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ORIGINAL PAPER

Florian Wichern · Torsten Müller · Rainer Georg Joergensen · Andreas Buerkert

Effects of manure quality and application forms on soil C and N turnover of a subtropical oasis soil under laboratory conditions

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Abstract Our knowledge of the agricultural sustainability of the millennia-old mountain oases in northern Oman is restricted in particular with respect to C and N turnover. A laboratory study was conducted (1) to analyse the effects of rewetting and drying on soil microorganisms after adding different manures, (2) to investigate the effects of mulching or incorporating of these manures, and (3) to evaluate the relationships between C and N mineralisation rates and manure quality indices. During the first 9-day rewetting and drying cycle, i.e. the "mulch" period, the content of extractable organic C decreased by approximately 40% in all four treatments. During the second 9-day rewetting and drying cycle, i.e. the "incorporation" period, this fraction decreased insignificantly in almost all treatments. The control and mature manure treatments form the first pair with a low percentage of total organic C evolved as CO_2 (0.3% in 18 days) and a considerable percentage of total N mineralised as NH_4 and NO_3 (1% in 18 days), the fresh and immature manure treatments form the second pair with a higher amount of total organic C evolved as CO_2 (0.5% in 18 days) and no net N mineralisation. During the first 9-day rewetting and drying cycle, the contents of microbial biomass C and biomass N increased by approximately 150% in all four treatments. During the second 9-day rewetting and drying cycle, no further increase was observed in the control and immature manure treatments and a roughly 30% increase in the other two treatments.

Keywords Rewetting · Drying · Microbial biomass · CO_2 evolution · N mineralisation

F. Wichern · T. Müller · R. G. Joergensen (✉)
Department of Soil Biology and Plant Nutrition,
University of Kassel, Nordbahnhofstrasse 1a,
37213 Witzenhausen, Germany
e-mail: joerge@wiz.uni-kassel.de
Tel.: +49-5542-981591
Fax: +49-5542-981596

A. Buerkert
Institute of Crop Science, University of Kassel,
Steinstrasse 19, 37213 Witzenhausen, Germany

Introduction

Under arid subtropical conditions where water is the most limiting factor for crop production, agriculture evolved as concentration agriculture on small areas near springs, wells or rivers (Mainguet 1998). Typical of this type of land-use system is the mountain oasis Balad Seet in northern Oman (Wichern et al. 2003). In the often millennia-old oases of this area organic material and nutrients were accumulated over time, raising the fertility of the man-made terrace soils. Under such conditions soil organic matter governs water holding and cation exchange capacity, thereby assuring the sustainability of cultivation at the fringe of drought and salinisation. The soil organic matter turnover can be considerably altered by the farmers through addition of organic fertilisers and also by the irrigation practice, which contrasts the situation in humid climates (Lal 1989). However, this dynamic situation enhances the risk of mismanagement, leading to a rapid decline in soil organic matter content and thus to a breakdown in soil fertility (Zech et al. 1997; Powlson et al. 2001). The decomposition rate of added organic material can be increased by drying and rewetting cycles (Van Gestel et al. 1993; Fierer and Schimel 2002), but it can also be decreased (Degens and Sparling 1995).

The terraces in Balad Seet are flooded regularly about every 9 days according to the crops' demand, leading to strong fluctuations of the soil water content and thus to repeated rewetting and drying cycles throughout the cropping season (Norman et al. 1998; Wichern et al. 2004). Organic fertilisers are added as a mulch layer on the soil surface during plant growth or by incorporation into the mineral soil after harvest of the crops. The soil organic matter turnover is controlled by the microbial performance in soil, which is caused by the interactions between water content and the placement of the organic fertilisers (Singh and Singh 1993). Another important factor is the quality and degradation status of the organic amendments, ranging from fresh manure and aged farmyard manure with all kinds of transitional stages preserved by desiccation. Common quality indices are the

total-C to total-N ratio (Powlson et al. 2001) or the lignin content (Seneviratne et al. 1998). Newer indices used for assessing litter and compost quality are the concentrations of extractable organic C and extractable organic N (Seneviratne et al. 1998; Wu et al. 2000) and the microbial-biomass-C to total-organic-C ratio (Mondini et al. 2002).

