

Effect of cattle faeces with different microbial biomass content on soil properties, gaseous emissions and plant growth

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Abstract A study was carried out to investigate the effects of different diets for heifers, low- and high-yielding cows on the microbial composition of their faeces and subsequently the impacts of these faeces on CO₂ and N₂O emissions, N mineralisation and plant N uptake. A diet low in N and high in acid detergent fibre offered to heifers resulted in faeces dominated by fungi. These faeces were characterised by a low content in microbial biomass C and N and a high ergosterol concentration in comparison to the faeces of high-yielding cows. Added to soil, faeces of heifers led to lower emission and stronger N immobilisation during a 14-day incubation in comparison to the faeces of high-yielding cows. Total N₂O emission was significantly ($P < 0.05$) correlated with faecal microbial biomass N. Rye grass yield and N uptake were lowest in the soil supplemented with faeces from heifers in a 62-day pot experiment. Plant N uptake was influenced by the faecal microbial biomass C/N ratio and the fungal C to bacterial C ratio. In conclusion, the faecal microbial biomass was affected to a high degree by the feeding regime and faecal microbial characteristics revealed higher impacts on plant N uptake than soil microbial properties.

Keywords Cattle faeces · Microbial biomass C and N · Ergosterol · Amino sugar · Gaseous emission · N mineralisation

Introduction

Solid manure from farm animals differs considerably in total N concentration and N availability to soil microorganisms and plants, due to differences in diet digestibility, diet conversion by different animal species, age of the animal and water intake (Chadwick et al. 2000). In faeces, more than 90 % of total N is present in organic forms (Jost et al. 2011), bound mainly in amino acids but also in heterocyclic components (Bosshard et al. 2011) or amino sugars (Rezaeian et al. 2004a, b, 2006; Jost et al. 2011). Mineralisation of these organic forms is a prerequisite for plant uptake. Solid manure low in total N content and high in plant cell wall components decreased net N mineralisation in laboratory incubation (Cusick et al. 2006; Morvan and Nicolardot 2009; Peters and Jensen 2011), greenhouse pot (Ikpe et al. 2003; Wu and Powell 2007) and field experiments (Sørensen et al. 2003; Reijs et al. 2007; Powell and Grabber 2009). The feeding regime also has significant impacts on the faeces-derived emissions of NH₃ (Merino et al. 2008; van der Stelt et al. 2008) and N₂O (Flessa et al. 2002a; Arriaga et al. 2010), causing serious N losses and atmospheric pollution.

Furthermore, differences in the chemical composition of the organic components affect the bacterial community of faeces (van Vliet et al. 2007). Size and community structure of faecal microorganisms may control the decomposition of the faeces and the release of inorganic N after entering the soil (Chadwick et al. 2000). It is well documented in various experiments with other organic material that the substrate

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colonising microbial community has an important influence on further decomposition processes (Flessa et al. 2002b) and directly adds significant amounts of microorganisms to the autochthonous soil microbial biomass (Rasul et al. 2008). Cattle faeces contain a highly dynamic community of bacteria, archaea and fungi which have not yet been qualified or quantified (Frostegård et al. 1997; Gattinger et al. 2007). Increased knowledge of the interactions between faecal composition and its behaviour in soil can lead to various strategies for livestock management and optimal utilisation, including method and time of application (Handayanta et al. 1997; Delve et al. 2001). As cattle diet and feed intake vary considerably between feeding regimes, influencing intestinal microbial parameters and direct measurements of the microbial community in faeces are an important source of information.

The objectives of this study were to assess the effects of different feeding regimes for heifers, low- and high-yielding cows on faecal composition and subsequently to assess the impacts of these faeces on N_2O emissions, N mineralisation and plant N uptake. Faeces quality has been characterised microbially by determining microbial biomass C and N using the chloroform fumigation extraction (CFE) method and also fungal ergosterol and the fungal cell wall components' muramic acid and glucosamine (Jost et al. 2011). The CFE method is suited to differentiate accurately between living and dead microbial tissue (Brookes et al. 1985; Vance et al. 1987). Fungal ergosterol is an important component of fungal cell membranes (Weete and Weber 1980). It has been repeatedly used as an index for fungal biomass in soil (Bååth and Anderson 2003), other solid substrates (Newell 1992) and in batch cultures of yeasts (Park et al. 1990). Fungal glucosamine and bacterial muramic acid were measured as independent control values for both CFE microbial biomass and ergosterol data (Appuhn and Joergensen 2006; Indorf et al. 2011; Jost et al. 2011).

