MICROBIAL IMMOBILISATION AND TURNOVER OF ^{13}C AND ^{15}N LABELLED SUBSTRATES AND MICROBIAL DIVERSITY IN TWO ARABLE SOILS UNDER FIELD AND LABORATORY CONDITIONS

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Dissertation 2003

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Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. B. Hock
Prüfer der Dissertation:

1. Univ.-Prof. Dr. J.C. Munch
2. Univ.-Prof. Dr. I. Kögel-Knabner

Die Dissertation wurde am 07.04.2003 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 25.06.2003 angenommen.

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1 Introduction

Soil microbial biomass (SMB) represents only 1-5 % of the total C- and N-pool in soil organic matter (SOM) (Jenkinson and Ladd, 1981; Smith and Paul, 1990; Sparling, 1985).

However, microorganisms play an essential role in ecosystem functioning as they are responsible for nutrient cycling, humus formation and building of soil structure along with many other functions. The microbial biomass is considered to be a dynamic source and sink of nutrients, and it is the driving force behind SOM transformations (Burger and Jackson, 2003; Jawson et al., 1989; Smith, 1994).

Most of the recent research in soil ecology has been done in laboratory microcosm studies (Kampichler et al., 2001). In spite of the importance of combining laboratory and field studies (Carpenter, 1996; Verhoef, 1996), this combination has very rarely been realised in soil-ecological research (Kampichler et al., 2001).

The work presented here was integrated into the FAM Research Network on Agroecosystems. The main goals of the FAM are to improve information about agroecosystems, to develop future strategies for environmentally compatible land use, and to achieve agricultural productivity and sustainability (Schroeder et al., 2002). Information about energy and matter fluxes in the agroecosystem is a central part in achieving these goals. The present work was realised within the sub-project "CN2: CN-Turnover", which focuses on measuring and modelling the C- and N-turnover.

Soil microbial C and N immobilisation and turnover were estimated for soils from a high yield and a low yield area of an agricultural field both under laboratory and field conditions. The objectives were:

- to determine if the different yield performance was reflected in
  - the size of soil microbial biomass,
  - the activity of soil microbial biomass and
  - the diversity of soil microbial communities.
- to clarify whether laboratory estimations agreed well with those under natural field conditions.

2 Literature Review

2.1 The Significance of Microorganisms in Soil

"Soil organic matter is composed of decomposing residues, by-products formed by organisms responsible for decomposition of the residues, the microorganisms themselves, and the more-resistant soil humates" (Paul and Clark, 1989). Soil microbial biomass (SMB) represents only 1-5 % of the total C- and N-pool in soil organic matter (SOM) (Jenkinson and Ladd, 1981; Smith and Paul, 1990; Sparling, 1985). However, it plays an important role as a dynamic source and sink of nutrients, and it is the driving force behind SOM transformations (Burger and Jackson, 2003; Jawson et al., 1989; Smith, 1994).

The cycling of nutrients in soil is related to the C cycling, since the SMB and its activity strongly depend on the amount and metabolic availability of C (van Veen et al., 1985). Also, C was reported to be the most limiting element for the SMB in most of the soil ecosystems studied (Smith, 1994).

N transformation by SMB occurs at rates capable of turning over the inorganic N pool several times a day (Coyne et al., 1998). Soil microbial N immobilisation may play an important role in regulating the soil N retention capacity (Bengtsson and Bergwall, 2000) and may thus prevent nitrogen loss through leaching of NO_3^- (Bengtsson et al., 2003). Singh et al. (1989) showed that SMB can be a source of plant nutrients in nutrient-poor tropical soils, where it acted as a sink of nutrients during the dry period (high biomass, low turnover) and as a source during the monsoon period of plant growth (low biomass, high turnover). N flux through SMB has been shown to be sufficient to supply plant N demand (Lethbridge and Davidson, 1982; Paul and Voroney, 1984).

Soil microorganisms also play an important role in the formation and stabilisation of soil structure (Lynch and Bragg, 1985). Hyphal threads and extracellular polysaccharides, produced by soil microorganisms, bind soil particles together. Humic substances, which mainly result from microbial action, form organic matter - clay complexes, which also contribute to soil aggregation (Paul and Clark, 1989). Soil aggregation reduces erosion,

allows good water infiltration and maintains soil aeration (Scheffer and Schachtschabel, 1984).

Soil bacteria and funghi have the potential to be used in biological pest control, which is the suppression of one pest by using its natural pest or antagonist (Kennedy, 1999). Several bacteria and funghi are already commercially available as bioherbicides, biofungicides and bioinsecticides. These biopesticides supplement or partially replace chemical agents and can be incorporated into composts, delivered directly to soil or used as seed-coating (Tengerdy and Szakács, 1998).

Microbial secondary metabolites have been used for medical, industrial and agricultural purposes, e.g. antibiotics, anticancer drugs, antifungal compounds, immunosuppressive compounds, enzyme inhibitors, antiparasitic agents, herbicides, insecticides and growth promoters. Most microbial secondary metabolites in use today come from soil-dwelling microorganisms, the most productive of which have been the actinomycetes (Rondon et al., 1999).

2.2 Estimating C and N Turnover through the Microbial Biomass

Transformation of organic residues is mediated by SMB, which is therefore considered to be a key compartment in various mathematical models dealing with organic matter and nutrient turnover in agricultural soils (Blagodatsky et al., 1998; Jenkinson and Rayner, 1977; van Veen et al., 1984). The importance of feeding the models with reliable and accurate estimates of the size of the biomass and its turnover rate has been emphasized by Chaussod et al. (1988).

Different approaches exist to estimate SMB turnover. Estimations can be based on data of the decline in a non-labelled biomass (McGill et al., 1986), on physiological parameters (Joergensen, 1995; van Veen et al., 1984) or on data of the decline in a labelled biomass (Chaussod et al., 1988; Jenkinson and Ladd, 1981).

Estimating SMB turnover rates by monitoring the decline of a non-labelled biomass is based on the idea that a decrease in microbial biomass results in mobilisation and an increase in biomass in immobilisation of nutrients. The sum of biomass losses (B_0 - B_t) over a given time period (t) in relation to

mean biomass content (B) results in microbial biomass turnover (k) (McGill et al., 1986):

$$k = \frac{1}{t} \times \frac{B_0 - B_t}{B}$$

However this procedure has several drawbacks. First, it is based on the classical assumptions of large annual biomass fluctuations and the synchronicity of growth and death processes, two concepts which have been put into question during the recent years (Joergensen et al., 1994). Secondly, only statistically significant losses should be used for the calculation, otherwise turnover will be overestimated. Consequently, the number of sampling events will influence the results (Harden and Joergensen, 2000). Thirdly, the method cannot account for turnover in the part of the microbial biomass in the state of "cryptic growth", i.e. the turnover which occurs even when biomass remains unchanged.

Turnover rates can be calculated as the product of physiological coefficients representing maintenance (M) and substrate utilisation (Y) efficiency (Joergensen, 1995; van Veen et al., 1984). Both efficiency values cannot be determined directly, but can be approximated by physiological ratios. Maintenance efficiency can be estimated by the metabolic quotient (qCO_2), which is the ratio of CO_2 respired and the mean of biomass C. An approximation of the microbial substrate utilisation efficiency is the ratio of the amount of substrate C converted to biomass C and to CO_2 (R), respectively:

$$k = M \times Y \quad \text{or} \quad k = qCO_2 \times R$$

The most critical point in calculating biomass turnover with these approximations, is the accurate estimation of substrate utilisation efficiency. The ratio seems to be influenced by factors like the amount and quality of substrate, soil conditions (e.g. clay content, nutrients) and temperature (Joergensen, 1995). Another problem is the correct assignation of non-biomass microbial products (Raubuch and Joergensen, 2002).

The turnover times of the microbial biomass can be estimated by isotope dilution technique (Hart and Myrold, 1996; Powlson and Barraclough, 1993). Soils are amended with a labelled substrate which is immobilised by the SMB and the change of labelled biomass is monitored subsequently.

Assuming that the death rate of the labelled biomass is equivalent to that of the whole biomass and that turnover rates are constant throughout the time interval (t) defined, turnover rates (k) can be estimated from the decrease of label in the SMB pool (B) by first-order kinetics (Chaussod et al., 1988; Jenkinson and Ladd, 1981; Joergensen, 1995):

$$B_t = B_0 \times e^{-k \times t}$$

This simple model can give a very good fit to the data, with the drawback that it may overestimate turnover times because it does not take the recycling of labelled substrate into account (Jenkinson and Parry, 1989).

Comparing the three methods, none can give fully satisfying results, as each has its own drawbacks. The restrictions of calculating turnover by the decline in a non-labelled biomass are prevailing and this method has been recommended to be abandoned (Harden and Joergensen, 2000). Only a few authors have used the physiological method so far (Joergensen, 1995; Raubuch and Joergensen, 2002), always pointing out the difficulty of estimating substrate utilisation efficiency. In contrast, the estimation of turnover times through the microbial biomass by measuring the decline in an isotopic label has been widely used in several studies (e.g. Chaussod et al., 1988; Jensen et al., 1997; Kouno et al., 2002; Ladd et al., 1981). Joergensen (1995) calculated the turnover times from data of the same experiment using both the physiological and the isotopic method. In his case the physiological method resulted in shorter turnover times than the isotopic method. As mentioned above, the isotopic method may overestimate turnover times, when the labelled element is subject to recycling (Jenkinson and Parry, 1989).

2.3 Estimating Microbial Diversity

Soil represents one of the most diverse habitats for microorganisms (Rondon et al., 1999). The analysis of soil microbial communities has traditionally been assessed by culture-dependent techniques, using a variety of culture media to maximise the recovery of microbial diversity. However, it has been estimated that less than 0.1% of the microorganisms found in typical agricultural soils are culturable using current culture media (Torsvik et al., 1990). To overcome these problems, culture-independent methods,

based on the extraction of phospholipid fatty acid (PLFA) or nucleic acids (DNA, RNA) from soils, have been introduced.

Phospholipids are found exclusively in cell membranes of microorganisms. Because cell membranes are rapidly degraded after cell death, phospholipid fatty acids can serve as indicators of active microbial biomass. Unique fatty acids have been identified to be indicative of specific groups of organisms (Hill et al., 2000). PLFA analysis is a useful method to determine gross community patterns and changes, but cannot characterise microorganisms to species level. Some important limitations of PLFA analysis are, that signature molecules are not known for all organisms in a soil sample and that the amounts and composition of fatty acids in a microorganisms cell wall may vary with growth conditions and environmental stresses (Haak et al., 1994).

Of all the cell component molecules tested to date, nucleic acids have been the most useful in providing information on the structure of microbial communities (Hill et al., 2000). A variety of genotyping methods are available, such as rep- (repetitive elements), AP- (arbitrary primed) and inter-LINE- (long interspersed elements) PCR (polymerase chain reaction), random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) analysis, and analyses of 16S/18S rDNA (Schloter et al., 2000).

Techniques which involve working with clone libraries are labour intensive. A faster and more inexpensive technique is the generation of distinct banding patterns or “fingerprints”. A widely used fingerprinting technique involves the amplification of the sequences of small subunit (SSU) 16S ribosomal RNA (rRNA) genes (encoded by rDNA) in prokaryotes or of 5S or 18S rRNA genes in eukaryotes. These rRNA molecules and the respective rDNA genes are found universally (Woese et al., 1990) and are composed of both highly conserved and variable regions (Woese, 1987). Using the conserved regions as annealing points for specific primers, the variable regions of SSU rDNA can be amplified by polymerase chain reaction (PCR). PCR will then generate copies of sequences similar in their number of base pairs, but differing in their sequence unique to each organism. This mix of PCR products from rDNA sequences of the various soil organisms can be separated by denaturing gradient or temperature gradient gel electrophoresis (DGGE/TGGE), generating a characteristic

banding pattern of the soil microbial population, which makes it possible to compare populations of different soils or after different treatments.

Some drawbacks have to be taken into account when interpreting DGGE profiles. First, single bacterial types may produce more than one band (Nübel et al., 1996) or bands of different sequences may show the same melting behaviour and therefore position in the gel (Vallaeys et al., 1997). Secondly, during PCR, selective amplification of certain targets may change the distribution in band intensities (Heuer and Smalla, 1997). And thirdly, due to the competitive nature of mixed-target PCR, only a range of numerically most dominant bacterial types will be detectable. These dominant bacterial groups are estimated to make up 0.1 to 1% of the total community (Heuer and Smalla, 1997; Muyzer et al., 1993). Changes in the structural diversity of soil microbial communities will therefore only be detected if predominant populations are affected. The presence or absence of bands does not necessarily signify the presence or absence of species, but their abundance above or below the detection limit.

However, generating fingerprints from 16S rDNA sequences has been successfully applied in studying the effects soil types (Gelsomino et al., 1999; Nakatsu et al., 2000), rhizosphere (Kandeler et al., 2002; Normander and Prosser, 2000; Smalla et al., 2001), heavy metals and antibiotics (Muller et al., 2002), and soil management (Donnison et al., 2000; Ibekwe et al., 2002; McCaig et al., 2001; Phillips et al., 2000) on soil microbial community composition.

3 Material and Methods

3.1 Site and Soil Material

The soils studied under field and laboratory conditions originated from a conventionally treated field at the experimental farm at Scheyern, which is located 40 km northeast of Munich, Germany (N 48° 30.0', O 11° 20.7'). Yield data of this agricultural field collected over four years (1995-1998) showed distinct areas of higher and lower yield. Soils in the high yield (HY) part were characterised as typic Udifluvent with sand, silt and clay fractions of 36%, 49% and 15% respectively. Soils in the low yield (LY) area were described as dystric Eutrochept with sand, silt and clay fractions of 29%, 51% and 20% (FAM data bases: http://www.gsf.de/FAM/adis.html; http://www.gsf.de/FAM/bis.html). For a summary of soil and site characteristics see Table 3.1.

Table 3.1. Summary of soil and site characteristics

	High Yield	Low Yield
Relative Yield (% of mean)	122%	85%
Soil type (US Soil Taxonomy)	typic Udifluvent	dystric Eutrochept
Landscape Position	erosion gully	hilltop
Sand/Silt/Clay fraction (%)	36/49/15 %	29/51/20 %
Soil organic C (C_{org})	1.7%	1.4%
Soil N (N_t)	0.17%	0.15%

The area chosen in the HY part of the field, had a mean yield of 122% and was situated between grid points 220-200 and 230-200. The LY area had a yield of 85% and was placed in the crossing of grid points 210-180, 220-180, 210-170 and 220-170.

In September 1999, maize was harvested followed by the sowing of winter wheat in the beginning of November. The winter wheat was harvested in August 2000 and mustard was sown as a catch crop. Fertiliser was applied at the end of March (50 kg N ha^{-1}), April (liquid manure 15 $m^3 ha^{-1}$), May (60 kg N ha^{-1}), June (40 kg N ha^{-1}), and September (20 kg N ha^{-1}).

3.2 Experimental Design

3.2.1 Field Experiment

The field experiment was started in October 1999 by the Institute of Soil Ecology, as part of the project 'CN-turnover' within the FAM Research Network on Agroecosystems (Jimenez et al., 2000). Two plots of 4x4 m², one amended with ^{13}C- and ^{15}N-labelled white mustard (*Sinapis alba*) and another non-amended control plot, were set up each in the HY and the LY area.

The labelled mustard was grown in 1998/99 in green houses of the GSF-National Research Centre under a ^{13}C-labelled CO_2-atmosphere and with ^{15}N-NO_3^- in the nutrient solution. Mustard material had a C:N ratio of 18, a ^{13}C-content of 101,08 ‰ PDB and a ^{15}N-content of 18 at% of dry matter.

The soil in the plots was removed to a depth of 15 cm and sieved (15 mm) through a compost sieving machine. Mustard material was chopped to 6 mm. Mustard and soil material were thoroughly mixed with a ratio of 1.37 mg mustard kg^{-1} dry soil (corresponding to 500 µg C g^{-1} dry soil and 30 µg N g^{-1} dry soil) and returned into the plots. Soil in the control plots was treated accordingly, without the addition of mustard straw. Within each plot, four adjacent stripes of 0.75 m width were defined as sampling replicates. A margin of 0.5 m width was spared from sampling as a transition to the untreated areas of the field.

Sampling started one day after amendment and was continued with a decreasing frequency from daily to twice a month until May 2001. Soil samples were taken from the upper 12 cm with a soil corer of 3 cm diameter and were processed the same day without sieving.

Of the extractions and analyses carried out on each sampling date, the following will be referred to in the present work:

1. microbial biomass-C and –N determined by chloroform fumigation extraction (CFE): the extracts obtained were stored at –18°C until isotope analysis;
2. dissolved organic carbon (DOC)
3. total soil C und N content.

Isotopic ratios were determined for microbial biomass C and N as well as for total N. For carbon this was done on 10 selected dates throughout the experiment, whereas N isotope ratios were determined on 10 sampling occasions prior to the first application of N fertilisers in April 2000.

