for CH_4 consumption under oxic conditions, which are unique in their ability to use CH_4 as sole carbon and energy source. By using molecular ecology techniques, it has become evident that methanotrophs are ubiquitous in nature and well adapted to high or low temperature, pH value, and salinity (Trotsenko and Khmelenina 2002). These bacteria are classified into two groups in dependence of morphological, physiological, biochemical, and phylogenetic characteristics. Type I methanotrophs belong to the gamma-subdivision of the *Proteobacteria* and use the ribulose monophosphate (RuMP) pathway for carbon incorporation into the cellular biomass. Type X methanotrophs may be partly distinguished from Type I organisms because they follow the RuMP and the serine pathway. Type II methanotrophs belong to the alpha-subdivision of the *Proteobacteria* and use the serine pathway for carbon assimilation. The key enzyme catalyzing the initial

oxidation of methane is the methane monooxygenase (MMO), which occurs in two different forms: a membrane-bound particulate form (pMMO) in all types of methanotrophs, and a cytoplasmatic or soluble form (sMMO) in Type II methanotrophs and only in *Methylococcus* of Type X methanotrophs that is expressed preferentially under copper-deficient conditions (summarized by Higgins *et al.* (1981), Conrad (1995; 1996), Hanson and Hanson (1996), and Lidstom (2001)). Although these enzymes exhibit similar function, they do not have any genetic or structural identity. Bender and Conrad (1992; 1995) revealed that there might be two types of CH_4 oxidizers. One population having a low affinity for CH_4 (K_m (apparent half saturation constant) in the range of 1000 nM CH_4), and another population having a high affinity for CH_4 (K_m in the range of 30 - 60 nM CH_4). In upland soils typically high affinity methanotrophs occur that consume atmospheric CH_4, whereas all methanotrophs in culture show low affinity for methane.

Beside methane-oxidizing bacteria, also ammonia (NH_3)-oxidizing bacteria are able to oxidize CH_4 (Hyman and Wood 1983; Jones and Morita 1983). The reaction is catalyzed by the ammonia monooxygenase (AMO), an enzyme that is very similar to MMO (Hanson and Hanson 1996). The oxidation processes of CH_4 and NH_3 catalyzed by the monooxygenases are:

$$CH_4 + O_2 + 2\,H^+ + 2\,e^- \xrightarrow{MMO} CH_3OH + H_2O$$

$$NH_3 + O_2 + 2\,H^+ + 2\,e^- \xrightarrow{AMO} NH_2OH + H_2O$$

Similarly, methanotrophs can also oxidize ammonia. The multiplicity of oxidation by methanotrophs and ammonia oxidizers reflects the lack of substrate specifity of both, the methane and the ammonia monooxygenase, respectively, resulting in a fortuitous metabolism of a large number of compounds (Dalton 1977; Bédard and Knowles 1989; King and Schnell 1994). However, the specific rates of ammonia oxidation by methanotrophs were two orders of magnitude lower than those of the nitrifiers (Bédard and Knowles 1989). Inhibition of CH_4 oxidation by NH_3 is of importance for the ecology of methanotrophic bacteria in arable, grassland, and forest soils. The most substantiated explanation for inhibition of CH_4 consumption due to ammonia is the competitive inhibition at the enzyme level (Dunfield and Knowles 1995; Gulledge and Schimel 1998). In addition, production of toxic nitrite from NH_3 oxidation and the depletion of the reducing equivalent pool has been discussed (King and Schnell 1994).

1.5.2 Methane Production

Non-water-saturated, generally oxic soils have the potential to produce CH_4 (Sexstone and Mains 1990; Koschorreck and Conrad 1993). Likewise, *in-situ* CH_4 emissions were observed in

temporarily wet or water-saturated upland soils (Steudler *et al.* 1989; Castro *et al.* 1995; MacDonald *et al.* 1996; Kamp *et al.* 2001; Merino *et al.* 2004). Since oxic and anoxic zones may co-exist in soil ecosystems, CH_4 production may also occur for short term in oxic soils where microniches provide oxygen-free conditions for methanogenesis (hot-spots). Production of CH_4 is performed under strict anoxybiosis by methanogenic *archaea* that belong to five different orders within the Euryarchaeota (Whitman *et al.* 2001; Hedderich and Whitman 2005). Methanogens occur ubiquitously and have a limited trophic spectrum comprised of a small number of simple substrates like H_2 and CO_2, acetate, formate, simple methylated compounds, and primary and secondary alcohols (Le Mer and Roger 2001). However, the two major pathways of CH_4 production in methanogenic ecosystems in nature seem to be acetoclastic and CO_2 reduction by H_2 (e.g. Chin and Conrad (1995).

$$CH_3COOH \Longrightarrow CH_4 + CO_2$$

$$CO_2 + 4\ H_2 \Longrightarrow CH_4 + 2\ H_2O$$

Methanogenic *archaea*, their phylogenetic and ecological diversity, and their function in soils have been reviewed by Conrad (1995; 1996) and Garcia *et al*. (2000).

1.6 Objectives and Setting of the Study

The objective of the present study was the comparative quantification of *in-situ* trace gas fluxes, primarily N_2O and CH_4, in organic cropping systems with and without livestock husbandry, respectively. Little is known about trace gas fluxes in organic agriculture in general and in particular when anaerobically fermented manures are used for fertilization. Possibly, trace gas fluxes would vary in various crops in dependence of the fertilizer regimes differing in intercrop and green manure management and the fermentation of respective organic materials such as cattle slurry and/or herbal organic matter. Therefore, different crops and several manuring treatments with fermented or non-fermented organic manures were selected within both organic cropping systems to investigate soil-derived trace gas fluxes. The same crops and manuring treatments were monitored during three years because N_2O production and CH_4 oxidation are affected by the amount and distribution of precipitation in conjunction with temporal temperature dynamics.

Since denitrification is an important, N_2O-producing, microbiological pathway, accompanying studies in the greenhouse and in the laboratory should contribute to clarify the impact of the different fermented and non-fermented organic manures on the soil bacterial denitrifying community composition and activity as well as on various microbiological soil parameters after fertilization.

Finally, after more than three years of different manuring management, field soil samples should be comparatively investigated regarding different biological and chemical characteristics related to the nitrogen and carbon metabolism to reveal possible effects and differences between the manuring treatments.

The present study was conducted within the framework of a collaborative project between the Professorship for Organic Farming, University of Giessen, the Institute for Energy and Environment, Leipzig, and the Institute for Applied Microbiology, University of Giessen, that was supported by a research grant from the Deutsche Bundesstiftung Umwelt (DBU), Osnabrück, Germany. Two organic cropping systems, with and without livestock husbandry, respectively, were performed operating different manuring systems. In some manuring treatments, cattle slurry and/or herbal biomass were fermented in a biogas plant, which then were used for fertilization in contrast to the common organic manuring practices in the respective cropping systems. Two Ph.D. students of the Giessen Organic Farming group mainly investigated classic cultivation parameters within the crop rotations like grain yield, nutrient contents, biomass of green manures, amount of straw, etc., whereas the Ph.D. student in Leipzig carried out an ecological and economical evaluation of organic model farms with or without the operation of biogas plants to demonstrate the operating profitability of biogas plants in organic agriculture under various determining conditions.

2 Material and Methods

2.1 Experimental Site

Two field trials conducted in organic agriculture as cropping system with and without livestock formed the basis of the experimental setup. Those field trials were conducted at the research station for organic farming "Gladbacherhof" of the Giessen University, 17 km east of Limburg in Hesse in the "Lahn" valley. The research station is located 140 - 230 m above sea level at the edge of the "Limburger Becken" in the foothills of the low mountain range "Taunus". There is a high variability of soil types, however most of the soils have silty loam texture derived from loess with pH values of 6.6 - 7.0 and are classified as Calcic Luvisols (Schmidtke 1997). Long-term annual temperature and annual precipitation average 9.3°C and 680 mm, respectively. Figure 2.1 reveals long-term means of monthly precipitation and air temperature as well as monthly precipitation and air temperature during the whole measurement period. Daily data of precipitation and air temperatures are shown in figure 2.2 according to the three measurement seasons. Further illustrations concerning data of daily precipitation, air temperature, and soil temperature in 5 cm and 20 cm depth are shown in Appendix A.1 - A.3. The field study started in 2001 and was conducted in plots of 6 x 12 m size comprising four replicates each.

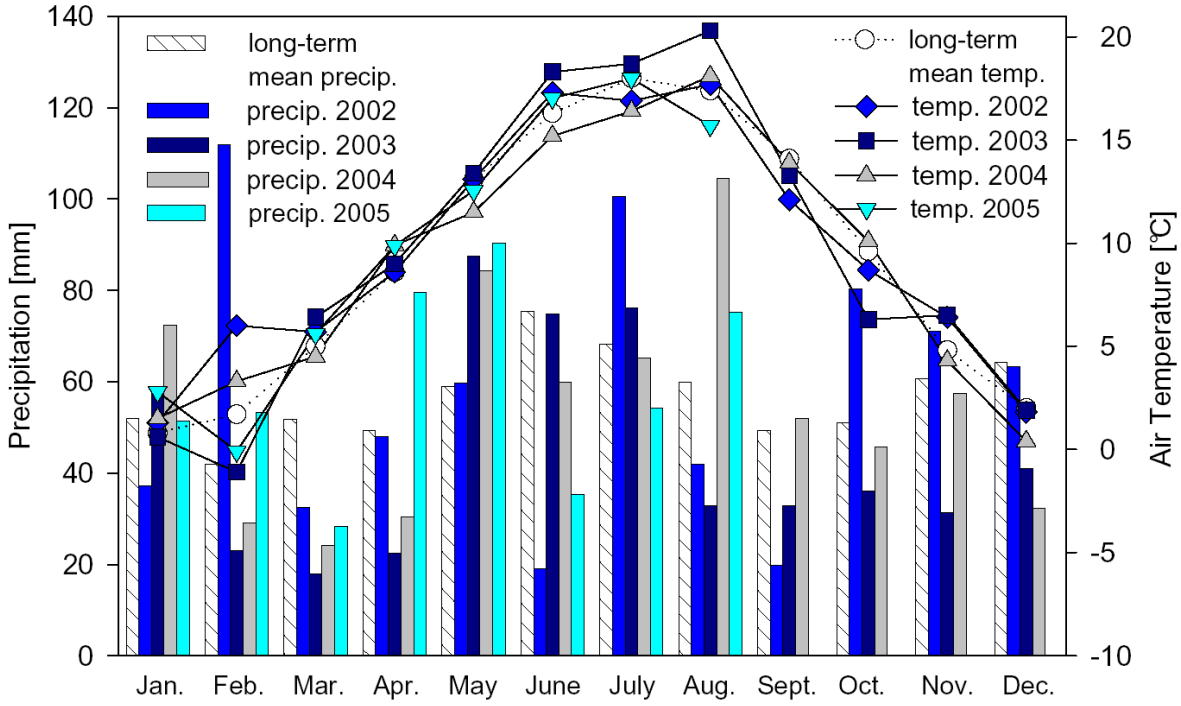


Figure 2.1: Long-term means of monthly precipitation and mean air temperature as well as monthly precipitation and mean air temperature during the measurement period. (Data: Franz Schulz, Villmar, personal communication)

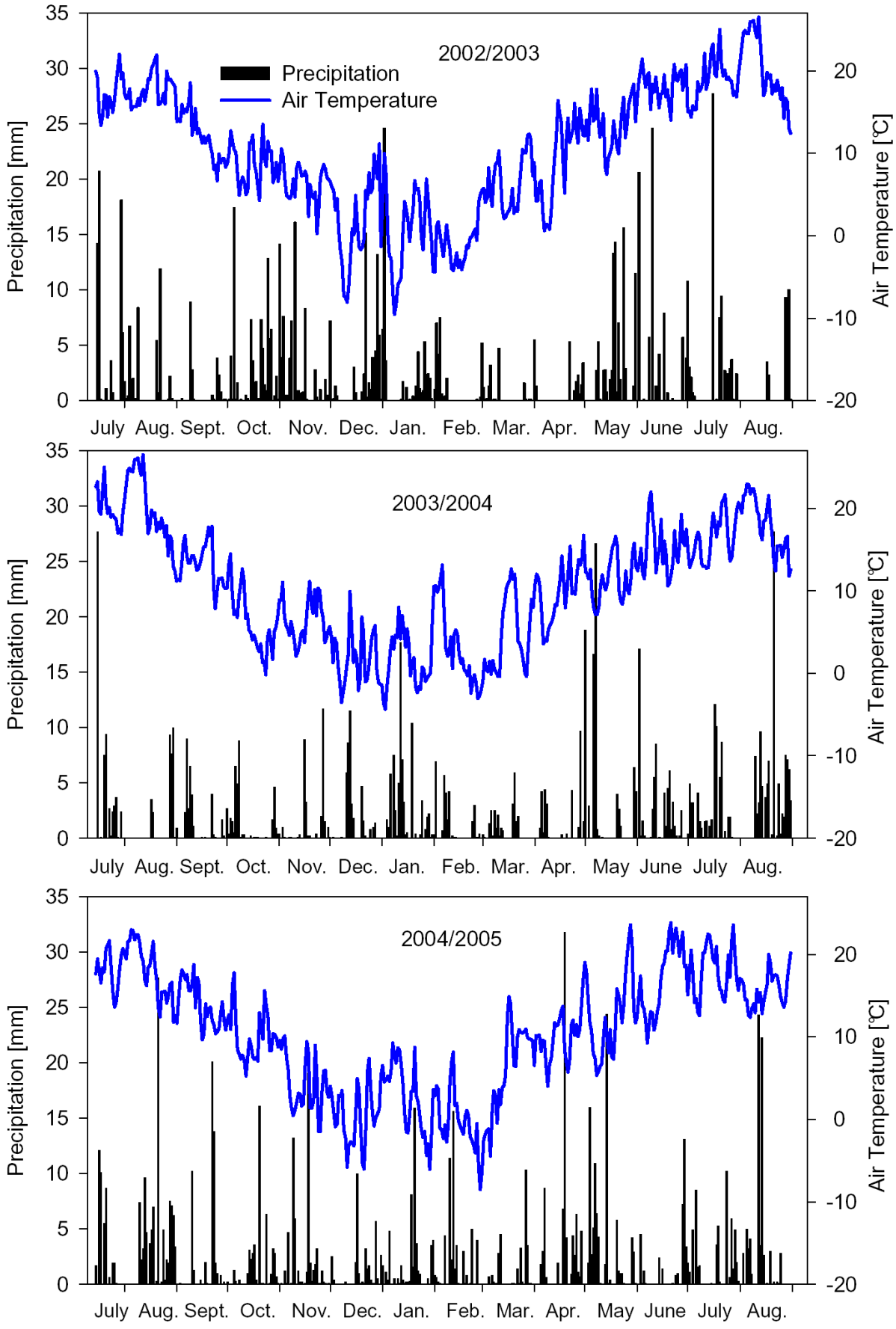


Figure 2.2: Daily data of precipitation and air temperature during the three measurement seasons. (Data: Franz Schulz, Villmar, personal communication)

2.1.1 Experimental Design of the Cropping System without Livestock

The cropping system without livestock consisted of a six-field crop rotation (table 2.1) in which the common mulching practice in organic agriculture without livestock (w/o L-M, control treatment) was compared to two manuring systems including a biogas plant. In both treatments, w/o L-FC and w/o L-FC+FE, the growth of lucerne-grass-mix, intercrops, and crop residues within the crop rotation were harvested (table 2.2), taken off the field and fermented in the biogas plant. In w/o L-FC+FE, external substrates (potatoes and spelt glumes or lucerne-grass) were additionally fermented in amounts according to the BIOLAND guideline resulting in a higher level of nitrogen input (max. + 40 kg N ha^{-1} a^{-1}) in this treatment.

Table 2.1: Six-field crop rotation of the cropping system without livestock (w/o L) (Stinner *et al.* 2006). X indicate which plant material was harvested for fermentation and which crops were fertilized by application of fermented plant material in manuring treatments w/o L-FC and w/o L-FC+FE.

	Crops	w/o L-FC and w/o L-FC+FE	
		Crops Used for Fermentation	Fertilized Crops
1. Year	**Lucerne-Grass-Mix**	X	
2. Year	**Potatoes**		X
3. Year	**Winter Wheat 3**		X
4. Year	Intercrops	X	
	Pea		
5. Year	Intercrops	X	
	Winter Wheat 5		X
6. Year	Intercrops	X	
	Spring Wheat undersown Lucerne-Grass-Mix		X

Table 2.2: Manuring treatments of the cropping system without livestock (w/o L) (Stinner *et al.* 2006).

Manuring Treatments "without Livestock" (w/o L)	Crop Residues, Intercrops & Lucerne-Grass
w/o L-M	Remain on Field (Mulched, M)
w/o L-FC	Fermented (FC)
w/o L-FC+FE	Fermented with Additional External Substrates (FC+FE)

Fermentation was carried out in a two-step percolation reactor (Edelmann *et al.* 1996) at mesophilic temperatures and a mean fermentation period of 5 - 7 days, depending on th e crop biomass. During the anaerobic degradation process, the complex organic compounds were converted in a series of microbial metabolic pathways by a consortium of bacteria (hydrolytic, acidogenic, and acetogenic bacteria, and methanogenic archaea) to CH_4 and CO_2. Beside the generation of biogas (CH_4), a renewable energy form, anaerobic fermentation resulted in products with lower carbon and dry mass contents, smaller C:N ratios, less organic nitrogen, higher concentration of mineral nitrogen (NH_4^+), and slightly elevated pH value.

Liquid of the fermentation process was applied in spring as fertilizer to the cashcrops within the crop rotation (table 2.1) in accordance with their nutrient demand. Manuring was performed by hand with watering cans near the soil surface simulating application via trail hose. Solid residues of the fermented crop material were turned back to the field prior to grubbing/ploughing before spring wheat and potatoes were sown and dibbled. Spreading of solid fermented crop residues were also accomplished by hand using dung forks. In contrast, in w/o L-M (control treatment) the growth of the lucerne-grass-mix, the plant material of the intercrops and the crop residues remained on the field, were mulched and grubbed/ploughed into the soil. Thus, due to operating the biogas plant, a mobile fertilizer was created in agriculture without livestock that was applied when demand emerged/arose. Additionally, nutrients were shifted within crop rotations of both biogas treatments, w/o L-FC and w/o L-FC+FE, away from legume crops advantaging cashcrops.

2.1.2 Experimental Design of the Cropping System with Livestock

In the cropping system with livestock consisting of an eight-field crop rotation (table 2.3), the common farmyard manure (wL-FYM) and raw slurry (wL-RS) manuring practices were compared to three manuring systems in which the cattle slurry was fermented in a biogas plant prior to field application (wL-FS; wL-FS+FC; wL-FS+FC+FE) (table 2.4). Fermentation of the cattle slurry was carried out in a batch reactor at mesophilic temperatures and a mean fermentation period of 30 days. Those biogas manuring systems differed in the handling of the intercrops and crop residues and in the additional fermentation of external substrates. In wL-FS, intercrops and crop residues remained on the field, e.g. they were mulched and grubbed into the soil. In contrast, in wL-FS+FC and wL-FS+FC+FE this plant material was harvested, taken off the field and fermented in the two-step percolation reactor as described above. External substrates like potatoes and spelt glumes or lucerne-grass were additionally fermented in wL-FS+FC+FE to increase the amount of nitrogen applied in this manuring treatment. However, not more than 40 kg N ha^{-1} a^{-1} accessory were fertilized in compliance to the BIOLAND guideline.

Table 2.3: Eight-field crop rotation of the cropping system with livestock (wL) (Möller *et al.* 2006). X indicate which plant material was harvested for fermentation and which crops were fertilized.

	Crops	Fertilized Crops (in all five Manuring Treatments)	Crops Used for Fermentation in wL-FS+FC and wL-FS+FC+FE
1. Year	**Lucerne-Grass-Mix**		
2. Year	**Lucerne-Grass-Mix**		
3. Year	**Winter Wheat**	X	
4. Year	Intercrops		X
	Potatoes	X	
5. Year	**Winter Rye**	X	
6. Year	Intercrops		X
	Pea		
7. Year	Intercrops		X
	Spelt	X	
8. Year	Intercrops		X
	Spring Wheat undersown Lucerne-Grass-Mix	X	

Table 2.4: Manuring treatments of the cropping system with livestock (wL) (Möller *et al.* 2006).

Manuring Treatments "with Livestock" (wL)	Cattle Excreta	Crop Residues & Intercrops
wL-FS	Fermented Slurry (FS)	Remain on Field (Mulched)
wL-FS+FC	Fermented Slurry (FS)	Fermented (FC)
wL-FS+FC+FE	Fermented Slurry (FS)	Fermented with Additional External Substrates (FC+FE)
wL-FYM	Farmyard Manure (FYM)	Remain on Field (Mulched)
wL-RS	Raw Slurry (RS)	Remain on Field (Mulched)

Slurry, fermented slurry and liquid of the crop fermentation were applied as fertilizer to the cashcrops within the crop rotation in spring (table 2.3) according to their nutrient demand. Simulating trail hose application technique, those fertilizers were applied by hand with watering cans near the soil surface. Before spring wheat and potatoes were sown and dibbled, solid residues of the fermented plant material were turned back to the field prior to grubbing/ploughing in wL-FS+FC. In wL-FYM the farmyard manure was applied and

incorporated into the soil prior to all crops other than peas and lucerne-grass. Solid fertilizers were also spread by hand using dung forks.

Climate data were collected by Franz Schulz, employee of the research station, and provided for illustration (figures 2.1 and 2.2; A.1 - A.3). Performance of manure management and fermentation processes were carried out by employees of the Professorship for Organic Farming. Crop cultivation and harvest at the field site were conducted by employees of the Professorship for Organic Farming and of the research station. Data concerning fertilization and cultivation operations (tables 2.5 and 2.6; A.4 - A.7) as well as soil mineral nitrogen contents in the depth profile (figures 3.5, 3.10, 3.16, and 3.20; tables 3.4. 3.6, 3.13, and 3.15), and total soil nitrogen and carbon concentrations at the field site (tables 3.5 and 3.14) were provided by Kurt Möller and Walter Stinner, employees of the Professorship for Organic Farming, for compilation and illustration.

2.2 Field Trial

2.2.1 *In-situ* Gas Flux Measurements

The principle of a static chamber system as described by Hutchinson and Mosier (1981) was employed to determine *in-situ* fluxes of N_2O, CH_4, and CO_2. From autumn 2002 until summer 2005 trace gas fluxes were measured with transparent chambers between 10:00 h and 12:00 h in selected combinations of crops and manuring treatments in both cropping systems in three field replicates. In the cropping system without livestock, fluxes were examined in winter wheat 5 and spring wheat with the prior intercrops in the control mulching treatment (w/o L-M) and in w/o L-FC. An overview of the agricultural activities carried out in winter wheat 5 and spring wheat including date and amount of fertilizer applications is given in table 2.5. In the cropping system with livestock, fluxes were investigated in spelt and potatoes with prior intercrops each fertilized with RS, FYM, FS, and FS+FC. Cultivation, tillage operations as well as manuring dates with respective fertilizer amounts in spelt and potatoes are shown in table 2.6.

Base frames of stainless steel (40 x 40 cm) were pressed into the soil and stayed permanently in the field. For drilling, tillage, mechanical weed control and harvest, base frames were removed and afterwards reinstalled at the same locations. For measurements, transparent chambers made of PETG equipped with battery-driven ventilator were placed on the frames enclosing the crops. Chambers could be adjusted in height to actual plant size (20 - 100 cm) resulting in varying covering periods between 60 and 100 minutes. During the cover period five gas samples were taken with 50 ml plastic syringes (Omnifix, B. Braun, Melsungen) and analyzed within 24 h by gas chromatography (2.7.1).

Table 2.5: Survey of cultivation, tillage, and manuring operations in investigated crops within the cropping systems without livestock. Amount of applied N_t in kg ha^{-1}. (Data: Kurt Möller and Walter Stinner, Giessen, personal communication)

	Winter Wheat 5 2002/2003	Winter Wheat 5 2003/2004	Winter Wheat 5 2004/2005	Spring Wheat 2002/2003	Spring Wheat 2003/2004	Spring Wheat 2004/2005
Manuring of Solid Ferm. Resid. (w/o L-FC)	-----	-----	-----	-----	-----	***4. Aug.*** (41)
Drilling of Intercrops	***8. Aug.*** Peas, Vetch & Oil Radish	***20. July*** Vetch & Oil Radish	***7. Aug.*** Vetch & Oil Radish	***8. Aug.*** Vetch & Oil Radish	***20. July*** Vetch & Oil Radish	***5. Aug.*** Vetch & Oil Radish
Harvest of Intercrops (w/o L-FC)	***7. Oct.***	***13. Oct.***	***4. Oct.***	***21. Nov.***	***24. Oct.***	***1. Nov.***
Manuring of Solid Ferm. Resid. (w/o L-FC)	-----	-----	***5. Oct.*** (95)	***6. Jan.*** (111)	***19. Feb.*** (115)	***7. Feb.*** (82) + liq.ferm.fert (35)
Ploughing	***10. Oct.***	***13. Oct.***	***5. Oct.***	***7. Jan.***	***20. Feb.***	***7. Feb.***
Drilling of Wheat	***10. Oct.***	***14. Oct.***	***5. Oct.***	***21. Mar.***	***20. Feb.***	***21. Mar.***
Manuring of Liquid Ferm. Fertil. (w/o L-FC)	***19. Feb.*** (91) ***13. Mar.*** (31) ***8. May*** (19)	***27. Feb.*** (75) ***25. Mar.*** (50) ***7. Apr.*** (1)	***31. Jan.*** (120) ***22. Feb.*** (62)	***27. May*** (10)	-----	***18. Apr.*** (120)
Rolling	***25. Mar.***	-----	-----	***25. Mar.***	-----	-----
Mechanical Weed Control	***29. Mar.*** ***24. Apr.***	***2. Apr.***	***21. Mar.***	***24. Apr.***	***2. Apr.*** ***27. Apr.***	***23. Apr.*** ***3. May***
Drilling of Undersown Crops	-----	-----	-----	***25. Apr.***	***28. Apr.***	***3. May***
Harvest of Wheat	***20. July***	***4. Aug.***	***8. Aug.***	***1. Aug.***	***9. Aug.***	***8. Aug.***

Table 2.6: Survey of cultivation, tillage, and manuring operations in investigated crops within the cropping systems with livestock. Amount of applied N_t in kg h
(Data: Kurt Möller, Giessen, personal communication)

	Spelt 2002/2003	Spelt 2003/2004	Spelt 2004/2005	Potatoes 2002/2003	Potatoes 2003/2004
Manuring of Liquid Fertilizer	-----	-----	***9. Aug.*** FS+FC (26)	***8. Aug.*** FS, FS+FC, RS (35)	***5. Aug.*** FS (110) FS+FC (146) RS (107)
Drilling of Intercrops	***16. Aug.*** Peas, Vetch & Oil Radish	***29. July*** Vetch & Oil Radish	***10. Aug.*** Vetch & Oil Radish	***8. Aug.*** Peas, Vetch & Oil Radish	***5. Aug.*** Vetch & Oil Radish
Harvest of Intercrops (FS+FC)	***7. Oct.***	***24. Sept.***	***11. Oct.***	***6. Nov.***	***29. Oct.***
Manuring of Solid Ferm. Residues (FS+FC) and FYM	***9. Oct.*** FYM (71)	***13. Oct.*** FS+FC (48) FYM (103)	***11. Oct.*** FS+FC (66) FYM (110)	***6. Jan.*** FYM (249) ***14. Apr.*** FS+FC (68) FYM (73)	***23. Jan.*** FS+FC (114) FYM (256) ***13. Apr.*** FS+FC (19) ***20. Apr.*** FYM (50)
Ploughing	***10. Oct.***	***14. Oct.***	***11. Oct.***	***7. Jan.***	***24 .Jan.***
Drilling of Spelt / Dibbling of Potatoes	***11. Oct.***	***14. Oct.***	***12. Oct.***	***15. Apr.***	***21. Apr.***
Manuring of Liquid Fertilizer	***20. Feb.*** FS (68) FS+FC (68) RS (49) ***2. Apr.*** FS (52) FS+FC (72) RS (81)	***5. Mar.*** FS (132) FS+FC (130) RS (132) ***25. Mar.*** FS+FC (37)	***23. Feb.*** FS (100) FS+FC (162) RS (108) ***18. Mar.*** FS+FC (47)	***29. Apr.*** FS (114) FS+FC (157) RS (103) ***30. May*** FS (48) FS+FC (50) RS (56)	***20. Apr.*** FS (52) FS+FC (37) RS (52) ***17. May*** FS (11) FS+FC (40) RS (10)
Mechanical Weed Control	-----	***2. Apr.*** ***12. May***	***21. Mar.***	***22. Apr.*** ***8. May*** ***31. May*** ***6. June***	***26. Apr.*** ***18. May*** ***24. May*** ***16. June***
Harvest of Spelt / Potatoes	***17. July***	***3. Aug.***	***4. Aug.***	***26. Aug.***	***6. Sept.***

2.2.2 Soil Mineral Nitrogen Measurements

Between October 2002 and April 2003, soil samples were taken additionally to the gas samples from all investigated plots and were analyzed for nitrate (NO_3^-) and ammonium (NH_4^+). N_{min}-analyses were continued in the cropping system without livestock in spelt until June 2004. Representative aliquots of 60 g soil derived from five sub-samples per plot in a depth of 30 cm (ploughed layer) were taken, immediately cooled and analyzed within 48 h (2.8.4).

2.3 Incubation Experiments

Incubation experiments with different organic fertilizers and arable soil were performed to determine in more detail the impact of the fertilizers on N_2O emissions. The investigated fertilizers were representative samples (well homogenized before taking aliquots) of the raw slurry (RS) and the fermented slurry (FS) from the cropping system with livestock, and of the fermented plant material (FC) from the cropping system without livestock that had been applied in spring 2003 in spelt and winter wheat 5, respectively. Arable soil derived from the upper soil layer of the field trial (field "Bremsberg 1") was air-dried, sieved ≤ 2 mm, and well homogenized. Soil aliquots of 20 g and 20 g of soil amended with 7.5 ml fertilizer, respectively, were weighted into 250 ml flasks (three replicates each) and were adjusted with deionized water to maximum soil water-holding capacity of 75%. In addition, 3 x 15 ml of the pure fertilizer were also filled in flasks. Bottles were sealed with butyl stoppers and the headspaces were flushed with N_2 using two needles to generate anoxic conditions under ambient air pressure. The flasks were incubated at 25°C on a rotary shaker (200 rpm, Swip, Edmund Bühler, Hechingen) and sampled over a period of 53 h by taking 0.3 ml headspace gas samples with gas-tight syringes (B-D Plastipak, 1 ml Luer Lock, BD Biosciences, Heidelberg). Gas samples were immediately analyzed by gas chromatography (2.7.2). In an additional set of flasks NO_3^- and NH_4^+ concentrations were measured in the fertilized soil in three replicates during the incubation (2.8.4). Anoxic incubation with 10% acetylene (C_2H_2) in the headspace was accessorily performed to determine N_2O emission rates under inhibition of the N_2O reductase. Therefore, 10% of the N_2 volume in the flask were replaced with C_2H_2 before incubation. Gas samples of 0.3 ml volume were taken with gas-tight syringes during an incubation period of 53 h and analyzed gas-chromatographically (2.7.2).

2.4 Greenhouse Studies

The manuring experiment in the greenhouse was performed for studies of both denitrification activity and soil bacterial denitrifying community under controlled conditions for 22 d after fertilizer application in comparison to an unfertilized control soil. Soil was picked from the field trial (topsoil, derived from field "Bremsberg 1"), was crushed, mixed, and filled into

microcosms (13 cm x 13 cm x 13 cm; 1.3 kg dw soil pot^{-1}). Spring wheat was sowed, cultivated, and adjusted to 25 plants pot^{-1} before manuring. Three organic fertilizers, raw cattle slurry (RS) and fermented cattle slurry (FS), both derived from the cropping system with livestock, and fermented crop material (FC) from the cropping system without livestock, respectively, were applied in amounts of 18.1 mmol N_t kg^{-1} dry soil (RS, FS) and 4.5 mmol N_t kg^{-1} dry soil (FC), each in three replicates. The reduced amount of N_t in manuring treatment FC was necessary due to toxic effects on the spring wheat. The differently fertilized soils and the unfertilized control soil were examined at 70% WHC and 20°C air temperature.

Before applying the fertilizers to the microcosms, pH-value (2.8.1), water content (2.8.2), amount of NH_4^+ and NO_3^- (2.8.4), N_t after Kjeldahl (2.8.5), most probable number of nitrate reducers by MPN (2.9.3), community composition of dominant and PCR-amplifiable *nirS* denitrifiers (2.10.5), and *nirK* and *nirS* gene target numbers (2.10.6) were determined within the fertilizers. Furthermore, *nirS* gene fragments of the three fertilizers as well as of the arable soil were cloned, sequenced and phylogenetically analyzed for diversity check (2.10.6.3 - 2.10.6.5).

Trace gas (N_2O, CH_4, and CO_2) flux rates were investigated in three replicates using the closed chamber technique by putting tranparent chambers made of PETG (20 cm x 20 cm x 25 cm) over the microcosms. During the cover period of 50 min five gas samples were taken with 50 ml plastic syringes (Omnifix, B. Braun, Melsungen) and were analyzed gas-chromatographically (2.7.1). Flux rates were determined daily in the first nine days after manuring.

Soil samples were taken 2 hours, 2, 4, 7, 10, 15, and 22 days after fertilizer application, at every sampling day from three new microcosms per treatment. After removing the wheat stems 5 - 6 cm of the upper soil were sampled, well mixed, subsampled, and, unless the investigations started at once, stored at 4°C. The solid residues of RS and FS that crusted on the soil surface, were scraped off prior to the sampling and thus were excluded from the investigations. Soil samples were analyzed for NH_4^+, NO_3^-, and NO_2^- contents (2.8.4), potential denitrifying activity (2.9.1), MPN of denitrifiers (2.9.3), number of *nirK* and *nirS* gene copies (real-time PCR, 2.10.7), and community composition of the dominant and PCR-amplifiable denitrifiers targeting the *nirS* genes using SSCP (2.10.5). Each of the three manuring replicates was investigated separately.

2.5 Investigations of Field Soil Samples after 3.5 Years of Different Manuring

After 3.5 years of differentiated manuring systems in both, the cropping system with and the cropping system without livestock, soil samples under spelt (wL) and under winter wheat 5 (w/o L), respectively, were comparatively analyzed on diverse chemical-physical and microbial soil parameters concerning the nitrogen and carbon metabolism. Representative aliquots per plot derived from four sub-samples in a depth of 30 cm (ploughed layer) were

taken in all four field replicates and immediately cooled for transport. Samples were well homogenized before subsampling and, unless the investigations started at once, stored temporarily at 4°C. Mineral nitrogen (NH_4^+, NO_3^-, and NO_2^-) contents (2.8.4), C_t and N_t concentrations (2.8.6), microbial biomass carbon (2.8.7), water-extractable carbon (2.8.8), potential denitrifying activity (2.9.1), potential nitrification activity (2.9.2), soil respiration (2.9.4), substrate-induced respiration (SIR, 2.9.5), carbon-source utilization (2.9.6), community composition of dominant and PCR-amplifiable *nirS* denitrifiers by SSCP (2.10.5), and amount of *nirK* and *nirS* gene targets via real-time PCR (2.10.7) were determined in the soil samples.

2.6 Chemicals, Gases, and Water

All chemicals used during this work exhibited the purity grade "purest" and "for analysis", respectively, and were purchased from the following companies: Fluka (Switzerland), Merck (Darmstadt), Peqlab (Erlangen), Riedel-de Haën (Seelze), Serva (Heidelberg), and Sigma-Aldrich (Seelze). The gas mixes used for calibration of the gas chromatographs were purchased as "ready-to-use" mixes from Deuste Steininger (Mühlhausen). The gases dinitrogen, FID-Mix, hydrogen, argon-methane, helium, and nitrogen/carbon dioxide had a purity grade of 4.5 (99.995%) until 5.0 (99.999%) and were provided by Air Liquide (Kassel-Kaufungen). The acetylene gas (Air Liquide) exhibited a purity grade of 2.6 and was used after purification according to Gross and Bremner (1992). All media, solutions, and buffers were made of deionized water derived from a pure-water-plant (Ultra-PurPlus, ELGA, Celle). Distilled, DNAse and RNAse free water used for all molecular investigations was purchased from Invitrogen (Karlsruhe).

2.7 Gas-Chromatographic Analyses

2.7.1 Gas Samples of 50 ml Volume

Air samples of 50 ml volume were analyzed using a gas chromatrograph (GC-14B, Shimadzu, Duisburg) equipped with an automated sampler and the respective software package Probe 65 (both LAL, Neu Eichenberg). Both, precolumn (length 0.8 cm) and main column (length 3.2 m) with a diameter of 1/8 inch were packed with Porapak Q mesh 80 to 100 (Millipore, Schwalbach) and heated at 65°C. The gas chromatograph had a flow rate of pure N_2 as carrier gas of approximately 25 ml min^{-1} and provided two detectors, an electron capture detector, ECD, 320°C and flame ionization detector, FID (fuel gas: H_2 and FID-Mix), 230°C to quantify simultaneously N_2O, CO_2, and CH_4. For detailed information about the autosampler, the control and data logging unit, and the gas chromatograhic system refer to Loftfield *et al.* (1997). Using various standard gas mixtures, peak areas were calculated, which were converted afterwards by means of the "gas law" and linear regression to gas flux rates for each chamber and subsequently per m^2 and h^1, respectively.

$$n = \frac{p * V_{ch} * c}{R * T}$$

n: amount of substance [µmol]

V_{ch}: volume chamber [l]

c: gas concentration within sample [ppmV = µl l^{-1}]

p: air pressure [mbar]

R = 83.14 [l mbar mol^{-1} K^{-1}]: gas constant

T: temperature [K]

2.7.2 Gas Samples of 1 ml Volume or Less

Air samples of one ml volume or less were gas-chromatographically analyzed with the Autosystem XL (PerkinElmer LAS, Rodgau-Jügesheim) including an ECD (350°C, make up gas: argon 95% - methane 5%) for N_2O detection and a TCD (temperature conductivity detector, 180°C) for CO_2 quantification. An FID (230°C) for CH_4 analysis was located in a second, adjoining gas chromatograph (Type 8500, PerkinElmer) that was connected with the other gas chromatograph. FID and ECD shared a precolumn (Porapak Q mesh 80 to 100 (Millipore), 1 m length, 1/8 inch) and a main column (Porapak Q mesh 80 to 100 (Millipore), 3 m length, 1/8 inch), both heated at 50°C and flushed with pure N_2 as carrier gas (flow rate approximately 30 ml min^{-1}). The TCD possessed a different column (Porapak Q mesh 80 to 100 (Millipore), 2 m length, 1/8 inch, 50°C) and used helium as carrier gas (approximately 28 ml min^{-1}). In case of gas samples containing 10% acetylene, the runtime per sample was elongated from 4 to 7 min directing the acetylene peak over the FID. Detector signals were evaluated with the Peak Simple Chromatography Data System (Model 202, Version 2.66, SRI Instruments, California, U.S.A.). Calculation of flux rates were processed as described above.

2.8 Chemical-Physical Analyses of Environmental Samples

Standard methods of soil investigations were applied for chemical-physical analyses (Schlichting *et al.* 1995) unless otherwise noted.

2.8.1 pH-Value

Soil pH-value measurements were performed with a pH electrode (CG 840 B, Schott, Mainz) in 0.01 M $CaCl_2$ after suspending 10 g of soil in 25 ml, whereas pH values of the liquid organic fertilizers was directly determined in the fertilizer.

2.8.2 Water Content

The water content was gravimetrically determined after drying at 105°C until the weight of the sample remained constant.

2.8.3 Water-holding Capacity

Soil water-holding capacity (WHC) was determined gravimetrically using ≤ 2 mm sieved dry soil and was given in % of maximum water-holding capacity.

2.8.4 Mineral Nitrogen

Mineral N contents (NH_4^+-, NO_2^--, and NO_3^--N) were photometrically determined in $CaCl_2$ extracts using the Spectrophotometer U-3200 (Hitachi, Düsseldorf). Soil samples of 60 g and fertilizer aliquots of 10 g, respectively, were extracted with 200 ml 0.0125 M $CaCl_2$ for 60 min on a horizontal shaker (VKS, Edmund Bühler, Hechingen) and filtered by type 595 fluted filter (Schleicher & Schuell, Dassel).

Ammonium: NH_4^+ contents of environmental samples were measured as indophenol complex at 660 nm (Kandeler and Gerber 1988).

Nitrite: NO_2^- concentrations were investigated via a pink diazo-complex at 535 nm (Keeney and Nelson 1982).

Nitrate: NO_3^- contents were either measured at 210 nm before and after the reduction of nitrate via nascent hydrogen (Navone 1964) or after reduction of nitrate to nitrite via a pink diazo-complex (Keeney and Nelson 1982) at 535 nm.

2.8.5 Total Nitrogen

Quantification of N_t was processed after Kjeldahl by colleagues of the Professorship for Organic Farming at the University of Giessen.

2.8.6 Total Carbon and Total Nitrogen

The contents of total C and total N were measured in dry, ground soil using the elementar analyser (vario MAX CNS, Hanau) by colleagues of the Institute for Plant Ecology at the University of Giessen.

2.8.7 Microbial Biomass Carbon

Soil microbial biomass carbon was estimated by the fumigation-extraction-method (Joergensen 1996). Principle of the determination is the comparison of the direct carbon extraction and the extraction of soil after chloroform-fumigation since due to the fumigation, microbes are killed and subsequently, the microbial biomass carbon can be easily extracted. A sub-sample of about 12.5 g fresh soil was incubated for 24 h in darkness under chloroform atmosphere at 25°C. Both, the incubated soil and a non-incubated sub-sample were extracted with 50 ml 0.5 M K_2SO_4 for 30 min on a horizontal shaker, filtered, and measured photometrically at 280 nm. After correction of blank value, soil water content, dilution factor, and soil mass, the difference of extinction between fumigated and non-fumigated sample

was determined. Using the slope calculated by Turner *et al.* (2001) that covers a range of soil organic matter between 2.9% and 8% and a range of clay content between 22% and 68%, extinction values were converted to microbial biomass C g^{-1} dw soil.

2.8.8 Water-Extractable Carbon

Water-extractable C fraction as a measure for easily degradable carbon was investigated in 30 g fresh soil after Burford and Bremner (1975). After soil extraction with 60 ml pure water on a horizontal shaker, addition of 10 ml Na_2SO_4 as flocculant, and filtration, an aliquot of 25 ml was evaporated at 80°C. Adhering the Lichterfelder method (Schlichting *et al.* 1995), the residues were dissolved in 9 ml concentrated H_2SO_4 and 5 ml 2 N $K_2Cr_2O_7$, oxidized at 120°C for 90 min, replenished with pure water to 50 ml and centrifugated (Labofuge, Heraeus Christ, Hanau) for 10 min at 1630 g. Extinction of the reduced Cr^{3+} ions was photometrically determined at 578 nm in comparison to a standard curve prepared with sodium oxalate.

2.9 Microbial Analyses of Environmental Samples

2.9.1 Potential Denitrifying Activity

Potential denitrification activities (Smith and Tiedje 1979; Luo *et al.* 1996) were determined in slurry experiments in anoxic atmosphere with 10% acetylene using 250 ml flasks sealed with butyl stoppers. Fresh sub-sampled soil (25 g) was enriched with 1.4 mM KNO_3 and 10 mM glucose-C and incubated at 25°C on a rotary shaker (Swip, Edmund Bühler, Hechingen). After 15, 30, and 45 min, gas samples of 1 ml volume (B-D Plastipak, Luer Lock, BD Biosciences, Heidelberg) were taken from the headspace using gas tight syringes and were analyzed by gas chromatography (2.7.2) for N_2O.

2.9.2 Potential Nitrifying Activity

Potential nitrification activities were measured after Berg and Rosswall (1985) modified by Schinner *et al.* (1991). For this purpose, 5 g fresh soil were enriched with 20 ml 1 mM $(NH_4)_2SO_4$ and 0.1 ml 1.5 M $NaClO_3$ and incubated at 25°C for 5 h on a horizontal shaker. Afterwards, the samples were extracted with 5 ml 2 M KCl and filtered. An aliquot of 5 ml filtrate was mixed with 3 ml 0.19 M NH_4Cl buffer (pH 8.5) and 2 ml dye solution (200 ml solution contain 2 g sulfanilamide, 0.1 g naphtyl-1-diethylene-dammonium-dichloride, and 20 ml conc. H_3PO_4) and incubated for 15 min in darkness. The generated NO_2^- was measured photometrically at 520 nm via a pink diazo-complex in comparison to a standard curve prepared with sodium nitrite (2.8.4).

2.9.3 MPN of Nitrate-Reducing Bacteria

The most probable number method (MPN) was used to enumerate nitrate reducers in the environmental samples. Five g of soil or fertilizer, respectively, were dispersed in 495 ml of 0.18% sodium pyrophosphate solution and subsequently 1:10 diluted with sterile 0.9% sodium chloride solution. 3 x 9 ml of the anoxic denitrifier medium were inoculated with 1 ml per dilution step, resulting in dilution series between 10^{-6} and 10^{-12} in three replicates. The headspace of the test tubes were immediately flushed with N_2/CO_2 (80:20 [vol/vol]) using the hungate technique, sealed with butyl stoppers and incubated at 25°C. Bacterial growth was verified per eye by turbidity of the medium every week. When changes in turbidity did not occur anymore in the dilution series, medium aliquots of the last tube with growth and the first tube without growth were taken to check the nitrate concentration. NO_3^- was analyzed by ion chromatography (IC-System S135, conductivity detector S3111, IC Modul S4260AB, autosampler S5200, Sykam, Fürstenfeldbruck) according to the modified method from Bak *et al.* (1991). Signals were evaluated with the Peak Simple Chromatography Data System (Model 202, Version 2.66, SRI Instruments, California, U.S.A.). Only if growth of the nitrate reducers could be confirmed by decrease of NO_3^-, the samples were assessed as positive. The highest three dilutions steps exhibiting growth were selected to read the combination of numbers that then were looked up in the corrected MPN table (de Man 1983) for 3 parallels. The resulting cell counts were converted to cells per g dw soil and per g fresh fertilizer, respectively.

Denitrifier medium

NaCl	1.0 g l^{-1}
$MgSO_4$ x 7 H_2O	0.486 g l^{-1}
$CaCl_2$ x 2 H_2O	0.15 g l^{-1}
KCl	0.5 g l^{-1}
KH_2PO_4	0.2 g l^{-1}
NH_4Cl	0.25 g l^{-1}
$NaNO_3$	0.425 g l^{-1}

The salts listed above were dissolved in demineralized water and autoclaved in a special flask (modified after Widdel and Bak (1992) for denitrifier). After the medium had cooled down under an N_2/CO_2 (80/20 [vol/vol]) atmosphere, the following components were added:

$NaHCO_3$-buffer (1M)	30 ml l^{-1}
Vitamin B12 (5 mg Cyanocobalamin 100 ml^{-1})	1 ml l^{-1}
Mix of 5 vitamins *	1 ml l^{-1}
Riboflavin (5 mg Riboflavin 100 ml^{-1} 20 mM acetic acid)	1 ml l^{-1}
Vitamin B1 (10 mg Thiamin-hydrochloride 100 ml^{-1} 25 mM sodium phosphate buffer pH 3,4)	1 ml l^{-1}
Solution of trace elements (EDTA)**	1 ml l^{-1}

* Mix of 5 vitamins:

Pyridoxine-dihydrochloride	15 mg 100 ml^{-1}
Nicotinic acid	10 mg 100 ml^{-1}
Calcium-D(+)-Pantothenate	5 mg 100 ml^{-1}
4-Aminobenzoic acid	4 mg 100 ml^{-1}
D(+)-biotine	1 mg 100 ml^{-1}

** Solution of trace elements (EDTA) (Widdel and Bak 1992):

Na_2-EDTA	5200 mg l^{-1}
$FeSO_4 \times 7\ H_2O$	2100 mg l^{-1}
$CoCl_2 \times 6\ H_2O$	190 mg l^{-1}
$ZnSO_4 \times 7\ H_2O$	144 mg l^{-1}
$MnCl_2 \times 4\ H_2O$	100 mg l^{-1}
$Na_2MoO_4 \times 2\ H_2O$	36 mg l^{-1}
H_3BO_3	30 mg l^{-1}
$NiCl_2 \times 6\ H_2O$	24 mg l^{-1}
$CuCl_2 \times 2\ H_2O$	2 mg l^{-1}
$CuSO_4 \times 5\ H_2O$	29 mg l^{-1}

After addition of the solutions, the pH-value was measured and, if necessary, adjusted with 1 M HCl or Na_2CO_3 to pH 7 - 7.2.

2.9.4 Basal Respiration

Determination of soil basal respiration was done using the OxiTop® Control BM (WTW Weilheim) analysis system that is based on pressure drop measurement in a closed system (Conzelmann 1996; Wagner and Fink 1996). Oxygen is consumed by respiration, and the produced CO_2 is absorbed by NaOH, thus creating a negative pressure. For this purpose, 100 g fresh soil were put in a 1 l preserving jar and were adjusted to approximately 63% WHC with 5 ml pure water. After two hours of pre-incubation at room temperature, a 50 ml plastic beaker filled with 50 ml 1 M NaOH was inserted into the jar without any contact to the soil sample by placing it on the loft underneath the cover. The jar was sealed gas-tightly with the special lid adapter including the measuring head equipped with a built-in pressure sensor and infrared interface. Incubation was done at 20°C for 66 h. By pointing the respective controller OC 110 at the measuring heads, the registered 360 data points over the incubation period were transferred via infrared interface to the controller and further by cable and the communication program Achat OC to the PC for evaluation. Linear pressure decrease was converted to oxygen consumption rate using the equation:

$$CR_{O2} = b\frac{(V_nv - Vs)}{R * T * ms} * 1000$$

CR_{O2}: oxygen consumption rate [mmol O_2 kg^{-1} dw soil h^{-1}]
b: pressure drop rate [mbar h^{-1}] evaluated by linear regression
V_nv: netto vessel volume without soil volume [l]
Vs: soil volume [l]
R = 83.14 [l mbar mol^{-1} K^{-1}]: gas constant
T = 293 [K]
ms: soil mass in the vessel [kg]
1000: conversion factor mol to mmol

Beside continuous pressure drop measurement an end-point quantification of CO_2 produced was done by titration of a 20 ml NaOH aliquot with 1 M HCl. HCl consumption was converted to the amount of absorbed CO_2 by the equation:

$$V_{NaOH} = \frac{(V_{aliquotNaOH} - V_{HCl}) * 50}{20}$$

V_{NaOH}: volume of neutralized 1 M NaOH through CO_2 absorption [ml]
$V_{aliquot\ NaOH}$ = 20: volume of titrated aliquot [ml]
V_{HCl}: volume of used 1 M HCl by titration [ml]
20 and 50: conversion factors from titrated NaOH aliquot to total amount of NaOH

Since 1 ml of 1 M NaOH corresponds to 1 mmol NaOH, and 1 mmol NaOH neutralizes 0.5 mmol CO_2, the amount of V_{NaOH} was subsequently devided by 2 to obtain the amount of absorbed CO_2 in mmol.

2.9.5 Substrate-Induced Respiration, SIR

Substrate-induced soil respiration was conducted as described for basal respiration (2.9.4) in principle. However, 140 mg glucose-C per 100 g fresh soil were added in 5 ml water and the incubation period was shortened to 10 h. The optimum amount of glucose was found out in a preliminary test.

2.9.6 BIOLOG Substrate Utilization Test

The carbon substrate utilization test was conducted as a rapid community-level method, whose principle was described by Garland and Mills (1991), to check differences of potential metabolic diversity between heterotrophic microbial communities in the differently fertilized soils. Microplates (Kämpfer 1988) were provided by Prof. Dr. Dr.-Ing. Peter Kämpfer, Giessen. Beside utilization of sole-carbon sources also acid production was determined via tetrazolium

redox dye and production of extracellular enzymes via decomposition of chromogen substrates. Microorganisms were detached from soil particles by dispersing 10 g fresh soil in 90 ml 0.18% [wt/vol] sodium pyrophosphate in a waring blendor (Breda Scientific, Cenco, Meerbusch) at low level for 30 s. After 10 min of sedimentation, the supernatant was removed, put into an ultrasonic bath (Sonorex RK 100 H, Bandelin, Berlin) for 30 s, and was diluted 1:10 with 0.9% [wt/vol] sodium chloride. Afterwards, soil extract was mixed in a ratio of 1:2 with tetrazolium redox dye (0.3% [wt/vol] iodnitrotetrazoliumchloride, INT), and was diluted 1:2 using pure water – to obtain the same soil dilution step in all wells – for verification of extracellular enzyme production, respectively. Wells were inoculated with 50 µl of the respective soil extract and sealed with a non-toxic film. Microplates were incubated at 20°C in darkness, since chromogen substrates were sensitive to light. Tests were performed in four independent replicates per manuring treatment. After 2, 3, and 5 days, plates were evaluated visually by means of color alteration. For examination, data after two incubation days were used since afterwards alterations could hardly be observed. Data analysis (Udo Jäckel, Giessen, personal communication) was performed by transforming color alteration in 0/1 matrices (Excel, Microsoft, Unterschleißheim) of each plate. Afterwards, a "master matrix" was generated for each manuring treatment to get a survey of well positions that could be considered in the following process. Only well positions with at least three similar numbers within the four replicates of one manuring treatment were labeled for further evaluation. Cluster analyses (WinSTAT® for Microsoft Excel) of the labeled wells in the different manuring treatments were performed using "Ward" as agglomeration method within the respective cropping system (without and with livestock, respectively). Additionally, all eight manuring treatments were simultaneously evaluated. However, logically, only well positions that were evaluable in all treatments and replicates compared could be included into the cluster analysis.

2.10 Molecular Biological Analyses

To investigate the impact of the different manuring systems on denitrification activity and the denitrifying bacterial community in more detail and in additional, using entirely different tools, several molecular biological techniques were applied. Targets of investigations were denitrifiers capable of reducing nitrite to nitric oxide, the key step in the denitrification pathway since it leads to the first gaseous product.

2.10.1 Genomic DNA Extraction from Environmental Samples

Various protocols for soil DNA extraction methods were tested to extract genomic DNA from soil samples. Efficient and reproducible cell lysis without rigorous shearing of the nucleic acids, sample homogenization, protein solubilization, and elimination of contaminants like humic acids had to be achieved. Several protocols varying in time of distinct extraction steps and

used chemicals were tested leading to best results obtained with the FastDNA Spin Kit for Soil (Bio 101, Qbiogene, Heidelberg). Therefore, extraction and purification of DNA from all environmental samples, fertilized and non-fertilized arable soil as well as different organic fertilizers, were performed with this commercially available DNA extraction kit in at least 3 replicates per sample according to the manufacturer´s instruction. Cell lysis by bead beating was done with a horizontal grinder (Retsch, Haan) for 30 s at maximum speed (approximately 1 m s^{-1}).

2.10.2 Genomic DNA Extraction from Pure Cultures

DNA was prepared from pure bacteria cultures (Henckel *et al.* 1999) that served as positive- or negative-control strains for *nirS* and *nirK* genes, respectively. Genomic DNA was obtained in duplicates by subsequent incubations of cell pellet with 100 µl lysozyme (10 mg ml^{-1}) at 37°C for 60 min and 15 µl proteinase K (20 mg ml^{-1}) with 567 µl sodium dodecyl sulfate (10% SDS) at 60°C for 15 min. Subsequently, 700 µl sodium-phosphate-buffer (120 mM; pH 8) and 40 vol % of cold ammonium acetate were added, chilled on ice for 5 min and centrifuged (Biofuge fresco, Heraeus, Hanau) at 13800 g for 5 min at 4°C. The supernatant was amended with 0.7 vol of 2-propanol and centrifugated again for 60 min. The DNA pellet was washed 2x with 600 µl of 70% ethanol, air-dried for 30 min, resuspended in Tris-EDTA-buffer, and stored at 4°C overnight.

2.10.3 Photometrical Quantification of Nucleic Acids

Nucleic acid concentration and purity of both, DNA extracts and PCR products, were determined photometrically (Ultrospec 4000, Amersham Biosciences, Freiburg) at 230, 260, and 280 nm, and stored in aliquots of 8 µl at -20°C.

2.10.4 Purification of PCR Products

PCR products were purified with the Qiaquick PCR Purification Kit (Qiagen, Hilden) as recommended by the manufacturer, however, finally, nucleic acids were eluted with PCR water.