Materials and methods

Soil

Soil samples were collected in March 2011 at 0–15-cm depth from the site “Saurasen”, located in the north of Hesse, Germany. The site is at 280 m above sea level, with an average annual precipitation of 625 mm and a temperature of 6.5°C. Developed from eroded loess overlying clayey sandstone, the soil is classified according to the WRB classification system as Stagnic Luvisol (Quintern et al. 2006). The particle size distribution was 6 % sand, 72 % silt and 22 % clay. Soil organic C and total N content were 8.2 and 0.89 mg g⁻¹, respectively. The soil had a pH (CaCl₂) of 6.4, and a water holding capacity of 50 %. After

sampling, the soil was homogenised, sieved (<2 mm) and stored moist in polyethylene bags at room temperature for 18 days before the experiments started.

Faeces sampling and quality determination

Faeces samples were taken once at the same time from 18 cattle, six heifers, six high-level and six low-level yielding dairy cows (*Bos primigenius taurus*, var. German Holstein) from a cattle breeding farm in Lower Saxony. Heifers were fed ad libitum with a silage mix of grass and straw. The low- and high-yielding dairy cows (approx. <25 and 25–40 kg milk cow⁻¹ day⁻¹, respectively) received the same silage within a total mixed ration but with different supplementary concentrate of 5 and 9 kg d⁻¹, respectively. Crude protein content was highest in the diet of the high-yielding cows, followed by the low yielding cow fodder (data not shown). The feeding ration for the heifers contained more neutral detergent fibre (NDF) than the other diets (491 versus 478 and 445 g kg⁻¹ for low- and high-yielding cows, respectively). Faeces samples were taken rectally, immediately homogenised, frozen in liquid nitrogen and stored at -18°C. This preservation technique has been previously tested; no significant differences were found between such preserved samples and fresh samples that were analysed immediately after sampling (data not shown). A preserved subsample was dried for 72 h at 65°C and finely ground for determination of faeces dry weight and chemical analyses. Total C, N, ammonium, crude ash and also the other organic components NDF, acid detergent fibre (ADF), acid detergent lignin (ADL), undigested dietary N (UDN) and undigested dietary C (UDC) were determined by near-infrared spectroscopy (FOSS 6500, Rellingen, Germany), as described by Althaus and Sundrum (University of Kassel, Witzenhausen, Germany, personal communication), after appropriate calibration and validation. Cellulose was calculated as the difference between ADF and ADL; hemicellulose was calculated as the difference between NDF and ADF. Easily decomposable C (C_E) and N (N_E) fractions in the feces were defined and calculated by subtraction of UDC and UDN from total faecal C and N, respectively. For further detail, see Sundrum et al. (2011). All data are shown as the mean of six cows for each treatment.

Incubation experiment

The experiment comprised three soil treatments and one control: (1) faeces from heifers, (2) faeces from low-yielding dairy cows, (3) faeces from high-yielding dairy cows and (4) control without faeces. Each treatment was replicated six times; faeces treatments comprised six individual faecal samples. The experiment was carried out in 1-l preserving jars. Each jar was filled with 50 g soil (on an

oven-dry basis) at a bulk density of 1.0 g cm^{-3} . In the treatments with faeces addition, freshly thawed faeces were thoroughly mixed with the soil before filling the jars. The application rate was 20 mg freshly thawed faeces g^{-1} soil dry weight (DW). This amount was equivalent to 0.4% of the soil DW and comprised an addition of 73 to 86 mg C per jar (18 – 21%) and 2.7 to 5.3 mg N per jar (6 – 12%).

The jars were incubated for 14 days at 22°C and kept in the dark. At day 0 , soil samples were removed from each jar to determine inorganic N. Soil respiration was measured as CO_2 and N_2O emissions. When starting the incubation, 10 ml of 0.5 M NaOH was placed at the jar bottom and the lid was closed immediately. The NaOH solution was changed at days 3 , 7 and 14 . Evolved CO_2 was determined by back titration to $\text{pH } 8.3$ of the excess NaOH with 0.5 M HCl after addition of 0.5 M BaCl_2 solution. Nitrous oxide emissions were measured at days 0 , 1 , 2 , 3 , 7 and 14 . A 10-ml gas sample was taken out of the jar through a three-layer silicone septum (Hamilton Company, NV, USA) with a plastic syringe. The samples were analysed immediately using a gas chromatograph GC-14B (Shimadzu Corporation, Kyoto, Japan). After 14 days, inorganic N and microbial biomass C and N were determined.

Pot experiment

The experiment had three soil treatments and one control: (1) faeces from heifers, (2) faeces from low-level dairy cows, (3) faeces from high-level dairy cows and (4) control without faeces. Each treatment was replicated six times; faeces treatments comprised six individual faecal samples. The experiment was carried out in plastic pots (2.7 l , $13 \times 13 \text{ cm}$). Each pot was filled with 2.4 kg soil (on an oven-dry basis) at a bulk density of 1.0 g cm^{-3} . In the three soil treatments, faeces were thoroughly mixed with the soil before filling the pots. The application rate was 20 g freshly thawed faeces kg^{-1} soil. This amount was equivalent to 0.4% of the soil DW and comprised an addition of 3.5 to 4.1 g C per pot (1.4 – 1.7 g C kg^{-1} soil) and 130 to 252 mg N per pot (54 – 105 mg N kg^{-1} soil).