The aims of the present study were (1) to analyse the effects of rewetting and drying on soil microbial biomass and activity after adding different manures, (2) to investigate the effects of a local practice where organic fertilisers are initially mulched and later incorporated into the soil, and (3) to evaluate the relationships between C and N mineralisation rates and quality indices for different types of manure, i.e. fresh, immature, and mature manure. This study was part of a larger project focussing on the sustainability of oases systems, especially with respect to the question of how the current levels of agricultural inputs affect C and nutrient balance under regular irrigation and high ambient temperatures (Wichern et al. 2004; Nagieb et al. 2004).

Materials and methods

Sampling site, soil and manure

The study site, the oasis of Balad Seet (23.19°N, 57.39°E; 1,000 m above sea level) is situated in the Hajar mountain range of northern Oman. The local climate is characterised by two distinct seasons, a very hot summer from May to September with maximum temperatures up to 45°C and a cool period from October to April with minimum temperatures as low as 5°C. Mean annual rainfall is about 100 mm. At the end of the cool season in March 2002 soil of harvested wheat plots was sampled at 0- to 20-cm depth. After sampling, the soil was sieved (<2 mm) and air-dried in the shade. The soil had a pH in water of 8.5, a cation exchange capacity of 218 $\mu mol_C\ g^{-1}$ soil and a carbonate C content of 44 mg g^{-1} soil. The particle size distribution was 18% clay, 58% silt and 24% sand. Three different types of manure qualities used for the subsequent incubation experiment were sampled from different places within the oasis and air-dried in the shade. Two consisted of cattle manure containing straw, one old and mature (mature manure), the other relatively young and less decomposed (immature manure). The third manure was a mixture of sheep and goat faeces (fresh manure). The three different manure qualities remained ungrounded for the incubation.

Incubation experiment

A laboratory experiment using 500 g (on an oven-dry basis) soil was carried out in open 1,000-ml glass jars (11 cm diameter) with the following four treatments: (1) +20 g (on an oven-dry basis) fresh manure, (2) +20 g (on an oven-dry basis) immature manure, (3) +20 g (on an oven-dry basis) mature manure, and (4) control. Prior to the incubation, soil and manures were analysed separately for microbial and chemical properties. From this analysis, initial values of the four treatments were calculated. At the beginning of the experiment, the three manures were placed on top of the soil surface as mulch. Soil and mulch were rewetted to 60% water holding capacity (WHC) by pouring water on the top and then dried slowly at 23°C in the dark over a period of 9 days simulating an on-field irrigation cycle (Wichern et al. 2004). The five replicates were placed in a randomised pattern in an incubation cupboard. Water loss was estimated daily by weighing the samples. CO_2 evolution was measured 4 h after the rewetting event over a period of 2 min and then every 24 h. After 9 days the amendments were mixed homogenously into the soil, a 30-g subsample was taken for measuring microbial biomass C and biomass N and the remaining sample was rewetted again to 60% WHC. Water loss and CO_2 evolution rate were measured as described above during this second 9-day period. Sampling was repeated at the end of the incubation.

Microbial and chemical analyses

Soil respiration was measured using a portable infrared gas analyser (Blanke 1996). The dynamic system consisted of a chamber (100 mm diameter, 150 mm height) coupled to a portable infrared gas analyser (IRGA) in a closed circuit (PP Systems, Hitchin, United Kingdom). The respiration chamber was placed on top of the jar, which was sealed with an elastic band. Using the surrounding air as a reference, the proportional increase in CO_2 concentration was measured [g $CO_2\ m^{-2}\ h^{-1}$] and recalculated to µg CO_2-C g^{-1} soil day^{-1}.