2.10.5 Single Strand Conformation Polymorphism (SSCP)

Bacterial denitrifying communities of the differently fertilized soils and the organic fertilizers were compared by the cultivation-independent fingerprinting technique "Single Strand Conformation Polymorphism" (SSCP) based on PCR amplification. Schwieger and Tebbe (1998) described for the first time the method derived from medical research for investigation of complex bacterial communities. PCR products were subjected to SSCP (Dohrmann and

Tebbe 2004) to generate genetic profiles, which corresponded to the diversity of the amplified *nirS* gene fragments.

Single-strand DNA molecules fold under non-denaturing conditions into secondary conformations that are primarily caused by the sequence of bases since complementary bases tend to form hydrogen bonds thus forming the secondary structure. As a result of PCR with primers binding to a gene of many different organisms in a community, products are generated that have a very similar size but different base sequences. These conformations encounter differential impedance within a polyacrylamide gel matrix and can therefore be separated by electrophresis.

2.10.5.1 PCR Assays for SSCP

PCR for SSCP was processed using the *nirS* primers cd3aF (GT(C/G) AAC GT(C/G) AAG GA(A/G) AC(C/G) GG (Michotey *et al.* 2000)) and R3cd (GA(C/G) TTC GG(A/G) TG(C/G) GTC TTG A (Throbäck *et al.* 2004)) resulting in PCR fragments of 425 bp length. A modification of the reverse primer, i.e. phosphorylation at the 5' end of R3cd, was necessary for subsequent single-strand digestion. PCR conditions comprising mastermix composition, amount of primers, annealing temperature and time, number of cycles, and "touchdown" program had to be optimized in depencence of the used primers. Finally, PCR was performed in a total volume of 25 µl containing 1 x PCR buffer, 0.16 mM of each desoxy-nucleotide, 3 mM $MgCl_2$, 2 U recombinant Taq Polymerase, 0.5 µM each primer (MWG Biotech, Ebersberg), and 0.25 µl bovine serum albumin (20 mg ml^{-1}, Fermentas, St. Leon-Rot) to enhance amplification of environmental DNA. The amount of template DNA was adjusted to 25 ng per reaction (2.10.1). DNA amplification was conducted in 6 replicates in the thermal cycler MyCycler (Bio-Rad, München). After a denaturation step of 3.5 min at 94°C, a "touchdown" PCR was performed that consisted for amplification of *nirS* fragments of a denaturation step of 30 s at 94°C, a primer-annealing step of 30 s, and an extension step of 60 s at 72°C. The annealing temperature was decreased by 0.5°C each cycle, starting at 63°C until it reached after 13 cycles a "touchdown" at 57°C. Additional 19 cycles were processed at an annealing temperature of 57°C. After 32 cycles, a final extension step of 15 min at 72°C was performed. The correct length of the amplification products was controlled by staining DNA with ethidium bromide after electrophoresis on 1.7% [wt/vol] agarose gels (Peqlab, Erlangen).

2.10.5.2 Single-Strand Removal

PCR products (2.10.5.1) were pooled, purified (2.10.4), and quantified (2.10.3). Double-stranded DNA (approximately 700 ng) were converted to single-stranded products by selective removal of the reverse phosphorylated strands with 2.5 U lambda-exonuclease (New England Biolabs, Frankfurt/Main) in 1 x exonuclease buffer (provided by the

manufacturer) at 37°C for 45 min. Digestion was stopped by starting the purification of the remaining single-strands using the MinElute PCR Purification Kit (Qiagen, Hilden) according to the manufacturers instruction. Single-stranded DNA was eluted with 10 µl EB buffer included in the kit.

Prior to loading on the polyacrylamid gel the single strands had to be denatured. Therefore, DNA was after spiking with 10 µl of SSCP loading buffer (10 mM NaOH, 0.25% [wt/vol] xylene cyanol, 0.25% [wt/vol] bromophenol blue, 95% [vol/vol] formamide) heated to 95°C for 2 min and immediately cooled on ice.

2.10.5.3 SSCP Gel Casting and Electrophoresis

Since the physicochemical environment during electrophoresis has an enormous effect on the electrophoretic mobility and the resolution of SSCP, composition of the polyacrylamide gel, buffer strength, temperature, and running time had to be optimized carefully depending on the DNA fragment length and the used primer. The electrophoretic separation was performed with the Protean II XL vertical electrophoresis cell (Bio-Rad, München) and the appropriate cooling module.

Two glass plates (20 x 20 x 0.4 cm and 22 x 20 x 0.4 cm) were cleaned three times with 70% [vol/vol] ethanol and polished with lint-free tissues before gel-casting procedure started. Spacers and comb were cleaned likewise. The smaller plate was impregnated with a few drops of repel-silane (SEA-Spray, CLP, England) by a lint-free tissue, and after 2-3 min it was polished with a fresh tissue, rinsed subsequently with 70% ethanol and polished again. In contrast, the larger plate was impregnated with 1 ml of bind-silane use solution (10 ml 96% [vol/vol] ethanol + 100 µl bind silane (Amersham Biosciences, Freiburg) + 100 µl 100% [vol/vol] acetic acid) in the same way as described for the other plate. A sandwich of both glass plates and the 0.35 mm spacers were prepared and clamped in the casting stand. The SSCP gel matrix consisted of 0.725 x MDE gel solution (Cambrex Bio Science, Belgium), 10% [wt/vol] formamide, 1 x TBE buffer, 4% [wt/vol] APS (ammonium persulfate), and 0.04% [wt/vol] TEMED (N, N, N', N' - tetramethylethylenediamine). When APS and TEMED had been added to the gel solution (careful mixing with a magnetic stir bar, no bubbles should emerge) the gel had to be poured quickly between the glass plates without air bubbles. The comb was ca. 1 cm inserted inversely, i.e. with the linear border into the gel matrix. Afterwards, the gel polymerized at least 2 h at room temperature. Coolant circulation within the cooling core was already turned on and the temperature was adjusted to 19.5°C to ensure this constant temperature of the cooling system right at the beginning of the electrophoretic separation. After polymerization the comb was reversed, the plate sandwich was placed in the electrophoresis cell, and 1 x TBE buffer was poured into the vessel. Before some drops of the SSCP loading dye were transferred to the sample wells, they were rinsed with buffer using a syringe and a needle, and a "pre-run" of about 15 min was startet. Thereafter, the denatured

samples were loaded into the wells (up to 8 µl per well), and the gel was run for 17 h at constant 450 V and 19.5°C.

2.10.5.4 Silver Staining of DNA

To visualize the SSCP-profiles, DNA within the polyacrylamide gels was silver-stained (Bassam *et al.* 1991) in carefully cleaned trays of stainless steel. The gel fixed to the glass plate was removed from the electrophoresis chamber, was transferred into a tray filled with 300 ml 10% [vol/vol] acetic acid and was incubated for 30 min under mild shaking. In the meantime, the staining solution (500 ml pure water + 0.5 g $AgNO_3$ + 0.75 ml 37% [wt/vol] formaldehyde) and the developer (500 ml pure water + 10.43 g Na_2CO_3 anhydrous) were prepared and stored in darkness and at 4°C, respectively. After the DNA fixation, the gel was washed three times for 5 min with pure water. For color impregnation, the gel was incubated for 30 min in the staining solution protected from light and afterwards rinsed for 10 s with pure water. The cooled developer solution was completed by adding 1 ml 37% [wt/vol] formaldehyde and 0.5 ml 0.2% [wt/vol] sodium thiosulfate, and the gel was then transferred to another tray filled with a small amount of the developer to rinse the gel for 20 s. In the residual developer, the gel was incubated until the patterns became clearly visible. To stop the staining process, the gel was transferred back to the first tray filled with 300 ml 10% [vol/vol] acetic acid for 4 min. After the color fixation, the gel was incubated in pure water for 30 min and subsequently dried at room temperature. Finally, the gel was scanned using the PowerLook 1120 (UMAX, Willich) scanner.

2.10.6 Cloning of *nir* Gene Fragments

2.10.6.1 PCR Assays for Cloning Real-Time PCR Standards

Positive-control DNA (2.10.2) was PCR-amplified firstly to confirm the respective correct primer hybridization and fragment length and secondly to generate PCR products that could be used after cloning and reamplification as real-time PCR standards. DNA of *Rhodopseudomonas palustris* (strain FLaA3, provided by Prof. Dr. Sylvia Schnell) served as positive-control for *nirK* gene amplification and was used to generate a *nirK* real-time PCR standard. As positive-control for *nirS* gene amplification and *nirS* real-time PCR standard, DNA of *Cupriavidus necator* DSMZ 530 was employed.

The PCR assay for the *nirS* real-time PCR standards was performed in 25 µl volume and in the thermal cycler MyCycler as described in 2.10.5.1. However, the protocol was slightly modified and optimized for amplification of positive-control DNA in the following way: PCR reactions were conducted without bovine serum albumine and with the non-phosphorylated reverse primer R3cd.

The *nirK* real-time PCR standards were produced using the degenerated primer nirK876 (AT(T/C) GGC GG(G/C/A) CA(T/C) GGC GA (Henry *et al.* 2004)) and nirK5R (GCC TCG ATC

AG(A/G) TT(A/G) TGG (Braker *et al.* 1998)). The composition of the mastermix corresponded to that described in 2.10.5.1 for *nirS* amplification, however, without addition of bovine serum albumine. The PCR protocol for *nirK* gene amplification was carried out as follows: After a denaturation step of 3.5 min at 94°C, a "touchdown" PCR was performed that consisted for amplification of *nirK* fragments of a denaturation step of 30 s at 94°C, a primer annealing step of 35 s, and an extension step of 35 s at 72°C. During the first 17 cycles, the annealing temperature was decreased by 0.5°C each cycle, starting at 63°C until it reached a "touchdown" at 55°C. Additional 15 cycles were performed at an annealing temperature of 55°C. After 32 cycles, a final incubation of 15 min at 72°C was carried out. The PCR amplification of *nirK* gene fragments was also performed in the thermal cycler MyCycler in 25 µl volume.

The amount of positive-control DNA was adjusted to 10 ng per reaction and DNA amplification was carried out in four replicates for both, the *nirK* and *nirS* gene fragment amplification. Correct amplification sizes were controlled via agarose gel (1.7%) electrophoresis and DNA staining with ethidium bromide.

2.10.6.2 Cloning of *nirK* and *nirS* Gene Fragments for Real-Time PCR Standards

Immediately after amplification check, *nirK* and *nirS* PCR products (2.10.6.1) were purified (2.10.3) and cloned using the TOPO TA Cloning® Kit (pCR2.1-TOPO vector, TOP10 cells, Invitrogen, Karlsruhe) according to the manufacturers instruction, however using 0.5 µl cloning vector instead of 1 µl. Ten clones each were analyzed by colony PCR using sterile toothpicks to pick up a small amount of cell material that was resuspended in 25 µl of the prepared PCR mix. In parallel, LB-masterplates (LB-medium: 1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% agar, adjusted with NaOH to pH 7 - 7.5; after autoclaving and cooling down addition of ampicillin with an end concentration of 100 mg l^{-1}) were inoculated with the toothpicks to conserve the cell material of the selected clone. Masterplates were incubated overnight at 37°C, sealed with parafilm and stored at 4°C. Accessorily to masterplates, glycerine stocks were prepared for longtime storage of clones freezing a mix of 700 µl overnight culture, grown in liquid LB-ampicillin medium, and of 300 µl sterile 99% glycerine at -80°C.

Colony PCR was performed using the M13-F (GTA AAA CGA CGG CCA G) and M13-R (CAG GAA ACA GCT ATG AC) primers (TOPO TA Cloning Kit) that targeted the surrounding sequence of the cloning site. The PCR mix consisted of 1 x PCR buffer, 0.16 mM of each desoxy-nucleotide, 2.5 mM $MgCl_2$, 2 U recombinant Taq Polymerase, and 0.2 µM of each primer. PCR was done in a T-Gradient Thermocycler (Biometra, Göttingen) under the following conditions: 5 min at 94°C, followed by 23 cyles of 30 s at 94°C, 50 s at 55°C, 60 s at 72°C, and finally 7 min at 72°C. Amplification specifity was analyzed on 1.7% [wt/vol] agarose gels and DNA staining with ethidium bromide. Insert of one clone each with the expected fragment length was PCR-amplified again as described before in 25 replicates and controlled

via agarose gel electrophoresis. PCR replicates were pooled, purified (2.10.5) and then fluorimetrically quantified (DyNA Quant 200, Hoefer, California) using Hoechst dye 33258 (Labarca and Paigen 1980) and stored at -20°C.

2.10.6.3 PCR Assays of Environmental DNA Samples for Cloning

PCR assays for cloning of *nirS* gene fragments derived from environmental DNA (2.10.1) were conducted as described in 2.10.5.1. However, PCR reactions were processed with the non-phosphorylated reverse primer R3cd.

2.10.6.4 Cloning of *nirS* Gene Fragments of Environmental Samples and Restriction Assays

Cloning procedure was processed as described for generation of real-time PCR standards (2.10.6.2) inclusive the first PCR amplification with M13 primers. However, at least 60 clones derived from each environmental sample were picked for diversity check of the plasmid inserts. Aliquots of 5 ml PCR product were enzymatically digested with endonucleases HpaII and Hin6I (Fermentas, St. Leon-Rot), respectively. Restriction assays were carried out separately at 37°C for 3.5 h in 1 x endonuclease buffer (provided by the manufacturer) using 0.1 µl of each enzyme, respectively. Separation of restriction fragments was achieved in 3% [wt/vol] TBE-agarose gels and visualized by DNA staining with ethidium bromide.
Gel images were processed using the GelCompar II software (version 3.5) as recommended by its designers (Applied Maths, Belgium). Restriction patterns of clones derived from one environmental sample digested with one endonuclease were normalized to a reference pattern which was loaded on four positions on each gel. After normalizing the respective restriction pattern each derived from one enzyme, both normalized gel images were adjusted in this way, that both restriction patterns of one clone (two gel lines) exactly fitted vertically. Cluster analysis of the composite data set was performed to obtain a dendrogram (UPGMA, Unweighted Pair Group Method using Arithmetic averages) revealing similar and different restriction patterns and hence probably similar and different inserts in the tested clones.

2.10.6.5. Sequencing of Selected *nirS* Clones and Phylogenetic Analysis

Plasmids selected for sequencing to represent preferably a high diversity among the clones were obtained by plasmid minipreparation using alkaline lysis with SDS (Sambrook and Russel 2001). Sequencing reactions were performed with the M13-R primer and the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Darmstadt) whose cycle-sequencing products were analyzed with an Abi Prism® 310 Genetic Analyzer (Applied Biosystems, Darmstadt). Sequencing procedures were conducted by collegues of the Institute for Microbiology and Molecular Biology of the University of Giessen.

Nucleotide sequences were restricted to *nirS* gene fragments by alignment with the *nirS* primers using the CLUSTALW program (Thompson *et al.* 1994) within MEGA version 3.1 (Kumar *et al.* 2004). The obtained *nirS* sequences were compared with sequences in the National Center for Biotechnology Information (NCBI) data bank by the use of the BLASTN program (http://www.ncbi.nih.gov/BLAST/). Thereafter, all generated clone sequences were aligned (CLUSTALW) together with additional 85 DNA sequences found in the database. Amino acid sequences were deduced on the base of described species also using MEGA. The phylogenetic trees based on DNA and amino acid sequences, respectively, were constructed by the neighbor-joining method. Bootstrap analyses with 1000 replicates were carried out to check robustness of the trees. Finally, the trees were designed and plotted using the COREL DRAW program.

2.10.7 Real-Time PCR

Real-time PCR assays were developed for a highly sensitive quantification of denitrifiers in environmental samples targeting *nirK* and *nirS* genes and were performed using the Rotor-Gene 3000 (Corbett Research, Australia) and the respective analysis software. Principles of the method have been described elsewhere (Becker *et al.* 2000; Raeymaekers 2000; Wilhelm and Pingoud 2003). The amount of PCR product was measured via the fluorescent signal of the fluorophore SybrGreen™ that was generated by binding to double-stranded DNA during the PCR reaction. Cycle number (C_T), at which the fluorescence signal crosses a certain threshold, was noted and used for calculation of target concentrations in the assay, since the Ct value is proportional to the logarithm of the target concentration. As calibration standards for real-time PCR, dilution series of positive-control DNAs with known concentration of target sequences were used.

First of all, several protocols, diverse mastermixes consisting of varying amounts of Sybr Green I and of different compositions of PCR chemicals, and the commercial ready-to-use reaction mix from Roche (LightCycler FastStart DNA Master SYBR Green I, Mannheim) were tested with different temperature profiles for an optimal real-time PCR performance. However, the outcome so far did not result in satisfying amplication curves, one distinct melting peak, and a unique DNA band on the agarose gel. In contrast, good results were obtained using the commercial kit ABsolute™ QPCR SYBR® Green Mix (ABgene®, Hamburg) after optimizing the amount of bovine serum albumine and primers in the mastermix and the amplification protocol. Therefore, all quantifications were carried out with the ABsolute™ QPCR SYBR® Green Mix.

2.10.7.1 Real-Time PCR Standards

Standard curves for *nirS* and *nirK* quantifications were created using a 10-fold dilution series of the cloned and reamplified PCR products of the positive-control DNAs of *Cupriavidus necator*

and *Rhodopseudomonas palustris*, respectively (2.10.6.2). Three independent dilution series were carried out resulting in DNA concentrations between 27.8 ng µl^{-1} and 0.0028 fg µl^{-1} for *nirS*, and 16.4 ng µl^{-1} and 0.0016 fg µl^{-1} for *nirK*. DNA concentrations were converted to target molecule numbers per µl using the equation:

$$C_S = \frac{C_W}{N * M_n} * N_A$$

C_s: target number [µl^{-1}]

C_w: DNA concentration [ng µl^{-1}]

N: number of bases in the amplicon

M_N = 649.5 [ng nmol^{-1}]: average molecular weight of a basepair

N_A = 6.23 x 10^{23} x 10^{-9} [nmol^{-1}]: Avogadro constant

Before quantification of target genes in the environmental samples, the three dilution series were run to confirm the accurateness of the dilutions and to determine the linear amplification range.

Performance of real-time PCR startet at 95°C for 15 min in order to activate the chemically modified Thermo-Start® DNA polymerase included in the real-time PCR kit. Afterwards, 40 cyles were run consisting of denaturation at 94°C for 20 s, primer annealing under stringent conditions at 64°C for 20 s, extension at 72°C for 15 s, and fluorescence data acquisition during an additional temperature step at 80°C for 15 s. The fourth step was included in the temperature profile to melt small unspecific PCR products and primer dimers prior to the data acquisition to minimize the false positive fluorescence.

Real-time PCR was processed in a total volume of 10 µl using the Strip Tubes 0.1 ml (VE1000, Corbett Research, Australia). The DNA template (2 µl of diluted PCR products) was added to 8 µl of master mix consisting of 5 µl of ABsolute™ QPCR SYBR® Green Mix (containing 3 mM $MgCl_2$), 0.5 µM primers, and 0.1 µl of bovine serum albumin (20 mg ml^{-1}).

Purity of amplified fragments was checked by observation of a single melting peak in the melt curve analysis and the presence of a unique DNA band of the expected size after agarose gel electrophoresis (1.7%) and staining with ethidium bromide.

2.10.7.2 Real-Time PCR Assays

DNA extracts of environmental samples (2.10.1) had to be tested in advance for inhibitory effects of coextracted substances by a dilution series of each sample with DNA amounts ranging from 1 to 30 ng µl^{-1}. The two lowest dilutions, which showed no inhibition in the PCR assay were then used for quantification. All environmental samples could be amplified in amounts of 2.5 ng and 1 ng DNA, each in three respective replicates, resulting in six replicates per sample. During each quantification run, a calibration curve was generated by amplifying dilutions steps from 10^{-5} to 10^{-9} of one dilution series of the respective positive-control DNA (2.10.7.1) in four replicates.

Quantification and evaluation of *nirK* and *nirS* gene targets in the environmental samples were done by the Rotor-Gene software comparing the values of the threshold cycles (C_T) of the environmental samples with the C_T values of the respectively performed standard curve. The calculated amount of targets per reaction was converted to target number g^{-1} dw sample using the equation:

$$C = \frac{Cr * Vu}{Vt * ms * dm} * 100\%$$

C: copies [g^{-1} dw sample]
Cr: copies per reaction
Vu: used volume of DNA extract [µl]
Vt = 150 [µl]: total volume of DNA extract
ms: mass of extracted environmental sample [g]
dm: dry matter content of extracted environmental sample [%]
100%: factor

2.11 Statistical Methods to Compare Collected Data

The pairwise t-test was performed to evaluate significances between two mean values as for example in the cropping system without livestock between the manuring treatments w/o L-M and w/o L-FC (Excel 2000, Microsoft, Unterschleißheim). When more than two mean values had to be checked for statistically significant differences, a one-way analysis of variance (ANOVA) was performed using SigmaStat 2.0 (Systat, Erkrath). Averages were compared using the Student-Newman-Keuls (SNK) method (all pairwise multiple comparison procedure). If normality test or equal variance test failed, a one-way analysis of variance on ranks was performed (Kruskal-Wallis test) with subsequent application of the SNK method.

2.12 Integration of Gas Flux Data Collected in the Field Trial

Gas flux data (N_2O and CH_4) collected in the field trial were integrated for each crop, manuring treatment, and season over 365 days using ModelMaker 4 (ModelKinetix, UK). Fluxes were cumulated during the complete cultivation period of the respective crop plus the cultivation time of the preceding intercrop. Consequently, "annual emissions from winter wheat 5 and spelt" consist of emissions determined in those winter cereals during their complete cultivation period of approximately ten months and of emissions from the previous intercrops during two months. "Annual fluxes from spring wheat and potatoes" are composed of emissions from the respective crop during its complete cultivation period of nearly half a year plus emissions from the preceding intercrops also during half a year.
CH_4 effluxes resulting from degassing of CH_4 dissolved in the applied fertilizer were only included for the day of measurement since experience showed a decay of emissions within approximately 24 hours. Likewise, elevated N_2O emissions after manuring were included into the integration only for the day of sampling.

In the season 2002/2003, gas sampling in wL-FYM (cropping system with livestock) started at the end of April 2003 in spelt and at the beginning of May 2003 in potatoes. Therefore, integration of N_2O emissions and CH_4 fluxes over 365 days was not performed in this manuring treatment since data of just two and four months, respectively, were available.
In 2004/2005, gas sampling in spelt could only be carried out until mid March 2005. Thus, integration was initially performed over this sampling time. Afterwards, the unavailable integration value over 365 days was extrapolated using data collected in 2003/2004 (integration over the same sampling period and over 365 days) by applying the rule of three. Additionally, the rule of three was performed with data from season 2002/2003 to extrapolate the missing value again. Both values obtained were averaged to estimate the value of integrated emissions over 365 days in spelt in 2004/2005. However, due to the missing integration value in wL-FYM in 2002/2003 extrapolation of integrated N_2O losses and CH_4 fluxes over 365 days in wL-FYM in 2004/2005 could only be performed in comparison to season 2003/2004.
The amount of N_2O emissions during the winter was calculated by integration of N_2O fluxes between December 1 and March 15 since temperatures < 0°C were only observed during this period in all three seasons.

2.13 Coefficients of Variation for Spatial and Temporal Variability of N_2O and CH_4 Fluxes in the Field Trial

Coefficients of variation (CV) for the mean spatial variability of N_2O and CH_4 fluxes were assessed as follows: CV values of every sampling day were determined demonstrating the ratio of the standard deviation to the average of the fluxes in percent derived from the three parallels. The calculated CV values were then averaged per crop and manuring treatment within a season to obtain the mean spatial variabiltiy of the N_2O and CH_4 fluxes, respectively.

$$CV\% = \frac{\text{standard deviation}}{\text{average}} * 100\%$$

CV values for temporal variabilty of the fluxes were achieved by calculation of the average and the standard deviation of the mean daily fluxes per season, crop, and manuring treatment, and subsequently by the percentage of standard deviation to average in accordance to the formula.

2.14 Coefficients of Correlation between CH_4 Fluxes and Temperatures in the Field Trial

The strength of correlation between CH_4 fluxes and air temperature as well as between CH_4 fluxes and soil temperature in 5 cm depth was described by the coefficient of correlation (r^2) that was performed with Excel 2000.

3 Results

3.1 Field Measurements in the Cropping System without Livestock

3.1.1 N_2O Fluxes and Soil Mineral Nitrogen Contents in Winter Wheat 5

During season 2004/2005, N_2O flux rates in winter wheat 5 and prior intercrops (vetch and oil radish) ranged from 5 to 358 µg N m^{-2} h^{-1} in the control treatment w/o L-M and from -1 to 254 µg N m^{-2} h^{-1} in the biogas treatment w/o L-FC, respectively (figure 3.1). Except the sampling period between mid February and end of March 2005, mostly lower N_2O losses were observed in w/o L-FC compared to w/o L-M that differed significantly on September 28, November 17, and December 8, 2004 (t-test, $P \leq 0.05$). Notably high N_2O effluxes occurred after tillage operations and drilling of wheat (October 5, 2004) in w/o L-M, where growth of intercrops remained on field, were mulched and were subjected to mineralization processes. However, higher N_2O emissions in w/o L-FC were found between February 24 and March 29, 2005 that were in part significanty different despite large standard deviations. Manuring of solid fermented residues on October 5, 2004 and liquid fermented fertilizer on January 31, 2005 in w/o L-FC did not result in elevated N_2O emissions. In contrast, on February 24, 2005, two days after application of liquid fermented fertilizer in w/o L-FC, significantly higher emission rates were detected (246 g N m^{-2} h^{-1}) than in w/o L-M. N_2O losses integrated over 365 days amounted to 4087 g N ha^{-1} in w/o L-M and 3056 g N ha^{-1} in w/o L-FC, respectively, representing a reduction of N_2O emissions by 25% (table 3.1) in w/o L-FC. The coefficient of variation (CV) for the spatial variability of N_2O emissions averaged 84% in w/o L-M and 101% in w/o L-FC (table 3.2). The CV value for the temporal variability of N_2O emissions accounted for 151% in w/o L-M and for 141% in w/o L-FC (table 3.2). During the winter period from December 1 and March 15, proportions of the annual N_2O losses of 25% and 34% were emitted in w/o L-M and w/o L-FC, respectively (table 3.3).

In 2003/2004, N_2O emissions in winter wheat 5 and prior intercrops (vetch and oil radish) were with 0 to 20 µg N m^{-2} h^{-1} (w/o L-FC) and 0 to 25 µg N m^{-2} h^{-1} (w/o L-M) relatively low in both manuring treatments (figure 3.2). A clear trend of lower N_2O emissions in w/o L-FC compared to w/o L-M was observed during the whole measurement period. After soil tillage, with which in w/o L-M growth of intercrops was incorporated into the soil, and wheat drilling on October 14, 2003, N_2O emission rates increased in w/o L-M. In January 2004, low emissions in both treatments occurred, whereas losses of N_2O increased at the end of February and March with highest fluxes on July 7, 2004. On October 23, November 19, December 2, January 15, and March 11, significantly less N_2O was evolved from w/o L-FC. N_2O emissions a few hours after fertilizer application on February 27 and March 25 in w/o L-FC were low (2 and 8 µg N m^{-2} h^{-1}, respectively) and even lower than in w/o L-M (6 and 11 µg N m^{-2} h^{-1}, respectively). However, two days after first manuring (February 29, 2004) more N_2O was emitted in w/o L-FC than in w/o L-M (not significant).

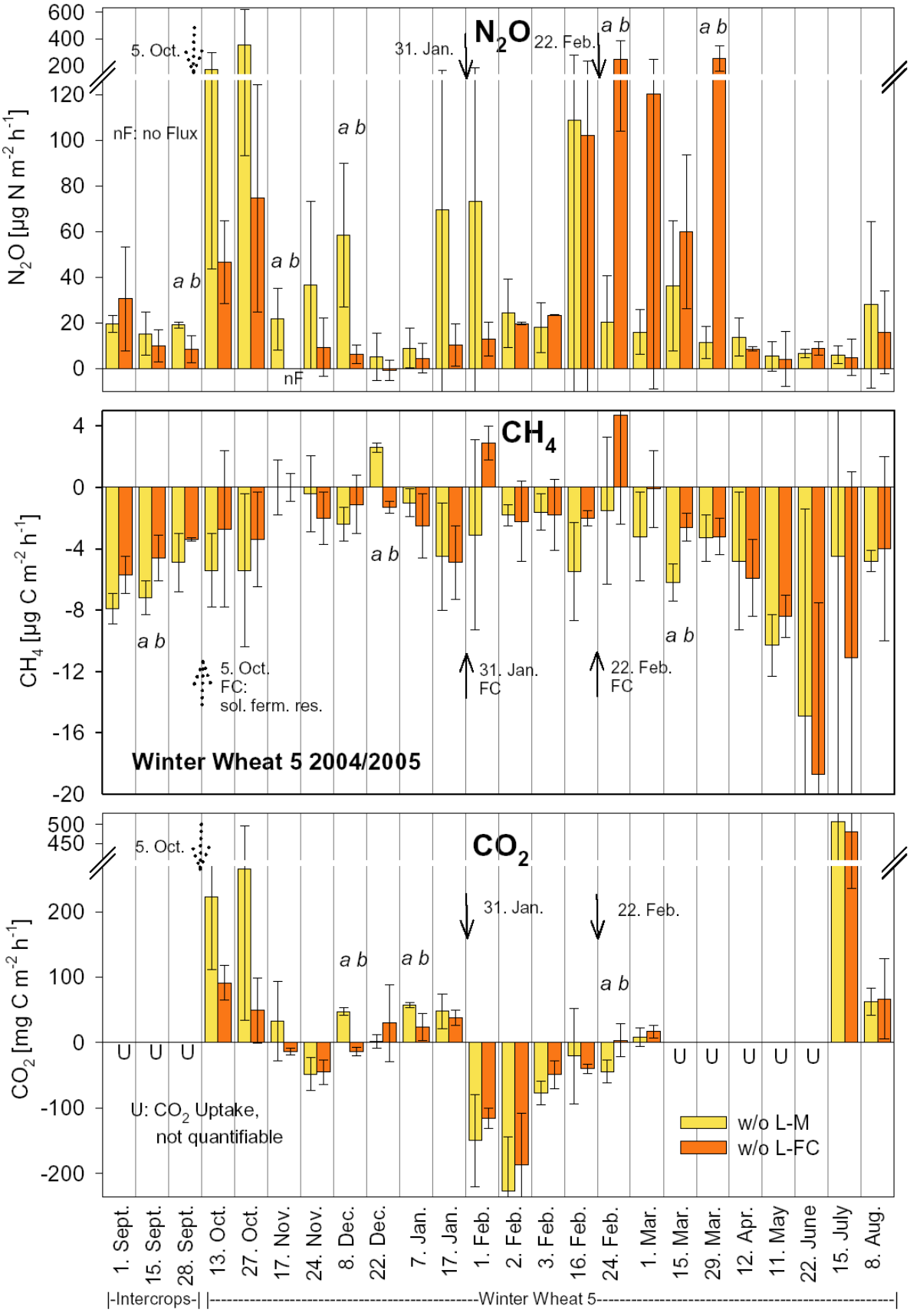


Figure 3.1: N_2O-, CH_4-, and CO_2- fluxes in winter wheat 5 and prior intercrops in the cropping system without livestock in season 2004/2005. Bold arrows: application of liquid fermented fertilizer, dotted arrows: application of solid fermented residues in w/o L-FC.

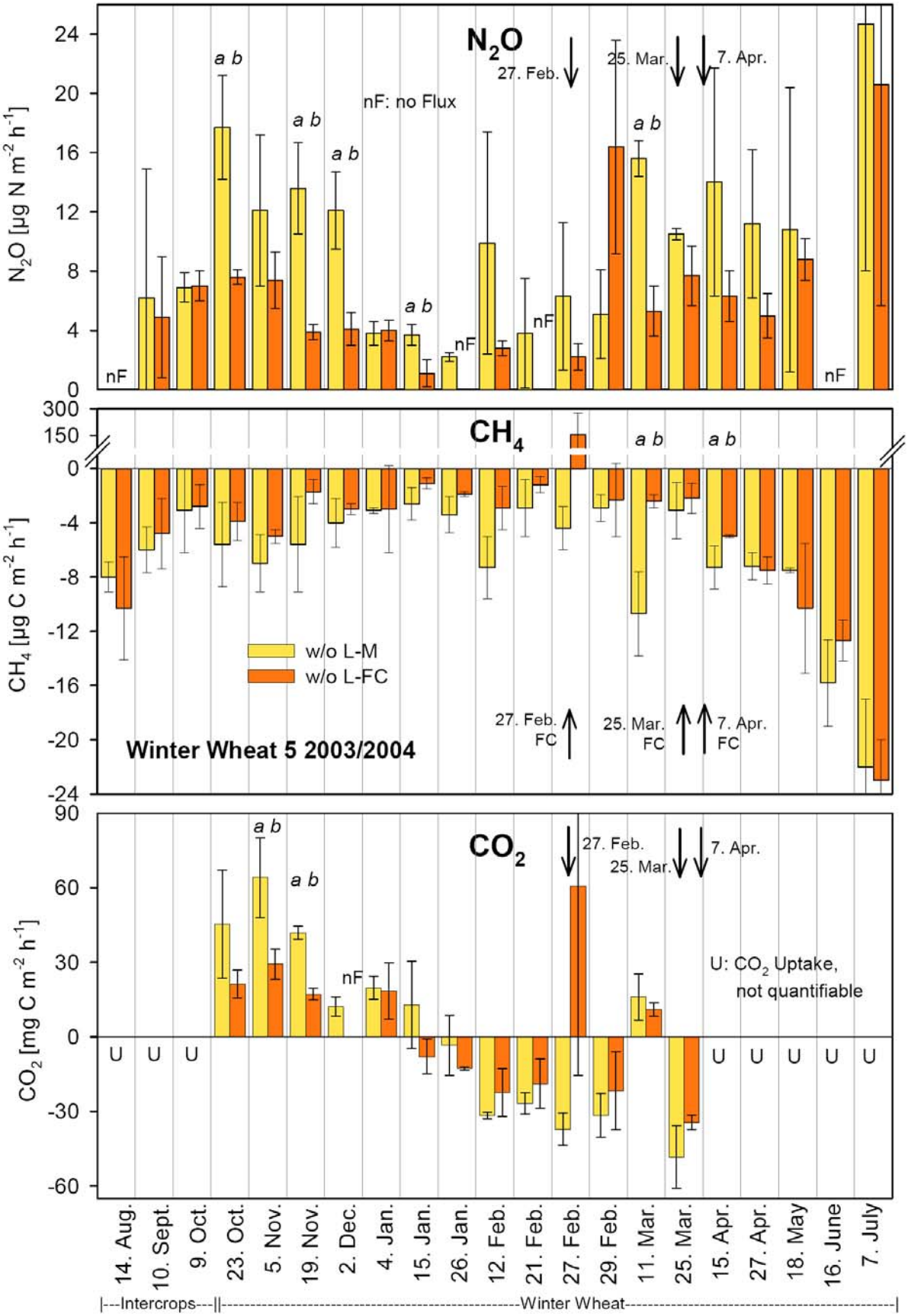


Figure 3.2: N_2O-, CH_4-, and CO_2- fluxes in winter wheat 5 and prior intercrops in the cropping system without livestock in season 2003/2004. Bold arrows: application of liquid fermented fertilizer in w/o L-FC.

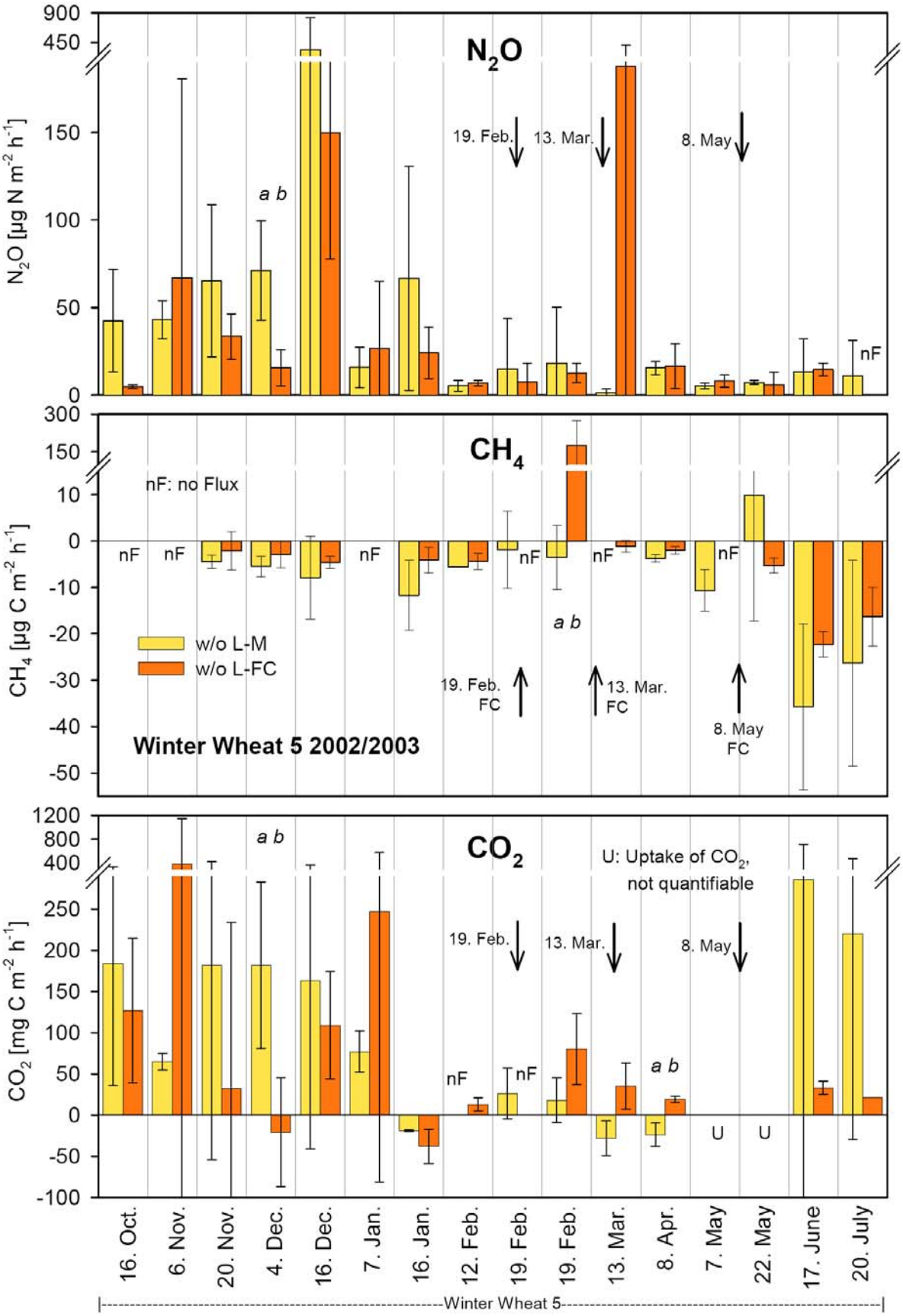


Figure 3.3: N_2O-, CH_4-, and CO_2- fluxes in winter wheat 5 in the cropping system without livestock in season 2002/2003. Bold arrows: application of liquid fermented fertilizer in w/o L-FC.

Table 3.1: Integrated N_2O emissions over 365 days in winter wheat 5 and spring wheat in the cropping system without livestock in w/o L-M and w/o L-FC, respectively.

Season	Crops	Manuring Treatments			
		w/o L-M		w/o L-FC	
		% of w/o L-M	g N ha^{-1}	% of w/o L-M	g N ha^{-1}
2002/2003	Winter Wheat 5	100	2684	56	1497
	Spring Wheat	100	699	146	1023
2003/2004	Winter Wheat 5	100	801	61	490
	Spring Wheat	100	1034	78	802
2004/2005	Winter Wheat 5	100	4087	75	3056
	Spring Wheat	100	765	184	1405

Table 3.2: Coefficients of variation (CV) for temporal and mean spatial variability of the N_2O emissions in winter wheat 5 and spring wheat in the cropping system without livestock in w/o L-M and w/o L-FC, respectively.

Season	Crops	Manuring Treatments			
		w/o L-M		w/o L-FC	
		CV % time	Ø CV % space	CV % time	Ø CV % space
2002/2003	Winter Wheat 5	178	92	150	71
	Spring Wheat	102	61	233	79
2003/2004	Winter Wheat 5	68	43	95	27
	Spring Wheat	118	63	125	71
2004/2005	Winter Wheat 5	151	84	141	101
	Spring Wheat	101	106	191	1581

Integration of N_2O fluxes over 365 days revealed a loss of 801 g N ha^{-1} in w/o L-M and 490 g N ha^{-1} in w/o L-FC that means a decrease of 39% in w/o L-FC (table 3.1). The coefficient of variation for the mean spatial variability of N_2O emissions amounted to 43% in w/o L-M and to 27% in w/o L-FC (table 3.2). The CV value for the temporal variability of N_2O emissions accounted for 68% in w/o L-M and for 95% in w/o L-FC (table 3.2). In the winter period between December 1 and March 15, 22% and 16% of the annual N_2O losses were emitted in w/o L-M and w/o L-FC, respectively (table 3.3).

N_2O emissions during 2002/2003 in winter wheat 5 varied between 2 and 343 µg N m^{-2} h^{-1} in w/o L-M and between 0 and 188 µg N m^{-2} h^{-1} in w/o L-FC (figure 3.3). The outstanding high N_2O loss on December 16, 2002 with 343 µg N m^{-2} h^{-1} in w/o L-M did not differ statistically from the emission rate of 150 µg N m^{-2} h^{-1} in w/o L-FC due to large standard deviation. Similarily, the

very high emission peak on March 13, 2003 (188 g N m^{-2} h^{-1}) in w/o L-FC was not significant. Emission levels in both treatments between February and July 2003 were relatively low (except March 13) varying from 0 to 18 g N m^{-2} h^{-1}. Application of liquid fermented fertilizer in w/o L-FC on February 19, 2003 did not result in elevated N_2O fluxes contrary to manuring on March 13, when the very high emission rate described before was observed. Only on December 4, 2002 a significant difference between the fluxes of both manuring treatments could be determined. Integration of N_2O losses over 365 days accounted for 2684 g N ha^{-1} in w/o L-M and 1497 g N ha^{-1} in w/o L-FC, thus revealing a flux reduction of 44% in w/o L-FC (table 3.1). The spatial variability of N_2O emissions averaged 92% in w/o L-M and 71% in w/o L-FC (table 3.2). The CV value for the temporal variability of N_2O emissions accounted for 178% in w/o L-M and for 150% in w/o L-FC (table 3.2). During the winter period 46% and 47% of the annual N_2O losses were emitted in w/o L-M and w/o L-FC, respectively (table 3.3).
Comparing annual N_2O losses in winter wheat 5 during the seasons 2004/2005, 2003/2004, and 2002/2003 showed lowest emissions in 2003/2004 in both, the biogas treatment w/o L-FC and the control treatment w/o L-M, respectively. In all three investigated seasons, w/o L-FC clearly revealed in comparison to w/o L-M lower N_2O emissions, where crop residues and intercrops were taken off the field, fermented in the biogas plant and applied as fertilizer when nutrient demand of the crops occurred. Neither an explicit pattern of N_2O fluxes over the course of a season that appeared in all measurement periods emerged nor a regularity of N_2O emission pattern after fertilizer application could be observed in the investigated seasons.

Table 3.3: Integrated N_2O emissions during the winter period (December 1 - March 15) in winter wheat 5 and in intercrops before spring wheat in the cropping system without livestock in w/o L-M and w/o L-FC, respectively.

Season	Crops	Manuring Treatments			
		w/o L-M		w/o L-FC	
		Winter %	Winter g N ha^{-1}	Winter %	Winter g N ha^{-1}
2002/2003	Winter Wheat 5	46	1244	47	704
	Intercrops	29	201	47	482
2003/2004	Winter Wheat 5	22	173	16	77
	Intercrops	36	377	19	151
2004/2005	Winter Wheat 5	25	1026	34	1028
	Intercrops	30	228	16	223

Soil NO_3^- concentrations in 2002/2003 in winter wheat 5 during the first five months of sampling were with 0.5 to 2 g N m^{-2} in 30 cm soil depth (ploughed layer) very low in w/o L-M and w/o L-FC, respectively (figure 3.4). However, lower amounts of NO_3^- were shown in w/o L-FC that were significantly different on December 16, 2002 and January 7, 2003. A significantly

higher concentration of NO_3^- in w/o L-FC was only observed on April 8, 2003. NH_4^+ concentrations were for all but one sampling time at the detection limit. No relationship was found between soil mineral nitrogen concentrations and the amount of emitted N_2O.

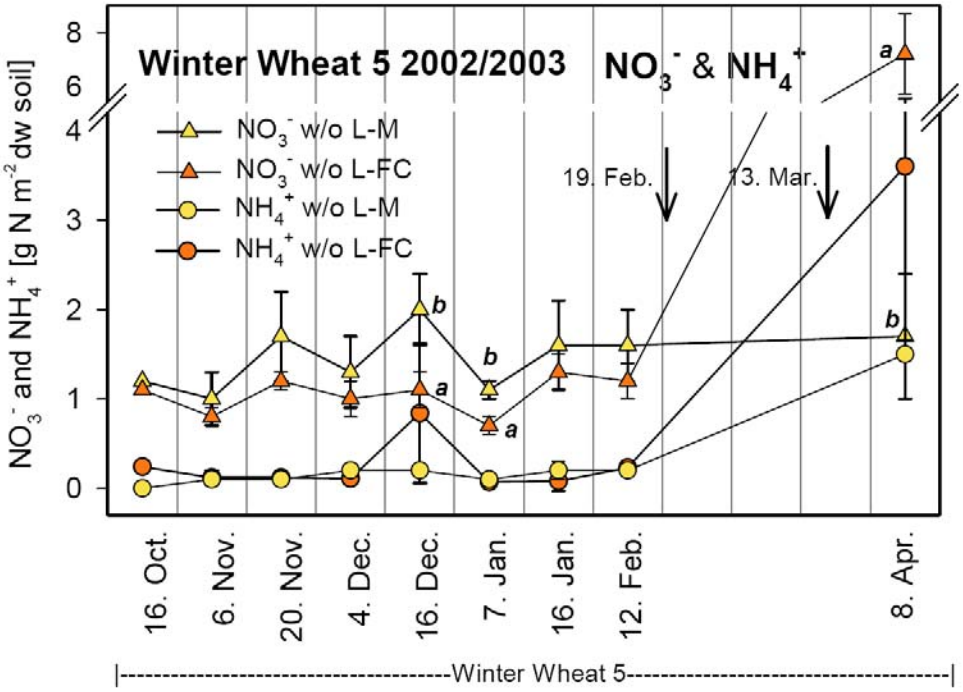


Figure 3.4: NO_3^--and NH_4^+- concentrations in winter wheat 5 in the cropping system without livestock in season 2002/2003.

NO_3^-- and NH_4^+- contents in soil depths of 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm in winter wheat 5 were investigated in fall/winter and spring revealing amounts of mineral nitrogen from 33 to 81 kg N ha^{-1} in w/o L-M and from 31 to 116 kg N ha^{-1} in w/o L-FC (figure 3.5). Each sampling in March showed (in part significantly) higher N_{min} or NO_3^- concentrations in w/o L-FC, whereas in November/December more N_{min} was found in w/o L-M except in 2003 when similar amounts were observed. Ammonium values were mostly relatively small and negligible. All significant differences found by comparison of NO_3^- and NH_4^+ concentrations in the different soil layers between the manuring treatments are given in table 3.4.

Contents of total nitrogen and total carbon were determined in all fields of the cropping system without livestock in the ploughed layer (30 cm soil depth) in spring 2001 (before starting the differentiated manuring) as well as in spring 2004. In table 3.5 the differences of total N and total C concentrations after three years of applying the different manuring treatments are shown. Fields are named with the first main crop cultivated in season 2001/2002 that was followed by consecutive crops according to the crop rotation (table 3.1). Differences of total N varied between -0.120 and 0.019 mg N g^{-1} and differences of total C accounted for -1.929 to 0.119 mg C g^{-1} over all fields and manuring treatments.

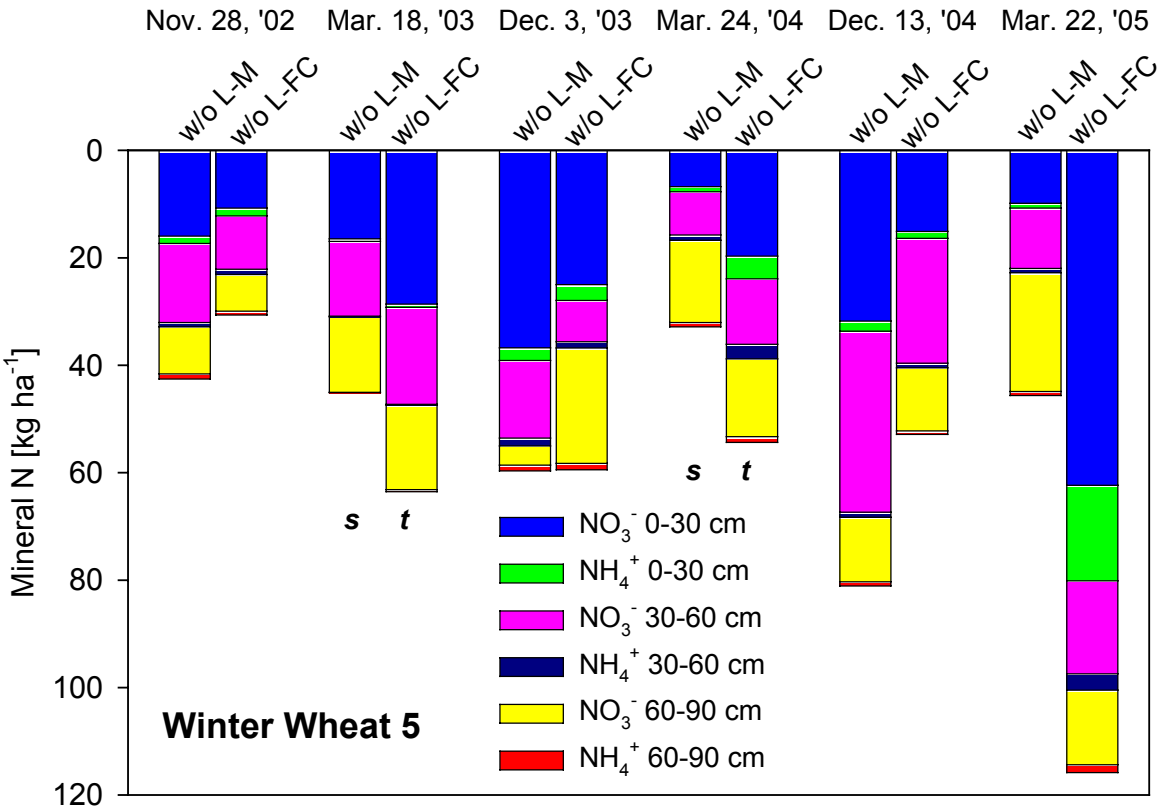


Figure 3.5: Amounts of NO_3^--N and NH_4^+-N in winter wheat 5 in the cropping system without livestock between November 2002 and March 2005 in soil depths of 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm, respectively, in the different manuring treatments. (Data: Kurt Möller and Walter Stinner, Giessen, personal communication)

Table 3.4: Significant differences in amounts of NO_3^-, NH_4^+, and N_{min} in winter wheat 5 in the cropping system without livestock between November 2002 and March 2005 in the different manuring treatments. (Data: Kurt Möller and Walter Stinner, Giessen, personal communication)

		w/o L-M	w/o L-FC
Mar. 18, 2003	NO_3^- 0 - 30 cm	a	b
	NO_3^- cumulative	a	b
	NH_4^+ 60 - 90 cm	x	y
	N_{min} total	s	t
Mar. 24, 2004	NO_3^- 0 - 30 cm	a	b
	NH_4^+ 0 - 30 cm	x	y
	N_{min} total	s	t
Dec. 13., 2004	NO_3^- 0 - 30 cm	a	b
	NO_3^- 30 - 60 cm	a	b
Mar. 22, 2005	NO_3^- 0 - 30 cm	a	b
	NO_3^- cumulative	a	b

However, no significant differences were found, neither between the treatments within one field (t-test) comparing nitrogen and carbon concentrations, respectively, nor between soil nitrogen and carbon contents, respectively, within a manuring treatment (ANOVA) comparing the six fields. Amounts of total N ranged in 2001 from 1.022 to 1.531 mg N g^{-1} dw soil and in 2004 from 0.543 to 1.606 mg N g^{-1} dw soil, concentrations of total C varied in 2001 between 8.148 and 13.432 mg C g^{-1} dw soil and in 2004 between 8.219 and 13.805 mg C g^{-1} dw soil.

Table 3.5: Differences of soil total nitrogen and total carbon concentrations in the cropping system without livestock after three years of processing differentiated manuring treatments. Soil sampling in spring 2004 in the denoted crops and in spring 2001 before start of the cropping system without livestock and different manuring treatments. Positive and negative numbers mean increase and decrease, respectively, of total N or total C between 2001 and 2004. Numbers in parentheses are standard deviations. Amounts of total N ranged in 2001 from 1.022 to 1.531 mg N g^{-1} dw soil and in 2004 from 0.543 to 1.606 mg N g^{-1} dw soil, concentrations of total C varied in 2001 between 8.148 and 13.432 mg C g^{-1} dw soil and in 2004 between 8.219 and 13.805 mg C g^{-1} dw soil. (Data: Kurt Möller and Walter Stinner, Giessen, personal communication)

Sampled Crops in Spring 2004	Differences between 2001 – 2004			
	Total Nitrogen [mg g^{-1} dw soil]		Total Carbon [mg g^{-1} dw soil]	
	w/o L-M	w/o L-FC	w/o L-M	w/o L-FC
Spring Wheat	-0.035 (± 0.038)	-0.073 (± 0.084)	-0.291 (± 0.488)	-0.632 (± 0.908)
Lucerne-Grass-Mix	0.019 (± 0.120)	-0.098 (± 0.164)	0.119 (± 0.721)	-0.705 (± 1.896)
Intercrops before Potatoes	-0.041 (± 0.088)	-0.028 (± 0.083)	0.078 (± 0.774)	-0.109 (± 0.780)
Winter Wheat 3	-0.030 (± 0.081)	-0.081 (± 0.038)	-0.130 (± 0.677)	-0.321 (± 0.714)
Pea	-0.023 (± 0.114)	-0.030 (± 0.057)	-0.255 (± 1.081)	-0.115 (± 0.779)
Winter Wheat 5	-0.120 (± 0,155)	-0.231 (± 0.342)	-1.215 (± 1.479)	-1.929 (± 3.219)

3.1.2 N_2O Fluxes and Soil Mineral Nitrogen Contents in Intercrops and Spring Wheat

During season 2004/2005 N_2O fluxes in intercrops (vetch and oil radish) and spring wheat ranged from 1 to 47 µg N m^{-2} h^{-1} in the control treatment w/o L-M and from 0 to 157 µg N m^{-2} h^{-1} in the biogas treatment w/o L-FC (figure 3.6). With exception of three sampling dates, emissions did not exceed 16 µg N m^{-2} h^{-1}. Harvest of intercrops in w/o L-FC on November 1, 2004 neither increased nor decreased N_2O losses in comparison to w/o L-M, also no explicit trend of elevated N_2O fluxes in one of the manuring treatments became apparent during the investigated period. Significantly higher emission rates in w/o L-FC compared to w/o L-M were observed after drilling of spring wheat on March 29 and on May 11, 2005. Integrated N_2O

fluxes over 365 days revealed a loss of 699 g N ha^{-1} in w/o L-M and 1023 g N ha^{-1} in w/o L-FC, meaning an elevated emission of 46% in w/o L-FC (table 3.1). The coefficient of variation for the spatial variability of N_2O emissions averaged 106% in w/o L-M and an exceedingly high value of 1581% in w/o L-FC (table 3.2). The CV value for the temporal variability of N_2O emissions accounted for 101% in w/o L-M and for 191% in w/o L-FC (table 3.2). During the winter period from December 1 and March 15, proportions of the annual N_2O losses of 30% and 16% were emitted in w/o L-M and w/o L-FC, respectively (table 3.3).