Italian ryegrass (*Lolium multiflorum*, breed Ligrande, from Deutsche Saatveredelung AG) was sown at a density of 250 seeds per pot (1.5 seeds cm^{-2}). The pots were arranged in a randomised complete block design and placed in a climate chamber with a $16/8 \text{ h}$ light/dark cycle and peak temperatures of 20°C (day) and 12°C (night). Air humidity varied between 40% (day) and 90% (night). Soil moisture was kept at 50% of the water-holding capacity by weighing and adding the water lost three times a week. Thirty days after germination, the ryegrass plants were cut 5 cm above ground, when the mean plant height was 15 cm . Total aboveground plants were subsequently harvested 62 days after germination. Samples were dried for 72 h at 40°C for

plant dry weight and then finely ground to determine total C and N by combustion in a CNS Analyser (Elementar Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). Mineral N was determined in soil subsamples after harvesting.

Microbial biomass C and N

Microbial biomass C and N were estimated by the “chloroform fumigation extraction method” (Brookes et al. 1985; Vance et al. 1987). All soil samples were fumigated, extracted and measured for total C and N as described below. Two subsamples of 10 g fresh soil were taken for the analysis. One subsample was fumigated at 25°C with ethanol-free CHCl_3 , which was removed after 24 h . Fumigated and non-fumigated portions were extracted with 40 ml $0.5 \text{ M K}_2\text{SO}_4$ for 30 min by horizontal shaking at 200 rev min^{-1} . Soil extracts were filtered (folded filter paper, hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was calculated as follows: microbial biomass C = E_C/k_{EC} , where E_C = (organic C extracted from fumigated faeces) – (organic C extracted from non-fumigated faeces) and k_{EC} = 0.45 (Wu et al. 1990; Joergensen 1996). Total N in the extracts was measured by chemoluminescence detection. Microbial biomass N was calculated as follows: microbial biomass N = E_N/k_{EN} , where E_N = (organic N extracted from fumigated faeces) – (organic N extracted from non-fumigated faeces) and k_{EN} = 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996).

Faeces samples were fumigated and measured for C and N as described above, while the extraction was carried out as described by Jost et al. (2011). Two freshly thawed subsamples equivalent to 0.5 g oven-dry faeces were taken for the analysis. Fumigated and non-fumigated portions were extracted with 100 ml of 0.05 M CuSO_4 for 30 min by horizontal shaking at 200 rev min^{-1} . Following centrifugation ($2,000 \times g$ for 10 min), faeces extracts were filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Carbon and N standards for calibration of the Dimatoc 100 analyser were prepared in 0.05 M CuSO_4 solution.

Ergosterol analysis

The fungal cell membrane ergosterol was extracted and measured in soil according to Djajakirana et al. (1996). In this method, 0.5 g of moist soil was extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 rev min^{-1} . After filtering, the soil extract was evaporated in a vacuum rotary evaporator at 40°C . The non-polar fraction was dissolved in 5 ml methanol and stored at 4°C until

measurement. For determination of ergosterol in faeces, the extraction method of Zelles et al. (1987) was used. Freshly thawed faeces equivalent to 0.5 g DW were placed into 30-ml test tubes and treated with 10 ml methanol, 2.5 ml ethanol and 1 g KOH. The samples were saponified for 90 min at 70°C under reflux. After cooling, ergosterol was extracted in two steps with 15+10 ml petroleum ether. From the supernatant, 15 ml was evaporated in a vacuum rotary evaporator at 40°C. The non-polar fraction was dissolved in 5 ml methanol and stored at 4°C until measurement (Jost et al. 2011). Ergosterol was determined by reversed-phase HPLC with 100 % methanol as the mobile phase and detected at a wavelength of 282 nm.

