MANURE APPLICATION EFFECTS ON NITROGEN POOLS IN A SUBTROPICAL SOIL OF OMAN

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Abbreviations

ape%	Atom percentage excess
at%	Atom percentage
C	Carbon
CFE	Chloroform-fumigation extraction
CM	Unfertilized control soil treated with labeled manure in the laboratory
C_{mic}	Microbial C
Co	Unfertilized control soil
DOC	Dissolved organic carbon
F1	Field-applied manure in year 1
F2	Field-applied manure in year 2
L+CH	Treatments with ^{15}N labeled manure applied in the first cropping season + 10% w/w charcoal
LL	Treatments with ^{15}N labeled manure applied in first and second cropping seasons
LU	Treatments with ^{15}N labeled manure applied in first cropping season and unlabeled manure applied in the second cropping season
LU+CH	Treatments with ^{15}N labeled manure applied only in the first cropping season and unlabeled manure applied in second cropping season + 10% w/w charcoal
M	Labeled manure
m	Unlabeled manure

$manure_{prev}$	Previous year manure application
$manure_{rec}$	Recent manure application
MM	Soil labeled with ^{15}N labeled manure in the first and second cropping seasons
Mm	Soil labeled with ^{15}N labeled manure in the first cropping season and unlabeled manure in the second cropping season
MMM	Soil labeled with ^{15}N manure in both cropping seasons and treated with labeled manure in the laboratory
MMm	Soil labeled with ^{15}N manure in both cropping seasons and treated with unlabeled manure in the laboratory and
Mmm	Soil labeled with ^{15}N manure in the first cropping, received unlabeled manure in the second cropping season and treated with unlabeled manure in the laboratory
N	Nitrogen
Ndfm	Nitrogen derived from manure
N_{mic}	Microbial nitrogen
OM	Organic matter
POM	Particulate organic matter
SOC	Soil organic carbon
SOM	Soil organic matter
WHC	Water holding capacity

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Summary

Agricultural productivity of the millennia-old oasis agriculture in Oman is based on the continuous application of high rates of goat and sheep manure combined with flood irrigation. The high nitrogen (N) and carbon (C) turnover in these systems as a result of year-around high temperatures have been well documented. However, knowledge is lacking on how N and C are transformed in the semi-arid soils. Therefore, this study aimed at investigating movements of N in different soil and plant pools and their ultimate turnover in gaseous form as ammonia (NH_3) and dinitrous oxide (N_2O). The study of N movement into different soil pools requires the utilization of isotopic N (^{15}N) as a marker to discriminate labeled N applied and N already available in the soil. The first two studies presented here focus on the development of an ammonia trap method to simultaneously trace minute amounts of NH_3, N_2O and CO_2 emissions in laboratory incubation studies, changes in soil and microbial N pools, and the last chapter focuses on the movement of labeled N from manure in soil and plant pools and the effect of charcoal application in a field study.

For field experiments ^{15}N labeled Rhodes grass (*Chloris gayana* Kunth) was produced by foliar spraying of 10 atom% (at%) urea after which labeled (L) or unlabeled (U) hay was fed to male Omani Batinah goats (*Capra aegagrus hircus*) to produce labeled (0.526 at% ± 0.003 SD) and unlabeled manure (0.369 at% ± 0.000 SD). Production of unlabeled manure along with labeled manure was necessary so that we could distinguish manure applied in the previous year and recent years. Sundried goat manure-N at the rate of 120 and 90 kg N ha^{-1} was applied in soil surface to cabbage (*Brassica oleracea* L.) followed by basil (*Ocimum basilicum* L.), respectively, for two seasons (2013/2014 and 2014/2015) with flood irrigation supplying 100% of the crop water requirement. Soil N, plant biomass N, ^{15}N at% and biomass yield were compared in two cropping seasons. In the first year, plots receiving the treatments: 1) labeled manure (L), 2) labeled manure and 10% w/w charcoal (L+CH), and 3) mineral fertilizer (Mn) were compared. In the second year, plots receiving the treatments: 1) labeled manure applied in two cropping seasons (LL), 2) labeled manure applied in the first cropping season and unlabeled manure applied in the second cropping season (LU), 3) LU + 10% w/w charcoal (LU+CH) and, 4) Mn applied plots were compared. Differently labeled soil samples were collected from the field after each cropping season for the subsequent laboratory soil incubation experiment.

Before the first soil incubation experiment, a modified method to trap volatilized ammonia directly into acid was tested. A standard NH_3 gas and volatilized NH_3 from NH_4Cl were trapped in acid ($KHSO_4$ and/or H_2SO_4) soaked glass fiber filters to verify the recovery rate of the acid trap system. The tested ammonia traps were hung tightly sealed glass jars packed with