In 2003/2004 N_2O emission rates in intercrops (vetch and oil radish) and spring wheat varied between -5 and 70 µg N m^{-2} h^{-1} in w/o L-M and 0 and 48 µg N m^{-2} h^{-1} in w/o L-FC (figure 3.7). Noticeably low or even no fluxes were observed at the beginning of the sampling period in intercrops that still continued after harvest of intercrops in w/o L-FC on October 24, 2003. Incorporating solid fermented residues in w/o L-FC on February 19, 2004 did not result in elevated N_2O emissions. After drilling of spring wheat on February 20, 2004, higher fluxes in w/o L-M compared to w/o L-FC were determined. Signifiantly decreased emissions were found on February 12, March 11, and May 18, 2004 in w/o L-FC. The trend of lower N_2O fluxes in w/o L-FC was confirmed by integrated emission rates over 365 days exhibiting a loss of 1034 g N ha^{-1} in w/o L-M compared to 802 g N ha^{-1} in w/o L-FC, an abatement of 22% N_2O (table 3.1). The mean spatial variability of N_2O emissions (CV) amounted to 63% in w/o L-M and to 71% in w/o L-FC (table 3.2). The CV value for the temporal variability of N_2O emissions accounted for 118% in w/o L-M and for 125% in w/o L-FC (table 3.2). In the winter period between December 1 and March 15, 36% and 19% of the annual N_2O losses were emitted in w/o L-M and w/o L-FC, respectively (table 3.3).

N_2O emission rates in 2002/2003 in intercrops (vetch and oil radish) and spring wheat ranged between 0 and 40 µg N m^{-2} h^{-1} in w/o L-M and 0 and 214 µg N m^{-2} h^{-1} in w/o L-FC (figure 3.8). With exception of outstanding high N_2O losses on December 16, 2002 notabely in w/o L-FC (214 µg N m^{-2} h^{-1}), emissions did not rise above 28 µg N m^{-2} h^{-1} in both manuring treatments. During the investigated period, no significant differences of N_2O fluxes could be observed, not even on December 16, 2002 due to very large standard deviations in both treatments. There was no obvious trend of increased or decreased emissions recognizable in one manuring treatment, however, integration of N_2O fluxes over 365 days showed losses of 765 g N ha^{-1} in w/o L-M and 1405 g N ha^{-1} in w/o L-FC, meaning an additional N loss of 84% in w/o L-FC (table 3.1). The spatial variability of N_2O emissions averaged 61% in w/o L-M and 79% in w/o L-FC (table 3.2). The CV value for the temporal variability of N_2O emissions accounted for 102% in w/o L-M and for 233% in w/o L-FC (table 3.2). During the winter period 29% and 47% of the annual N_2O losses were emitted in w/o L-M and w/o L-FC, respectively (table 3.3).

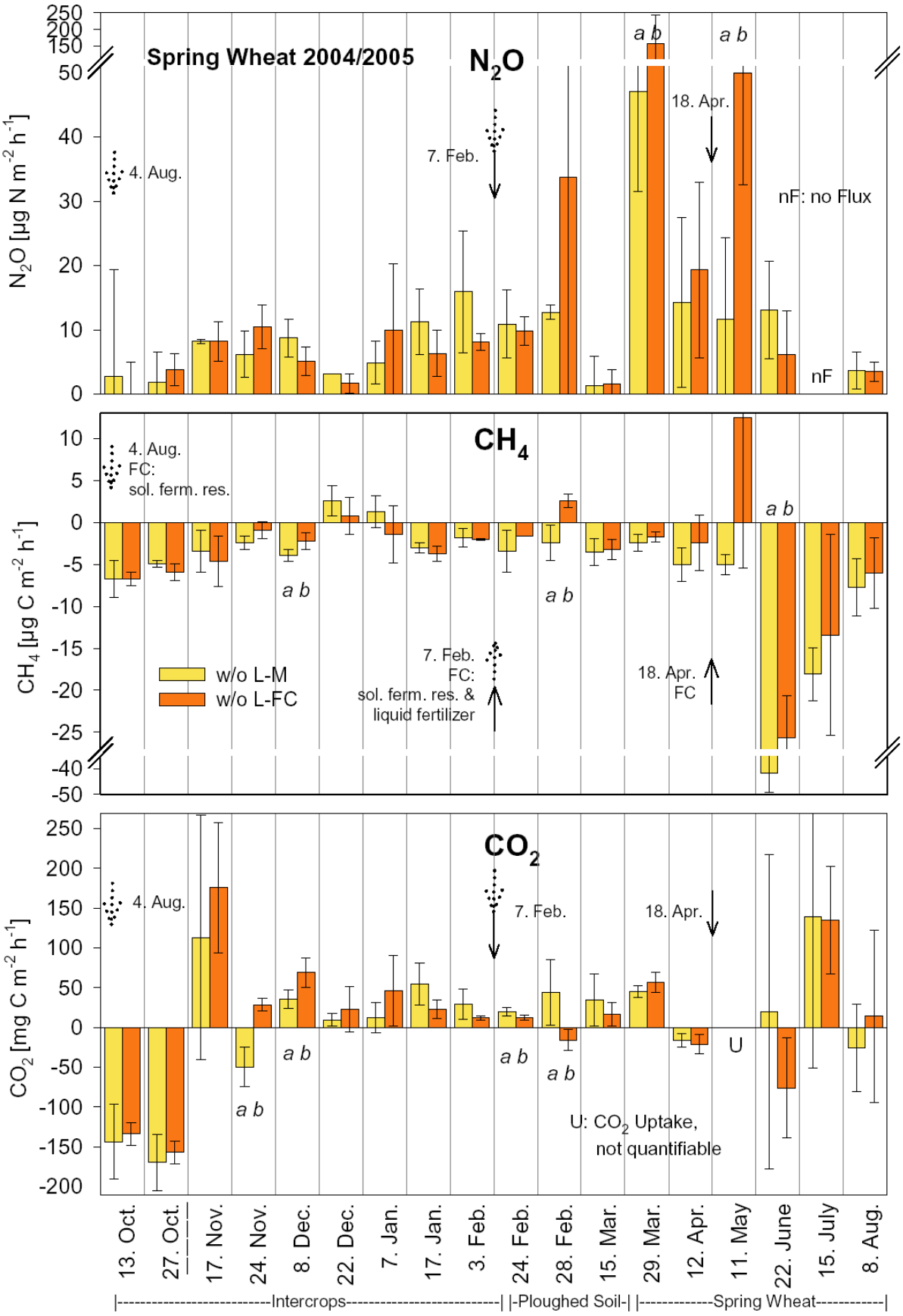


Figure 3.6: N_2O-, CH_4-, and CO_2- fluxes in intercrops and spring wheat in the cropping system without livestock in season 2004/2005. Bold arrows: application of liquid fermented fertilizer, dotted arrows: application of solid fermented residues in w/o L-FC.

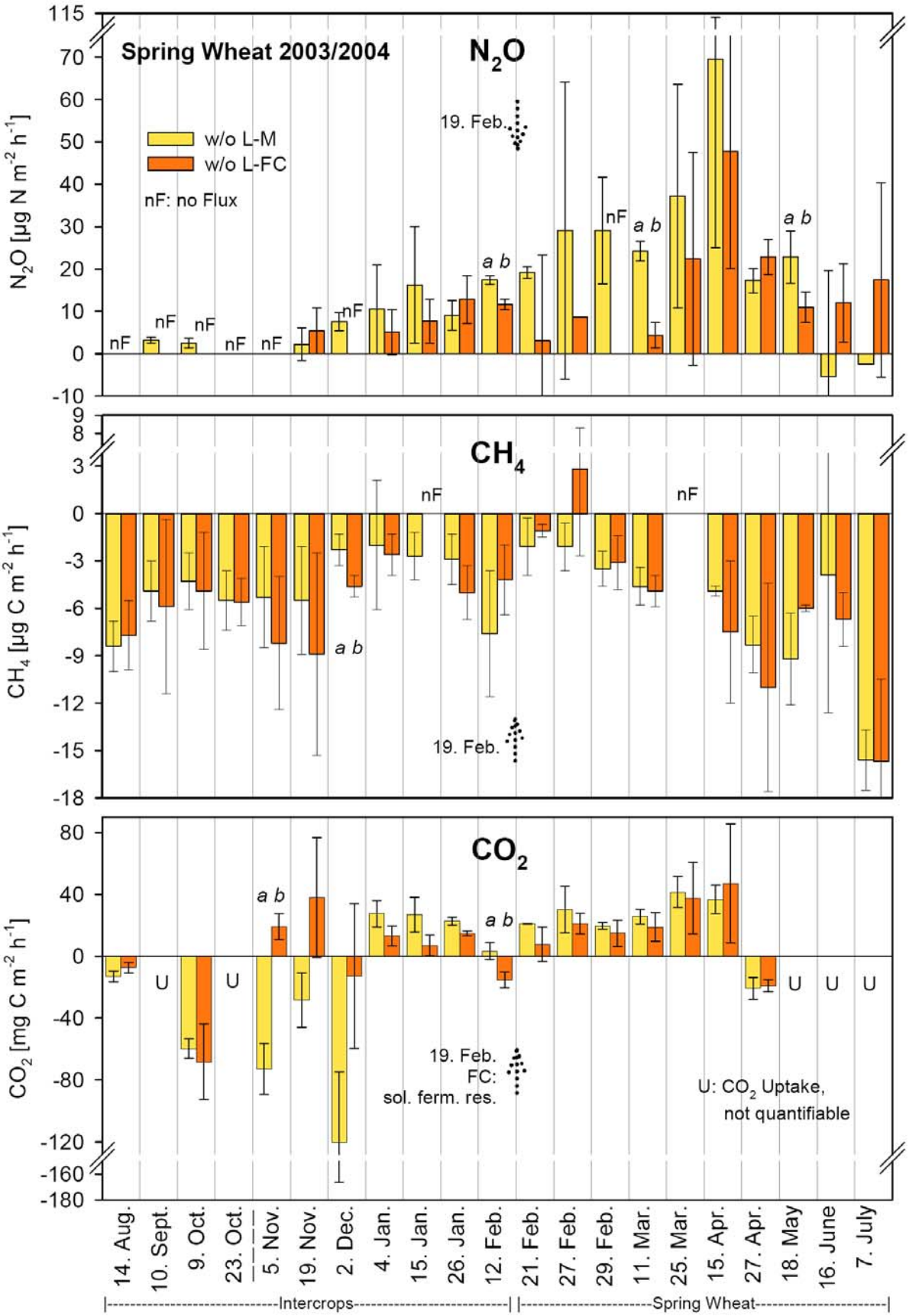


Figure 3.7: N_2O-, CH_4-, and CO_2- fluxes in intercrops and spring wheat in the cropping system without livestock in season 2003/2004. Dotted arrows: application of solid fermented residues in w/o L-FC.

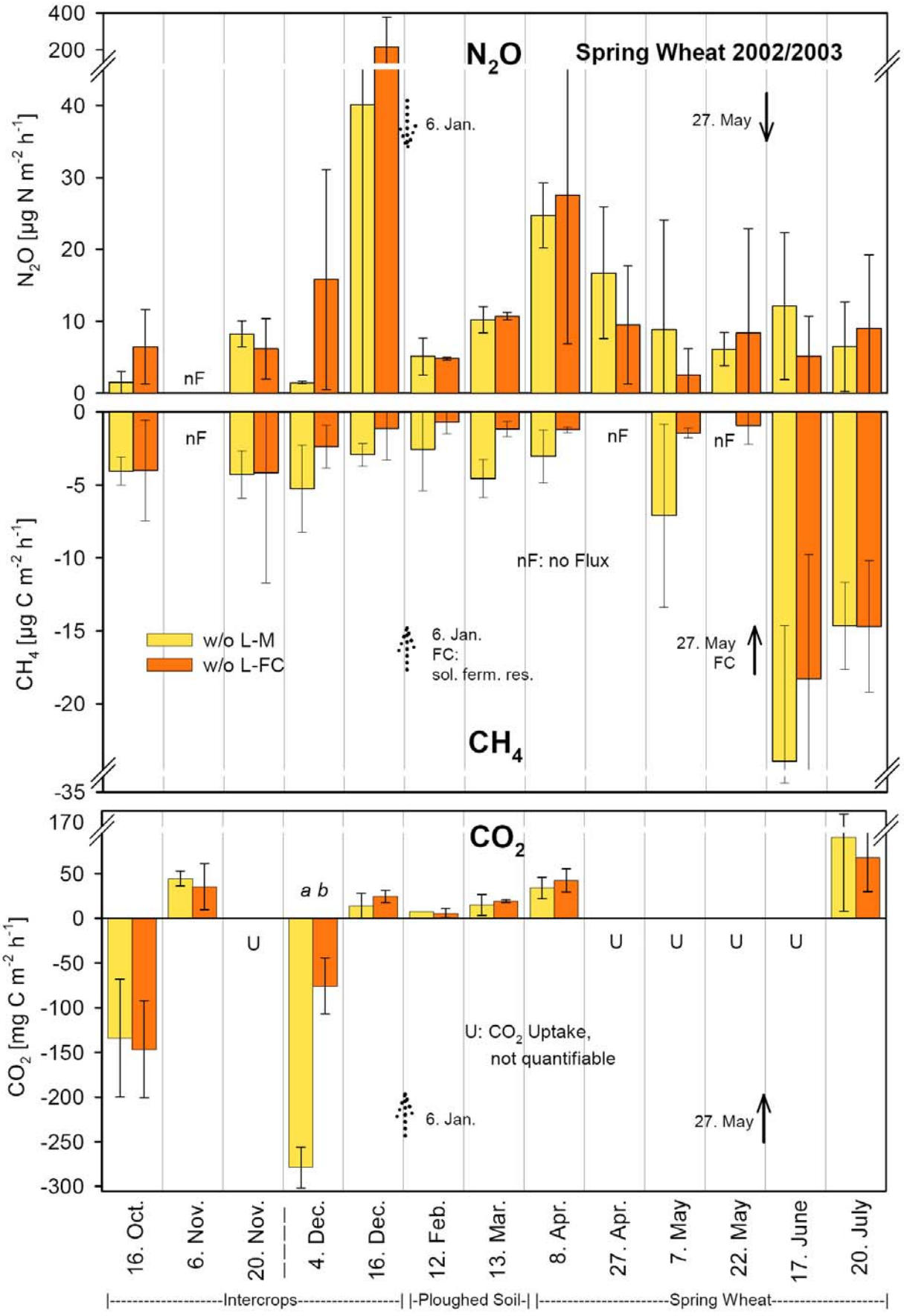


Figure 3.8: N_2O-, CH_4-, and CO_2- fluxes in intercrops and spring wheat in the cropping system without livestock in season 2002/2003. Bold arrows: application of liquid fermented fertilizer, dotted arrows: application of solid fermented residues in w/o L-FC.

The comparison of results of annual N_2O losses during the seasons 2004/2005, 2003/2004, and 2002/2003 in intercrops and spring wheat revealed in 2003/2004 lowest emissions in the biogas treatment w/o L-FC, but highest emissions in the control treatment w/o L-M. This finding is in contrast to observations made in winter wheat 5 also within the cropping system without livestock. In 2002/2003 and 2004/2005, w/o L-FC showed increased N_2O emissions compared to w/o L-M, whereas in 2003/2004 reduced N_2O fluxes were observed in w/o L-FC. However, in winter wheat 5 an abatement of N losses could be confirmed in all three investigation periods in w/o L-FC. As in winter wheat 5 no definite pattern of N_2O fluxes over the course of a season became apparent in all measurement periods.

Soil NO_3^- concentrations between October 2002 and April 2003 in intercrops and spring wheat were slightly lower at the majority of samplings in w/o L-FC than in w/o L-M, however without significance (figure 3.9). Amounts of NO_3^- ranged between 0.6 and 6.2 g N m^{-2} in 30 cm soil depth (ploughed layer). NH_4^+ concentrations were mostly at the detection limit. On April 8 significantly more NH_4^+ was found in w/o L-FC (2.3 g N m^{-2}) than in w/o L-M (0.5 g N m^{-2}). Thus, approximately similar observations concerning soil mineral nitrogen concentrations were made in intercrops and spring wheat compared to winter wheat 5, both within the cropping system without livestock, during the first months of season 2002/2003. Again, there was no relationship between NO_3^- and NH_4^+ concentrations and the amount of emitted N_2O.

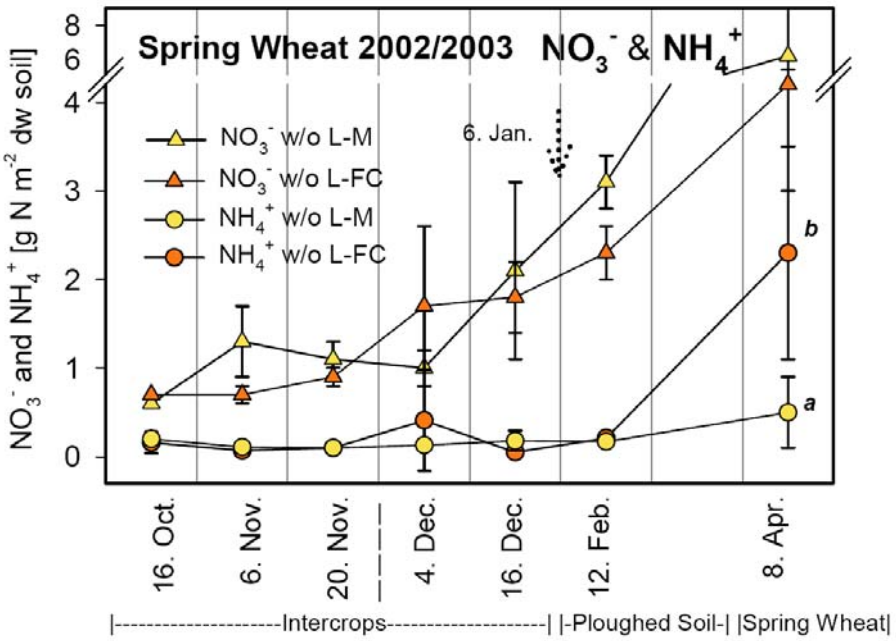


Figure 3.9: NO_3^--and NH_4^+- concentrations in intercrops and spring wheat in the cropping system without livestock in season 2002/2003.

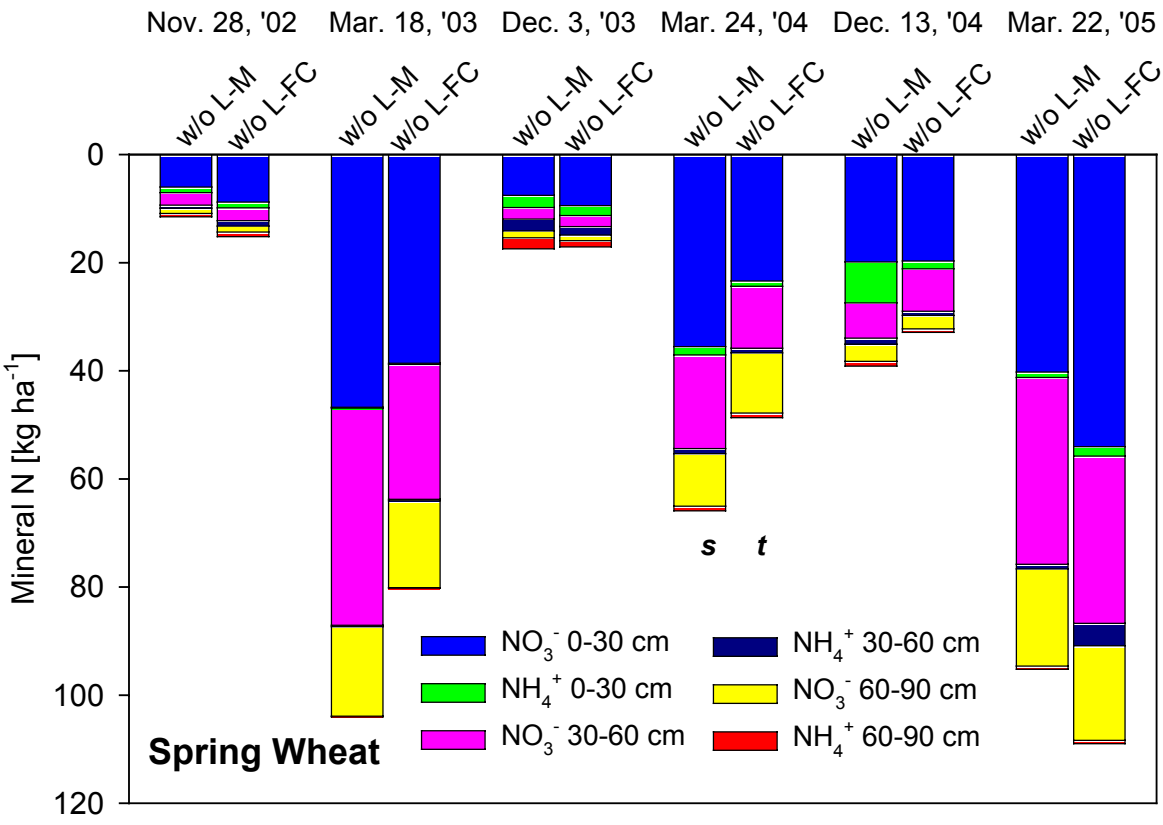


Figure 3.10: Amounts of NO_3^--N and NH_4^+-N in spring wheat and intercrops in the cropping system without livestock between November 2002 and March 2005 in soil depths of 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm, respectively, in the different manuring treatments. (Data: Kurt Möller and Walter Stinner, Giessen, personal communication)

Table 3.6: Significant differences in amounts of NO_3^-, NH_4^+, and N_{min} in spring wheat and intercrops in the cropping system without livestock between November 2002 and March 2005 in the different manuring treatments. (Data: Kurt Möller and Walter Stinner, Giessen, personal communication)

		w/o L-M	w/o L-FC
Mar. 24, 2004	NO_3^- 0 - 30 cm	a	b
	NO_3^- 30 - 60 cm	a	b
	NO_3^- cumulative	a	b
	N_{min} total	s	t
Dec. 13, 2004	NH_4^+ 30 - 60 cm	x	y
	NH_4^+ cumulative	x	y

Amounts of soil mineral nitrogen in 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm soil depth in intercrops and spring wheat investigated in fall/winter and spring ranged between 12 and 106 kg N ha^{-1} in w/o L-M and between 15 and 109 kg N ha^{-1} in w/o L-FC (figure 3.10). Ammonium values were mostly relatively small and negligible. Sampling in fall/winter showed lower NO_3^-- and NH_4^+- concentrations than in March, however, no trend of increased or

decreased mineral nitrogen could be observed comparing the manuring treatments. Only on December 13, 2004 a significant difference between N_{min} values of w/o L-M and w/o L-FC was determined. All significant differences found by comparison of NO_3^- and NH_4^+ concentrations in the different soil layers between the manuring treatments are given in table 3.6.

3.1.3 CH_4 Fluxes in Winter Wheat 5

As expected, the arable soil functioned as CH_4 sink during the whole season 2004/2005 in winter wheat 5 and prior intercrops with only three excepted sampling dates (figure 3.1). CH_4 oxidation rates ranged between 0 and 15 µg C m^{-2} h^{-1} in w/o L-M and between 0 and 19 µg C m^{-2} h^{-1} in w/o L-FC. Highest CH_4 uptake rates of the soil occurred in May, June, and July 2005. CH_4 emissions were observed in w/o L-FC on February 1 (2.9 µg C m^{-2} h^{-1}) and February 24, 2005 (4.7 µg C m^{-2} h^{-1}), i.e. one and two days after application of liquid fermented fertilizer, which derived from CH_4 dissolved in the fertilizer. However, two days after manuring end of January, no CH_4 emissions could be measured anymore but CH_4 uptake. The only efflux of CH_4 in w/o L-M was detected in winter wheat 5 on December 22, 2004 (2.6 µg C m^{-2} h^{-1}), that differed significantly from CH_4 uptake in w/o L-FC. Further significant differences were determined in intercrops on September 15, 2004 and in winter wheat 5 on March 15, 2005. CH_4 fluxes integrated over 365 days resulted in similar carbon uptakes of 479 g C ha^{-1} in w/o L-M and 478 g C ha^{-1} in w/o L-FC, respectively (table 3.7). Generally, emissions derived from CH_4 outgassing of the fertilizer (here on February 1 and 24, 2005) were included into the integration for the day of measurement and into the calculation of the coefficients of variation. Mean spatial variabilities of CH_4 fluxes of 256% and exceedingly high 905% were assessed in w/o L-M and in w/o L-FC, respectively (table 3.8). The CV values for temporal variability of the CH_4 fluxes amounted to 85% in w/o L-M and 131% in w/o L-FC (table 3.8). Coefficients of correlation (r^2) between CH_4 fluxes in winter wheat 5 and air temperature and between CH_4 fluxes and temperature in 5 cm soil depths accounted for 0.37 and 0.51, respectively (table 3.9).

CH_4 fluxes measured in 2003/2004 in winter wheat 5 and prior intercrops similarily showed a continuous CH_4 uptake of the soil with rates between 2.6 and 22 µg C m^{-2} h^{-1} in w/o L-M and between 1.1 and 23 µg C m^{-2} h^{-1} in w/o L-FC (figure 3.2). High CH_4 oxidation rates were observed in June and July 2004. A single CH_4 emission peak occurred a few hours after application of liquid fermented fertilizer on February 27, 2004 in w/o L-FC, when 153 µg C m^{-2} h^{-1} (CH_4 dissolved in the fertilizer) were evolved. However, this efflux event was not significant. Manuring on March 25 and April 7, 2004 did not result in CH_4 emissions at the investigated dates. The only significantly decreased CH_4 oxidation rates in w/o L-FC were found on March 11 and April 15, 2004. Integration of CH_4 fluxes over 365 days led to amounts of carbon uptake of 663 g C ha^{-1} in w/o L-M and 546 g C ha^{-1} in w/o L-FC, thus a reduction of

18% in w/o L-FC (table 3.7). The spatial variability of CH_4 fluxes averaged 37% in w/o L-M and 42% in w/o L-FC (table 3.8). The CV value for the temporal variability of CH_4 fluxes accounted for 71% in w/o L-M and for exceedingly high 1592% in w/o L-FC (table 3.8). Coefficients of correlation of 0.37 and 0.51 between CH_4 fluxes and air and soil temperature in 5 cm soil depth, respectively, were calculated (table 3.9).

During season 2002/2003, CH_4 oxidation rates in winter wheat 5 accounted for 0 to 36 µg C m^{-2} h^{-1} in w/o L-M and for 0 to 22 µg C m^{-2} h^{-1} in w/o L-FC (figure 3.3). The large CH_4 influxes occurred in June and July 2003. Due to manuring of liquid fermented fertilizer in w/o L-FC on February 19, 2003, a very high CH_4 emission peak of 175 µg C m^{-2} h^{-1} was observed approximately six hours after application (significant), whereas determination of a CH_4 flux directly after fertilizing was not possible. However, manuring on March 13, 2003 did not result in CH_4 efflux. A single observation of a non-significant CH_4 emission in w/o L-M was made on May 22, 2003 when 10 µg C m^{-2} h^{-1} evolved. No CH_4 fluxes could be observed on October 16, November 6, 2002, and January 7, 2003 in both treatments, on February 19 and May 7, 2003 in w/o L-FC, and on March 13, 2003 in w/o L-M. Integration of CH_4 fluxes over 365 days showed a carbon uptake of 855 g C ha^{-1} in w/o L-M and 476 g C ha^{-1} in w/o L-FC, representing a decrease of 44% in w/o L-FC (table 3.7). CH_4 emission on May 22, 2003 in w/o L-M was included in integration for one day. Mean spatial variabilities of CH_4 fluxes of 84% and 45% were assessed in w/o L-M and in w/o L-FC, respectively (table 3.8). The CV values for temporal variability of the CH_4 fluxes amounted to 163% in w/o L-M and 661% in w/o L-FC (table 3.8). Correlation coefficients (r^2) between CH_4 fluxes in winter wheat 5 and air temperature and between CH_4 fluxes and temperature in 5 cm soil depths accounted for 0.34 and 0.31, respectively (table 3.9).

Table 3.7: Integrated CH_4 fluxes over 365 days in winter wheat 5 and spring wheat in the cropping system without livestock in w/o L-M and w/o L-FC, respectively.

Season	Crops	Manuring Treatments			
		w/o L-M		w/o L-FC	
		% of w/o L-M	g C ha^{-1}	% of w/o L-M	g C ha^{-1}
2002/2003	Winter Wheat 5	100	-855	56	-476
	Spring Wheat	100	-585	90	-529
2003/2004	Winter Wheat 5	100	-663	82	-546
	Spring Wheat	100	-502	109	-547
2004/2005	Winter Wheat 5	100	-479	100	-478
	Spring Wheat	100	-714	90	-644

Table 3.8: Coefficients of variation (CV) for temporal and mean spatial variability of the CH_4 fluxes in winter wheat 5 and spring wheat in the cropping system without livestock in w/o L-M and w/o L-FC, respectively.

Season	Crops	Manuring Treatments			
		w/o L-M		w/o L-FC	
		CV % time	Ø CV % space	CV % time	Ø CV % space
2002/2003	Winter Wheat 5	163	84	661	45
	Spring Wheat	121	38	110	73
2003/2004	Winter Wheat 5	71	37	1592	42
	Spring Wheat	68	56	77	47
2004/2005	Winter Wheat 5	85	256	131	905
	Spring Wheat	159	47	205	76

Table 3.9: Correlation coefficients (r^2) between CH_4 fluxes and air temperature as well as between CH_4 fluxes and soil temperature in 5 cm depth. CH_4 emissions resulting from fertilizer application were excluded for correlation analysis.

Season	Crops	CH_4 Fluxes – Air Temp.	CH_4 Fluxes – Soil Temp.
2002/2003	Winter Wheat 5	0.34	0.31
	Spring Wheat	0.47	0.50
	Spelt	0.57	0.58
	Potatoes	0.48	0.44
2003/2004	Winter Wheat 5	0.37	0.51
	Spring Wheat	0.46	0.44
	Spelt	0.57	0.64
	Potatoes	0.29	0.27
2004/2005	Winter Wheat 5	0.37	0.51
	Spring Wheat	0.42	0.51
	Spelt	0.28	0.46

Comparing results of annual CH_4 fluxes in winter wheat 5 and prior intercrops during the seasons 2002/2003, 2003/2004, and 2004/2005, the arable soil acted continuously as CH_4 sink. Oxidation rates in intercrops slightly decreased from first to third sampling in 2004/2005 and 2003/2004. Highest CH_4 uptake rates were always observed at the end of the investigation periods, thus in summer. Manuring of liquid fermented fertilizer in w/o L-FC mostly entailed CH_4 emissions of CH_4 dissolved in the fertilizer with different duration. Except 2004/2005 with similar amounts of carbon uptake, less CH_4 was oxidized in w/o L-FC compared to w/o L-M, but in

different proportions. The level of uptake rates in w/o L-M decreased from 2002/2003 to 2004/2005, whereas the level in w/o L-FC was higher in 2003/2004 than in the other two seasons.

3.1.4 CH_4 Fluxes in Intercrops and Spring Wheat

During season 2004/2005 in intercrops and spring wheat, the arable soil showed CH_4 uptake rates between 1.8 and 42 µg C m^{-2} h^{-1} in w/o L-M and between 0.9 and 26 µg C m^{-2} h^{-1} in w/o L-FC, respectively (figure 3.6). Large CH_4 influxes were determined in June and July 2005. Unexpected CH_4 emissions were observed at following sampling days: on December 22, 2004 in both manuring treatments (2.6 µg C m^{-2} h^{-1} in w/o L-M and 0.8 µg C m^{-2} h^{-1} in w/o L-FC), on January 7, 2005 in w/o L-M (1.3 µg C m^{-2} h^{-1}, significant), as well as on February 28 (2.6 µg C m^{-2} h^{-1}) and May 11, 2005 (13 µg C m^{-2} h^{-1}) in w/o L-FC. Significant differences in CH_4 uptake rates were found on December 8, 2004 and June 22, 2005, whereas the outstanding high emission peak in w/o L-FC in May was not significant. Integration of the observed CH_4 fluxes over 365 days led to a total carbon uptake of 714 g C ha^{-1} in w/o L-M and 644 g C ha^{-1} in w/o L-FC, thus a decrease of 10% in w/o L-FC (table 3.7). CH_4 emission on February 28, 2005 was used until February 28, CH_4 efflux on May 11, 2005 was only calculated for that day. The spatial variability of CH_4 fluxes averaged 47% in w/o L-M and 76% in w/o L-FC (table 3.8). The CV value for the temporal variability of CH_4 fluxes accounted for 159% in w/o L-M and for 205% in w/o L-FC (table 3.8). Coefficients of correlation of 0.42 and 0.51 between CH_4 fluxes and air and soil temperature in 5 cm soil depth, respectively, were calculated (table 3.9).

In 2003/2004, CH_4 oxidation rates in intercrops and spring wheat were found between 0 and 16 µg C m^{-2} h^{-1} in both manuring treatments (figure 3.7). An exception of the continuous CH_4 uptake represented February 27, 2004 when 3 µg C m^{-2} h^{-1} were emitted in w/o L-FC but without significance. No CH_4 fluxes could be determined on January 15, 2004 in w/o L-FC and on March 25, 2004 in both manuring treatments. In the majority of sampling dates CH_4 uptake rates were slighly higher in w/o L-FC than in w/o L-M, however just on December 2, 2004 with significant difference. Carbon uptake of 502 g C ha^{-1} in w/o L-M and 547 g C ha^{-1} in w/o L-FC, respectively, were calculated integrating CH_4 fluxes over 365 days that corresponded to an increase of 9% in w/o L-FC (table 3.7). CH_4 emission on February 27, 2004 was calculated for that day in the integration. Mean spatial variabilities of CH_4 fluxes of 56% and 47% were determined in w/o L-M and in w/o L-FC, respectively (table 3.8). The CV values for temporal variability of the CH_4 fluxes amounted to 68% in w/o L-M and 77% in w/o L-FC (table 3.8). Coefficients of correlation (r^2) between CH_4 fluxes air temperature and between CH_4 fluxes and temperature in 5 cm soil depth accounted for 0.46 and 0.44, respectively (table 3.9).

CH_4 uptake rates measured in 2002/2003 in intercrops and spring wheat ranged between 0 and 24 µg C m^{-2} h^{-1} in w/o L-M and between 0 and 18 µg C m^{-2} h^{-1} in w/o L-FC, respectively (figure 3.8). The higher oxidation rates were determined in June and July 2003. At the majority

of sampling dates larger CH_4 influxes in the soil were analyzed in w/o L-M compared to w/o L-FC. On November 6, 2002 and April 27, 2003 neither CH_4 uptake nor CH_4 emission could be observed in both manuring treatments. On May 22, 2003 no flux was found in w/o L-M. During this season, no significant differences between the manuring treatments in CH_4 fluxes could be determined. Integration of CH_4 oxidation over 365 days showed a carbon uptake of 585 µg C m^{-2} h^{-1} in w/o L-M and 529 µg C m^{-2} h^{-1} in w/o L-FC, meaning a reduction of 10% in w/o L-FC (table 3.7). Mean spatial variabilities of CH_4 fluxes of 38% and 73% were assessed in w/o L-M and in w/o L-FC, respectively (table 3.8). The CV values for temporal variability of the CH_4 fluxes amounted to 121% in w/o L-M and 110% in w/o L-FC (table 3.8). Coefficients of correlation (r^2) between CH_4 fluxes and air temperature and between CH_4 fluxes and temperature in 5 cm soil depths accounted for 0.47 and 0.50, respectively (table 3.9).

Comparison of annual CH_4 fluxes during the seasons 2004/2005, 2003/2004,and 2002/2003 in intercrops and spring wheat revealed that the arable soil acted continuously as sink for CH_4 with oxidation rates mainly varying between 2 and 8 µg C m^{-2} h^{-1}. Elevated uptake rates were found in the summer months. CH_4 emissions were rarely observed without identifiable reason and without statistical significance. With exception of 2003/2004, when an increase of carbon uptake (9%) integrated over 365 days was observerd, less CH_4 was taken up and was oxidized by the soil in w/o L-FC in comparison to w/o L-M (10%). Comparing integrated CH_4 fluxes of winter wheat 5 with prior intercrops on the one hand and intercrops and spring wheat on the other hand higher carbon uptake was displayed in w/o L-M in winter wheat 5 in 2002/2003 and 2003/2004, whereas in 2004/2005 in this manuring treatment less CH_4 oxidized up compared to intercrops and spring wheat. Regarding w/o L-FC, less carbon uptake occurred in winter wheat 5 in comparison to intercrops and spring wheat in 2002/2003 and 2004/2005, but in 2003/2004 similar amounts of CH_4 were oxidized in both crops.

3.1.5 Net CO_2 Fluxes in Winter Wheat 5

Since the trace gas measurements were carried out in the soil-plant system with transparent chambers using the closed chamber method, the observed net CO_2 fluxes represent an overlap of CO_2 production and consumption processes. Quantification of CO_2 flux rates was not possible when the photosynthetic activity was so high that the amount of CO_2 enclosed within the chamber plus any CO_2 production were not sufficient for a linear regression analysis over at least three of the five sampling time points. Due to the non-quantifiable uptake rates, integration of CO_2 fluxes over 365 days (as done with N_2O and CH_4 fluxes) was not performed.

During season 2004/2005 in winter wheat 5 and prior intercrops, CO_2 flux rates were observed in w/o L-M between -227 and 508 mg C m^{-2} ha^{-1} and in w/o L-FC between -187 and 481 mg C m^{-2} ha^{-1} (figure 3.1). After drilling of winter wheat CO_2 emissions were determined in both manuring treatments in October, on December 22, 2004, in January, on March 1, in July, and

August 2005. Relatively high efflux rates occurred in w/o L-M in October (up to 265 mg C m^{-2} ha^{-1}) and highest emissions in July in both treatments (508 mg C m^{-2} ha^{-1} in w/o L-M, 481 mg C m^{-2} ha^{-1} in w/o L-FC). At the remaining sampling dates quantifiable and not quantifiable CO_2 uptakes were found in both manuring treatments or in one of the treatments, respectively. During the season a trend of slightly decreased CO_2 emissions and reduced CO_2 uptake in w/o L-FC compared to w/o L-M became apparent. Significant differences of CO_2 fluxes between the manuring treatments were shown on December 8, 2004, January 7 and February 24, 2005. The very small emission of 4 mg C m^{-2} ha^{-1} in w/o L-FC possibly resulted from degassing of dissolved CO_2 in liquid fermented fertilizer that was applied two days before. However, one day after fertilizer application in w/o L-FC at the end of January, strong CO_2 uptake was observed.

In 2003/2004 CO_2 fluxes in winter wheat 5 and prior intercrops accounted for -48 to 64 mg C m^{-2} ha^{-1} in w/o L-M and for -34 to 61 mg C m^{-2} ha^{-1} in w/o L-FC, respectively (figure 3.2). After drilling of winter wheat on October 14, 2003, CO_2 was evolved from both, w/o L-M and in lower amounts from w/o L-FC until mid January. Significantly higher emissions in w/o L-M were found on November 5 and 19, 2003. In January 2004, CO_2 emission switched to CO_2 uptake in both treatments that was mostly lower in w/o L-FC. The emission peak in w/o L-FC on February 27, 2004 (61 mg C m^{-2} h^{-1}, not significant) was probably due to degassing of CO_2 dissolved in the liquid fermented fertilizer that was applied a few hours before. Further manuring on March 25 and April 7, 2004 did not result in CO_2 emissions. Non-quantifiable CO_2 uptake rates were found in the intercrops (August 14 until October 9, 2003) and in winter wheat 5 as of April 15, 2004.

In winter wheat 5 during season 2002/2003 CO_2 fluxes ranged between -28 and 286 mg C m^{-2} ha^{-1} in w/o L-M and between -40 and 378 mg C m^{-2} ha^{-1} in w/o L-FC, frequently with high standard deviations (figure 3.3). No fluxes could be observed on February 12 and February 19, 2003 in w/o L-M and w/o L-FC, respectively. Quantifiable CO_2 uptake rates were determined in both manuring treatments on January 16, 2003 and in w/o L-M on March 13 and April 8, 2003. Uptake rates in May were not quantifiable. Significances between the treatments were found on December 4, 2002 and April 8, 2003. CO_2 emissions in w/o L-FC on February 19 and March 13, 2003 were probably due to degassing of CO_2 dissolved in the liquid fertilizer applied to the soil a few hours before.

Comparison of CO_2 fluxes in winter wheat 5 and prior intercrops in all three seasons showed a trend of reduced CO_2 emissions in w/o L-FC excluding emission peaks caused by fertilizer application, notabely in 2004/2005 and 2003/2004. In cases of quantifiable CO_2 uptake mostly lower uptake rates occurred in w/o L-FC compared to w/o L-M.

3.1.6 Net CO_2 Fluxes in Intercrops and Spring Wheat

In 2004/2005, CO_2 fluxes in intercrops and spring wheat ranged from -170 to 139 mg C m^{-2} ha^{-1} in w/o L-M and from -157 to 176 mg C m^{-2} ha^{-1} in w/o L-FC (figure 3.6). Sampling on October 13 and 27, 2004 in intercrops revealed largest CO_2 uptake rates during the season in both manuring treatments. Furthermore, CO_2 uptake was observed in November 24, 2004 and August 8, 2005 in w/o L-M, and in February 28 and June 22, 2005 in w/o L-FC. CO_2 uptake rates found in May 11, 2005 were not quantifiable in both treatments. At the other sampling dates, CO_2 emissions occurred with notably high emission peaks on November 17, 2004 and July 15, 2005, however without any trend of increase or decrease in w/o L-FC compared to w/o L-M. Significant differences between the treatments were determined on November 24 and December 8, 2004 as well as on February 24 and 28, 2005.

During season 2003/2004 CO_2 fluxes in intercrops and spring wheat accounted for -120 to 26 mg C m^{-2} ha^{-1} in w/o L-M and for -68 to 47 mg C m^{-2} ha^{-1} in w/o L-FC (figure 3.7). Except February 12, 2004, CO_2 emissions were observed between January and April 15, 2004 in both treatments, exhibiting a trend of reduced CO_2 emissions in w/o L-FC. CO_2 uptake (quantifiable and not quantifiable) occurred in both manuring treatments until October 23, on December 2, 2003, and as of April 27, 2004 until end of sampling period in July 2004. In November 2003 CO_2 uptake in w/o L-M and CO_2 efflux in w/o L-FC was found demonstrating the effect of cutting and harvesting the intercrops in w/o L-FC. CO_2 fluxes on November 5, 2003 and February 12, 2004 were significantly different.

CO_2 fluxes between -279 and 91 mg C m^{-2} ha^{-1} in w/o L-M and between -147 and 48 mg C m^{-2} ha^{-1} in w/o L-FC were determined in 2002/2003 in intercrops and spring wheat (figure 3.8). On November 6, 2002, from December 16, 2002 to April 8, 2003, and on July 20, 2003 CO_2 emissions in both manuring treatments occurred. However, no trend of reduced or elevated fluxes between the treatments emerged. At the remaining sampling dates, quantifiable and non-quantifiable CO_2 uptake was found. Only on December 4, 2002 a significance between the treatments could be observed.

3.2 Field Measurements in the Cropping System with Livestock

3.2.1 N_2O Fluxes and Soil Mineral Nitrogen Contents in Spelt

During season 2004/2005 N_2O emissions in spelt and prior intercrops ranged between 1 and 299 µg N m^{-2} h^{-1} in wL-FS, 1 and 164 µg N m^{-2} h^{-1} in wL-FS+FC, 2 and 436 µg N m^{-2} h^{-1} in wL-FYM, and 4 and 235 µg N m^{-2} h^{-1} in the control treatment wL-RS (figure 3.11). Notably high N_2O losses occurred in all treatments on October 27, 2004, low emissions in all treatments were determined on September 15 and 28, 2004 (intercrops) as well as on January 7, 2005. N_2O fluxes often exhibited high standard deviations entailing significant differences between the manuring treatments only at three sampling dates (October 27, 2004, February 26 and March 1, 2005; multiple comparison of averages using Student-Newman-Keuls method, $P \leq 0.05$). A trend of increased or decreased emissions in a distinct treatment in comparison to wL-RS did not become apparent, the emission patterns were very heterogenous. Elevated N_2O fluxes on October 13, 2004 in wL-FS+FC and wL-FYM could be linked to application of solid fermented residues and farmyard manure, respectively. Integration of emission data over 365 days (refer to 3.12) showed a nitrogen loss of 5449 g N ha^{-1} in wL-FS (147%), 2239 g N h^{-1} in wL-FS+FC (60%), and 3709 g N ha^{-1} (100%), thus a reduction in wL-FS+FC and an increase in wL-FS compared to wL-RS (table 3.10). Extrapolation of N_2O emissions in wL-FYM during season 2004/2005 was only performed in comparison to wL-FYM of the previous season 2003/2004 because second reference data of season 2002/2003 were not available in this manuring treatment. Coefficients of variation for the mean spatial variability of N_2O emissions amounted to 70% in wL-FS, 81% in wL-FS+FC, 70% in wL-FYM, and 68% in wL-RS (table 3.11). The CV value for the temporal variability of N_2O emissions accounted for 160% in wL-FS, 127% in wL-FS+FC, 196% in wL-FYM, and 146% in wL-RS (table 3.11). In the winter period between December 1 and March 15, 12%, 27%, 11%, and 17% of the annual N_2O losses were emitted in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.12).

In 2003/2004 N_2O fluxes in spelt and prior intercrops varied in wL-FS from -2 to 62 µg N m^{-2} h^{-1}, in wL-FS+FC from -5 to 17 µg N m^{-2} h^{-1}, in wL-FYM from 0 to 41 µg N m^{-2} h^{-1}, and in wL-RS from 0 to 23 µg N m^{-2} h^{-1} (figure 3.12). No fluxes were determined in some of the manuring treatments on August 14 and October 9, 2003, and in all treatments in January and on March 7, 2004. Elevated N_2O emissions in wL-FYM were observed after drilling of spelt on October 14 until December 2003, however just on December 2 with statistical significance. Further significant differences were only found on February 21, 2004 due to high standard deviations. Application of the respective liquid fertilizers on March 5, 2004 in wL-FS, wL-FS+FC, and wL-RS resulted in non-significant, higher emissons in those treatments that day. However, manuring in wL-FS+FC on March 25, 2004 did not increase N_2O losses in this treatment that day. The seldom observation of N_2O uptake was made on January 26 in wL-FS and on June 11, 2004 in wL-FS+FC. A clear pattern of emission with continuously reduced or elevated N_2O fluxes in a

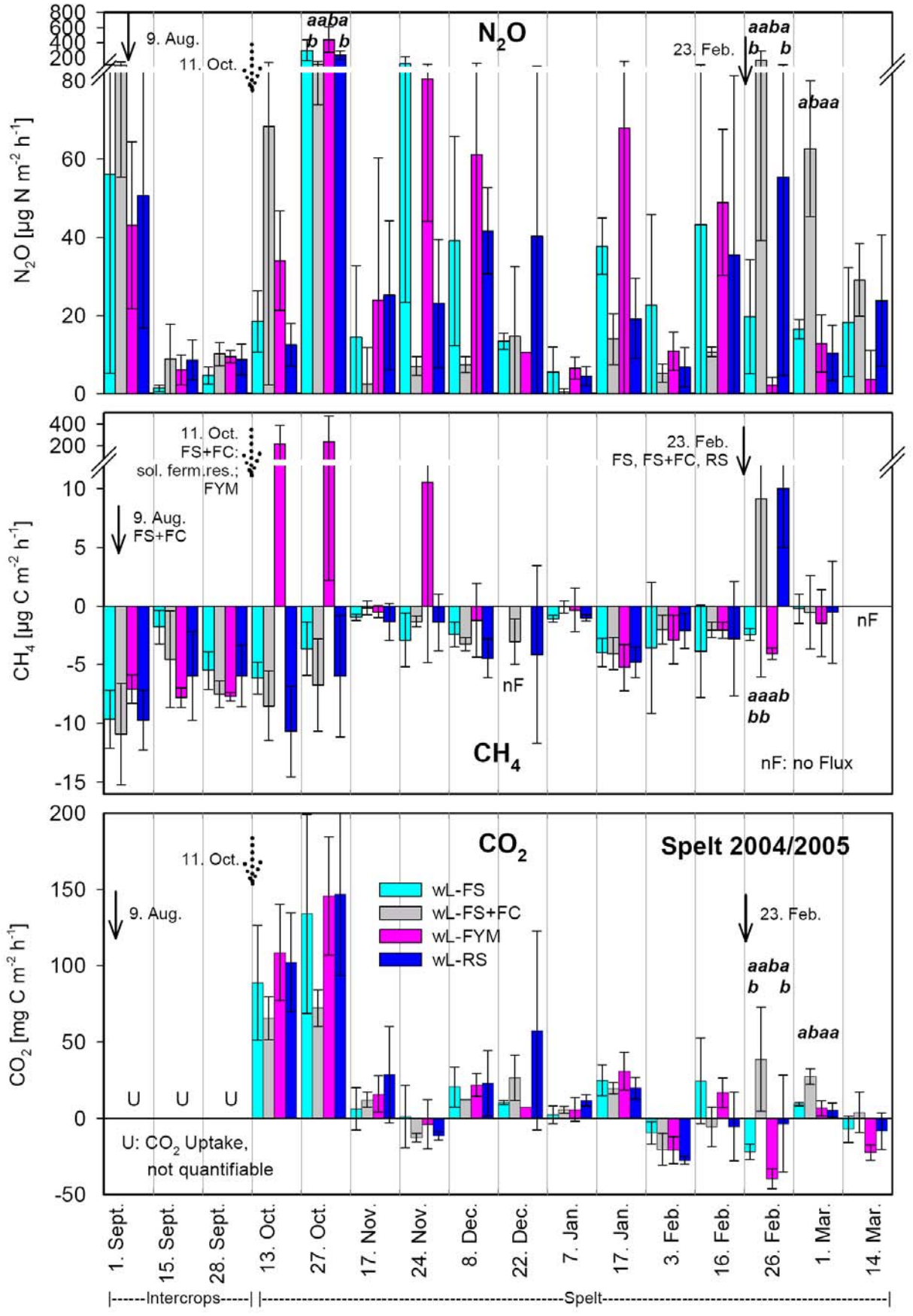


Figure 3.11: N_2O-, CH_4-, and CO_2- fluxes in spelt and prior intercrops in the cropping system with livestock in season 2004/2005. Bold arrows: application of liquid fertilizers, dotted arrows: application of solid fermented residues or farmyard manure in the respective manuring treatments.

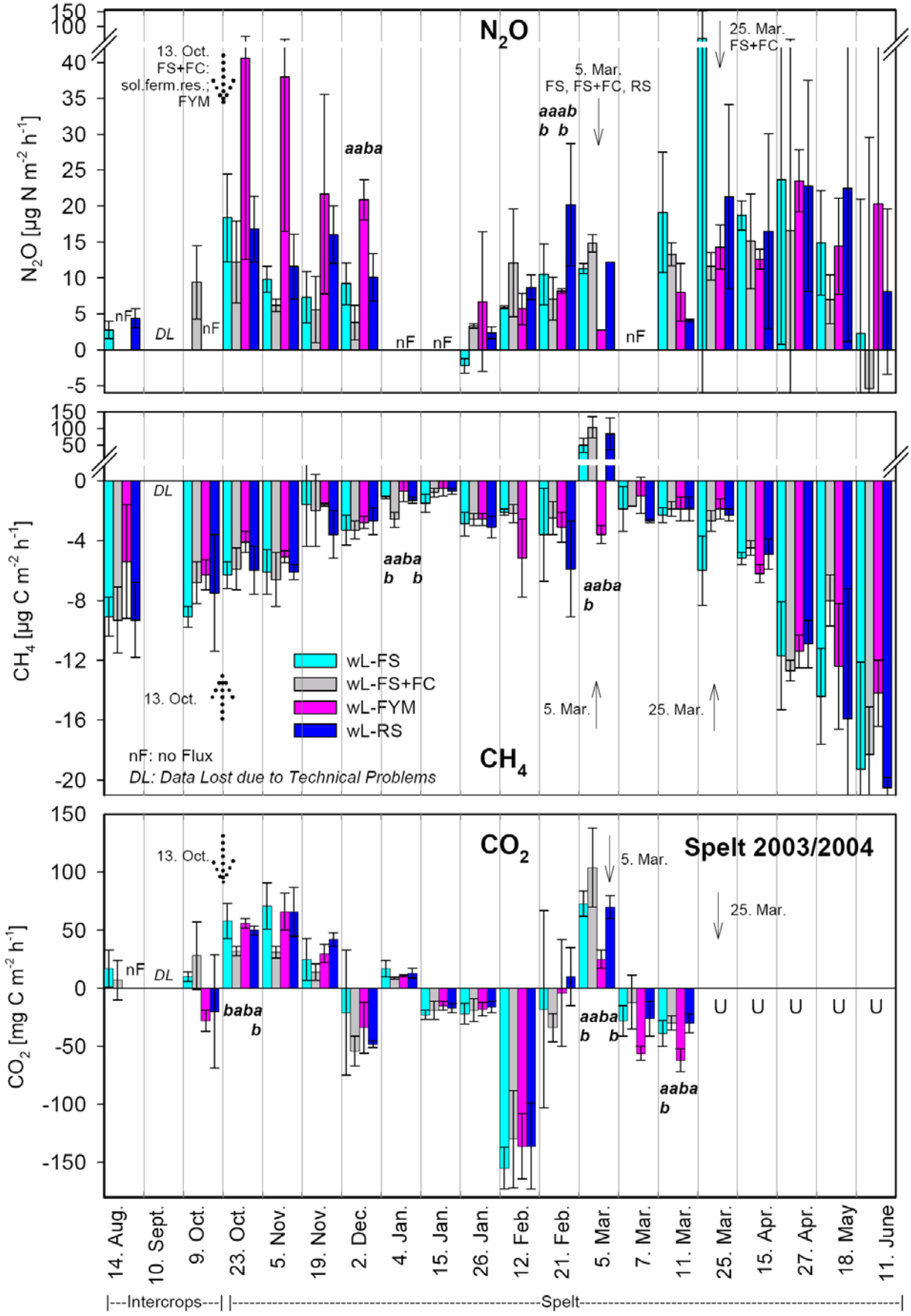


Figure 3.12: N_2O-, CH_4-, and CO_2- fluxes in spelt and prior intercrops in the cropping system with livestock in season 2003/2004. Bold arrows: application of liquid fertilizers, dotted arrows: application of solid fermented residues or farmyard manure in the respective manuring treatments.

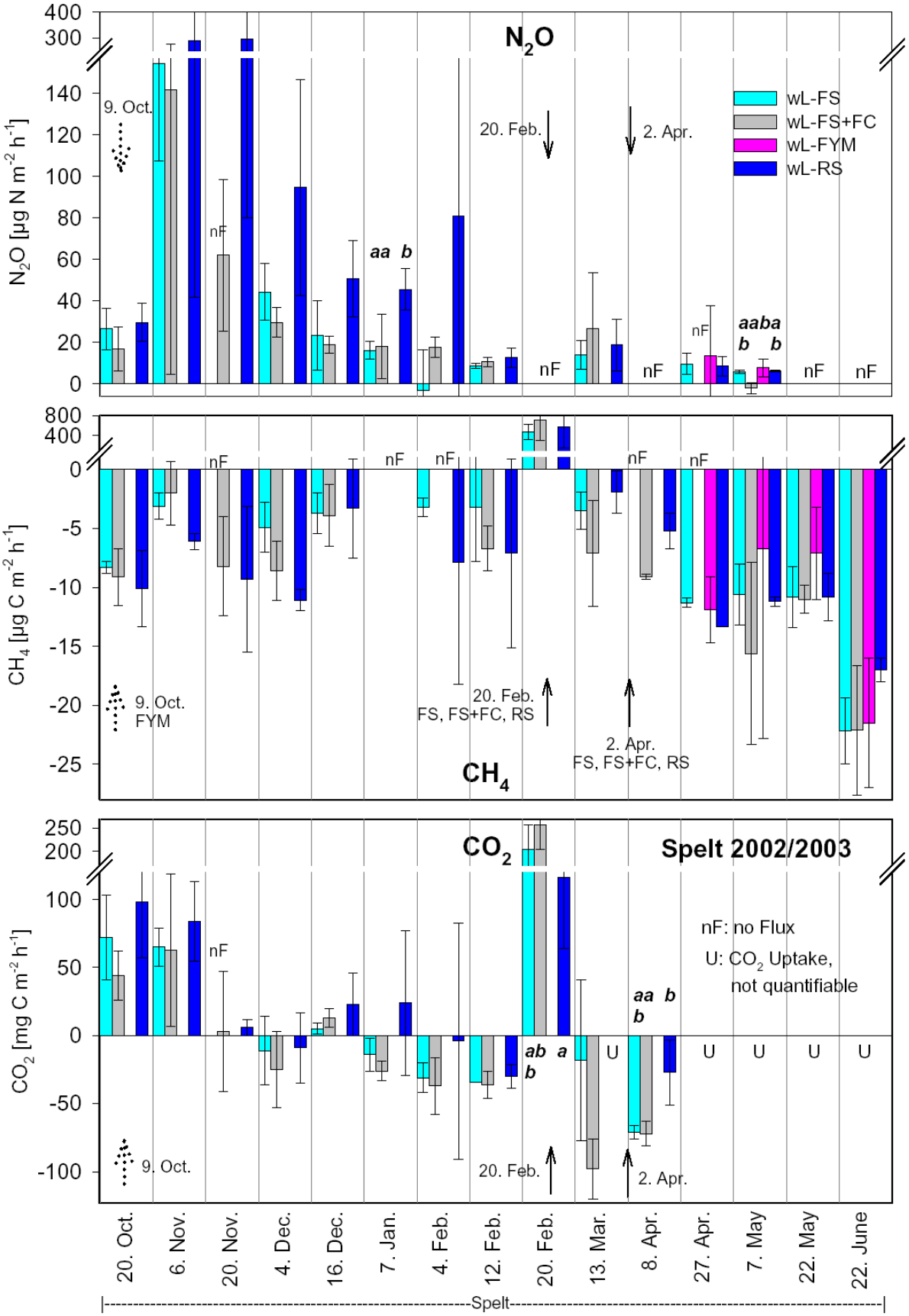


Figure 3.13: N_2O-, CH_4-, and CO_2- fluxes in spelt in the cropping system with livestock in season 2002/2003. Start of gas sampling in manuring treatment wL-FYM on April 27 2003. Bold arrows: application of liquid fertilizers, dotted arrows: application of solid fermented residues or farmyard manure in the respective manuring treatments.

treatment did not emerge. The nitrogen loss over 365 days amounted to 838 g N ha^{-1} in wL-FS, to 456 g N ha^{-1} in wL-FS+FC, to 1017 g N ha^{-1} in wL-FYM, and to 834 g ha^{-1} in wL-RS, meaning a decrease of 45% in wL-FS+FC, a very small increase of 1% in wL-FS, and an increase of 22% in wL-FYM compared to wL-RS (table 3.10). Coefficients of variation for the spatial variability of N_2O emissions averaged 76% in wL-FS, 75% in wL-FS+FC, 36% in wL-FYM, and 37% in wL-RS (table 3.11). The CV value for the temporal variability of N_2O emissions accounted for 129% in wL-FS, 90% in wL-FS+FC, 99% in wL-FYM, and 79% in wL-RS (table 3.11). During the winter proportions of the annual N_2O losses of 16%, 28%, 17%, and 20% were emitted in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.12).