Amino sugar analysis

The amino sugars muramic acid, glucosamine and galactosamine were determined in faeces samples according to Indorf et al. (2011). Two grams of moist fresh faeces was weighed into 20-ml test tubes, mixed with 10 ml of 6 M HCl and heated for 2 h at 105°C. HCl was removed from the filtered hydrolysates in a vacuum rotary evaporator at 40°C, and the samples were centrifuged ($5,000 \times g$) and then transferred to vials and stored at -18°C until the HPLC measurements. Chromatographic separations were performed at 35°C on a Phenomenex (Aschaffenburg, Germany) HyperClone C₁₈ column (125-mm length \times 4-mm diameter), protected by a Phenomenex C₁₈ security guard cartridge (4-mm length \times 2-mm diameter). The HPLC system consisted of a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS-3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445-nm emission and 330-nm excitation wavelengths with medium sensitivity. *o*-Phthalaldehyde (OPA) reagent (Merck, Darmstadt), sample and preparation vials were stored in the autosampler at 15°C. For the automated precolumn derivatisation, 30 μl of sample was mixed with 50 μl of OPA in a preparation vial and then 15 μl of the indole derivatives was injected after 120-s reaction time. The mobile phase consisted of two eluents and was delivered at a flow rate of 1.5 ml min⁻¹. Eluent A was a 97.8/0.7/1.5 (v/v/v) mixture of an aqueous phase, methanol and tetrahydrofuran (THF). The aqueous phase contained 52 mmol sodium citrate and 4 mmol sodium acetate, adjusted to pH 5.3 with HCl, to which the methanol and THF were then added. Eluent B consisted of 50 % water and 50 % methanol (v/v).

Fungal C (in milligrams per gram dry weight) was calculated as an index of fungal residues by subtracting bacterial glucosamine from total glucosamine, assuming that muramic acid and glucosamine occur at a 1 to 2 molar ratio in bacterial cells (Engelking et al. 2007): milligrams fungal C per gram dry weight = (mmol glucosamine–mmol

muramic acid) \times 179.2 gmol⁻¹ \times 9, where 179.2 is the molecular weight of glucosamine and 9 the conversion value of fungal glucosamine to fungal C (Appuhn and Joergensen 2006). Bacterial C (in micrograms per gram dry weight) was calculated as an index for bacterial residues by multiplying the concentration of muramic acid in micrograms per gram dry weight by 45 (Appuhn and Joergensen 2006).

Inorganic N

Non-fumigated K₂SO₄ soil extracts were analysed for NH₄⁺-N and NO₃⁻-N by colorimetric analysis with a Continuous Flow Analyser (Evolution2, Alliance Instruments, Friedrichsdorf) at 540 nm. Net mineralised N from soil organic N in each treatment was calculated as the sum of the inorganic N forms at the end of the incubation time minus the initial inorganic N in soil.

Statistical analysis

The results of tables are arithmetic means and expressed on an oven-dry basis (about 72 h at 60°C). The significance of difference was tested by one-way analysis of variance. All statistical analyses were performed using JMP 7.0 (SAS Inst. Inc.).

Results

Differences in faeces characteristics

Differences in the feeding regime between the three treatments resulted in clear differences in the composition of their faeces (Table 1). The concentration of total N, the C/N ratio, N_E and hemicellulose content differed significantly ($P < 0.05$) between the faeces treatments. In contrast, the concentration of UDN, ADF and cellulose showed no significant differences between treatments. Easily decomposable carbon was higher in the faeces of heifers than in the other groups. In faeces of high-yielding cows, the faecal C/N ratio was lower with a higher dietary N concentration compared to the other treatments. Concerning faecal chemical properties, negative correlation coefficients were found between total N and ADF ($r = -0.72$, $P < 0.001$) and between N_E and ADL ($r = -0.80$, $P < 0.001$).

Microbial biomass C, microbial biomass N and bacterial muramic acid were higher in the faeces of high-yielding cows in comparison to the faeces of heifers ($P < 0.05$) (Table 2). Microbial indices revealed no significant differences between the faeces of low- and high-yielding cows, except for ergosterol. This fungal biomarker was significantly ($P < 0.05$) lower in the faeces of low-yielding cows than in the other faeces. The concentrations of glucosamine, galactosamine and

Table 1 Elemental composition and organic components in different cattle feeding regimes and in cattle faeces

	N	NH ₄ ⁺	C/N	N _E	UDN	UDC	NDF	ADF	Cellulose	Hemicellulose	Crude ash
	(mg g ⁻¹ DW)			(mg g ⁻¹ DW)							
Feeding (TMR)											
Heifer	21						491	342	267	149	185
Low yielding	21						478	246	240	232	106
High yielding	25						445	214	234	231	92
Faeces											
Heifer	16 a	1.7 a b	28 a	0.93 a	6.3 a	25 a	472 a	383 a	281 a	89 a	196 a
Low yielding	22 b	1.5 a	20 b	1.52 b	7.1 a	29 a	512 a b	379 a	288 a	133 b	158 b
High yielding	28 c	2.1 b	16 c	2.17 c	6.8 a	30 b	522 b	353 a	281 a	169 c	150 b
CV (±%)	12	19	10	17	9.3	3.4	6.0	5.8	5.4	16	6.5

Different letters indicate a significant difference (Tukey–Kramer, $P < 0.05$)