Microbial biomass C and biomass N were estimated by fumigation-extraction (Brookes et al. 1985; Vance et al. 1987) in samples removed from the incubation jars. One portion of 10 g (on an oven-dry basis) soil was rewetted to 50% WHC and was then immediately fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the sample was extracted with 40 ml 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered through a folded filter paper (Schleicher & Schuell 595 1/2). The non-fumigated 10-g portion was extracted similarly at the time when fumigation commenced. Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatec 100 automatic analyser. Microbial biomass C was calculated as follows: microbial biomass C=E_C /k_{EC}, where E_C= (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and k_{EC}=0.45 (Wu et al. 1990). Total N in the extracts was measured as NO_2 by chemoluminescence detection after combustion at 850°C using a Dimatec 100 automatic analyser. Microbial biomass N was calculated as follows: microbial biomass N=E_N /k_{EC}, where E_N=(total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and k_{EN}=0.54 (Brookes et al. 1985; Joergensen and Mueller 1996). Due to a very high content of soluble organic C in the original soil, reliable measurements of microbial biomass C could not be obtained for the control before rewetting. Therefore, initial microbial biomass C was estimated from biomass N assuming a constant C-to-N ratio of the microbial biomass throughout the experiment in the control treatment (Joergensen and Mueller 1996).

Total C and total N were determined by gas chromatography after combustion at 1,200°C using a Carlo Erba ANA 1400 analyser. The carbonate content was measured gas-volumetrically after treatment with 10% HCl. Total organic C was calculated as the difference between total C and carbonate C. In the K_2SO_4 extracts of the non-fumigated samples, NO_3-N and NH_4-N were determined using segmented flow analysis. The K_2SO_4-extractable organic N was calculated as the difference between total extractable N minus inorganic N (NO_3-N and NH_4-N).

Statistics

The results presented in the tables are arithmetic means and are expressed on an oven-dry basis (about 24 h at 105°C). The significance of treatment effects per sampling date was tested with a one-way analysis of variance (ANOVA) using the Tukey/Kramer post-hoc test. All statistical analyses were performed using StatView 5.0 (SAS Institute).

Results

Soil moisture

During the first 9-day rewetting and drying cycle, the soil moisture content decreased from 24% to 16% dry matter in the control treatment, and to 20% in the three manure treatments, which formed a mulch layer on top of the soil surface (Fig. 1). During the second 9-day rewetting and drying cycle after incorporation of manures, the mean water content of all four treatments decreased from 23% to 17%.

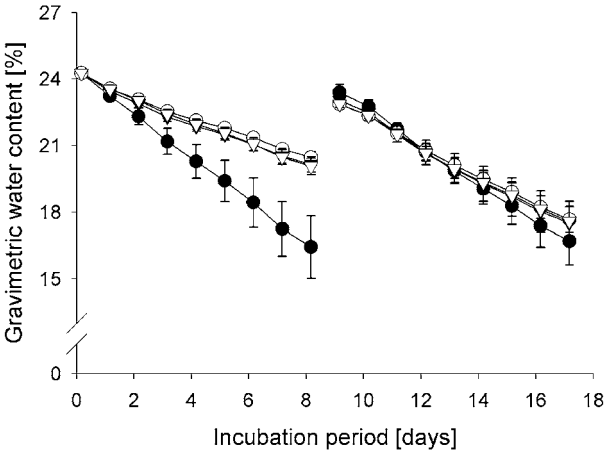

Fig. 1 Gravimetric water content (%) of the soils of the four treatments (*open circle* fresh manure, *open triangle* mature manure, *filled circle* control) during the two rewetting and drying cycles (0–9 days, 10–18 days) of the incubation experiment at 23°C. *Bars* indicate ± standard error of mean

Manure properties

The three manures contained more than 30% organic matter (Table 1). The contents of total organic C, extractable organic C and the total-organic-C to total-N ratio of the three manures decreased in the order fresh > immature > mature manure. The contents of total N and extractable organic N were highest in the immature manure and lowest in the mature manure. The same was true for extractable organic N and extractable organic C as percent of total N and of total organic C, respectively.

