Nitrogen turnover in a repeatedly manured arid subtropical soil: Incubation studies with ^{15}N isotopes

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Abstract

Under the hot and moist conditions of irrigated agriculture in the arid subtropics, turnover of organic matter is high, which can lead to considerable carbon (C) and nitrogen (N) losses. Therefore, sustainable use of these soils requires regular manure application at high rates. To investigate the contribution of consecutive manure applications to an arid sandy soil to various soil N pools, goat manure was isotopically labeled by feeding ^{15}N-enriched Rhodes grass hay and applied to the soil during a two-year field experiment. In the first year, soils received ^{15}N-labeled manure to distinguish between soil-derived and manure-derived N. In the second year, these plots were split for the application of either ^{15}N-labeled or unlabeled manure to discriminate N derived from previous (first year) and recent (second year) manure application. Soil samples (of control and ^{15}N-manured soil) were collected at the end of the first and the second year, and incubated in two laboratory experiments with labeled or unlabeled manure. At the beginning of Experiment 1, 7% of total N, 11% of K_2SO_4 extractable N, and 16% of microbial biomass N were derived from previously field-applied manure. While the application of manure during incubation increased microbial biomass N by 225% and 410% in the control soil and the previously field-manured soil, respectively, N_2O emissions were more affected on the control soil, releasing considerable amounts of the soil N-pool (80% of total emissions). In Experiment 2, 4% of total N, 7% of K_2SO_4 extractable N, and 7% of microbial biomass N derived from previously applied manure, and 4%, 8%, and 3% from recently applied manure, respectively. Microbial biomass N and N_2O-N derived from manure declined with time after manure application, whereas in Experiment 1 this tendency was only observed for microbial biomass N.

Key words: ^{15}N-labeled manure / C and N turnover / oasis agriculture / repeated manure application

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1 Introduction

The interaction between high temperatures, frequent irrigation, and tillage often leads to high microbial activity and a subsequent rapid organic matter turnover and nutrient losses in the tropics and subtropics (*Austin* et al., 2004; *Conant* et al., 2011; *Fiedler* et al., 2016). Under the hot climatic and continuously moist conditions of irrigated agriculture in arid environments, up to 95% of organic matter applied as animal manure to soils may disappear within 12 weeks (*Esse* et al., 2001; *Ouédraogo* et al., 2004; *Ingold* et al., 2017). In addition, considerable carbon (C) and nitrogen (N) volatilization losses in irrigated semi-arid and arid soils have been documented (*Wichern* et al., 2004a; *Buerkert* et al., 2010; *Siegfried* et al., 2011; *Goenster* et al., 2014). Despite these reported losses, the repeated application of animal manure can stabilize or increase soil organic C (SOC) and N concentrations of top-soils (*Craswell* and *Lefroy*, 2001; *Lal*, 2006; *Siegfried* et al., 2011; *Ingold* et al., 2015), which is important for soil fertility and sustainable crop production. However, the application of animal manures to soil does not only target enhancement of soil organic C stocks but also contributes to fertilizing crops. In animal manure 70% to 90% of N are present in organic form (*Parker* and *Castellanos*, 1981), which are not immediately plant-available but need to be mineralized. High mineralization rates at times of low plant demand may lead to the above mentioned high N losses, whereas slow mineralization rates at times of high demand can lead to crop nutrient deficiencies. Mineralization of organic matter depends on soil temperature, moisture, texture, microbial activity, and manure characteristics (*Zech* et al., 1997; *Wichern* et al., 2004b). Frequent wet–dry cycles induced by flood-irrigation can further intensify min-

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eralization processes (*Austin* et al., 2004; *Borken* and *Matzner*, 2008). Addition of organic matter may also increase the mineralization of native C and N, which has been termed as 'priming effect' (*Bingemann* et al., 1953; *Jenkinson* et al., 1985). This priming effect was found to be larger in soils rich in C and N compared to soils poor in these elements (*Hart* et al., 1986; *Kuzyakov* et al., 2000).

The effects of repeated manure applications to irrigated sandy subtropical soils on mineralization of organic matter have rarely been investigated. The relevance of these effects, however, is growing as climate change and increasing population pressure will likely cause extension of cultivated areas under arid and semi-arid conditions in the future. To distinguish between C and N derived from different sources, labelling approaches based on isotope (^{13}C/^{12}C or ^{15}N/^{14}N) enrichments of manures are useful (*Barraclough*, 1995; *Dittert* et al., 1998; *Robinson*, 2001; *Smith* and *Chalk*, 2018). The approach of labeling manure *via* ^{15}N-labeled foddergrass as animal feed is rare because it is costly and time consuming (*Powell* et al., 2004), however, it has been shown to be a very good tool for the detection of manure derived N entering the soil in organic forms (*Sørensen* et al., 1994; *Sørensen* and *Jensen*, 1998; *Wachendorf* and *Joergensen*, 2011). To date isotopic labelling approaches have rarely been adopted to investigate N cycling in subtropical soils. In a first methodological report it was shown that ^{15}N from low labeled manure could be used as a tracer in N_2O, NH_3, and soil N pools in an incubation experiment with soil from Oman (*Ingold* et al., 2018).

The objective of this study was to investigate the contribution of one to three consecutive manure applications to a subtropical soil to different soil and gaseous N pools in a laboratory incubation system. Our hypothesis, that the proportion of N derived from manure in soil N pools and in N_2O-N after repeated application of manure declines with time after application, was tested in two incubation experiments with soil from a field experiment manured in one and in two years with ^{15}N labeled manure.