N_E easily decomposable N, *UDN* undigested dietary N, *UDC* undigested dietary C, *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *DW* dry weight, *TMR* total mixed ration, *CV* pooled coefficient of variation between feeding regimes (cow replicates $n = 6$)

microbial C did not differ between faeces of the three treatments. Faecal microbial biomass C/N ratio and the ratio of fungal C to bacterial C showed a strong positive correlation with $r = 0.79$ ($P < 0.001$). Both ratios significantly ($P < 0.05$) declined with increasing N content (Table 3). The correlation coefficients were $r = -0.76$ and $r = -0.74$, respectively ($P < 0.001$). The ratio of ergosterol to microbial biomass C was lowest in the faeces of heifers, which in contrast revealed the highest ratio of fungal glucosamine to ergosterol. Faecal UDC correlated negatively with the microbial biomass C/N ratio ($r = -0.74$, $P < 0.001$) and with the fungal C to bacterial C ratio ($r = -0.88$, $P < 0.001$).

Effects of faeces types on soil microorganisms and grass growth

The addition of the cattle faeces to the soil significantly ($P < 0.05$) increased the contents of soil microbial biomass N and fungal ergosterol (Table 4). However, the increase in soil microbial biomass C was only significant ($P < 0.05$) in the

case of faeces from the low-yielding cows. Faeces addition increased the amounts of CO₂ evolved; CO₂ emission was higher when faeces of the high-yielding cows were added in comparison with the other two faeces types ($P < 0.05$). The release of CO₂-C from faeces was negatively correlated with the faecal microbial biomass C/N ratio ($r = -0.69$, $P = 0.002$) and positively correlated with soil ergosterol ($r = 0.78$, $P < 0.002$). Addition of faeces to soil generally led to immobilisation of inorganic N, i.e. negative net N mineralisation. Nitrogen immobilisation in soil was significantly higher when faeces of heifers were supplemented, compared with the faeces of the other two treatments. Faeces addition generally increased N₂O emission rates. A clear increase of N₂O emission occurred especially in the treatment with faeces of high-yielding cows (Fig. 1). Total N₂O emission was significantly correlated with faecal microbial biomass N ($r = 0.58$; $P < 0.05$, $n = 24$).

In the pot experiment, inorganic N was immobilised in all the treatments, lowest in the control and highest in the treatment with faeces of heifers. Consequently, the highest

Table 2 Microbial biomass indices, amino sugar indices and microbial C in cattle faeces from different feeding regimes

Faeces type	Microbial biomass	N	Ergosterol	MurN	ManN	GlcN	GalN	Microbial C
	C							
	(mg g ⁻¹ DW)		(µg g ⁻¹ DW)	(mg g ⁻¹ DW)				
Heifer	10 a	1.3 a	4.4 a	0.22 a	0.07 a	1.5 a	0.79 a	21 a
Low yielding	14 ab	2.0 ab	2.2 b	0.41 b	0.15 b	2.0 a	1.15 a	31 a
High yielding	18 b	3.1 b	3.8 a	0.45 b	0.09 ab	2.0 a	0.83 a	32 a
CV (±%)	22	25	23	23	49	26	29	14

Different letters indicate a significant difference (Tukey–Kramer, $P < 0.05$)

MurN muramic acid, *ManN* mannosamine, *GlcN* glucosamine, *GalN* galactosamine, *DW* dry weight, *CV* pooled coefficient of variation between feeding regimes (cow replicates $n = 6$)

Table 3 Microbial indices in cattle faeces from different feeding regimes

Faeces type	Microbial biomass C/N	Ergosterol/microbial biomass C (‰)	Fungal C/bacterial C	Fungal glucosamine/ergosterol
Heifer	7.9 a	0.45 a	1.10 a	296 a
Low yielding	6.8 b	0.15 b	0.66 b	672 b
High yielding	5.6 c	0.27 a b	0.60 b	385 a b
CV ($\pm\%$)	12	11	11	12

Different letters indicate a significant difference (Tukey–Kramer, $P < 0.05$)

CV pooled coefficient of variation between feeding regimes (cow replicates $n=6$)

ryegrass yield and N uptake were found in the control treatment without the addition of faeces, and the lowest, in the treatment with faeces of heifers (Fig. 2). However, there was no significant difference in N immobilisation between the three faeces treatments. The strongest correlations with N uptake were linear relationships with faecal NE and total N content ($r=0.84$ and 0.81 , $P < 0.001$), followed by a negative relationship with the faecal microbial biomass C/N ratio ($r=-0.76$, $P < 0.001$). The ratio of faecal fungal C to bacterial C also showed a significant negative relationship with plant N uptake.