Table 1 Chemical properties of the three different manure qualities (fresh, immature, mature) from the oasis Balad Seet (Oman) used for the incubation experiment [*HSD* honestly significant difference (Tukey/Kramer P <0.05, n =3)]

Property	Fresh	Immature	Mature	HSD
Total C [mg g^{-1}]	464	359	327	18
Total N [mg g^{-1}]	19.0	21.3	20.9	1.2
Total C/total N	24.4	16.9	15.6	0.8
Extractable organic C [mg g^{-1}]	8.0	7.3	2.5	3.3
Extractable organic N [mg g^{-1}]	0.45	0.71	0.23	0.34
Extr. org. C/extr. org. N	17.8	10.3	10.8	1.4
Extr. organic C [% total C]	1.7	2.0	0.8	
Extr. organic N [% total N]	2.4	3.3	1.1	
Microbial biomass C [mg g^{-1}]	1.3	7.8	3.3	3.3
Microbial biomass N [mg g^{-1}]	0.4	1.0	0.3	0.4
Microbial biomass C/N	3.2	7.9	9.8	4.2
Biomass C [% total C]	0.3	2.2	1.0	
Biomass N [% total N]	2.2	4.6	1.6	
NO_3-N [μg g^{-1}]	1	3	83	19
NH_4-N [μg g^{-1}]	59	117	47	70

The sum of inorganic N did not differ significantly between the three manures. However, virtually no NO_3-N was found in the immature and fresh manure samples in contrast to the mature manure where NO_3-N made up nearly two-thirds of inorganic N.

Extractable organic C and extractable organic N

The soil of the control treatment contained considerable amounts of 0.5 M K_2SO_4 extractable organic C initially, which were further increased by manure addition (Table 2). During the first 9-day rewetting and drying cycle, the content of extractable organic C decreased by approximately 40% in all four treatments. The absolute decrease in extractable organic C formed two pairs of treatments, the control and mature manure treatments with –175 μg g^{-1} soil, maintaining the absolute difference of 90 μg g^{-1} soil between these two treatments throughout the experiment, and the fresh and immature manure treatments with –270 μg g^{-1} soil. During the second 9-day rewetting and drying cycle, the content of extractable organic C barely changed in the control and mature

Table 2 Initial total organic C content and the total-organic-C to total-N ratio in the soils of the four treatments, the content of 0.5 M K_2SO_4-extractable organic C and the ratio of extractable-organic-C to extractable-organic-N before (day 0) and after the first (9 days) and second rewetting and drying cycle (18 days) of the incubation experiment at 23°C [*HSD* honestly significant difference (Tukey/Kramer P <0.05, n =5)]

Treatment	Total organic C (mg g^{-1} soil)	C/total N Total org.	Extractable organic C			Extr. org. C/extr. org. N		
			0 days	9 days	18 days	0 days	9 days	18 days
			(μg g^{-1} soil)					
Fresh manure	42.6	13.0	670	420	390	15.1	13.8	13.9
Immature manure	38.6	11.4	650	360	310	11.9	11.7	11.4
Mature manure	37.3	11.3	470	296	290	12.9	12.1	12.6
Control	26.4	9.7	390	209	210	13.3	14.1	16.0
HSD	10.2	3.2	130	130	130	1.9	1.9	1.9