differently labeled soil-manure mixtures. These mixtures were incubated for 31 days (21 days after manure application) at 25°C. In the first laboratory incubation experiment conducted with the soil collected after the first cropping season, the treatments were: (1) unfertilized control soil (Co), (2) unfertilized control soil treated with labeled manure in the laboratory (CM), (3) ^{15}N labeled soil treated with labeled manure in the laboratory (MM) and (4) ^{15}N labeled soil treated with unlabeled manure in the laboratory (Mm). In the second incubation experiment conducted with the soil collected after the second cropping season, the treatments were: (1) unfertilized control soil (Co), (2) soil labeled with ^{15}N manure in both cropping seasons and treated with labeled manure in the laboratory (MMM), (3) soil labeled with ^{15}N manure in both cropping seasons and treated with unlabeled manure in the laboratory (MMm), and (4) soil labeled with ^{15}N manure only in first cropping season and treated with unlabeled manure in the laboratory (Mmm). In both incubation experiments, total N and organic C, K_2SO_4 extractable N, and C, microbial N (N_{mic}), and C (C_{mic}) in the soil, N_2O-N and CO_2–C emission rates as well as the ^{15}N concentration in N pools were analyzed.

The filter trap method allowed to recover 81-87% of the standard NH_3 gas, and 63-78% of the NH_3 volatilized from an ammonium chloride (NaCl) solution, thus showing limitations in the accuracy of the system. The modified methodology was adapted for field level emissions concentration of NH_3 (4.6 µg N) inside the vessels unlike the original method that was designed for sample masses of 50-200 ug N. The modified method can be improvised to be more accurate. In the first incubation experiment, cumulative N_2O-N emission for one-time manure applied soil (CM) was 141 µg N kg^{-1} after manure application, of which only 22% was derived from manure and which was more than three times higher than for MM. N_{mic} in CM increased by 120%, of which only 32.5% was derived from manure. At the same time, K_2SO_4 extractable N was 37.48 µg g^{-1} for CM and 45.67 µg g^{-1} for MM after the fertilizer application that decreased by 38.6% and 7%, respectively, at the end of the incubation. This indicated larger soil N availability for microorganism than manure N in CM. No significant changes were observe in the second incubation experiment except the K_2SO_4 extractable N was low in all treatments. However, the ^{15}N derived from the manure was in the order MMM>MMm>Mmm at the end of the experiment as expected. Our study showed that freshly applied goat manure on unfertilized soil triggered microbial growth and N_2O emissions, causing a ‘priming effect’ compared to repeated application of manure.

In the field experiment, cabbage dry matter yields in the first season amounted to 2.9 (SD ± 0.6), 1.6 (SD ± 0.2) and 7.2 (SD ± 1.2) Mg ha^{-1} for the L, L+CH and Mn treatments each significantly different from another. The dry mater yields increased by 70%-167% for manured plots while Mn yields remained similar and still significantly different. Basil yields were 0.9 (SD ± 0.2), 0.8 (SD ± 0.1), 1.2 (SD ± 0.3) Mg ha^{-1} for the L, L+CH and Mn in the first season that

increased by 71% for manured plots and 26% for Mn in the second season. The yields were not significantly different for manured or mineral fertilizer applied plots in both the season. Charcoal application significantly reduced cabbage biomass yield by 44% for cabbage in the first cropping season. However, it increased the total C % of the soil significantly higher than the Mn plots at the end, showing its potential implication to increase soil organic carbon. The leftover N derived from labeled manure (% N_{dfm}) at the end of the second cropping season was 9.9%, 5.5%, and 5.4% for LL, LU, and LU+CH, respectively. This indicated a significant portion of manure applied was recalcitrant for at least two seasons.

Zusammenfassung

Die seit Jahrtausenden anhaltende Produktivität der Oasenlandwirtschaft im Oman beruht sowohl auf einer kontinuierlichen Anwendung von hohen Ziegen- und Schafmistapplikationsraten als auch einer intensiven Überstaubewässerung. Der hohe Stickstoff- (N) und Kohlenstoffumsatz (C) in diesen Systemen in Folge der ganzjährig hohen Temperaturen ist gut dokumentiert, jedoch ist über die Umwandlung von N und C in den semi-ariden Böden wenig bekannt. Daher zielte die vorliegende Studie darauf ab, die Bewegungen von N in unterschiedlichen Boden- und Pflanzenpools und seinem endgültigen Umsatz in Form von Ammoniak (NH_3) und Distickstoffoxid (N_2O) zu untersuchen.

Die Untersuchung der N Bewegung in verschiedene Boden-Pools erfordert die Nutzung von isotopischem N (^{15}N) als Marker, um den markierten, frisch applizierten N von schon im Boden vorhandenen N zu unterscheiden. Die ersten zwei hier präsentierten Studien befassen sich mit der Entwicklung einer Ammoniak-Fallenmethode, die es ermöglicht sehr geringe Mengen an NH_3-, N_2O- und CO_2-Emissionen in Laborinkubationsversuchen zeitgleich zu verfolgen, sowie mit den Veränderungen im Boden- und mikrobiellen N Pool. Das letzte Kapitel ist auf die Translokation von markiertem N von Dung in den Boden- und Pflanzen-Pool sowie den Effekt von Kohle in einem Feldversuch fokussiert.