Discussion

Microbial indices

The concentrations of all microbial indices were in the middle of the range described by Jost et al. (2011) in differently fed dairy cows and heifers. The range of the data for microbial biomass C, N, and ergosterol obtained by these two studies was larger than that for the amino sugars muramic acid, glucosamine, galactosamine and mannosamine. These differences between the studies are most likely due to differences in diet composition. An N-balanced or protein-rich diet that is low in crude fibre, NDF and ADF generally supports faecal microbial and especially faecal bacterial biomass (van Vliet et al. 2007). This might also be the reason for the different contribution of microbial

biomass C obtained by fumigation extraction to total microbial C, calculated on the basis of bacterial muramic acid and fungal glucosamine. According to Amelung (2001); Amelung et al. 2008), as cell wall components, amino sugars have a tendency to be accumulated in microbial residues, suggesting that roughly 50 % of the faecal microbial C in the present study belonged to the living fraction and the other 50 % was from the remains of dead fungi and bacteria. Jost et al. (2011) reported that the living fraction contributed roughly 30 %. It cannot be completely excluded that this low percentage was caused by the different sample treatments, shock freezing in liquid N_2 in the current study and slow freezing at -18°C in the previous study by Jost et al. (2011).

In the highly dynamic situation of C and N supply in the gut, rapid microbial growth is accompanied by concomitant microbial death. Consequently, total microbial C, calculated on the basis of bacterial muramic acid and fungal glucosamine, should exceed microbial biomass estimated by fumigation extraction. However, the differences between the microbial indices should not be stressed too much due to several methodological uncertainties: It is not known (1) whether the glucosamine and muramic acid concentrations are identical in living and dead microbial tissue or (2) whether the several conversion values for microbial biomass C by the fumigation extraction method and for microbial C by amino sugar analysis may lead to the correct estimation of the respective microbial indices (Jost et al. 2011). The

Table 4 Microbial indices, CO_2 emissions and mineral N in different soil treatments after 14 days of incubation

Soil treatment	Microbial biomass			Respiration	
	C	N	Ergosterol	$\text{CO}_2\text{-C}$	N_{\min}
	($\mu\text{g g}^{-1}$ soil)				
Heifer	227 a b	62 a	0.64 a	246 a	-8.6 a
Low yielding	261 a	71 a	0.71 a	262 a	-5.5 b
High yielding	234 ab	64 a	0.67 a	330 b	-4.3 b
Control	187 b	36 b	0.38 b	52 c	3.2 c
CV ($\pm\%$)	14	24	18	10	26

Different letters indicate a significant difference (Tukey–Kramer, $P < 0.05$)

CV pooled coefficient of variation between feeding regimes ($n=6$)

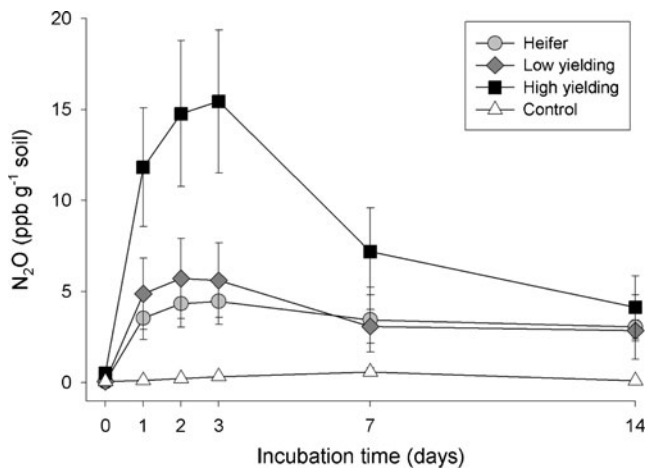


Fig. 1 Nitrous oxide emissions from different soil treatments during 14 days of incubation at 22°C; bars indicate ± 1 standard error; n=6