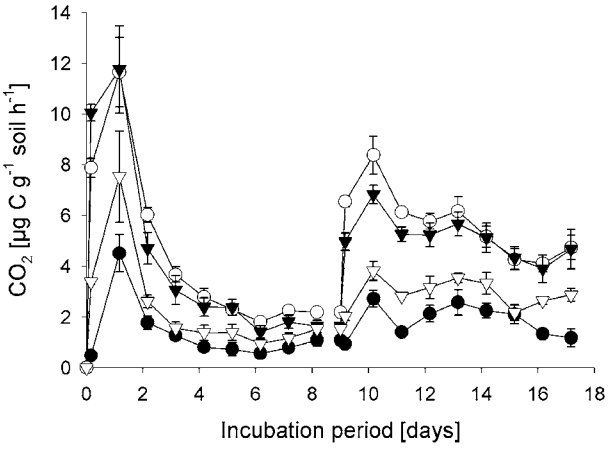

Fig. 2 CO_2 evolution rate from the soils of the four treatments (*open circle* fresh manure, *filled triangle* immature manure, *open triangle* mature manure, *filled circle* control) during the two rewetting and drying cycles (0–9 days, 10–18 days) of the incubation experiment at 23°C. *Bars* indicate ± standard error of mean

manure treatments and changed by approximately a further 10% in the fresh and immature manure treatments. The ratio of extractable-organic-C to extractable-organic-N remained roughly constant in almost all treatments throughout the incubation and this ratio was always above the total-organic-C to total-N ratio, except for the immature manure treatments where both ratios were identical.

CO_2 evolution

The CO_2-C evolution rate increased rapidly within 4 h of rewetting and reached a maximum after 24 h, followed by a rapid decline over the following 5 days (Fig. 2). Differences between the treatments were obvious throughout the incubation. Highest CO_2-C evolution rates were measured in the fresh and immature manure treatments followed at a considerable distance by the mature manure and control treatments. After incorporation of the manures into the soil followed by a second rewetting and drying cycle, the CO_2-C evolution rate increased again rapidly within 4 h and reached another maximum 24 h later. This reached, however, a much lower level than the first maximum. The following decline in CO_2-C evolution rates was less pronounced, so that the sum of CO_2-C evolved during the second rewetting and drying cycle was on average 25% larger in the four treatments compared to the first rewetting and drying cycle (Table 3). The percentage of total organic C evolved as CO_2-C was roughly 0.3% in the control and mature manure treatments and, thus, significantly lower than the 0.5% measured in the fresh and immature manure treatments.

N mineralisation

The addition of the manures significantly increased the initial contents of extractable nitrate or ammonium (Table 3). The amount of net N mineralised increased during the first 9-day rewetting and drying cycle in all four treatments. The amount of net N mineralised in the control treatment significantly exceeded the fresh and immature manure treatments, but was only 60% of the mature manure treatment. The ratio of CO_2-C evolved to net N mineralised was approximately 13 in the control and mature manure treatments, i.e. very similar to the extractable-organic-C to extractable-organic-N ratio. In contrast, the ratio of CO_2-C evolved to net N mineralised was around 60 in the fresh and immature manure treatments, i.e. more than 5 times the extractable-organic-C to extractable-organic-N ratio. During the second 9-day rewetting and drying cycle, net N mineralisation was observed in the control treatment only, but at a 5 times smaller rate. In the three manure treatments, increasing amounts of N were immobilised in the order mature < fresh < immature manure. Consequently, no net N mineralisation was observed in the fresh and immature manure treatments during the whole 18-day incubation period. In contrast, approximately 1% of total N was mineralised in the control and mature manure treatments.

Microbial biomass

The addition of manures increased the contents of soil microbial biomass C (Fig. 3a) and microbial biomass N

Table 3 Initial content of 0.5 M K_2SO_4-extractable inorganic N, net N mineralised and cumulated ΣCO_2-C evolved during the two rewetting and drying cycles (0–9 days, 10–18 days) of the incubation experiment at 23°C [*HSD* honestly significant difference (Tukey/Kramer $P < 0.05$, $n = 5$)]

Treatment	Extractable inorganic N		Net N mineralised			Cumulated ΣCO_2-C evolved		
	Nitrate	Ammonium	0–9 days	10–18 days	0–18 days (% total N)	0–9 days	10–18 days	0–18 days (% total organic C)
	(µg N g^{-1} soil at day 0)		(µg N g^{-1} soil)			(µg C g^{-1} soil)		
Fresh manure	8.9	5.2	15.4	−14.1	0.04	910	1100	0.50
Immature manure	8.9	7.3	16.9	−17.0	0.00	850	1000	0.51
Mature manure	11.8	4.8	38.8	−7.5	0.93	500	640	0.31
Control	8.8	3.0	23.7	4.9	1.08	290	400	0.27
HSD	0.3	1.2	5.1	5.0	0.20	190	160	0.10