Für einen Feldversuch wurde ^{15}N markiertes Rhodesgras-Heu (*Chloris gayana* Kunth) durch Blattapplikation von 10 Atom-% (at%) Harnstoff hergestellt. Anschließend wurde markiertes (L) oder unmarkiertes (U) Heu an männliche Omani Batinah-Ziegen (*Capra aegagrus hircus*) verfüttert, um markierten (0,526 at%) und unmarkierten Dung herzustellen (0,369 at%). Die Herstellung von unmarkiertem Dung war notwendig, um zwischen Dungapplikationen aus dem ersten und zweiten Anbaujahr zu unterscheiden. In einer Fruchtfolge von Kohl (Brassica oleracea L.) gefolgt von Basilikum (*Ocimum basilicum* L.) wurde der getrockneter Ziegendung oberflächlich mit einer Applikationsmenge von 120 bzw. 90 kg N ha^{-1} in zwei aufeinanderfolgenden Vegetationsperioden (2013/14 und 2014/15) ausgebracht. Die Bewässerung wurde zur vollständigen Deckung des Wasserbedarfs der Pflanzen angepasst. Der N-Vorrat im Boden, die N-Aufnahme der Pflanzen, die ^{15}N-Markierung in Boden und Pflanzen sowie der Biomasseertrag , wurden in den beiden Vegetationsperioden verglichen. Im ersten Jahr wurden die Parzellen mit folgenden Behandlungen gedüngt: 1) markierter Dung (L), 2) markierter Dung und 10% w/w Kohle (L+CH) und 3) Mineraldünger (Mn). Im zweiten Jahr erhielten die Plots folgende Behandlungen: 1) Düngung mit ^{15}N-markiertem Dung für zwei Anbausaisons (LL), 2) Düngung mit ^{15}N-markiertem Dung in der ersten und mit

unmarkiertem Dung in der zweiten Anbausaison (LU), 3) wie LU + 10% Kohle (LU+C) sowie 4) Mineraldünger (Mn). Die somit unterschiedlich markierten Böden wurden nach jeder Anbausaison beprobt und für anschließende Laborinkubationsversuche verwendet.

Vor dem ersten Inkubationsexperiment wurde die Wiederfindungsrate eines modifizierten Ammoniak-Säurefallsystems (mit Säure ($KHSO_4$ und / oder H_2SO_4) getränkte Glasfaserfilter) mittels eines Standard-NH_3-Gases und flüchtigem NH_3 aus NH_4Cl überprüft. Diese Ammoniak-Fallen wurden im Anschluss in Bodeninkubationsversuchen in dicht verschlossene Glasgefäßen mit unterschiedlich markierten Bodenmistgemischen angewendet. Die Bodenmistgemische wurden 31 Tage (21 Tage nach dem Düngemittelauftrag) bei 25 °C inkubiert. Im ersten Inkubationsversuch wurden folgende Behandlungen mit dem Boden, der nach dem ersten Jahr beprobt wurde, durchgeführt: (1) ungedüngter Kontrollboden (Co), (2) Kontrollboden, der im Labor mit markiertem Dung (CM) behandelt wurde, (3) ^{15}N markierter Boden, der im Labor mit markiertem Dung behandelt wurde (MM) und (4) ^{15}N-markierter Boden, der im Labor mit unmarkiertem Dung behandelt wurde (Mm). Im zweiten Inkubationsversuch wurden folgende Behandlungen mit dem Boden, der nach dem zweiten Jahr beprobt wurde, untersucht: (1) ungedüngter Kontrollboden (Co), (2) ^{15}N markierter Boden, der in beiden Anbauperioden und im Labor mit markiertem Dung (MMM) behandelt wurde, (3) Boden, der in beiden Anbauperioden mit markiertem Dung und im Labor mit unmarkiertem Dung (MMm) behandelt wurde sowie (4) Boden, der in der ersten Anbauperiode mit markiertem Dung und danach ausschließlich mit unmarkiertem Dung (Mmm) behandelt wurde. In beiden Inkubationsversuchen wurden der Gesamtstickstoff und der organische C, der K_2SO_4-extrahierbare N und C, und der mikrobielle N (Nmic) und C (Cmic) im Boden, die N_2O-N und CO_2-C Emissionsraten sowie die ^{15}N-Konzentrationen in den N-Pools analysiert.

Gases und 63-78% des aus einer Ammoniumsulfat-Lösung verflüchtigten NH_3, wodurch die Genauigkeit des Systems eingeschränkt ist. Im Gegensatz zur Originalmethode, die für N-Mengen von 50-200 µg entwickelt wurde, wurde die modifizierte Methode für niedrige, in Feldmessungen übliche Ammoniakkonzentrationen (4,6 µ N im Inkubationsgefäß) angepasst. Für eine höhere Präzision sind jedoch weitere Modifikationen notwendig. Im ersten Inkubationsversuch betrugen die kumulativen N_2O-N-Emissionen für CM nach der Dungapplikation 141 µg N kg^{-1}, wovon lediglich 22% aus dem Dung stammten, und dreimal so hoch waren wie für MM. In CM erhöhte sich der N_{mic} um 120%, wovon lediglich 32,5% aus dem Dung aufgenommen wurden. Gleichzeitig betrug der K_2SO_4 extrahierbare N 37,5 µg g^{-1} für CM und 45,3 µg g^{-1} für MM nach der Düngeranwendung, die am Ende des Inkubationsexperimentes um 38,6% bzw. 7% abnahmen. Dies deutet auf eine höhere N-Verfügbarkeit für Mikroorgansimen des Dungstickstoffs aus CM an. Im zweiten Inkubationsexperiment gab es keine signifikanten Veränderungen, wobei der K_2SO_4-