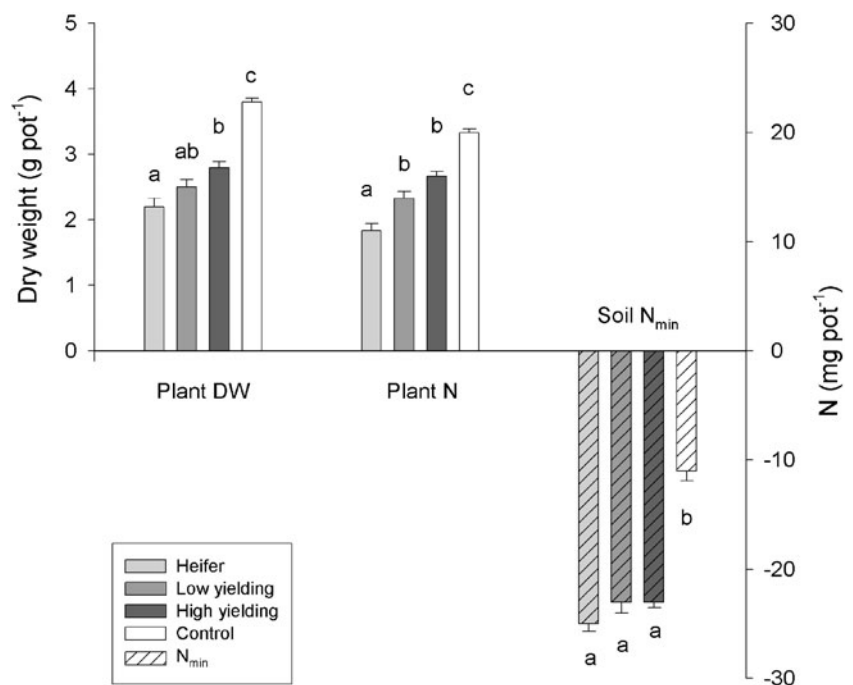
comparison is even more complex as archaea contribute a significant percentage to the microbial biomass of cattle faeces. Gattinger et al. (2007) estimated that the archaeal phospholipid etherlipids added approximately 16 % to the total phospholipid chain content in dairy cow manure. Archaea most likely add to the fraction of CHCl₃-labile C. However, they do not contain muramic acid but galactosamine, glucosamine and a variety of other rare amino sugars such as N-acetyl-L-talosaminuronic acid (Kandler and König 1998). Considering these uncertainties, the similarity in results of the different microbial indices is amazing and absolutely satisfactory (Jost et al. 2011).

Bacterial and fungal contribution to microbial tissue

In the current study, the mean faecal concentration of ergosterol was 3.5 µg g⁻¹ DW. Klamer and Bååth (2004) obtained a factor of 190 when calculating the fungal biomass C from the ergosterol concentration in 11 compost fungi species. Taking this factor into account would result in a mean fungal biomass C content of 0.67 mg C g⁻¹ DM in the present faeces samples, leading to the suggestion that ergosterol-containing fungi contributed 5 % to total microbial biomass C, which is in the range obtained by Jost et al. (2011). However, this value is considerably below the value obtained by amino sugar analysis. Of the anaerobic fungal populations, yeasts contain high concentrations of ergosterol (Aguilera et al. 2006; Ahmad et al. 2010). In contrast, chytridiomycetes contain no ergosterol but do have high concentrations of cholesterol (Weete et al. 1989; Kagami et al. 2007). Information is still lacking on the ergosterol concentration of anaerobic fungal species found in the cattle rumen such as *Anaeromyces*, *Orpinomyces*, *Caecomycetes* or *Piromyces* (Griffith et al. 2009).

On the other hand, amino sugar analysis, comprising all fungi, revealed a higher proportion of fungi in the current study. The observed fungal C to bacterial C ratio of 0.60 to 1.1 corresponds to a fungal contribution between 37 and 52 % to microbial C. These percentages are similar or slightly above the values obtained by Jost et al. (2011) for heifers from the same farm. Chitin, the polymer of glucosamine, has been previously used as an indicator for fungal biomass in rumen fluid of cattle (Sekhavati et al. 2009) and

Fig. 2 Harvest of Italian ryegrass after growing for 62 days on different soil treatments. Plant dry weight per pot, plant N content and mineral N immobilised from the soil per pot; bars indicate ± 1 standard error; different letters indicate a significant difference (Tukey–Kramer, P<0.05; n=6)



in sheep faeces (Rezaeian et al. 2004a, b). The chitin concentration in sheep faeces was $10.2 \text{ mg}^{-1} \text{ DW}$ (Rezaeian et al. 2004a). One reason for the markedly higher chitin concentration in comparison to the glucosamine concentration in the present study might be the differences in rumen and intestine physiology between sheep and cattle. An additional possible reason for this difference could be the colorimetric assay of Chen and Johnson (1983) used by Rezaeian et al. (2004a, 2006), which cannot distinguish between fungal glucosamine, bacterial glucosamine and galactosamine, and this may have overestimated fungal tissue.

Effects of feeding regime and faeces composition

Feeding regimes affected not only the chemical but also the microbial composition of cattle faeces. A diet low in N and high in ADF which was offered to heifers resulted in faeces dominated by fungi (calculated from fungal glucosamine). The faeces were low in microbial biomass C and N and also had a wide microbial biomass C/N ratio and a high ergosterol concentration, especially in comparison with the faeces produced from the high-lactating cows. This effect is common because a higher proportion of roughage in the feed promotes fungal growth (Rezaeian et al. 2006) due to fungi preferring C-rich cell wall material and needing less N concentration in the substrate.