2 Material and methods

2.1 ^{15}N labeled manure and soil

Soil and manure samples used in two laboratory incubation experiments were collected from a two-year field experiment conducted from October 2013 to April in 2015 in the Al-Batinah coastal plain near Sohar (24.2°N, 56.7°E), Sultanate of Oman. The local soil was characterized as hyperthermic Typic Torrifluvent (*Al-Farsi* and *Cookson*, 2002) with 82% sand, 16% silt, and 2% clay in the upper 15 cm, a pH of 8.8, $CaCO_3$ content of 5.3%, and bulk density of 1.8 g cm^{-3}. ^{15}N labeled and unlabeled manure were applied at an annual rate of 210 kg N ha^{-1} to a flood irrigated cabbage (*Brassica oleracea* L.) and basil (*Ocimum basilicum* L.) crop rotation (*Ingold* et al., 2018). To obtain uniformly labeled manure, ^{15}N-labeled Rhodes grass (*Chloris gayana* Kunth) hay was produced by foliar application of ^{15}N labeled urea at the Agricultural Experiment Station of Sultan Qaboos University in Al-Khoud (22°36′ N, 58°10′ E), near Muscat, Sultanate of Oman (*Ingold* et al., 2018). The aboveground biomass was harvested after 35 days, sun dried for two days, and stored as hay bundles at room temperature. The ^{15}N isotope concentration of produced hay was 0.675 at% ^{15}N (± 0.002 SD) compared to 0.369 at% ^{15}N (± 0.000 SD) in unlabeled Rhodes grass hay from a nearby field. The labeled or unlabeled Rhodes grass hay was fed to male Northern Omani goats (*Capra aegagrus hircus*) in combination with crushed barley (*Hordeum vulgare* L.) at the ratio 60:40. After an adaptation period of seven days, manure was collected twice a day in specially constructed fabric bags attached to the goat's back (*Schlecht* et al., 2011) and was air-dried before storage. Labeled manure (M) had an isotopic label of 0.526 at% ^{15}N (± 0.003 SD), total C concentration of 45.65%, and total N concentration of 1.84% compared to unlabeled manure (m) with 0.369 at% ^{15}N (± 0.000 SD), 45.29% C, and of 1.76% N.

In the first year, four 3 × 3 m^2 irrigated plots were amended with labeled manure (M), hence referred to as field application F1. At the beginning of the second cropping season, the plots were split in two, each half either receiving labeled (M) or unlabeled manure (m) at similar rates as in year 1 (field application F2). In addition, eight plots were established on adjacent fallow land, of which four plots were left unfertilized as a control (Co) and four plots received M. Depending on soil water status, the crops were flood irrigated with 17 to 22 mm per irrigation event. Soil samples were collected from the upper 15 cm with the help of a pair of spades from four randomly selected spots per plot as bulked samples at the end of the first year (October 2014) after a hot and dry fallow and before the crop harvest at the end of the second year (April 2015; Fig. 1). Samples were air-dried and sieved to 2 mm prior to storage at room temperature in the dark for nine and five month until analysis.

2.2 Incubation experiments

Two incubation experiments were conducted with the soil collected at the beginning and at the end of the second year, subsequently referred to as 'year 1' and 'year 2'. In the first experiment, the unfertilized control soil and the ^{15}N manured soil (M) from year 1 were mixed with ^{15}N manure (M), unlabeled manure (m) or left unfertilized (Co) to generate the following four treatments: Co1, CoM, Mm, and MM (*Ingold* et al., 2018). The soil used in the second experiment remained either unfertilized in both years of the field experiment (Co), was manured with labeled manure in the first and with unlabeled manure in the second year (Mm), or was amended with labeled manure in both years (MM). These soils were treated with or without M and m to create the following four treatments: Co2, Mmm, MMm, and MMM (Tab. 1).

For the incubation experiment, 150 g air-dry soil was incubated in air-tight glass jars (1.6 L) with two sealed vents placed in an incubation chamber at 25°C for 31 days. The soil was adjusted to a bulk density of 1.8 g cm^{-3} and 50% water holding capacity (WHC) throughout the experimental period. Soil samples were pre-incubated for 10 days at 50% WHC before manure application (equivalent to 100 kg N ha^{-1}) and further incubated for 21 days thereafter. Soil gas fluxes were

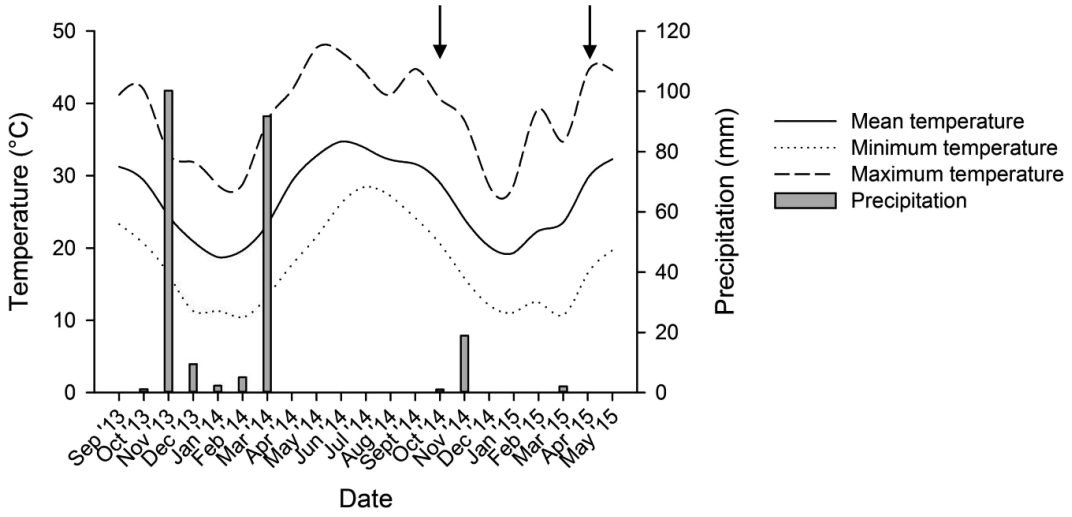

Figure 1: Monthly mean, minimum and maximum temperatures and precipitation at the experimental site in the Al-Batinah coastal plain near Sohar, Sultanate of Oman, during the field experiment in 2013 to 2015. Arrows indicate sampling dates after the first and second cropping season.