Table 3.10: Integrated N_2O emissions over 365 days in spelt and potatoes in the cropping system with livestock in the respective manuring treatments.

Season	Crops	Manuring Treatments							
		wL-FS		wL-FS+FC		wL-FYM		wL-RS	
		% of wL-RS	g N ha^{-1}	% of wL-RS	g N ha^{-1}	% of wL-RS	g N ha^{-1}	% of wL-RS	g N ha^{-1}
2002/2003	Spelt	38	1474	40	1535	n.d.	n.d.	100	3868
	Potatoes	72	1524	105	2234	n.d.	n.d.	100	2128
2003/2004	Spelt	101	838	55	456	122	1017	100	834
	Potatoes	92	2824	85	2586	105	3194	100	3054
2004/2005	*Spelt$^{\surd}$*	*124*	*2400*	*81*	*1570*	*157*	*3048*	*100*	*1943*
	Spelt	147	5449	60	2239	174	6465*	100	3709

Spelt$^{\surd}$ 2004/2005: Integrated N_2O emissions during sampling period from September 2004 until March 2005

*Extrapolated value calculated only in comparison to season 2003/2004 due to missing value in 2002/2003

n.d.: not determined

Table 3.11: Coefficients of variation (CV) for temporal and mean spatial variability of the N_2O emissions in spelt and potatoes in the cropping system with livestock in the respective manuring treatments.

Season	Crops	Manuring Treatments							
		wL-FS		wL-FS+FC		wL-FYM		wL-RS	
		CV % time	Ø CV % space	CV % time	Ø CV % space	CV % time	Ø CV % space	CV % time	Ø CV % space
2002/2003	Spelt	189	68	163	43	124	57	158	38
	Potatoes	177	52	203	36	120	55	159	62
2003/2004	Spelt	129	76	90	75	99	36	79	37
	Potatoes	107	49	97	60	122	94	152	40
2004/2005	*Spelt$^{\surd}$*	*160*	*70*	*127*	*81*	*196*	*70*	*146*	*68*

Spelt$^{\surd}$ 2004/2005: Coefficients of variation during sampling period from September 2004 until March 2005

Table 3.12: Integrated N_2O emissions during winter period (December 1 - March 15) in spelt and in intercrops before potatoes in the cropping system with livestock in the respective manuring treatments.

Season	Crops	Manuring Treatments							
		wL-FS		wL-FS+FC		wL-FYM		wL-RS	
		Winter %	Winter g N ha^{-1}	Winter %	Winter g N ha^{-1}	Winter %	Winter g N ha^{-1}	Winter %	Winter g N ha^{-1}
2002/2003	Spelt	23	333	29	439	n.d.	n.d.	29	1131
	Intercrops	38	580	33	738	n.d.	n.d.	18	388
2003/2004	Spelt	16	131	28	129	17	172	20	163
	Intercrops	26	732	31	808	22	696	12	357
2004/2005	Spelt	12	653	27	594	11*	708*	17	623

*Extrapolated value of annual emission calculated only in comparison to season 2003/2004 due to missing value in 2002/2003

N_2O fluxes in spelt and prior intercrops during 2002/2003 were observed in ranges of -3 to 155 µg N m^{-2} h^{-1} in wL-FS, -2 to 142 µg N m^{-2} h^{-1} in wL-FS+FC, 0 to 14 µg N m^{-2} h^{-1} in wL-FYM (sampling in this treatment started end of April), and 0 to 296 µg N m^{-2} h^{-1} in wL-RS (figure 3.13). Until February 2003 highest N_2O emissions were found in wL-RS compared to the fermented slurry treatments, however only on January 7, 2003 with significant difference. On May 7, 2003 flux rates also differed significantly. Notably high N_2O emissions occurred in November 2002. No fluxes were determined on November 20, 2002 in wL-RS and on April 27, 2003 in wL-FS+FC as well as on February 20 (a few hours after fertilizer application), April 8, May 22, and June 22, 2003 in all manuring treatments. Integration of flux rates over 365 days showed a nitrogen loss of 3868 g N ha^{-1} (100%) in wL-RS, 1474 g N ha^{-1} (38%) in wL-FS, and 1535 g N ha^{-1} (40%) in wL-FS+FC (table 3.10). No integration was carried out in wL-FYM since data of just two months were available. The mean spatial variability of N_2O emissions amounted to 68% in wL-FS, 43% in wL-FS+FC, 57% in wL-FYM, and 38% in wL-RS (table 3.11). The CV value for the temporal variability of N_2O emissions accounted for 189% in in wL-FS, 163% in wL-FS+FC, 124% in wL-FYM, and 158% in wL-RS (table 3.11). CV values in wL-FYM were achieved from existing data of only two months. During the winter period 23%, 29%, and again 29% of the annual N_2O losses were emitted in wL-FS, wL-FS+FC, and wL-RS, respectively (table 3.12).

Comparison of N_2O fluxes in spelt and prior intercrops in 2004/2005, 2003/2004, and 2002/2003 revealed highest nitrogen losses during season 2004/2005 and a level of low emissions in 2003/2004. Looking at the emission patterns, no regularity of increased or reduced N_2O fluxes in the different manuring treatments appeared in three years of sampling. However, concerning integrated N fluxes over 365 days, wL-FS+FC showed in all seasons clearly decreased N losses (19 - 60%), and wL-FYM exhibited elevated N_2O emissions of 122% in 2003/2004, and of 157% during sampling period from September 2004 to March 2005 compared to wL-RS.

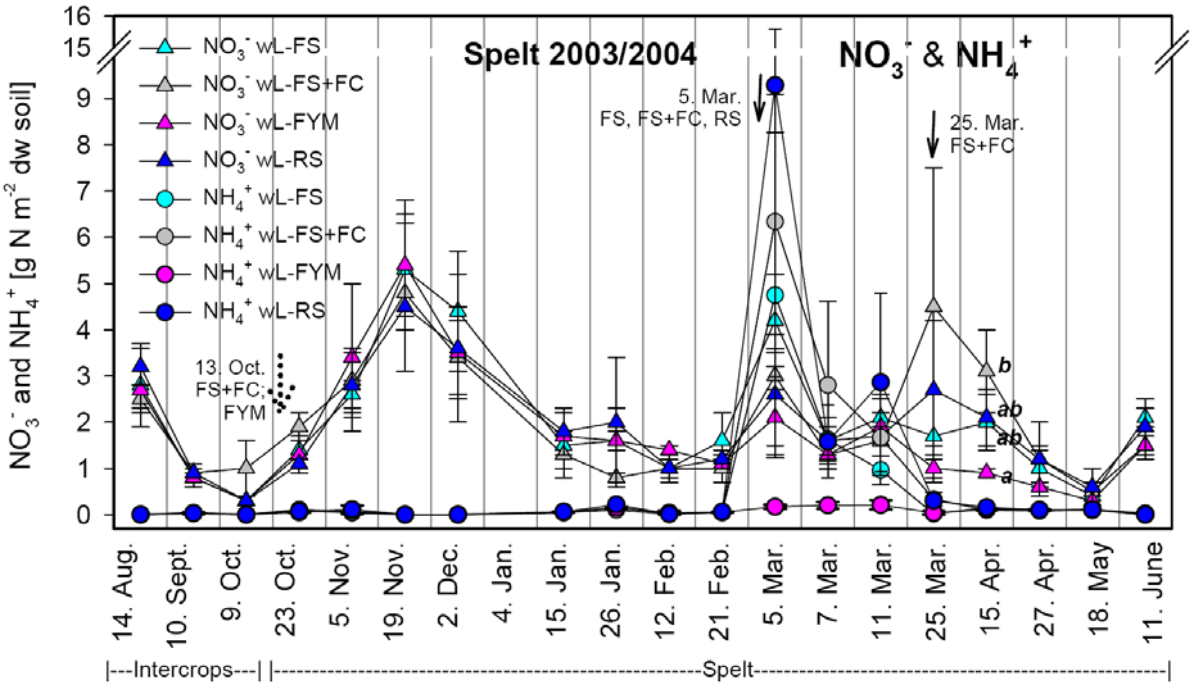


Figure 3.14: NO_3^--and NH_4^+- concentrations in spelt and prior intercrops in the cropping system with livestock in season 2003/2004.

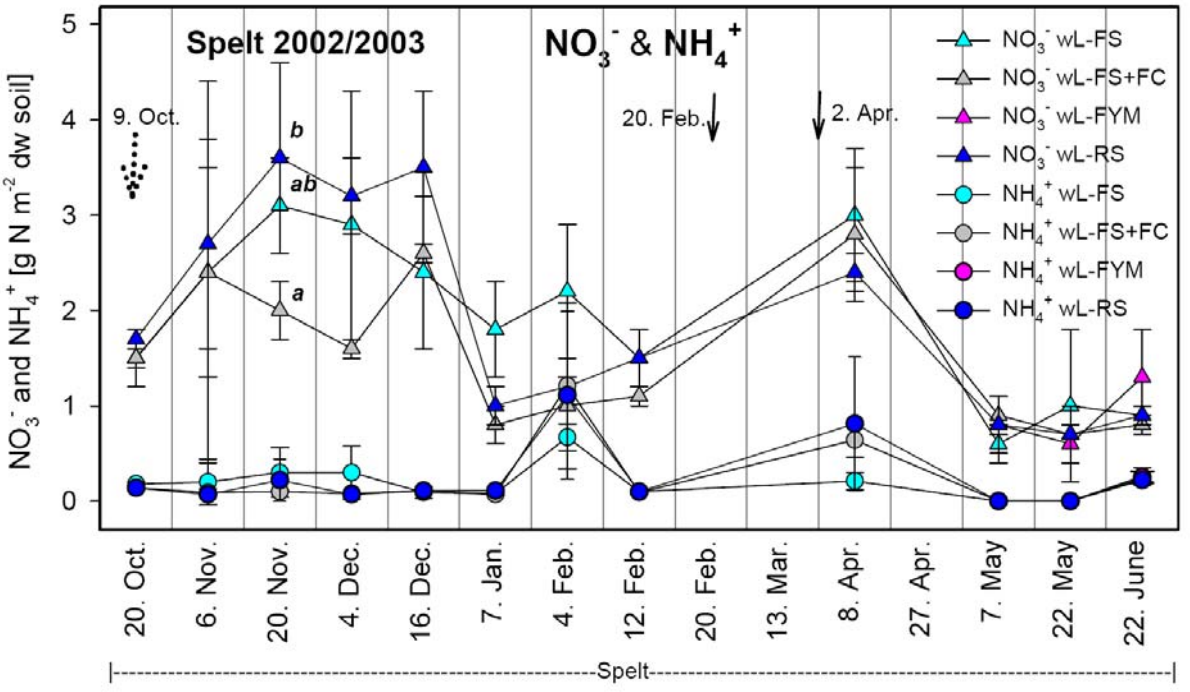


Figure 3.15: NO_3^--and NH_4^+- concentrations in spelt in the cropping system with livestock in season 2002/2003.

Soil NO_3^- concentrations in spelt and prior intercrops varied in 2003/2004 between 0.3 and 5.3 g N m^{-2} in 30 cm soil depth (ploughed layer) in wL-FS, 0.5 and 4.8 g N m^{-2} in wL-FS+FC, 0.3 and

5.4 g N m^{-2} in wL-FYM, and 0.3 and 4.5 g N m^{-2} in wL- RS (figure 3.14). After drilling of spelt on October 14, 2003, NO_3^- concentrations increased in all manuring treatments up to 5 g N m^{-2} on November 19, 2003 and decreased afterwards. In part elevated amounts of NO_3^- were detected after manuring on March 5 and 25, 2004 in the fertilized treatments, however, signifiant differences were only found on April 15, 2004. NH_4^+ concentrations were mostly at the detection limit with exception of sampling dates in March 2004 due to fertilizer application in wL-FS, wL-FS+FC, and wL-RS. Up to 4.8 g NH_4^+-N m^{-2} in wL-FS, 6.3 g NH_4^+-N m^{-2} in wL-FS+FC, and 9.3 g NH_4^+-N m^{-2} in wL-RS were observed on March 5, 2004 but without statistical significance. No relationship was found between soil mineral nitrogen concentrations and the amount of emitted N_2O.
In 2002/2003 soil NO_3^- concentrations in spelt ranged in 30 cm soil depth (ploughed layer) between 0.6 and 3.1 g N m^{-2} in wL-FS, 0.7 and 2.8 g N m^{-2} in wL-FS+FC, and 0.7 and 3.5 in wL-RS (figure 3.15), hence varied on a comparable level as in 2003/2004. Sampling in wL-FYM started on May 7 2003 and revealed NO_3^- amounts of 0.6 to 1.3 g N m^{-2}. The only signifcant difference of NO_3^- concentrations was found on November 20, 2002 between wL-FS+FC and wL-RS. NH_4^+ concentrations were for many sampling days at the detection limit, slightly elevated amounts of NH_4^+ were observed on February 4 and April 8, 2003. As during season 2003/2004 no indication of related N_2O emissions and soil mineral nitrogen concentrations became apparent.

NO_3^-- and NH_4^+- contents in soil depths of 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm in spelt investigated in fall/winter and spring revealed amounts of mineral nitrogen from 66 - 152 kg N ha^{-1} in wL-FS, 48 - 149 kg N ha^{-1} in wL-FS+FC, 42 - 84 kg N ha^{-1} in wL-FYM, and 62 - 95 kg N ha^{-1} in wL-RS (figure 3.16). Ammonium values were relatively small and negligible at most of the sampling times. On March 18, 2004, December 10, 2004, and March 21, 2005 significant differences of N_{min} between the manuring treatments were found, and all further significances found by comparison of NO_3^- and NH_4^+ concentrations in the different soil layers between the manuring treatments are shown in table 3.13. During the investigation period no pattern of elevated or reduced NO_3^- and NH_4^+ in distinct treatments became apparent.

In spring 2001 (before start of the differentiated manuring systems) as well as in spring 2004 contents of total nitrogen and total carbon were determined in the cropping system with livestock in the ploughed layer (30 cm soil depth) in the fields "Pfaffengraben 3" and "Pfaffengraben 4". Rye, peas, and spelt were subsequently cultivated in field "Pfaffengraben 3", and peas, spelt, and spring wheat were consecutively grown in field "Pfaffengraben 4" between both soil sampling dates. Table 3.14 exhibits the differences of total N and total C concentrations after three years of processing the different manuring treatments. Over both fields and all manuring treatments differences of total N amounts varied from -0.077 to 0.006

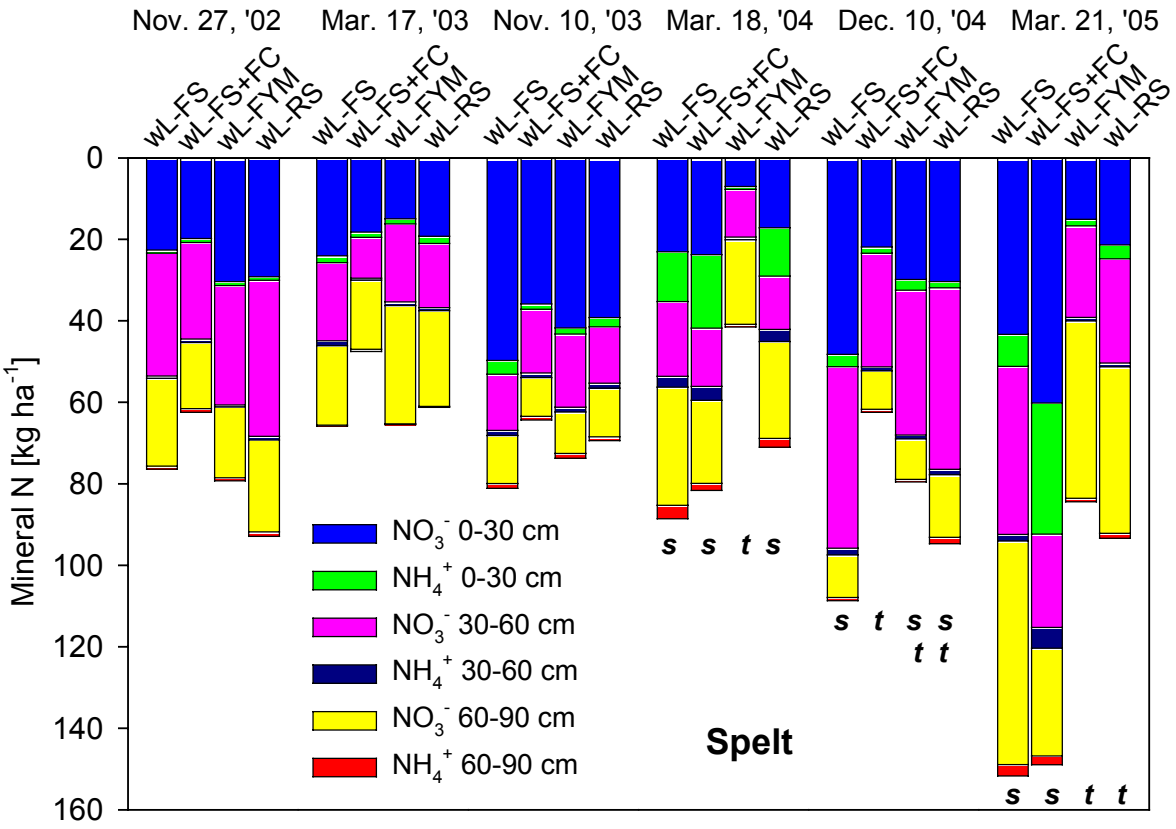


Figure 3.16: Amounts of NO_3^--N and NH_4^+-N in spelt in the cropping system with livestock between November 2002 and March 2005 in soil depths of 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm, respectively, in the different manuring treatments. (Data: Kurt Möller, Giessen, personal communication)

Table 3.13: Significant differences in amounts of NO_3^-, NH_4^+, and N_{min} in spelt in the cropping system with livestock between November 2002 and March 2005 in the different manuring treatments. (Data: Kurt Möller, Giessen, personal communication)

		wL-FS	wL-FS+FC	wL-FYM	wL-RS
Mar. 18, 2004	NO_3^- 0 - 30 cm	a	a	b	ab
	NO_3^- cumulative	a	ab	b	ab
	NH_4^+ 0 - 30 cm	x	x	y	x
	NH_4^+ cumulative	x	x	y	x
	N_{min} total	s	s	t	s
Dec. 10, 2004	NO_3^- 60 - 90 cm	a	a	a	b
	NO_3^- cumulative	a	b	ab	ab
	NH_4^+ 60 - 90 cm	x	x	x	y
	N_{min} total	s	t	st	st
Mar. 21, 2005	NO_3^- 0 - 30 cm	a	b	c	c
	NO_3^- 60 - 90 cm	a	b	ab	ab
	NO_3^- cumulative	a	ab	b	b
	NH_4^+ 0 - 30 cm	x	y	z	z
	NH_4^+ 30 - 60 cm	x	y	x	x
	NH_4^+ cumulative	x	y	z	z
	N_{min} total	s	s	t	t

mg N g^{-1} and differences of total C between -0.878 and 0.092 mg C g^{-1}. However, no significant differences could be determined, neither between the five manuring treatments in 2001 and 2004 (ANOVA), respectively, comparing both, nitrogen and carbon concentrations, nor between soil nitrogen and carbon contents, respectively, within a manuring treatment (t-test) comparing year 2001 with year 2004. Amounts of total N ranged in 2001 from 1.001 to 1.415 mg N g^{-1} dw soil and in 2004 from 1.009 to 1.405 mg N g^{-1} dw soil, concentrations of total C varied in 2001 between 8.824 and 12.541 mg C g^{-1} dw soil and in 2004 between 8.185 and 12.053 mg C g^{-1} dw soil.

Table 3.14: Differences of soil total nitrogen and total carbon concentrations in the cropping system with livestock after three years of processing differentiated manuring treatments. Soil sampling in spring 2001 (before start of the different manuring treatments) and in spring 2004. Positive and negative numbers mean increase and decrease, respectively, of total N or total C between 2001 and 2004. Numbers in parentheses are standard deviations. Amounts of total N ranged in 2001 from 1.001 to 1.415 mg N g^{-1} dw soil and in 2004 from 1.009 to 1.405 mg N g^{-1} dw soil, concentrations of total C varied in 2001 between 8.824 and 12.541 mg C g^{-1} dw soil and in 2004 between 8.185 and 12.053 mg C g^{-1} dw soil. (Data: Kurt Möller, Giessen, personal communication)

Field	Difference 2001 – 2004	wL-FS	wL-FS+FC	wL-FS+FC+FE	wL-FYM	wL-RS
Pfaffen-graben 3	Total Nitrogen [mg g^{-1} dw soil]	0.000 (± 0.111)	0.006 (± 0.057)	-0.011 (± 0.063)	-0.045 (± 0.038)	-0.002 (± 0.073)
	Total Carbon [mg g^{-1} dw soil]	-0.516 (± 0.986)	-0.370 (± 0.632)	-0.637 (± 0.130)	-0.878 (± 0.197)	-0.158 (± 0.487)
Pfaffen-graben 4	Total Nitrogen [mg g^{-1} dw soil]	-0.072 (± 0.091)	-0.077 (± 0.112)	-0.035 (± 0.113)	-0.028 (± 0.097)	-0.036 (± 0.114)
	Total Carbon [mg g^{-1} dw soil]	-0.336 (± 1.160)	-0.780 (± 1.478)	-0.352 (± 0.855)	-0.283 (± 0.775)	0.092 (± 0.962)

3.2.2 N_2O Fluxes and Soil Mineral Nitrogen Contents in Intercrops and Potatoes

During season 2003/2004 N_2O emissions in intercrops and potatoes amounted up to 170 µg N m^{-2} h^{-1} in wL-FS, to 69 µg N m^{-2} h^{-1} in wL-FS+FC, to 149 µg N m^{-2} h^{-1} in wL-FYM, and to 251 µg N m^{-2} h^{-1} in wL-RS (figure 3.17). N_2O effluxes increased partly after soil ploughing on January 24 and notabely when potatoes were dibbled on April 21, 2004 and when the ridges were curried later on. A distinct emission pattern of the manuring treatments did not become apparent, however, as of February 21, 2004, lowest nitrogen losses always occurred in wL-FS+FC. Despite five times of fertilizer application (liquid manure as well as solid fermented residues and farmyard manure) no significant differences in N_2O emissions between the treatments could be determined. No fluxes in part or in all treatments were found in October, on November 19, 2003, on January 4 and 15, and on February 21, 2004. Integrated emissions over 365 days accounted for N losses of 3054 g N ha^{-1} in wL-RS, 2824 g N ha^{-1} in wL-FS, 2586 g N ha^{-1} in wL-FS+FC, and 3194 g N ha^{-1} in wL-FYM, hence reductions of 8% in wL-FS and 15% in

wL-FS+FC, and an increase of 5% in wL-FYM, respectively, compared to wL-RS (table 3.10). Coefficients of variation for the mean spatial variability of N_2O emissions amounted to 49% in wL-FS, 60% in wL-FS+FC, 94% in wL-FYM, and 40% in wL-RS (table 3.11). The CV value for the temporal variability of N_2O emissions accounted for 107% in wL-FS, 97% in wL-FS+FC, 122% in wL-FYM, and 152% in wL-RS (table 3.11). In the winter period between December 1 and March 15, 26%, 31%, 22%, and 12% of the annual N_2O losses were emitted in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.12).

In 2002/2003, N_2O emissions in intercrops and potatoes achieved up to 216 µg N m^{-2} h^{-1} in wL-FS, 388 µg N m^{-2} h^{-1} in wL-FS+FC, 175 µg N m^{-2} h^{-1} in wL-FYM (start of investigation on May 7, 2003), and 201 µg N m^{-2} h^{-1} in wL-RS (figure 3.18). Effluxes increased with dibbling of potatoes on April 15, 2003 with notabely high flux rates on May 22 and June 5, 2003. Outstanding high N_2O emissions were observed on December 16, 2002 in intercrops (wL-FS and wL-RS) and harvested intercrops (plant stubbels, wL-FS+FC) in all three investigated manuring treatments, whereas no effluxes occurred on April 8 and August 14, 2003. During the sampling period, no significances between the treatments could be determined although liquid and solid manures were applied at five time points. Integration of N_2O flux rates over 365 days revealed nitrogen losses of 2128 g N ha^{-1} in wL-RS, 1524 g N ha^{-1} in wL-FS, and 2234 g N ha^{-1} in wL-FS+FC, exhibiting a reduction of 28% in wL-FS and an increase of 5% in wL-FS+FC compared to wL-RS (table 3.10). Integration over 365 d in wL-FYM was not carried out since emission data of just four months were available. Coefficients of variation for the spatial variability of N_2O emissions averaged 52% in wL-FS, 36% in wL-FS+FC, 55% in wL-FYM, and 62% in wL-RS (table 3.11). The CV value for the temporal variability of N_2O emissions accounted for 177% in wL-FS, 203% in wL-FS+FC, 120% in wL-FYM, and 159% in wL-RS (table 3.11). CV values in wL-FYM were calculated from existing data of around four months. During the winter period proportions of the annual N_2O losses of 38%, 33%, and 18% were emitted in wL-FS, wL-FS+FC, and wL-RS, respectively (table 3.12).

Comparison of season 2003/2004 and 2002/2003 revealed a higher level of N_2O emissions in 2003/2004. In both seasons, less nitrogen was evolved in wL-FS (8% and 28%) compared to respective wL-RS. In contrast, in wL-FS+FC 5% more N_2O was emitted in 2002/2003 but in 2003/2004 a decrease of N losses of 15% occurred. Except elevated emissions after dibbling of potatoes and no significant differences between the manuring treatments, no similarities or distinct flux pattern could be assessed. Due to the completely different chronology of tillage, intercrop management, cultivation and harvest technique of spelt and potatoes, elevated N_2O emissions occurred with dibbling of potatoes in April and May whereas increased N_2O effluxes in spelt were determined in October and November after drilling, if any distinct trend could be noticed. The level of integrated emissions over 365 days varied in 2002/2003 and 2003/2004 in potatoes between 1524 and 3194 g N ha^{-1} and in spelt between 456 and 3868 g N ha^{-1} over all investigated manuring treatments.

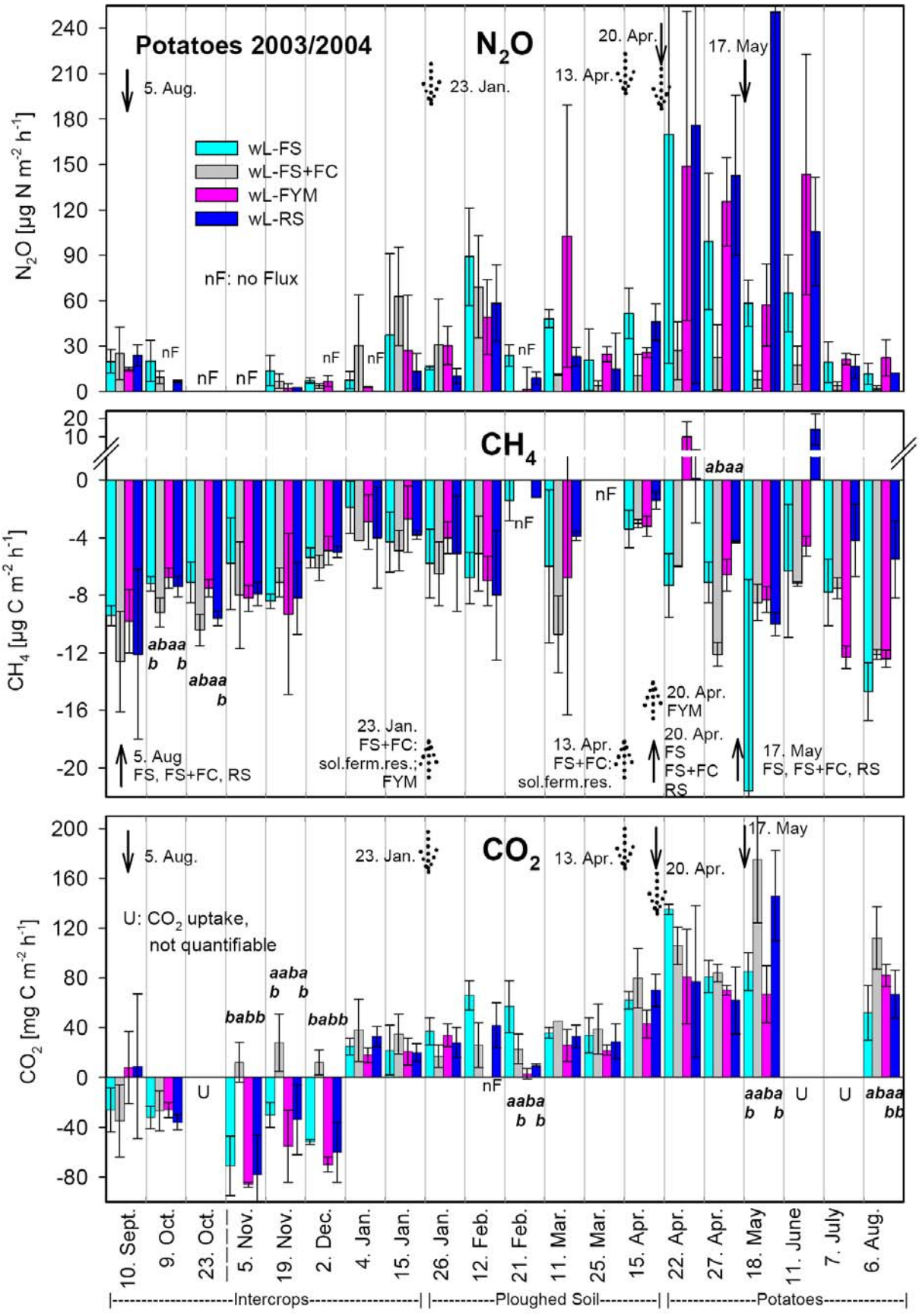


Figure 3.17: N_2O-, CH_4-, and CO_2- fluxes in intercrops and potatoes in the cropping system with livestock in season 2003/2004. Bold arrows: application of liquid fertilizers, dotted arrows: application of solid fermented residues or farmyard manure in the respective manuring treatments.

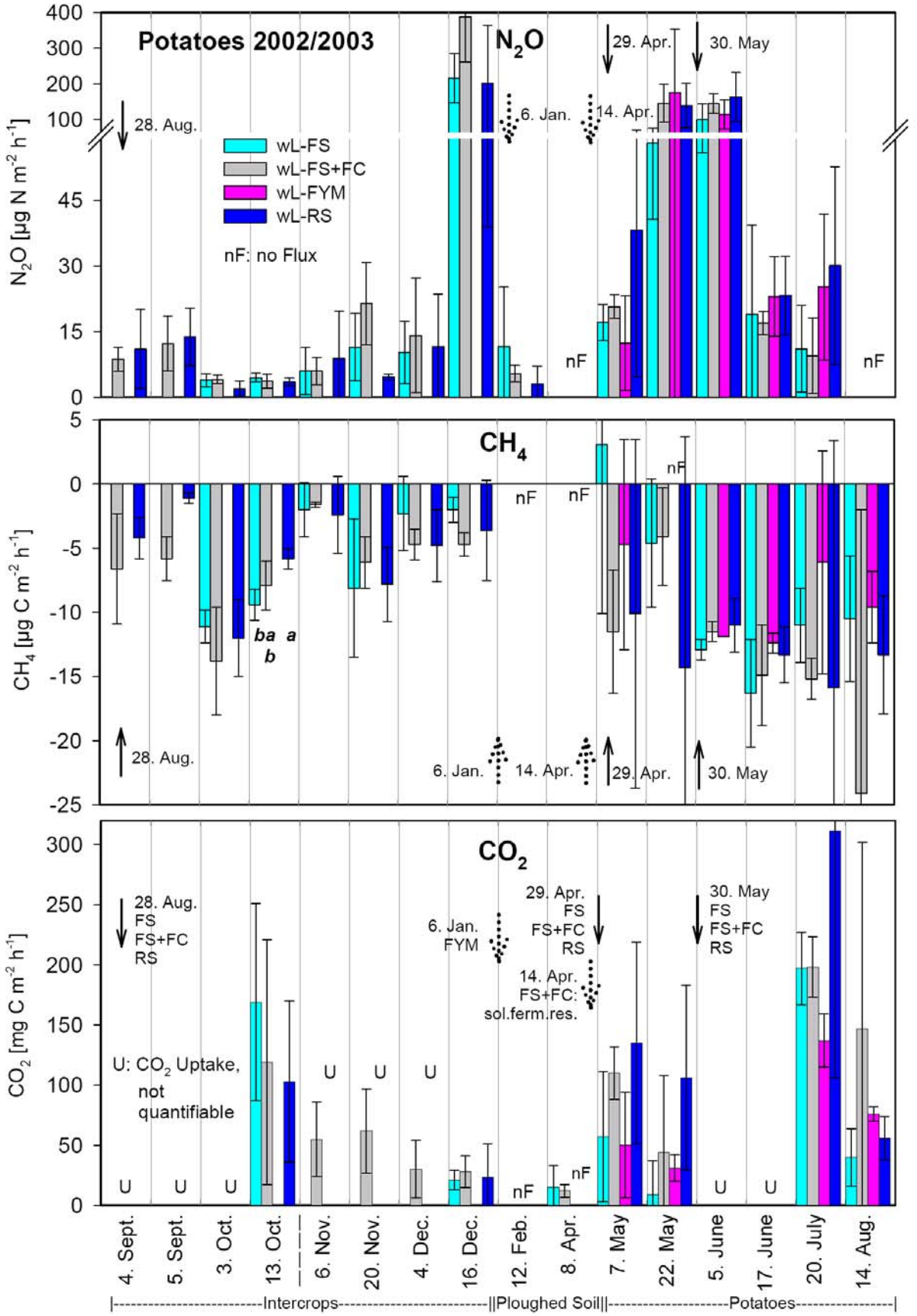


Figure 3.18: N_2O-, CH_4-, and CO_2- fluxes in intercrops and potatoes in the cropping system with livestock in season 2002/2003. Bold arrows: application of liquid fertilizers, dotted arrows: application of solid fermented residues or farmyard manure in the respective manuring treatments.

Soil NO_3^- concentrations varied between 0.4 and 1.5 g N m^{-2} in 30 cm soil depth (ploughed layer) in all three investigated manuring treatments until December 4, 2002 and increased also in all treatments up to 5 g N m^{-2} on April 8, 2003 without statistical significance (figure 3.19). NH_4^+ concentrations were for all sampling times at the detection limit. Similar observations concerning course and height of mineral nitrogen were made in the same cropping system in spelt in the same and the following season.

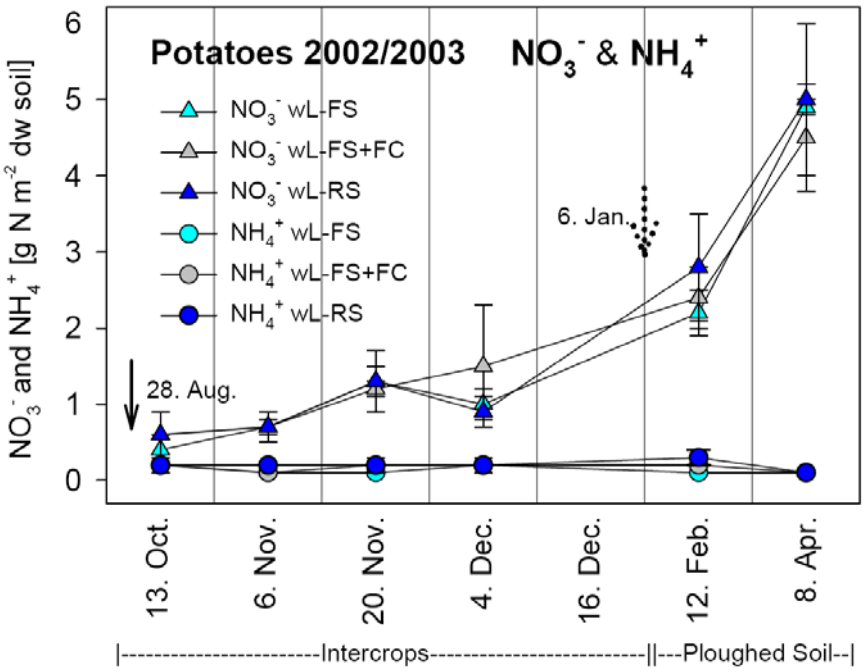


Figure 3.19: NO_3^--and NH_4^+- concentrations in intercrops and ploughed soil before potatoes in the cropping system with livestock in season 2002/2003

Amounts of soil NO_3^--N and NH_4^+-N in 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm soil depth investigated in fall/winter and spring in intercrops and potatoes exhibited contents of mineral nitrogen from 13 - 148 kg N ha^{-1} in wL-FS, 19 - 145 kg N ha^{-1} in wL-FS+FC, 21 - 157 kg N ha^{-1} in wL-FYM, and 12 - 159 kg N ha^{-1} in wL-RS (figure 3.20). Ammonium values were always relatively small and negligible. Within both seasons, 2002/2003 and 2003/2004, respectively, lowest amounts of N_{min} were found in November and highest contents in May in all manuring treatments. On March 18, 2003 and November 11, 2003 significant differences of mineral nitrogen between the manuring treatments were observed. All further significances found by comparison of NO_3^- and NH_4^+ concentrations in the different soil layers between the manuring treatments are given in table 3.15. During the investigation period no trend of elevated or reduced NO_3^- and NH_4^+ in distinct treatments could be determined.

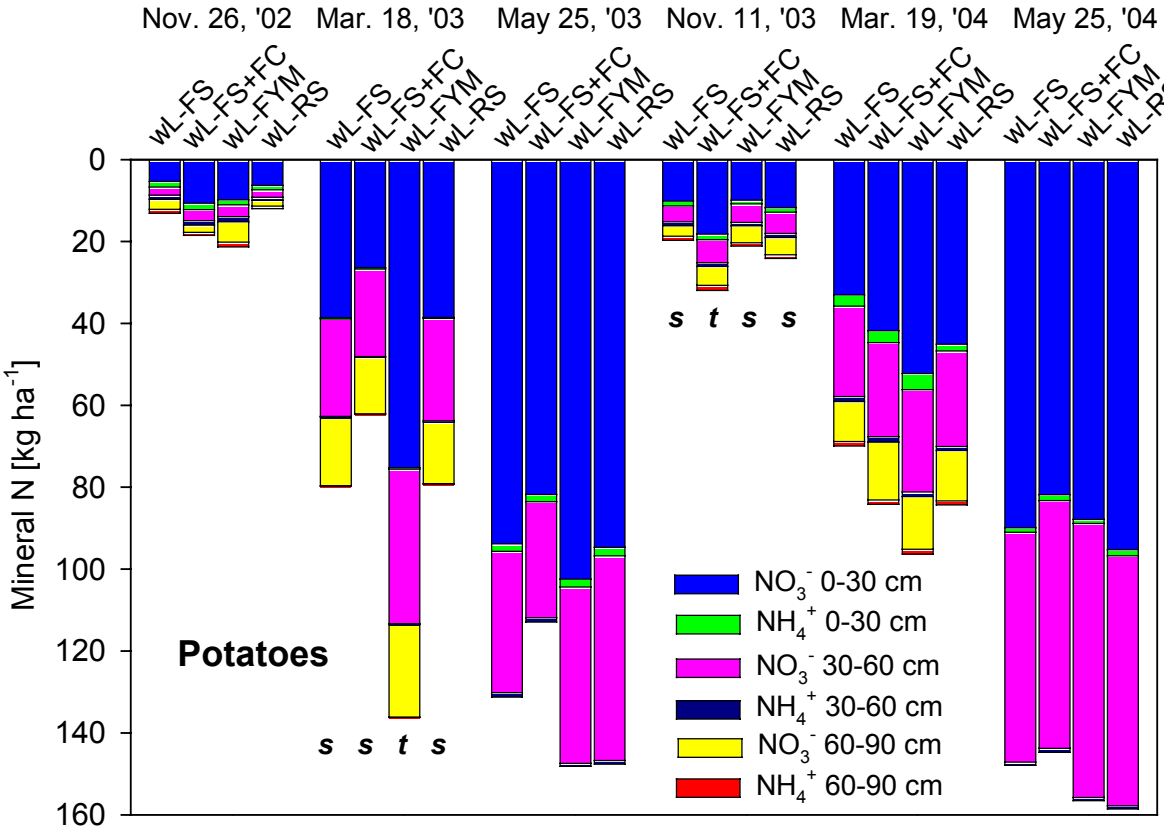


Figure 3.20: Amounts of NO_3^--N and NH_4^+-N in potatoes and intercrops in the cropping system with livestock between November 2002 and May 2004 in soil depths of 0 - 30 cm, 30 - 60 cm, and 60 - 90 cm, respectively, in the different manuring treatments. In May 2003 and 2004 sampling down to 60 cm soil depth. (Data: Kurt Möller, Giessen, personal communication)

Table 3.15: Significant differences in amounts of NO_3^-, NH_4^+, and N_{min} in potatoes and intercrops in the cropping system with livestock between November 2002 and May 2004 in the different manuring treatments. (Data: Kurt Möller, Giessen, personal communication)

		wL-FS	wL-FS+FC	wL-FYM	wL-RS
Nov. 26, 2002	NO_3^- 0 - 30 cm	a	b	b	ab
Mar. 18, 2003	NO_3^- 0 - 30 cm	a	a	b	a
	NO_3^- 30 - 60 cm	a	a	b	a
	NO_3^- cumulative	a	a	b	a
	N_{min} total	s	s	t	s
Nov. 11, 2003	NO_3^- 0 - 30 cm	a	b	a	a
	NO_3^- cumulative	a	b	a	a
	N_{min} total	s	t	s	s

3.2.3 CH_4 Fluxes in Spelt

During season 2004/2005 CH_4 flux rates varied in spelt and prior intercrops between -10 and 0.2 µg C m^{-2} h^{-1} in wL-FS, -11 and 9 µg C m^{-2} h^{-1} in wL-FS+FC, -8 and 235 µg C m^{-2} h^{-1} in wL-FYM, and -11 and 11 µg C m^{-2} h^{-1} in wL-RS (figure 3.11). Albeit single CH_4 emission events were observed in October, on November 24, 2004, and on February 26, 2005, soil acted neverthless as sink for methane. Only on February 26, 2005 a significant emission peak could be determined that was eventually caused by fertilizer application three days before. Partly high CH_4 effluxes in wL-FYM, that could be favored by incorporation of FYM on October 11, 2004, were not significantly different due to similarly high standard deviations. No fluxes occurred on March 14, 2005 in all treatments and on December 8, 2004 in wL-FS and wL-RS. Over the sampling time no trend of elevated or reduced uptake rates in a manuring treatment became apparent. Integration over the sampling period until March 2005 in wL-FYM revealed a carbon efflux 6.8 times higher (1645 g C ha^{-1}) than carbon uptake in wL-RS. Including the CH_4 effluxes in October and on November 24 only for the day of sampling in wL-FYM resulted in a carbon uptake of 42 g C ha^{-1} within the sampling time, thus a reduction of 78%. Integration of CH_4 flux rates over 365 days was performed as described in 3.12. Extrapolation of CH_4 fluxes in wL-FYM during season 2004/2005 was only performed in comparison to wL-FYM of the previous season 2003/2004 because the second reference data of season 2002/2003 were not available in this manuring treatment. Calculation showed soil carbon uptake of 669 g C ha^{-1} in wL-RS compared to 752 g C ha^{-1} in wL-FS, 799 g C ha^{-1} in wL-FS+FC, and 128 g C ha^{-1} in wL-FYM, representing an increased uptake of 12% in wL-FS and of 19% in wL-FS+FC, and a decreased CH_4 oxidation of 81% in wL-FYM (table 3.16). Mean spatial variabilities of CH_4 fluxes of 77%, 134%, 101%, and 122% were assessed in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.17). The CV values for temporal variability of the CH_4 fluxes amounted to 75% in wL-FS, 160% in wL-FS+FC, 286% in wL-FYM, and 153% in wL-RS (table 3.17). Coefficients of correlation (r^2) between CH_4 fluxes in spelt and air temperature and between CH_4 fluxes and temperature in 5 cm soil depths accounted for 0.28 and 0.46, respectively (table 3.9).

In 2003/2004 CH_4 oxidation rates ranged in spelt and prior intercrops between 1.1 and 19 µg C m^{-2} h^{-1} in wL-FS, 0.8 and 18 µg C m^{-2} h^{-1} in wL-FS+FC, 0.5 and 14 µg C m^{-2} h^{-1} in wL-FYM, and 0.7 and 21 µg C m^{-2} h^{-1} in wL-RS with exception of March 5, 2004 when after manuring 49, 105, and 86 µg C m^{-2} h^{-1} were emitted in wL-FS, wL-FS+FC, and wL-RS, respectively (figure 3.12). However, no CH_4 was evolved after fertilizer application on March 25, 2004. CH_4 uptake decreased in fall/winter and increased markedly as of April 2004. Significant differences in CH_4 fluxes between the treatments were found on January 4 as well as on March 5, 2004 a few hours after manuring. Integration of CH_4 flux rates over 365 days (table 3.16) revealed soil carbon uptake of 664 g C ha^{-1} in wL-RS (100%), 677 g C ha^{-1} in wL-FS (102%), 591 g C ha^{-1} in wL-FS+FC (89%), and 523 g C ha^{-1} in wL-FYM (79%). The spatial variability of CH_4 fluxes averaged 38% in wL-FS, 27% in wL-FS+FC, 36% in wL-FYM, and 28% in wL-RS (table 3.17). CV

values for the temporal variability of CH_4 fluxes were in part very high and accounted for 442%, 4779%, 84%, and 2069% in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.17). Coefficients of correlation of 0.57 and 0.64 between CH_4 fluxes in spelt and air and soil temperature in 5 cm soil depth, respectively, were calculated (table 3.9).

Table 3.16: Integrated CH_4 fluxes over 365 days in spelt and potatoes in the cropping system with livestock in the respective manuring treatments.

Season	Crops	Manuring Treatments							
		wL-FS		wL-FS+FC		wL-FYM		wL-RS	
		% of wL-RS	g C ha^{-1}	% of wL-RS	g C ha^{-1}	% of wL-RS	g C ha^{-1}	% of wL-RS	g C ha^{-1}
2002/2003	Spelt	96	-649	102	-687	n.d.	n.d.	100	-675
	Potatoes	88	-498	113	-644	n.d.	n.d.	100	-568
2003/2004	Spelt	102	-677	89	-591	79	-523	100	-664
	Potatoes	120	-618	129	-662	114	-585	100	-514
2004/2005	*Spelt*√	*83*	*-158*	*88*	*-169*	*22*	*-42*‡	*100*	*-191*
						8.6x†	*1645*†		
	Spelt	112	-752	119	-799	19	-128*	100	-669

Spelt√ *2004/2005: Integrated CH_4 fluxes during sampling period from September 2004 until March 2005*

‡*CH_4 emissions included in integration but each only for one day*

†*8.6 times more CH_4 emitted than CH_4 taken up in wL-RS if usual integration of CH_4 emissions performed*

*Extrapolated value calculated only in comparison to season 2003/2004 due to missing value in 2002/2003

n.d.: not determined

Table 3.17: Coefficients of variation (CV) for temporal and mean spatial variability of the CH_4 fluxes in spelt and potatoes in the cropping system with livestock in the respective manuring treatments.

Season	Crops	Manuring Treatments							
		wL-FS		wL-FS+FC		wL-FYM		wL-RS	
		CV % time	Ø CV % space	CV % time	Ø CV % space	CV % time	Ø CV % space	CV % time	Ø CV % space
2002/2003	Spelt	463	30	457	36	58	86	491	47
	Potatoes	94	72	78	32	64	59	72	56
2003/2004	Spelt	442	38	4779	27	84	36	2069	28
	Potatoes	68	39	51	27	93	32	125	1315
2004/2005	*Spelt*√	*75*	*77*	*160*	*134*	*286*	*101*	*153*	*122*

Spelt√ *2004/2005: Coefficients of variation during sampling period from September 2004 until March 2005*

CH_4 oxidation rates in spelt in 2002/2003 reached up to 22 µg C m^{-2} h^{-1} in wL-FS and wL-FS+FC, 21 µg C m^{-2} h^{-1} in wL-FYM (start of investigation on April 27, 2003), and 17 µg C m^{-2} h^{-1} in wL-RS (figure 3.13). After application of liquid fertilizer on February 20, 2003, CH_4 emissions of 472, 719, and 571 µg C m^{-2} h^{-1} in wL-FS, wL-FS+FC, and wL-RS, respectively, were determined. No fluxes could be found on November 20, 2002, in January, on February 4, on April 8 and 27, 2003 in parts or in all manuring treatments. Integrating CH_4 fluxes over 365 days showed a carbon uptake of the arable soil of 675 g C ha^{-1} in wL-RS, 649 g C ha^{-1} in wL-FS, and 687 g C ha^{-1} in wL-FS+FC, representing a decrease of 4% in wL-FS and an increase of 2% in wL-FS+FC compared to wL-RS (table 3.16). Oxidation rates in wL-FYM were not integrated since only results of three months were available. Mean spatial variabilities of CH_4 fluxes of 30%, 36%, 86%, and 47% were assessed in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.17). CV values for temporal variability of the CH_4 fluxes amounted to 463% in wL-FS, 457% in wL-FS+FC, 58% in wL-FYM, and 491% in wL-RS (table 3.17). CV values in wL-FYM were obtained by calculation with the existing data of about three months. Coefficients of correlation (r^2) between CH_4 fluxes in spelt and air temperature and between CH_4 fluxes and temperature in 5 cm soil depths accounted for 0.57 and 0.58, respectively (table 3.9).

The comparison of CH_4 fluxes during season 2004/2005, 2003/2004, and 2002/2003 in spelt and prior intercrops exhibited that the arable soil acted continuously as methane sink, in summer as stronger, in winter as minor sink. No explicit pattern of elevated or reduced oxidation rates in a distinct manuring treatment became apparent. This determination was confirmed considering the integrated values over 365 days, when increased and decreased CH_4 uptake was found in wL-FS and wL-FS+FC compared to the respective control treatment wL-RS in the three seasons.

3.2.4 CH_4 Fluxes in Intercrops and Potatoes

In intercrops and potatoes during season 2003/2004 soil CH_4 uptake rates were found of up to 22 µg C m^{-2} h^{-1} in wL-FS, 13 µg C m^{-2} h^{-1} in wL-FS+FC, and 12 µg C m^{-2} h^{-1} in wL-FYM and wL-RS (figure 3.17). CH_4 emission events were observed on April 22 in wL-FYM and on June 11, 2004 in wL-RS without prior fertilizer application, however without statistical significance. Significant differences between the manuring treatments were determined on October 9 and 23, 2003 in intercrops as well as on April 27, 2004 in potatoes. No fluxes occurred on February 21 in wL-FS+FC and wL-FYM as well as on March 25, 2004 in all treatments. Manuring of solid and liquid fertilizer did not show an obvious impact on CH_4 flux rates at the sampling times. Integration of CH_4 fluxes over 365 days resulted in soil carbon uptake of 514 g C ha^{-1} in wL-RS compared to 618 g C ha^{-1} in wL-FS, 662 g C ha^{-1} in wL-FS+FC, and 585 g C ha^{-1} in wL-FYM, hence an increase of 20% in wL-FS, 29% in wL-FS+FC, and 14% in wL-FYM (table 3.16). Emissions on April 22 and on June 11, 2004 were only for the day of measurement included into the integration. Mean spatial variabilities of CH_4 fluxes of 39%, 27%, 32%, and exceedingly high

1315% were assessed in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.17). CV values for temporal variability of the CH_4 fluxes amounted to 68% in wL-FS, 51% in wL-FS+FC, 93% in wL-FYM, and 125% in wL-RS (table 3.17). Coefficients of correlation (r^2) between CH_4 fluxes in potatoes and air temperature and between CH_4 fluxes and temperature in 5 cm soil depths accounted for 0.29 and 0.27, respectively (table 3.9).

In season 2002/2003 soil CH_4 influxes in intercrops and potatoes accounted for 0 to 16 µg C m^{-2} h^{-1} in wL-FS and wL-RS, 0 to 24 µg C m^{-2} h^{-1} in wL-FS+FC, and 0 to 12 µg C m^{-2} h^{-1} in wL-FYM (start of sampling on May 7, 2003) (figure 3.18). Only on May 7, 2003 in wL-FS a CH_4 emission was observed (3 µg C m^{-2} h^{-1}). Significant differences between the oxidation rates were just found on October 13, 2002. On February 12 and April 8, 2003 no CH_4 fluxes could be detected in all treatments, on May 22, 2003 no flux occurred in wL-FYM. Integration of CH_4 fluxes over 365 days revealed that soil carbon uptake amounted to 568 g C ha^{-1} in wL-RS (100%) in comparison to 498 g C ha^{-1} in wL-FS (88%), and 644 g C ha^{-1} in wL-FS+FC (113%) (table 3.16). The efflux on May 7, 2003 in wL-FS was included into the integration for the day of sampling. In wL-FYM integration of oxidation rates over 365 days were not performed since only data of four month were available. The spatial variability of CH_4 fluxes averaged 72% in wL-FS, 32% in wL-FS+FC, 59% in wL-FYM, and 56% in wL-RS (table 3.17). CV values for the temporal variability of CH_4 fluxes accounted for 94%, 78%, 64%, and 72% in wL-FS, wL-FS+FC, wL-FYM, and wL-RS, respectively (table 3.17). CV values in wL-FYM are based on the existing data of about four months. Coefficients of correlation of 0.48 and 0.44 between CH_4 fluxes in spelt and air and soil temperature in 5 cm soil depth, respectively, were calculated (table 3.9).

Comparison of CH_4 fluxes in intercrops and potatoes in seasons 2003/2004 and 2002/2003 revealed the arable soil as continuous CH_4 sink with only single, non-significant exceptions. In both seasons an increase of soil CH_4 uptake was observed in wL-FS+FC (113% in 2002/2003, and 129% in 2003/2004). However wL-FS exhibited in 2002/2003 a reduced soil carbon uptake of 22%, whereas in 2003/2004 an elevated carbon uptake of 20% was determined compared to respective wL-RS. The comparison of the CH_4 fluxes within the cropping system with livestock over all seasons did not result in an explicit trend of elevated or reduced CH_4 oxidation in a manuring treatment.