extrahierbare N in allen Behandlungen niedrig war. Jedoch konnte am Ende des Experiments gezeigt werden, dass der markierte N aus dem Dung in der erwarteten Reihenfolge MMM>MMm>Mmm vorlag. Unsere Studie zeigte, dass im Vergleich zu wiederholter Ziegendungapplikation, frisch applizierter Ziegendung auf ungedüngtem Boden das Wachstum von Mikroben und die N_2O-Emissionen anregen, und einen „Priming-Effekt“ auslösen kann.

Im Freilandversuch betrugen die Biomasseerträge des Kohls in der ersten Saison 2,9 (SD ± 0,6), 1,6 (SD ± 0,2), und 7,2 (SD ±1,2) Mg TM ha^{-1} für die L, L+CH- und Mn Behandlungen, welche signifikant verschieden waren. Die Trockenmasseerträge stiegen in der zweiten Saison um jeweils 79% bis 167% in den mit Ziegendung gedüngten Behandlungen, während die Erträge für Mn konstant blieben und signifikant von den anderen Behandlungen verschieden waren. Die Basilikumerträge erreichten in der ersten Saison 0,9 (SD ± 0,2), 0,8 (SD ± 0,1) und 1,2 (SD ± 0,3) Mg ha^{-1} für die L, L+CH und Mn Behandlungen, welche in der zweiten Saison in den Ziegendung-gedüngten Behandlungen um 71% und für Mn um 26% anstiegen. Die Erträge zwischen Ziegendung-gedüngten und Mineraldünger-gedüngten Plots waren in beiden Anbaujahren nicht signifikant verschieden. Die Anwendung von Aktivkohle reduzierte die Kohlerträge signifikant in der ersten Saison um 44%. Jedoch wurde der Gesamt-Kohlenstoffgehalt im Boden signifikant erhöht im Vergleich zu den Mn-Plots, wodurch sein Potential, den organischen Kohlenstoff im Boden zu erhöhen, gezeigt wurde. Der im Boden verbleibende markierte Dung-N lag am Ende der Saison bei 9,9%, 5,5% bzw. 5,4% für LL, LU und LU+C, was darauf hindeutet, dass ein deutlicher Anteil des applizierten Dungs für mindestens zwei Anbauperioden abbaustabil war.

1. Chapter
General introduction and research objectives

1.1 Agriculture in Oman

The sustainability of agriculture in arid and semi-arid regions depends on the careful utilization and management of available resources. More than 2 billion people live in drylands and face everyday challenges with agricultural practices such as water scarcity, land degradation, and salinity (*Koohafkan,* 2008).

As a Gulf Country, the Sultanate of Oman covers an area of 309,500 km^2 that extends from the United Arab Emirates (UAE) to Yemen along the Gulf of Oman and the Arabian Sea. It has a population of 4.16 Mio (*MoSPI*, 2016), who are primarily concentrated around the Al-Hajar Mountains that extend along the coastline of the Gulf of Oman and the Arabian Sea in the northern and eastern side of the country. About 80 % of Oman's surface is covered by desert, and only around 0.3 % of the total land area is used for irrigated oasis agriculture (*MoSPI*, 2016), which contributes 2% to the national GDP. However, oasis agriculture defines to a substantial degree of Omani culture, which is reflected in the food, building style, mode of communication, and infrastructure. People take pride in having a green garden/orchard or agricultural field called 'Majra' in the local Arabic language.

The widespread traditional canal irrigation system called 'Falaj' (singular) or 'Aflaj' (plural) developed in the area since 500 AD allowed Omani farmers for centuries to channel water from surface springs in the often distant mountains to agricultural lands through gravity flow (*Abdel Rahmnn and Omezzine*, 1996; *Al-Marshudi*, 2001; *Nagieb et al.*, 2004). As an example of centuries-old irrigation agriculture, this system has been intensively studied in the past (*Luedeling et al.*, 2005; *Siebert et al.*, 2005). However, the move towards modern agricultural practices since Oman's opening to the outside world in 1970, has made traditional sustainable agricultural practices less attractive compared to the expansion of intensive drip-irrigation practices around the planes of the Al-Hajar Mountains, especially in the AL-Batinah region. Irrigation agriculture in Al-Batinah depends exclusively on rapidly dwindling groundwater resources, whereby it has been estimated that Oman now uses 93% of its groundwater for agriculture (*FAO*, 2009). Permanent tree crops (date palm-*Phoenix dactylifer*a, acid lime - *Citrus aurantifolia* and mango- *Mangifera indica*), perennial forage crops (alfalfa – *Medicago sativa*, Rhodes grass- *Chloris gayana*, elephant grass- *Pennisetum puipureum*), field crops (wheat- *Triticum aestivum and T. durum*, barley- *Hordeum vulgare*, oat – *Avena sativa*, maize- *Zea mays*, sorghum - *Sorghum bicolor*) and vegetable crops (cucumber- *Cucumis sativus*,

garlic- *Allium sativum*, onion- *Allium cepa*, tomato- *Lycopersicon esculentum*) are widely grown in Oman (*FAO*, 2008). However, their production is not sufficient to meet local demands.