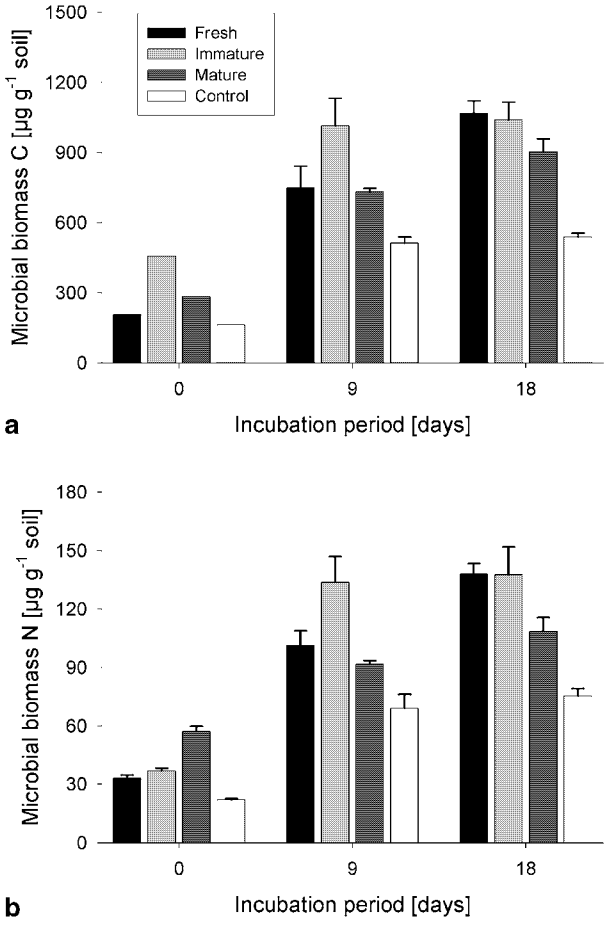

Fig. 3 Contents of microbial biomass C (**a**) and microbial biomass N (**b**) in the soils of the four treatments before and after the first (9 days) and second rewetting and drying cycle (18 days) of the incubation experiment at 23°C. *Bars* indicate ± standard error of mean

(Fig. 3b). During the first 9-day rewetting and drying cycle, both biomass indices increased by approximately 150% in all four treatments. During the second 9-day rewetting and drying cycle, no further increase was observed in the control and immature manure treatments and a roughly 30% increase in the other two treatments. As a result, the three manure treatments all reached a similar level at the end. The microbial biomass C-to-N ratio was roughly constant at 7.7 throughout the experiment in all four treatments.

Discussion

A total organic C content of more than 2% in the oasis soil from Balad Seet is high for arable soils under arid tropical conditions (Jenkinson et al. 1999; Friedel et al. 2000; Dominy et al. 2002) and considerably higher than those in the intensively irrigated soils of the Batinah region in Oman (Cookson and Lepiece 1996). The reason might be the continuous application of large amounts of organic matter through plant residues and manure (Ghoshal and Singh 1995). The microbial biomass C contents of the control treatment observed throughout the incubations are in the range of arable soils from non-irrigated (Jenkinson et al. 1999; Dominy et al. 2002) and irrigated semi-arid and arid subtropical soils (Friedel et al. 2000). Our results are in accordance with Wardle (1998) who reported that neither the total organic C content nor the microbial biomass C content of subtropical soils are necessarily below those of temperate humid regions. This is amazing considering the fact that the soil water content is able to change from completely air-dry for long periods to nearly water-saturated during the irrigation cycles. The fluctuations of the water content during our two simulated rewetting and drying cycles are similar to the field situation (Wichern et al. 2004).