estimated 8, 4, and 1 day(s) before, and 0, 1, 2, 3, 4, 5, 6, 8, 11, 14, 18, and 21 days after manure application in Experiment 1, and 8, 6, and 3 days before, and 0, 1, 2, 3, 4, 6, 8, 10, 13, 16, 20, and 23 days after manure application in Experiment 2, by flushing the incubation jars with fresh air and sealing them with a gas-tight lid until the next sampling date. Gas samples were collected at the beginning and at the end of each accumulation period with pre-evacuated gas flasks (50 mL) for CO_2 and N_2O analyses by gas chromatography (GC-14B Analysis System TCD/FID and ECD, Shimadzu Corp., Kyoto, Japan). At each gas sampling, a second gas sample was taken in a 100-mL glass flask sealed with a gray butyl stopper for isotope analysis of N_2O. To cover the isotopic composition during peak emissions in Experiment 1, samples from all gas accumulation periods until day 7 after manure amendment were analysed by an Isotope Mass Spectrometer Delta XP and GC interface coupled with a PreCon [Thermo Electron (Bremen) GmbH, Bermen, Germany], whereas in Experiment 2 samples from two accumulation periods during pre-incubation and three accumulation periods during main incubation were used. Soil samples were taken directly after manure application at the beginning of main-incubation (t0) and at the end of the main incubation (t1) for laboratory analyses.

Total C and N contents were estimated in dried sample aliquots, pulverized in a ball mill, by a CN analyser (VarioMax® CHN, Elementar Analysesysteme GmbH, Langenselbold, Germany). The chloroform fumigation-extraction method was used to analyse soil microbial biomass C and N (*Brookes*

Table 1: Experimental set-up of two laboratory incubation experiments in which manure was added to unfertilized (Co) and field manured soil from a two year field experiment near Sohar, Sultanate of Oman and initial soil C and N concentrations in total, K_2SO_4 extractable, and microbial biomass pools (C_{mic} and N_{mic}).

		Experiment 1				**Experiment 2**			
Field year 1	F1	Co	Co	M	M	Co	M	M	M
Field year 2	F2	–	–	–	–	Co	m	M	M
Laboratory	L	Co	M	m	M	Co	m	m	M
Treatment ID		**Co1**	**CoM**	**Mm**	**MM**	**Co2**	**Mmm**	**MMm**	**MMM**
SOC	g kg^{-1}	4.2		7.8		5.9	8.5		8.4
Total N	mg kg^{-1}	406.2		583.5		402.0	568.2		557.6
K_2SO_4 extr. C	mg kg^{-1}	72.3		114.8		44.2	65.0		57.7
K_2SO_4 extr. N	mg kg^{-1}	7.3		13.1		2.1	4.0		3.5
C_{mic}	mg kg^{-1}	34.4		62.1		55.7	65.7		69.3
N_{mic}	mg kg^{-1}	3.2		4.9		5.1	4.0		4.5

[a]F1 = field-applied manure in year 1, F2 = field-applied manure in year 2, L = manure application during laboratory experiment, M = labeled manure, m = unlabeled manure, and Co = unfertilized control.

et al., 1985). An aliquot of 10 g of fumigated (24 h with $CHCl_3$ at 25°C) and non-fumigated soil were extracted in 40 mL 0.05 M K_2SO_4 solution for 30 min in a horizontal shaker at 200 rev min^{-1}. Organic C and total N in the filtered soil extracts were measured using an automatic analyser (Multi N/C® 2100, Analytic Jena GmbH, Germany). The results were used to calculated microbial biomass C and N according to *Sradnick* et al. (2014; *Brookes* et al., 1985; *Joergensen* et al., 2011). Aliquots of the extracts were freeze-dried and analyzed by an Isotope Mass Spectrometer Delta V Advantage and a Conflo III interface [Thermo Electron (Bremen) GmbH, Bremen, Germany] coupled with an Elemental Analyser Flash 2000 (Thermo Fisher Scientific Inc., Cambridge, UK) for the isotopic composition of N.

For calculation of the ^{15}N enrichment in microbial biomass, *at% excess* of fumigated (ape_f) and non-fumigated extracts (ape_{nf}) were multiplied with the respective N concentration of the extracts, subtracted and divided by the difference of N between fumigated and non-fumigated extracts as follows (*Wachendorf* and *Joergensen*, 2011):

$$at\%\ excess_{Nmic} = (N_f \times ape_f - N_{nf} \times ape_{nf})/(N_f - N_{nf}). \quad (1)$$

N_2O and CO_2 emissions were calculated as follows (*Siegfried* et al., 2011; *Ingold* et al., 2018):

$$\begin{aligned} &Gas_{N_2O\ or\ CO_2}\left[mg\ h^{-1}\ kg^{-1}\right) \\ &= \left[\left(\frac{C_2}{(273,15+T_2)}\right) - \left(\frac{C_1}{(273,15+T_1)}\right)\right] \\ &\times \frac{\left(V \times \frac{3600}{t} \times 298,15\right)}{\left(\frac{8.3143 \times 298,15}{101325}\right) \times 1000000 \times W} \times m_{C\ or\ N}, \end{aligned} \quad (2)$$

whereby C_1 and C_2 represent the measured N_2O or CO_2 concentrations (ppb), T_1 and T_2 the temperature measured at the sampling events (°C), V the volume of the incubation jars, t the time between the two sampling events (s), W the DM weight of the soil (kg), and m_C the mass of C or m_N the mass of N. The constant *298.15* is the standardized temperature of 25°C in °K, the term $\frac{8.3143 \times 298.15}{101325}$ stands for the gas constant at ambient temperature and pressure. Measured ^{15}N concentrations in air samples were corrected for dilution with ambient air used to flush incubation jars before subsequent calculations.