3.2.2 Laboratory Experiment

Soil material from HY and LY areas was sampled in April 2000 (winter wheat) from the upper 18 cm, sieved (< 5 mm) and stored air-dry for three (LY) and six (HY) months. The soil fraction >5 mm was 3.1% in the HY and 1.9% in the LY soil. The maximum water holding capacity (WHC) of the sieved soil material, was 56.6% and 48.1% for HY and LY, respectively.

Before starting the incubation, the soil water content was adjusted to 40% WHC and the soils were conditioned at 22°C for two weeks.

Batch incubations were carried out in PE-tubes (4.5 cm in diameter, 25 cm long), at a water content increased to 50% WHC at 14°C for a period of 98 days. Tubes were filled with an amount of soil equivalent to 300 g dry soil. Soil was treated in three ways: 1) control; 2) white mustard ($^{13}C/^{15}N$ labelled, ground mustard); 3) glucose/nitrate solution (same C/N ratio and isotope labelling as mustard). Mustard and glucose/nitrate solution were added in the amounts and with the ^{13}C and ^{15}N label corresponding to those in the field experiment mentioned above, i.e. 500 µg C g^{-1} dry soil and 30 µg N g^{-1} dry soil, 101,08 ‰ PDB ^{13}C and 18 at% ^{15}N. For each treatment and sampling date, three PE tubes were filled as replicates. Additional tubes were filled for gas sampling, making up a total of 99 tubes. During incubation, tubes were covered with plastic bags (Melitta, Germany) to minimise evaporation.

Incubation of LY soil material was conducted between July 14th and October 24th 2000, followed by the incubation of HY soil material between November 14th and February 20th 2000/01. During the three months of incubation, samples were taken on 10 sampling dates with a decreasing frequency from a few days up to three weeks.

On each sampling date the following extractions and analyses were carried out:

1. gravimetric water content;
2. microbial biomass-C and –N by chloroform fumigation extraction (CFE): the extracts obtained were stored at –18°C until analysis;
3. dissolved organic carbon (DOC) and dissolved nitrogen (N_{min}) by $CaCl_2$- extraction: the extracts obtained were stored at –18°C until analysis;
4. CO_2- and N_2O-emissions: gas samples were stored in air-tight glass vials, which could be stored for several weeks until measurements.
5. total soil C und N content: soil aliquotes were stored at –18°C until analysis.
6. molecular microbiological parameters: soil aliquots were stored in 2 ml Eppendorf cups at –18°C until DNA extraction

3.3 Physical Soil Parameters

3.3.1 Maximum Water Holding Capacity

Maximum water holding capacity (WHC) was determined according to Nehring (1960) using glass containers with a permeable bottom. Through this bottom, soil material within the containers absorbed water until saturation and afterwards released surplus water until reaching WHC. WHC was expressed as % weight.

3.3.2 Gravimetric Water Content

The gravimetric water content of soil material was determined by drying an aliquot of about 10 g soil at 105°C until weight constancy. W_{grav} was calculated from the difference between moist (*mw*) and dry soil (*dw*).

$$W_{grav} = mw - dw \quad (1)$$

3.4 Microbial Biomass Carbon and Nitrogen

Biomass C and N were measured by the chloroform fumigation extraction (CFE) method (Brookes et al., 1985; Vance et al., 1987). The amount of chloroform labile C and N (*FlushC*, *FlushN*) was calculated from the

difference between the amounts in extracts of fumigated (*fum*) and non-fumigated (*nfum*) soil samples. No conversion factor was used to calculate soil microbial biomass C and N from extractable C- and N-flush, as the intention was to compare the soils among each other.

$$FlushC = fumC - nfumC \quad \text{or} \quad FlushN = fumN - nfumN \tag{2}$$

Chloroform fumigation of soil samples was conducted in a desiccator during 24 h at 24°C. Fumigated and non-fumigated soil samples of 50 g wet soil were homogenised with 160 ml 0.5 M K_2SO_4 for 30 min and passed through paper filters (595 ½, Schleicher&Schuell, Dassel). Extracts were analysed for DOC (DIMA-TOC, Dimatec, Essen), dissolved nitrogen (N_{dis}), ammonium (NH_4^+) and nitrate (NO_3^-) (continuous flow analyzer, SA 20/40 Skalar Analytical, Erkelenz), as well as ^{13}C and ^{15}N contents (see 3.8.2 and 3.8.3).

3.5 Dissolved Organic Carbon (DOC) and Dissolved Nitrogen (N_{ges})

Soil DOC was extracted with 0.01 M $CaCl_2$ at a soil to solution ratio of 1:2 (W:V). After homogenisation for 30 min the samples were centrifuged at 4000 rev min^{-1} for 10 min (J2-21 Centrifuge, Beckmann) and the supernatant was filtered through 0.45 µm membrane filters (Nuclepore® Polycarbonate, Whatman). Organic C in filtrates was measured with a TOC-analyser (DimaToc, Dimatec, Essen), $N_{dis,}$ NH_4^+ and NO_3^- were determined at the continuous flow analyser (SA 20/40 Skalar Analytical, Erkelenz) and isotopic labelling was analysed as described in chapter 3.8.2 and 3.8.3.

3.6 CO_2- and N_2O-Fluxes in the Laboratory Experiment

A closed chamber method was used to measure CO_2 and N_2O evolution in the laboratory approach. Two PE tubes were put into a gastight chamber of 5 l volume. Each of the three treatments was measured in triplicates. Gas samples were taken with evacuated 100 ml glass containers through a two-way valve connected to the chamber. Prior to each sampling 100 ml of helium were added with a syringe through a septum in the chamber's lid to avoid low pressure in the chamber. Gas samples were taken after closing the chamber and hourly 3 times after. After the first and third hour, samples for

^{13}C analysis were taken through the septum with a syringe, injected into evacuated 12 ml glass vials and analysed as described in chapter 3.8.4. CO_2 and N_2O were quantified with a gas chromatograph (Shimadzu GC 14-A) with a ^{63}Ni electron capture detector (ECD) and gas flow rates were calculated as follows:

$$F = k \times \frac{T_0}{T_1} \times \frac{V}{dw} \times \frac{dc}{dt} \qquad (3)$$

k = molar weight * molar volume^{-1} [µg µl^{-1}]

k_{CO2} = 0,536 µg µl^{-1} k_{N2O} = 1,250 µg µl^{-1}

T = temperature [K] T_0 = 273 K T_1 = 287 K

V = headspace [l] V = 4,24 l

dw = dry weight [g]

dc/dt = gradient of gas accumulation [ppm CO_2 h^{-1}] or [ppb N_2O h^{-1}]

Cumulative emissions were calculated by extrapolating the gas flow rate of a given sampling date over the time interval until the next sampling event.

3.7 Total Carbon and Nitrogen

Soil samples for total carbon and nitrogen analysis were sieved (<2 mm), air dried and ground with a ball mill (Retsch, Switzerland). Aliquots of ca. 70 mg soil were weighted into tin cups (Lüdi AG, Switzerland). Total C and N content were determined with a CN-Analyser (NA 1500, Carlo Erba, Italy). Isotope analysis was carried out as described in chapter 3.8.1.

3.8 Isotope Analyses

3.8.1 Analysis of ^{13}C and ^{15}N in Solid Samples

The CN-Analyser (NA1500) was connected to an Isotope-Ratio-Mass-Spectrometer (Barrie and Prosser, 1996) making it possible to determine ^{13}C and ^{15}N simultaneously with total C and N analysis (see chapter 3.7).

3.8.2 Analysis of ^{13}C in Liquid Samples

For ^{13}C analysis of liquid samples, an especially designed glass container with two chambers, each possessing a gastight closure, was used (Potthoff et al., 2003). The smaller chamber contained 600 mg of the oxidant $K_2S_2O_8$. In the larger chamber an aliquot of the extract containing approximately 100 µg C was given. 2-5 ml diluted H_2SO_4 (pH 1-2) were added to remove inorganic C from the extract while evacuating the chambers. After evacuation the oxidant was transferred to the larger chamber and organic C was oxidised to CO_2 by heating the mixture for 20 minutes at 130°C in an oil bath. CO_2 was purified from H_2O and other interfering gases (e.g. O_2) in a glass equipment with a system of cryo traps. Purified CO_2 was analysed for ^{13}C in the double inlet system of the Isotope-Ratio-Mass-Spectrometer, IRMS (Delta E, Finnigan MAT, Bremen, Germany).

The chlorine in DOC-extracts was precipitated with $AgNO_3$ prior to sample preparation in order to avoid the formation of chlorine gases during oxidation which could not be removed by cryo traps. 0.1 ml ml^{-1} of 0.2 *M* $AgNO_3$ were given to $CaCl_2$-extracts in a 50 ml Falcon tube (Falcon, Germany). AgCl was precipitated and the tubes were centrifugated for 10 min at 4000 rev min^{-1} (J2-21 Centrifuge, Beckmann). The supernatant free of chlorine ions was then given into the larger chamber of the glass container.

3.8.3 Analysis of ^{15}N in Liquid Samples

For ^{15}N analysis of liquid samples the diffusion method according to Jensen (1991) was used. This method is based on the conversion of dissolved mineral N into $(NH_4)_2SO_4$. Devarda's alloy reduces NO_3^- to NH_4^+, which at an alkaline pH volatilises in the form of NH_3 and can be recaptured in an acid trap as $(NH_4)_2SO_4$.

Since NH_4^+ concentrations were very low, this N-fraction could not be determined accurately with the continuous flow analyser and therefore all mineral N was considered as a single pool for ^{15}N analysis of $CaCl_2$ extracts. Prior to the analysis of K_2SO_4 extracts from CFE, organic N was

oxidised to NO_3^- by adding 500 mg of the oxidation agent Oxisolv® (Merck, Darmstadt) including $K_2S_2O_8$, and autoclaving for 25 min at 145°C and 2.6 bar. Aliquots of liquid samples containing 150-250 µg N were transferred into PE-flasks (Nalgene) with 0,4 g Devarda's alloy reducing NO_3^- to NH_4^+. 2 ml 1 M NaOH were added to increase the pH over 10 causing the NH_4^+ to volatilise as NH_3. NH_3 then was captured in acid traps, consisting of glasfibre filters with 30 µl $KHSO_4$ in a tin cup. The traps were placed suspended into to the flasks directly after addition of NaOH and the flaks were shut airtight. After an incubation period of seven days at room temperature, the traps were removed and dried for three days in a desiccator with H_2SO_4. The tin cups were then closed and analysed for ^{15}N as described in chapter 3.8.1 for solid samples.

3.8.4 Analysis of $^{13}CO_2$-C in Gaseous Samples

^{13}C in gas samples from the laboratory incubation experiment (chapter 3.6) was measured on-line at the GC-IRMS (Delta Plus, Finnigan MAT, Bremen, Germany).

3.8.5 Calculations

Allegation Alternate

The recovery of isotopes originating from added mustard, glucose and nitrate in different soil compartments was calculated using allegation alternate. The isotopic label (I_{mix}) of total soil C and N, biomass C and N or CO_2-C is obtained by a composition of soil derived (I_{Soil}) and substrate derived (I_{Subs}) isotopes of C and N. Assuming that:

$$I_{mix} = x \times I_{Soil} + y \times I_{Subs} \quad \text{and} \quad x + y = 1 \tag{4}$$

leads to:

$$y = \frac{I_{mix} - I_{Soil}}{I_{Subs} - I_{Soil}} \tag{5}$$

y = proportion of substrate derived C or N

x = proportion of soil derived C or N

I_{mix} = δ^{13}C [‰ PDB] or ^{15}N [at%] of the respective soil compartment

I_{Soil} = δ^{13}C [‰ PDB] or ^{15}N [at%] of the soil (controls)

I_{Subs} = δ^{13}C [‰ PDB] or ^{15}N [at%] of the added substrate

Isotopic Labelling of Biomass

The isotopic label of the biomass was calculated from the C and N content and the corresponding isotopic label of fumigated and non-fumigated extracts using the following form of allegation alternate:

$$I_{Biom} = \frac{c_{fum} \times I_{fum} - c_{nfum} \times I_{nfum}}{c_{fum} - c_{nfum}} \quad (6)$$

I_{Biom} = isotopic label δ^{13}C [‰ PDB] or ^{15}N [at%] of the biomass

I_{fum} = δ^{13}C [‰ PDB] or ^{15}N [at%] in fumigated extracts

I_{nfum} = δ^{13}C [‰ PDB] or ^{15}N [at%] in non-fumigated extracts

c_{fum} = concentration [µg ml^{-1}] of C or N in fumigated extracts

c_{nfum} = concentration [µg ml^{-1}] of C or N in non-fumigated extracts

Isotopic Labelling of Gas Fluxes

CO_2 sampled from the headspace is a mixture of CO_2 already present in the air and CO_2 emitted by the soil. The δ^{13}C of the soil derived CO_2 (I_{em}) results from an allegation alternate. CO_2–concentrations in the small 14°C-chamber changed substantially during sampling, therefore the values of the first sampling after one hour were used in spite of initial CO_2 concentrations and label in the air.

$$I_{em} = \frac{c_{mix2} \times I_{mix2} - c_{mix1} \times I_{mix1}}{c_{mix2} - c_{mix1}} \quad (7)$$

I_{em} = δ^{13}C [‰ PDB] of CO_2-emissions from the soil

I_{mix1} / I_{mix2} = $\delta^{13}C$ [‰ PDB] of CO_2 sampled from the headspace at times 1 and 2

c_{mix1} / c_{mix2} = CO_2-concentration [ppm] sampled from the headspace at times 1 and 2

Turnover Times

The net turnover rate k [d^{-1}] of biomass C and N was calculated from the decline of substrate derived biomass C or N using a first order equation:

$$B_t = B_0 \times e^{-k \times t} \quad (8)$$

$B_{t/0}$ = amount of substrate derived biomass [µg g^{-1}] at time t = t and t = 0

k = turnover rate constant

The net turnover time T [y] was calculated from T = $(365\ k)^{-1}$ (Chaussod et al., 1988; Jenkinson and Ladd, 1981; Kouno et al., 2002).

3.9 Molecular Microbiological Analyses

3.9.1 DNA-Extraction

Total DNA was extracted from 500 mg of HY and LY soil material from the three laboratory treatments (mustard, glucose/nitrate and control) of 5 sampling dates (day 0, 13, 20, 34 and 98) as well as from a field sample collected on the day of sampling for the laboratory experiment. The soil material was stored at -18°C until DNA extractions. All samples were extracted in triplicates. For extractions the FastDNA® SPIN Kit for Soil (BIO 101, Vista, USA) was used. Briefly, 200 m*M* phosphate buffer and 10% SDS were added to the samples which were then homogenised in a bead beater. After proteins were removed with a protein precipitation solution (PPS®) the DNA was bound to a silica matrix (Binding Matrix®), washed with an ethanol-salt solution (SEWS®) and then desorbed into sterile water. According to the manufacturers manual a minimum of 0.02 µl DNA ml^{-1} were extracted. The quality of DNA extraction was verified by electrophoresis in a 1% agarose gel (Biozym, Germany) using ethidium

bromide staining. DNA extracts which were not used immediately for PCR reactions, were divided in aliquots and stored at –18°C.

3.9.2 Polyacrylamid Gel Elektrophoresis (PAGE)

For a first analysis of differences in the genomic DNA extracted from 5 sampling dates in 3 replicates each, DNA was amplified with random primers. The primer in use was a 22 bp part of the ERIC (Enterobacterial Repetitive Intergenic Consensus) sequence (ATGTAAGCTCC TGGTGATTCAC). The use of ERIC sequences in PCR at low temperatures (52°C and 65°C) has been shown to not necessarily amplify genuine ERIC sequences, but to produce complex banding patterns from various bacteria, fungi and plants (Gillings and Holley, 1997).

For PCR, 1 µl of DNA extract was added to 48.5 µl PCR reaction mix composed of 5 µl 10x PCR Buffer (Gibco BRL, Germany), 2.5 µl 50 m*M* $MgCl_2$ (Gibco BRL, Germany), 5 µl 5 *M* Betaine (Sigma, Germany), 5 µl 2 m*M* dNTPs (MBI Fermentas, Germany), 2 µl 100µ*M* random primer and 29 µl pure water (Sigma, Germany). DNA was amplified in a Biometra T3 thermal cycler (Biometra, Germany). After the first 10 min at 95°C (hotstart), 0.5 µl Taq polymerase (10 U μl^{-1}, Gibco BRL, Germany) was added. The PCR reaction consisted of 36 cycles of 1 min denaturation at 94°C, 1 min at 52°C for primer annealing and 2 min at 65°C for elongation. In the last cycle the elongation phase was extended to 16 min. Successful amplification was verified by electrophoresis in a 1.7% agarose gel (Biozym, Germany) with ethidium bromide stain. PCR products were then applied to a ready polyacrylaminde gel (CleanGel DNA Analysis Kit, Amersham Bioscience, Germany) according to the manufacturers manual. The gel was stained with ethidium bromide and digitised (Intas, Germany).