1.2 Nutrient management of Omani soils

The lack of vegetation cover, sparse precipitation, and year-round high temperatures lead to low soil organic carbon (SOC) contents in Omani soils. Moreover, intensive agriculture has raised the risk of soil fertility decline, overuse of water resources and soil and water salinity (*Abdel-Rahman and Abdel-Magid*, 1993; *FAO*, 2009; *MAF and ICBA,* 2012*)*. The predominantly sandy soils are well-draining but require frequent straw incorporation, root debris, and manure application, mainly from the widely kept goat herds (*Buerkert et al.*, 2010). Its regular application is vital for sustainable traditional oasis agriculture in Oman. However, under the prevailing conditions, the turnover rate of SOC is high (*Bationo et al.*, 2007), and the residual effect of manure can decreases rapidly (*Freschet et al.*, 2008). The application efficiency is also low because of substantial gaseous and leaching losses under irrigated conditions (*Wichern et al.*, 2004b; *Buerkert et al.*, 2010; *Siegfried et al.*, 2011). The traditional practices of manure incorporation in agricultural soil have played a crucial role in the sustainability of the semi-arid mountains of Oman and forging animals like goats have explicitly been crucial as they bring in nutrients to the farm (*Buerkert et al.*, 2005; *Schlecht et al.*, 2011). Goats are particularly well adapted to the arid climate, and their role may even increase in the future as climate changes (*Silanikove and Koluman*, 2015). In 2009, 66% of the total ruminant raised in Oman were goats (*MAF*, 2014).

Net mineralization of manure in irrigated tropical sandy soils such as those predominating in Oman is typically high leading to rapid losses of C and N as NH_3 and N_2O volatilization and NO_3^- leaching (*Zech et al.*, 1997; *Sierra*, 2002; *Wichern et al.*, 2004a, 2004b; *Buerkert et al.*, 2010; *Lompo et al.*, 2012; *Siegfried et al.*, 2011; *Ingold et al.*, 2015; *Al-Rawahi et al.*, 2017). In addition to that, alkaline soil conditions could exacerbate volatilization losses. From the Al-Batinah Plain in northern Oman average gaseous N losses (NH_3 and N_2O emissions) of 50-55 kg N ha^{-1} and N leaching of 10-56 kg N ha^{-1} have been reported over 24 months experimental period under vegetable cropping system after buffalo manure application (*Siegfried et al.*, 2011). Similarly, emission losses of 109 and 157 kg N ha^{-1} yr^{-1}have been reported under vegetable cropping system after goat manure application in the Hajar Mountains in northern Oman (*Al-Rawahi et al.*, 2017). However, a solid understanding of the C and N turnover in the agriculture system is lacking.

1.3 Soil organic carbon (SOC) and the importance of manure incorporation

Globally carbon (C) is stored in five principal pools: (1) the oceanic pool (38,000 Pg), (2) the geological pool (coal 4000 Pg + oil 500 Pg + gas 500 Pg), (3) the pedologic pool SOC 1220 to 1550 Pg + soil inorganic C 695 to 748 Pg at 1-m depth), (4) the atmospheric pool (760 Pg) and (5) the biological pool (560 Pg; *Lal*, 2004). Management of terrestrial (pedologic and biotic) C pool has a huge potential to address issues of sustainable agriculture and climate change as oxidation of SOC due to agricultural activities is of major concern (*IPCC*, 2014).

Incorporation of plant litters and faunal residues in agricultural soil is a general practice and critical to stabilize or increase soil organic matter (SOM) content. SOM can consist of a mixture of various organic components like undecomposed or partially decomposed plant and animal tissues, particulate organic matter (POM), microorganisms, plant roots, and relatively stable humus (*Johnston et al.*, 2009). During the decomposition of organic material, three main carbon fractions are formed, that is the liable, intermediate, and passive fraction, with resident times ranging from 0.1 to 2200 years. Most of the organic C in the soil belongs to the passive fraction humus, which is amorphous, has a large surface area, and has a high charge density (*Lal*, 2004) formed during the decomposition of organic material in the soil through the process of humification (*Zech et al.*, 1997).

SOM consists of up to 46-58% C (*Stockmann et al.*, 2013) and is vital to the soil fertility, crop productivity, and sustainable agriculture system (*Johnston et al.*, 2009). SOM is even more important in fragile soil as it contributes to the formation of organo-mineral complexes and soil micro- (<250 µm) and macro-aggregates (>250 µm; *Christensen*, 2001) thus affecting soils' physical strength against environmental disturbances. However, agricultural practices like tillage, irrigation, and land conversion can accelerate the disruption of soil aggregates (*Six et al.*, 2000; *Denef et al.*, 2001; *Wang et al.*, 2014). These agricultural practices disrupt and distribute soil aggregates exposing it to environmental breakdown by microorganisms or physicochemical deterioration.