3.2.5 Net CO_2 Fluxes in Spelt

During season 2004/2005 quantifiable CO_2 fluxes in spelt and prior intercrops were found in ranges of -22 to 134 mg C m^{-2} h^{-1} in wL-FS, -20 to 72 mg C m^{-2} h^{-1} in wL-FS+FC, -40 to 146 mg C m^{-2} h^{-1} in wL-FYM, and -28 to 147 mg C m^{-2} h^{-1} in wL-RS (figure 3.11). Highest emissions were determined after drilling of spelt in October 2004 with slightly reduced CO_2 effluxes in wL-FS-FC. Non-quantifiable CO_2 uptake rates were detected in all manuring treatments in September 2004 in the intercrops. Beside sampling days when in all treatments emission or

uptake was observed, respectively, emission and uptake occurred at the same sampling date on November 24, 2004, February 16 and 26, and on March 14, 2005. Significant differences, however, were only confirmed on February 26 (3 days after manuring) as well as on March 1, 2005. Integration of CO_2 fluxes over 365 days (as done with N_2O and CH_4 fluxes) were not carried out due to the non-quantifiable uptake rates.

In 2003/2004 quantifiable CO_2 fluxes in spelt and prior intercrops varied between -155 and 73 mg C m^{-2} h^{-1} in wL-FS, -130 and 104 mg C m^{-2} h^{-1} in wL-FS+FC, -136 and 66 mg C m^{-2} h^{-1} in wL-FYM, and -136 and 70 mg C m^{-2} h^{-1} in wL-RS (figure 3.12). After drilling of spelt on October 14 emissions in all treatments were observed until November 2003, with reduced effluxes in wL-FS+FC (significant on October 23). Significantly elevated CO_2 emissions were found on March 5, 2004 a few hours after fertilizer application in wL-FS, wL-FS+FC, and wL-RS, when CO_2 dissolved in the fertilizer degassed. On March 11 significances in uptake rates between the treatments were determined. As of March 25, 2004 only not quantifiable CO_2 uptake was detected in all treatments.

Quantifiable CO_2 fluxes in spelt during season 2002/2003 ranged between -71 and 204 mg C m^{-2} h^{-1} in wL-FS, -98 and 257 mg C m^{-2} h^{-1} in wL-FS+FC, and -30 and 116 mg C m^{-2} h^{-1} in wL-RS (figure 3.13). Sampling in wL-FYM started on April 27, 2003 and revealed in the remaining time only not quantifiable CO_2 uptake. After drilling of spelt on October 11, 2002 CO_2 emissions in all manuring treatments occurred until the beginning of November. Very high emissions were observed in all treatments a few hours after manuring on February 20, 2003 (significant) when degassing of CO_2 dissolved in the fertilizers occurred. On April 8 CO_2 uptake rates differed significantly.

Comparison of CO_2 fluxes in spelt and intercrops during season 2004/2005, 2003/2004, and 2002/2003 showed a trend of elevated emissions after soil tillage and drilling of spelt in fall over few weeks. Likewise, a trend of reduced effluxes in wL-FS+FC was shown probably caused by harvesting of intercrops in this manuring treatment entailing a decreased amount of substrate for mineralization. When sampling at the day of manuring, very high emissions could be observed due to degassing of dissolved CO_2 in the fertilizer.

3.2.6 Net CO_2 Fluxes in Intercrops and Potatoes

In season 2003/2004 quantifiable CO_2 fluxes in intercrops and potatoes accounted for -71 to 135 mg C m^{-2} h^{-1} in wL-FS, -35 to 175 mg C m^{-2} h^{-1} in wL-FS+FC, -86 to 82 mg C m^{-2} h^{-1} in wL-FYM, and -78 to 146 mg C m^{-2} h^{-1} in wL-RS (figure 3.17). Until December 2003 CO_2 uptake was observed in all manuring treatments with exception of wL-FYM and wL-RS on September 10 and wL-FS+FC after harvest of intercrops on October 29, 2003. Sampling in November and December 2003 revealed significant differences between the treatments. As of January 2004 in all treatments CO_2 emissions were determined (except June and July with not quantifiable CO_2 uptake rates) that differed significantly on February 21, on May 18, and on August 6,

2004. The significance found on May 19 could have been caused by fertilizer applicaton one day before. The level of CO_2 effluxes increased in April 2004 with dibbling of potatoes on April 21. No explicit trend of elevated or reduced emissions in a distinct manuring treatment became apparent even if slightly higher emission rates were often found in wL-FS+FC.

During season 2002/2003 CO_2 emissions in intercrops and potatoes amounted up to 197 mg C m^{-2} h^{-1} in wL-FS, 198 mg C m^{-2} h^{-1} in wL-FS+FC, 137 mg C m^{-2} h^{-1} in wL-FYM (start of investigation on May 7, 2003), and 311 mg C m^{-2} h^{-1} in wL-RS (figure 3.18). CO_2 effluxes in all investigated treatments were determined on October 13 and on December 16, 2002 in intercrops, on May 7 and 22, on July 20, and on August 14, 2003 in potatoes. Not quantifiable CO_2 uptake rates were found in all treatments until October 3, 2002 and in June 2003, as well as in wL-FS and wL-RS in November and on December 4, 2002 in contrast to wL-FS+FC, where intercrops had been harvested for fermentation. No fluxes could be determined on February 12 in all treatments and on April 8, 2003 in wL-RS.

Comparing CO_2 fluxes measured in intercrops and potatoes in 2003/2004 and 2002/2003, CO_2 uptake was mostly found until December with exception of wL-FS+FC after cutting the intercrops. Elevated CO_2 emissions were notabely observed after dibbling of potatoes.

3.3 Incubation Experiments

Incubation experiments were performed to investigate in more detail N_2O production after application of different fertilizers on arable soil. Investigations were performed with unplanted soil to exclude any crop effects and under optimized conditions for denitrification, thus in anoxic atmosphere. N_2O production after anoxic incubation of the differently fertilized soils, the bare arable soil, and the pure fertilizers revealed clearly different production pattern (figure 3.21). None of the pure fertilizers raw slurry (RS), fermented slurry (FS), and fermented crops (FC) showed N_2O production during the incubation time. In contrast, the bare soil exhibited high production up to 257 nmol N_2O g^{-1} soil after 30 hours of incubation that had disappeared after 48 hours. In the fertilized soils N_2O values of up to 11, 12, and 14 nmol N_2O g^{-1} weighted sample were found in treatments of soil amended with RS, FS, and FC, respectively. After 24 hours N_2O was not detectable anymore in each differently fertilized soil. Thus, N_2O losses of the fertilized soils varied in similar range and took an intermediate position between production of the pure fertilizers and the bare soil. Since gas sampling was not performed in higher frequency, e.g. hourly, maximum values of emitted N_2O could be underestimated. Time course of N_2O effluxes and production pattern were in the fore instead of maximum production values.

Evaluation of NO_3^- values measured in the differently fertilized soils (figure 3.22) showed a similar decrease of NO_3^- starting from 2300 nmol in Soil & RS, 2350 nmol in Soil & FS, and 1970 nmol g^{-1} soil and fertilizer in Soil & FC. As of 23 hours NO_3^- concentrations remained at constant levels (exact time point when concentration did not decrease anymore was not determined) that were around 90, 380, and 20 nmol g^{-1} soil and fertilizer in Soil & RS, Soil & FS, and Soil & FC, respectively.

Considering investigation of CH_4 fluxes (figure 3.21), the highest amount of methane was found at the end of the sampling period in pure RS with 2404 nmol CH_4 g^{-1} fertilizer. Pure FS and pure FC revealed CH_4 effluxes of 1262 and 196 nmol CH_4 g^{-1} fertilizer, respectively. CH_4 values obtained in the bare arable soil and in soil fertilized with FC ranged at the detection limit. CH_4 emissions in soils amended with RS and FS amounted to 39 and 223 nmol CH_4 g^{-1} weighted sample, respectively.

Regarding CO_2 emissions, CO_2 concentrations accounted for 21 µmol CO_2 g^{-1} fertilizer in pure RS and pure FS and for 15 µmol CO_2 g^{-1} fertilizer in pure FC after 54 hours of incubation (figure 3.22). The smallest amount of emitted CO_2 was observed in the bare arable soil with around 3 µmol CO_2 g^{-1} soil. CO_2 effluxes in the differently fertilized soils ranged between pure fertilizers and bare soil and exhibiting 6, 10, and 8 µmol CO_2 g^{-1} weighted sample in soil amended with RS, FS, and FC, respectively.

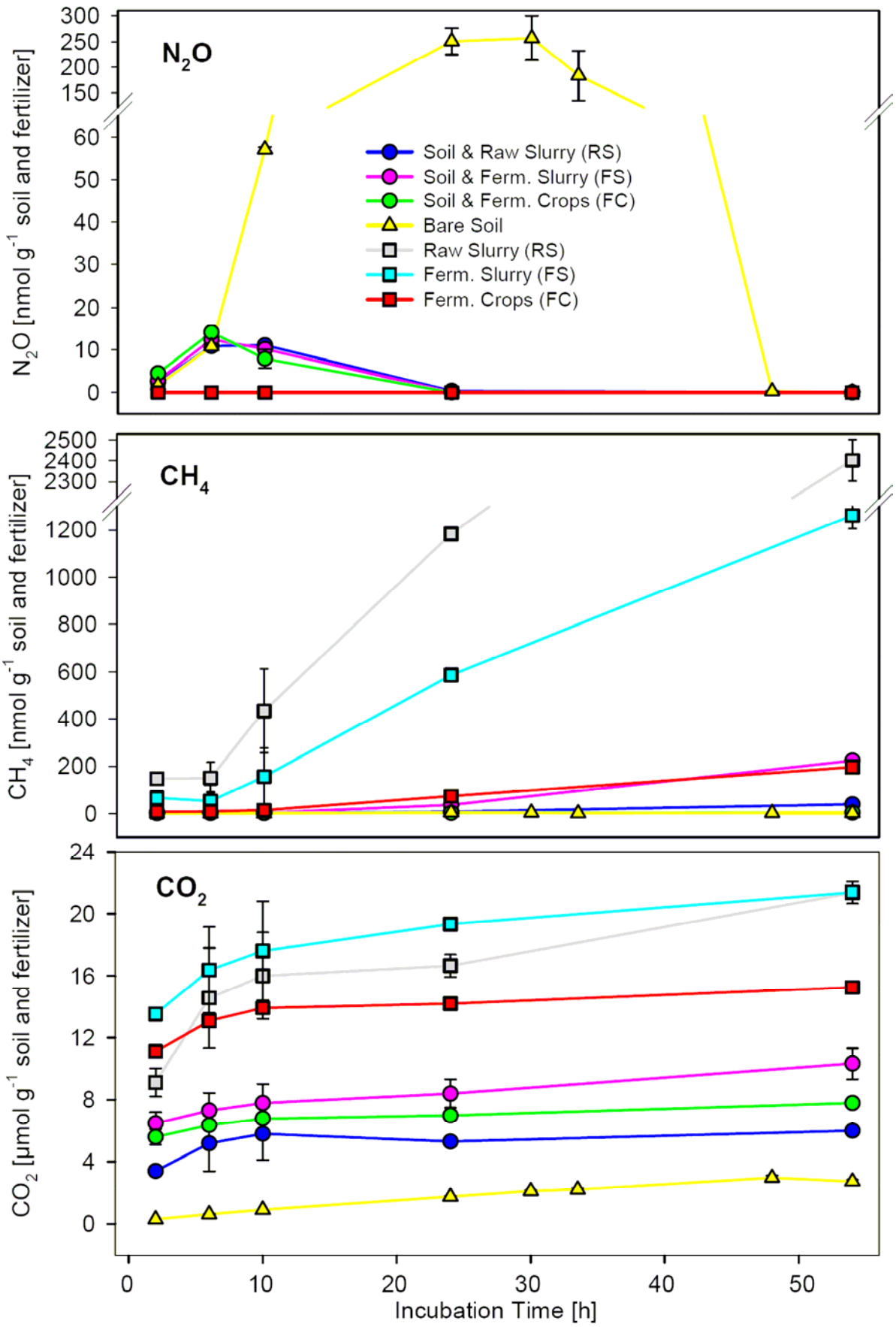


Figure 3.21: Amounts of N_2O-, CH_4-, and CO_2 emitted of the differently fertilized soils, the bare soil, and the pure fertilizers during anoxic incubation.

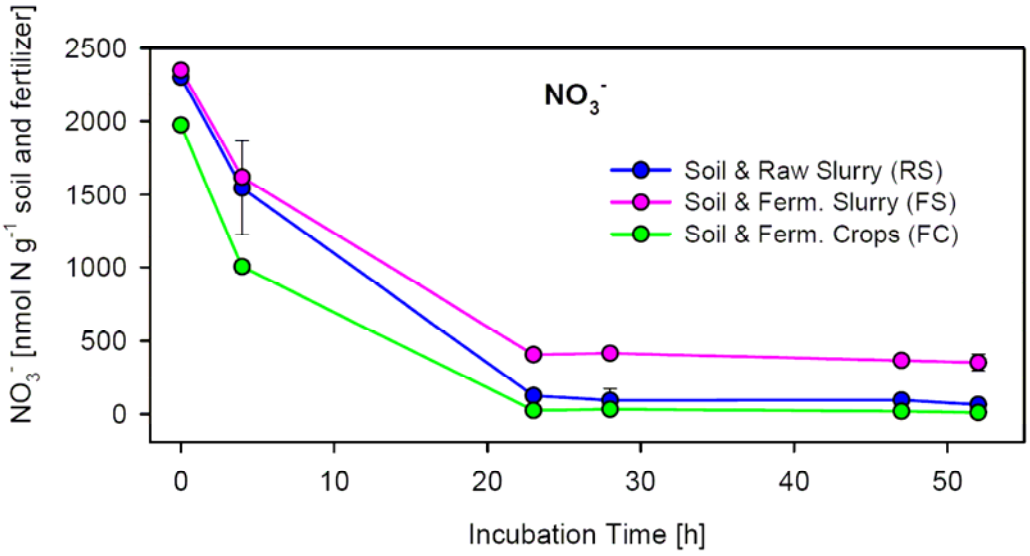


Figure 3.22: NO_3^- contents in the differently fertilized soils during anoxic incubation.

N_2O production was investigated again in the bare arable soil as well as in the soil amended with the three different fertilizers, however with 10% acetylene in the anoxic atmosphere to inhibit the enzyme N_2O reductase and hence to accumulate the produced N_2O. At the beginning, most N_2O was produced in soil amended with FC, whereas N_2O production was lowest in the bare soil (figure 3.23). After 24 hours, amounts of 469, 510, and 502 nmol N_2O g^{-1} soil had been emitted in soils fertilized with RS, FS, and FC, respectively, the bare soil exhibited a loss of 440 nmol N_2O g^{-1} soil. The fertilized soils showed thereafter no further increase of the N_2O concentrations in the headspaces, whereas in the bare soil the amount of N_2O still increased. After 53 hours a loss of 505 nmol N_2O g^{-1} soil was determined in this treatment.

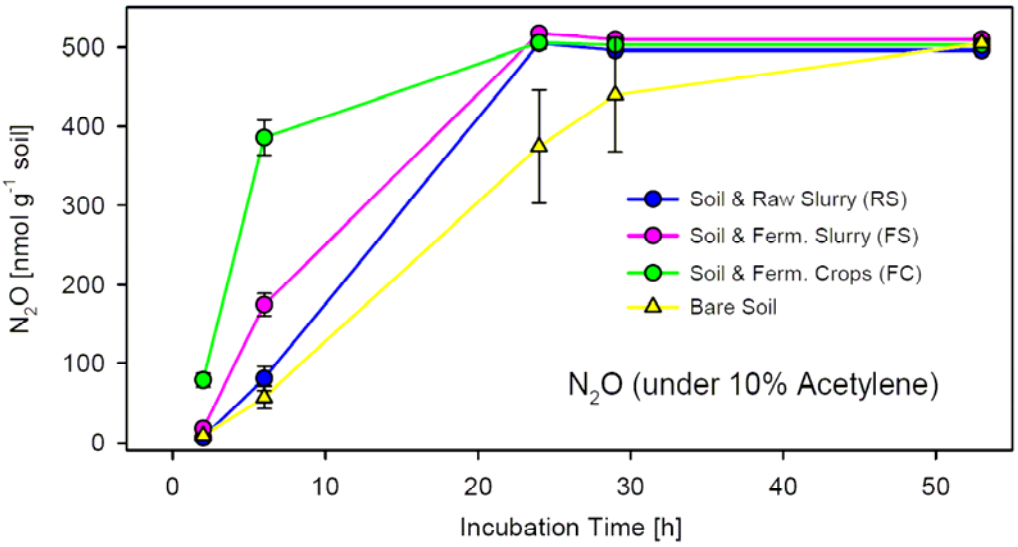


Figure 3.23: Accumulation of emitted N_2O during incubation under anoxic atmosphere containing 10% acetylene in the differently fertilized soils and the bare soil.

3.4 Greenhouse Studies

The aim of the greenhouse studies was to investigate the impact of manuring on the denitrifying activity and the soil bacterial denitrifying community in more detail in planted soil and under comparable, standardized conditions. For characteristics of applied raw slurry (RS), fermented slurry (FS), and fermented crops (FC) to the microcosms refer to table 3.18.

Table 3.18: Characteristics of the different fertilizers. MPN numbers in parentheses are 95% confidence limits multiplied by the respective order of magnitude. *NirK* and *nirS* gene target numbers in parentheses are standard deviations multiplied by the respective order of magnitude.

	Raw Slurry (RS)	**Fermented Slurry (FS)**	**Fermented Crops (FC)**
Total Nitrogen Applied [µmol g^{-1} Soil]	18.1	18.1	4.5
NH_4^+ Applied [µmol N g^{-1} Soil]	5.56	4.5	2.45
NO_3^- Applied [µmol N g^{-1} Soil]	0.0004	0.0003	0.0003
Total Nitrogen [µmol g^{-1} Fertilizer]	230	252	80
NH_4^+ [µmol N g^{-1} Fertilizer]	70.8	62.7	43.1
NO_3^- [µmol N g^{-1} Fertilizer]	0.005	0.005	0.006
Dry Matter Content [%]	10.9	9.8	2.5
pH	7.0	7.1	5.9
MPN of NO_3^- Reducers [g^{-1} Fertilizer]	2.4×10^8 (0.4 – 9.9)	2.3×10^7 (0.5 – 9.4)	2.3×10^7 (0.5 – 9.4)
***nirS* Gene Targets [g^{-1} Fertilizer]**	4.7×10^7 (± 1.2)	3.9×10^7 (± 1.4)	2.1×10^7 (± 0.3)
***nirK* Gene Targets [g^{-1} Fertilizer]**	2.4×10^6 (± 0.3)	1.6×10^6 (± 0.3)	7.5×10^6 (± 0.8)
Potential Denitrifying Activity [nmol N_2O g^{-1} h^{-1}]	4.2	2.2	0.01

Soil mineral nitrogen concentrations (figure 3.24) revealed two hours after manuring high NH_4^+ concentrations in the soil fertilized with FS (9290 nmol g^{-1} soil) and RS (6512 nmol g^{-1} soil) and lower amounts in FC (1762 nmol g^{-1} soil) in comparison to NH_4^+ concentrations ranging at the detection limit in the unfertilized soil. After seven days amounts of NH_4^+ had decreased in all manuring treatments to the detection limit. NO_3^- concentrations raised up to 900, 869, and 823 nmol g^{-1} soil on day 4 in RS, FS, and FC, respectively, decreased subsequently and were not detectable anymore as of day 15 (RS) or day 22 (FS and FC). In the unfertilized soil

amounts of NO_3^- and NO_2^- fluctuated around the detection limit during the sampling period. NO_2^- concentrations in the fertilized soils increased faster but to a lesser extent (26 nmnol g^{-1} soil in RS, 22 nmnol g^{-1} soil in FS, and 29 nmnol g^{-1} soil in FC) and had declined in all treatments to the detection limit on day 10.

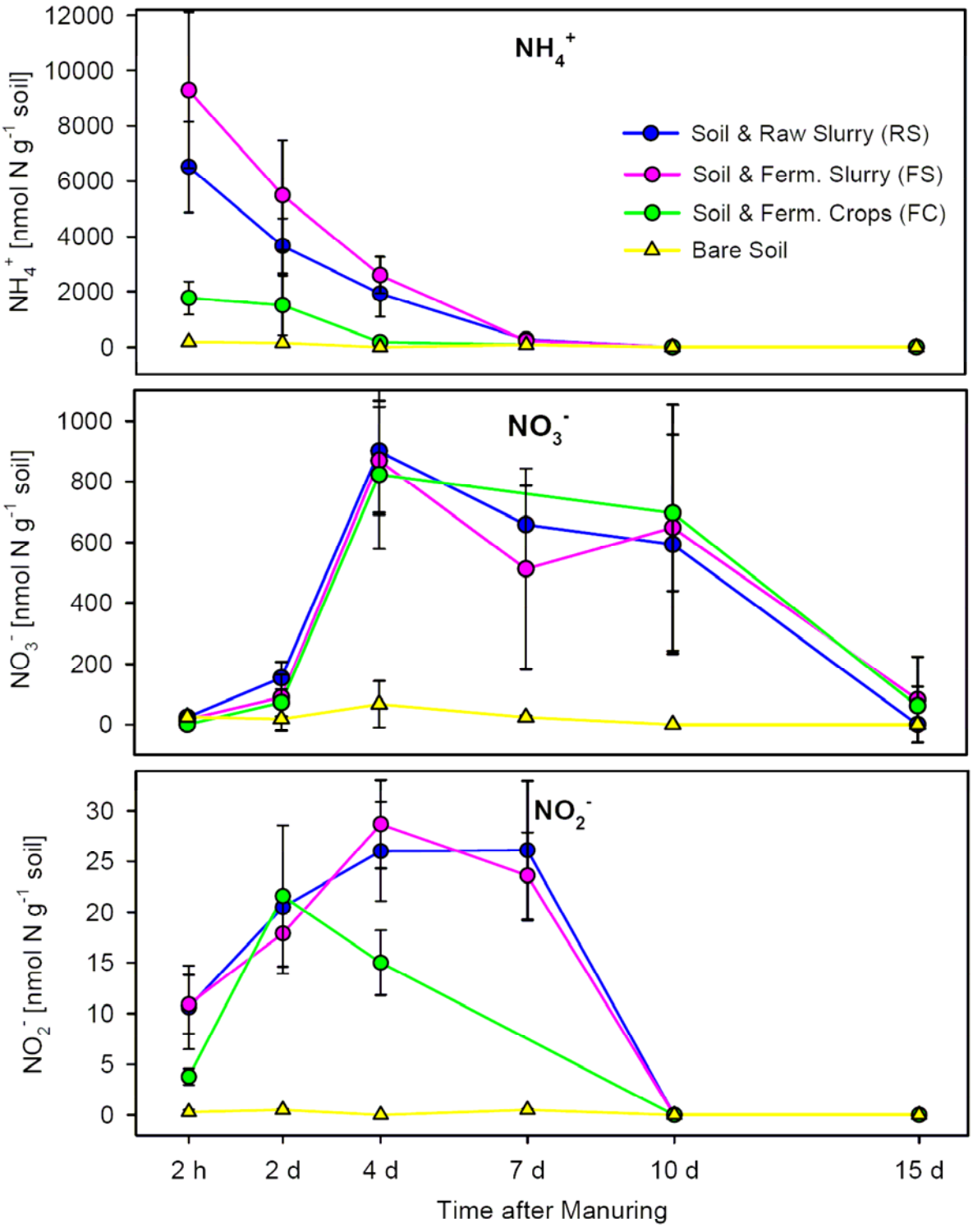


Figure 3.24: Soil mineral nitrogen contents of the differently fertilized soils and the unfertilized soil in the upper soil layer (~6 cm). After 15 days changes did not occur anymore.

The fresh weight of shoots and leaves in the different manuring treatments was assessed 22 days after fertilization and revealed significantly higher values for the RS and FS amended crops (23.4 and 24.7 g pot^{-1}) compared to the unfertilized crops (16.2 g pot^{-1}) and the crops fertilized with FC (17.8 g pot^{-1}).

All fertilizers applied caused an increase of N_2O emissions during the first five - six days (figure 3.25), but after seven days changes of emission did not occur anymore. Very high flux rates (up to 29 µmol N_2O m^{-2} h^{-1}) were observed in the first two days in treatment FC, whereas emission rates in RS and FS remained on a constant elevated level of about 2.3 µmol N_2O m^{-2} h^{-1} in RS and 4 µmol N_2O m^{-2} h^{-1} in FS for four and three days, respectively, before decreasing. N_2O effluxes differed significantly between the manuring treatments until day 3.

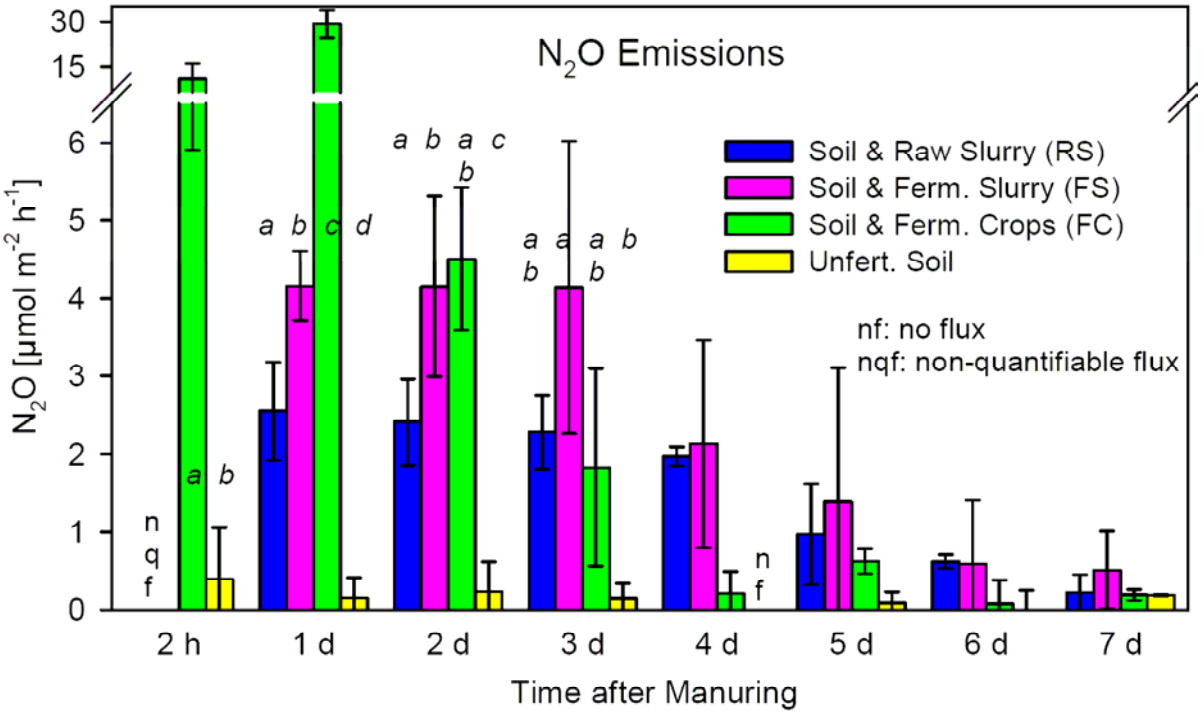


Figure 3.25: *In-situ* N_2O emission rates of the differently fertilized soils and the unfertilized soil. After seven days no changes of emission occurred anymore.

For easier comparableness denitrifying enzyme activities investigated in the different manuring treatments are presented in % of the respective N_2O production rate at the same day in the unfertilized control soil. Absolute values of N_2O production rates varied in soil fertilized with RS between 4.1 and 16.2 nmol N_2O, with FS between 4.1 and 14.5 nmol N_2O, with FC between 4.9 and 10.4 nmol N_2O, and in the unfertilized soil between 4.8 and 10 nmol N_2O g^{-1} soil h^{-1}. Evaluation of the potential denitrifying activites (figure 3.26) did not reveal a significantly elevated activity in FC during the sampling period in contrast to N_2O emissions. However, the unaffected activity agreed with the very low denitrification activity (0.01 nmol g^{-1} fertilizer h^{-1}) in the pure FC fertilizer (table 3.18). Manuring of RS and FS resulted after two hours in significantly decreased and after two days in significantly stimulated denitrification

activities. At day 4 a significantly elevated denitrifying enzyme activity was still observed in RS, whereas at day 7 RS and FS differed only significantly of each other but not from the unfertilized control soil. Denitrification activities in the pure fertilizers RS and FS exhibited an emission rate of 4.2 and 2.2 nmol N_2O g^{-1} fertilizer and h^{-1}, respectively (table 3.18). After ten days significant changes in potential denitrifying activity were not assessed anymore.

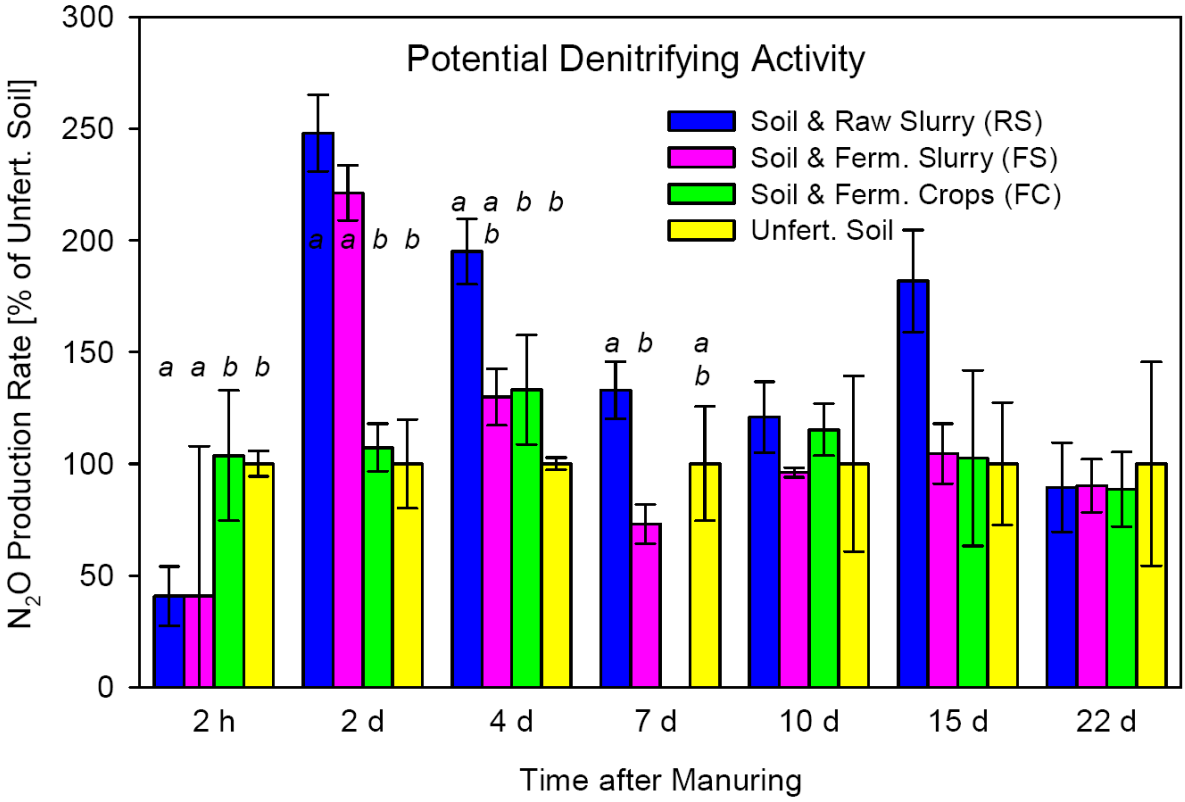


Figure 3.26: Potential denitrifying activity of the differently fertilized soils and the unfertilized soil in the upper soil layer (~6 cm).

Enumeration of nitrate reducers by most probable number technique (MPN) in three parallels resulted in cell numbers g^{-1} dw soil between 2.6 x 10^8 and 6.6 x 10^9 in RS, 1.2 x 10^8 and 2.9 x 10^{10} in FS, 1.8 x 10^8 and 1.9 x 10^9 in FC, and 2.7 x 10^8 and 2.9 x 10^9 in the unfertilized soil (table 3.19). Thus, notably when considering the 95% confidence limits, no broad numerical differences could be observed between the manuring treatments as well as during investigation. Nitrate reducers in pure fertilizers amounted to 2.4 x 10^8 in pure RS and 2.3 x 10^7 in pure FS and pure FC (table 3.18).

Quantification of gene copies of the two alternative nitrite reductases by real-time PCR, however, led to frequent, mostly significant increases of *nirK* copies in the fertilized soils compared to the unfertilized soil, notably in RS and FS (figure 3.27). As done with results of the denitrifying enzyme activities, numbers of *nirK* and *nirS* gene copies in the manuring treatments are shown in % of the respective *nirK* and *nirS* copy number at the same day in the unfertilized control soil. Absolute values of *nirK* copies were determined in soil fertilized with RS

between 6.4 x 10^6 and 2.4 x 10^7, with FS between 5.8 x 10^6 and 2.5 x 10^7, with FC between 5.5 x 10^6 and 2.0 x 10^7, and in the unfertilized soil between 3.7 x 10^6 and 1.7 x 10^7 targets g^{-1} soil. Thus, *nirK* copies ranged in RS between 142 and 382%, in FS between 99 and 276%, and in FC between 90 and 245% of respective *nirK* copies in the unfertilized soil. *NirS* copies varied between 112 and 320% in RS, 106 and 293% in FS, and 39 and 325% of *nirS* copies in the unfertilized soil (figure 3.27). Significant increases of *nirS* copies in the soils amended with manure were observed from day 10 on after fertilization, i.e. in all manuring treatments at day 10, in RS and FS at day 15, and in FS and FC at day 22. The absolute values of *nirS* copy numbers ranged in RS from 7.4 x 10^6 to 4.3 x 10^7, in FS from 8.7 x 10^6 to 4.3 x 10^7, in FC from 2.8 x 10^6 to 3.2 x 10^7, and in the unfertilized soil from 6.6 x 10^6 to 2.4 x 10^7 targets g^{-1} soil.

Table 3.19: MPN enumeration of nitrate reducers in the differently fertilized soils and the unfertilized soil in the upper soil layer (~6 cm). Numbers in parentheses are 95% confidence limits multiplied by the respective order of magnitude.

MPN of NO_3^- Reducers [g^{-1} dw soil]				
Time after Manuring	**Manuring Treatments**			
	Soil & Raw Slurry	**Soil & Ferm. Slurry**	**Soil & Ferm. Crops**	**Unfertilized Soil**
2 h	6.5 x 10^8 (1.3 – 28)	3.3 x 10^8 (0.7 – 14)	5.8 x 10^8 (1.2 – 24)	5.7 x 10^8 (1.2 – 24)
2 d	1.5 x 10^9 (0.4 – 4.6)	3.1 x 10^9 (0.5 – 13)	1.9 x 10^8 (0.6 – 4.7)	3.5 x 10^8 (1.1 – 12)
4 d	2.6 x 10^8 (0.6 – 5.0)	2.9 x 10^{10} (0.6 – 12)	1.8 x 10^8 (0.6 – 4.7)	2.7 x 10^8 (0.6 – 5.1)
7 d	6.6 x 10^9 (1.7 – 19)	2.3 x 10^8 (0.5 – 9.6)	----	2.4 x 10^9 (0.5 – 9.8)
10 d	9.6 x 10^8 (2.2 – 26)	----	1.2 x 10^9 (0.2 – 4.6)	1.2 x 10^9 (0.2 – 4.7)
15 d	2.9 x 10^8 (0.6 – 12)	1.2 x 10^8 (0.2 – 4.5)	1.9 x 10^9 (0.6 – 12)	1.2 x 10^9 (0.2 – 4.6)
22 d	1.8 x 10^9 (0.6 – 4.6)	5.3 x 10^9 (1.1 – 22)	4.6 x 10^8 (1.1 – 13)	2.9 x 10^9 (0.6 – 12)

Evaluating SSCP fingerprinting patterns of *nirS* gene fragments during the sampling period, impact of fertilizer application on the denitrifier composition became visible directly after manuring (figure 3.28). Distinct bands had appeared two hours after fertilization, notably in RS and FS, which were mostly not present in the fertilizers. Until day 10, when the arisen bands of the nucleic acids in those two manuring treatments had disappeared, patterns of RS and FS altered between day 0 (two hours after application) and day 7. In contrast, manuring with FC did not result in broad changes in SSCP patterns during the investigation period so that patterns of the unfertilized soil and of the soil amended with FC remained similar.

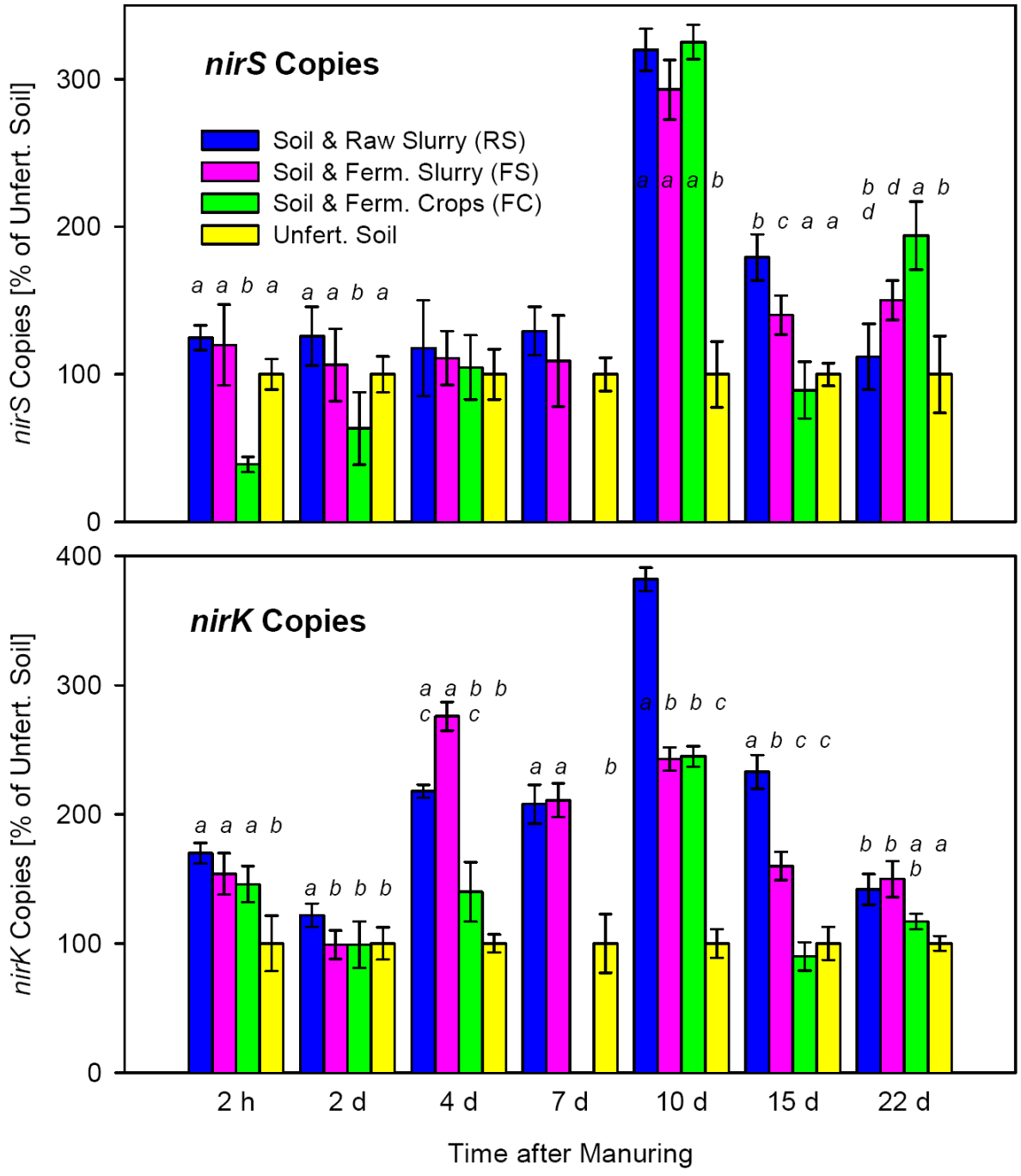
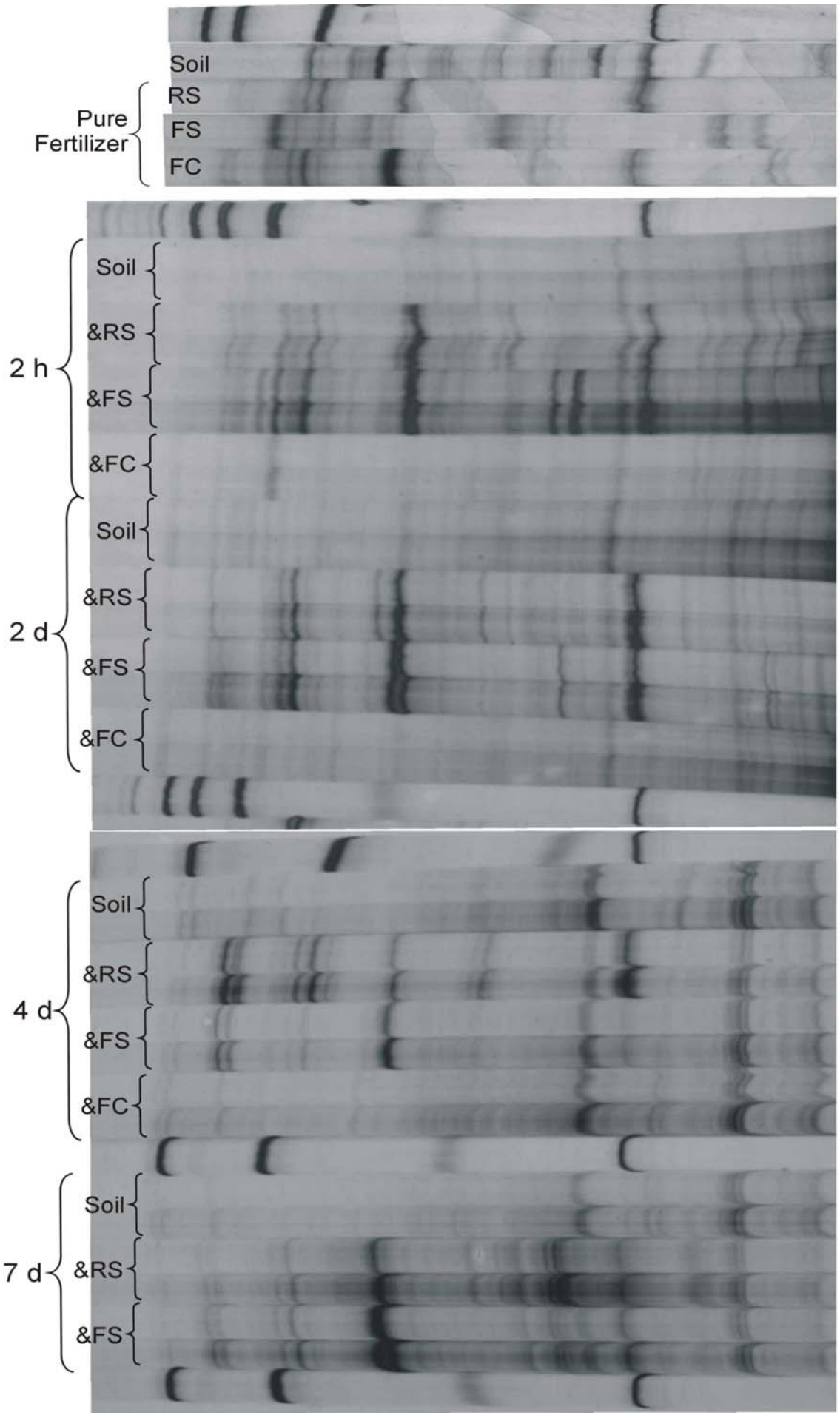


Figure 3.27: *nirS* and *nirK* gene copies encoding the two alternative nitrite reductases quantified by real-time PCR in the differently fertilized soils in % of the respective unfertilized soil in the upper soil layer (~6 cm).


Soil
RS
Pure
Fertilizer
FS
FC
2 h
Soil
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&FS
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4 d
Soil
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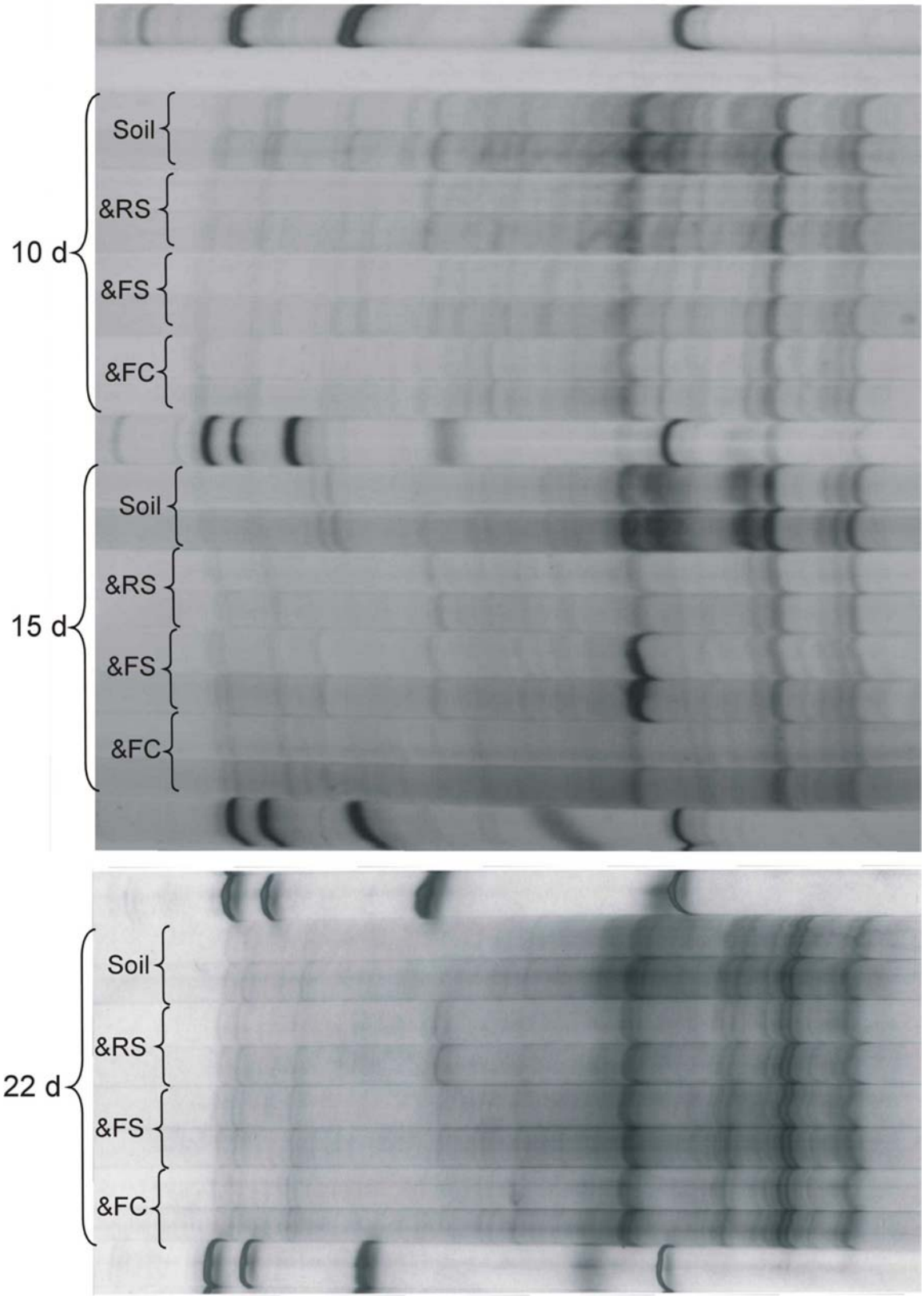

Figure 3.28: SSCP patterns of *nirS* nitrite reductase gene fragments in the differently fertilized soils 2 hours, 2, 4, 7, 10, 15, and 22 days after manuring.

Partial *nirS* sequences (410 - 425 bp) were obtained from 34, 21, 23, and 21 clones derived from arable soil, raw slurry, fermented slurry, and fermented crop material, respectively. They were confirmed as *nirS* genes except clone FS 40, for which only very few and very short matching segments could be found by BLAST search. For both dendrogram calculations, regions of insertion or deletion were omitted from the analysis because of uncertain alignment and the sequence of clone FS 40 was disregarded. In the DNA-based tree appeared to be four major clusters consisting exclusively of clones derived from arable soil, from manures, or from manure and soil, respectively (figure 3.29). However, bootstrap analysis did not support those major clusters with high values in contrast to several subclusters. Within the mixed cluster, all soil clones grouped closely in a subcluster anyway. *Thauera* sp., *Alcaligenes faecalis*, and *Pseudomonas stutzeri* branched in this mixed cluster, whereas only *Cupriavidus necator* (formerly *Ralstonia eutropha*) coincided with the soil cluster. *Acidovorax* sp., *Pseudomonas* sp., *Comamonas* sp., *Roseobacter denitrificans*, and *Paracoccus denitrificans* clustered in the RS+FC group. *Pseudomonas migulae*, *P. lini*, *P. mandelii*, *P. fluorescens*, *P. aeruginosa*, and *P. qianpuensis* branched in the manure cluster. Comparing identities of denitrifying strains with environmental clones, *Thauera* sp. exhibited the highest nucleotide identity of 100% with clone FC 15. The highest nucleotide identities between the other isolates and the clones ranged between 93.3% (*Pseudomonas qianpuensis* - clone FC 25) and 62.9% (*Roseobacter denitrificans* - clone FS 64). Frequently, clones derived from manures branched with clones or strains isolated from wastewater treatment plants or activated sludge. A clone sequence derived from an arable soil (clone US6A) grouped with clones derived from the arable soil. Nucleotide sequences of isolated strains often clustered rather with DNA sequences of other isolates than with sequences of the new environmental *nirS* clones.

The dendrogram based on the deduced amino acid sequences tended to reveal similar major clusters even if *Roseobacter denitrificans* and *Paracoccus denitrificans* formed a separate cluster (figure 3.30). The topology differed slightly due to deeper branching of several (sub-)clusters. Nevertheless, within the mixed cluster of soil and manure clones, the soil clones grouped closely, and within the manure cluster, similar subclusters of FC and RS clones were recovered as in the DNA dendrogram. The soil cluster and the manure and soil cluster exhibited in both dendrograms a similar grouping of isolated strains, whereas in the amino acid tree the cluster of *Acidovorax* sp., *Pseudomonas* sp., and *Comamonas* sp. coincided now with the manure cluster. High bootstrap values of all major clusters were lacking, too. *Roseobacter denitrificans* and *Paracoccus denitrificans* showed the greatest distance to the environmental clones and the other strains exhibiting highest amino acid identities of only 60.5% (*R. denitrificans* - e.g. clone FS 64) and 67.8% (*P. denitrificans* - e.g. clone AS 27). As on DNA level, the highest sequence identity was confirmed for *Thauera* sp. and clone FC 15. Identities between the remaining strains and environmental clones varied from 97.9% (*Pseudomonas qianpuensis* - clone FC 45) - 85.8% (*Pseudomonas lini* - clone FC 33).

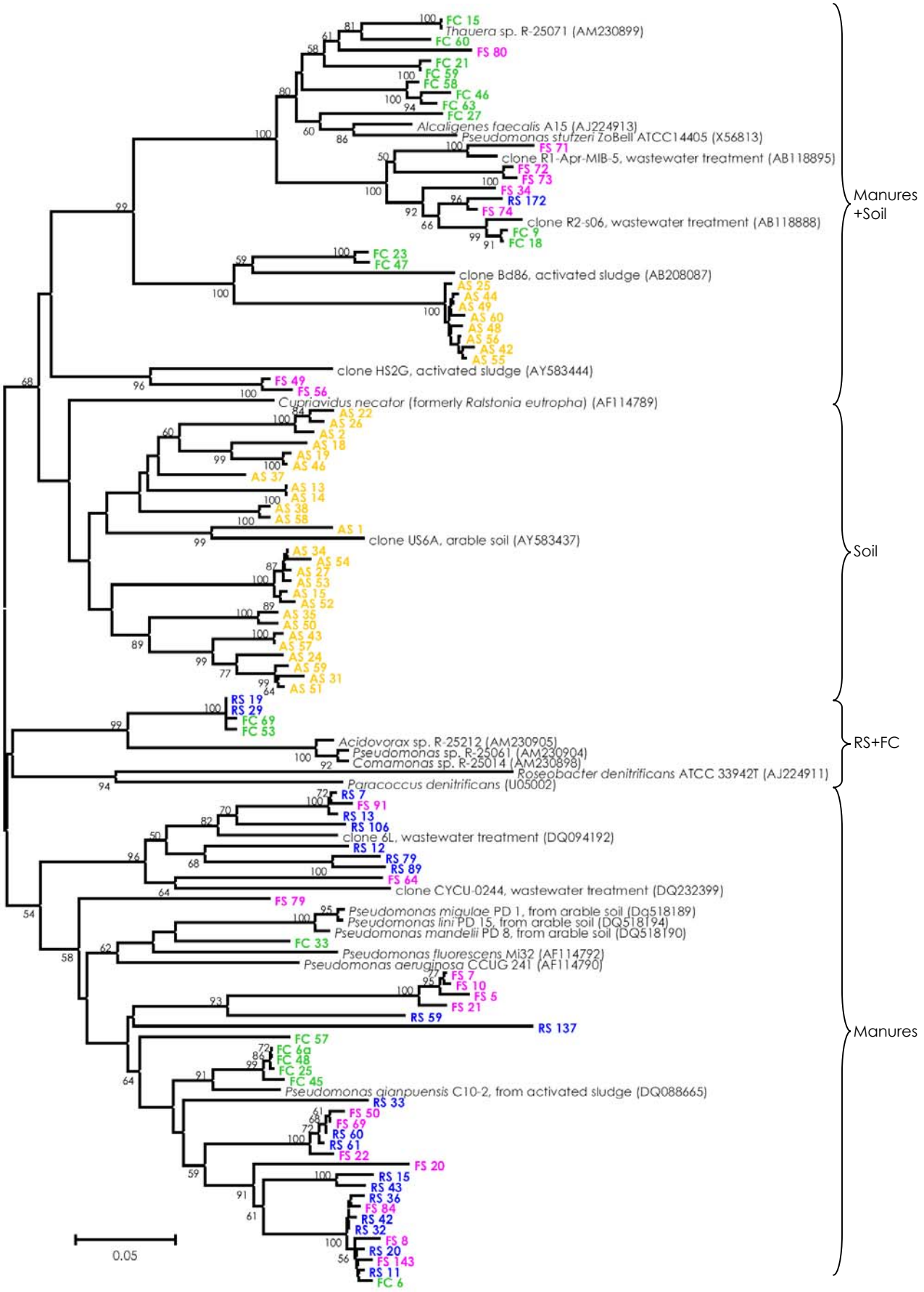

Figure 3.29: Neighbor-joining analysis of partial *nirS* gene fragments (410 - 425 bp) cloned from the three different manures (RS, FS, FC) and the arable soil (AS). Bootstrap values indicate the percentage of 1000 replicate trees supporting the branching order, values below 50 are omitted. Scale bar: 5 base pairs difference in 100 sequence positions. Only *Roseobacter denitrificans* is a type strain.