3.9.3 Denaturing Gradient Gel Electrophoresis (DGGE)

For the analysis on phylogenetic level, denaturing gradient gel electrophoresis was applied to generate 16S rDNA fingerprints of the different treatments and sampling times. 16S rDNA was amplified using the primer pair F-U968GC and R-L1401 (Nübel et al., 1996). A 2 µl volume of

pooled DNA extract from three replicates added to 97.5 µl PCR mixture, was amplified in a Biometra T3 thermal cycler (Biometra, Germany). The PCR reaction mix consisted of 10 µl 10x buffer (Applied Biosystems, Germany), 6 µl 25 m*M* $MgCl_2$ (Applied Biosystems, Germany), 10 µl 3% BSA (Sigma), 5 µl 2 m*M* dNTPs (MBI Fermentas, Germany), 2 µl of each primer (10 µ*M*), 5 µl 100% DMSO (Sigma) and 57.5 µl pure water (Sigma, Germany). 0.5 µl Ampli*Taq*® polymerase (10 U μl^{-1}, Applied Biosystems, Germany) were added to each sample after the hotstart (10 min, 95°C). The PCR reaction consisted of 30 cycles of 1 min denaturation at 94°C, 1 min at 54°C for primer annealing and 2 min at 72°C for elongation. In the last cycle the elongation phase was extended to 10 min. After successful amplification, which was verified by electrophoresis in a 1.7% agarose gel (Biozym, Germany) with ethidium bromide stain, PCR products were purified from primers, nucleotides and polymerases using the QIAquick PCR Purification Kit (Qiagen, Germany). DGGE was performed with 10% acrylamide gels with a denaturing gradient of 45-62% (100% solution contained 7 *M* urea and 40% formamide) in 1x TAE buffer at 60°C and 100 V for 16 h (D-Code® System, Bio-Rad, Germany). A mixture of 16S rDNA PCR products of six pure cultures (*Arthrobacter citreus*, *Cytophaga xanta*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Burkholderia cepacia* and *Azospirillum brasiliense*) was used as a reference on each gel. After electrophoresis, gels were stained with silver nitrate (slightly modificated after Heukeshoven and Dernick, 1986), dried and digitised (Scanner HP7400c).

3.9.4 Analysis of banding patterns

Digitised PAGE gels were analysed for presence (1) or absence (0) of bands in each lane, which ran the same distance. Presence and absence of bands on DGGE gels were analysed directly over transmitted light. Dendrograms were constructed from the binary data matrices of banding patterns by simple matching similarity coefficients and hierarchical cluster analysis, using the SPSS 11.0.1 (Lead Technologies, Germany) software.

3.10 Statistical Analyses

All results are given on an oven-dry basis (drying at 105°C for 24 h) and are expressed as means of 3 replicates with the respective standard deviation. Statistical analysis of the means of two or more independent samples, was done using non-parametric tests (U-Test, H-Test) with a significance level of 95% ($p < 0,05$).

4 Immobilisation and Turnover of Carbon

4.1 Results

4.1.1 Mineralisation of glucose C and mustard C in the laboratory

During the 98 days of incubation, 51 ±4% of the added glucose C and 25±4% of added mustard C were mineralised in both high (HY) and low yield (LY) soils (Fig. 4.1 and Fig. 4.2). The rates of mustard C mineralisation were very similar in both soils, whereas the mineralisation of glucose C was faster in the LY soil compared to the HY soil during the first week but decreased more rapidly thereafter.

Total CO_2 evolved in control treatments amounted to 378.5 ±26.0 µg CO_2-C per g dry soil (µg g^{-1}) in both, HY and LY soils. The treated soils however differed markedly in their mineralisation response. After glucose amendment the HY soil CO_2-C respired totalled 917.2 ±48.2 µg g^{-1} for the 98 days of incubation, whereas in the LY soil CO_2-emissions were 588.8 ±55.4 µg g^{-1} (Fig. 4.1). These differences were caused by a prominent positive priming effect shortly after glucose addition to the HY soil. No priming effect was detected after glucose addition to LY soil. In the mustard treatment (Fig. 4.2), total CO_2 evolved amounted to 610.2 ±7.3 µg g^{-1} and 950.9 ±26.4 µg g^{-1} after 98 days in HY and LY soils, respectively. A positive priming effect was revealed after 20 days in both soils, being much higher in the LY soil.

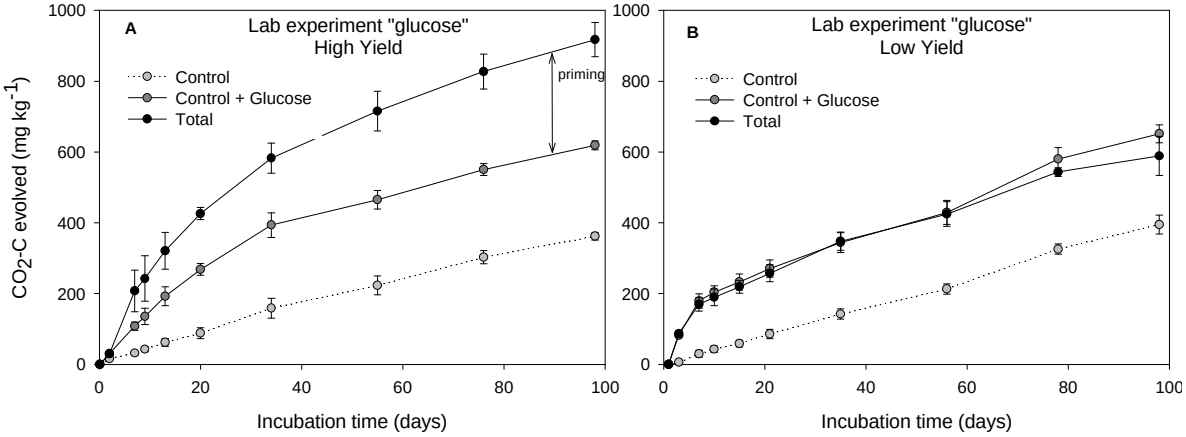

Fig. 4.1. Cumulative CO_2 evolution from glucose treated soil material from **A**: high yield and **B**: low yield areas, incubated over 98 days in the laboratory. Control: CO_2 measurements from controls; Total: CO_2 measurements from glucose amended soils; Control + Glucose: control CO_2 measurements plus glucose derived CO_2 from treatments calculated from isotopic enrichment.

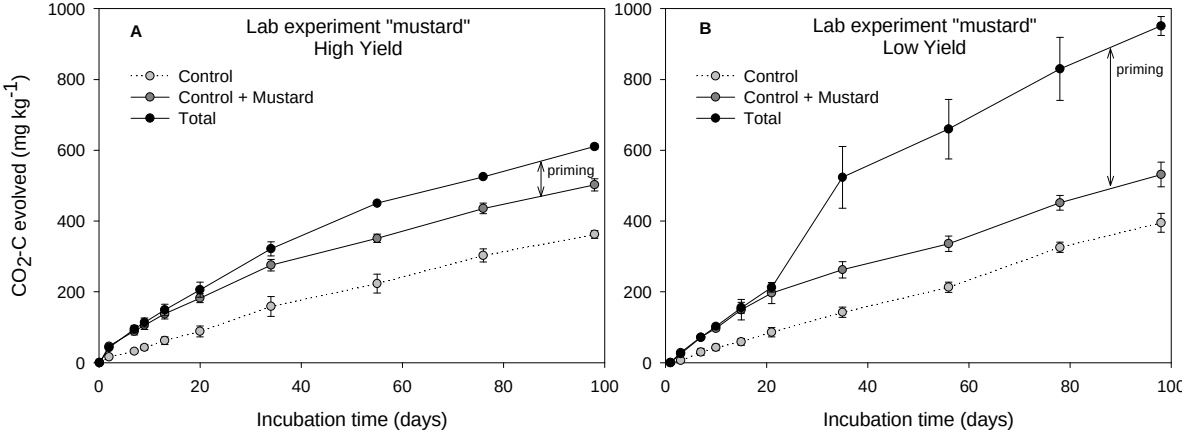

Fig. 4.2. Cumulative CO_2 evolution from mustard treated soil material from A: high yield and B: low yield areas incubated over 98 days in the laboratory. Control: CO_2 measurements from controls; Total: CO_2 measurements from mustard amended soils; Control + Mustard: control CO_2 measurements plus mustard derived CO_2 from treatments calculated from isotopic enrichment.

4.1.2 DOC dynamics following substrate addition

In the field experiment no significant differences in total DOC contents between treated and control plots were revealed. The DOC content over the whole experiment averaged 8.9 ± 2.6 µg C g^{-1} dry soil in the HY and 8.5 ±3.1 µg g^{-1} in the LY areas. Only in the HY soil, a slight increase of DOC content was detected during the first week in the treated plots compared to the control plots.

In the laboratory experiment, the DOC content of controls in the HY soil averaged 11.9 ±3.2 µg g^{-1} and was significantly higher than in the LY soil (7.1 ±1.7 µg g^{-1}; $p<0.05$). An increase in DOC levels was measured after substrate incorporation, with the highest values in the glucose treatment. After one week of incubation, the DOC content in all treatments was on the level of the control. Isotope analysis of DOC-^{13}C (Fig. 4.3) showed that added glucose C disappeared 6 days after addition, whereas mustard C was detected until the end of the experiment, accounting for 4 to 5% of total DOC-C.

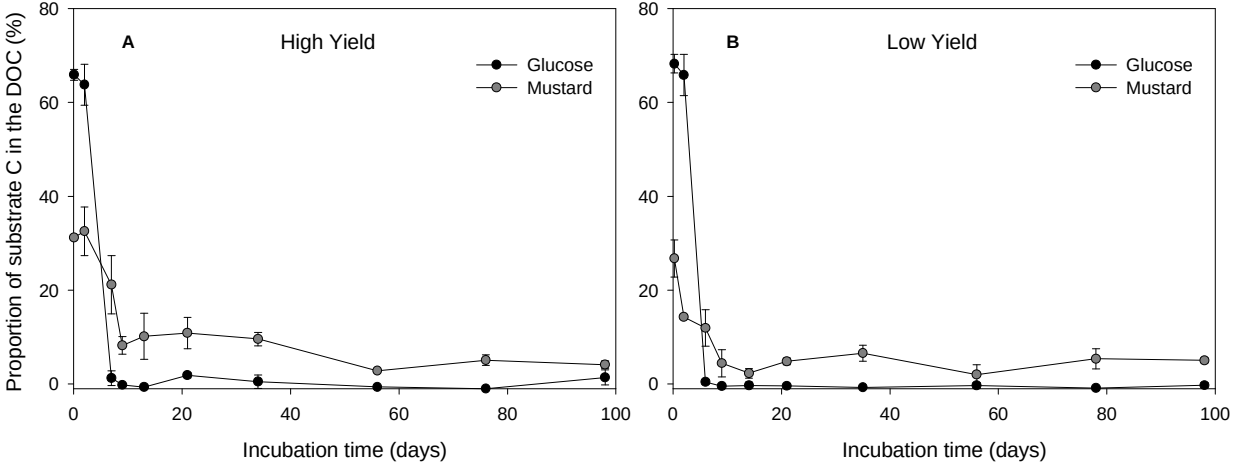

Fig. 4.3. Percentage of glucose and mustard derived C in the DOC in **A:** high yield and **B:** low yield soil material incubated under laboratory conditions over 98 days.

4.1.3 Total C content

Total C content was significantly higher in the HY soil compared to the LY soil ($p<0.05$) under field and laboratory conditions and averaged 1.6% and 1.3%, respectively. ^{13}C data of the laboratory soils showed that added glucose could be retrieved only to 45-50% up to two days after addition and decreased continually to about 34% in the HY and 27% in the LY soil during the 98 days of the experiment (Table 4.1). The recovery rate for mustard C was 90-100% in the HY soil during the first three weeks and in the LY soil until the end of the first week. Subsequently, the retrieval of added mustard C within total soil C decreased constantly during the course of the experiment to 55% and 47% in HY and LY soils, respectively.

Table 4.1. Recovery [%] of added substrate C in soil samples at the 10 sampling dates in the laboratory (day 0, 2, 6, 13, 20, 34, 55, 76, 98); Standard deviation is given in brackets.

Sample	Laboratory experiment			
	Glucose-C		Mustard-C	
N°	HY	LY	HY	LY
1	44.8 *(2.5)*	30.9 *(2.0)*	90.9 *(2.2)*	90.6 *(12.6)*
2	51.2 *(13.0)*	46.9 *(12.8)*	96.5 *(8.1)*	96.1 *(6.2)*
3	34.0 *(1.5)*	40.1 *(4.9)*	88.0 *(5.2)*	97.9 *(15.6)*
4	32.0 *(2.0)*	38.0 *(5.1)*	83.3 *(3.5)*	70.7 *(10.9)*
5	31.3 *(1.2)*	36.9 *(0.9)*	93.8 *(2.2)*	74.1 *(12.1)*
6	32.6 *(4.3)*	33.5 *(6.5)*	91.6 *(6.1)*	76.0 *(22.8)*
7	25.1 *(4.5)*	35.9 *(3.3)*	70.6 *(4.9)*	80.1 *(13.7)*
8	23.8 *(3.8)*	33.9 *(2.1)*	67.2 *(3.0)*	66.5 *(11.2)*
9	25.6 *(6.8)*	29.1 *(3.6)*	61.9 *(4.3)*	65.1 *(11.3)*
10	33.7 *(1.4)*	26.6 *(2.6)*	55.1 *(6.2)*	47.0 *(6.8)*

4.1.4 Biomass C dynamics following substrate addition

No conversion factor was used to calculate microbial biomass C from extractable biomass C, so the biomass C referred to is in fact the flush-C.

In the field experiment, a significant increase in biomass C compared to control plots of 54% in HY and 49% in LY areas was detected 12 days after incorporation of mustard residues. However, this increase did not derive wholly from incorporation of added mustard C (Fig. 4.4). Over the entire period of time, biomass C in HY mustard and control plots was relatively stable and averaged 267.8 ±62.4 µg g^{-1} and 255.2 ±58.9 µg g^{-1}, respectively (Fig. 4.4 A). HY biomass C values were significantly higher than those in LY area plots, which averaged 210.8 ±45.0 µg g^{-1} and 198.5 ±50.8 µg g^{-1} (Fig. 4.4 B). Biomass C derived from mustard C reached a maximum of 13% and 16% in HY and LY, respectively, on day 8 after incorporation and was still detectable one and a half years later making up 3% and 5% of the biomass C.

In the laboratory experiment, biomass C content of the LY soil exceeded that of the HY soil (Fig. 4.5 and Fig. 4.6). The average biomass C in LY controls of 190.7 ±39.6 µg g^{-1} was significantly higher than that in HY controls of 131.2 ±39.2 µg g^{-1}. HY biomass C contents in the laboratory were significantly lower than the corresponding contents in the field, whereas the differences in the LY soils were not significant. Shortly after glucose addition, biomass C in the HY soil increased by about 54%, while in the LY soil this was 18% initially and 38% after three weeks (Fig. 4.5). In the HY soil the increase was partially due to enhanced incorporation of soil derived C. After mustard addition, an increase by 50% occurred in HY biomass C, but only a minor part of this was derived by added mustard C (Fig. 4.6 A). During the rest of the experiment, amounts of biomass C in amended soils declined slightly, but did not differ significantly from that in the controls. At the same time the amount of biomass C comprised by mustard C declined in the same order of magnitude, making up 14 ±1 % of the total biomass C. In the LY soil, biomass C increased until day 21 while the amount of mustard C in the biomass only increased until day 7 and decreased thereafter (Fig. 4.6 B). In the LY soil, more added substrate (glucose or mustard) C was incorporated into microbial biomass, but the amount of substrate C incorporated per unit biomass C was less than in HY soil.

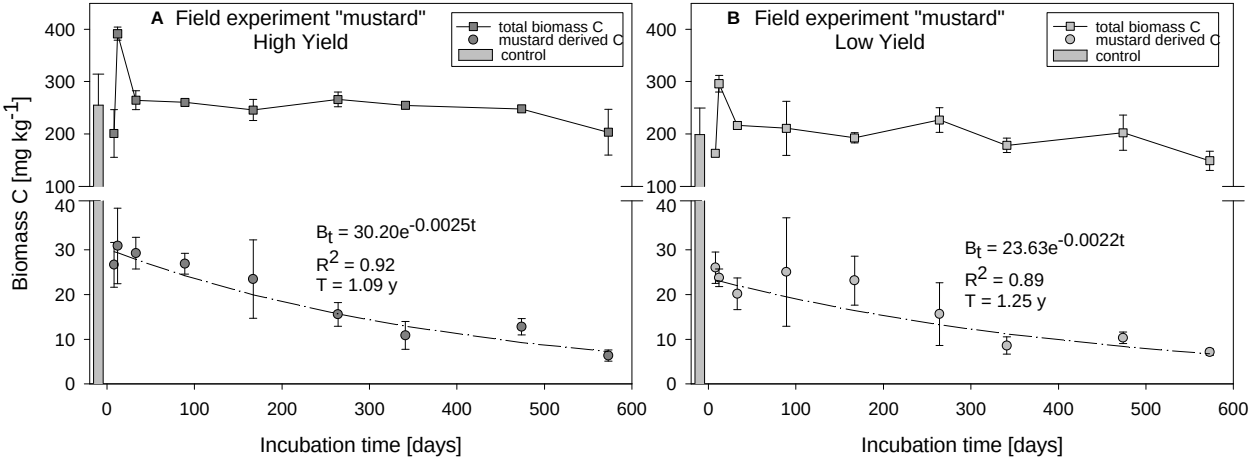

Fig. 4.4. Biomass C dynamics in **A**: high yield and **B**: low yield soil material after mustard addition in the field experiment: mean total biomass C in controls (column), total biomass C (¦) and estimation of turnover times T [y] through the decline in mustard derived biomass C (?) using a first order exponential function (– · –).