The combination of high temperature and moisture in intensive agriculture leads to even faster SOC turnover under arid tropical conditions such as in Oman (*Wichern et al.*, 2004b; *Buerkert et al.*, 2010; *Siegfried et al.*, 2011). SOC storage declines with increasing mean annual temperature. Thus in an arid tropical climate without much vegetation, SOC is much lower than in temperate organic soil at the same depth (*Post et al.*, 1982).

Continuous incorporation of plant litters and faunal residues and changes in farm management practices such as mulching, no-till, and conversion to organic farming can increase SOC (*Six et al.*, 2000; *Pagliai et al.*, 2004; *Artemyeva and Kogut*, 2016). However, this is a slow process,

as the turnover of incorporated organic matter is rather fast. Only 10-20% of biomass-incorporated C remains in the soil after 5-10 years which largely depends upon the recalcitrant C in plant material (*Gill and Jackson*, 2000; *Lehmann et al.*, 2006).

Animal manure has always been a vital source of C and nutrients in agriculture. Repeated application of manure in the soil has often resulted in higher SOC, and it can be particularly beneficial for tropical and sub-tropical soils with initial low SOC (*Kihanda et al*., 2006; *Hemmat et al*., 2010; *Chen et al*., 2018). Since microorganisms discriminate added substrate based on the complexity of C molecules, larger macromolecules, including cellulose, hemicellulose, and lignin, are degraded slowly (*Berg and McClaugherty*, 2003). As the manure itself is a heterogeneous mixture of fully or partially digested plant material, gut bacteria, and animal proteins, it consists of a labile fraction and a more recalcitrant fraction, which takes time for mineralization, often releasing nutrients even to the succeeding crops. This residual effect of manure on SOC and productivity can last for years (*Kihanda et al*., 2006; 2005).

According to a recent study by *Ingold et al.* (2018), under the conditions of irrigated agriculture in northern Oman, around 50% of goat manure is decomposed within 4-6 weeks after application, followed by lower rates of decomposition and nutrient release. The study also reported that despite high N losses, repeated application of goat manure increased the N stock in soil (*Ingold et al.*, 2015). The study verifies the importance of manure application in Omani soils as reported by previous studies (*Buerkert et al*., 2010; *Siegfried et al.*, 2011). This also partially explains the success of age-old sustainable farming in the mountain Oasis using goat manure (*Buerket et al*., 2005). However, there is still limited information on the N loss pathways from various pools and the effect of repeated manure applications on N turnover in Omani soils. Also unknown is the effect of residual N from previously applied manure on subsequent N mineralization.

The mineralization of manure is determined by physical, chemical and biological factors such as temperature, moisture, pH, the initial chemical composition of manure (concentration of lignin, the C/N ratio, and tannins), storage of manure, the soil's nutrient status, soil properties (texture, pH), tillage and soil biological activity. The C/N ratio of the incorporated manure is particularly important for the N release on nutrient-poor soils (*Manzoni et al.*, 2010). N mineralization or immobilization of N, for example, depends upon the C/N ratio of the soil and the incorporated manure. As mineralization is affected by many factors, supplying enough nutrients during periods of higher crop demand is sometimes tricky. Untimely release of excess N in the soil could lead to heavy losses due to volatilization or leaching. Besides the direct loss of N from manure, N losses may also occur from the soil as a consequence of

increased mineralization of soil due to a priming effect (*Jenkinson et al.*, 1985; *Kuzyakov*, 2010).

1.4 Manure N and losses through N_2O and NH_3

N is one of the most essential plant nutrients and plays a critical role in the nutrient turnover of soils. Although some plants may effectively absorb a simpler form of organic N (*Nasholm et al.*, 2009), they mainly utilize N in the inorganic forms ammonium (NH_4^+) and nitrate (NO_3^-). However, losses of N from the field after the application are both economically and environmentally hazardous. N volatilizes as NH_3 and N_2O, leaches as NO_3^- or gets lost in runoffs and erosion (*Cameron et al.,* 2013). While the emission of greenhouse gases such as N_2O can also have a larger environmental impact (*IPCC*, 2007), re-deposition of N in terrestrial environments can alter species composition, eutrophication, and overall degradation of biodiversity (*Aneja et al.*, 2001).

Most of the N excreted by animals (60-90%) is present in urine as labile urea, which is difficult to handle (*Marschner and Rengel*, 2007). Animal manure, on the other hand, is convenient to handle but has a lower N concentration, mostly present as insoluble organic N bound to indigested feed, microorganisms, and N secretion in the digestive tract (dead cells and mucus) with a tiny fraction in ammonia (NH_3). In the soil, microorganisms help to break down organic N to NH_4^+ by ammonification, which is further oxidized to NO_3^- by a two-step process called 'nitrification' controlled by specific groups of bacteria and archaea (*Canfield et al.*, 2010). The oxidation process can continue until N is finally released to the atmosphere as N_2, which is called 'denitrification' (Fig. 1.1).