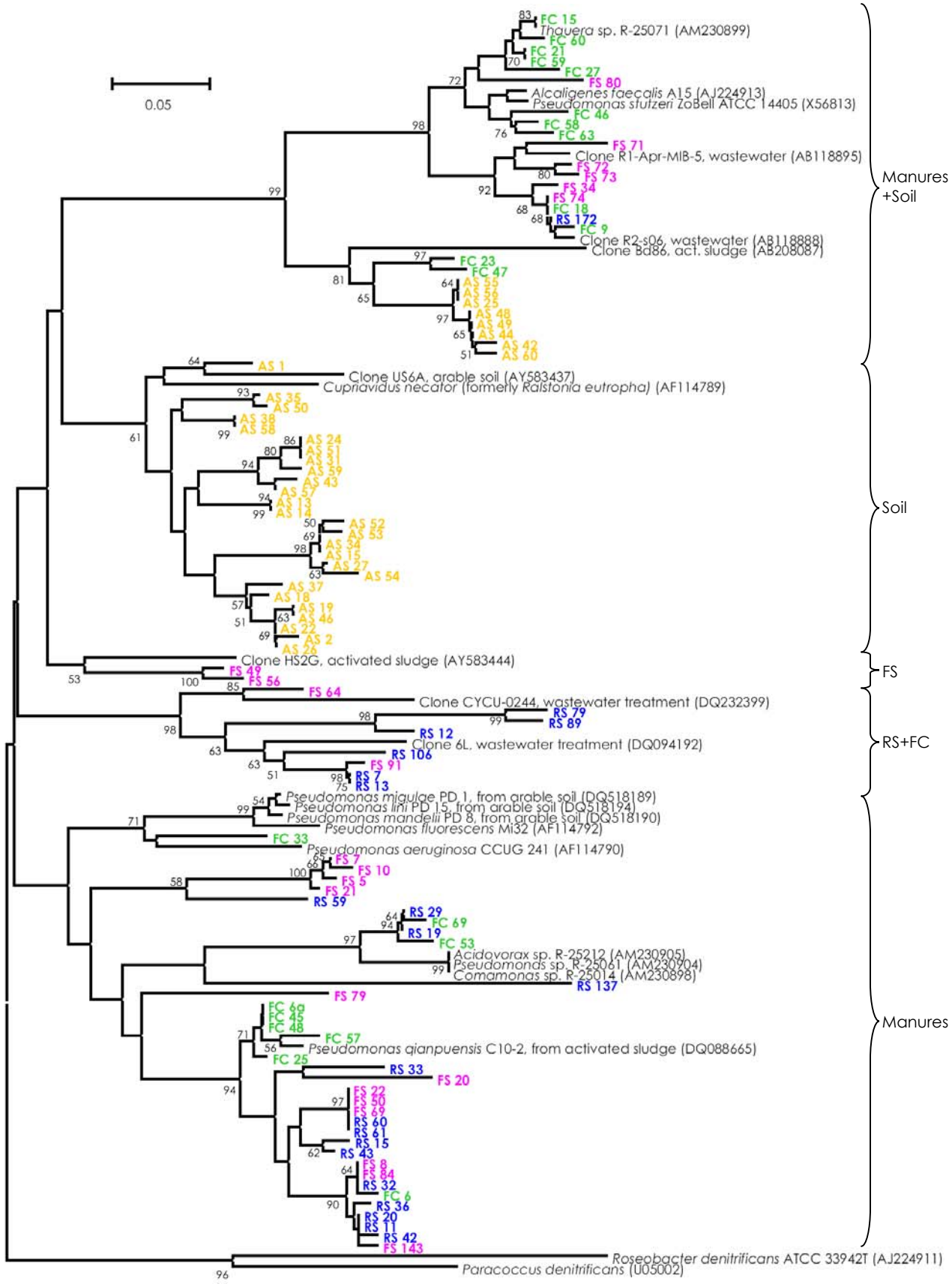

Figure 3.30: Neighbor-joining analysis of partial *nirS* gene products (136 - 141 deduced amino acids) cloned from the three different manures (RS, FS, FC) and the arable soil (AS). Bootstrap values indicate the percentage of 1000 replicate trees supporting the branching order, values below 50 are omitted. Scale bar: 5 amino acids difference in 100. Only *Roseobacter denitrificans* is a type strain.

3.5 Investigations of Field Soil Samples after 3.5 Years of Different Manuring

After 3.5 years of performing different manuring treatments including differentiated handling of intercrops, green manures, and crop residues in both, the cropping system with and the cropping system without livestock, soil samples were comparatively analyzed on diverse parameters concerning the nitrogen and carbon metabolism.

Evaluating soil mineral nitrogen contents, significantly lower amounts of NH_4^+, NO_3^-, and NO_2^- were found in wL-FYM in the cropping system with livestock and in w/o L-M in the cropping system without livestock, probably due to no fertilizer application in those treatments three weeks before soil sampling (table 3.20). Over all treatments amounts of NH_4^+ ranged between 0.1 and 5.3 g N m^{-2}, NO_3^- concentrations varied between 0.5 and 3.9 g N m^{-2}, and NO_2^- contents occurred between 0.01 and 0.14 g N m^{-2} referring to ploughed layer of 30 cm soil depth.

Soil analyses concerning the N metabolism (table 3.20) like potential nitrification and potential denitrification activity showed slightly lower activities in the respective control treatments wL-RS and w/o L-M, but without any statistical significance. Potential nitrification activites amounted to 96 to 208 ng N g^{-1} soil h^{-1} over all treatments, potential denitrification activities ranged between 67 and 142 ng N g^{-1} soil h^{-1}. Similarly, total N contents of the soils were the same (1.3 - 1.7 mg N g^{-1} soil). C/N ratios of the soils were determined between 6.9 and 8.4 exhibiting a significant higher ratio in w/o L-FC and w/o L-FC+FE in comparison to w/o L-M.

Real-time PCR quantification of denitrifiers resulted in significantly lower copy numbers of *nirK* and *nirS* genes in the different manuring treatments compared to the respective control treatments wL-RS and w/o L-M (table 3.20). Between 36% and 77% of *nirK* copies, and 47% and 83% of *nirS* copies found in the respective control treatments were observed in the different fermented slurry treatments (wL-FS, wL-FS+FC, wL-FS+FC+FE) and farmyard manure treatment (wL-FYM) as well as in treatments of fermented crops in the cropping system without livestock (w/o L-FC and w/o L-FC+FE). Absolute values of *nirS* gene copy numbers ranged in the cropping system with livestock from 1.1 - 2.3 x 10^6, and in the cropping system without livestock from 4.2 - 8.9 x 10^5 targets g^{-1} soil. *NirK* copies varied in both cropping systems between 3.2 and 8.4 x 10^6 gene targets g^{-1} soil.

Table 3.20: Survey of results compassed by several investigations of arable soil that had been differently fertilized for 3.5 years. Numbers in parentheses are standard deviations.

	wL-FS	wL-FS+FC	wL-FS+FC+FE	wL-FYM	wL-RS	w/o L-M	w/o L-FC	w/o L-FC+FE
NO_3^- [µg N g^{-1}]	6.63 (0.48)	5.36 (1.24)	5.10 (1.25)	2.32 (1.29)	3.71 (2.25)	1.39 (0.82)	9.89 (2.26)	9.15 (6.04)
	a	ab	ab	b	ab	y	z	z
NH_4^+ [µg N g^{-1}]	5.27 (2.29)	8.35 (9.32)	6.81 (7.01)	0.18 (0.02)	0.75 (0.53)	0.18 (0.01)	13.65 (13.12)	2.25 2.11)
	a	a	a	b	a	y	z	z
NO_2^- [µg N g^{-1}]	0.23 (0.06)	0.22 (0.08)	0.22 (0.09)	0.03 (0.01)	0.19 (0.16)	0.04 (0.02)	0.36 (0.16)	0.30 (0.11)
	a	ab	ab	b	ab	y	z	z
Total Nitrogen [mg g^{-1}]	1.43 (0.09)	1.46 (0.10)	1.31 (0.28)	1.53 (0.09)	1.50 (0.19)	1.67 (0.18)	1.37 (0.22)	1.37 (0.18)
C/N Ratio	7.4 (0.30)	7.4 (0.25)	6.9 (0.58)	7.5 (0.47)	7.0 (0.80)	6.9 (0.22)	8.4 (0.39)	8.1 (0.55)
	a	a	a	a	a	y	z	z
Potential Nitrification [ng N g^{-1} h^{-1}]	115.0 (78.2)	126.2 (81.4)	208.0 (75.8)	187.1 (81.1)	101.9 (74.3)	95.5 (40.2)	121.7 (28.7)	100.3 (85.2)
Potential Denitrification [ng N g^{-1} h^{-1}]	92.1 (58.9)	116.3 (65.7)	133.7 (39.8)	90.9 (38.6)	67.3 (59.4)	85.5 (67.1)	104.7 (66.8)	142.4 (84.2)
***nirS* Copies [% of Respective Control Treatm.]**	68.5 (16.4)	57.2 (16.1)	50.2 (21.5)	73.6 (13.4)	100 (13.8)	100 (13.1)	82.8 (16.4)	47.3 (17.1)
	ab	ab	b	a	c	y	y	z
***nirK* Copies [% of Respective Control Treatm.]**	66.5 (11.9)	77.4 (18.0)	69.6 (10.6)	62.4 (17.3)	100 (8.2)	100 (23.6)	70.0 (11.3)	37.8 (20.9)
	ab	a	ab	b	c	x	y	z
Total Carbon [mg g^{-1}]	10.61 (0.89)	10.82 (1.09)	9.15 (2.62)	11.56 (1.30)	10.46 (2.38)	11.45 (1.62)	11.47 (1.93)	11.14 (2.02)
Microb. Biomass [µg C g^{-1}]	1088 (424)	995 (381)	905 (255)	1175 (254)	1353 (363)	758 (293)	558 (133)	575 (117)
Water-Extract. Carbon [µg g^{-1}]	82.3 (28.4)	96.9 (29.8)	88.9 (40.0)	39.0 (14.7)	50.6 (17.2)	39.3 (23.5)	54.4 (8.5)	61.5 (25.7)
Basal Respiration* [nmol CO_2 g^{-1} h^{-1}]	82.9 (24.6)	71.0 (39.2)	89.2 (37.6)	74.5 (11.4)	77.7 (48.8)	77.5 (9.7)	51.0 (6.0)	84.1 (23.0)
	a	a	a	a	a	y	z	y
Basal Respiration [nmol O_2 g^{-1} h^{-1}]	-90.3 (9.8)	-57.0 (14.6)	-79.4 (22.6)	-66.9 (20.6)	-84.1 (13.1)	-54.8 (24.9)	-92.3 (42.1)	-67.0 (22.8)
SIR* [nmol CO_2 g^{-1} h^{-1}]	1662 (75)	1526 (474)	2047 (100)	2309 (98)	1110 (224)	896 (292)	987 (112)	772 270)
	ab	ab	b	c	a	z	z	z
SIR [nmol O_2 g^{-1} h^{-1}]	-472 (50)	-456 (135)	-479 (124)	-231 (44)	-367 (90)	-283 (82)	-483 (97)	-377 (37)
	b	b	b	a	ab	y	z	y

*CO_2 emission rate calculated under assumption of linear CO_2 production rate during sampling period

Also SSCP fingerprinting technique targeting the *nirS* nitrite reductase gene partly exhibited different patterns of *nirS* gene fragments in the differently fertilized soils with partly striking single bands (figure 3.31). However, beside those additional distinct bands, many bands of nucleic acids were found in all manuring treatments, sometimes in varying intensity. Lighter patterns like in wL-FS probably resulted from lower amounts of DNA applied to the SSCP gel despite determination of DNA concentrations in advance and thus should not necessarily lead to assumption of less diversity of denitrifying bacteria in this treatment.

Figure 3.31: SSCP patterns of *nirS* nitrite reductase gene fragments in the differently fertilized soils.

All investigations related to the C metabolism (table 3.20) did not reveal any significant differences between the manuring treatments indicating neither an increase nor a decrease of those C fractions through the different manuring management. Microbial biomass carbon ranged between 905 - 1353 µg C g^{-1} soil in the cropping system with livestock and amounted to 558 - 758 µg C g^{-1} soil in the cropping system without livestock. Over all treatments the

concentration of water-extractable carbon varied from 39 to 97 µg C g^{-1} soil and the amount of total carbon accounted for 9.2 - 11.6 mg C g^{-1} soil. Partly high standard deviations were responsible for missing significances.

Regarding basal soil respiration (table 3.20), 3.4 - 5.9 µmol CO_2 g^{-1} soil were produced during 66 hours of incubation in the different manuring treatments representing a rate of 51 - 89 nmol CO_2 g^{-1} h^{-1} by assumption of linear production rate. Oxygen consumption rates amounted to 55 - 92 nmol O_2 g^{-1} soil h^{-1} meaning a reduction of 3.6 - 6.1 µmol O_2 g^{-1} in 66 hours postulating linearity. Significant differences could only be determined in CO_2 production in the cropping system without livestock.

Substrate-induced respiration (SIR) by addition of glucose (table 3.20) revealed CO_2 production of 11 - 23 µmol CO_2 g^{-1} soil in the cropping system with livestock and 7.7 - 9.9 µmol CO_2 g^{-1} in the cropping system without livestock during ten hours of incubation, thus rates of 1110 - 2309 nmol CO_2 g^{-1} h^{-1} and 772 - 987 nmol CO_2 g^{-1} h^{-1}, respectively, in the two cropping systems assuming linearity. However, oxygen consumption only ranged between 231 and 483 nmol O_2 g^{-1} soil h^{-1} over all treatments meaning amounts of 2.3 - 4.8 µmol O_2 g^{-1} soil produced during the incubation period of ten hours when linear consumption was supposed. In both cropping systems significances between the manuring treatments were partly noticed.

The BIOLOG substrate utilization test to check differences of potential metabolic diversity in the differently fertilized soils did not exhibit distinct clusters of the particular manuring treatments (figure 3.32). Cluster analysis of all eight manuring treatments did neither result in separation of the treatments nor in separation of the two different cropping systems. Cluster analyses within both, the cropping system with livestock and without livestock also revealed no partition between the five and three manuring treatments, respectively. Likewise, if wells of acid production and sole carbon sources, respectively, were compared separately, distinct clusters of the particular manuring treatments could not be observed, neither by comparison of all treatments nor by comparison of the treatments within the cropping systems.

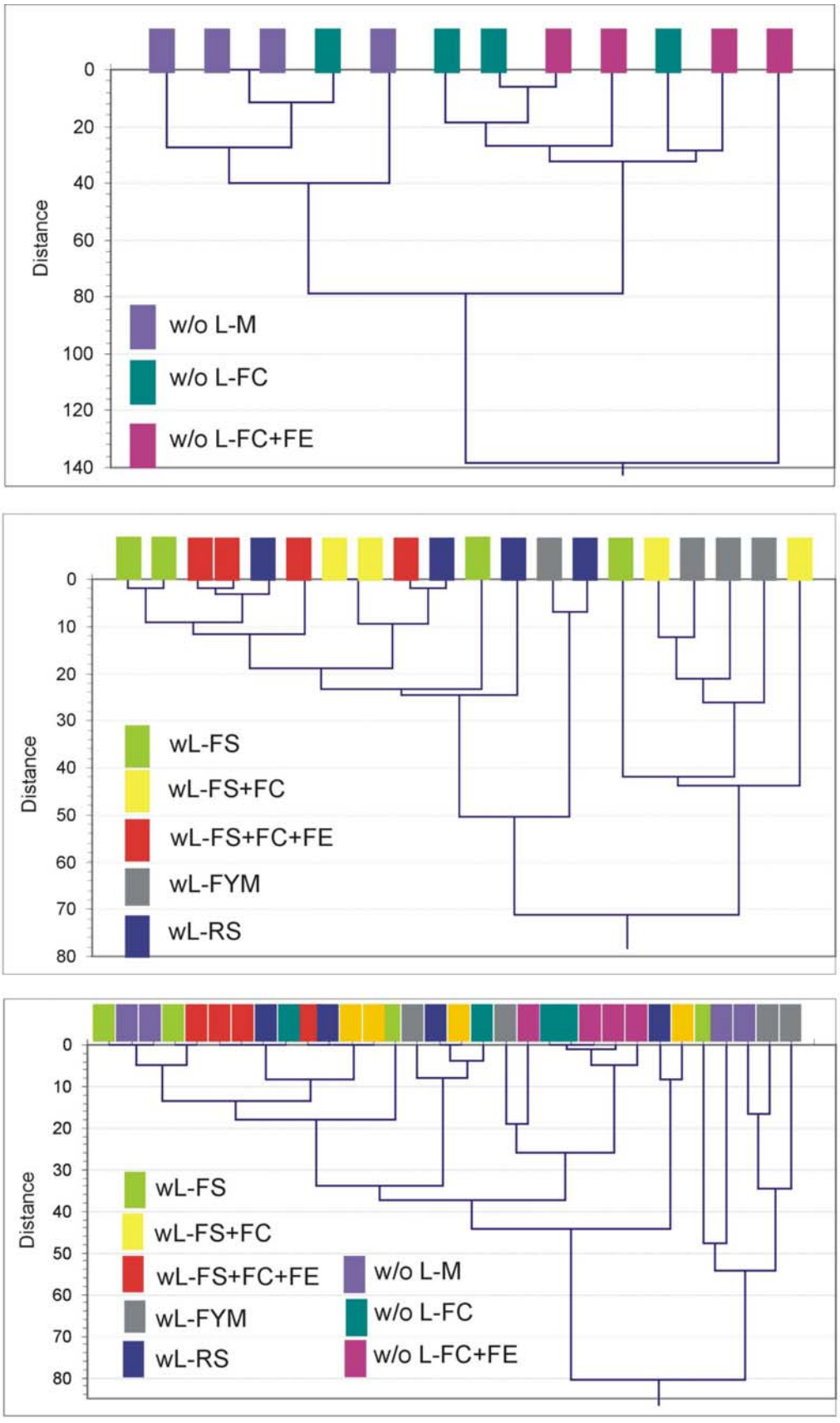

Figure 3.32: Cluster analysis (method: Ward) of the BIOLOG substrate utilization test within the cropping system without livestock, with livestock, and within both cropping systems.

4 Discussion

4.1 General Remarks to *in-situ* Gas Flux Measurements

Performing investigations at the field site, courses and sums of temperature and precipitation, soil drying and wetting, freeze-thaw cycles, and duration of freezing vary in part considerably from year to year which affect the soil moisture content, the water and air filled pore space, the soil temperature, the release of available nutrients and hence the activity of soil microorganisms. Weather conditions also influence time of tillage, drilling, manuring, and harvest. Other important factors that should be considered for data interpretation are the spatial and temporal variabilities of trace gas fluxes. Crop cultivation, manuring, and tillage intensify spatial variability due to rhizosphere effects, uneven fertilizer application/ incorporation, and non-uniform soil loosening have to be taken into account as well. Comprehensive reviews on the interactions among N_2O fluxes from agricultural soils and influencing factors can be found by Eichner (1990), Williams *et al.* (1992), Granli and Bøckman (1994), Ramos (1996), Mosier *et al.* (1998a), Barton *et al.* (1999), and Bouwman et al. (2002). Factors that may affect *in-situ* CH_4 fluxes from agricultural soils have been reviewed by Mosier *et al.* (1998b), Hütsch (2001a), and Bodelier and Laanbroek (2004).

Comparison and discussion of gas flux data in the present field trial are additionally complicated by the fact that (slightly) modified cultivation and manuring operations during the three investigated seasons have to be considered between the years such as varying amounts of applied nitrogen in the different seasons within one manuring treatment and different times of fertilizer application. The trace gas fluxes were interpreted with regard to precipitation data.

The amount of published data concerning field investigations of N_2O and CH_4 fluxes in different habitats is huge. Many studies were performed on diverse forest soils, grassland soils, organic soils, meadows, and conventionally managed arable soils. However, only few results have been published on *in-situ* flux rates in organic agriculture, where cropping regime and manuring managements and not mineral fertilizer and pesticide applications are the only management options. Therefore, this field study aimed to obtain estimates of trace gas fluxes from organically managed cropping systems that are currently getting more important. Due to few data of N_2O and CH_4 fluxes in organic agriculture, data collected during this field study were mainly compared with flux measurements from conventionally managed arable soils. Primarily, emission rates of organically fertilized crops, i.e. application of dung, slurry, liquid manure and, if available, of fermented animal excreta, in conventional agriculture were used for discussion.

4.2 *In-situ* N_2O Fluxes

4.2.1 Annual N_2O Fluxes

Cumulative N_2O losses in the cropping system without livestock ranged in winter wheat 5 from 0.49 to 4.09 kg N ha^{-1} a^{-1} and in spring wheat from 0.70 to 1.41 kg N ha^{-1} a^{-1} (table 3.1) over the three years of investigation. In the cropping system with livestock annual emissions varied between 0.46 and 6.47 kg N ha^{-1} in spelt and from 1.52 to 3.19 kg N ha^{-1} in potatoes (table 3.6). The calculated losses in the field trials are in line with other *in-situ* investigations of N_2O emissions in arable soils: Bouwman (1990) gave a broad range of annual N_2O fluxes from -0.6 to 41.8 kg N ha^{-1} for mineral soils. Kaiser and Ruser (2000) found by comparison of six long-term field experiments in Germany emission ranges from 0.5 to 16.8, from 1.4 to 12.9, and from 0.5 to 8.6 kg N ha^{-1} a^{-1} in treatments amended with mineral+organic fertilizers, exclusively with organic manures, and exclusively with mineral fertilizers, respectively. In contrast, annual N_2O emissions only ranged between 1.1 and 3.1 kg N ha^{-1} in unfertilized winter wheat and winter barley on two different soils (Kaiser and Heinemeyer 1996; Röver *et al.* 1998).

Model (2003) measured N_2O emissions exclusively from organic agriculture in cropping systems with and without livestock in a three field crop rotation (lucerne-grass, spring wheat, and maize) with vetch-rye-mix as intercrop between wheat and maize, respectively. Annual emissions in the cropping system with livestock accounted for 1.5 kg N ha^{-1} in vetch-rye-mix, 2.2 kg N ha^{-1} in lucerne-grass and spring wheat, and 4.4 kg N ha^{-1} in maize. In the cropping system without livestock N_2O effluxes amounted to 2.0 kg N ha^{-1} a^{-1} in lucerne-grass, 2.1 kg N ha^{-1} a^{-1} in spring wheat, 2.4 kg N ha^{-1} a^{-1} in vetch-rye-mix, and 5.4 kg N ha^{-1} a^{-1} in maize.

4.2.2 Spatial and Temporal Variability of N_2O Emissions

Generally, N_2O emissions determined in field trials are characterized by high variability in time and space (Cates Jr. and Keeney 1987; Firestone and Davidson 1989; Christensen *et al.* 1990; Kaiser *et al.* 1996; Velthof *et al.* 1996; Ball *et al.* 2000; Mathieu *et al.* 2006). Flessa *et al.* (1995) noted that the spatial variability of N_2O fluxes within one site can be as high as the temporal variability, but on average temporal variability was higher. They calculated coefficients of variation (CV) for one site at one sampling day of 12% to 230% performing five simultaneous flux measurements from individual chambers. Röver *et al.* (1999) observed high spatial and temporal variability over the whole experimental area (60 x 63 m) as well as in the reduced sampling scale (7 x 7 m). Data evaluation in this field study confirmed such a large spatial heterogeneity with high standard deviations of all gas flux measurements during three seasons in different crops and different manuring treatments (figures 3.1 - 3.3, 3.6 - 3.8, 3.11 - 3.13, and 3.17 - 3.18). Coefficients of variation for spatial heterogeneity of N_2O emissions varied between 27% and 106% (except outlier in spring wheat in 2004/2005: 1581%) across all seasons and crops (tables 3.2 and 3.11). High variance of N_2O emissions was the reason that

differences in flux rates were seldom statistically significant between manuring treatments, even if averages of daily fluxes were clearly different.

Parkin (1987) introduced the concept of organic hot-spots, in which the intensity of microbial respiration may create an O_2 demand that exceeds the diffusive supply for a limited time. The extreme variability of denitrification activity observed under field conditions suggests that a substantial amount of denitrification may be associated with such hot-spots.

High temporal variations of N_2O emission were observed over the course of a season in the investigated crops. Due to seasonal temperature changes, rainfall, freeze-thaw cycles, tillage operations, and fertilizer applications CVs for temporal heterogeneity of N_2O fluxes ranged between 68% and 233% in all seasons and investigated crops (tables 3.2 and 3.11). However, no explicit emission pattern became apparent in any of the investigated crops during the entire measurement period. The calculated CV values are in accordance with coefficients of variation in several field studies ranging from 86% - 350% (Flessa *et al.* 1995; Kaiser and Heinemeyer 1996; Kaiser *et al.* 1998; Kamp *et al.* 1998; Model 2003).

Temporal heterogeneity was further assessed comparing cumulated N_2O emissions (tables 3.1 and 3.10) and CV of temporal variability (tables 3.2 and 3.11) in one crop during three seasons. For example, N_2O losses in winter wheat 5 in the cropping system without livestock (manuring treatment w/o L-M) accounted for 2.68 kg N ha^{-1} a^{-1} in 2002/2003, 0.80 kg N ha^{-1} a^{-1} in 2003/2004, and 4.09 kg N ha^{-1} a^{-1} in 2004/2005. Hence, annual emissions varied in one crop during three years up to a factor of five. Kaiser *et al.* (1998) studied N_2O emissions in crops (winter wheat, winter barley, sugar beet, and winter rape) cultivated during three successive seasons with three respective N application rates. They found cumulative N_2O losses that differed up to three fold in the same crop and fertilizer rate within three years. Additionally, the factor "year" was identified as a significant influence on N_2O emissions in both, the winter period and the whole year, respectively.

4.2.3 Impact of Climate Conditions on N_2O Emissions

Amount and temporal distribution of precipitation in combination with course and sum of air and soil temperature are important to interpret trace gas fluxes in field studies as mentioned in 4.1. Notably intense rain, soil drying and wetting (Jørgensen *et al.* 1998; Priemé and Christensen 2001), freeze-thaw cycles, and duration of freezing (Cates Jr. and Keeney 1987; Christensen and Tiedje 1990; Röver *et al.* 1998; Teepe *et al.* 2004) promote high N_2O emissions. In all investigated seasons, amounts of annual precipitations were smaller (637 mm in 2002/2003 (August - July), 539 mm in 2003/2004, 567 mm in 2004/2005) than the long-term mean (682 mm). However, several months exhibited higher precipitations compared to the long-term mean of the respective monthly rainfall (figure 2.1). Daily precipitations during the three years are illustrated in figure 2.2 and demonstrate the variability and differences in amount and course between the seasons. Sums of monthly mean air temperatures did not

differ greatly between 2002/2003 (112.2°C), 2003/2004 (110.6°C), 2004/2005 (112.6°C), and the long-term mean of monthly mean air temperatures (111.4°C). However, monthly mean air temperatures showed in part clear differences between the seasons and the long-term mean (figure 2.1). Illustrations of daily temperatures in air and soil (in 5 cm and 20 cm soil depth) partly exhibit differences of the temperature course with decreasing temperature fluctuations in the order air > 5 cm soil depth > 20 cm soil depth in all three seasons (Appendix A.3). The smaller amount of precipitation in 2003/2004 in combination with partly decreased monthly mean air temperatures may have contributed to low emissions in all treatments analyzed in winter cereals spelt (cropping system with livestock) and winter wheat 5 (cropping system without livestock) (tables 3.1 and 3.10). Additionally, these crops and manuring treatments showed also the smallest CV values for temporal variability during this season. Only in January and May 2004 monthly rainfall rose above the long-term mean and monthly mean air temperatures of seven months were lower in comparison to the respective monthly long-term mean.

The comparison of precipitation data with sampling days revealed that e.g. on March 29, 2005 in spring wheat increased N_2O emissions in both manuring treatments were possibly induced by 14 mm rainfall during the preceding two days. Significantly elevated N_2O losses on the same day in winter wheat 5 in w/o L-FC could also have been caused by precipitation and eventually spatially limited higher concentrations of nitrogen and carbon in hot-spots due to fertilizer application a month before. The increase of N_2O emissions after rainfall has frequently been observed in agricultural soils (Cates Jr. and Keeney 1987; Ball *et al.* 1999). Notably high N_2O fluxes often occurred when the soil water content was high shortly after N fertilization (Smith *et al.* 1998b; Ruser *et al.* 2001). Model (2003) observed a second pulse of N_2O losses after 22 mm of rain within 24 hours three days after application of liquid manure and slurry in spring wheat that accounted for half of the emissions after fertilizer application. Furthermore, precipitation of 26 mm within 48 hours after fertilizing liquid manure and slurry in maize induced N_2O emission rates that were 10 times higher than N_2O rates measured directly after manuring. Sehy *et al.* (2003) found 85% of all elevated N_2O flux rates (> 50 µg N m^{-2} h^{-1}) in maize at soil water contents between 55% and 90% water-filled pore space (WFPS), with maximum emissions at 65% WFPS. They detected highest emissions in spring and summer following N fertilization associated with rainfall.

Many studies have indicated that considerable N_2O might be emitted during winter periods in particular during freezing-thawing events. Although emissions are mostly limited to a few days, they might contribute as much as 70% of the total annual emissions from agricultural soil (Flessa *et al.* 1995; Wagner-Riddle *et al.* 1997; Wagner-Riddle and Thurtell 1998; Kaiser and Ruser 2000). Processes responsible for large N_2O emissions in winter are mainly attributed to an enhanced supply of nutrients, which is caused by bacteria killed during freezing (Skogland *et al.* 1988; Teepe *et al.* 2001). Furthermore, nutrients become available when soil aggregates are disrupted as result of ice crystals expanding in pores between particles (Christensen and

Christensen 1991; van Bochove *et al.* 2000) and when fine roots die in the freezing soil (Priemé and Christensen 2001). Physical release of N_2O upon thawing which was produced in the unfrozen subsoil and accumulation below the frozen layer have also been discussed as possible mechanisms (Burton and Beauchamp 1994; Teepe *et al.* 2004). The term "winter period" is not an accurately defined time period and may last between two and six months. In this field study "winter period" is considered to be between December 1 and March 15 when temperatures below 0°C were observed during this period in all three seasons. Winter emissions in the cropping system with livestock accounted for 12% - 29% in spelt and 12% - 38% in intercrops before potatoes, and amounted to 16% - 47% in winter wheat 5 and intercrops before spring wheat in the cropping system without livestock (tables 3.3 and 3.12). Kaiser *et al*. (1998) observed half of annual emissions between October and February. Ruser *et al.* (2001) determined winter emissions between October and March and measured on average 49% of N_2O losses during that time. Röver *et al.* (1998) even assessed 70% of annual N_2O effluxes between December and February, whereas Sehy *et al.* (2003) only observed 10 - 20% of N_2O emissions between October and March. In contrast, no elevated flux rates have been found by Model (2003) during two winter periods, neither in lucerne-grass nor in vetch-rye-mix, although soil freeze-thaw cycles occurred.

Strikingly high N_2O emissions as observed on December 16, 2002 in winter wheat 5 (up to 343 µg N m^{-2} h^{-1}, figure 3.3) and intercrops previous to spring wheat (up to 214 µg N m^{-2} h^{-1}, figure 3.8) and potatoes (up to 388 µg N m^{-2} h^{-1}, figure 3.18) were associated with a freezing-thawing cycle. The soil temperature in 5 cm depth was below the freezing point during six days prior to the date but the air temperature varied between -8°C and 5°C. However, it remains unclear why elevated N_2O emissions have not been observed in spelt at the same day. Sehy *et al.* 2003 determined in a field study N_2O flux rates up to 120 µg N m^{-2} h^{-1} during freeze-thaw cycles, whereas Teepe *et al.* 2004 observed N_2O emission rates up to 600 µg N m^{-2} h^{-1} in undisturbed soil columns of agricultural soils. Ruser *et al.* (2001) even measured peak emissions of > 900 µg N m^{-2} h^{-1} on a low fertilized wheat plot. Further peak emissions during the investigation period that could be explicitly and exlusively attributed to events of freezing and thawing have not been ascertained. This observation is supported by Model (2003) who did not find any elevated N_2O emissions in conjunction with soil freezing and thawing during two years. However, elevated N_2O emissions in this study were also observed in winter wheat 5 and spelt mid-end of February and on March 1, 2005 when air and soil temperature in 5 cm depth ranged at the freezing point. But manuring was also processed end of February so that soil freezing and thawing in combination with fertilizer application (change of soil conditions by addition of nitrogen, carbon, water,…) have most likely provoked higher emissions.

Amount and temporal distribution of precipitation as well as the course and sum of air and soil temperatures may vary conspicuously between the years. Thus, field studies should be conducted at least during two, better more years to reduce possible strong influences of

weather conditions on parameters of interest. Ideally, trace gas emissions in similar crops should be investigated over several years, whereas emissions in different crops should be determined both, simultaneously (during the same season) and over multiple years.

4.2.4 Impact of Crop Species on N_2O Emissions

Crop species may also affect N_2O losses. Related to the management of the cultivated crop, the time of tillage as well as tillage operations may differ in the way green manures and crop residues are incorporated and the seed-bed is prepared. Generally, tillage may promote soil aeration, affect evaporation, and enhance accessability of crop residues for soil microbes (Granli and Bøckman 1994). Fertilization and hence addition of nutrients to the soil should correspond to the nutrient demand of the respective cultivated crop in time. Consequently, tillage and manuring affect conditions for biological processes, they may vary notedly in time (season) between different crop species and thus, are performed under different soil and climate conditions entailing different nitrogen dynamics. Furthermore, crop species themselves may influence soil biological processes, since production rates of trace gases are affected by substrate availability (Tate III 1995). According to Janzen (1990), root exudates influence nutrient dynamics and organic matter turnover in soil. Differences in root exudates between diverse plant species as well as during the vegetation period have been demonstrated (Gransee and Wittenmayer 2000; Hütsch *et al.* 2002). Bachman and Kinzel (1992) verified the impact of six different plant species on colonization and enzymatic activites in the rhizosphere. Höflich *et al.* (2000) provided evidence for stimulated microbial activity under legumes (pea and lupine) but not under cereals and oil flax. Tate III (1995) also pointed out that the availability of nutrients for microorganisms in the rhizosphere is dependent on plant species and the stage of plant development leading to differences in dynamic that may be the reason for the different temporal course of trace gas emissions under different crop species. Clays-Josserand *et al.* (1995) revealed a different distribution of fluorescent *Pseudomonas* spp. in dependence of crop species: according to their ability to dissimilate nitrogen, different proportions of non-dissimilatory and dissimilatory strains, nitrate reducers and true denitrifier with or without N_2O reductase were observed in the rhizosphere, rhizoplane, and root tissue of tomato and flax.

Over all three seasons in the present field study, no distinct order of N_2O emissions in terms of the different crops has been observed. However, similar ranges of N_2O effluxes were determined in winter wheat 5 (cropping system without livestock) and spelt (cropping system with livestock) in biogas treatments w/o L-FC and wL-FS+FC, respectively, within a season. Those winter cereals are comparable with respect to crop rotation (tables 2.1 and 2.3), similar cultivation strategies, manuring regimes (tables 2.5 and 2.6), and similar treatment of intercrops (tables 2.2 and 2.4) where biomass of intercrops was harvested in fall for fermentation and applied as fertilizer in spring. Amounts of approximately 1.5 kg N ha^{-1} a^{-1} in

2002/2003 and roughly 0.5 kg N ha^{-1} a^{-1} in 2003/2004 were emitted in both, w/o L-FC in winter wheat 5 and wL-FS+FC in spelt, respectively. Ruser *et al.* (2001) assessed N_2O emissions from 0.3 to 6.9 kg N ha^{-1} a^{-1} based on two year averages in the order fallow < maize, wheat < potatoes. On a scottish arable soil Smith *et al.* (1998a) measured annual N_2O losses in the order winter wheat < spring barley < potatoes with emission ranges between 0.3 and 1.2 kg N ha^{-1}. Kaiser *et al.* (1998) revealed a significant impact of cultivated crops on mean N_2O emissions with respect to the measurement period (winter, vegetation period or whole year). Annual losses in winter wheat and winter barley (1.1 kg N ha^{-1}) differed significantly from N_2O emissions in winter rape and sugar beet (1.3 kg N ha^{-1}). On the other hand, Hénault *et al.* (1998) described silmilar amounts of emitted N_2O during a seven months investigation period in wheat and rape.

However, comparing similar crops over several years the fertilizer regime consisting of fertilizer amount, fertilizer splitting, kind of manure (green manure, dung, slurry, fermented, not-fermented, liquid, solid), viscosity, amount of applied total nitrogen and ammonium-nitrogen, application time (growth phase of crops), and soil and weather conditions before, during, and after manuring has to be taken into account. To identify the crop effect on trace gas emissions, it would be advantageous to keep the fertilizer regime the same over the three year study period. But considering manuring operations (tables 2.5, 2.6, Appendix A.4 - A.7) in more detail, partly distinct differences emerged between the investigated seasons. The fertilizer and cropping regimes were carried out as good as possible, however they were not standardized. For example, all intercrops in 2002/2003 consisted of peas, vetch, and oil radish but in the successive two seasons peas were omitted. Only in season 2004/2005 the following manures were applied compared to the previous two seasons under investigation: solid fermented residues in w/o L-FC before drilling of intercrops prior to spring wheat (41 kg N_t ha^{-1}), solid fermented residues in w/o L-FC before drilling of winter wheat (95 kg N_t ha^{-1}) (table 2.5), and liquid fermented fertilizer in wL-FS+FC before drilling of intercrops prior to spelt (26 kg Nt ha^{-1}) (table 2.6). Moreover, amounts of total nitrogen or ammonium-nitrogen applied in a distinct manuring treatment of a distinct crop varied in part considerably between the years. In w/o L-FC in winter wheat 5, for example, input of nitrogen amounted to 162 kg N_t ha^{-1} in 2002/2003, 144 kg N_t ha^{-1} in 2003/2004, and 305 kg N_t ha^{-1} in 2004/2005 through liquid and solid fermented fertilizer, green manuring and pea straw (Appendix A.4). Additionally, in 2004/2005 manuring of liquid fermented fertilizer was already completed on February 22, thus earlier than in the preceding seasons (table 2.5).

Examining influences of crop species in this study on annual N_2O emissions the follwing notes should be considered: The cultivation period of the respective crops differed considerably in terms of the time of intercrop incorporation, soil ploughing, and sowing. For instance, the winter cereals spelt and winter wheat were sown in fall while spring wheat and potatoes were drilled and dibbled, respectively, in spring. As a result of temporally diverse tillage operations and cultivation periods, the amount and distribution of mineral nitrogen in the soil profile

varied in spring and fall between the crops (figures 3.5, 3.10, 3.16, and 3.20). Furthermore, nutrient demands differed between winter and spring cereals and notably potatoes entailing varying manuring times. In this study integration of N_2O losses from crops of interest with the respective intercrops were carried out over 365 days. Thus, N_2O emitted from spring wheat contributed to a lesser extent to annual emissions than N_2O losses from winter wheat because of the shorter cultivation period. The alternative method to integrate N_2O emissions over the cultivation period and extrapolate those to 365 days pretending cultivation of spring wheat (and potatoes) during the whole year would have resulted in different yearly emissions because possibly high winter emissions would have been excluded. In the literature often information of the integration method, time periods, and the extrapolation of emission data are missing.

Differences in cultivation techniques and the way N_2O emissions are measured can result in different trace gas estimates. For instance, potatoes in contrast to cereals are usually cultivated using ridge culture leading to different bulk densities and pore size distributions in the ridge soil, the uncompacted interrow soil, and the tractor-compacted interrow soil (Ruser *et al.* 1998b). The spatial variability in soil properties should be considered for estimating total N_2O effluxes. However, in the present field study the facilities were not available to monitor all areas with the respective replicates during the investigation period. Thus, only the ridge soil was sampled. Annual N_2O emissions in potatoes and prior intercrops ranged in this field study between 1.52 and 3.19 kg N ha^{-1} over all manuring treatments and the investigated seasons. Smith *et al.* (1998a) measured N_2O fluxes in the ridge soil and found losses of 1.2 - 3.2 kg N ha^{-1} dependent on the year and the applied fertilizer. However, they observed high N_2O emissions in the post-harvest period (2.0 kg N ha^{-1}) which was not sampled in this study. Ruser *et al.* (1998b) investigated separately N_2O effluxes on the ridges, on the interrow areas, and on the compacted interrow areas. Assuming that two-thirds of the field area are ridge soils, one-sixth uncompacted and one-sixth compacted interrow soils, they estimated that 16.0 kg N ha^{-1} were emitted in average during the year (sampling during last two months (November and December) already in winter wheat). The major part (72%) of the total N_2O release during the cropping period (May - September) evolved from the compacted interrow soil, while 21% and 7% were emitted from the ridges and the uncompacted interrow soil, respectively. Flessa *et al.* (2002) determined separately N_2O emission rates in the different field areas during two cropping periods (May - September/October) and found total mean losses of 1.6 and 2.0 kg N ha^{-1}, respectively. They calculated mean shares of 66%, 10%, and 24% for the ridges, uncompacted interrows, and compacted interrows, respectively, of the total *in-situ* emissions. Moreover, Gattinger *et al.* (2002) found in the same field experiment a more denitrifying microbial community in the compacted interrow soil by analyzing the phospholipid fatty acids (PLFA) and phospholipid etherlipids (PLEL). Based on the results of other field studies, N_2O emissions in the present study are possibly underestimated.

4.2.5 Impact of Different Handling of the Intercrops on N_2O Emissions

Studies on arable land differ markedly whether they consider intercrop cultivation and the impact of different intercrop residues on mineralization or not (McKenney *et al.* 1993; 1995; Baggs *et al.* 2000a; Baggs *et al.* 2000b; Rosecrance *et al.* 2000; Burger *et al.* 2005). In the present field experiment intercrops were grown in all manuring treatments. However, treatments of intercrops varied among treatments within both cropping systems: The w/o L-FC and wL-FS+FC intercrops were harvested in fall for fermentation and were brought back to the field as fermented fertilizer in non-legume crops within the crop rotation at a different, later time point. In contrast, intercrops in w/o L-M, wL-FS, wL-FYM, and wL-RS were incorporated to the soil as green manure before main crops were cultivated.

With intercrops, green manures, and crop residues which were kept on the field after harvest, considerable amounts of organic carbon and nitrogen were incorporated into the soil (Appendix A.4 - A.7). As revealed by Wagner-Riddle and Thurtell (1998) the impact of the preceding crop and green manures should be taken into account examining *in-situ* N_2O emissions in field trials since they may affect the soil nitrogen status even after months (mineralization, release of nitrogen, C/N ratio of crop residues).

Apart from a few exceptions, N_2O emissions were clearly reduced through the harvest of intercrops in w/o L-FC and wL-FS+FC prior to winter crops after tillage and drilling of winter wheat and spelt in October, respectively (figures 3.1 - 3.3 and 3.11 - 3.13). Due to the harvest less organic material was available for mineralization, hence reducing the substrate for microbial metabolism and N_2O production via nitrification and denitrification. Data of soil mineral nitrogen concentrations within 30 cm soil depth in 2002/2003 supported this theory with lower amounts of nitrate in w/o L-FC (figure 3.4) and wL-FS+FC (figure 3.15). In winter wheat 5, significantly lower nitrate concentrations were exhibited in fall in w/o L-FC compared to w/o L-M in 0 - 30 cm and 30 - 60 cm soil depth in all three seasons (figure 3.5). Similarly, in spelt lower amounts of total N_{min} as well as lower nitrate contents in 0 - 30 cm and 30 - 60 cm soil depth were observed in fall of all three seasons (figure 3.16). However, removal of intercrops (end of October or in November) prior to spring wheat and potatoes in w/o L-FC and wL-FS+FC did not entail such clear reductions of N_2O losses. In contrast to winter cereals, intercrops or plant stubbles of harvested intercrops were not removed but incorporated into the soil not until January. Tillage operations for preparation of sowing (spring wheat) and dibbling (potatoes) were processed in February/March and April, respectively (tables 2.5, 2.6). Thus, tillage, incorporation of plant biomass, sowing/dibbling, and manuring were processed at different time points with different soil and weather conditions in comparison to winter cereals, possibly leading to different N_2O emission patterns. The N_{min} values determined in fall in the cropping system with livestock were conspicuously lower in all manuring treatments in intercrops prior to potatoes (figure 3.20) than in spelt (figure 3.16). Likewise, the N_{min} concentrations in the cropping system without livestock observed in fall in intercrops prior to spring wheat (figure 3.10) showed lower amounts than in winter wheat 5 (figure 3.5). The

consideration of nitrate contents also in deeper soil depths appears to be reasonable since Müller *et al.* (2004b) verified N_2O production in soil depths down to 50 cm using ^{15}N labeled fertilizers. They unambiguously exhibited elevated ^{15}N labeled N_2O concentrations in 50 cm depth that had been derived from ^{15}N labeled nitrate.

4.2.6 Impact of Manuring on N_2O Emissions

During this field study, several fertilizers were applied beside green manure and straw incorporation in dependence of cropping system and manuring treatment. In agro-ecosystems application of organic manures marks an interesting and important event for analyzing N_2O emissions. Manuring may lead to formation of hot-spots in the soil due to high concentrations of (available) organic carbon (Paul and Beauchamp 1989) which can sustain denitrification for extended periods (Thompson *et al.* 1987; Thompson 1989). In the cropping system without livestock, liquid of fermented crops as well as corresponding solid fermented crop residues were used for fertilization in w/o L-FC whereas in the control treatment w/o L-M the respective crop material was mulched and remained on field. Consequently, in that cropping system manuring in w/o L-FC could not be compared with applications of different fertilizers on N_2O emissions, but the impact of fertilization in w/o L-FC was compared between the investigated seasons. In the cropping system with livestock diverse fertilizers were applied: raw slurry in wL-RS, fermented slurry in wL-FS, fermented slurry, liquid of fermented crops as well as solid fermented crop residues in wL-FS+FC, and farmyard manure in wL-FYM. Fermented slurry (wL-FS and wL-FS+FC) and liquid of fermented crops (wL-FS+FC) were applied simultaneously to raw slurry (wL-RS), whereas solid fermented crop residues (wL-FS+FC) were spread at the same time as farmyard manure (wL-FYM). In that cropping system, a direct comparison of manuring effects of the different fertilizers on N_2O emissions was possible as well as a comparison between the investigated years.

Anaerobic digestion in a biogas plant results in fermented products that differ from the respective "raw material" in the following way: increase of NH_4^+-N content, increase of NH_4^+-N proportion to total nitrogen content, increase of pH value, decrease of dry matter content, decrease of total carbon content, decrease of organic dry matter content, decrease of biological oxygen demand (BOD), decrease of viscosity, smaller C/N ratio, no alteration concering total nitrogen content, potassium content, and phosphate content (Field *et al.* 1984; Asmus *et al.* 1988; Wulf *et al.* 2001). The elevated pH value and the increased amount of NH_4^+-N may potentially lead to an increased production of ammonia resulting in higher nitrogen losses in form of ammonia volatilization. However, the reduced viscosity of the fermented fertilizers may entail faster penetration into the soil with subsequently lower ammonia emissions but eventually increased N_2O effluxes (Wulf *et al.* 2002b). On the other hand, the reduced amount of easily decomposable carbon in the fermented slurry may result in a lower rate of microbial activity (lower BOD). As a consequence, less or smaller

anoxic areas might emerge in the soil entailing lower denitrifying activity and less N_2O emissions (Clemens and Huschka 2001).

Considering the days following fertilizer applications, no distinct patterns of N_2O emissions became apparent as exemplified by data collected in winter wheat 5 (cropping system without livestock) after manuring in all three years. On January 31, 2005 liquid of fermented crops (120 kg N ha^{-1}) was manured in w/o L-FC. After one, two, and three days *in-situ* N_2O fluxes were investigated without noticably elevated N_2O emissions in the fertilized treatment. In contrast, application of the liquid fermented crops (62 kg N ha^{-1}) on February 22, 2005 resulted in a significantly increased N_2O flux rate (246 µg N m^{-2} h^{-1}) two days after manuring although less nitrogen (total N and NH_4^+-N) was applied. Elevated N_2O fluxes were still present during the next three samplings in March that were characterized by high spatial variability. In 2003/2004, an elevated N_2O emission rate (but on lower level) was observed in winter wheat 5 on February 29 in w/o L-FC (17 µg N m^{-2} h^{-1}) two days after manuring of liquid fertilizer of 75 kg N ha^{-1}. Examining N_2O effluxes on the date of fertilizer application as on February 27, 2004 and February 19, 2003 in winter wheat 5 in w/o L-FC, no increased emissions were observed after manuring of 75 kg N ha^{-1} and 91 kg N ha^{-1}, respectively, compared to the non-fertilized treatment w/o L-M. Only on March 13, 2003 a highly elevated flux (188 µg N m^{-2} h^{-1}) was found after manuring in w/o L-FC, but it was not significant due to large standard deviation.

The concomitant application of water by manuring (slurry often contains > 90% water) may also affect N_2O emissions (Davidson 1992; Jørgensen *et al.* 1998; Model 2003). The soil water content has a strong influence on the process that leads to N_2O emissions and the amount of N_2O emitted (Granli and Bøckman 1994). However, the amount of water alone does not explain the different observations of N_2O emissions after manuring in winter wheat 5 in w/o L-FC in January and February 2005. Less water was dispensed on February 22 (19 m^3 ha^{-1}) than on January 31, 2005 (36 m^3 ha^{-1}), but higher N_2O emissions were observed in February.

Applying organic fertilizers, the input of considerable amounts of readily available organic carbon and the impacts it exerts on N_2O production has to be considered. Clemens and Huschka (2001) assumed that the microbially available organic carbon determined the amount of N_2O emitted shortly after slurry application. According to Paul and Beauchamp (1989), different carbon constituents of the manure revealed strong relationship on denitrification. However, liquid of fermented crop residues applied in spring and winter wheat in the cropping system without livestock during the whole investigation period did not differ greatly in C/N ratios (total carbon/total nitrogen) with values between three and five. Additionally, only fermented biomass and hence material with no or few easily degradable organic carbon was used for manuring in winter wheat 5. Consequently, different amounts of readily available organic carbon applied with the fermented manure in January and February 2005 might not be responsible for different N_2O dynamics as described above.

N_2O is a highly water soluble gas that is produced and released in varying amounts from slurry during storage and application in dependence of the pH value of the slurry (Oenema *et al.* 1993). During and after slurry application, previously dissovled N_2O might be slowly released and thus cannot be distinguished from a fast production in soil. Apart from a highly increased N_2O efflux in winter wheat 5 on March 13, 2004 in one replicate leading to a large standard deviation of the mean emission, no elevated N_2O emissions were observed on the day of fertilizer application.

Elevated N_2O emissions after fertilization on February 22, 2005 in winter wheat 5 coincided with air and soil temperature in 5 cm depth below the freezing point. Temperatures after fertilization on January 31, 2005, in contrast, ranged slightly above 0°C (section 4.2.3), when no elevated N_2O emissions were observed.

In the cropping system with livestock, four different fertilizers were investigated. Comparing simultaneous manuring of liquid fertilizer (raw slurry, fermented slurry, and fermented slurry + liquid of fermented crops), no distinct order of N_2O emissions became apparent in both, spelt and potatoes during the investigation period. Similarly, no trend was noticeable concerning application of the solid fertilizers farmyard manure in wL-FYM and solid fermented crop residues in wL-FS+FC. For example, in spelt incorporation of farmyard manure (103 kg N_t ha^{-1}) on October 13, 2003 seemed to increase N_2O emissions for weeks, but this observation could not be confirmed with other fertilizer applications of farmyard manure, neither in spelt nor in potatoes. Thus, the missing explicit order of N_2O emissions between the different manuring treatments in this cropping system during the sampling period was continued with the determination that a fertilizer-induced trend of N_2O losses between the treatments was not detectable either. Furthermore, application of fermented slurry and fermented plant biomass (wL-FS and wL-FS+FC) did not result in explicit elevated or reduced N_2O emissions compared to fertilization of raw slurry (wL-RS). However, integration of N_2O emissions over 365 days in spelt showed in all three investigated seasons reduced N_2O losses in wL-FS+FC compared to wL-RS. Thus, harvest of intercrops in fall and fermentation of cattle slurry and intercrop biomass entailed decreased N_2O emissions although overall higher amounts of nitrogen (total N and NH_4^+-N) were applied in this treatment. Manuring treatment wL-FS, for comparison, in which only fermentation of cattle slurry was carried out, did not reveal in spelt decreased N_2O emissions in all seasons.

Elevated N_2O emissions directly or during the first few days after application of manure as observed in the field study were often described in literature (Christensen 1983; Comfort *et al.* 1990). Model (2003) determined on the day of application of liquid manure and slurry, respectively, peak N_2O emissions of up to 183 µg N m^{-2} h^{-1} in spring wheat. Clemens *et al.* (2006) also analyzed suddenly elevated N_2O emissions after manuring of mineral fertilizer, cattle slurry, and fermented cattle slurry, respectively, that had decreased during the subsequent one to three days to the level of the control plot. Rochette *et al.* (2000) assessed 18 hours after spreading of pig slurry even a peak emission rate of 1260 µg m^{-2} h^{-1}. Whalen

(2000) detected four hours after fertilization of liquid swine waste a significant increase of the N_2O emission rate that was highest two days after manuring (103 µg N m^{-2} h^{-1}).

In the cropping system with livestock, no definite effect of fermentation of cattle slurry on N_2O emissions could be determined with the feasible frequency of measurements. Wulf *et al.* (2001) and Clemens *et al*. (2006) did not assess significances in cumulated N_2O losses during five weeks after application of raw cattle slurry and fermented cattle slurry and during one year, respectively. Petersen (1999) observed significantly lower mean rates of N_2O evolution with and without irrigation before sampling in soils fertilized with fermented compared to raw slurry. However, no significant differences could be observed between the treatments a year before. Wulf *et al*. (2002b) found up to the second week after fertilization significantly higher cumulated emissions in raw slurry compared to fermented slurry amended soils that was ascribed to the reduced amount of degradable carbon in the fermented slurry. Similarly, Petersen *et a*l. (1996) determined consistently lower soil denitrification rates in hot-spots derived from digested slurry compared to raw slurry in a laboratory experiment.

The variability of results reflects the complex interplay of factors like fertilizer type, content of nitrogen and carbon, amount of water, fertilizer viscosity, application technique, soil moisture, temperature, precipitation,… that affect production and release of *in-situ* N_2O emissions.

4.2.7 Further Factors Influencing *in-situ* N_2O Emissions

In the literature, several further parameters are discussed in connection with *in-situ* N_2O emissions in arable soils. However, since those factors did not differ in this field study, they cannot have contributed to or cannot have provoked differences of N_2O effluxes in the diverse treatments.

Soil texture and drainage play an important role because of their impact on oxygen and moisture status as well as on gas diffusion. Fine-textured clay soils can maintain a higher WFPS for longer periods due to more capillary pores within soil aggregates than coarse-textured soils. Thus, the potential for anoxic N_2O formation via denitrification is higher in clay soils (Granli and Bøckman 1994; Bouwman *et al.* 2002). However, with decline of air filled porosity in heavier textured soil, gas diffusion becomes increasingly restricted and reduction of N_2O to N_2 might be favored. In this field study all *in-situ* measurements were performed in neighboring field plots at one location with similar soil texture.

Soil pH values may also affect N_2O production, but impact of pH is complex and results of diverse studies differ (for review refer e.g. to Granli and Bøckman (1994)). Bouwman *et al.* (2002) revealed by evaluation of more than 800 N_2O emission measurements in agricultural fields that neutral to slighlty acidic conditions favor N_2O effluxes. Nägele and Conrad (1990) showed that the N_2O release decreased when pH_{H2O} was raised from 4 to 7 in an acid forest soil. The release of N_2O increased when pH_{H2O} in an alkaline agricultural soil was reduced from

7.8 to 6.5, but decreased when pH_{H2O} was further reduced to 4. Differences of soil pH values in this field trial are negliglible due to proximity of the plots.

In several studies different application techniques of slurry like splash plate, band-spreading, trail hose, trail shoe with and without immediate shallow incorporation, and injection have been assessed with respect to N_2O emissions (Dosch and Gutser 1996; Clemens *et al.* 1997; Flessa and Beese 2000). Greater contact of slurry with soil, e.g. after incorporation or injection, could induce conditions favorable for N_2O production, whereas splash plate and band-spreading could activate microorganisms in larger soil areas. Results of N_2O emissions obtained so far in field studies on arable soil differ. Weslien *et al.* (1998) 1998 reported slightly but not significantly higher emissions after band-spreading followed by harrowing compared with band-spreading, trenching, and shallow injection. Wulf *et al*. (2002b) observed significantly increased N_2O losses by injection compared to trail hose with and without incorporation or splash plate. In contrast, Clemens *et al.* (1997) did not determine any effects of application technique on N_2O emissions with injection depths of 5 and 10 cm. In the present field trial, all slurries were applied by trail hose.

4.3 *In-situ* CH_4 Fluxes

4.3.1 Annual CH_4 Fluxes

Integrated CH_4 uptake rates in the cropping system without livestock varied in winter wheat 5 between 0.48 and 0.86 kg C ha^{-1} a^{-1} and in spring wheat from 0.50 to 0.71 kg C ha^{-1} a^{-1} during the investigation period of three years (table 3.7). In the cropping system with livestock, the amount of annual CH_4 oxidation ranged in spelt from 0.13 to 0.80 kg C ha^{-1} and in potatoes from 0.50 to 0.66 kg C ha^{-1} (table 3.16). Consequently, the methane uptake rates are in line with published CH_4 oxidation activities in arable soils ranging from 0.13 - 0.66 kg C ha^{-1} a^{-1} (Flessa *et al.* 1995; Schmädeke *et al.* 1998; Kamp *et al.* 2001). Model (2003) observed in both, an organic cropping system with and without livestock CH_4 uptake rates of 0.27 - 1.54 kg C ha^{-1} a^{-1} in lucerne-grass- and vetch-rye-mixes. For spring wheat and maize in both cropping systems, the integration of CH_4 fluxes was performed with and without consideration of immediate CH_4 emissions after fertilizer applications. Excluding CH_4 emissions after manuring, annual soil CH_4 uptakes amounted to 0.57 - 1.2 kg C ha^{-1} in spring wheat and maize in both cropping systems. Including fertilizer-related CH_4 emissions, soil CH_4 oxidation activities accounted for 0.03 - 0.61 kg C ha^{-1} a^{-1} in spring wheat in both systems. In contrast, the annual balances in maize exhibited CH_4 losses between 0.72 and 1.76 kg C ha^{-1} in both cropping systems when CH_4 emissions after fertilization were considered.