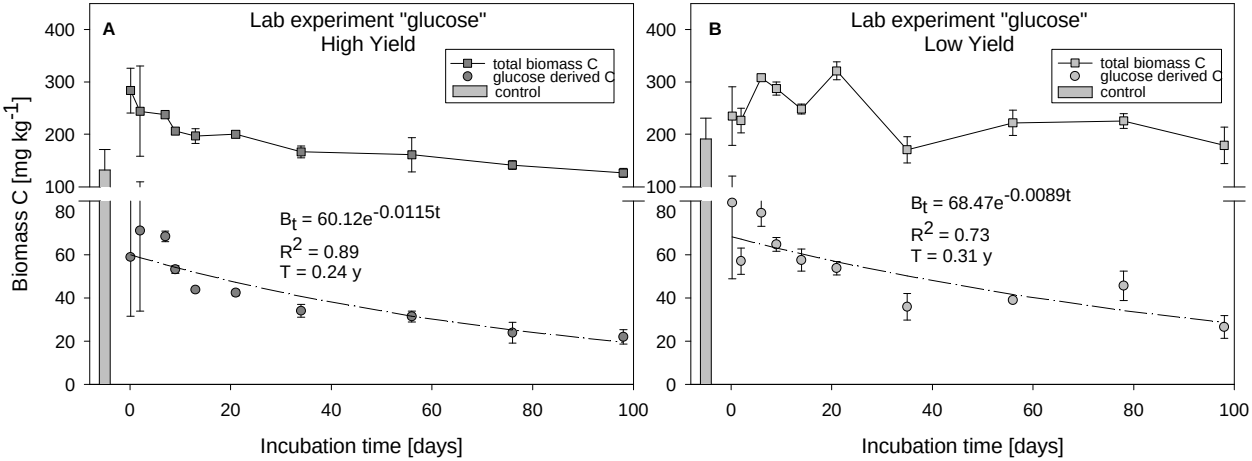

Fig. 4.5. Biomass C dynamics in **A**: high yield and **B**: low yield soil material after glucose addition in the laboratory experiment: mean total biomass C in controls (column), total biomass C (¦) and estimation of turnover times T [y] through the decline in glucose derived biomass C (?) using a first order exponential function (– · –).

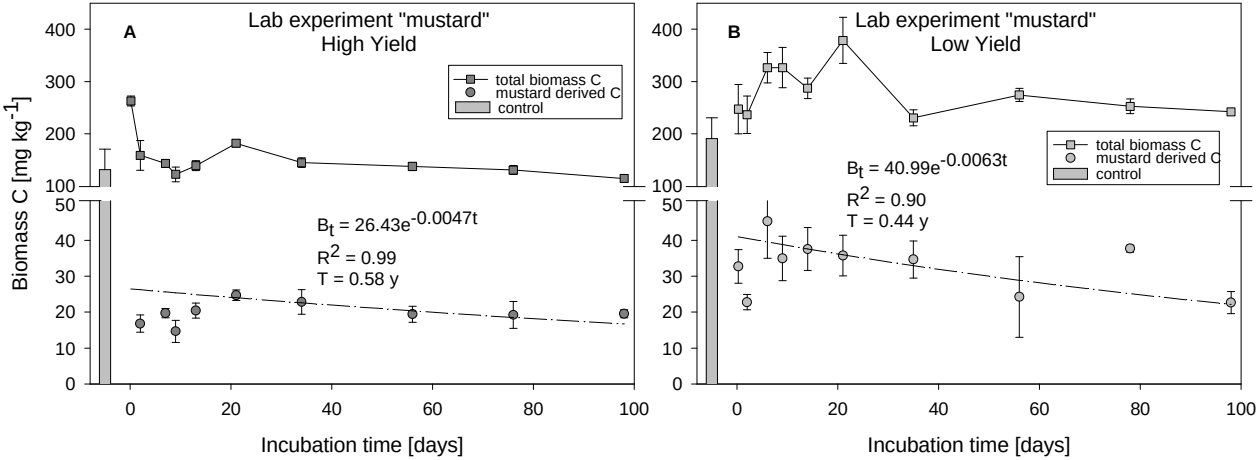

Fig. 4.6. Biomass C dynamics in **A**: high yield and **B**: low yield soil material after mustard addition in the laboratory experiment: mean total biomass C in controls (column), total biomass C (¦) and estimation of turnover times T [y] through the decline in mustard derived biomass C (?) using a first order exponential function (– · –).

4.1.5 Net turnover times of microbial biomass C

In the field experiment, estimated turnover times of microbial biomass C were 1.09 y in the HY and 1.25 y in the LY soil (Fig. 4.4). After addition of glucose in the laboratory experiment, the fastest turnover times of 0.24 y and 0.31 y were reached in HY and LY soils, respectively (Fig. 4.5). Field mustard and laboratory glucose treatments showed a similar relationship between turnover in HY and LY soils, the first being approximately 1.14 to 1.3 times faster than the latter. Turnover of mustard C in the field (Fig. 4.6) took 4.1 to 4.6 times longer than that of glucose C in the laboratory. Turnover times and ratios after mustard addition in the laboratory were reverse with about 0.58 y in the HY and 0.44 y in the LY soil.

4.2 Discussion

4.2.1 C mineralisation in the laboratory experiment

Until the end of the experiment after 98 days, about 50% of added glucose C and 25% of added mustard C were mineralised to CO_2. The value for glucose mineralisation lies within the range described in previous studies over periods of 35 to 185 days (Mary et al., 1993; Saggar et al., 1999; Stenger et al., 2001). The mineralisation of mustard residues was slower, due to the higher complexity and lower solubility of the mustard C of 15 to 25% after water extraction and acid hydrolysis, respectively. This mineralisation rate corresponds with rates measured by Trinsoutrot (2000) after an incubation time of 100 days for rapeseed (*Brassica napus*) roots with a similar C:N ratio and soluble C content as the mustard residues.

Glucose caused a positive priming effect in the high yield (HY) soil shortly after addition which was measurable over nearly the whole length of the incubation. On the contrary, the increase in CO_2 emissions in the low yield (LY) soil could be shown to come exclusively from the mineralisation of added glucose C. Priming also occurred after mustard addition from the third week on, lasting for about 5 weeks and being much more pronounced in the LY soil.

A positive priming effect was defined as the acceleration of mineralisation of soil-derived C caused by the addition of substances to the soil (Kuzyakov et al., 2000). The mechanisms causing priming are not well understood. Evolution of additional CO_2 may be caused by enhanced mineralisation of soil organic matter by soil microorganisms reactivated by the addition of easily decomposable material (Dalenberg and Jager, 1987; Mary et al., 1993). Another hypothesis proposed by Dalenberg and Jager (1987) is that the primed C comes from the turnover of soil derived microbial biomass C. This has been supported to different extends by several authors (De Nobili et al., 2001; Mary et al., 1993; Wu et al., 1993). Hamer and Marschner (2002) agreed to the contribution of microbial biomass turnover to priming but also stated that solid organic matter seems to be the main CO_2-source.

In the present laboratory experiment, priming was induced after a significant increase of soil-derived C in the biomass. Thereafter, the total C loss through priming was always smaller than or equal to the decline in microbial biomass C when converting the measured flush-C with a k_{EC} factor ranging between 0.26, as proposed by Jimenez (2000) for soils of the same area, and 0.35 (Sparling et al., 1990). It seems that the addition of substrate C caused an increase in immobilisation of soil organic C into the biomass which was then subject to accelerated turnover and measured as primed C. The absence of priming in the LY glucose treatment would then indicate that microbial C turnover was increased proportionately to the available glucose C present. In agreement with the findings by Dalenberg and Jager (1987), the C loss due to priming did not exceed the C input to the soil in this study.

4.2.2 Microbial biomass C immobilisation and C turnover

Immobilisation of glucose C by the microbial biomass occurred faster and to a greater extend than that of mustard C. While a maximum of incorporated substrate C was measured shortly after glucose addition, it was delayed for 2-3 weeks in the mustard amendments, both in the laboratory and field. This behaviour can be attributed to the higher availability and degradability of glucose in comparison to the more complex mustard C compounds, as has been reported in literature (Amato and Ladd, 1992; Bremer and Kessel, 1992; Chotte et al., 1997).

The size of microbial biomass C differed significantly for the high yield soil in the field compared to the laboratory. This might have been caused by the pre-treatment of soils prior to laboratory incubations. While some authors state that sieved and dried soils compare favourably to field-moist intact soil cores (Barkle et al., 2001; Franzluebbers, 1999) others report that disturbance can alter microbial community structure and microbial C and N dynamics (Lundquist et al., 1999; McLean and Huhta, 2000; Van Gestel et al., 1993). Van Gestel et al. (1993) pointed out, that rapidly growing types of bacteria and young cells in active growth phase were more susceptible to drying than less active cells, due to their cell wall properties. This concept is consistent with the observations made for the microbial biomass in high yield soil. Incorporation of substrate C per unit biomass occurred faster and to a greater extend in the HY soils and it showed a faster turnover in two of the experimental treatments, indicating a more active microbial population which was more susceptible to drying and rewetting events.

A first order exponential function was used to estimate net turnover times through the decline in labelled biomass C (Chaussod et al., 1988; Jenkinson and Ladd, 1981). A limitation to this method is the possibility that the nutrients, when transformed by microbial processes, remain in the soil and re-enter the biomass turnover repeatedly leading to an overestimation of turnover times (Jenkinson and Parry, 1989). With C the problem is supposed to be least serious, as a large proportion of the labile C is rapidly lost as CO_2 (Kouno et al., 2002), but in the laboratory experiment it was obvious that the amount of substrate-C loss through mineralisation depended on the solubility and degradability of the substrate. Under field conditions, it was also possible that substrate C was lost through leaching. While glucose C disappeared rapidly from soil DOC in the laboratory, soluble mustard C was present to some extend until the end of the experiment, these values being slightly higher in the HY soil compared to the LY soil.

Estimated turnover rates for mustard in the field and glucose in the laboratory showed some similarities. In both treatments, turnover in the HY soil exceeded that in the LY soil, with the net turnover time T being 4 to 4.5 times higher in the laboratory compared to the field. A similar relationship has been reported by Chaussod et al. (1988), who found values of 0.41 y and 1.62 y for glucose turnover under laboratory and field conditions, respectively, which they accounted to the average temperature in the field.

A positive relationship between turnover and temperature in the laboratory and the field could be observed in the present experiments, as the average temperature at the experimental site in Scheyern was 7.8°C, about half of the temperature in the laboratory.

The turnover times T for glucose were comparable to that of 0.22 y and 0.26 y for glucose and ryegrass, respectively, found by Kouno et al. (2002). They observed that the biomass turnover times did not appear to depend upon the nature of the substrate. In our experiment turnover of mustard C in the laboratory experiment was markedly slower than that of glucose. Furthermore the estimated turnover of mustard C was slower in the HY soil compared to the LY soil. Due to the reasons mentioned above, concerning recycling of labelled microbial biomass and residuals, it is possible that turnover rates in the laboratory mustard treatment have been underestimated, in the HY soil possibly to a greater extend than in the LY soil, considering the higher amounts of labelled C in the DOC. Therefore caution has to be taken when estimating turnover rates of complex substrates in batch incubations.

Turnover times in the field-mustard and laboratory-glucose treatments as well as overall microbial biomass dynamics indicated that the HY soil possessed a more active microbial population with a higher C turnover than the LY soil. Carbon and nutrient cycles in soils are closely linked, with C being the energy source and thus the most limiting element to the soil microbial population in most ecosystems (Smith, 1994; van Veen et al., 1984). N mineralisation can be limited by the lack of a labile carbon source, such as DOC (Magill and Aber, 2000). Plant productivity is dependent on the mineralisation of macronutrients such as N, P and S from the soil organic matter (Smith, 1994). Smith and Paul (1990) introduced the hypothesis that the N available for plant uptake comes directly from the SMB and is dependent on the turnover of the SMB pool, and this has been supported by several studies (Lethbridge and Davidson, 1982; Paul and Voroney, 1984; Singh et al., 1989). Thus the higher C turnover rates in HY soil may involve a better nutrient supply of the plants resulting in the higher agricultural yield observed in these areas.

5 Immobilisation and Turnover of Nitrogen

5.1 Results

5.1.1 Dynamics of substrate N in soil fractions

In the field experiment about 74 to 113 % of the added mustard N could be retrieved in soil samples up to one week after addition (Table 5.1). The proportion of added mustard N in the soil decreased continuously to 50 % until the end of field sampling after 166 days. There was no significant difference found between substrate recovery in high and low yield soils.

Added nitrate-N could be recovered to about 80 % during the first 2 days of laboratory incubation of HY and LY soil (Table 5.1). Rates in the LY soil did not differ from this ratio until the end of the experiment, while in the HY soil a significant loss of added nitrate-N from the batch system was measured. Nitrate N losses amounted to 20 % in the LY soil and up to 57 % in the HY soil. In the laboratory mustard treatment (Table 5.1), HY and LY soil did not differ significantly in their recovery rates of nearly 100 % during the first 3 weeks of incubation. Thereafter rates in the LY soil were maintained unchanged, while in the HY soil a slight but significant loss of 12 % of mustard N was measured.

In the laboratory control soils the amount of dissolved mineral N in $CaCl_2$ extracts in the HY soil averaged 112.4 $\pm$8.9 µg g^{-1} and was significantly higher than in the LY soil (49.6 $\pm$16.8 µg g^{-1}). Added nitrate N was recovered entirely in the soluble fraction directly after addition in both soils, decreasing rapidly thereafter (Fig. 5.1). During the rest of the incubation 29.0 $\pm$5.6 % of added nitrate N was recovered as mineral N in extracts of the HY soil, which was significantly less than in the LY soil, where it was 43.9 $\pm$7.2 %. Of the added mustard N only a small fraction was retrieved as mineral nitrogen in the $CaCl_2$ extracts of both soils. In the HY soil this averaged 16.3 $\pm$3.7 % during the first 2 days of incubation and increased to an average of 28.9 $\pm$3.6 % after that. In the LY soil these amounts were slightly higher and the increase of mustard derived N as soluble mineral N only occurred after two weeks (22.2 $\pm$4.1 % until day 13, 32.7 $\pm$5.9 % thereafter).

Table 5.1. Recovery [%] of added substrate N in soil samples at the 10 sampling dates in the field (day 1, 4, 8, 25, 32, 40, 61, 89, 145, 166) and laboratory (day 0, 2, 6, 9, 13, 20, 34, 55, 76, 98); Standard deviation is given in brackets.