To calculate the percentage of N derived from applied manure for each treatment ($Ndfm_{treatment}$) the following equation from *Nason* and *Myrold* (1991) was used whereby *atom % excess* (*ape*) is the difference between the ^{15}N concentration of samples and the natural abundance measured in controls (Co1 or Co2):

$$\begin{aligned} &Ndfm\ treatment\% \\ &= {}^{15}N\ ape\ sample\ (gas,\ soil,\ microorganism)/ \\ &{}^{15}N\ ape\ goat\ manure \times 100. \end{aligned} \quad (3)$$

To estimate the amount of N in the total N, K_2SO_4 extractable N, microbial biomass N, and N_2O-N pools derived from soil (soil Co, soil Mm, and soil MM), field-applied manure in year 1 (F1), field-applied manure in year 2 (F2) and manure applied during the laboratory experiment (L) the following calculations were conducted for Experiment 1. Mean ($\bar{x}$) concentrations of the N pools from the treatments Mm and MM in Experiment 1 and Mmm, MMm, and MMM in Experiment 2 were used under the statistically validated assumption that unlabeled (m) and labeled manure (M) had similar effects on the N pools.

$$Ndf\ L_{CoM} = \frac{Ndfm_{CoM}\% \times N_{CoM}}{100}, \quad (4)$$

$$Ndf\ Soil\ Co = N_{CoM} - N_{dfm}\ L, \quad (5)$$

$$Ndf\ F1_{MM} = \frac{Ndfm_{Mm}\ \% \times \bar{x}N_{Mm+MM}}{100}, \quad (6)$$

$$Ndf\ L_{MM} = \frac{(Ndfm_{MM}\% - \bar{x}Ndfm_{Mm}\%) \times \bar{x}N_{Mm+MM}}{100}, \quad (7)$$

$$Ndf\ Soil\ M = \bar{x}N_{Mm+MM} - Ndf\ F1_{MM} - Ndf\ L_{MM}. \quad (8)$$

For the calculation of the results from Experiment 2 the following equations were used:

$$Ndf\ F1_{MMM} = \frac{Ndfm_{Mmm}\% \times \bar{x}N_{Mmm+MMm+MMM}}{100}, \quad (9)$$

$$Ndf\ F2_{MMM} = \frac{(Ndfm_{MMm}\% - \bar{x}Ndfm_{Mmm}\%) \times \bar{x}N_{Mmm+MMm+MMM}}{100}, \quad (10)$$

$$Ndf\ L_{MMM} = \frac{(Ndfm_{MMM}\% - \bar{x}Ndfm_{MMm}\%) \times \bar{x}N_{Mmm+MMm+MMM}}{100}, \quad (11)$$

$$Ndf\ Soil\ MM = \bar{x}N_{Mmm+MMm+MMM} - Ndf\ F2 - Ndf\ F1 - Ndf\ L. \quad (12)$$

2.3 Statistical analysis

Normality of data residuals was examined using the Shapiro–Wilk test and homogeneity of variances was analyzed by the Levene's test at $p < 0.05$. Statistical analysis was performed using the Generalized Linear Mixed Model (GLMM) procedure in SPSS (SPSS 20.0) taking into account the relatedness of soil samples collected from the same plots in the field experiment, plot was a random factor. Treatment and sampling time were considered fixed factors. The significance of changes of estimated Ndf manure pools during the laboratory experiment was tested by paired t-tests at $p < 0.05$.

3 Results

In Experiment 1, concentrations of SOC, K_2SO_4 extractable C, and microbial biomass C increased with increasing number of manure applications (Co1 < CoM < Mm = MM; Tab. 2) and significantly changed during the incubation experiment. While SOC generally decreased during the experiment, K_2SO_4 extractable C increased on control soils and remained relatively constant on field-manured soils, whereas microbial biomass C increased in all treatments. During pre-incubation,

Table 2: Mean and standard deviation (in parentheses) of SOC, K_2SO_4 extractable C, microbial biomass C (C_{mic}) after pre- and main-incubation and cumulative CO_2-C emissions during pre- and main-incubation of two laboratory incubation experiments with soils from a field experiment in the Sultanate Oman mixed with ^{15}N labeled (M) or unlabeled (m) manure. The statistical results of a mixed model ANOVA with treatment (tr) as a fixed factor and sampling time (ti) as repeated measure variable are given below. Letters indicate significant differences between treatments at $p < 0.05$ for each sampling time separately.

Sampling time (d)		SOC		K_2SO_4 extractable C		C_{mic}		Cumulative CO_2-C pre- / main-incubation	
		(g kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})	
		0	21	0	21	0	21	–10 to 0	0 to 21
Exp. 1[a]	Co1	3.7 a (0.77)	3.4 a (0.38)	87.0 a (9.93)	123.5 a (11.03)	43.5 a (16.19)	45.3 a (12.42)	61.9 a (16.16)	55.9 a (5.98)
	CoM	5.4 b (0.60)	4.6 a (0.38)	111.1 a (16–02)	128.1 ab (7.12)	67.3 ab (16.19)	129.5 b (40.82)	61.0 a (19.34)	239.2 b (14.43)
	Mm	8.8 c (0.74)	8.1 b (1.25)	147.5 b (18.01)	147.8 b (10.77)	122.4 bc (30.04)	174.9 b (31.09)	121.9 b (34.39)	279.5 c (30.62)
	MM	10.1 c (0.96)	8.7 b (1.44)	167.2 b (21.29)	141.7 ab (10.10)	149.5 c (57.89)	159.6 b (44.87)	125.1 b (19.34)	330.1 d (29.38)
Exp. 2	Co2	5.7 ab (1.83)	6.0 (1.85)	25.3 a (1.34)	27.8 a (3.24)	61.6 a (15.74)	34.7 a (13.80)	33.6 a (3.91)	50.2 a (7.95)
	Mmm	8.9 ab (0.48)	8.8 (0.95)	52.1 b (11.84)	45.2 c (2.60)	140.5 b (26.35)	136.9 b (9.00)	89.7 b (10.70)	342.0 b (31.85)
	MMm	8.6 a (0.18)	7.5 (1.27)	51.9 b (5.87)	47.1 c (7.97)	124.4 b (5.80)	118.5 b (32.90)	90.0 b (5.13)	332.0 b (38.36)
	MMM	9.6 b (0.30)	8.7 (0.45)	57.5 b (13.56)	38.4 b (2.30)	134.3 b (31.21)	133.9 b (12.49)	83.1 b (4.39)	356.7 b (51.85)
Exp. 1[a]	Tr	0.001		< 0.001		< 0.001		0.008	< 0.001
	Ti	0.018		0.049		0.004			
	Tr × Ti	0.515		< 0.001		< 0.001			
Exp. 2	Tr	< 0.001		< 0.001		< 0.001		< 0.001	< 0.001
	Ti	0.274		0.013		0.224			
	Tr × Ti	0.553		0.055		0.576			