CH_4 oxidation activities in cultivated, arable soils are on average lower than in grassland soils and in forest soils (Hütsch 2001a). Boeckx *et al.* (1998) calculated overall CH_4 uptakes of 1.03 kg C ha^{-1} in a deciduous forest soil, 0.71 kg C ha^{-1} in a natural grassland soil, 0.33 kg C ha^{-1} in a fertilized pasture soil, and 0.34 to 0.37 kg C ha^{-1} in arable soils with different fertilizer treatment. Merino *et al.* (2004) assessed average annual *in-situ* soil CH_4 oxidation activities of 4.7, 0.9, and 0.2 kg C ha^{-1} in forest, grassland, and cropfield soils, respectively. Willison *et al.* (1997) found up to ten times lower CH_4 uptake rates in an arable than a grassland or woodland soil.

4.3.2 Spatial and Temporal Variability of CH_4 Fluxes

Coefficients of variation (CV) for the mean spatial heterogeneity of the CH_4 fluxes ranged in both, the cropping system with and without livestock, between 27% and 122% (tables 3.8, 3.17) with exception of conspicuously higher values in potatoes 2003/2004 (wL-RS) and in winter wheat 5 2004/2005 (w/o L-M and w/o L-FC). The high standard deviations of the average CH_4 fluxes in all crops and manuring treatments during the three seasons indicated high spatial variabilities and were the reason that flux rates among the manuring treatments were seldom statistically different. However, the CVs observed in this study are similar to those reported from other studies on arable soil that encompass a range between 13% and 663% (Flessa *et al.* 1995; Ruser *et al.* 1998b; Kamp *et al.* 2001).

Temporal variability of CH_4 fluxes in all investigated crops and manuring treatments ranged in this field study between 51% and 661% (tables 3.8, 3.17) without consideration of the

exceptional high CV values in spelt 2003/2004 (wL-FS+FC and wL-RS) and winter wheat 5 2003/2004 (w/o L-FC). Those relatively high coefficients of variation resulted from a single, high CH_4 emission rate after application of the respective organic fertilizer and are in line with the reported CVs (176% - 2200%) from studies in organic agriculture with and without livestock where crops have been fertilized with various organic manures (Model 2003). When CH_4 emissions after fertilization were omitted from the calculations, CV values ranged between 70% and 157% for various crops and manuring treatments (Model 2003). Alternating oxidation-emission periods in winter might have contributed to the temporal variability. Furthermore, it seemed that CH_4 fluxes had a less pronounced seasonality compared to N_2O and CO_2 (Flessa *et al.* 1995; Model 2003) which is in accordance with observations in the present field study. Comparison of cumulated CH_4 fluxes in winter wheat 5 in manuring treatment w/o L-M during the three investigated seasons revealed only a variation of annual fluxes by a factor of 1.8, whereas N_2O emissions differed by a factor of five (see 4.2.2). Moreover, elevated CH_4 consumption rates were often determined in this field trial during the summer months which is in line with other *in-situ* investigations on arable land (Flessa *et al.* 1995; Ruser *et al.* 1998b). In contrast, Dobbie and Smith (1996) did not find significant differences between CH_4 oxidation rates in winter wheat throughout a year.

4.3.3 Impact of Climate Conditions on CH_4 Fluxes

The response of *in-situ* CH_4 oxidation to soil moisture has been frequently reported with an inverse relationship between CH_4 comsumption rate and moisture content for various soils (Steudler *et al.* 1989; Whalen *et al.* 1991; Dörr *et al.* 1993; Castro *et al.* 1995; MacDonald *et al.* 1996; Singh *et al.* 1999) and has also been observed in a potato field for the interrow soil (Ruser *et al.* 1998b). However, no significant correlation could be determine for the ridges that was ascribed to the low percentage of the WFPS in the ridge soil. Highest CH_4 uptake was exhibited during the summer months when soil temperatures were high and soil moisture relatively low. Merino *et al.* (2004) showed that the soil moisture was the main factor influencing *in-situ* CH_4 fluxes (negative relationship) in a cropland soil with highest CH_4 uptakes during the summer months, coinciding with low soil moisture contents throughout two years of investigation. Soil temperature was found to be a secondary factor in many studies for CH_4 fluxes. Flessa *et al.* (1995) found negative correlations between CH_4 consumption rates and soil moisture and positive correlations of CH_4 consumption rates with temperature in three arable soils. Those soils oxidized also more CH_4 towards midsummer when soil temperatures were high, however, heavy rainfall in the middle of July markedly reduced CH_4 uptake. The fourth site under investigation showed a different behavior with generally higher CH_4 consumption rates and without significant relationships between CH_4 fluxes and soil moisture or soil temperature.

Looking at CH_4 fluxes determined in this field study in all crops and manuring treatments, seasonal changes in CH_4 oxidation rates were dependent on crop and year. Notably in the winter cereals spelt and winter wheat and particularly during season 2003/2004, highest soil uptake rates occurred in June and July. Lower CH_4 consumption were observed during the winter period from December 1 to March 15. On a few sampling dates in winter, CH_4 emissions occurred that were not related to fertilizer applications. For example, on December 22, 2004 a significant amount of CH_4 was emitted in winter wheat 5 in manuring treatment w/o L-M. Also, both treatments in intercrops prior to spring wheat showed CH_4 losses on this day. Similarly, on January 7, 2005 w/o L-M exhibited CH_4 emission, but it was not significantly different from CH_4 oxidation in w/o L-FC due to high standard deviations. Precipitation of 11.4 mm during the preceding five days of December 22, 2004 and 3.2 mm on the day of sampling could have increased the water-filled pore space leading to anoxic conditions in the soil and hence to CH_4 production surpassing CH_4 consumption. Descriptions of net *in-situ* emissions of CH_4 from arable soils were reported after heavy precipitation events which caused a sudden reduction in gas diffusivities (Merino *et al.* 2004). Kamp *et al*. (2001) found occasionally CH_4 emissions from soil monoliths of a loamy sand that were attributed to frequently high soil moisture contents and saturated conditions in the upper horizon due to clay accumulation and hence stagnent water. Decreases of CH_4 oxidation and even CH_4 losses with increasing WFPS were also assessed in forest soils and peat soils (Steudler *et al.* 1989; Castro *et al.* 1995; MacDonald *et al.* 1996). Apart from reduced gas diffusivities, a slower consumption rate could also be the result of an initiation of *in-situ* CH_4 production in anoxic zones that counterbalances consumption (Mosier *et al.* 1998b).

Soil temperature often correlated (positively) with CH_4 oxidation rates as mentioned above. It should be noted that CH_4 uptake in aerobic soils does not cease in winter. Even in subalpine mountain soils under a snow pack (Sommerfeld *et al.* 1993) or when surface soils weere frozen (Mosier *et al.* 1991) CH_4 oxidation continued. In winter soil temperature and biological activity are low, but as temperature rises in spring, biotic activity increases until the microbes become substrate-limited (Crill 1991; Castro *et al.* 1995). Demand surpasses supply and consumption rates eventually plateau at a diffusion-contolled maximum. However, if soils become very dry, oxidation rates can fall again since moisture stress affects the biological activity (Mosier *et al.* 1991). However, some authors did not find any or only a weak relationship between *in-situ* CH_4 uptake and soil temperature (Steudler *et al.* 1989; Crill *et al.* 1994; Dobbie and Smith 1996). King and Adamsen (1992) as well as Born *et al.* (1990) concluded that microbial activity was mainly controlled by gas transport (diffusion). In the present field study, correlation coefficients (r^2) between CH_4 oxidation rates and soil temperature in 5 cm depth or air temperature varied from 0.51 to 0.80 and from 0.53 to 0.76, respectively (table 3.9). Schmädeke *et al*. (1998) calculated correlation coefficients for CH_4 fluxes and soil temperature in 2.5 cm depth in winter rape, winter wheat, and winter barley for the winter and summer periods and obtained coefficients between 0.44 and 0.76.

4.3.4 Impact of Crop Species on CH_4 Fluxes

Different crop species may require different cultivation and tillage operations, different management and manuring strategies. Those activities may be performed at different time points in the season with varying soil and climatic conditions as described in more detail in section 4.2.4. Comparability of influences of different crops on trace gas fluxes is only given if investigations are performed during the same season on the same site under similar soil and climatic conditions. Ideally, comparative studies should also be conducted throughout at least two, better several years to minimize the possible strong impact of weather conditions. Only limited data on the effect of vegetation on *in-situ* CH_4 oxidation in arable soils are available. Schmädeke *et al.* (1998) measured CH_4 fluxes in three different crops with two different levels of nitrogen fertilizer during two years. However, the amount of applied N was reduced in the second year in both fertilizer levels. During the first summer period, in part significant differences occurred between the crop species, but they could not be confirmed in the following summer period. Ruser *et al.* (1998a) found in the first investigation season higher mean annual CH_4 uptakes in potatoes than in winter wheat, but in the second season inverse results. Consequently, no crop species related influences on soil CH_4 oxidation activities were determined. In the present field trial, mean annual CH_4 uptakes of the four investigated crops across the manuring treatments varied between 479 and 740 g C ha^{-1}, but no significant impact of the crop species on CH_4 oxidation rates was observed. In incubation studies no differences in soil CH_4 uptake was found between maize cropping and an unplanted control soil (Syamsul Arif *et al.* 1996).

Indirect effects on CH_4 fluxes may also emerge from cultivation of different crop species. Hütsch (1996) compared in incubation experiments CH_4 oxidation rates of soils under continuous winter rye and maize and determined remarkably higher CH_4 comsumption under rye than under maize. The results were probably caused by an indirect influence of different pesticides that had been applied to the monocultures for many decades and/or the lower soil pH value under maize could have been responsible for the observed differences in CH_4 oxidation activities.

As mentioned in section 4.2.4, cultivation of potatoes differs markedly from other crops because of the ridge culture. Ruser *et al*. (1998b) quantified CH_4 fluxes in an intensively and extensively fertilized potato field separately for the ridges, the uncompacted and the tractor-traffic-compacted interrow soils. During the potato cropping period of five months (May - September), the ridge soils revealed the highest CH_4 uptakes (98 and 143 g ha^{-1} (intensively and extensively fertilized, respectively)) followed by the interrow areas (30 and 25 g ha^{-1}). Soil compaction by tractor traffic changed the soils from a sink to a source for atmospheric CH_4 (56 and 72 g ha^{-1}) that pointed to an O_2 limitation in the compacted soil. This is in line with the study by Hansen *et al*. (1993) who determined a 52% reduction in CH_4 oxidation rates in an arable soil following compaction through tractor traffic. Thus, determining total *in-situ* CH_4 fluxes in potatoes, the particularity of the cropping area with different soil properties,

especially areas of soil compaction have to be considered. However, due to missing facilities to monitor all different areas with the respective replicates throughout the investigation period in this field study, only the ridges were sampled. Consequently, the calculated capacity of soil CH_4 oxidation under potatoes (498 - 662 g C ha^{-1} a^{-1}) is presumably overestimated.

4.3.5 Impact of Different Handling of the Intercrops on CH_4 Fluxes

In the present field trial, different intercrop managements were carried out. In manuring treatments w/o L-FC and wL-FS+FC intercrops were harvested for fermentation and were applied to the field as fermented fertilizer to non-legume crops at a later time point. In contrast, in treatments w/o L-M, wL-FS, wL-FYM, and wL-RS the growth of the intercrops was incorporated into the soil as green manure before main crops were cultivated. Cumulated CH_4 uptakes in the cropping system with livestock tended to be higher in wL-FS+FC than in the other manuring treatments (table 3.16), whereas in the cropping system without livestock soil CH_4 uptake tended to be lower in treatment w/o L-FC compared to w/o L-M (table 3.7).

With the incorporation of intercrops, green manures, and crop residues, considerable amounts of organic carbon and nitrogen remained on the field (Appendix A.4 - A.7). The impact of organic residue amendements on CH_4 oxidation seems to depend on their C/N ratio. Crop residues with a wide C/N ratio (e.g. straw) stimulated N immobilization and did not affect CH_4 oxidation, whereas residues with a narrow C/N ratio (e.g. sugar beet leaves) enhanced N mineralization resulting in a strong inhibition of CH_4 oxidation, occasionally up to almost 100% (Boeckx and Van Cleemput 1996). Hütsch (1998) observed after application of fresh sugar beet leaves to a loamy arable soil an immediate inhibition of CH_4 oxidation, however, with a reduction of only 20%. Application of wheat straw, in contrast, did not cause any changes of soil CH_4 uptake.

In the present study, incorporation of intercrops prior to spelt and winter wheat was conducted in October directly before drilling of the winter cereals, whereas intercrop incorporation prior to potatoes and spring wheat was performed in January/February. No explicitly increased or decreased CH_4 oxidation rates were observed after incorporation of the intercrops or the intercrop stubbles in the various manuring treatments. Possibly, the variability at the field site masked an effect of the organic residues, but the C/N ratios of the incorporated intercrops varied in a range between 9 and 43 (Appendix A.4 - A.7) during the three seasons that might have led to variable, non-comparable effects and observations. The difference to the results by Boecks and Van Cleemput (1996) and Hütsch (1998) is possibly related to a more even incorporation of the organic matter in the soil in their laboratory studies and the higher sampling frequencies.

4.3.6 Impact of Manuring on CH_4 Fluxes

The application of the different liquid organic fertilizers resulted mainly in high short-term CH_4 emissions (figures 3.1, 3.2, 3.12, and 3.13). As revealed by incubation studies (figure 3.21), the CH_4 was probably derived from the release of dissolved CH_4 in the manures and not produced via methanogenesis in the soil (Sommer *et al.* 1996; Chadwick *et al.* 2000). CH_4 emissions directly after application of liquid organic manures were also observed by e.g. Wulf *et al*. (2002b) and Model (2003). Incorporation of solid fertilizers like solid fermented residues in w/o L-FC and wL-FS+FC or farmyard manure in wL-FYM did not seem to affect CH_4 fluxes in the different crops (figures 3.7 and 3.12). However, after application of farmyard manure in spelt in season 2004/2005 elevated but non-significant CH_4 emissions were assessed in wL-FYM during several weeks (figure 3.11).

As discussed in 4.3.5, the C/N ratio of organic amendments might have influenced the soil CH_4 oxidation rate, but no distinct pattern of reduced or increased CH_4 fluxes was observed due to spreading of organic fertilizers in the subsequent days. The C/N ratios varied between 3 and 5 in the liquid fermented fertilizers and between 11 and 26 in the solid fermented crop residues in the cropping system without livestock (Appendix A.4 and A.5). In the cropping system with livestock C/N values ranged from 4 - 13 in the different liquid fertilizers and from 12 - 47 in the solid manures (Appendix A.6 and A.7).

Generally, nitrogen fertilization is widely recognized as one of the key factors influencing CH_4 oxidation in agricultural soils whose impacts have to be distinguished in short-term and long-term effects. It was shown that oxidation of CH_4 and NH_4^+ exclude each other, and nitrification of the applied NH_4^+ has to be almost completed before CH_4 consumption commences (Hütsch 1998). Due to the low substrate specificity of the methane monooxygenase (Hubley *et al.* 1974; Colby *et al.* 1977; Dalton 1977; Higgins *et al.* 1979; Burrows *et al.* 1984), methanotrophic bacteria may metabolize NH_4^+ as soon as it is applied and oxidize CH_4 afterwards. Dalton (1977) and O´Neill and Wilkinson (1977) concluded that NH_4^+ acted as a competitive inhibitor whose effect was not completely reversible. In contrast, Dunfield and Knowles (1995) found in incubation studies a complete reversible inhibitory effect of NH_4^+ postulating a NH_4^+ concentration depending and time depending ability of methanotrophs to recover. However, different investigations under field conditions did not reveal any impact of NH_4^+ fertilizers or urea on CH_4 uptake of fertile agricultural soils (Bronson and Mosier 1993; Hütsch *et al.* 1993; Dobbie and Smith 1996).

Surprisingly, Bodelier *et al*. (2000b) showed that ammonium-based fertilization did not necessarily inhibit CH_4 consumption in the root zone of rice plants but rather stimulated activity and growth of methanotrophic bacteria. CH_4 oxidation and numbers of methanotrophs were stimulated by the fertilizer addition and by the presence of rice plants. Without fertilization, nitrogen limiting conditions for the CH_4 consuming bacteria, that will be the normal situation in the rice rhizosphere, restrict CH_4 oxidation (Bodelier *et al.* 2000a). Dan

et al. (2001) observed stimulated CH_4 production and consumption in the top soil of a rice field during nine days following a modest urea fertilization, but major changes in CH_4 emission were missing. Rice plants took up ammonium so fast that a direct impact of NH_4^+ on CH_4 oxidation was hardly possible.

As mentioned in 4.2.6, fermentation in a biogas plant leads to products with increased NH_4^+-N contents and a higher proportion of NH_4^+-N of the total nitrogen content in comparison to the respective "raw material" (Field *et al.* 1984; Asmus *et al.* 1988; Wulf *et al.* 2001). In the cropping system with livestock, where the effects of non-fermented (wL-RS) and fermented slurry (wL-FS) could be compared, no definite influences on *in-situ* CH_4 fluxes were determined.

The soil moisture status is an important factor influencing CH_4 fluxes. Physiological water stress of methanotrophic bacteria was shown to limit atmospheric CH_4 consumption rates in a forest soil at low water contents (Schnell and King 1996). Maximum rates of atmospheric CH_4 oxidation occurred at a gravimetric soil water content of 25% corresponding to a water potential of about -0.2 MPa. CH_4 uptake was strongly depressed by water potentials of -3 to -4 MPa indicating that soil methanotrophs require a certain amount of free water. Soil drying may reduce the CH_4 oxidation and the capacity of soils to act as sink for CH_4 (Nesbit and Breitenbeck 1992). Soil water not only affects the moisture availability to microorganisms but also influences the gaseous diffusion and the supply of CH_4 (Born *et al.* 1990; Whalen *et al.* 1990). The capacity of methylotrophic bacteria to consume CH_4 commonly exceeds the potential of CH_4 to diffuse from the atmosphere to the consumers. The maximum rate of uptake of atmospheric CH_4 by soil is limited by diffusion and can be calculated from soil physical properties and the CH_4 concentration gradient (Striegl 1993). Elevated soil moisture contents, on the other hand, may also lead to lower soil CH_4 uptake rates of atmospheric CH_4 due to limited CH_4 diffusion into the soil and enhanced CH_4 production (Steudler *et al.* 1989; Nesbit and Breitenbeck 1992).

Investigations on acid forests soil by Schnell and King (1994) exhibited that exogenous nitrite (NO_2^-) was an even more effective inhibitor of CH_4 oxidation than NH_4^+. Although NO_2^- is the end product of NH_4^+ oxidation by methanotrophic bacteria (Bédard and Knowles 1989) it may accumulate and strongly restrict activities under low pH values (Schnell and King 1994). Probably, NO_2^- was a more effective and more persistent inhibitor because it is present in soil solution and not like NH_4^+ to 65% adsorbed to soil particles (Hütsch 1998). In soils with good conditions for nitrification (well aerated, approximately neutral pH value) inhibitory effects of NH_4^+ of urea via NO_2^- is unlikely since NO_2^- is oxidized immediately after its production and only minor accumulation occurs (Hütsch 1998).

The effect of NO_3^- on CH_4 oxidation is not clear. Many studies revealed that NO_3^- fertilization had no impact on soil CH_4 uptake (Boeckx and Van Cleemput 1996; Hütsch 1998), whereas other investigations showed an adverse effect on the CH_4 oxidation ability (Adamsen and King 1993; Bronson and Mosier 1994; Hütsch 1996). The discrepancy may be explained by

different NO_3^- application rates. If NO_3^- salts were applied in common agricultural amounts, CH_4 oxidation rates did not differ from the unamended control soil (Hütsch 1998), but much higher fertilizer doses may cause osmotic stress to the methanotophic bacteria (Dunfield and Knowles 1995). Consequently, NO_3^- may effect indirectly soil CH_4 uptake due to salinity effects. Furthermore, if potassium salts like KNO_3 were used for fertilization, K^+ is able to exchange NH_4^+ from surfaces and interfaces of clay particles which may inhibit CH_4 consumption through release of NH_4^+ (King and Schnell 1998).

4.3.7 Further Factors Influencing *in-situ* CH_4 Fluxes

Further impact factors on soil CH_4 oxidation activities are discussed in the literature, but they did not differ in the present field study and hence cannot have provoked different CH_4 oxidation rates in the investiated manuring treatments.

DeVisscher *et al*. (1998) pointed out that the cation exchange capacity (CEC) of a soil is an important parameter for the degree of inhibition of CH_4 oxidation through NH_4^+ that could partly explain contradictory observations in the literature. They revealed that on soils with high CEC rather large amounts of NH_4^+ entailed only small effects on CH_4 uptake, whereas on soils with low CEC already small NH_4^+ applications caused strong inhibitory influences on CH_4 consumption. Consequently, only soils with the same CEC are directly comparable in their reaction of different fertilizers on CH_4 oxidation activity. Since the present field trial was performed in neighboring field plots at one location without big differences in soil texture, the same CEC of the plots may be taken for granted.

Long-term effects resulting from repeated N applications during several years and decades are also of interest and have been studied in arable soils. Various studies have been conducted in regularly fertilized arable soils that exhibited strong inhibitory influences of NH_4^+ on soil CH_4 uptake (Bender and Conrad 1994; Flessa *et al.* 1996; Hütsch 1996; 1998; 2001b). Hütsch *et al*. (1993) observed in an incubation experiment with soil cores from the "Broadbalk Wheat Experiment" at Rothamsted, UK (began in 1843) highest CH_4 oxidation rates in the unfertilized control treatment. The soil CH_4 oxidation ability decreased progessively with increases in mineral N application (up to 144 kg N ha^{-1} a^{-1}), whereas the soil amended with farmyard manure (nearly 240 kg N ha^{-1} a^{-1}) exhibited almost the same CH_4 oxidation activity as the unfertilized plot. Likewise, investigations with soil cores from the "Rye Experiment" at Halle, Germany (start in 1878), revealed enormous declines in the soil CH_4 uptake rates due to mineral N application (60 kg N ha^{-1} a^{-1}) (Hütsch 1996). Manuring of the same nitrogen amount with farmyard manure (12 t FYM ha^{-1} a^{-1}) reduced also the soil CH_4 consumption, but to a lesser extent. Investigations carried out on soil samples derived from the "Static Fertilization Experiment" at Bad Lauchstädt, Germany (established in 1902) resulted in a decreased CH_4 uptake of soil fertilized with mineral N, whereas application of farmyard manure entailed an increased CH_4 oxidation rate (Willison *et al.* 1996). In contrast, Hütsch (1996) found no

differences among soils of the "Static Fertilization Experiment" without any nitrogen addition, with mineral N fertilizer, and with farmyard manure application. However, in a different subplot which did not receive periodically lime contrary to the first subplot and hence exhibiting lower pH values, mineral N application reduced CH_4 oxidation significantly compared to the no-N treatments. In summary, the ability to oxidize CH_4 seems to decrease in arable soils that had been amended with inorganic nitrogen for many decades in dependence of the amount and kind of applied nitrogen fertilizer as key determinants.

Long-term applications of nitrogen fertilizer could cause changes in microbial ecology and probably a shift in microbial populations (Adamsen and King 1993). Seghers *et al.* (2005) determined in an incubation experiment three times higher CH_4 oxidation rates in soils amended with organic fertilizer (compost) for five years than in soil fertilized with mineral nitrogen. The larger consumption rates were positively reflected in a significantly enhanced abundance of methanotrophic bacteria in the organically fertilized soil. Seghers *et al*. (2003) showed in a long-term field study that the composition of the Type I methanotrophic community differed between an agricultural soil receiving organic or mineral fertilizer, respectively. Since CH_4 fluxes in soil are not only determined by methanotrophic but also by methanogenic activities, impact of manuring on CH_4 producing community should be considered, too. Sheppard *et al*. (2005) exhibited marked differences in community patterns of methanogenic *archaea* between a long-term fermented sludge amended grassland soil and an untreated soil with reduced diversity in the fertilized soil.

4.4 Net *in-situ* CO_2 Fluxes

As minor part of the study, also net CO_2 fluxes were determined in the soil-plant system using the closed chamber technique with transparent chambers that included plants. This experimental setup allowed the observation of differences in net CO_2 fluxes among the manuring treatments, encompassing possible plant-stimulated direct effects on the soil respiration via the rhizosphere in the soil-plant system (Domanski *et al.* 2000; Ehrensberger and Kuzyakov 2000; Siniakina *et al.* 2000). It should be noted that the net CO_2 flux is the result of various CO_2 producing and CO_2 consuming processes. The amount of CO_2 enclosed within the chamber plus any CO_2 production were not sufficient when the photosynthetic activities were high so that net CO_2 rates could not be determined. Due to the above-mentioned problems, annual balances of CO_2 fluxes in the different crops and manuring treatments were not assessed, and the net CO_2 fluxes reported in this study should be regarded with the appropriate care.

In other studies, opaque chambers were used to determine *in-situ* flux rates of trace gases (Sehy *et al.* 2003; Dörsch *et al.* 2004; Merino *et al.* 2004). Those chambers prevent photosynthetic activity, hence only respiratory processes are considered during the investigation period regardless of plant CO_2 uptake. Subsequently, net CO_2 emissions of the site are overestimated.

In the present study, mostly lower CO_2 emissions were observed in the winter cereals winter wheat and spelt in the manuring treatments w/o L-FC and wL-FS+FC, respectively, after intercrop incorporation (figures 3.1, 3.2, 3.11, and 3.12). Thus, the lower amount of degradable organic matter due to the intercrop harvest for fermentation (Appendix A.4 - A.7) entailed lower CO_2 emission rates. However, significant differences were seldom assessed. CO_2 flux patterns determined in intercrops prior to spring wheat and potatoes differed from those in winter cereals because the intercrops were incorporated to the soil not until January or February. Harvest of intercrops prior to potatoes in fall in wL-FS+FC resulted in CO_2 emissions in the crop stubbles in contrast to CO_2 uptake in the other manuring treatments with intact intercrops (figures 3.17 and 3.18). Comparable observations were made in intercrops prior to spring wheat (figure 3.7). Flux measurements in the ploughed soil prior to potatoes until May and later in summer revealed net CO_2 emissions in both seasons. In all investigated crops and manuring treatments CO_2 emission rates amounted up to 508 mg C m^{-2} h^{-1}. Elevated CO_2 emissions were partly observed after application of liquid manure due to degassing of dissolved CO_2 (e.g. figures 3.2, 3.12, and 3.13).

The CO_2 emissions determined in this field study are in line with other field trials exhibiting CO_2 flux rates between 5 and 447 mg C m^{-2} h^{-1} (Model 2003; Sehy *et al.* 2003; Merino *et al.* 2004). Drury *et al*. (2006) even found CO_2 emission rates up to 150 kg C ha^{-1} d^{-1} (625 mg C m^{-2} h^{-1}) in dependence on tillage, season, and depth of nitrogen fertilizer placement.

Several factors may influence CO_2 emissions such as soil moisture content, temperature, tillage, soil compaction, fertilization, C availability, and C/N ratio of organic amendments. Kessavalou *et al*. (1998b) assessed a general increase of mean annual CO_2 emissions with intensity and degree of tillage. Largest CO_2 emissions occurred during spring and decreased in the order spring > summer > fall > winter, regardless of cropping and tillage management. Furthermore, CO_2 fluxes of the interrow soil in wheat increased linearly with soil temperature. Drury *et al*. (2006) observed a significant site - year effect in maize as well as greatest CO_2 emissions in July and August, when soil temperatures were high. Significant effects of tillage, soil water content, depth of N placement, or interactions between tillage and depth of N placement with respect to seasonal CO_2 emissions were not determined. Ball *et al*. (1999) found that *in-situ* CO_2 fluxes were not strongly influenced by tillage a few weeks after sowing of spring barley. Periods of low or zero CO_2 fluxes (and very high N_2O emissions) under no tillage were associated with reduced gas diffusion and air-filled porosity, both caused by heavy rainfall.

Kessavalou *et al*. (1998a) observed short-term elevated CO_2 emissions in fall after subtillage (V-blade) and in summer after discing. In summer, CO_2 losses remained reduced in comparison to the averaged CO_2 loss before discing, suggesting an instantaneous release of entrapped CO_2 in soil pores. Presumably, the prolonged reduction in CO_2 loss was partly caused by an altered soil microenvironment, such as a reduced soil water content resulting in reduced microbial activity. Overall, wetting induced an increase of CO_2 emissions after 24 h and 72 h in all tillage treatments, indicating elevated microbial activity due to a more favorable soil environment from improved soil water conditions. Magid *et al*. (2001) investigated the decomposition of *Medicago lupulina*, *Melilotus alba*, and *Poa pratensis* at 3°C, 9°C, and 25°C during four weeks and noticed an increased mineralization activity with rising temperature. Additionally, different proportions of the crop-bound C were respired as CO_2 by the end of the experiment pointing to differences in substrate quality (highest proportions of mineralized C in the *Melilotus alba* treatment). Analysis of biochemical characteristics revealed that this crop material contained the highest nitrogen concentration, the lowest C/N ratio, and the lowest proportion of cellulose and lignin.

4.5 Incubation Experiments

The incubation experiments were performed to investigate in more detail the N_2O emissions of the fertilized arable soils and of the different pure fertilizers themselves under favorable conditions for denitrification activity. Although the investigation of nitrite reductase gene fragments (*nirK* and *nirS*) by real-time PCR, the enumeration of nitrate reducers by MPN, and the determination of the potential denitrifying enzyme activity confirmed the presence of denitrifying bacteria in the different fertilizers in the greenhouse experiment (table 3.18), no N_2O production was observed during the anoxic incubation of the pure fertilizers (figure 3.21). Consequently, the substrates for denitrifying activity, in particular low nitrate contents, might have prevented high N_2O production in the pure fertilizers, but stimulated the potential denitrification activity after addition of nitrate and glucose (table 3.18).

The unfertilized arable soil exhibited the highest N_2O production, whereas all fertilized soil samples revealed similar patterns of N_2O production with only small amounts of emitted nitrous oxide (figure 3.21). Thus, the denitrifiers could have been either inhibited by addition of the fertilizers or the ratio of the denitrification products N_2O and N_2 was in favor of N_2 production. An inhibition of the denitrification activity could be excluded by incubation of the fertilized soils and the bare soil under anoxic atmosphere containing 10% acetylene to repress the N_2O reductase so that only N_2O was produced. All fertilized soil samples showed a similar or even faster N_2O production than the bare soil (figure 3.23). After 53 h of incubation, similar N_2O concentrations were found in all investigated samples. Consequently, the ratio of N_2O and N_2 must have been decreased by fertilizer addition during the anoxic incubation without acetylene (figure 3.21). Firestone and Davidson (1989) and Granli and Bøckman (1994) compiled in their reviews the impact of different factors on the N_2O/N_2 ratio during denitrification. Since temperature, absence of oxygen, soil moisture content, and nitrate content remained more or less unaffected by fertilization in comparison to the unfertilized soil, the addition of organic carbon through the fertilizers might have led to the shift of end products towards N_2. The assumption is in accordance with the literature where easily degradable organic carbon is considered to promote full reduction to N_2, i.e. low N_2O/N_2 ratios (Elliott *et al.* 1990; Weier *et al.* 1993). Furthermore, the faster increase of N_2O in the fertilized soils suggested a higher denitrification rate presumably also provoked by the added organic carbon through the manures (Paul and Beauchamp 1989; Drury *et al.* 1991). The question whether the soil autochtonous or the imported denitrifiers by the manures caused this result remains open. However, observations made during the greenhouse experiment investigating the *nirS* gene fragments of denitrifiers after manuring indicated a possible stimulation of fertilizer derived denitrifying bacteria (figure 3.28).

As expected, a fast decrease of nitrate, the substrate for denitrification, in the fertilized soils was observed during the incubation (without acetylene) (figure 3.22). The concentration of nitrate remaining in the soil amended with fermented slurry might be due to interference of

the photometrical determination by turbidity caused by the manure. The difference between the amount of nitrate (up to 2350 nmol N g^{-1} soil and fertilizer) present in the fertilized soils and the recovered N_2O concentration using the acetylene inhibition technique (maximum 1020 nmol N g^{-1} soil and fertilizer), however, remains unclear. Possibly, nitrate assimilation by bacteria, dissolved N_2O in the soil water phase, dissimilatory nitrate reduction to ammonia (DNRA), and measuring inaccuracy might have contributed to the discprepancy.

Highest CH_4 concentrations were found in the raw slurry (2400 nmol g^{-1} fertilizer), as anticipated. Surprisingly, the fermented slurry exhibited also a clearly elevated CH_4 concentration in the headspace (1262 nmol g^{-1} fertilizer), even if it was lower than in the raw slurry. The methane could either originate from degassing of already produced and dissolved CH_4 in the fertilizer and/or it was produced during the incubation period suggesting an incomplete fermentation process in the biogas plant. The methane concentration in the headspace of the fermented crop material was comparatively low (196 nmol g^{-1} fertilizer) and might also have been derived from degassing or methanogenesis during the incubation. When the different fertilizers were applied at the field site, CH_4 emissions were frequently observed directly after manuring (e.g. figures 3.2, 3.3, 3.12) indicating the degassing of already produced and dissolved methane because of the dramatic and abrupt increase of the surface area. Further comparisons of N_2O and CH_4 fluxes between the incubation experiment and the field observations are difficult due to differences between 1. anoxic and oxic conditions, 2. determination of gas accumulation and flux rates, and 3. determination in respect of the soil volume and the soil surface area, respectively.

4.6 Greenhouse Studies

The aim of the greenhouse studies was to investigate the effect of the different organic fertilizers on the denitrifying activity and the soil bacterial denitrifying community in more detail. Due to unknown and unidentified toxic effects of the fermented crop material (FC) on the cultivated summer wheat only 25% of the planned fertilizer amount could be applied in comparison to raw slurry (RS) and fermented slurry (FS). Furthermore, the consistency of FC differed from RS and FS through lower viscosity and lower dry matter content favoring a fast and spacious distribution in the soil compared to RS and FS (table 3.18). Consequently, a larger soil zone was probably activated in the FC manuring treatment. The penetration of RS and FS into the soil was obviously slower, and solid residues within the manures formed a crust on the soil surface. However, since the upper soil layer of about 6 cm was sampled for the diverse investigations in the laboratory, the investigated soil zones amended with RS and FS might have received higher fertilizer concentrations than the soil fertilized with FC.

The soil mineral nitrogen concentrations followed typical dynamics. The NH_4^+ concentrations decreased continuously and the NO_2^- dynamics were characterized by an initial increase followed by a decline between seven and ten days after fertilizer application. NO_3^- contents followed the NO_2^- dynamics with a delayed response and in higher concentrations (figure 3.24). About two times more NH_4^+ was applied through RS and FS than through FC, but surprisingly, concentrations of nitrate and nitrite were similar in all manuring treatments during the sampling period. The different amounts of NH_4^+ originally applied were possibly compensated by diverse "losses" so that similar NH_4^+ amounts could have been available for subsequent microbial nitrification and denitrification activities in the different manuring treatments. Various proportions of the applied ammonium were likely lost through ammonia volatilization after manuring as revealed in similar studies (Dosch and Gutser 1996; Wulf *et al.* 2002a; Amon *et al.* 2006; Clemens *et al.* 2006). This was probably associated with higher pH values in RS and FS compared to FC. In addition, more NH_4^+ might have been adsorbed to the soil matrix in RS and FS treatments. More ammonium might also have been directly assimilated by crops and/or soil microorganisms. In fact, the RS and FS manuring treatments revealed significant higher crop biomasses. However, the interpretation of N_{min} data is complicated by the fact that the nitrite pool merges the intermediates of the overlaying processes nitrification and denitrification (and others).

Nitrite and nitrate concentrations indicated a fast initiation of nitrification with seemingly similar metabolic rates in the differently fertilized soils. Nitrifiers (ammonium and nitrite oxidizers) are known to be slow-growing bacteria (Belser 1979) with only limited population size increases in the field (Okano *et al.* 2004). In the greenhouse experiment, NH_4^+ concentrations in all manuring treatments had declined by day 7 to an amount similar to that of the unfertilized control treatment. Thus, nitrification of NH_4^+ was probably performed by residing bacteria whose metabolic rate might have been at maximum in all manuring

treatments assuming a non-limitation of NH_4^+. Alternatively, the nitrifying bacteria might have been similarily affected by oxygen diffusion in the wet soils (Belser 1979). However, the observed N_{min} dynamics are related to the sampling frequencies and therefore, it cannot be excluded that they do not represent the actual temporal dynamics. Possibly, maximum amounts of nitrite and nitrate differed between the treatments in height and time point.

The elevated pool sizes of nitrate and nitrite in the manuring treatments did not coincide with the respective N_2O fluxes (figure 3.25) (Müller *et al.* 2004b) indicating that nitrate was not the limiting factor for N_2O production. During the first two days, highest N_2O emission rates were observed in the soil fertilized with FC. This observation was probably linked to the rapid penetration of FC into the soil compared to RS and FS, and hence a faster activation of a larger soil zone. Not significantly elevated N_2O fluxes were still measured till day 5 - 6 in the soils amended with RS and FS, respectively. The decrease of N_2O emissions despite that nitrate was available may have been related to a reduced ratio of the denitrification products N_2O and N_2 and/or to the decline of available organic carbon.
The observed N_2O emission rates in the greenhouse experiment expressed in µg N m^{-2} h^{-1} as in the field study amounted to 71 in RS, 116 in FS, and 820 µg N m^{-2} h^{-1} in FC. Thus, the N_2O effluxes in the soil amended with FC in the greenhouse were clearly higher than after fertilization in the field. But the applied amount of RS and FS in the greenhouse was with 200 kg N_t ha^{-1} higher than in the field study. In contrast, the amount of FC fertilized in the greenhouse accounted just for 50 kg N_t ha^{-1} (due to toxic effects of the respective fertilizer charge on the crops) and hence was approximately in the range that had been applied on the field site. Although the amount of N_t in FC was only a quarter of RS and FS, the applied fertilizer volume was higher than in RS and FS due to the relatively low N_t concentration of the respective FC charge. This high amount of fertilizer liquid and its fast penetration into the relatively small soil volume in the greenhouse experiment might have contributed to the high N_2O emission rates in the FC manuring treatment in the greenhouse compared to RS and FS as well as in comparison to the *in-situ* fluxes.

Contrary to the high N_2O emission rates, the potential denitrifying activity was not significantly affected by application of FC compared to the unfertilized soil. In contrast, fertilization with RS and FS induced a significant stimulation of the potential denitrifying activity two days after manuring (figure 3.26). Those observations agreed with the very low denitrification potential in the pure fermented crop material and the higher potential denitrifying activities in the pure raw and fermented slurry, respectively (table 3.18). Moreover, the significantly increased denitrification potentials in RS and FS coincided well with the significantly increased N_2O effluxes in those manuring treatments two days after manuring (figure 3.25). However, the potential denitrification activities of the soils fertilized with RS and FS were significantly inhibited two hours after manuring. Possible reasons like nitrite accumulation or a drop of

pH value, triggering the decrease of the potential denitrifying activities, might not have been responsible in this case. Two and four days after manuring, when in part significantly elevated denitrification potentials were determined in the RS and FS treatments, even higher nitrite concentrations were measured than two hours after fertilization. Furthermore, a comparison of pH values of the applied fertilizers exhibited even higher pH values in RS and FS than in FC.

Surprisingly, studies that compassed the impact of (organic) fertilizers on the denitrifying enzyme activity directly after manuring are rare. Hashimoto and Niimi (2001) found an enhanced denitrifying activity in surface soils with increasing slurry amounts. Hashimoto *et al.* (2002) reported on the effect of different slurry applications in spring and autumn 0 - 85 days after manuring and revealed a general increase of soil potential denitrification activities along with the increased levels of slurry applications in the autumn survey. However, as in the previous study, no data were shown, and the reading of N_2O production rates on the basis of the graphs with log scale identifying the alterations of the denitrification potential between 0 and 85 days was difficult. Moreover, it was only mentioned that slurry application enhanced the potential denitrifying activity.

From long-term studies it is evident that organic fertilizers such as cattle slurry and sewage sludge promote higher denitrification rates from soil than mineral or unfertilized treatments (Wolsing and Priemé 2004; Enwall *et al.* 2005). The observed range of potential denitrification activities from 114 to 454 ng N_2O-N g^{-1} dw soil h^{-1} in the greenhouse experiment was similar to the rates exhibited in the mentioned long-term studies (up to 550 ng N_2O-N g^{-1} soil h^{-1}).

Considering the enumeration of nitrate reducers by MPN technique, no broad numerical differences of cell numbers occurred within a manuring treatment during the investigation period and between the different manuring treatments at the same time point, notably regarding the 95% confidence limits (table 3.19). Thus, mostly activity changes and not bacterial growth during the experiment must have been responsible for the observed elevated N_2O fluxes (figure 3.25) and potential denitrifying activities (figure 3.26). Generally, the numbers of nitrate reducers across all manuring treatments were relatively high varying between 1.2 x 10^8 and 2.9 x 10^{10} g^{-1} dw soil. For comparison, enumeration of nitrate reducers by MPN in different soils resulted in cell numbers ranging from 10^4 to 10^8 g^{-1} dw soil (Chèneby *et al.* 1998; Cannavo *et al.* 2002; Hashimoto *et al.* 2002; Cannavo *et al.* 2004). However, the inherently reduced resolution, the generally large uncertainty between replicates (Cannavo *et al.* 2002), and the dependence of culturability of the respective nitrate reducers reduce the explanatory power of the MPN method.

Quantification of denitrifiers targeting the two alternative nitrite reductase genes by real-time PCR exhibited results varying between 2.8 x 10^6 - 4.3 x 10^7 g^{-1} dw soil. Especially in soils fertilized with RS and FS (significant) increases of *nirK* copy numbers were frequently found, whereas

significant increases of *nirS* copies were observed from day 10 on after manuring (figure 3.27). Significant alterations in denitrifier populations were not noticed by the MPN technique. Currently, it is assumed that *nirK* and *nirS* genes are single copy genes (Philippot and Hallin 2005), hence indicating denitrifier growth during the investigation period. The soil was homogenized as good as possible before soil DNA extraction, however, the results are based on the extraction of 3 x 0.5 g soil from the replicate pots that contained each 1.3 kg dw soil. Furthermore, the amplified nitrite reductase gene fragments rely on extracted DNA that might entail amplification of not functional genes, since DNA represents rather the potential of existing denitrifiers and not the metabolizing, active denitrifiers.

In many cases, observations of elevated or reduced *nirK* and *nirS* copy numbers did not correspond well to increased or decreased N_2O emissions, potential denitrification activities, or MPN enumeration. Possibly, homogenization of a very inhomogeneous substrate at the microscale like soil was not as successful as assumed maybe due to the small soil aliquots. However, the obviously and significantly elevated copy numbers of *nirK* and *nirS* genes ten days after manuring were determined when soil nitrite concentrations of the fertilized treatments had decreased to the detection limit. It is likely that denitrifying bacteria were sensitive to nitrite and hence were restricted in growth and metabolism until the soil nitrite concentrations decreased (Chèneby *et al.* 1998).

Previously, it was assumed that *nirS* denitrifiers are predominant in marine ecosystems, whereas *nirK* denitrifiers are preferentially found in soils (Braker *et al.* 2000; Avrahami *et al.* 2002). Sharma *et al*. (2005) did not succeed in *nirS* amplification with DNA and cDNA for any of the three different rhizosphere soils, while amplification of *nirK* genes derived from the same soil samples was successful. Likewise, Priemé *et al.* (2002) were not able to detect *nirS* copies from a mixed deciduous upland forest soil. Wolsing and Priemé (2004) detected *nirS* nitrite reductase genes at only one time point from soil sites receiving mineral fertilizer. However, Throbäck *et al*. (2004) revealed by re-evaluation and in part new developing of PCR primers targeting *nirS* and *nirK* genes that this conclusion should be revised. They demonstrated that *nirS* denitrifiers were common in many different soils and identified environmental habitats harboring a substantial diversity of *nirS* denitrifying bacteria. Since it is believed that nitrite reductases encoded by *nirS* are more widespread but less conserved among bacteria than *nirK* nitrite reductases, it was more difficult to design broad-range primers or probes for *nirS* than for *nirK*. The *nirK* gene occurs in bacteria of totally unrelated systematic affiliation but is apparently conserved throughout the bacterial world (Coyne *et al.* 1989; Bothe *et al.* 2000). The quantification of *nirK* and *nirS* gene copies in the present greenhouse study with gene target numbers between 10^6 and 10^7 g^{-1} dw soil are in line with estimations by Throbäck *et al.* (2004).

Different studies report denitrifier quantification in several environments using real-time PCR assays via different target genes. Targeting *nirS* genes in various environmental habitats, gene copy numbers between 0 and 1.1 x 10^{11} μg^{-1} DNA were determined (Grüntzig *et al.* 2001;

Wallenstein and Vilgalys 2005). The quantification of *nirK* gene fragments in different soils revealed 9.7 x 10^4 - 3.9 x 10^6 gene copies g^{-1} soil (Henry *et al.* 2004) and 3,5 x 10^5 - 1,2 x 10^6 μg^{-1} soil DNA (Wallenstein and Vilgalys 2005). Target numbers of the *nosZ* genes in forest soils were found in the range of 4.7 x 10^4 μg^{-1} soil DNA (Wallenstein and Vilgalys 2005). In various soils and freshwater sediments *narG* gene copy numbers varied betweeen 5.08 x 10^9 and 1.14 x 10^{11} copies g^{-1} dw sample (López-Gutiérrez *et al.* 2004). Thus, *nirK* and *nirS* target numbers determined during the greenhouse experiment in the different manuring treatments fit in size to published *nirK* and *nirS* gene copy numbers.

Comparing cell numbers revealed by MPN technique and the number of nitrite reductase gene fragments generated by real-time PCR assuming one gene copy per cell, a discrepancy of 2 - 3 orders of magnitude emerges. But it has to be noted that using the real-time PCR assay denitrifying bacteria were quantified by targeting the two alternative nitrite reductase genes, whereas performing the MPN technique nitrate reducing bacteria were enumerated. It is known that the majority of bacteria selected by anaerobic growth with nitrate as electron acceptor only reduces nitrate as far as nitrite ("nitrate respirers") (Gamble *et al.* 1977). A relatively small fraction of nitrate reducing bacteria is capable of producing gaseous nitrogen products via denitrification ("denitrifiers"). Thus, in addition to denitrifying bacteria, also bacteria capable only of nitrate respiration were enumerated by MPN. Higher *narG* than *nirK* and *nirS* copy numbers in diverse environmental samples support the observation with real-time PCR (Grüntzig *et al.* 2001; Henry *et al.* 2004; López-Gutiérrez *et al.* 2004) even if 1 - 3 copies of the *narG* gene could be present per cell (Philippot 2002).

In arable soils, portions of 21% and 2.7% of nitrate respirers that were able to denitrify were estimated finding cell numbers of denitrifiers between 10^5 and 10^7 g^{-1} dw soil (Chèneby *et al.* 1998). However, those numbers could eventually not be true measures of the relative abundance because denitrifying bacteria might be more sensitive to nitrite concentrations. Enumeration of nitrate reducing bacteria in estuarine sediments exhibited 4 x 10^6 and 3.5 x 10^7 cells g^{-1} dw sediment, while denitrifying bacteria were detected in amounts of 4 x 10^2 and 3.5 x 10^3 cells g^{-1} dw sediment (Nogales *et al.* 2002). Cannova *et al*. (2002) revealed in upper vadose zone layers higher cell numbers of nitrate reducers (~10^6 g^{-1} dw soil) compared to denitrifiers (~10^4 g^{-1} dw soil) using the MPN technique.

The impact of the different organic fertilizer on the composition of the dominant denitrifiers was investigated by SSCP fingerprinting patterns targeting the *nirS* genes. After fertilizer application different community patterns were found between the manuring treatments indicating a shift within the dominant denitrifying bacteria (figure 3.28). Different, distinct bands appeared, notably in treatments with addition of RS and FS, which were in part also present in the pure fertilizers. Consequently, either the denitrifying bacteria imported through the manures or the soil autochthonous denitrifiers were promoted by fertilization. The influence of FC on the denitrifier composition was weaker, but only 25% of FC were applied

compared to RS and FS on the basis of N_t. Thus, the apparently minor impact of FC on the dominant denitrifying bacteria could at least partly result from the lower fertilizer amount that had been applied. Nevertheless, it cannot be excluded that neither the FC manure itself was able to stimulate the soil autochthonous denitrifying bacteria by fertilization nor that the imported denitrifiers through FC were able to establish themselves. Not later than ten days after manuring, all emphasized bands had disappeared and only small differences between single bands in the *nirS* community patterns of the manuring treatments were observed. The *nirS* denitrifier community patterns were based on DNA extraction and hence on the gene pool level. The presence of a gene, however, does not imply its expression. Which denitrifying bacteria were activated by manuring and hence were responsible for e.g. the altered composition of the dominant denitrifiers, the elevated N_2O emissions, the changed potential denitrification activity, or the higher *nirK* and *nirS* gene target numbers, the soil autochthonous or the imported denitrifying bacteria, could not be clarified.

The influence of fertilizer application or rhizodeposition on the composition of soil denitrifying bacterial communities was the subject of several studies. Avrahami *et al*. (2002) observed a community shift of *nirK* denitrifiers in the soils amended with medium and high NH_4^+ concentrations. Thus, the elevated nitrogen level simulating fertilization entailed an adapted composition of the dominant *nirK* denitrifiers comparably to the *nirS* denitrifying bacteria in the greenhouse experiment. In a field study, Wolsing and Priemé (2004) revealed a wide and significant dispersion of *nirK* community patterns between differently fertilized soils indicating that the fertilizer type was an important determinant for the *nirK* population structure which might have been linked to the potential denitrifying activity, the nitrous oxide reductase activity, and their ratio between the soils. Those findings seem to support the observation that the *nirS* communities in the greenhouse experiment were temporarily altered due to different fertilizer application. Sharma *et al*. (2005) analyzed the diversity of *nirK* gene transcripts, thus targeting the active denitrifiers, in the rhizospheres of *Vicia faba*, *Lupinus albus*, and *Pisum sativum*. Each of the three legume rhizospheres produced a distinct molecular profile of *nirK* gene fragments. *Vicia* and *Lupinus* rhizosphere samples were more similar to each other than to the *Pisum* profile which was attributed to similar N rhizodeposition values between these two legumes compared to the value in *Pisum*. The rhizodeposits and the different organic manures in the greenhouse study might have similar influences on the denitrifier composition. Mounier *et al*. (2004) showed that a maize mucilage amendment of two weeks was enough to elevate the soil denitrifying activity, but only few stronger or additional bands could be observed with the RFLP fingerprint patterns of the *narG* gene fragments. No differences between the communities occurred within the *nosZ* gene fragment patterns. Consequently, the increase in the denitrifying activity did not reflect important changes in the diversity of this functional community. Moreover, Enwall *et al*. (2005) achieved related findings exhibiting clear influences of different fertilizers on both, the activity and the composition of the denitrifying communities in the long-term. However, fertilizer treatments entailing the most

different activities did not correspond to treatments in which the denitrifying community composition differed the most, pointing out that the activity was not coupled to the community composition. Similar results were obtained in the greenhouse experiment, where different SSCP community patterns of *nirS* gene fragments in the four manuring treatments were assessed up to seven days after manuring, notably through application of RS and FS. But the altered population composition was not necessarily reflected by e.g. activity investigations like nitrous oxide emissions and denitrification potential. With exception of the study by Sharma *et al*. (2005), all other investigations examined the functional diversity of denitrifiers on the gene pool (DNA) level that does not provide information about the active, metabolizing denitrifying bacteria. In a recent publication, Sharma *et al*. (2006) demonstrated that the most dominant denitrifiers (revealed by DNA-derived profiles) were not the most active ones (exhibited by RNA-derived profiles). In consequence of the relatively good agreement between changes in RNA-community patterns and N_2O emissions, DNA-derived community patterns can only be used as an indication for changes of the active denitrifiers.

Sequence analysis of cloned *nirS* gene fragments derived from the different origins showed that the arable soil as well as the investigated organic fertilizers coverd a substantial inherent *nirS* diversity (figures 3.29 and 3.30). Similar topologies emerged in both, the DNA- and the amino acid- based dendrogram, resulting in comparable major clusters consisting exclusively of clones derived from arable soil, from manures, or from manure and soil, respectively. However, *Roseobacter denitrificans* and *Paracoccus denitrificans* formed a distant cluster in the amino acid tree. The major clusters were not supported by high bootstrap values, neither in the DNA nor in the amino acid tree, in contrast to several subclusters. Striking (sub-)clusters between the three organic manures and the arable soil partly appeared in the dendrograms indicating special community compositions despite high diversity within the different origins. Consequently, the altered composition of dominant *nirS* denitrifying bacteria after fertilization revealed by SSCP (figure 3.28) was possibly due to imported denitrifiers by the manures. However, the possibility that the soil autochthonous denitrifying bacteria were stimulated by manuring cannot be excluded by the sequence analysis.

In the literature and databases, *nirS* clones from soil, notably from arable soil, are rare, but no sequences matched *nirS* sequences derived from organic fertilizers performing the BLAST search. Prieme *et al.* (2002) studied the diversity of *nirS* gene fragments in forested upland and wetland soils, however, they were not able to amplify any *nirS* gene fragments from the upland soil. Rösch *et al.* (2002) investigated the diversity of *nirS* denitrifying bacteria in an acid forest soil, but of 15 sequenced clones ten provided false, non-nitrite reductase sequences. The remaining five showed distinct homology to *Azospirillum brasilense* and *Ralstonia eutropha*, respectively. In the present study, *Cupriavidus necator* (formerly *Ralstonia eutropha*) branched not closely but nearest to various arable soil clones. However, reassessing known PCR primers and developing new PCR primers for *nirS* genes, Throbäck *et*

al. (2004) succeeded in amplification of numerous diverse *nirS* gene fragments in arable soils. They exhibited that the environmental clones were scattered within many phylogenetic denitrifier lineages and demonstrated that *nirS* denitrifying bacteria are common in soils rebuting earlier assumptions that marine ecosystems are preferential habitats for *nirS* denitrifiers. The findings of Throbäck and co-workers were confirmed by the high diversity of *nirS* denitrifiers determined in the arable soil and the manures in the present study. The lack of similar sequences to numerous generated environmental clones suggested community compositions within the different origins of high and seemingly undiscovered diversity of *nirS* denitrifying bacteria.

4.7 Investigations of Field Soil Samples after 3.5 Years of Different Manuring

A number of parameters related to the nitrogen and carbon metabolism have been obtained in field soil samples derived from both, the cropping system with and without livestock after 3.5 years of different manuring management. It is generally assumed that a change in agricultural mangement practices such as alterations of tillage, cultivation strategies, or fertilizer regimes take many years until the effects become manifested. However, this does not exclude that the different organic manuring strategies with and without utilization of biogas plants have led to measurable differences in various soil parameters. All results have to be considered against the background of this single "snap-shot". It cannot be excluded that further samplings and investigations at different time points during the year with different soil conditions and after shorter or longer intervals to manuring or incorporation of crop biomass would entail different results.

The soil mineral nitrogen contents were afflicted with in part high standard deviations, but still significant differences were observed (table 3.20). Lower amounts of nitrate, nitrite, and ammonium were found within one cropping system in the manuring treatments wL-FYM and w/o L-M, respectively. Those treatments had not received any fertilizer application three weeks before soil sampling compared to all other manuring treatments which presumably caused the differences.

The potential denitrification and nitrification activities as well as the total nitrogen contents of the soils did not show significant differences (table 3.20). The rates of the potential denitrifying activities varied between 67.3 and 133.7 ng N g^{-1} dw soil h^{-1} and hence were not as high as in the greenhouse experiment (114 - 454 ng N g^{-1} dw soil h^{-1}). Possibly, the impact of elevated N application rates (200 kg N_t ha^{-1}) on a conspicuously lower soil volume in the greenhouse study contributed to the higher denitrifying potential. In several long-term fertilization studies where different organic and mineral fertilizers have been applied, denitrifying enzyme activities were investigated showing N_2O production rates between 14.8 - 550 ng N g^{-1} h^{-1} (Simek *et al.* 2000; Enwall *et al.* 2005; Dambreville *et al.* 2006). The generally higher rates in the study of Enwall and co-workers were possibly caused by the use of thawed soil samples and the longer incubation time without inhibition of the synthesis of new denitrifying enzymes (Luo *et al.* 1996; Murray and Knowles 1999).

Potential nitrification activities in the soil samples ranged between 95.5 and 208.0 ng N g^{-1} dw soil h^{-1} and hence were in line with published nitrifying data in other arable soils. Nitrification activities in different and differently fertilized soils varied betweeen 30 and 650 ng N g^{-1} h^{-1} (Kandeler 1988; Bollmann 1996; Fortuna *et al.* 2003).