Sample	Field experiment		Laboratory experiment			
	Mustard-N		Nitrate-N		Mustard-N	
N°	HY	LY	HY	LY	HY	LY
1	92.9*(25.9)*	80.0*(6.7)*	79.1*(4.6)*	67.2*(0.8)*	96.6*(1.1)*	96.0*(1.6)*
2	112.6*(17.6)*	73.6*(16.9)*	83.1*(10.4)*	86.2*(5.7)*	100.6*(2.4)*	98.6*(20.3)*
3	85.4*(18.1)*	80.7*(15.6)*	51.0*(2.9)*	80.2*(8.7)*	99.5*(4.3)*	112.5*(13.0)*
4	78.8*(8.6)*	88.6*(nd)*	36.3*(15.4)*	79.3*(5.7)*	95.6*(1.2)*	88.8*(9.1)*
5	72.5*(14.5)*	68.6*(23.7)*	43.3*(15.8)*	86.1*(4.6)*	96.5*(5.8)*	96.1*(11.1)*
6	66.4*(13.5)*	71.3*(14.9)*	49.2*(15.8)*	70.2*(11.4)*	94.1*(1.5)*	104.8*(20.1)*
7	70.3*(19.0)*	75.1*(13.1)*	34.1*(19.4)*	84.6*(5.6)*	87.7*(9.0)*	114.7*(6.2)*
8	59.2*(11.56)*	60.9*(13.4)*	42.0*(18.4)*	84.5*(7.5)*	90.3*(7.4)*	100.1*(12.0)*
9	55.4*(3.8)*	68.2*(20.0)*	39.0*(26.7)*	90.1*(13.3)*	83.4*(2.5)*	112.4*(11.4)*
10	45.6*(9.0)*	53.3*(7.4)*	72.5*(7.3)*	74.6*(7.6)*	81.3*(1.2)*	91.1*(5.1)*

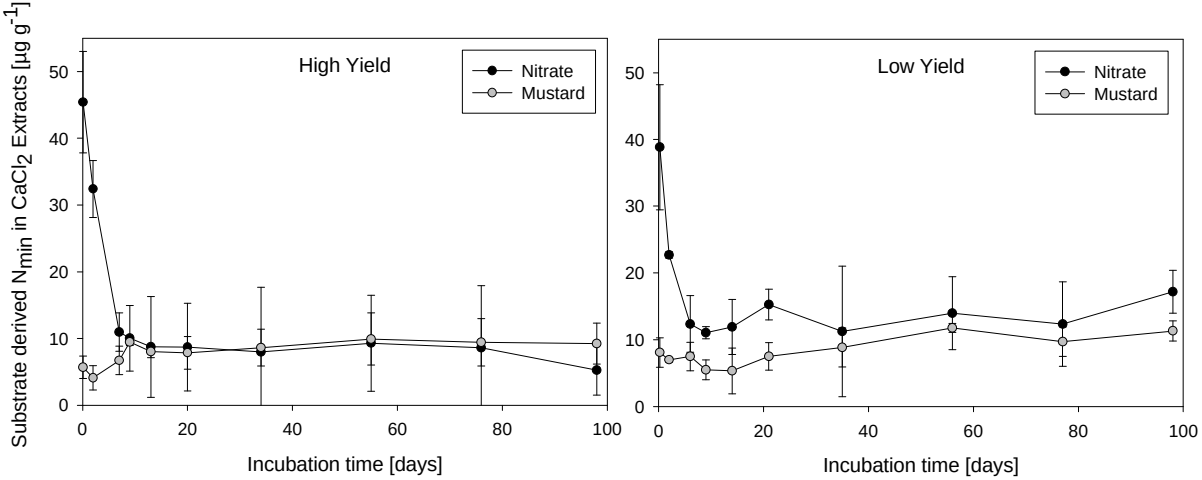

Fig. 5.1. Amount of nitrate and mustard derived mineral N in $CaCl_2$ extracts of high yield (HY) and low yield (LY) soil material incubated under laboratory conditions over 98 days.

Total N_2O losses during the incubation period were markedly higher in the HY compared to the LY soil in all treatments, including controls (Fig. 5.2). Elevated N_2O emissions in the HY soil were measured up to 9 days after nitrate and 21 days after mustard amendment (data not shown). In the LY soil slightly elevated N_2O fluxes from soils were only measured during the first 2 days after nitrate application and up to one week after mustard addition. In the HY soil highest cumulative emissions were calculated for mustard treatments followed by nitrate and controls. In the LY soil emissions from nitrate amendments exceeded that from mustard treatments.

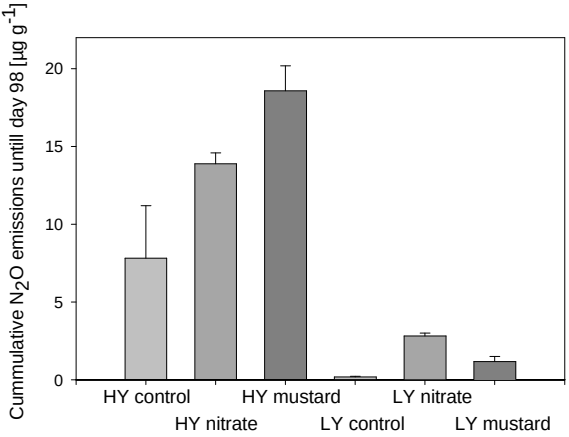

Fig. 5.2. Cumulative N_2O emissions over 98 days in control, nitrate and mustard treatments of high yield (HY) and low yield (LY) soil material in the laboratory experiment.

5.1.2 Biomass N dynamics following substrate addition

No conversion factor was used for the measurements of flush-N from CFE, as they were used for comparisons between soils and treatments, therefore the biomass N referred to in the text is in fact the flush-N.

Field plots showed a significantly higher microbial biomass N in HY soils compared to LY soils (Fig. 5.3). After addition of mustard residues, microbial immobilisation of mustard N (Fig. 5.3) increased in both soils until day 4, followed by a rapid decrease and another increase leading to a second maximum at day 40. Thereafter the amount of mustard derived N immobilised decreased continuously.

Laboratory controls showed a significantly lower microbial biomass N content in the HY soil compared to the LY soil (Fig. 5.4 and Fig. 5.5). The lower amounts in laboratory HY soil also differed significantly from SMB N values in the field HY plots.

After nitrate addition in both soils total biomass N increased during the first week and decreased continuously thereafter (Fig. 5.4). The amount of immobilised nitrate N in the microbial biomass in LY soil already reached a maximum of 7.0 $\pm$2.6 µg g^{-1} after the first few hours of incubation (Fig. 5.4 B). In the HY soil (Fig. 5.4 A), immobilisation of labelled N was slower and a maximum of 5.1 $\pm$1.2 µg g^{-1} was reached only after 9 days. The dynamics of total biomass N were caused by the uptake and loss of added nitrogen N as well as soil derived unlabelled N.

Following mustard addition in the HY soil (Fig. 5.5A), the total microbial biomass increased until day 7 and rapidly decreased thereafter, whereas in the LY soil (Fig. 5.5B) fluctuations were not as pronounced and significant increases compared to control values were measured after two and eight weeks. Immobilisation of labelled mustard N reached a maximum of 2.6 +0.1 µg g^{-1} on day 7 in HY soil. In LY soil this was on day 21 when 3.7 +0.7 µg g^{-1} were found immobilised (Fig. 5.5).

In both amendments microbial populations in the LY soil immobilised more of the substrate N than those in the HY soil throughout the incubation. When data were expressed per units biomass this was also true from day 35 onwards (data not shown).

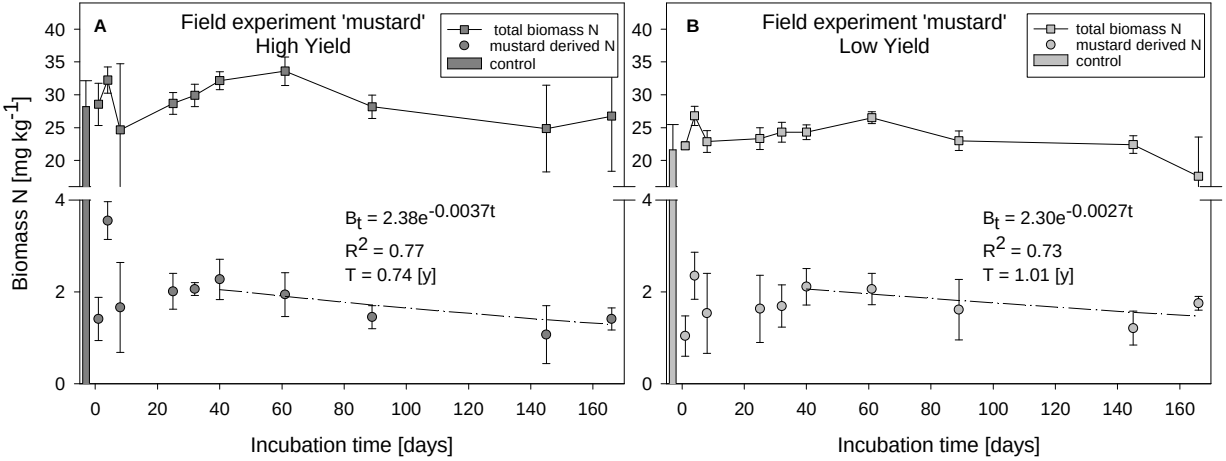

Fig. 5.3. Biomass N dynamics in **A**: high yield and **B**: low yield soils after mustard addition in the field experiment: mean total biomass N in controls (column), total biomass N (¦) and estimation of turnover times T [y] through the decline in mustard derived biomass N (?) using a first order exponential function (– · –).

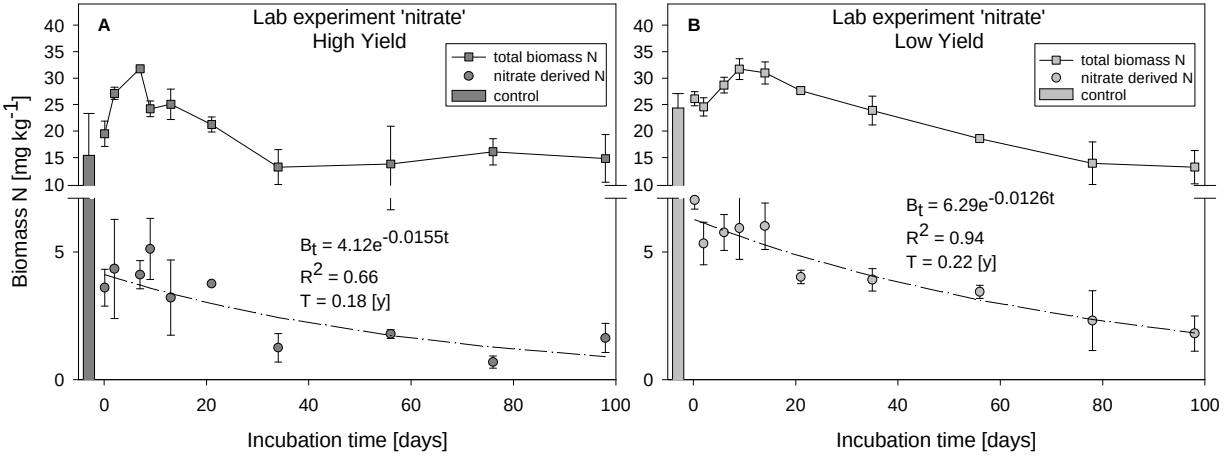

Fig. 5.4. Biomass N dynamics in **A**: high yield and **B**: low yield soils after nitrate addition in the laboratory experiment. mean total biomass N in controls (column), total biomass N (¦) and estimation of turnover times T [y] through the decline in nitrate derived biomass N (?) using a first order exponential function (– · –).

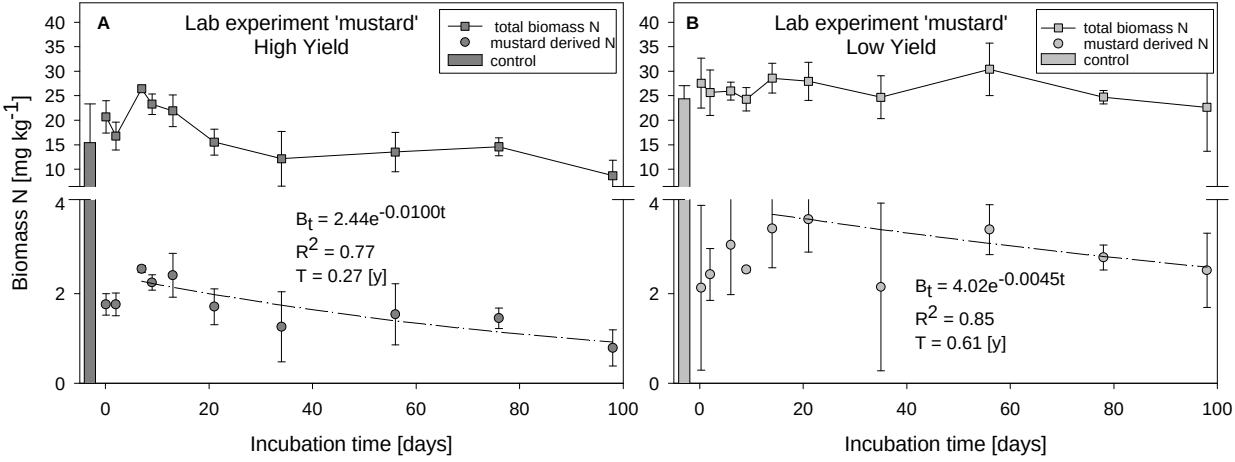

Fig. 5.5. Biomass dynamics in **A**: high yield and **B**: low yield soils after mustard addition in the laboratory experiment. mean total biomass N in controls (column), total biomass N (¦) and estimation of turnover times T [y] through the decline in mustard derived biomass N (?) using a first order exponential function (– · –).

5.1.3 Net turnover times of microbial biomass N

Net turnover times in all experimental treatments were higher for HY soils than for LY soils. The longest turnover times were calculated for field amendments resulting in 0.74 y for HY and 1.01 y for LY soils (Fig. 5.3). In the laboratory mustard treatment the respective times were 0.27 y and 0.61 y (Fig. 5.5) and in the nitrate treatment the shortest turnover times of 0.18 y and 0.22 y were estimated (Fig. 5.4). Field mustard and laboratory nitrate treatments showed a similar relationship between turnover in HY and LY soils, the first being approximately 1.3 times faster than the latter. Turnover of mustard N in the field took 4.2 to 4.7 times longer than that of nitrate N in the laboratory.

5.2 Discussion

5.2.1 Dynamics of substrate N in soil

In the field experiment, between 74 % and 112 % of labelled mustard N was retrieved during the first days after addition. This was most probably due to the fact, that a homogeneous distribution of the chopped mustard residues in the soil was difficult to achieve. In the laboratory about 80 % of labelled nitrate N were retrieved. Substrate N losses during the course of the experiment were highest in the field and the HY laboratory treatments. N-losses were most probably caused by denitrification to gaseous N and, in the field, also by N leaching. Isotopic labelling of N_2O emissions in the laboratory were not quantified, but overall N losses through N_2O were markedly higher in the HY treatments. N_2O emissions are positively related to soil nitrate content (Ruser et al., 2001). Accordingly, highest N_2O emissions occurred in the laboratory HY treatments, where a higher mineral N pool was determined. Maximum cumulative N_2O emissions were determined after mustard addition to HY soil. In this case, recoveries of labelled N only decreased by 11 % until the end of the experiment, implying that in this case high N_2O emissions must have reduced soil derived N.

5.2.2 Microbial biomass N immobilisation and N turnover

In the laboratory, less amounts of added substrate N were immobilised by the SMB in HY soils compared to LY soils, whereas in the field immobilisation of added N by SMB was higher in HY soils initially and slightly lower after 40 days of incubation. The turnover of added substrate N was always faster in HY soils.

Immobilisation and turnover of nutrients by the SMB has been reported to be influenced by a variety of factors, like soil texture, soil C availability, N availability and temperature. The influence of soil type on N immobilisation depends mainly on soil clay content. Clay content was positively correlated with the amount of C and $^{13/14}C$ as well as N and ^{15}N in the microbial biomass (Amato and Ladd, 1992; Franzluebbers et al., 1996; Ladd et al., 1981; Thomsen et al., 1996). The effect of clay on SMB may lie in its capacity to protect biomass, to provide an environment for closer interaction and to retain more decay products in the vicinity of the active SMB (van Veen et al., 1985). In the laboratory experiment lower substrate N immobilisation and faster turnover in HY soils went along with a 5% lower clay content compared to the LY soils. However, it is not likely that the small difference in clay content had a significant influence on the size of the biomass or the amount of substrate immobilised.

Nutrient cycling in the soil is highly dependent on the energy supply to SMB (van Veen et al., 1985), therefore N immobilisation can be limited by C availability (Burger and Jackson, 2003; Trinsoutrot et al., 2000). Addition of glucose enhanced the microbial uptake of NH_4^+ and NO_3^- (Christie and Wasson, 2001) and soil carbon content was shown to explain 58 % of the variation in potential nitrogen immobilisation in grassland soils (Barrett and Burke, 2000). C availability and mineralisation in the laboratory experiment presented here did not coincide with substrate N immobilisation by SMB. The HY soil had a significantly higher DOC content than the LY soil during the laboratory incubation (see chapter 1.1.1). Cumulative CO_2 evolution in the HY soil compared to the LY soil did not show a consistent pattern and was lower in the mustard treatment, but higher after glucose/ nitrate addition (see chapter 4.1.1).

Microbial growth during decomposition is also substrate dependent (Mary et al., 1993; Nicolardot et al., 1994; Trinsoutrot et al., 2000). Mineral N, mainly in the form of NH_4^+, is supposed to be the source for microbial

immobilisation (Corre et al., 2002; Paul and Clark, 1989). In the absence of ammonium, nitrate has been shown to be as good a substrate for microbial assimilation (Recous et al., 1990; Recous et al., 1992). Burger and Jackson (2003) even observed that microbes assimilated more NO_3^- than NH_4^+ in two arable soils. In the present laboratory experiment, added nitrate N available in the $CaCl_2$ extractable mineral N pool made up 29 % in the HY soil and 44 % in the LY soil after the first 2 to 14 days of the incubation. Higher availability of added N in the N_{min} pool of the LY soil agreed with more labelled nitrate N immobilised by the SMB.