Effects of drying and rewetting on soil micro-organisms

A large percentage of the microbial biomass increase after the first rewetting and drying cycle is probably due to the metabolism of dead microbial tissue by surviving micro organisms (Amato et al. 1984; Van Gestel et al. 1993). An unknown percentage of this increase could also be caused by rehydration as observed by the rapid increase in ATP from initially 16% to 82% within 4 h of rewetting an air-dry clay soil that could not be attributed to growth processes (Ahmed et al. 1982). Microbial death leads to an enormous loss of energy corresponding to approximately 60% to 80% of the biomass (Jenkinson 1988). Therefore, the observed strong increase in microbial biomass after rewetting must be due to further mobilisation of non-biomass soil organic matter during drying (Appel 1998). This process is strongly indicated by the C-to-N ratio of K_2SO_4-extractable organic matter, indicating the mobilisation of an overproportionate percentage of carbohydrates during drying (Bottner 1985).

Organic fertilisers as a mulch layer or incorporated into the soil

Evaporation was significantly reduced in our experiment by the presence of a mulch layer, which is a well-documented phenomenon in arid climates (Zaongo et al. 1997; Buerkert and Lamers 1999). Reducing the immobilisation of inorganic N in the soil microbial biomass, but especially in soil organic matter, is another important effect of applying manures as a mulch layer.

The decomposition rates of manures on the soil surface was roughly half the rate in comparison to those in the soil when the flush of CO_2 evolution caused by rewetting and mixing is over. A similar difference was observed by Flessa et al. (2002) looking at the decomposition of mulched or incorporated rye grass. They attributed this result to an improved regulation of the water supply to the microbial decomposer community. In our study, the enhanced contact between manures and soil particles after

incorporation may have led to a considerable decrease in inorganic N during the second rewetting period, possibly due to microbial transformation into non-biomass amino acids (Appel 1998). NH_3 volatilisation could not be completely excluded at a pH of 8.5, as in our soil, although more than 95% of the inorganic N fraction was transformed into nitrate at the end of the incubation. During the second rewetting and drying cycle, N immobilisation occurred in all three manure treatments and net N mineralisation also decreased in the control treatment. This indicates a considerable shift in microbial substrate utilisation from soluble components to solid soil organic matter and probably also a shift in the microbial community structure (Lundquist et al. 1999).

Substrate quality indices and microbial decomposition

The quality differences might have had different effects during the first "mulch" and the second "incorporation" period as the four treatments form two pairs with similarity in the percentages of total organic C evolved as CO_2 and total N mineralised as NH_4 and NO_3. The control and mature manure treatments form the first pair with a low percentage of total organic C evolved as CO_2 and a considerable percentage of total N mineralised as NH_4 and NO_3, the fresh and immature manure treatments form the second pair with a high amount of total organic C evolved as CO_2 and no net N mineralisation during the 18-day incubation with two rewetting events.

The only property of the manures reflecting future CO_2 evolution and N immobilisation rates, i.e. the formation of the two treatment pairs, is the content of extractable organic C in % total organic C. This fraction decreases as biological decomposition proceeds (Wu et al. 2000). The importance of extractable organic C for the immobilisation of N has been shown repeatedly (Seneviratne et al. 1998; Martin-Olmedo and Rees 1999), but not in comparison to the substrate quality indices explained above. No N immobilisation should occur after adding substrates with a total-C to total-N ratio <15 (Powlson et al. 2001). In our immature manure and fresh manure samples, N was probably immobilised at relatively low total-C to total-N ratios of between 17 and 24 in combination with high percentages of extractable organic N. This indicates that extractable organic C could be more important than extractable organic N to predict N immobilisation.

Conclusions

The application of manures as surface mulch reduces water loss and might be one reason for the high water use efficiency of the oasis systems in Oman. N immobilisation may play an important role after incorporation of the manure. After subsequent irrigation events this immobilised N is likely to be available for the growing plants. This temporal N immobilisation during times of low crop demand helps to prevent leaching losses, thereby increasing nutrient use efficiency.

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