[a]Results for K_2SO_4 extractable C and C_{mic} of Experiment 1 have been published in *Ingold* et al. (2018).

cumulative CO_2-C emissions were twice as high in field-manured soil (Mm and MM) compared with the control soil and increased two- to four-fold after fresh manure application, while emission from un-manured control slightly declined during main incubation. In Experiment 2 SOC, K_2SO_4 extractable C, microbial biomass C, and cumulative CO_2-C emissions were significantly higher in manured treatments compared with the unmanured control. Significant changes during the incubation experiment were only observed for K_2SO_4 extractable C, with 10 to 30% reductions until the end of the experiment. Cumulative CO_2-C emissions were about four fold higher during the main incubation compared to the pre-incubation.

Similar to the C pools, total N, K_2SO_4 extractable N, and microbial biomass N at the beginning of Experiment 1 were higher with increasing number of manure applications (Co1 < CoM < Mm = MM; Fig. 2), while at the end of the incubation this was only true for total N. In the repeatedly manured soils Mm and MM, changes in K_2SO_4 extractable N and microbial biomass N during main incubation were only minor, whereas in CoM K_2SO_4 extractable N declined by 40% and microbial biomass N increased by 220%. Cumulative N_2O-N emissions were 2.5-fold higher in previously field-manured soil compared with control soil during pre-incubation and increased 10-fold in CoM during main incubation. Remarkably, one-time manured soil (CoM) emitted three-fold more N_2O-N during the main incubation compared with two-times manured soils Mm and MM. Nitrogen derived from labeled manure (Ndfm) was significantly higher in MM compared with CoM for total N and K_2SO_4 extractable N at the end of the main incubation period, whereas for cumulative N_2O-N emissions CoM was 5-fold higher than Mm. In Experiment 2, three time manuring increased total N, K_2SO_4 extractable N, and microbial biomass N compared with the control Co2 (Fig. 2). Nitrogen derived from labeled manure (Ndfm) in total N, K_2SO_4 extractable N, and microbial biomass N increased with the number of ^{15}N manure applications (Co2 < Mmm < MMm < MMM) at the beginning and at the end