The amount of added mustard N immobilised by the microbial biomass in the field experiment did not differ much in the HY and LY plots. Nicolardot et al. (1994) showed that N immobilisation was positively correlated with temperature and Fierer and Schimel (2002) demonstrated that drying and rewetting events can influence microbial C and N dynamics significantly over up to several weeks after the stress. In the field, temperature and moisture regime were highly variable and will have mainly influenced SMB N immobilisation.

The amount of total microbial biomass N in the HY soil was significantly lower in the laboratory treatment compared to field values. The same effect was observed for biomass C (see chapter 4.1.4). This may have been an effect of soil treatment prior to incubations. While some authors reported that sieved and dried soils were comparable to field-moist intact soils (Barkle et al., 2001; Franzluebbers, 1999), others found that disturbance can alter microbial community structure and microbial C and N dynamics (Lundquist et al., 1999; McLean and Huhta, 2000). Especially active, rapidly growing bacteria are susceptible to drying, due to their cell wall properties (Van Gestel et al., 1993). SMB in the HY soil showed a faster turnover of added substrate N, indicating a higher microbial activity and thus an increased susceptibility to drying.

SMB turnover of nitrogen was estimated by fitting a first order exponential function to the decline in labelled microbial biomass N (Chaussod et al., 1988; Jenkinson and Ladd, 1981). A limitation of this approach is the possibility of recycling of labelled N to the microbial biomass by decomposition of ^{15}N-labelled humus or release of ^{15}N labelled compounds by microbial cells leading to an overestimation of turnover times (Jenkinson and Parry, 1989), but currently there does not seem to exist a satisfactory

way to address it (Kouno et al., 2002). In the field experiment, remineralised nitrogen may have been removed by leaching, whereas in the laboratory batch incubations the only way of nitrogen loss was by denitrification to N-gases. A substantial amount of added nitrogen was retrieved as mineral N throughout the incubation, therefore it has to be kept in mind that turnover times may have been shorter than estimated from laboratory data. Turnover times for SMB reported in literature vary widely depending on substrate and experimental conditions. Jensen et al. (1997) and Jensen (1994) estimated turnover times of 3.9 y for straw derived and 1.2 to 2.3 y for pea residue derived microbial biomass N in different field experiments, while shorter turnover times of 0.4 y were reported for *Medicago littoralis* in the field (Ladd et al., 1981). Smith and Paul (1990) estimated SMB turnover time to range from 0.2 to 0.6 y for several global vegetation types. The results presented here therefore agree well with turnover times found by others, ranging among the lower values.

Turnover times of mustard N in the field took about 4.5 times longer than of nitrate N in the laboratory. A similar relationship has been found for C turnover during the same experiment (see chapter 4.1.5): here turnover of the complex substrate mustard in the field took about 4.3 times longer than the easily available glucose in the laboratory. Chaussod et al. (1988) found that turnover of labelled glucose in the field took 1.62 y and was 4 times slower than under constant laboratory conditions (0.41 y), which they accounted to the average temperature in the field. In accordance, mean temperature during the sampling period in the field experiment from October 1999 to March 2000 was 2.9°C, 4.9 times lower than during the laboratory incubations.

Estimated N turnover times through the microbial biomass were always shorter in HY compared to LY soils. The differences in SMB turnover in HY and LY soils were also reflected in the turnover of particulate organic matter (POM), which was degraded more rapidly in soils from the HY area (Kögel-Knabner and Munch, 2002).

Smith and Paul (1990) introduced the hypothesis that the N available for plant uptake comes directly from the SMB and is dependent on the turnover of the SMB. Singh et al. (1989) showed that SMB can act as a nutrient source for plants during the monsoon period of plant growth in tropical soils. Other authors demonstrated that the N flux through SMB acting as the

sole source of N was sufficient to supply crop demands (Lethbridge and Davidson, 1982; Paul and Voroney, 1984). Comparing N pools on a global basis Smith (1994) concluded that the SMB turnover rate must be less than one year to satisfy plant uptake. The turnover times estimated from the field and laboratory experiments were all in the range of maximal one year in the field to much shorter times in the laboratory, indicating that SMB turnover could indeed supply crop N requirements. Faster turnover in the HY soils would thus involve a better nutrient supply of the plants, which correlates positively with the higher agricultural yield observed in these areas.

6 Microbial Community Patterns

6.1 Results

6.1.1 Analysis of genomic DNA using random primer

For a first analysis of differences in the genomic DNA extracted from the five sampling dates and their replicates, DNA was amplified by random primers. Profiles generated by random primers revealed fourteen different bands, of which a minimum of four were found in field soil patterns and a maximum of ten in LY controls. Two bands were consistent in all soils and treatments, while one band was characteristic for most HY and LY soils, respectively (Fig. 6.3).

Triplicate DNA extractions from HY and LY experiments were compared within each treatment (control, glucose/nitrate, mustard) (Fig. 6.1). Triplicate extractions of all samples showed a high reproducibility and had a mean of 96,8% conformity, with the highest variation of 86% occurring between banding patterns of HY glucose/nitrate treatments at day 13. Sampling dates within one treatment also showed very consistent patterns. However, clear differences were seen between samples from HY and LY soil material in each laboratory treatment, as patterns revealed a relationship of 56.2-63.8% between HY and LY soil material.

As sampling dates within treatments showed a high similarity, one sampling date (day 13) was chosen randomly to compare the three laboratory treatments with field soil (Fig. 6.2). Cluster analysis showed a separation of treatments and soils and a high similarity of replicates. Banding patterns obtained from laboratory LY soils differed most from the other patterns, while profiles from laboratory HY treatments, especially controls, were more closely related to the field soils

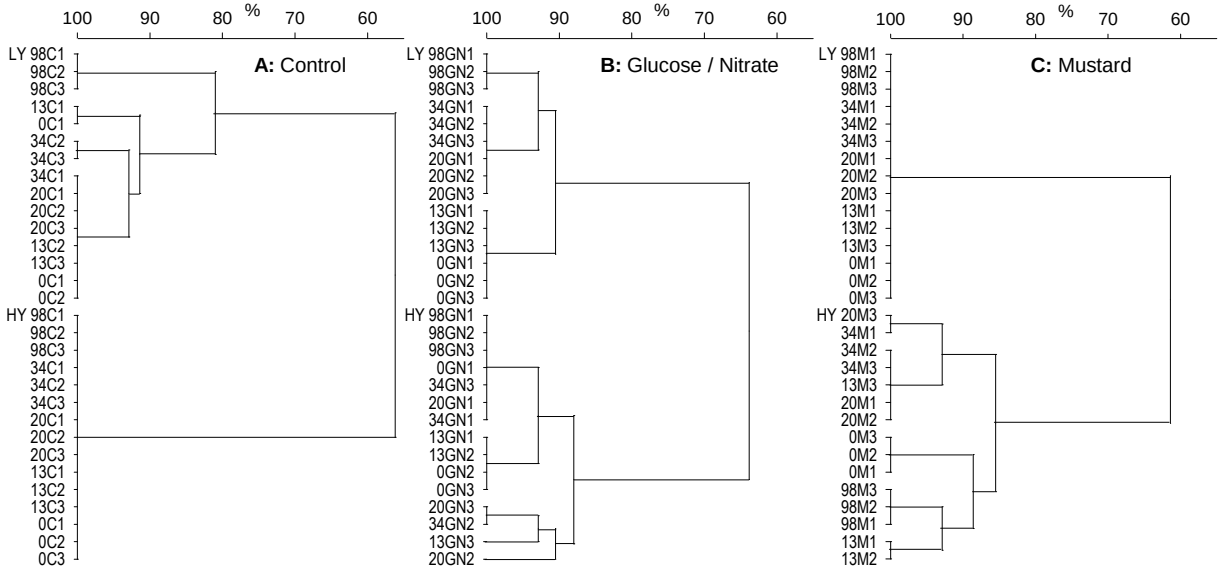

Fig. 6.1. Genetic similarity of community fingerprints obtained with random primer polymerase chain reaction (ERIC PCR) – PAGE from the target DNA extracted in triplicates from five sampling dates (day 0, 13, 20, 34 and 98) of each laboratory treatment (C: control, M: mustard, GN: glucose/nitrate) with high yield (HY) and low yield (LY) soil material.

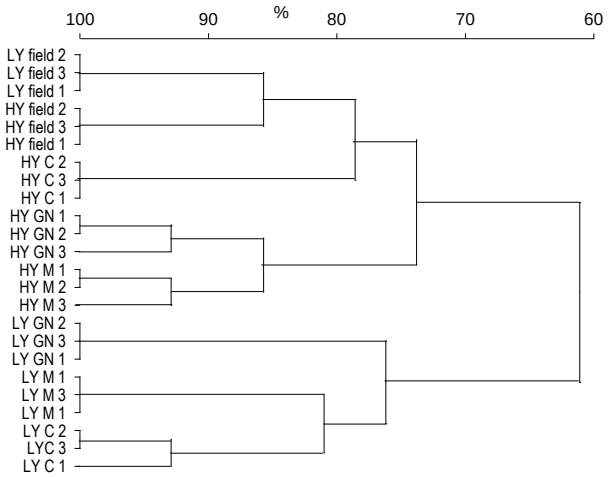

Fig. 6.2. Genetic similarity of community fingerprints obtained with random primer polymerase chain reaction (ERIC PCR) – PAGE from the target DNA extracted from high yield (HY) and low yield (LY) soil material from the field and from three experimental treatments (C: control, GN: glucose/nitrate, M: mustard) on the 13th day of incubation. All treatments were extracted in triplicates.

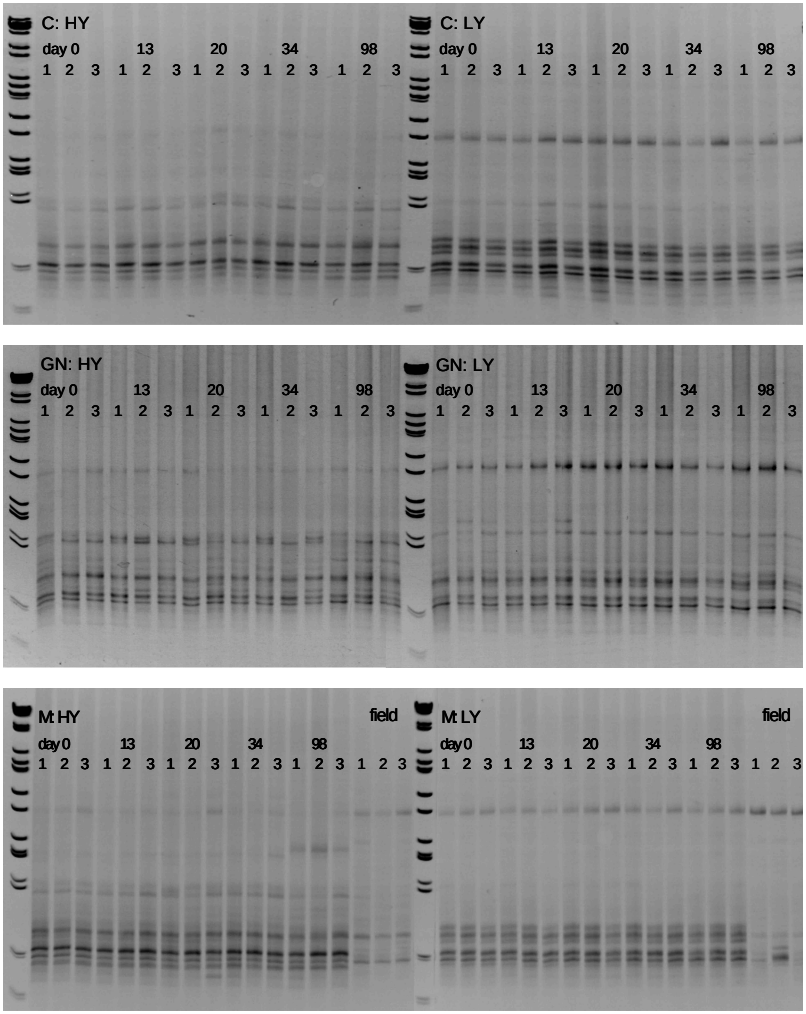

Fig. 6.3. PAGE pattern of DNA fragments obtained by PCR with random primers from high yield (HY) and low yield (LY) soil material from the field and from three laboratory treatments (C: control, G/N: glucose/nitrate, M: mustard) at five sampling dates (day 0, 13, 20, 34 and 98) in three independent replicates (1, 2, 3).

6.1.2 Analysis of 16S rDNA

Correspondence between the sampling dates of each treatment was also checked using denaturing gradient gel electrophoresis with 16S rDNA products. Cluster analysis of profiles revealed a high similarity between sampling dates of each treatment and a clear separation between HY and LY soils (Fig. 6.4).

The DGGE gel comparing PCR products of laboratory treatments of one sampling date (day 13) and of field soil (Fig. 6.6), revealed a total of 27 different bands, 10 of which all treatments had in common. The maximum number of 23 bands was generated from the DNA extracted from HY glucose/nitrate treated soils, while a minimum of 15 bands occurred in LY mustard and control treatments and HY field soil. The HY glucose/nitrate treated soil also revealed the highest number of three unique bands (Fig. 6.6, 'U'). One distinct band, which ran about the same distance as *Pseudomonas fluorescens* in the reference lane, was more pronounced in both HY and LY glucose/nitrate treatments (Fig. 6.6, 'GN'). Four bands were found solely in laboratory treatments (Fig. 6.6, 'L1/2'), of which two bands were restricted to the HY (Fig. 6.6, 'L2').

Cluster analysis of banding patterns (Fig. 6.5) revealed three clusters separating HY laboratory, LY laboratory and field soils. Within both the HY and LY laboratory group, the glucose/nitrate treatment separated from the mustard and control treatments, while differences between treatments were more pronounced within the HY group. Banding patterns in the LY treatments were more closely related to the field patterns that those in the HY treatments.

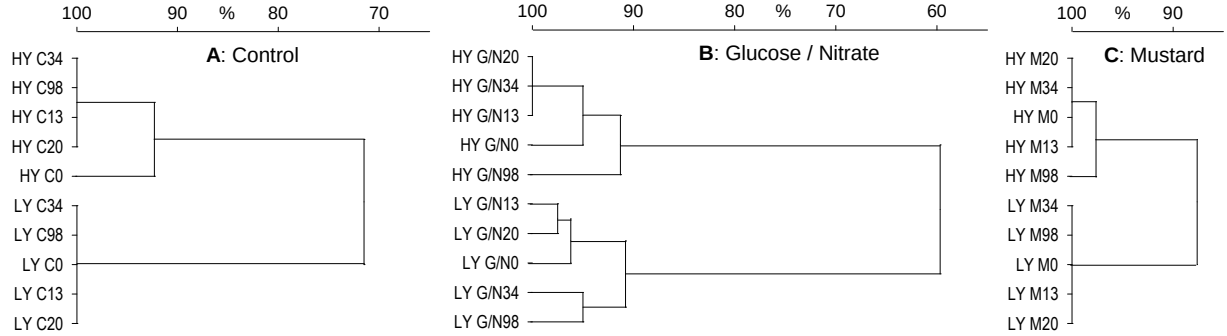

Fig. 6.4. Genetic similarity of community fingerprints obtained with polymerase chain reaction (PCR) – DGGE from the target DNA extracted from high yield (HY) and low yield (LY) soil material of three experimental treatments (A: control, B: glucose/nitrate, C: mustard) on five sampling dates (day 0, 13, 20, 34 and 98).

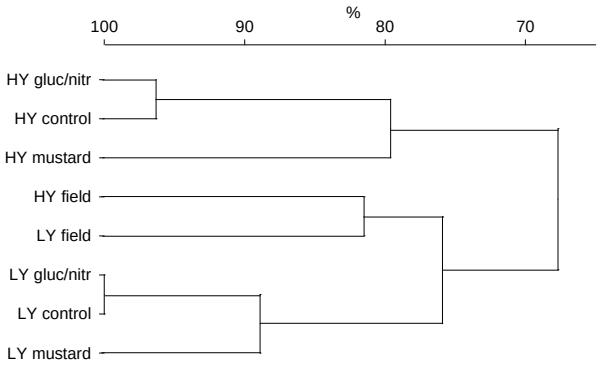

Fig. 6.5. Genetic similarity of community fingerprints obtained with polymerase chain reaction (PCR) – DGGE from the target DNA extracted from high yield (HY) and low yield (LY) soil material from the field and from three laboratory treatments (control, glucose/nitrate, mustard) on the 4th sampling date (day 13).

6.2 Discussion

The banding patterns derived by random primers and 16S rDNA fragments showed little variation between replicates of one sample (random primers) and between different sampling dates of one soil treatment (both). Both however, indicated that soil treatments, substrate additions or soil type may have had an effect on the composition of the soil microflora.

The use of a part of the ERIC sequences as a random primer in the present study, was suitable to give an overall picture of the composition of the genomic DNA extracted directly from soil samples. DGGE profiles of 16S rDNA gave more detailed information about changes on phylogenetic level.