The soil total nitrogen contents in all manuring treatments amounted to 1.37 - 1.67 mg N g^{-1} dw soil. Comparable N concentrations in organically managed soils ranging from 1.2 - 2.0 mg N g^{-1} dw soil have been observed in several studies (Fließbach and Mäder 1997; 2000; Friedel and Gabel 2001; van Diepeningen *et al.* 2006).

Surprisingly, denitrifier quantification by real-time PCR targeting the *nirK* and *nirS* genes encoding the two alternative nitrite reductases revealed more gene copy numbers in the respective control treatments wL-RS and w/o L-M (table 3.20). However, absolute values only varied within one order of magnitude and hence in similar ranges. The *nirS* gene copy numbers ranged in the cropping system with livestock from 1.1 - 2.3 x 10^6, and in the cropping system without livestock from 4.2 - 8.9 x 10^5 targets g^{-1} dw soil. In both cropping systems *nirK* copies varied between 3.2 and 8.4 x 10^6 gene targets g^{-1} dw soil. For comparison, 2.8 x 10^6 - 4.3 x 10^7 *nirS* gene copies g^{-1} dw soil and 3.7 x 10^6 - 2.5 x 10^7 *nirK* gene copies g^{-1} dw soil were determined in the greenhouse study 0 - 22 days after manuring. Thus, the numbers of the nitrite reductase genes were approximately in the same order even if slightly less targets were assessed in the field soil samples. In the cropping system without livestock, the unfertilized control treatment w/o L-M exhibited signficantly higher *nirK* and *nirS* copy numbers and lower amounts of nitrate, nitrite, and ammonium (table 3.20). In the cropping system with livestock, highest numbers of nitrite reductase genes were found in the control treatment, but wL-RS had received a manure application in contrast to w/o L-M entailing neither significantly reduced N_{min} values in this treatment (for further discussion see section 4.6).

PCR-SSCP targeting the *nirS* gene of denitrifiers exhibited more or less different patterns of the dominant denitrifying bacteria in the differently fertilized soils (figure 3.31). Bands that emerged in all treatments were observed, but some single bands appeared only in one or a few manuring treatments. Possibly, manure applications three weeks before had a dominant impact on the denitrifier composition. However, the greenhouse experiment revealed comparatively similar *nirS* profiles of the different manuring treatments three weeks after fertilization. Unlike the greenhouse study, samples were taken in a soil depth of 0 - 30 cm hence investigating a more heterogeneous soil volume. Furthermore, the soil of the different manuring treatments had experienced different fertilizer applications, a different cover crop, intercrop, and crop residue management for 3.5 years that might have contributed to alterations in the *nirS* community composition. But, as mentioned above, it has to be scrutinized if the profiles can be considered as representative regarding the big soil sample volume in comparison to the marginal soil mass that had been extracted for PCR-SSCP. Which of the denitrifiers were active and contributed to the nitrite turnover in the soil could not be elucidated using this DNA-PCR based fingerprinting technique.

Various authors (Wolsing and Priemé 2004; Enwall *et al.* 2005; Sharma *et al.* 2005) investigated the effect of different fertilizers or rhizodeposits of different crops on the composition of the dominant denitrifying bacteria. Different DNA-derived profiles of functional denitrifying genes were observed in different soil samples in comparison to the field samples. However, the investigations as well as the present study have to be interpreted against the backgound of a single "snap-shot", with the exception of the study of Wolsing and Priemé (2004). They took soil samples at three different time points during the year and showed a significant seasonal shift in the community structure of *nirK*-containing bacteria despite the fact that no significant

relation to any single abiotic parameter could be determined. Seasonal fluctuations were also observed in the population of cultured, denitrifying bacteria in a Norway spruce forest (Mergel *et al.* 2001). Thus, seasonal impacts on community profiles may in part contribute to differences between manuring treatments.
However, the different patterns of *nirS* denitrifiers in the manuring treatments may also point to an alteration on community compositions due to the different manuring management. Different denitrifying populations may have contrasting physiological characteristics such as growth kinetics or sensitivity of enzymes to oxygen, which are not taken into account when potential denitrifying activity measurements are being studied on the field soil samples (Cavigelli and Robertson 2000; Holtan-Hartwig *et al.* 2000; Cavigelli and Robertson 2001).

The investigations related to the soil carbon metabolism, i.e. total C content, water-extractable C, soil microbial biomass C, and C substrate utilization test did not reveal any significant differences between the manuring treatments and therefore were in line with soil nitrogen parameters (table 3.20). Total C contents of the differently fertilized soils varied between 9.15 - 11.56 mg C_t g^{-1} dw soil. Other organically managed arable soils exhibited comparable carbon contents in the range of 11.2 - 16.9 mg C_t g^{-1} soil (Fließbach and Mäder 2000; Tu *et al.* 2006a). Likewise, the range of water-extractable carbon in the field study with 39.0 - 96.9 µg C g^{-1} dw soil was in line with published sizes of water-extractable carbon concentrations (Tu *et al.* 2006b). The contents of soil microbial biomass carbon (MBC) in all manuring treatments (558 - 1353 µg MBC g^{-1} dw soil) seemed to be higher than in other organically cultivated agricultural soils which are often in the range between 149 - 451 µg MBC g^{-1} soil (Fließbach and Mäder 1997; 2000; Friedel and Gabel 2001; Böhme and Böhme 2006; Tu *et al.* 2006a; Tu *et al.* 2006b). Increases of the soil microbial biomass often occur following increased amounts of organic inputs (Ocio *et al.* 1991; Wyland *et al.* 1996; Gunapala and Scow 1998), and higher stable amounts of microbial biomass may accumulate after several years of increased organic inputs (Schnürer *et al.* 1985; Collins *et al.* 1992; Gunapala and Scow 1998). Consequently, the elevated amounts of MBC might have resulted from the organic fertilizer application three weeks before sampling. Sarathchandra *et al.* (2006) assessed 32 days after the first application of dairy factory high carbon effluent (corresponding to four days after the last application) to a pasture soil values of up to 1150 µg MBC g^{-1} soil that decreased with time but remained higher than in the untreated soil. A further explanation for the high soil microbial biomass content in the present field study might be a generally elevated biomass content of the arable soil caused by organic cultivation since 1989 which is supported by mean MBC values (1450 - 1600 µg MBC g^{-1} dw soil) in soil samples derived from another field study at the same site (Stefan Ratering, Giessen, personal communication).
The carbon substrate utilization test was performed to achieve rapidly an overview about differences in the potential metabolic diversity in the differently fertilized soils. However, the

utilization test did not yield a formation of distinct clusters of particular manuring treatments (figure 3.32) indicating similar community-level catabolic profiles. Prior to cluster analyses, differences within a treatment between the four replicates became apparent possibly due to high heterogeneity within a manuring treatment. Therefore, the lack of clustering was not surprising. Using the sole-carbon-source utilization approach, distinctive patterns among microbial habitats, different soil types, and spatial gradients within soil and estuarine sites can be observed (Garland and Mills 1991). Moreover, various land uses cause differences in catabolic capability of soil microbial communities (Graham and Haynes 2005). Different compost additions to two soils in laboratory scale experiments modified the community-level physiological profiles after ten days compared to the untreated soils (Pérez-Piqueres *et al.* 2006). Repeated applications (four applications in four weeks) of dairy factory high carbon effluent to a pasture soil in a pot experiment led to significant differences in microbial functional diversity patterns and activities 32 days after the first (four days after the last) application (Sarathchandra *et al.* 2006). However, the effects were not detectable anymore after 130 (102) days. Fließbach and Mäder (1997) exhibited in carbon substrate utilization profiles of soil samples derived from a long-term field trial an often higher variation along with field replicates of the same treatments than between different treatments. Furthermore, the utilization patterns showed distinct overlapping and not distinct grouping which was explained by the variation within the field replicates (soil inhomogeneities).

In summary, the use of substrate utilization assays to investigate community-level physiologic profiles seems to be appropriate to discriminate microbial communities derived from different habitats, different soil types, and spatial gradients within habitats. Differences of soil community-level catabolic profiles in homogenized soil samples and elevated fertilizer application rates could be revealed in the first few hours and days after N application. However, the differentiation of microbial communities at field scale with high spatial and temporal heterogeneity in the soil profile appear to be more difficult to analyze. Therefore, the sampling in the field three weeks after manuring and 3.5 years after performance of the different manuring treatments was possibly not ideal to identify differences in clustering. Community-level physiologic profiles focus on substrate utilization patterns of the cultivable fraction of soil communities that grows on various C sources. Smalla *et al*. (1998) demonstrated that the functional potential of the numerically dominant members of the microbial community was not necessarily reflected by carbon-source utilization profiles. Fast-growing bacteria adapted to the high substrate concentrations dominated the wells and may have been responsible for the patterns. Furthermore, the limited number of samples used for the determination of the potential metabolic diversity may not have been adequate to reflect real differences in substrate utilization of soil microorganisms (Monokrousos *et al.* 2006).

The determination of soil respiration and substrate (glucose) induced respiration (SIR) partly exhibited little differences between the treatments (table 3.20). Soil respiration rates of the

differently fertilized soils determined during 66 hours of incubation varied between 51.0 - 89.2 nmol CO_2 g^{-1} soil h^{-1} CO_2 evolution and 54.8 - 92.3 nmol O_2 g^{-1} soil h^{-1} O_2 consumption, respectively. Thus, the observed respiration rates were similar within the range reported from organically managed arable soils (5 - 191 nmol CO_2 g^{-1} h^{-1}) (Fließbach *et al.* 2000; Pérez-Piqueres *et al.* 2006; Tu *et al.* 2006a; van Diepeningen *et al.* 2006). The substrate (glucose) induced soil respiration rates varied between 772 - 2309 nmol CO_2 g^{-1} h^{-1} CO_2 evolution and 231 - 483 nmol O_2 g^{-1} h^{-1} O_2 consumption, respectively. Various compost amended soils revealed SIR rates from 187 - 872 nmol CO_2 g^{-1} h^{-1} (Pérez-Piqueres *et al.* 2006; Saison *et al.* 2006). Consequently, the determined SIR rates are in line with data found in the literature, maybe with the exception of some elevated CO_2 values in the cropping system with livestock.

The OxiTop methodology can only provide a rough survey of respiration activities and is not an appropriate method to determine soil O_2 consumption and CO_2 production rates accurately. The differences between O_2 consumption and CO_2 production rate analyzing the substrate-induced respiration indicate the shortcoming of the indirect determination of the oxygen concentration via pressure decrease. The pressure decrease due to respiration could have been masked by the production of gaseous products derived from anaerobic respiration processes in anoxic microsites, e.g. denitrification, consequently underestimating the O_2 consumption rate. Denitrification could also have contributed in low amounts to elevated CO_2 concentrations without O_2 consumption. Another methodical shortcoming is the quantification of the produced CO_2 by end point titration, so that the amount of CO_2 had to be converted into a CO_2 production rate assuming a linear production during the incubation period.

To sum up, the results of the diverse investigations related to the soil nitrogen and carbon metabolism showed that the different organic manuring treatments performed during the last 3.5 years did not affect measurably the nitrogen or carbon metabolism. The soil N_t and C_t contents prior to and after three years of the differentiated fertilization regimes did not differ significantly, too (tables 3.5 and 3.14). The differences between the eight organic manuring treatments performed in this field study are presumably smaller than differences between e.g. organic and inorganic fertilization regimes with and without cultivation of intercrops and green manures. Consequently, the lack of significances between the treatments of the various investigation parameters is not surprising taking the soil heterogeneity across the area and in the soil depth profile into account. Moreover, the time period processing the different manuring treatments was possibly not long enough so that the manuring management caused a measurable change. The molecular biological methods revealed some differences among the manuring treatments. However, even more than for the remaining

microbiological investigations, the crucial question is how much soil is needed to adequately address the heterogeneity at the field scale. Moreover, and as mentioned at the beginning of the section, it cannot be excluded to obtain different results at another sampling time point during the season or after several years of different manuring.

5 Summary

The aim of the present study was to quantify *in-situ* N_2O and CH_4 fluxes in organic agriculture. Trace gas fluxes were observed in winter wheat and spring wheat within an organic cropping system without livestock as well as in spelt and potatoes within an organic cropping system with livestock. Thereby, the impact of different manuring treatments based on the fermentation of cattle slurry and/or plant biomass in biogas reactors was investigated in comparison to the respective, common manuring practices. In the cropping system without livestock, green manures, intercrops, and crop residues were anaerobically fermented and used for manuring in contrast to the common mulching practice. In the cropping system with livestock, cattle slurry or cattle slurry and intercrops and crop residues were fermented and then applied as fertilizer in comparison to the common slurry and farmyard manure practice. The same crops and manuring treatments were investigated during three years (2002/2003 - 2004/2005) due to the high temporal variability of N_2O production and CH_4 oxidation which is partly caused by different precipitation patterns and temperature dynamics each year.

The total annual N_2O losses amounted to 490 - 4087 g N ha^{-1} in winter wheat and spring wheat of the cropping system without livestock and varied with crop types, manuring treatments, and year. In the cropping system with livestock (i.e. in spelt and potatoes), the annual N_2O emissions ranged between 456 and 6465 g N ha^{-1}. The integrated, annual soil CH_4 uptakes amounted to 476 - 855 g C ha^{-1} in the system without livestock and to 128 - 799 g C ha^{-1} in the system with livestock. Consequently, the N_2O and CH_4 fluxes observed in organic agriculture were not different from those measured in conventional farming systems.

In the cropping system without livestock, N_2O emissions in the biogas treatment with the fermented crop material were 44%, 39%, and 25% lower than in the mulching control treatment during the three observation periods. Likewise, in the cropping system with livestock, reduced N_2O emissions of 60%, 45%, and 40%, respectively, were observed in the biogas treatment with fermented slurry and fermented crop material compared to the raw slurry control treatment. Thus, harvest of pea straw (preceding crop) or rather harvest of intercrop biomass in fall seemed to mitigate the N_2O losses. However, these observations were only made for the winter cereals, when the incorporation of intercrops or intercrop stubbles was already processed in October, but not for spring wheat and potatoes. The proportions of the total annual N_2O losses during the winter period (December 1 - March 15) accounted for 11% to 47%, hence are comparable to those reported in the literature. The winter losses were dependent on the cultivated crop type, the manuring treatment, and the year.

Comparing the annual soil CH_4 uptakes in the cropping system without livestock during the three-year investigation period, overall lower soil CH_4 oxidation activities were observed in the biogas treatment (56 - 90%) than in the mulching treatment. In the cropping system with

livestock, no trend of decreased (up to 19%) or increased (up to 129%) CH_4 uptake was found.

Peak N_2O emissions of 358 and 436 µg N m^{-2} h^{-1} were observed in the cropping system without and with livestock, respectively. The CH_4 oxidation rates ranged between 0 and 42 µg C m^{-2} h^{-1} in the system without livestock, and between 0 and 24 µg C m^{-2} h^{-1} in the system with livestock. Elevated N_2O emission rates as well as occasional CH_4 emissions were related to fertilizer applications, freeze-thaw-periods, heavy rainfall, or incorporation of organic material. CH_4 emissions after manuring were caused by degassing of dissolved CH_4 and had declined after 24 h.

Incubation experiments were carried out to assess the N_2O and CH_4 evolution from the fertilizers themselves, i.e. from raw slurry, fermented slurry, and fermented crop material under optimum conditions for denitrification. However, no N_2O production was determined in any of the manures. CH_4 evolutions were relatively high from the fermented slurry (1262 nmol g^{-1} fertilizer) but lower than those from the non-fermented raw slurry (2400 nmol g^{-1} fertilizer).

As part of the laboratory study, a three-week fertilization experiment was conducted in the greenhouse with spring wheat to investigate in more detail the impact of the different organic manures (raw slurry, fermented slurry, and fermented crop material) on the N_2O emissions and the soil denitrifiers. The focus was on denitrifying bacteria because they are to a great extent responsible for N_2O productions in soils. Within the first three days after application of the different organic fertilizers, considerably increased N_2O emissions were observed. Moreover, the PCR-SSCP (Single Strand Conformation Polymorphism) fingerprint method targeting the *nirS* nitrite reductase gene of denitrifiers exhibited different community compositions of the dominant and PCR-amplifiable denitrifying bacteria in the different manuring treatments during the first week. Mostly higher amounts of nitrite reductase gene fragments (*nirK* and *nirS*) were observed by real-time PCR in the differently fertilized soils than in the unfertilized control soil at the different sampling dates. This points to a stimulation of the imported denitrifiers by manures and/or to growth of the soil autochthonous denitrifying bacteria. Generally, *nirK* and *nirS* gene copy numbers ranged between 2.8×10^6 - 4.3×10^7 g^{-1} dw soil. Furthermore, a clone library of *nirS* gene fragments derived from the pure fertilizers and the arable soil was generated. Sequence analysis exhibited a relatively high diversity of the clones and only seldom high sequence identities to known denitrifying strains. The clone sequences often grouped in the dendrogram according to their origins possibly indicating special communitiy compositions in the different organic manures and the arable soil, hence supporting the different *nirS* gene fragment patterns via SSCP.

After 3.5 years of different manuring regimes, field soil samples were compared with respect to the microbial nitrogen and carbon metabolism. The investigations exhibited distinct

differences only of some N and C parameters between the manuring treatments, which partly relied on the timing of fertilizer application. For instance, significantly lower concentrations of NO_3^-, NO_2^-, and NH_4^+ were observed in the previously not amended mulching treatment (cropping system without livestock) and the farmyard manure treatment (cropping system with livestock). SSCP fingerprint patterns of *nirS* gene fragments showed in part different community compositions of dominant denitrifying bacteria with partly striking single DNA bands in the differently fertilized soils. Real-time PCR quantification of denitrifiers targeting the *nirS* and *nirK* nitrite reductase genes resulted in all manuring treatments in approximately similar gene fragment copy numbers (*nirS*: 4.2 x 10^5 - 2.3 x 10^6 g^{-1} dw soil; *nirK*: 3.2 - 8.4 x 10^6 g^{-1} dw soil). Soil potential denitrification and nitrification activities as well as total N contents did not differ significantly between the manuring treatments. Moreover, investigations of soil respiration, microbial biomass C, water-extractable C, total C content, and the BIOLOG carbon-source utilization assay were similar in all treatments. In summary, the modified fertilization management performed during 3.5 years had no measurabe influence on the investigated parameters related to the soil N and C metabolism.

6 Zusammenfassung

Im Rahmen der vorliegenden Arbeit sollten N_2O- und CH_4-Flüsse in einem Winter- und Sommerweizenbestand eines viehlosen ökologischen Betriebssystems und in einem Dinkel- und Kartoffelbestand eines viehhaltenden ökologischen Betriebssystems *in-situ* quantifiziert werden. Dabei wurden die Einflüsse von Düngevarianten basierend auf der Vergärung von Rindergülle und/oder pflanzlicher Biomasse in Biogasanlagen auf die Spurengasflüsse vergleichend zu den sonst gängigen Düngestrategien untersucht. Im viehlosen Betriebssystem wurden im Vergleich zur üblichen Mulchwirtschaft die Schnitte der Gründüngungsrotation, die Zwischenfruchtaufwüchse sowie die Erntereste fermentiert und zur Düngung genutzt. Im viehhaltenden Betriebssystem wurde Rindergülle bzw. Rindergülle, Zwischenfruchtaufwüchse, Ernte- und Futterreste in Biogasanlagen vergoren und als Dünger appliziert und mit der üblichen Gülle- und Stallmistwirtschaft verglichen. Da bei Feldstudien die Niederschlagsverteilung und -menge im Zusammenhang mit dem Temperaturverlauf einen entscheidenden Einfluss auf die N_2O-Produktion und CH_4-Oxidation des Bodens ausüben, wurden über drei Jahre (2002/2003 - 2004/2005) die gleichen Bestände und Düngesysteme beprobt, um die Effekte unterschiedlicher Jahre besser einschätzen zu können.

Die jährlichen N_2O-Verluste beliefen sich auf 490 bis 4087 g N ha^{-1} in den Sommer- und Winterweizenbeständen des viehlosen Betriebssystems in Abhängigkeit von Düngesystem, Pflanzenbestand und Jahr. Im viehhaltenden Betriebssystem variierten die jährlichen N_2O-Emissionen zwischen 456 und 6465 g N ha^{-1} in den Dinkel- und Kartoffelbeständen. Die jährlichen CH_4-Aufnahmen bewegten sich im viehlosen Betriebssystem zwischen 476 und 855 g C ha^{-1} und im viehhaltenden Betriebssystem zwischen 128 und 799 g C ha^{-1}. Damit unterschieden sich die im ökologischen Landbau gemessenen N_2O- und CH_4-Flüsse nicht in ihrer Höhe von denen in konventioneller Landwirtschaft.

Der Vergleich der Düngevarianten zeigte im Winterweizenbestand (viehloses Betriebssystem) während der drei Jahre 44%, 39% bzw. 25% geringere N_2O-Verluste aus der Variante mit vergorenem Pflanzenmaterial als aus der Kontrollvariante. Ebenso wurden im viehhaltenden Betriebssystem um 60%, 45% bzw. 40% geringere N_2O-Emissionen im Dinkelbestand der Düngevarianten mit vergorener Gülle und vergorenem Pflanzenmaterial als in der Rohgüllevariante festgestellt. Damit scheint die Ernte des Erbsenstrohs (Vorfrucht) bzw. eher noch die Ernte des Zwischenfruchtaufwuchses im Herbst die N_2O-Verluste zu reduzieren. Allerdings wurden diese Beobachtungen nur für die Wintergetreidebestände, bei denen das Einarbeiten der Zwischenfrucht bzw. der Zwischenfruchtstoppeln bereits im Oktober stattfand, nicht aber für den Sommerweizen- und Kartoffelbestand gemacht. Die Anteile der N_2O-Winteremissionen an den Jahresemissionen lagen mit 11% bis 47% je nach Pflanzenbestand, Jahr und Düngevariante in Bereichen, die aus der Literatur bekannt sind.

Im Vergleich der jährlichen CH_4-Oxidationsleistungen des Bodens innerhalb des viehlosen Betriebssystems waren meist geringere CH_4-Aufnahmen in der Düngevariante mit

vergorenem Pflanzenmaterial (56% - 90%) als in der Mulchwirtschaft zu beobachten. Im viehhaltenden Betriebssystem dagegen wurde kein Trend reduzierter (bis 19%) oder erhöhter (bis 129%) CH_4-Aufnahmen festgestellt.
Im viehlosen und im viehhaltenden Betriebssystem wurden N_2O-Emissionsraten bis 358 bzw. 436 µg N m^{-2} h^{-1} gemessen. Die CH_4-Oxidationsraten rangierten zwischen 0 und 42 µg C m^{-2} h^{-1} im viehlosen und zwischen 0 und 24 µg C m^{-2} h^{-1} im viehhaltenden Betriebssystem. Einzelne, erhöhte N_2O-Flussraten sowie gelegentliche CH_4-Emissionen konnten häufig besonderen Ereignissen wie Düngerapplikation, Frost-Tau-Perioden, starkem Niederschlag und Einarbeitung von organischem Material zugeordnet werden. Die Methanemissionen nach Düngerapplikation beruhten auf der Ausgasung von im Dünger gelöstem Methan und waren nach 24 h abgeklungen.

Die Feldmessungen ergänzend wurde in einem Inkubationsexperiment unter optimalen Denitrifikationsbedingungen die N_2O- und CH_4-Entwicklung aus den reinen Düngern Rohgülle, vergorene Gülle und vergorenes Pflanzenmaterial gemessen. Dabei wurde nachgewiesen, dass in den Düngern selbst kein N_2O gebildet wurde. Bei Betrachtung der CH_4-Entwicklung wurde festgestellt, dass aus der vergorenen Gülle noch relativ viel Methan freigesetzt (1262 nmol g^{-1} Dünger) wurde, wenn auch in geringerem Maße als aus der Rohgülle (2400 nmol g^{-1} Dünger).

Im Gewächshaus wurde ein dreiwöchiges Düngungsexperiment mit Sommerweizen durchgeführt, um unter gleichen und kontrollierten Bedingungen die Wirkungen von Rohgülle, vergorener Gülle und vergorenem Pflanzenmaterial auf die bodenbürtigen N_2O-Emissionen sowie auf die denitrifizierenden Bakterien detaillierter zu untersuchen. Die Gruppe der Denitrifizierer wurde genauer betrachtet, da sie einen großen Teil der bodenbürtigen N_2O-Emissionen verursacht. Alle drei organischen Dünger stimulierten signifikant die N_2O-Emissionen bis drei Tage nach Applikation. Außerdem war eine veränderte Zusammensetzung der dominanten und PCR-amplifizierbaren Denitrifizierer, die über Genfragmente der *nirS*-Nitritreduktase auf DNA-Ebene mittels SSCP (Single Strand Conformation Polymorphism)-Fingerprintmethode abgebildet wurde, während der ersten sieben Tage zu beobachten. Die molekulare Quantifizierung der denitrifizierenden Bakterien durch *real-time* PCR zu unterschiedlichen Beprobungsterminen zeigte meist eine höhere Anzahl an Nitritreduktasen-Genfragmenten (*nirS* und *nirK*) der gedüngten Böden im Vergleich zum ungedüngten Boden. Dies könnte auf eine Populationszunahme der durch die Dünger eingetragenen Denitrifizierer hinweisen, jedoch ist auch eine Vermehrung der bodenbürtigen Denitrifizierer durch die Düngung nicht auszuschließen. Die Anzahl der *nirS*- und *nirK*-Genkopien variierte in allen Varianten über die Zeit zwischen 2.8 x 10^6 - 4.3 x 10^7 g^{-1} TB. Des weiteren wurde eine Klonbibliothek aus *nirS*-Genfragmenten aus den verschiedenen Düngern sowie aus dem ungedüngten Ackerboden erstellt. Die Sequenzanalyse zeigte eine relativ große

Klonsequenzdiversität und nur selten hohe Sequenzähnlichkeiten zu bekannten, denitrifizierenden Stämmen. Die Sequenzen fielen im Stammbaum häufig in gemeinsame Gruppen entsprechend ihrer Herkunft, was auf unterschiedliche Zusammensetzungen der Denitrifizierergemeinschaften in den verschiedenen organischen Düngern und dem Ackerboden hindeuten könnte und die unterschiedlichen DNA-Bandenmuster der dominanten *nirS*-Denitrifizierer mittels SSCP unterstützt.

Nach 3,5 Jahren differenzierten Düngemanagements wurden verschiedene biologische und chemische Parameter, die in Beziehung zum mikrobiellen Stickstoff- und Kohlenstoff-metabolismus stehen, in Feldbodenproben aus allen Düngesystemen analysiert. Die Untersuchungen ergaben nur in wenigen N- und C-Parametern signifikante Unterschiede zwischen den Düngesystemen, die meist auf die drei Wochen vorher stattgefundene Düngung zurückgeführt wurden. Beispielsweise wurden signifikant geringere Nitrat-, Nitrit- und Ammoniumkonzentrationen in der zuvor ungedüngten Mulchwirtschaft-Variante im viehlosen und der Stallmist-Variante im viehhaltenden Betriebssystem gefunden. Die Zusammensetzung der dominanten *nirS*-Denitrifizierer ermittelt über SSCP differierte teilweise zwischen den verschiedenen Düngevarianten und wies zum Teil distinkte DNA-Banden auf. Die Quantifizierung der Denitrifizierer über die *nirS*- und *nirK*-Genfragmente mittels *real-time* PCR resultierte in allen Düngesystemen in Targetzahlen ähnlicher Größenordnung (*nirS*: 4.2 x 10^5 - 2.3 x 10^6 g^{-1} TB; *nirK*: 3.2 - 8.4 x 10^6 g^{-1} TB). Keine signifikanten Unterschiede wurden in der potenziellen Denitrifikations- und Nitrifikationsaktivität sowie im N_t-Gehalt der unterschiedlich gedüngten Böden festgestellt. Ebenso ergaben die Bodenuntersuchungen auf Bodenatmungsaktivität, mikrobiellen Biomasse-Kohlenstoff-, wasserlöslichen Kohlenstoff-, Gesamt-Kohlenstoffgehalt sowie der BIOLOG C-Quellen-Verwertungstest keine Signifikanzen zwischen den Düngesystemen. Damit lässt sich resümieren, dass die untersuchten Parameter des N- und C-Metabolismus durch die unterschiedlichen Düngestrategien während der letzten 3,5 Jahre scheinbar nicht messbar beeinflusst wurden.

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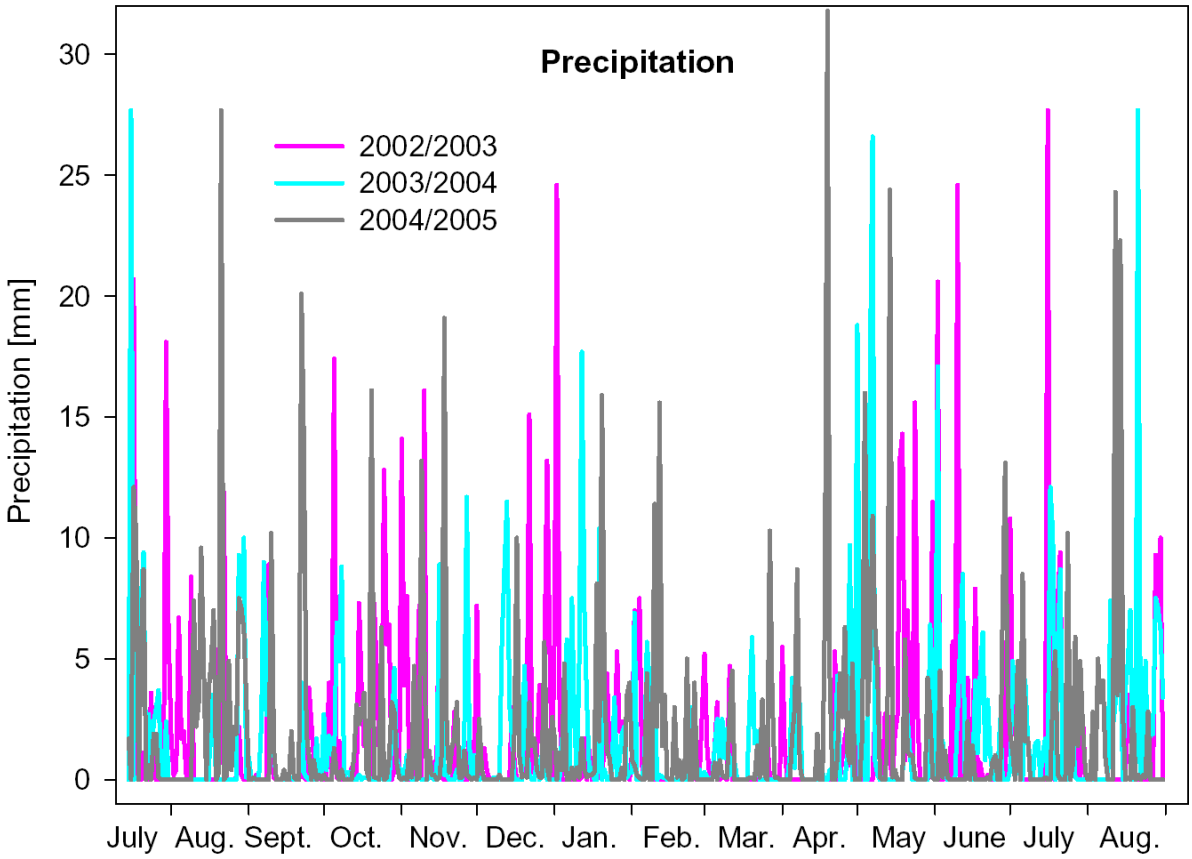
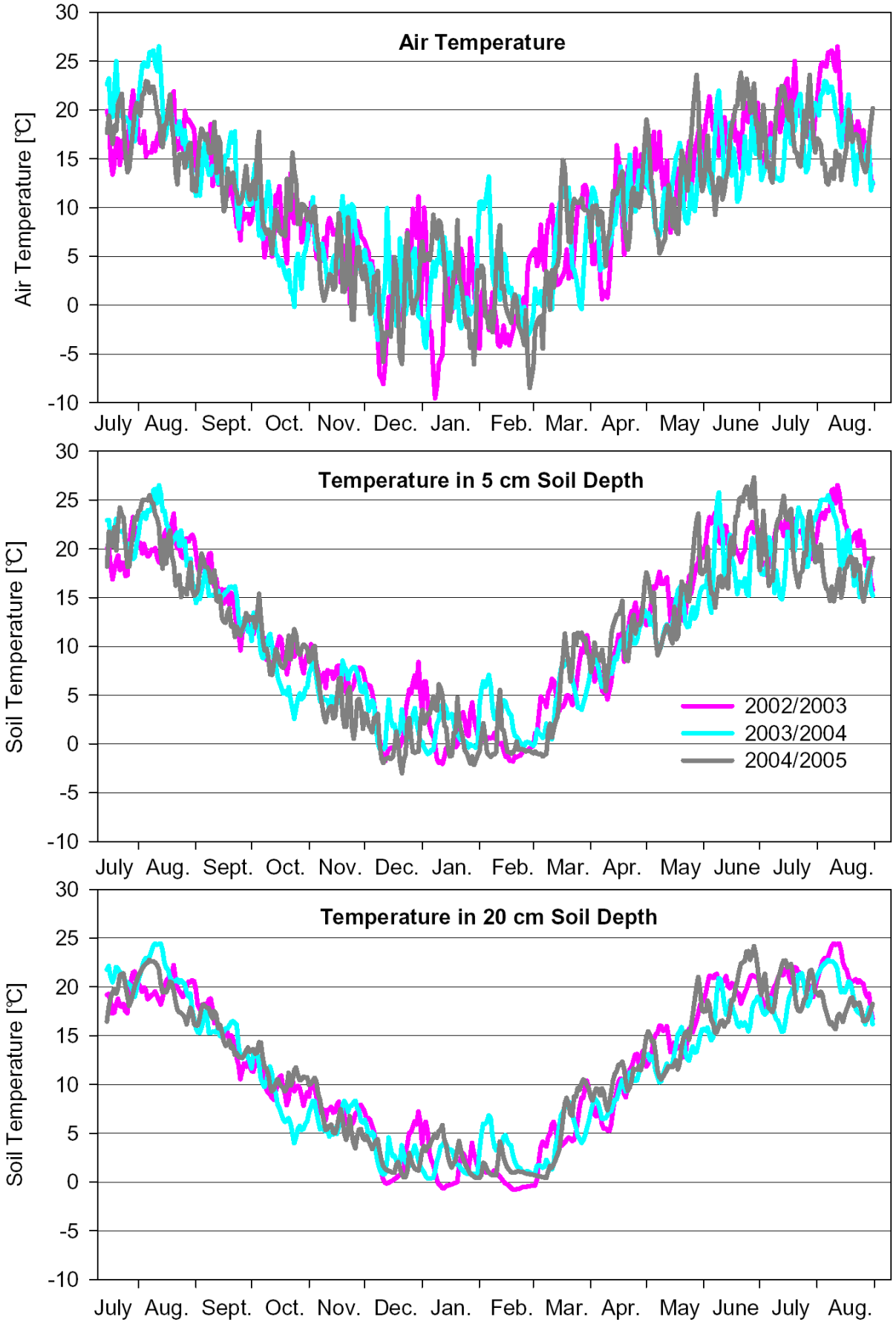
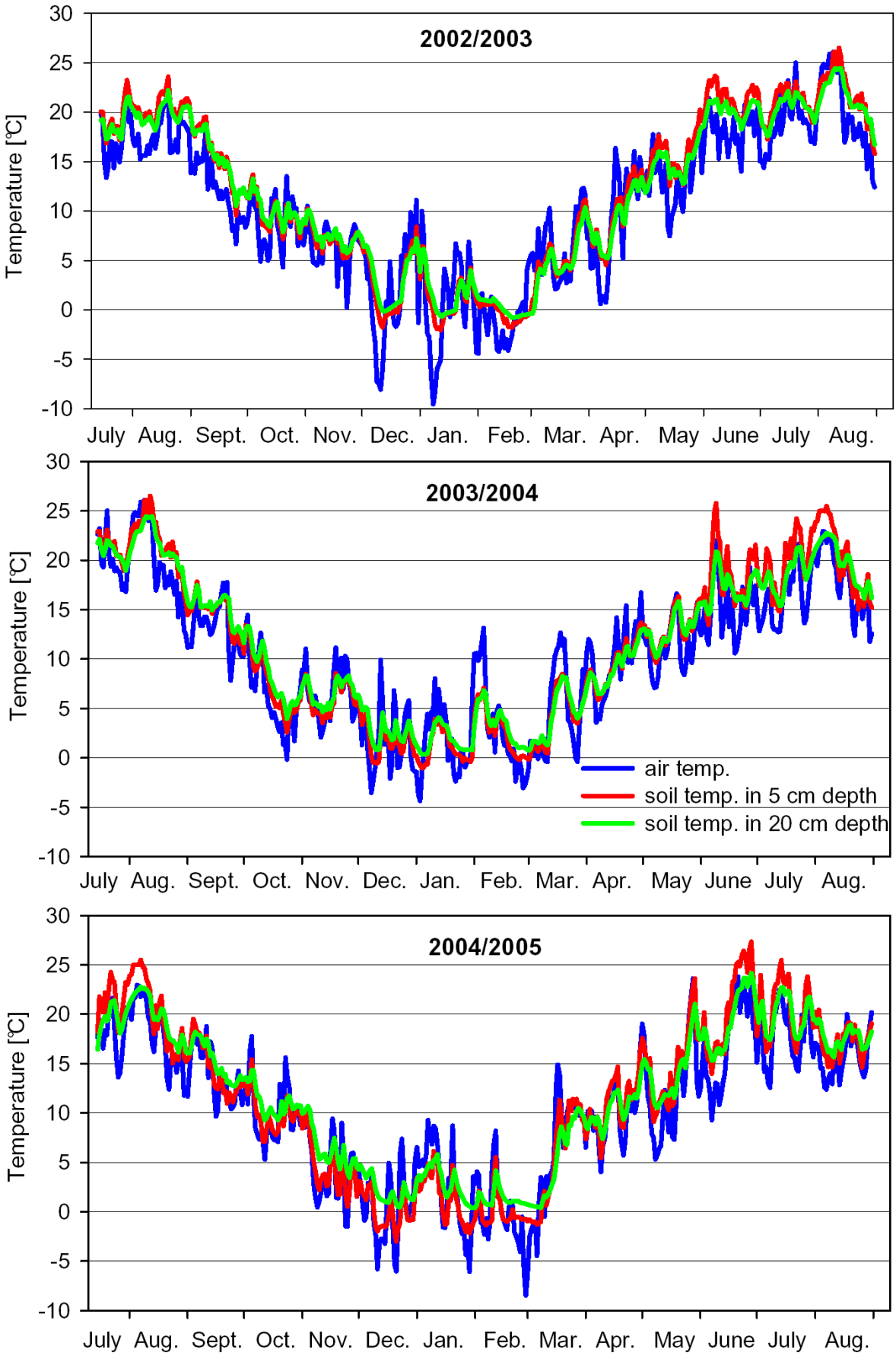
Appendix



A.1: Daily precipitation during the seasons 2002/2003, 2003/2004, and 2004/2005. (Data: Franz Schulz, Villmar, personal communication)



A.2: Daily temperature data during the seasons 2002/2003, 2003/2004, and 2004/2005 illustrated for air, soil in 5 cm, and soil in 20 cm depth, respectively. (Data: Franz Schulz, Villmar, personal communication)



A.3: Data of daily air temperatures and temperatures in 5 cm and 20 cm soil depth during the seasons 2002/2003, 2003/2004, and 2004/2005. (Data: Franz Schulz, Villmar, personal communication)

A.4: Amounts and characteristics of the applied fertilizers in both manuring treatments in winter wheat 5 and prior intercrops in the cropping system without livestock between 2002 and 2005.

***WINTER WHEAT 5* – Cropping System without Livestock**								
Season	**Date**	**Manuring Treat-ment**	**Manure**	**N_t [kg ha^{-1}]**	**NH_4^+-N [kg ha^{-1}]**	**Org. Dry Matter [kg ha^{-1}]**	**C_t [kg ha^{-1}]**	**C/N Ratio**
2002/2003	2. Aug.	w/o L-M	Pea-Straw$^{\Delta}$	20	n.d.	2374	1169	58
		w/o L-FC		6	n.d.	523	260	46
	9. Oct.	w/o L-M	Intercrops$^{\theta}$	118	n.d.	2820	1560	13
		w/o L-FC		15	n.d.	763	423	28
	19. Feb.	w/o L-FC	Liquid‡	91	70	414	270	3
	13. Mar.			31	19	148	96	3
	8. May			19	14	112	70	4
	Sum	*w/o L-M*		*138*	*n.c.*	*5194*	*2729*	*(20)*
		w/o L-FC		*162*	*n.c.*	*1960*	*1119*	*(7)*
2003/2004	19. July	w/o L-M	Pea-Straw$^{\Delta}$	41	n.d.	3259	1564	38
		w/o L-FC		8	n.d.	613	301	37
	13. Oct.	w/o L-M	Intercrops$^{\theta}$	74	n.d.	2549	1317	18
		w/o L-FC		10	n.d.	640	326	34
	27. Feb.	w/o L-FC	Liquid‡	75	52	556	295	4
	25. Mar.			50	34	354	245	5
	7. Apr.			1	1	9	4	3
	Sum	*w/o L-M*		*115*	*n.c.*	*5808*	*2882*	*(25)*
		w/o L-FC		*144*	*n.c.*	*2172*	*1171*	*(8)*
2004/2005	6. Aug.	w/o L-M	Pea-Straw$^{\Delta}$	53	n.d.	5132	2534	48
		w/o L-FC		10	n.d.	914	453	46
	5. Oct.	w/o L-M	Intercrops$^{\theta}$	116	n.d.	2341	1236	11
		w/o L-FC		18	n.d.	690	369	21
		w/o L-FC	Solid*	95	6	3085	1632	18
	31. Jan.	w/o L-FC	Liquid‡	120	86	661	428	4
	22. Feb.			62	37	474	303	5
	Sum	*w/o L-M*		*169*	*n.c.*	*7474*	*3770*	*(22)*
		w/o L-FC		*305*	*n.c.*	*2739*	*3186*	*(10)*

Pea-Straw$^{\Delta}$: incoporation of complete pea-straw in w/o L-M, and straw stubbles in w/o L-FC
Intercrops$^{\theta}$: green manuring through incorporating of intercrops into the soil; whole plants in w/o L-M, and plant stubbles in w/o L-FC
Liquid‡: liquid of crop fermentation in w/o L-FC
Solid*: solid fermented crop residues in w/o L-FC
n.d.: not determined
n.c.: not calculable

A.5: Amounts and characteristics of the applied fertilizers in both manuring treatments in spring wheat and prior intercrops in the cropping system without livestock between 2002 and 2005.

***SPRING WHEAT* – Cropping System without Livestock**								
Season	**Date**	**Manuring Treatment**	**Manure**	**N_t [kg ha^{-1}]**	**NH_4^+-N [kg ha^{-1}]**	**Org. Dry Matter [kg ha^{-1}]**	**C_t [kg ha^{-1}]**	**C/N Ratio**
2002/2003	14. Aug.	w/o L-M	Wheat-Straw$^{\int}$	21	n.d.	5403	2597	122
		w/o L-FC		5	n.d.	843	402	87
	6. Jan.	w/o L-M	Intercrops$^{\theta}$	120	n.d.	2247	1139	9
		w/o L-FC		17	n.d.	802	402	23
		w/o L-FC	Solid*	111	19	2551	1306	12
	27. May	w/o L-FC	Liquid‡	10	7	57	36	4
	Sum	*w/o L-M*		*142*	*n.c.*	*7651*	*3736*	*(26)*
		w/o L-FC		*143*	*n.c.*	*4253*	*2147*	*(15)*
2003/2004	10. Aug.	w/o L-M	Wheat-Straw$^{\int}$	38	n.d.	4830	2371	62
		w/o L-FC		7	n.d.	1146	564	81
	19. Feb.	w/o L-M	Intercrops$^{\theta}$	78	n.d.	2885	1567	20
		w/o L-FC		10	n.d.	766	415	43
		w/o L-FC	Solid*	115	9	3093	1651	15
	Sum	*w/o L-M*		*115*	*n.c.*	*7715*	*3939*	*(34)*
		w/o L-FC		*132*	*n.c.*	*5005*	*2631*	*(20)*
2004/2005	4. Aug.	w/o L-M	Wheat-Straw$^{\int}$	51	n.d.	7693	3713	72
		w/o L-FC		8	n.d.	1890	920	120
		w/o L-FC	Solid*	40	2	2070	1067	26
	7. Feb.	w/o L-M	Intercrops$^{\theta}$	113	n.d.	2604	1377	12
		w/o L-FC		18	n.d.	873	461	26
		w/o L-FC	Solid*	82	18	1674	893	11
			Liquid‡	35	26	232	158	5
	18. Apr.	w/o L-FC	Liquid‡	120	80	699	453	4
	Sum	*w/o L-M*		*164*	*n.c.*	*10297*	*5090*	*(31)*
		w/o L-FC		*302*	*n.c.*	*7437*	*3953*	*(13)*

Wheat-Straw$^{\int}$: incorporation of complete winter wheat-straw in w/o L-M, and straw stubbles in w/o L-FC
Intercrops$^{\theta}$: green manuring through incorporating of intercrops into the soil; whole plants in w/o L-M, and plant stubbles in w/o L-FC
Liquid‡: liquid of crop fermentation in w/o L-FC
Solid*: solid fermented crop residues in w/o L-FC
n.d.: not determined
n.c.: not calculable

A.6: Amounts and characteristics of the applied fertilizers in all manuring treatments in spelt and prior intercrops in the cropping system with livestock between 2002 and 2005.

***SPELT* – Cropping System with Livestock**								
Season	**Date**	**Manuring Treatment**	**Manure**	**N_t [kg ha^{-1}]**	**NH_4^+-N [kg ha^{-1}]**	**Org. Dry Matter [kg ha^{-1}]**	**C_t [kg ha^{-1}]**	**C/N Ratio**
2002/2003	14. Aug.	wL-FS	Pea-Straw$^{\theta}$	25	n.d.	2117	1057	42
		wL-FS+FC		6	n.d.	549	274	44
		wL-FYM		25	n.d.	2218	1111	45
		wL-RS		26	n.d.	2088	1044	40
	9. Oct.	wL-FS	Intercrops$^{\nabla}$	110	n.d.	2278	1223	11
		wL-FS+FC		16	n.d.	686	367	23
		wL-FYM		119	n.d.	2479	1326	11
		wL-RS		115	n.d.	2364	1283	11
		wL-FYM	Solid$^{\surd}$	71	8	2288	986	14
	20. Feb.	wL-FS	Liquid$^{\#}$	68	33	1533	819	12
		wL-FS+FC		68	33	1533	819	12
		wL-RS		49	24	776	432	9
	2. Apr.	wL-FS	Liquid$^{\#}$	52	26	821	428	8
		wL-FS+FC		72	40	841	474	7
		wL-RS		81	38	1578	845	10
	Sum	*wL-FS*		*256*	*n.c.*	*6750*	*3527*	*(14)*
		wL-FS+FC		*162*	*n.c.*	*3609*	*1935*	*(12)*
		wL-FYM		*215*	*n.c.*	*6985*	*3423*	*(16)*
		wL-RS		*271*	*n.c.*	*6807*	*3604*	*(13)*
2003/2004	27. July	wL-FS	Pea-Straw$^{\theta}$	54	n.d.	3923	2088	36
		wL-FS+FC		11	n.d.	840	109	37
		wL-FYM		50	n.d.	3663	1869	36
		wL-RS		53	n.d.	3964	2015	37
	13. Oct.	wL-FS	Intercrops$^{\nabla}$	83	n.d.	2417	1086	14
		wL-FS+FC		12	n.d.	722	323	28
		wL-FYM		80	n.d.	2169	1032	13
		wL-RS		79	n.d.	2171	1019	13
		wL-FS+FC	Solid$^{\surd}$	48	1	1508	817	17
		wL-FYM		103	9	2285	1244	12
	5. Mar.	wL-FS	Liquid$^{\#}$	132	62	1986	1083	8
		wL-FS+FC		130	68	1601	899	7
		wL-RS		132	63	2376	1313	10
	25. Mar.	wL-FS+FC	Liquid$^{\#}$	37	26	344	233	6
	Sum	*wL-FS*		*269*	*n.c.*	*8326*	*4153*	*(15)*
		wL-FS+FC		*238*	*n.c.*	*5016*	*2695*	*(11)*
		wL-FYM		*233*	*n.c.*	*8117*	*4171*	*(18)*
		wL-RS		*264*	*n.c.*	*8511*	*4253*	*(16)*

A.6 continued

Season	Date	Manuring Treatment	Manure	N_t [kg ha^{-1}]	NH_4^+-N [kg ha^{-1}]	Org. Dry Matter [kg ha^{-1}]	C_t [kg ha^{-1}]	C/N Ratio
2004/2005	8. Aug.	wL-FS	Pea-Straw$^{\theta}$	47	n.d.	4401	2148	46
		wL-FS+FC		12	n.d.	1083	530	43
		wL-FYM		58	n.d.	5004	25848	45
		wL-RS		46	n.d.	4374	2163	47
	9. Aug.	wL-FS+FC	Liquid$^{\#}$	26	14	320	180	7
	11. Oct.	wL-FS	Intercrops$^{\nabla}$	137	n.d.	2773	1402	10
		wL-FS+FC		21	n.d.	882	449	22
		wL-FYM		154	n.d.	2771	1514	10
		wL-RS		141	n.d.	2830	1430	10
		wL-FS+FC	Solid$^{\surd}$	66	8	1725	922	13
		wL-FYM		110	18	2999	1557	14
	23. Feb.	wL-FS	Liquid$^{\#}$	100	54	1121	642	7
		wL-FS+FC		162	101	1777	1027	6
		wL-RS		108	48	1986	1076	10
	18. Mar.	wL-FS+FC	Liquid$^{\#}$	47	33	281	186	4
	Sum	*wL-FS*		*284*	*n.c.*	*8295*	*4193*	*(15)*
		wL-FS+FC		*334*	*n.c.*	*6068*	*3294*	*(10)*
		wL-FYM		*322*	*n.c.*	*10773*	*5174*	*(16)*
		wL-RS		*295*	*n.c.*	*9190*	*5150*	*(17)*

Pea-Straw$^{\theta}$: incorporation of complete pea-straw in wL-FS, wL-FYM, and wL-RS, and straw stubbles in wL-FS+FC

Intercrops$^{\nabla}$: green manuring through incorporating of intercrops into the soil; whole plants in wL-FS, wL-FYM, and wL-RS, plant stubbles in wL-FS+FC

Liquid$^{\#}$: fermented cattle slurry (FS) in wL-FS; fermented cattle slurry (FS) and fermented crops (FC) in wL-FS+FC; raw cattle slurry (RS) in wL-RS

Solid$^{\surd}$: solid fermented crop residues in wL-FS+FC; farmyard manure in wL-FYM

n.d.: not determined

n.c.: not calculable

A.7: Amounts and characteristics of the applied fertilizers in all manuring treatments in potatoes and prior intercrops in the cropping system with livestock between 2002 and 2004.

***POTATOES* – Cropping System with Livestock**								
Season	**Date**	**Manuring Treat-ment**	**Manure**	**N_t [kg ha^{-1}]**	**NH_4^+-N [kg ha^{-1}]**	**Org. Dry Matter [kg ha^{-1}]**	**C_t [kg ha^{-1}]**	**C/N Ratio**
2002/2003	6. Aug.	wL-FS	Wheat-Straw[∫]	5	n.d.	1169	574	114
		wL-FS+FC		5	n.d.	1061	521	113
		wL-FYM		4	n.d.	1013	495	125
		wL-RS		4	n.d.	986	487	127
	28. Aug.	wL-FS	Liquid[#]	35	20	468	265	7
		wL-FS+FC		35	20	468	265	7
		wL-RS		35	19	465	264	8
	6. Jan.	wL-FS	Intercrops[▽]	125	n.d.	3016	1609	13
		wL-FS+FC		15	n.d.	785	422	27
		wL-FYM		137	n.d.	3294	1770	13
		wL-RS		117	n.d.	2990	1604	14
		wL-FYM	Solid[√]	249	40	6106	3176	13
	14. Apr.	wL-FS+FC	Solid[√]	68	12	1621	827	12
	29. Apr.	wL-FS	Liquid[#]	114	59	2058	1123	10
		wL-FS+FC		157	95	2227	1229	8
		wL-RS		103	51	2489	1371	13
	30. May	wL-FS	Liquid[#]	48	25	719	392	8
		wL-FS+FC		50	26	748	407	8
		wL-RS		56	26	1019	564	10
	Sum	*wL-FS*		*327*	*n.b.*	*7431*	*3962*	*(12)*
		wL-FS+FC		*336*	*n.b.*	*6910*	*3672*	*(11)*
		wL-FYM		*390*	*n.b.*	*10412*	*5441*	*(14)*
		wL-RS		*315*	*n.b.*	*7949*	*4290*	*(14)*

A.7 continued

Season	Date	Manuring Treatment	Manure	N_t [kg ha^{-1}]	NH_4^+-N [kg ha^{-1}]	Org. Dry Matter [kg ha^{-1}]	C_t [kg ha^{-1}]	C/N Ratio
2003/2004	3. Aug.	wL-FS	Wheat-Straw[∫]	4	n.d.	864	425	105
		wL-FS+FC		6	n.d.	1061	524	81
		wL-FYM		4	n.d.	814	401	110
		wL-RS		5	n.d.	921	454	84
	5. Aug.	wL-FS	Liquid[#]	110	52	1993	1008	9
		wL-FS+FC		146	70	2361	1301	9
		wL-RS		107	35	2343	1312	12
	6. Jan.	wL-FS	Intercrops[∇]	108	n.d.	2744	1418	13
		wL-FS+FC		20	n.d.	1038	533	26
		wL-FYM		59	n.d.	1225	645	11
		wL-RS		88	n.d.	1967	1024	12
	23. Jan.	wL-FS+FC	Solid[√]	114	21	2833	1507	13
		wL-FYM		256	23	7653	4015	16
	13. Apr.	wL-FS+FC	Solid[√]	19	6	1773	883	47
	20. Apr.	wL-FS	Liquid[#]	52	27	902	502	10
		wL-FS+FC		37	19	649	361	10
		wL-FYM	Solid[√]	50	8	1999	1041	21
		wL-RS	Liquid[#]	52	23	1037	553	11
	17. May	wL-FS	Liquid[#]	11	6	164	92	8
		wL-FS+FC		40	21	589	329	8
		wL-RS		10	5	183	100	10
	Sum	*wL-FS*		*285*	*n.c.*	*6667*	*3444*	*(12)*
		wL-FS+FC		*383*	*n.c.*	*10304*	*5438*	*(14)*
		wL-FYM		*368*	*n.c.*	*11691*	*6102*	*(17)*
		wL-RS		*263*	*n.c.*	*6451*	*3442*	*(13)*

Wheat-Straw[∫]: incorporation of straw stubbles in all manuring treatments
Intercrops[∇]: green manuring through incorporating of intercrops into the soil; whole plants in wL-FS, wL-FYM, and wL-RS, plant stubbles in wL-FS+FC
Liquid[#]: fermented cattle slurry (FS) in wL-FS; fermented cattle slurry (FS) and fermented crops (FC) in wL-FS+FC; raw cattle slurry (RS) in wL-RS
Solid[√]: solid fermented crop residues in wL-FS+FC; farmyard manure in wL-FYM
n.d.: not determined
n.c.: not calculable

Ich erkläre:

Ich habe die vorliegende Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.
Die Wetterdaten wurden von Franz Schulz, Mitarbeiter der Versuchsstation, erhoben und zur Darstellung zur Verfügung gestellt (Abb. 2.1 und 2.2; A.1 - A.3). Die Düngemaßnahmen wurden von den Mitarbeitern der Professur für Organischen Landbau ausgeführt. Alle Kultivierungs- und Erntearbeiten auf dem Feld wurden von Mitarbeitern der Professur für Organischen Landbau und der Versuchsstation durchgeführt. Die Daten zu Düngung- und Kultivierungsmaßnahmen (Tab. 2.5 und 2.6; A.4 - A.7) sowie die N_{min}-Messwerte im Tiefenprofil (Abb. 3.5, 3.10, 3.16 und 3.20; Tab. 3.4. 3.6, 3.13 und 3.15) und N_t- und C_t-Gehalte (Tab. 3.5 und 3.14) wurden von Kurt Möller und Walter Stinner, Mitarbeitern der Professur für Organischen Landbau, zur Zusammenstellung und Darstellung zur Verfügung gestellt.
Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Giessen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Giessen, im Juli 2006