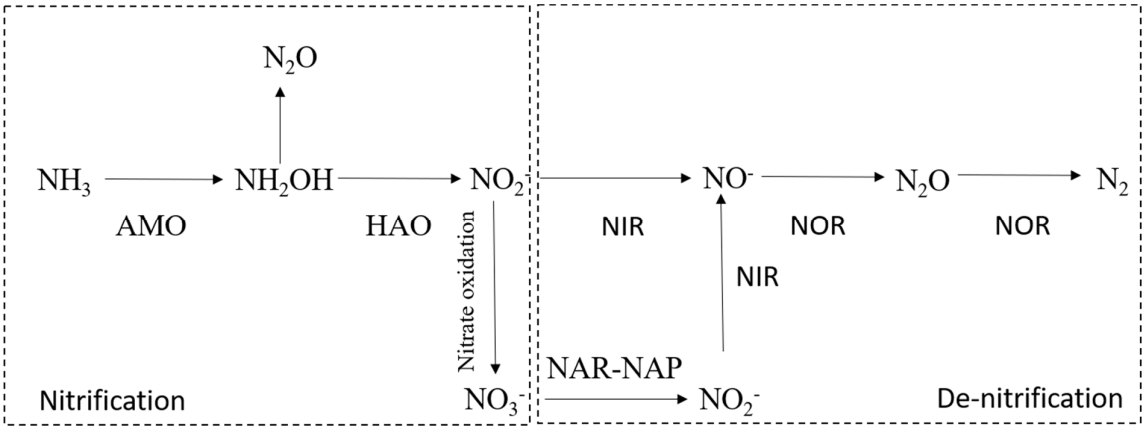

Figure 1.1 Nitrification and Denitrification. Enzymes AMO=Ammonia mono-oxygenase enzyme, HAO= Hydroxylamine oxidoreductase, NIR=Nitrite reductase, NOR=nitrous oxide reductase. NAR= the membrane-bound nitrate reductase, NAP= periplasmic nitrate reductase. Adapted from *Baggs* (2011) and *Baggs and Philippot,* (2011).

During nitrification and denitrification, N_2O is released to the atmosphere (Fig. 1.1), which has the global warming potential (CO_2 equivalent) of 298 x CO_2. Emission from soil alone is responsible for ~70% of the global N_2O emissions of which agricultural systems contribute 6.8 Tg N_2O-N $year^{-1,}$ making it the biggest contributor (*IPCC*, 2007). An estimated 0.1% to 7% of N applied to the field can be emitted as N_2O after fertilizer application (*Skiba and Smith*, 2000). The estimation has a considerable variation because the emission of N_2O is determined by agro-ecological conditions and agricultural practices, N and C availability, soil moisture content/oxygen partial pressure and pH (*Baggs and Philippot*, 2011). Application of fresh manure is likely to cause large N_2O emissions as it creates favourable conditions for nitrification with the supply of N and C together with increase in soil moisture content.

Globally, about 80% of the volatilized NH_3 is of anthropogenic origin, and in 2005 emissions from manure management and its field application contributed 34% of total volatilization compared to 47% from synthetic fertilizer (*Behera et al.*, 2013). Dry and wet deposition of NH_3 and NH_4 salts (formed after NH_3 reacts with acids compounds like sulfuric acid, nitric acid, and hydrochloric acid) can cause eutrophication and biodiversity degradation (*Aneja et al.*, 2001; *Krupa*, 2003; *Behera et al.*, 2013). NH_4^+ released from manure can convert to NH_3. Both NH_4^+ and NH_3 are present in the manure, but the NH_4^+/NH_3 equilibrium in manure depends upon the ionic strength of aqueous NH_4^+ and aqueous NH_3 at the source which is affected by pH and temperature. Higher pH or higher temperature means higher NH_3 volatilization (*Sommer et al.*, 1991; *Rochette et al.*, 2013). Some NH_3 can get re-absorbed to the aqueous surface, but a high wind intensity can cause significant losses. The formation of NH_3 depends upon the equilibrium between aqueous NH_3 in the manure and gaseous NH_3 (*Behera et al.*, 2013).

Losses of N through N_2O and NH_3 emissions from both manure applied irrigated mountain oasis agriculture and from coastal agricultural land in Oman are high. *Siegfried et al*. (2011) reported that 25% of the total gaseous losses were N_2O, and 75% NH_3. *Al-Rawahi et al*. (2017) reported that 63-48% of the total gaseous losses were through $NH_{3,}$ and the emissions were typically high during the initial days of manure application. While the emissions were measure after the application of manure, the emissions could very well be soil-derived. *Buerkert et al*. (2010) suggested losses of NH_3 were soil-derived rather than manure derived.