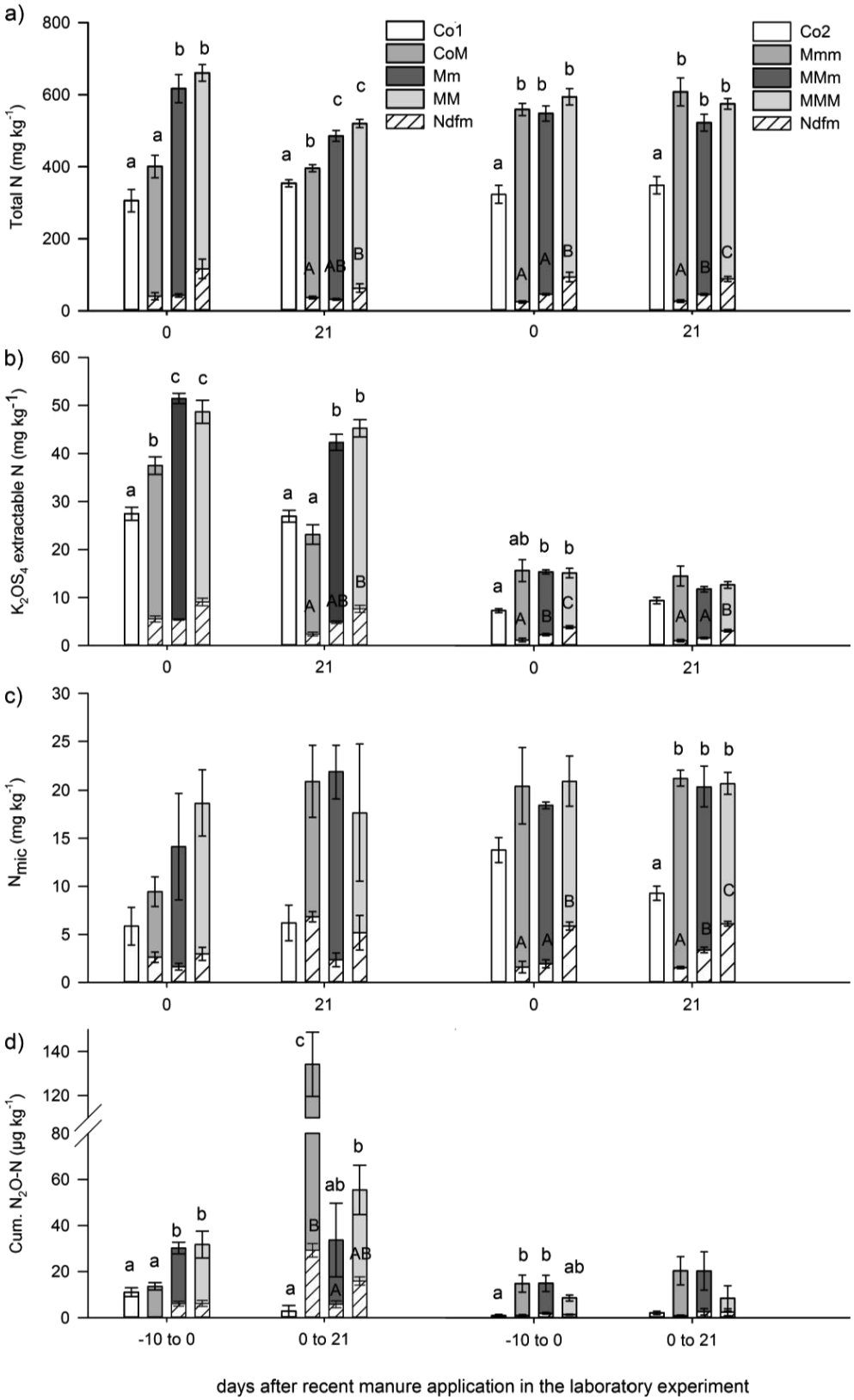

Figure 2: Mean total N (a), K_2SO_4 extractable N (b), and microbial biomass N (N_{mic}; c) at the beginning (0 days) and at the end (21 days) of the main incubation and cumulative N_2O-N emissions (d) during pre- (–10 to 0 days) and main-incubation (0 to 21 days) of two laboratory incubation experiments with soils from a field experiment in the Sultanate Oman mixed with ^{15}N labeled (M) or unlabeled (m) manure. Whiskers show standard errors of mean. Small letters indicate significant differences between treatments for overall N pools, and capital letters for Ndfm at significance levels of $p < 0.05$.

of the main incubation, although differences between Mmm and MMm were not always significant. Cumulative N_2O-N emissions were only significantly higher in manured soil compared with CO_2 during pre-incubation and Ndfm did not change with increasing number of labeled manure applications.

To distinguish between N derived from soil pool and manure applications F1 and L, the above described equations resulted in contributions to total N, K_2SO_4 extractable N, and microbial biomass N of about 80 to 90% from soil (Co and M) at the beginning of the main incubation of Experiment 1. 7%, 11%, and 16% of the three N pools derived from F1 in soil M, respectively, and 10%, 8 to 15%, and 2 to 21% from L in soils Co and M, respectively (calculated from Tab. 3). During the main incubation of previously field-manured soil, total N, K_2SO_4 extractable N, and microbial biomass N derived from soil and F1 decreased by 4 to 26%, although these changes were not always significant. Changes were strongest in N derived from L with 40% reduction of K_2SO_4 extractable N and increases of 400% of microbial biomass N. Average daily N_2O-N emission rates of recently manured control soil (CoM) increased four-fold after manure application compared to the pre-incubation, with L contributing only 22% of daily emissions. In contrast, repeatedly manured soil (M) had a 40% lower N_2O-N emission rate after fresh manure application and F1 contributed 22% and L 9% to daily N_2O-N emissions.

In Experiment 2, soil-derived N was estimated to 84%, 75%, and 71% in total, K_2SO_4 extractable N, and microbial biomass N, respectively. About similar amounts of N derived from F1 and F2 with 4% of total N and 7–8% in K_2SO_4 extractable N, whereas fresh manure L contributed 7%, 11%, and 18% to total, K_2SO_4 extractable and microbial biomass N, respectively. While in unmanured soils K_2SO_4 extractable N increased by 29% and microbial biomass N decreased by 33% in soils manured three times, K_2SO_4 extractable N derived from soil, F1, F2, and L decreased, whereas microbial biomass N remained relatively constant. The N_2O emission rates were generally lower in Experiment 2 compared to Experiment 1 and N_2O-N originating from soil, F1, and F2 declined after manure application. During the main incubation period, N_2O-N derived from soil was highest with 71%, followed by L with 20%, F2 with 5% and F1 with 4%.

4 Discussion

Our soils had a low SOC and total N content, as typically observed for this soil type and climate region (*Siegfried* et al., 2011; *Ingold* et al., 2015). Previous studies showed that soil C and N stocks of semi-arid and arid soils can be maintained or increased by repeated applications of animal manure at rates of 2–8 t ha^{-1} y^{-1} and 134–350 kg N ha^{-1} y^{-1} (*Siegfried* et al., 2011; *Ingold* et al., 2015; *Yang* et al., 2016). However, the same studies revealed quick mineralization of soil-applied manure and high annual C and N losses *via* gaseous emissions. Yet, studies investigating N-turnover processes under arid and semi-arid conditions, particularly using isotope tracing techniques, are scarce. In the current study, due to dilution by unlabeled fertilizer N and high soil N mineralization

Table 3: Means and standard errors (in parentheses) of total N, K_2SO_4 extractable N, microbial biomass N (N_{mic}) and daily N_2O-N emission rates derived from soil and manure applications during pre- and main-incubation of two laboratory incubation experiments with soils from a field experiment in the Batinah plain of Sultanate Oman mixed with ^{15}N labeled (M) or unlabeled (m) manure. Significant changes of N pools during the experiment are indicated by small letters.