Gaseous emissions from faeces added to soil were significantly ($P < 0.05$) influenced by the composition and characteristics of faeces. Release of $\text{CO}_2\text{-C}$ and N_2O was highest in the soil amended with the faeces of high-yielding cows, providing a higher amount of easily available C and N substrates than the other faeces. This is known to increase gaseous emissions (Flessa et al. 2002a; Arriaga et al. 2010).

The addition of heifer faeces, characterized by low N and high fibre content, to soil led to a stronger N immobilisation than the other treatments. An increased microbial biomass C/N ratio in combination with an increased fungal C to bacterial C ratio indicates that the shift in the microbial community structure towards fungi reduced N storage of faecal microorganisms, i.e. less N is incorporated into microbial components. In soil, an increasing microbial biomass C/N ratio together with an increasing fungal biomass has not often been found (Heinze et al. 2010; Joergensen et al. 2010).

The N immobilisation found in this study has been repeatedly observed after direct application of cattle faeces to soil (Chadwick et al. 2000; Griffin et al. 2005; Cusick et al. 2006; Peters and Jensen 2011). This was to be expected because the heifer faeces C/N ratio of 28 was higher than the threshold of 25, indicating restricted N availability to soil microorganisms (Powlson et al. 2001). However, the strong N immobilisation in the faeces of high-yielding cows is surprising, considering the fact that the C/N ratio was far below 20, where an N release to the soil solution usually

occurs (Janssen 1996; Seneviratne 2000; Peters and Jensen 2011). The current study suggests that the faecal organic N fractions are hardly available to soil microorganisms, in contrast to the N-free faecal organic C fractions. A poor availability of faecal organic N to soil microorganisms and consequently to subsequent crops was also observed by Bosshard et al. (2009). However, solid-state cross polarisation ^{15}N nuclear magnetic resonance spectroscopy and Curie-point pyrolysis–gas chromatography–mass spectrometry also failed to give clear further information on the chemical reasons for the poor availability of faecal organic N (Bosshard et al. 2011). The authors assumed that some N compounds present in plants were not digested in the gut and also that some excreted recalcitrant N compounds were de novo synthesised by gut microorganisms. An N fixation capacity like that found in the cattle faeces in the present study has been observed for highly decomposed sugarcane filter press mud, which led to N immobilisation even at an initial C/N ratio of 12 (Rasul et al. 2008). In the long term, studies have shown that N from immobilising faeces is also released by the microbial decomposition processes into the soil solution (Morvan and Nicolardot 2009; Peters and Jensen 2011) and taken up by plants (Chadwick et al. 2000).

Nitrogen uptake by ryegrass increased with faecal N concentration and decreased with higher faecal crude fibre content. Plant N uptake also decreased with a higher ratio of fungal C to bacterial C and a higher microbial biomass C/N ratio. A negative relationship between diet composition, faecal N concentration and N mineralisation has been repeatedly observed for sheep faeces (Kyvsgaard et al. 2000) and also for cattle faeces (Sørensen et al. 2003; Wu and Powell 2007; Morvan and Nicolardot 2009). However, none of these investigations considered the microbial quality of faeces. In the current study, closer relationships have been observed between plant N uptake and faecal microbial properties than between plant N uptake and soil microbial properties. A high microbial biomass C/N ratio and a high fungal C to bacterial C ratio in cattle faeces had a strong negative impact on plant N uptake. The highest CO_2 evolution and lowest N immobilisation suggest a stronger turnover of the microbial biomass in the high-yielding faeces treatment with high N and N_E concentration and low ADF concentration. The increased microbial turnover might be the reason why the increase in microbial biomass did not consistently differ between the three faeces types. The relationships between faecal and soil processes might be masked by the autochthonous microbial community. The use of ^{13}C - and ^{15}N -labelled cattle faeces would make it possible to test these assumptions using an identical approach to that presented in this study (Sørensen and Jensen 1998; Jensen et al. 1999; Bosshard et al. 2011; Wachendorf and Joergensen 2011). Hence, it would be possible to investigate whether microbially incorporated C or N is derived from faeces or soil.

Conclusions

This study showed that a feeding regime low in protein and high in fibre content resulted in less microbial but higher fungal biomass. Gaseous emissions from faeces added to soil were not only significantly ($P < 0.05$) influenced by the composition and characteristics of faeces but also by the faecal microbial indices. Composition of faeces showed a clear impact on N mineralisation in soil and consequently the N supply to plants, with faecal microbial properties revealing closer relationships to plant N uptake than soil microbial properties. This is a factor which is not taken into account frequently. The considerable impact of faeces-derived microorganisms on emission potential and plant growth in this study requires further investigation. Moreover, the addition of faeces from heifers with a high C/N ratio and a microbial community dominated by fungi may contribute to the soil N supply in the longer run, which should also be considered. Further studies will help to identify the best management practices to reduce gaseous emissions and will also allow farmers to develop good practices for efficient nutrient use.

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