Duineveld et al. (1998) and Gelsomino et al. (1999) supported the hypothesis that each soil type has its own set of dominant microbial groups, which mainly determines the DNA based profiles of bacterial communities. Differences between profiles from field soils of HY and LY areas showed a resemblance of 81%, indicating that the small differences in main soil

characteristics (Table 3.1) of the two areas did not induce the evolution of distinct communities.

At the same time, banding patterns generated from DNA of the soil material used in laboratory treatments differed from that of the original soil sampled in the field. The profiles of HY and LY soils during the laboratory experiments were also separated clearly by cluster analysis. Some bands in the DGGE profiles were unique to laboratory treatments. Pre-treatment of the soil material prior to incubations, namely sieving, drying and rewetting, was likely to have influenced the microbial community structure (Lundquist et al., 1999; McLean and Huhta, 2000). Especially active, rapid growing bacteria are susceptible to drying, due to their cell wall properties (Van Gestel et al., 1993). It seems that pre-treatment promoted a few species in the laboratory experiment, which then raised above the detection limit, but it did not decrease the abundance of any of the species already detectable in the field soil. Patterns of the LY soil were more related to the field soil profiles than HY soil patterns, indicating that the microbial population of LY soil was less affected by the pre-treatment.

The influence of substrate additions was most visible in the glucose/nitrate treatment. In the HY soil the highest number of unique bands was determined after glucose and nitrate addition and in both HY and LY soils an increased intensity in one specific band, which ran the same distance as *P. fluorescens* in the reference lane, was observed, indicating that this bacterial type profited especially from the kind of substrate added. Changes in the microbial community have also been observed as effects of the addition of nitrogen (Bardgett et al., 1999), synthetic root exudates (Griffiths et al., 1999) and of the proximity to plant roots, which was mostly attributed to their input of exudates (Kandeler et al., 2002; Normander and Prosser, 2000; Smalla et al., 2001).

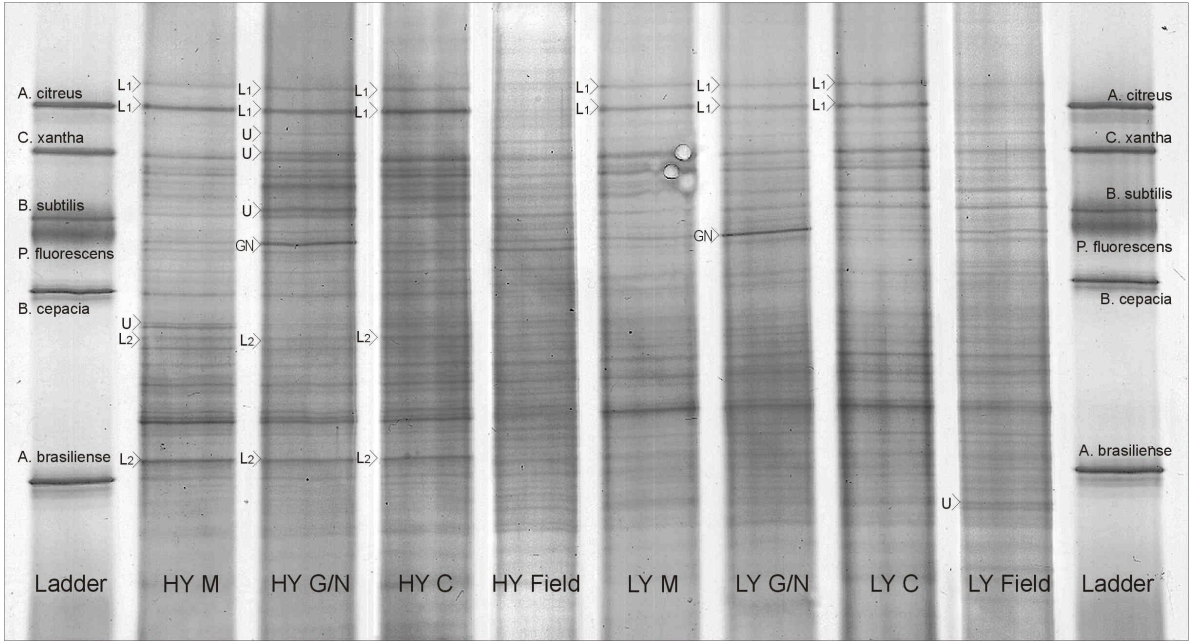

Fig. 6.6. DGGE patterns of 16SrDNA fragments obtained by polymerase chain reaction (PCR) from the target DNA extracted from high yield (HY) and low yield (LY) soil material from the field and three laboratory treatments (C: control, G/N: glucose/nitrate, M: mustard) of the 4th sampling date (day 13). U: unique bands, L1: laboratory specific bands, L2: HY laboratory specific bands, G/N: enhanced bands after glucose/nitrate treatment. Ladder: 16S rDNA PCR products of six pure cultures: *Arthrobacter citreus*, *Cytophaga xanta*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Burkholderia cepacia* and *Azospirillum brasiliense*.

7 Summary Discussion

7.1 CN turnover and ratios

C and N cycling in soils is tightly linked and the rates of N transformations have been shown to depend on the availability of labile C as an energy source (Bengtsson et al., 2003; Chaussod et al., 1988; Christie and Wasson, 2001; Magill and Aber, 2000; Trinsoutrot et al., 2000).

Estimated turnover times of C and N through the microbial biomass in the different experiments showed several similarities. Both were generally shorter in HY soils compared to LY soils. Fastest turnover always occurred in laboratory glucose/nitrate amendments, followed by laboratory mustard and field mustard treatments. Turnover of mustard C as well as N in the field took about 4.5 times longer than that of glucose C and nitrate N in the laboratory. Within a given treatment, the turnover times for N were shorter than those for C in all but one case (LY laboratory mustard). In the field mustard as well as the laboratory glucose/nitrate treatments, N turnover was approximately 1.4 times faster than C turnover. Likewise, Kouno et al. (2002) found that turnover of biomass P was faster than that of biomass C, which they reasoned with the location of these elements within the cell. C is the major element in all classes of macromolecules, including resistant forms like cell walls and storage polymers. P is required by cells primarily for synthesis of nucleic acids and phospholipids and N is a major constituent of proteins and enzymes, which are changed constantly in response to physiological requirements (Beck et al., 1997; Schlegel, 1992).

During the field experiment, both the amounts of biomass C and biomass N were higher in the HY soil compared to the LY soil, while during laboratory incubations, SMB C and N were significantly lower in the HY soil material. Calculating C:N ratios ($(C:N)_{mic}$) of the SMB-flush (Table 7.1) revealed an average of 9.5 for all treatments. The values of $(C:N)_{mic}$ within treatments ranged from a minimum of 6.3 to a maximum of 14.4. Joergensen (1995) compared the $(C:N)_{mic}$ of 27 arable soils, which lay well below the values for HY and LY soils, ranging from 5.2 to 10.4 and averaging 6.9. In a study by Zagal and Persson (1994), mean $(C:N)_{mic}$ of unamended soil was also 6.9. After they added glucose and four rates of N, the $(C:N)_{mic}$ increased to up to 25.9 in low-N treatments. When comparing $(C:N)_{mic}$ ratios from

different studies the conversion factors (k_{EC} and k_{EN}) used to calculate biomass C and N values from flush C and N measurements have to be kept in mind. Joergensen (1995) used a k_{EC} of 0.45 (Joergensen, 1996) and a k_{EN} of 0.54 (Joergensen and Mueller, 1996), while Zagal and Persson (1994) calculated with a k_{EC} of 0.41 (Zagal, 1993) and a k_{EN} of 0.45 (Jenkinson, 1988). Depending on the relationship between k_{EC} and k_{EN}, the $(C:N)_{mic}$ will be slightly higher or lower than if calculated directly from flush-C and flush-N. Using the conversion factors of 0.45 and 0.54, would increase the $(C:N)_{mic}$ values for HY and LY soils by 1.2.

C and N availability have been reported to play an important role in the determination of microbial C:N ratios (Anderson and Domsch, 1980; Zagal and Persson, 1994). C availability has been shown to be positively correlated with fungal C:N ratios in laboratory cultures (Anderson and Domsch, 1980). Zagal and Persson (1994) measured high $(C:N)_{mic}$ in agricultural soils after low N additions (38 and 76 µg N g^{-1}), which they accounted for with unbalanced microbial growth and the production of exopolysaccharides during growth as a physiological response of microorganisms to nitrogen limitation in the presence of an abundant C source (Sutherland, 1977). In the present study, a high $(C:N)_{mic}$ was also measured in control soils and the addition of substrate C and N did not alter these values. Soil C:N ratios in the HY and LY soils were constant during

Table 7.1. Mean C:N ratios (C:N), standard deviation (*sd*), minimum (min) and maximum (max) C:N ratios of the microbial biomass in HY and LY soils in laboratory and field experiments (G/N – glucose/nitrate, M – mustard, C – control)

	High Yield					Low Yield					
	Laboratory			Field		Laboratory			Field		Mean
	G/N	M	C	M	C	G/N	M	C	M	C	Ø
C:N	9.8	10.3	9.3	9.0	9.0	10.5	10.4	8.0	9.7	9.4	9.5
sd	*2.5*	*4.0*	*3.0*	*1.7*	*1.7*	*2.4*	*1.7*	*2.2*	*1.8*	*2.3*	*0.8*
min	7.5	5.3	5.7	5.5	5.9	7.2	8.7	5.4	6.8	5.4	6.3
max	14.6	16.3	13.8	13.5	13.8	14.3	13.5	13.5	14.3	16.3	14.4

field and laboratory experiments, being 10 and 9.3, respectively. As discussed in chapter 5.1.2, C availability did not restrict the immobilisation of substrate N by SMB. Therefore the above-average values of $(C:N)_{mic}$ in HY and LY soils may have been caused by a relatively better C availability.

Influenced by soil C and N parameters, the amount of fungi and bacteria, their physiological state and adaptation strategies determine the value of $(C:N)_{mic}$. The C:N ratio of fungi can range between 4.5 and 15, while that of bacteria is relatively constant between 3 and 5 (Paul and Clark, 1989). Fungal populations dominated in a range of soils tested by Anderson and Domsch (1980), making up 75% of the microbial biomass. The dominance of fungal or bacterial biomass may fluctuate during an incubation. Scheu and Parkinson (1994) determined fungal and bacterial biomass after rewetting air-dried forest soils and observed an initial dominance of bacteria during the first 10 days, followed by an increase in fungal biomass between days 10 to 40. Assuming that in the present study, bacteria had a C:N of 5 and fungi ranged between a ratio of 10 to 15, the amount of bacterial biomass, calculated by allegation alternate, will have ranged between 11% and 58% to reach a $(C:N)_{mic}$ of 9.5.

7.2 Field and Laboratory estimates

Estimations of turnover rates in the field and laboratory experiments showed similar trends, namely that HY soils had a faster turnover of C and N than LY soils. A direct relationship could be seen between turnover times of the complex substrate in the field experiment and those of the readily available substrates in the laboratory experiment, the former being 4.5 times slower than the latter for both C and N. In contrast, turnover of mustard C and N in the laboratory did not show a consistent relationship, neither between field and laboratory nor between HY and LY soils. For example, mustard C turnover times were estimated to be shorter in LY compared to HY soils. As discussed in chapter 4.2.2, these results may have been biased by the higher amount of labelled C persisting as DOC in the HY soil. Incubation of easily available substrates in closed systems of batch incubations, may be more recommendable for estimations of turnover rates, than that of complex substrates, due to long residence times and recycling of labelled products of the latter.

It is difficult to compare absolute values of turnover times from laboratory and field experiments, because of the highly variable and sometimes extreme temperature and moisture conditions in the field. Differences in turnover times were associated with differences in mean temperatures. For instance the mean temperatures during the period used for estimations of N turnover showed the same relationship between laboratory and field turnover times (4.5) and temperatures (4.9). Temperature dependence of C and N transformation processes have been widely explored (Dalias et al., 2001; Dalias et al., 2002; Kirschbaum, 1995). The overall consensus is, that data from different experimental conditions cannot simply be converted by introducing a temperature dependence function. Functions describing temperature dependence of biological C and N transformation processes are usually based on the Arrhenius or Van't Hoff function. The main element of these functions is the constant Q_{10}, describing the increase in a given process rate for a temperature increase of 10°C (Rodrigo et al., 1997). The reported values of Q_{10} vary widely and have been shown to depend on the substrate, experimental conditions and the temperature range utilised (Dalias et al., 2001; Dalias et al., 2002; Kirschbaum, 1995).

However, results of laboratory and field data allow to draw the general conclusion, that turnover times of C and N were shorter in the HY soil compared to the LY soil.

7.3 Microbial diversity and C N turnover

Bacterial diversity has been considered to be critical to system functioning because of the diversity of processes which bacteria are responsible for (Kennedy, 1999). Kandeler et al. (2002) determined a relationship between changes in community composition and changes in enzyme activity in the presence of maize roots during microcosm experiments. However, changes in species composition of the soil microbial community do not necessarily alter soil functional capability, because most microbial processes can be carried out by diverse taxa (redundance) (Hill et al., 2000). Donnison et al. (2000) found that enzyme activities were ubiquitous to changed fungal communities in differently managed haymeadows. Furthermore, a great taxonomic diversity is not necessary to ensure functional diversity, because although taxonomically related, organisms can have a high functional

diversity, as has been shown for ammonia oxidisers in agronomic systems (Phillips et al., 2000).

In the present study no direct relationship could be seen between diversity and function (as determined by C and N turnover) of the microbial community. C and N turnover rates were shown to be generally higher in HY soils compared to LY soils, under field as well as laboratory conditions. At the same time microbial community patterns based on 16S rDNA analysis showed a high similarity between HY and LY soils (81%) in the field, while in the laboratory experiment they showed clear differences.

The analyses of community patterns are based on 16S rDNA fingerprints, which can give information about the overall potential of a soil, but not about the DNA sequences actually in use. The results imply that the genetic settings of a soil do not explain differences in its nutrient turnover. Another feature that cannot be detected by the analysis of 16S rDNA is the so called microdiversity (Schloter et al., 2000). Microdiversity describes the phenomenon of structural and functional diversity below species level, caused by the evolution of different geno- and phenotypes within one species.

The differences in community patterns of laboratory HY and LY treatments were most probably induced by the pre-treatment of soil material. 16S rDNA patterns of LY soils were more closely related to that of field soils than those of HY soils, indicating that the microbial community in the LY soil was less affected by sieving, drying and storage of the soil. This assumption was also supported by the finding that SMB C and N contents in HY soils during laboratory incubations were significantly lower than in the field, while no differences were found between SMB C and N contents in LY field and laboratory treatments. SMB in the HY soil showed a faster turnover of added substrate C and N, indicating a higher microbial activity and thus an increased susceptibility to drying (Van Gestel et al., 1993).

A possible explanation for the different responses of HY and LY SMB to soil drying is the landscape position of HY and LY areas, which may have influenced stress resistance in microbial populations. HY areas in the field are situated in a former erosion gully, while LY areas are localised on a rounded hilltop. Landscape position influences soil hydrology (Huggett, 1975), and different yield performance in the HY and LY areas of the agricultural field investigated, are supposed to be highly related to water

availability (Schmidthalter et al., 2002). Thus it can be assumed that the microbial population in the LY area was more exposed to desiccation stress than that in the HY area. Therefore LY microbial populations may have been in a different physiological state than HY microbial populations and were more adapted to survive desiccation. Common responses to desiccation and other stresses are the reduction of cell size and the production of dormancy stages (van Overbeek and van Elsas, 1997). The ability to produce resting stages even under optimal conditions constitutes a risk-adverse strategy for a species population (Bakken, 1997).

Bacteria are known to survive for many decades in stored dried soils (Paul and Clark, 1989). Drying and rewetting events have often been reported to result in a decrease in microbial biomass (Pulleman and Tietema, 1999; Van Gestel et al., 1992; Van Gestel et al., 1993). In contrast, Fierer and Schimel (2002) found that the pool of microbial biomass C was increased by frequent exposure to drying-rewetting events in soils from a Mediterranean climate, which they contributed to a better adaptation of the soil microbes to desiccation stress. In their study the effects of stress history induced changes in microbial C and N dynamics, which lasted for more than a month after the stress, even when control and treated soils were incubated at the same temperature and moisture content. Assuming that LY microbial population had a different stress history than HY populations, LY populations were more adapted to stress induced by pre-treatments and the effects on microbial biomass C and N did not cease during pre-incubations, but may have lasted throughout the period of laboratory incubations.

7.4 Conclusions

The different yield performance in HY and LY areas of the agricultural field was partially reflected in the size of the SMB. Under field conditions the HY area had a higher microbial biomass compared to the LY area, but under laboratory conditions this was reversed.

A positive relationship was seen between yield performance and the turnover of C and N through SMB in field as well as laboratory treatments, with the one exception of mustard C turnover in the laboratory.