Sampling time (d)		Total N		K_2SO_4 extractable N		N_{mic}		Daily N_2O-N emission pre- / main-incubation	
		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(μg kg^{-1} d^{-1})	
		0	21	0	21	0	21	–10 to 0	0 to 21
Experiment 1[a]	Soil0 (Co1)	306 a (18.1)	354 b (8.3)	27.4 (1.4)	26.9 (1.2)	5.9 (2.0)	6.2 (1.9)	1.24 b (0.12)	0.13 a (0.12)
	Ndf Soil Co	359 (11.0)	359 (3.5)	31.9 b (0.5)	20.8 a (0.2)	7.5 a (0.7)	14.4 b (0.6)	1.24 a (0.12)	4.98 b (0.10)
	Ndf L_{CoM}	42 (11.0)	37 (3.5)	5.5 (0.5)	2.4 (0.2)	2.0 (0.7)	6.5 (0.6)		1.41 (0.10)
	Ndf Soil M	528 (25.4)	442 (11.0)	40.7 b (0.7)	36.4 a (0.7)	13.6 (0.8)	13.0 (1.8)	2.48 b (0.07)	1.48 a (0.13)
	Ndf $F1_{MM}$	44 b (3.0)	33 a (1.9)	5.3 (0.2)	5.1 (0.3)	2.6 (0.6)	2.1 (0.5)	0.62 b (0.07)	0.46 a (0.07)
	Ndf L_{MM}	67 (22.7)	28 (11.2)	4.0 b (0.7)	2.4 a (0.6)	0.9 a (0.4)	4.6 b (1.4)		0.18 b (0.07)
Experiment 2	Soil0 (Co2)	323 a (24.7)	349 b (24.1)	7.3 a (0.4)	9.4 b (0.7)	13.8 b (1.3)	9.3 a (0.7)	0.09 (3.91)	0.10 (0.03)
	Ndf Soil MM	478 (9.9)	482 (6.4)	11.7 b (0.4)	9.8 a (0.3)	14.2 (0.8)	14.6 (0.4)	0.82 b (10.70)	0.50 a (0.05)
	Ndf $F1_{MMM}$	25 (2.8)	25 (2.5)	1.0 (0.2)	0.8 (0.1)	1.4 (0.4)	1.5 (0.1)	0.08 b (5.13)	0.03 a (0.01)
	Ndf $F2_{MMM}$	23 (1.9)	25 (3.2)	1.3 b (0.2)	0.9 a (0.1)	0.7 (0.5)	2.0 (0.3)	0.19 b (0.18)	0.07 a (0.02)
	Ndf L_{MMM}	41 (9.4)	38 (8.1)	1.6 (0.3)	1.4 (0.1)	3.6 (0.5)	2.6 (0.2)		0.17 (0.07)

[a]Results for total, K_2SO_4 extractable N and N_{mic} of Experiment 1 have been published in *Ingold* et al. (2018). F1 = field-applied manure in year 1, F2 = field-applied manure in year 2, L = manure application during laboratory experiment, M = labeled manure, m = unlabeled manure, and Co = unfertilized control.

rates, the labeling of Rhodes grass resulted in a ^{15}N labeling of 0.675 at%, which is relatively low for ^{15}N tracing approaches. Nevertheless, ^{15}N labeling in manure, in manured soil N pools (total N, K_2SO_4 extractable N, and microbial biomass N), and in volatile N (N_2O-N and NH_3-N) were significantly different from natural abundance measured in unlabeled manure and N pools of control soils under laboratory conditions (*Ingold* et al., 2018). Under field conditions in Oman, 57% of N derived from previously field applied manure was left in the soil one year after manure application at rates equivalent to 210 kg N ha^{-1} and 30% after a further year. In a litterbag experiment conducted in a comparable soil in Northern Oman, 60 to 70% of applied manure N remained in manure placed in litterbags in the soil at 10 cm depth after 6 to 12 weeks in regularly irrigated soil (*Ingold* et al., 2017). The study showed that organic matter disappearance from litterbags strongly declined after four to six weeks, being best described by an asymptotic model, indicating that the remaining organic matter in soil applied manure was more stable and less prone to decomposition processes. In general, field-applied manure contributed to a higher extent to the more labile and active N pools of K_2SO_4 extractable N and microbial biomass N than soil. This was expected as the soil N pool mainly comprises a large humus N pool with slow mineralization rates (*Beauchamp* et al., 2011). In Experiment 1, previously field applied manure ($F1_{MM}$) contributed 7% of total N, 11% of K_2SO_4 extractable N, and 16% to microbial biomass N in soil sampled at the end of the first year of a field experiment. As expected, after a further cropping period the contribution of this manure (Ndf $F1_{MMM}$) to total N, K_2SO_4 extractable N, and to microbial biomass N declined to 4%, 7%, and 7%, respectively. The decline of ^{15}N-labeled manure derived microbial biomass from 2.6 mg kg^{-1} in MM to 1.4 mg kg^{-1} in MMM is in accordance with results of the Broadbalk wheat experiment, in which a 60% decrease of microbial biomass N derived from ^{15}N-labeled ammonium nitrate was observed after three years (*Shen* et al., 1988). The reduction of manure derived microbial biomass is with 46% in the same order as the 43% reduction of total N, indicating similar long-term turnover rates of both pools. However, within three weeks after fresh manure application during the two laboratory incubation experiments, both decrease and increase of microbial biomass N derived from previously applied manure could be observed. Thereby, the direction of the changes seemed to be influenced by the immediate history of the soils used, although these changes should be interpreted carefully as the ^{15}N labeling in manure was relatively low. The soil used in Experiment 1 was collected after an extremely hot and dry fallow period of five months following the harvest of the crops cultivated during the first cropping season, whereas for Experiment 2 the soil was collected one week before crop harvest. The possible decomposition of residual manure, harvest residues from the first year, and dead microbial biomass during the hot and dry fallow period may have led to the release of easily decomposable solutes after rewetting of dry soil (*Birch*, 1958; *Zech* et al., 1997; *Borken* and *Matzner*, 2008). The 1.9-fold higher K_2SO_4 extractable C and 3.8-fold higher K_2SO_4 extractable N found in the initial soil of Experiment 1 indicated a higher presence of labile C and N fractions compared with the soil used in Experiment 2. This can explain the 33 to 88% higher CO_2 emissions and 2 to 10-fold higher N_2O emissions during pre-incubation in Experiment 1 compared with Experiment 2, even though the two incubation experiments were conducted under similar laboratory conditions (25°C, 50% water holding capacity, 1.8 g cm^{-3} bulk density, durations of pre- and main incubation). The two major processes emitting N_2O from soil are nitrification and denitrification, which are mainly affected by soil moisture, temperature and the presence of labile C and N (*Bateman* and *Baggs*, 2005; *Butterbach-Bahl* and *Dannenmann*, 2011). It is likely that the stronger microbial growth, the higher CO_2 emissions during pre-incubation and the considerably higher N_2O emissions during the whole incubation experiment were triggered by the higher availability of easily mineralizable C and N sources (*Murphy* et al., 2000; *Jones* et al., 2004; *Velthof* and *Mosquera*, 2011). Interestingly, microbial biomass C and N increased during Experiment 1 after manure application, whereas during Experiment 2 they remained constant or even decreased. This in turn may have resulted in higher mineralization rates and a flush of microbial activity, CO_2, and N_2O emissions (*Harrison-Kirk* et al., 2013), called priming or "Birch effect" (*Birch*, 1958; *Kuzyakov* et al., 2000). This effect was much higher after the application of manure to unfertilized control compared with previously field-manured soil, which has been discussed by *Ingold* et al. (2018). In accordance to previous studies (*Austin* et al., 2004; *Borken* and *Matzner*, 2008), our results show that in arid, subtropical environments the distinct hot and dry fallow period strongly affects microbial activity and mineralization processes after rewetting of exposed soils. This can lead to considerable C and N losses *via* gaseous emissions. Particularly on previously unfertilized soils, N-losses *via* N_2O can be substantial, whereby the more recalcitrant soil N pool is significantly affected. However, even a single application of organic manure in the field led to a considerable increase in soil organic C and N pools, and reduced N_2O emissions by 67% compared with the unfertilized control soil. This emphasises the importance of regular manure applications to irrigated sandy soils in arid and subtropical environments.