Soil microbial diversity as determined by 16S rDNA analysis, did not correspond with neither yield nor turnover patterns and differences in soil microbial communities of HY and LY soil in the laboratory experiment were most probably related to soil pre-treatment.

Comparing laboratory and field data, the estimated turnover times in the laboratory were always faster than those in the field. A direct relationship could be seen between turnover times of mustard in the field and glucose/nitrate in the laboratory, the former being 4.5 times slower than the latter. Although the differences in turnover times seemed to be at least partially related to temperature differences in the laboratory and the field, it is difficult to compare absolute values of turnover time. However, the results of laboratory and field data allow to draw the general conclusion, that turnover times of C and N were higher in the HY soil compared to the LY soil.

The assumption that the N available for plant uptake comes directly from the SMB (Smith, 1994), implies that a faster turnover of N through the microbial biomass, supported by a faster turnover of C as an energy source, involves a better nutrient supply of the plants. In the HY soil this correlates positively with the higher agricultural yield observed in these areas.

8 Summary

Soil microbial biomass (SMB) C and N dynamics, as well as microbial community patterns were studied under laboratory and field conditions in soils of high yield (HY) and low yield (LY) areas in an agricultural field. Soils were amended with ^{13}C and ^{15}N labelled mustard (*Sinapis alba*) residues (both experiments) and labelled glucose/nitrate solution (laboratory only) at 500 µg C g^{-1} and 30 µg N g^{-1} dry soil. SMB C and N, dissolved organic carbon (DOC), mineral N (N_{min}) and total C and N content were monitored in both the field and the laboratory experiments. CO_2 and N_2O efflux were additionally measured in laboratory treatments. Isotope ratios were determined for SMB in both experiments, for all other parameters only in the laboratory treatments. For the analysis of microbial community patterns, total DNA was extracted from HY and LY soil material from the three laboratory treatments (mustard, glucose/nitrate, control) of 5 sampling dates (day 0, 13, 20, 34 and 98) as well as from a field sample collected on the day of sampling for the laboratory experiment. DNA fingerprints were generated from amplification of genomic DNA with random primers and from amplification of 16S rDNA with specific primers.

A positive priming effect was measured in three of four laboratory treatments. Priming was induced after a significant increase of soil derived C in the biomass and C loss through priming was always smaller than or equal to the decline in microbial biomass C. Total N_2O losses during laboratory incubations were significantly higher in HY soils compared to LY soils in all treatments including controls.

During the field experiment, biomass C and N contents in the HY plots exceeded those in the LY plots, whereas this was reverse during the laboratory experiment. In the laboratory, biomass C and N contents in the HY soil were significantly lower than in the field HY plots, while no differences were determined between biomass C and N contents in LY field and laboratory treatments.

In the laboratory experiment, SMB in the HY soil immobilised less of the added substrate C than LY soil SMB, whereas in the field experiment HY SMB immobilised more. Calculated turnover times in the laboratory

glucose, laboratory mustard and field mustard amendments were 0.24, 0.58 and 1.09 years (HY) and 0.31, 0.44 and 1.25 years (LY), respectively.

Less amounts of added substrate N were immobilised by SMB in HY soils compared to LY soils during the laboratory experiment. In the field, immobilisation of added N by SMB was higher in HY soils initially and slightly lower after 40 days of incubation. Calculated turnover times in the laboratory nitrate, laboratory mustard and field mustard amendments were 0.18, 0.27 and 0.74 years (HY) and 0.22, 0.61 and 1.01 years (LY), respectively.

The turnover of C as well as N in the field mustard and laboratory glucose/nitrate treatments showed a similar relationship between turnover in HY and LY soils, the first being 1.14 to 1.3 times faster than the latter. Turnover in the field took an average of 4.4 times longer than that of glucose C in the laboratory.

Microbial community patterns, derived by 16S rDNA analysis, showed a high similarity between HY and LY soils in the field experiment (81%), while those in the laboratory experiment showed clear differences. Patterns of LY soils were more closely related to that of field soils than those of HY soils.

DGGE banding patterns indicated that the microbial community in the LY soil was less affected by sieving, drying and storage of the soil than the HY soil. This assumption was also supported by the finding that SMB C and N contents in HY soils during laboratory incubations were significantly lower than in the field, while no differences were found between SMB C and N contents in LY field and laboratory treatments. SMB in the HY soil showed a faster turnover of added substrate C and N, indicating a higher microbial activity and thus an increased susceptibility to drying.

The turnover times for C and N through the microbial biomass estimated from field and laboratory data were generally shorter in HY compared to LY soils. A faster turnover of nutrients in the HY soils may involve a better nutrient supply of the plants, which coincides with the higher agricultural yield observed in these areas.

9 Zusammenfassung

Abbau und Umbau organischer Substanz im Boden erfolgt hauptsächlich durch dessen mikrobiellen Gemeinschaften. Die mikrobielle Biomasse ist zudem ein dynamischer Speicher von Nährstoffen. Sie spielt eine wichtige Rolle in der Nährstoffnachlieferung. In Labor- und Feldversuchen wurde unter Einsatz stabiler Isotope der C und N Umsatz durch die mikrobielle Biomasse in Bodenmaterial von Hoch- (HE) und Niedrigertragsbereichen (NE) eines konventionell bewirtschafteten Ackers untersucht. Zusätzlich wurde die Zusammensetzung der mikrobiellen Populationen mit molekularbiologischen Methoden (DNA fingerprints) untersucht, um diese in Zusammenhang mit den Stoffumsätzen zu stellen.

Dazu wurde das Bodenmaterial aus den beiden Bereichen des Ackers in Feld- und in Laborversuchen mit ^{13}C und ^{15}N markierter Senf (*Sinapis alba*), sowie, ausschließlich im Laborversuch, markierte Glukose-Nitrat-Lösung versetzt. Das zugegebene Substrat hatte einen C und N Gehalt von 500 g C g^{-1}, bzw. 30 µg N g^{-1} Trockenboden, bei einem Isotopengehalt von 101,8 ‰ PDB ^{13}C und 18 at% ^{15}N. Im Feld- wie im Laborversuch wurden der C und N Gehalt der mikrobiellen Biomasse, der gelöste organische C (DOC), gelöster N (N_{dis}), sowie der gesamte C- und N-Gehalt des Bodens gemessen. Im Labor wurden zusätzlich CO_2 und N_2O Emissionen bestimmt. Isotopengehalte wurden im Laborversuch für alle Parameter außer dem N_2O bestimmt, im Feldversuch nur für den mikrobiellen Biomasse-C und -N, sowie für den gesamten C-Gehalt des Bodens. Die Analyse der mikrobiellen Diversität erfolgte über DNA fingerprints. Die DNA Extraktion erfolgte aus Bodenmaterial von HE und NE aus den drei Laborvarianten (Kontrolle, Glukose-Nitrat, Senf) von 5 Probenahmen (Tag 0, 13, 20, 34, 98), sowie aus einer Feldprobe von dem Tag der Bodenentnahme für den Laborversuch. DNA fingerprints wurden durch die PCR (Polymerase Chain Reaction) - Amplifikation der genomischen DNA mit Zufallsprimern und der 16S rDNA mit spezifischen Primern erstellt.

Ein positiver Priming Effekt konnte in drei der vier Laborvarianten nachgewiesen werden. Priming entstand immer nach einer signifikanten Zunahme von bodenbürtigem C in der mikrobiellen Biomasse. Der C-Verlust durch Priming war dabei immer geringer oder gleich der Abnahme von C in der mikrobiellen Biomasse. Kumulierte N_2O Emissionen während

des Laborversuchs waren in allen Versuchsvarianten, einschließlich Kontrollen, im HE signifikant höher als im NE.

Im Feldversuch waren die C- und N-Gehalte der mikrobiellen Biomasse im HE stets höher als im NE, währen dies im Laborversuch umgekehrt war. Die Biomasse-C und –N Gehalte des HE im Labor waren signifikant niedriger als im Feld, währen jene im NE keine Unterschiede zwischen Labor und Feld aufwiesen.

Im Laborversuch bauten die Mikroorganismen des NE mehr substratbürtigen C in ihre Biomasse ein als die des HE, während dies im Feldversuch umgekehrt war. Errechnet aus der Isotopenverdünnung in der mikrobiellen Biomasse, betrugen die Umsatzzeiten in den Laborvarianten „Glukose-Nitrat“ und „Senf“ und in der Feldvariante „Senf“ 0,24, 0,58 und 1,09 Jahre im HE, bzw. 0,31, 0,44 und 1,25 Jahre im NE.

Auch vom substratbürtigen N wurde im Labor im NE mehr in die mikrobielle Biomasse aufgenommen als im HE. Im Feldversuch wurde im HE anfangs mehr N immobilisiert als im NE, nach 40 Tagen war dies etwas weniger. Die Berechnung der Umsatzzeiten für die Laborvarianten „Glukose-Nitrat“ und „Senf“ und die Feldvariante „Senf“ ergaben im HE 0,18, 0,27 und 0,74 Jahre und im NE 0,22, 0,61 und 1,01 Jahre.

Die Umsatzzeiten für C und N in der Feldvariante „Senf“ und der Laborvariante „Glukose-Nitrat“ waren im HE 1,14-1,3 mal kürzer als im NE. Der Umsatz im Feld war dabei im Durchschnitt 4,4 mal langsamer als im Labor. Nach Zugabe von Senf in Feld- und Laborversuch waren zwischen den Umsatzzeiten in HE und NE allerdings keine Zusammenhänge erkennbar.

Die Analysen der 16S rDNA fingerprints ergaben, dass die genetische Zusammensetzung der mikrobiellen Populationen im Feld in HE und NE sehr ähnlich war (81 %). Im Labor hingegen zeigten sich deutliche Unterschiede zwischen Populationen aus HE und NE. Dabei waren die Bandenmuster des NE denen aus dem Feld ähnlicher als die des HE.

Die DGGE Bandemuster wiesen darauf hin, dass die mikrobielle Population im NE durch die Vorbehandlung des Bodenmaterials für den Laborversuch (sieben, trocknen) weniger beeinflusst wurde als die Population im HE. Die Unterschiede in den Biomasse-C und –N Gehalten in HE und NE in Labor und Feld unterstützen diese Annahme. Die mikrobielle Biomasse im HE

setzte den zugegebenen C und N schneller um, zeigte also eine höhere mikrobielle Aktivität und ist daher anfälliger für den Trockenstress bei der Bodenvorbehandlung gewesen.

Die Umsatzzeiten von C und N durch die mikrobielle Biomasse, welche für die Feld- und Laborvarianten berechnet wurden, waren generell im HE kürzer als im NE. Ein schnellerer Umsatz von Nährstoffen, bedingt durch eine bessere Energieversorgung aus einem schnelleren C-Umsatz, kann eine bessere Nährstoffversorgung der Pflanzen gewährleisten. Im Fall der Hoch- und Niedrigertragsbereiche stimmten die Umsatzraten mit den unterschiedlichen Ertragspotentialen überein.

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Tables and Figures

Fig. 5.1. Amount of nitrate and mustard derived mineral N in $CaCl_2$ extracts of high yield (HY) and low yield (LY) soil material incubated under laboratory conditions over 98 days.

Fig. 5.2. Cumulative N_2O emissions over 98 days in control, nitrate and mustard treatments of high yield (HY) and low yield (LY) soil material in the laboratory experiment.

Fig. 5.3. Biomass N dynamics in **A**: high yield and **B**: low yield soils after mustard addition in the field experiment: mean total biomass N in controls (column), total biomass N ( ) and estimation of turnover times T [y] through the decline in mustard derived biomass N ( ) using a first order exponential function (– · –).

Fig. 5.4. Biomass N dynamics in **A**: high yield and **B**: low yield soils after nitrate addition in the laboratory experiment. mean total biomass N in controls (column), total biomass N ( ) and estimation of turnover times T [y] through the decline in nitrate derived biomass N ( ) using a first order exponential function (– · –).

Fig. 5.5. Biomass dynamics in **A**: high yield and **B**: low yield soils after mustard addition in the laboratory experiment. mean total biomass N in controls (column), total biomass N ( ) and estimation of turnover times T [y] through the decline in mustard derived biomass N ( ) using a first order exponential function (– · –).

Fig. 6.1. Genetic similarity of community fingerprints obtained with random primer polymerase chain reaction (ERIC PCR) – PAGE from the target DNA extracted in triplicates from five sampling dates (day 0, 13, 20, 34 and 98) of each laboratory treatment (C: control, M: mustard, GN: glucose/nitrate) with high yield (HY) and low yield (LY) soil material.

Fig. 6.2. Genetic similarity of community fingerprints obtained with random primer polymerase chain reaction (ERIC PCR) – PAGE from the target DNA extracted from high yield (HY) and low yield (LY) soil material from the field and from three experimental treatments (C: control, GN: glucose/nitrate, M: mustard) on the 13th day of incubation. All treatments were extracted in triplicates.

Fig. 6.3. PAGE pattern of DNA fragments obtained by PCR with random primers from high yield (HY) and low yield (LY) soil material from the field and from three laboratory treatments (C: control, G/N: glucose/nitrate, M: mustard) at five sampling dates (day 0, 13, 20, 34 and 98) in three independent replicates (1, 2, 3).

Fig. 6.4. Genetic similarity of community fingerprints obtained with polymerase chain reaction (PCR) – DGGE from the target DNA extracted from high yield (HY) and low yield (LY) soil material of three experimental treatments (A: control, B: glucose/nitrate, C: mustard) on five sampling dates (day 0, 13, 20, 34 and 98).

Fig. 6.5. Genetic similarity of community fingerprints obtained with polymerase chain reaction (PCR) – DGGE from the target DNA extracted from high yield (HY)

and low yield (LY) soil material from the field and from three laboratory treatments (control, glucose/nitrate, mustard) on the 4th sampling date (day 13).

Fig. 6.6. DGGE patterns of 16SrDNA fragments obtained by polymerase chain reaction (PCR) from the target DNA extracted from high yield (HY) and low yield (LY) soil material from the field and three laboratory treatments (C: control, G/N: glucose/nitrate, M: mustard) of the 4th sampling date (day 13). U: unique bands, L1: laboratory specific bands, L2: HY laboratory specific bands, G/N: enhanced bands after glucose/nitrate treatment. Ladder: 16S rDNA PCR products of six pure cultures: *Arthrobacter citreus*, *Cytophaga xanta*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Burkholderia cepacia* and *Azospirillum brasiliense.*

Danksagung

Die vorliegende Arbeit wurde am Institut für Bodenökologie des GSF Forschungszentrums für Umwelt und Gesundheit, Neuherberg, im Rahmen des vom BMBF finanzierten Forschungs-verbundes Agrarökosysteme München (FAM) erstellt.

An erster Stelle möchte ich Herrn Prof. Dr. Jean Charles Munch danken, welcher mir dieses Thema zur Bearbeitung überlassen hat. Ich danke ihm besonders für die wissenschaftliche Betreuung, die vielseitige Unterstützung und die Diskussionsbereitschaft im Verlauf der Arbeit.

Frau Prof. Kögel-Knabner und Herrn Prof. Hock sei für die bereitwillige Übernahme des Co-Referats bzw. Prüfungsvorsitzes gedankt.

Franz Buegger hat mir die Isotopenmessung unzähliger Proben ermöglicht, ich danke ihm für seine geduldige Einführung in die Welt der stabilen Isotope und für die MS-Telefonhotline. Den MitarbeiterInnen der AG Adam Zsolnay danke ich für die Hilfe bei Analysen am TOC und Skalar. Der AG von Michael Schloter danke ich für die Möglichkeit den molekularbiologischen Teil der Arbeit durchzuführen und für die großartige Unterstützung im Labor. Hier sei auch diversen Hiwis, Zivis und Praktikanten gedankt, die mir im Laufe der Zeit im Labor zur Hand gegangen sind.

Ich freue mich über die guten Freundschaften, die während dieser drei Jahre entstanden sind und möchte all meinen KollegInnen danken, die für eine schöne Arbeitsatmosphäre am IBÖ gesorgt haben: meine Bürogenossinnen Karin Pritsch und Uli Sehy, Viviane Barbosa da Silva und Marc Marx (vamos tomar um cafezinho?), Alex Hagn, Jens Junkers, Heidrun Karl, Kerstin Goerke, Petra Jäckel, Andreas Gattinger, Miguel Jimenez, Arndt Embacher, Reiner Ruser und Michael Schloter. Sie alle haben mir durch fachliche Diskussionen, kritische Durchsicht von Manuskripten oder einfach nur ein Schwätzchen, in dieser Zeit weitergeholfen.

Meinen Eltern und Geschwistern und Marlon danke ich für viele Dinge, besonders aber für das schöne Gefühl, immer auf ihre Unterstützung zählen zu können. Wie kein anderer hat Marlon alle Höhen und Tiefen während dieser drei Jahre mit mir durchlebt, hat sich mit mir gefreut oder mich wieder aufgebaut und motiviert. Dafür meinen allerherzlichsten Dank.

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