Our hypothesis, that the proportion of N derived from repeated manure applications in the measured N pools declines with time, was only partly confirmed. Studies on manure turnover in Oman revealed quick mineralization of organic matter and high gaseous C and N losses in irrigated agriculture (*Buerkert* et al., 2010; *Siegfried* et al., 2011). The organic matter of goat manure applied in a litterbag experiment in Oman declined within two to four weeks after soil application and remained relatively stable thereafter (*Ingold* et al., 2017). Depending on feed composition, animal manure contains different proportions of undigested N, bacterial and endogenous debris N, and water-soluble N (*Al-Kindi* et al., 2015), which are decomposed at different rates (*Sørensen* and *Jensen*, 1998). This decline of organic matter in the litterbag experiment followed an asymptotic exponential model with a stable organic matter fraction of about 60%, which likely comprised of undigested feed components (*Sørensen* and *Jensen*, 1998). Thus, it was expected that easily decomposable organic compounds from goat manure are quickly mineralized after application leaving the more stable compounds in the soil for slow mineralization during the following years (*Dittert* et al., 1998). This would lead to a reduction of

the contribution of manure to measured N pools with time in the order $F2_{MMM} < F1_{MMM} < L_{MMM}$, which was observed at the end of Experiment 2 and is in agreement with a review by *Smith* and *Chalk* (2018). Because of the smaller labile fraction of K_2SO_4 extractable N in manure with time, a reduced utilization of N derived from $F2_{MMM}$ and $F1_{MMM}$ for microbial biomass N and N_2O emissions was anticipated. This was, however, only true for N_2O emissions, whereas microbial biomass N was mainly promoted by $F1_{MMM}$. It must, however, be taken into consideration that the total microbial biomass C and N did not significantly change during the main incubation of Experiment 2, and calculated differences in N derived from manure were not statistically significant. At the end of Experiment 1, K_2SO_4 extractable N and N_2O-N emission rates were higher in $F1_{MM}$ (field-applied manure in first year) compared with L_{MM} (applied during laboratory incubation) although its contribution to total N was lower. Though this appears to contradict our hypothesis, it should be kept in mind that the late sampling date and concurrent longer exposure of the field-applied manure to very hot temperatures during the fallow period seemed to have led to an accumulation of more labile C- and N-forms in manure and soil pools and subsequently to higher N_2O emission rates. In addition, the soils used in the two experiments were stored for nine and five month under air dry conditions and room temperatures, which might have affected the soils differently. The microbial biomass in Experiment 1, however, preferably utilized freshly applied manure leading to an increase of L_{MM} derived microbial biomass N by 400%, whereas microbial biomass N derived from $F1_{MM}$ and from soil remained relatively constant. The reason for this contradictory effect of manure application on microbial biomass N and N_2O emissions in Experiment 1 is unclear and merits further research. Despite the differences between the two experiments, repeated manure applications seemed to stabilize soil N pools compared with the unfertilized control soil.

5 Conclusions

Despite similar laboratory conditions during the two experiments, effects of repeated manure applications on total, K_2SO_4 extractable, and microbial biomass N, as well as CO_2 and N_2O emissions were ambiguous and only partly confirmed our hypothesis. At the end of Experiment 2, the contribution of manure applications to total N, K_2SO_4 extractable N, and microbial biomass N as well as N_2O-N during main incubation followed the expected order $F2_{MMM} < F1_{MMM} < L_{MMM}$, which could not be confirmed in Experiment 1. The sampling of soil for Experiment 1 after a long, hot and dry fallow period seemed to be one of the major factors leading to high K_2SO_4 extractable C and N and consequentially to high CO_2 and N_2O emissions, particularly during pre-incubation, and a strong microbial growth after manure application. On previously field-manured soils, microbial biomass N uptake originated mainly from recent manure applications, whereas no further microbial biomass was built upon soil-derived N. Already a single manure application in the field led to higher SOC and total N, and 67% lower N_2O-N emissions during the laboratory incubation. This indicated a stabilization of soil N-pools compared to unfertilized soil, and emphasizes the importance of regular organic matter applications in irrigated agriculture of the arid Subtropics.

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