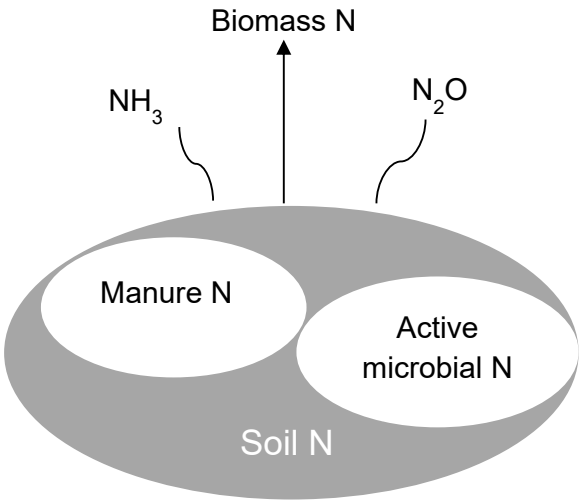

Figure 1.2 Nitrogen (N) pool investigated in the presented research

1.5 Tracing nitrogen movement with isotopic N (^{15}N)

Isotopes of elements have different numbers of neutrons in their nuclei, but equal numbers of protons are yielding the same chemical properties. If isotopes are stable enough, they allow studying various biological and chemical processes as the isotopes of the elements can be identified in the system. Isotopic studies have helped to understand the movements of important agricultural elements such as C, N, S, and P in the ecosystem (*Peterson and Fry*, 1987; *Di et al.*, 1997; *Dawson et al.*, 2002). Nitrogen has two stable isotopes (^{14}N and ^{15}N), which has helped to study N movement in the ecosystem. There are additional isotopes of N, but they are not useful for ecological studies because they only last for a few milliseconds to minutes (^{13}N lasts as long as 10 minutes). Generally, the abundance of 0.3662 atom % ^{15}N (atom % is the percentile contribution of the heavy isotope to the total number of atoms of that element in the sample) is considered as standard. The ratio of $^{14}N/^{15}N$ isotopes varies due to isotopic fractionation during physical, chemical, and biological processes (*Mariotti*, 1983). Isotope fractionation is defined as 'changes in the partitioning of heavy and light isotopes between a source, substrate, and the product(s)' (Dawson et al., 2002). The variation due to fractionation is not random; thus, it is essential to consider them during the calculation after careful sampling and accurate measurement. Furthermore, mass spectrometry (MS) allows the most precise analysis and has advantages over other methods of analysis at low concentrations of tracer N in the substrate.

Both, naturally occurring abundances and artificially increased abundances of ^{15}N are used in studying N cycles in the soil (*Robinson*, 2001). Studies relating N cycling and nutrient availability to crops through manure utilize artificial labeling with a high concentration of ^{15}N to

trace and study N transformation (*Dittert et al.*, 1998; *Chalk et al.*, 2013). Manures are labeled either post-excretion by labeling NH_4 pools (in manure) or by feeding ^{15}N labeled feedstocks to animals to produce labeled manure. The later is time-consuming and expensive (*Powell et al.*, 2004) but gives more homogenous labeling of all manure N pools, which is a major advantage over other methods (*Sørensen et al.*, 1994; *Sørensen and Jensen*, 1998; *Wachendorf and Joergensen*, 2011).

The direct measurement of NH_3 under field conditions is difficult, especially when the concentration is low or the field size is small (*Shah et al*., 2006). Furthermore, there are very few studies tracing volatilized NH_3-N directly after fertilizer application (*Zhao et al.*, 2016). The methodologies to trace applied ^{15}N in the soil N, microbial N, N_2O N, and biomass N are, however, established and regularly used.

1.6 Potential of biochar in Omani soil

An age-old practice of incorporating charcoal into the soil (*Glaser*, 2007) can potentially sequester C in agricultural soils while restoring soil quality (*Lehmann*, 2007). Recently termed as biochar, this carbon-rich compound is charcoal produced by pyrolysis of biomass in the complete or partial absence of oxygen (O_2) at relatively low temperatures (<700°C) after which it is applied to the soil (*Lehmann and Joseph*, 2009). The end product is usually a high surface area, high pH, recalcitrant C product with high cation exchange capacity (*Krull et al.*, 2009; *Novak et al.*, 2009). In earlier studies, they were termed charcoal, soot, char, graphite carbon, ash, coal, activated charcoal, black carbon, etc. (*Spokas*, 2010). Because of the recalcitrant nature of biochar, incorporation is likely to increase SOC over time. Nevertheless, fresh biochar can be decomposed by microorganisms over time - especially the labile fraction, dissolved organic carbon (DOC) in biochar can leach, and biochar can be transported horizontally by runoff, erosion or translocate vertically (*Major et al*., 2010). In five years of field condition, only up to 40% of incorporated biochar was reported to be lost from 0-20 cm soil (*Dong et al*., 2017). The quality of biochar depends upon the feedstock used, the peak temperature inside the pyrolysis reactor, the residence time of feedstock inside the pyrolysis reactor, the heating rate inside the pyrolysis reactor, and pre- and post-handling (*Brown*, 2009; *Cheng and Lehmann*, 2009; *Bruun et al.*, 2012; *Mukome et al.*, 2013; *Jindo et al.*, 2014).

It has been well documented that biochar affects the nitrogen cycle in various ways. In general, biochar increases the production of biomass, increases symbiotic biological N_2 fixation, improves plant uptake, reduces N_2O emission, decreases soil N leaching and poses a risk of increased soil NH_3 volatilization when applied with various manure and fertilizers (*Biederman and Harpole*, 2012; *Liu et al.*, 2018). Nevertheless, the mechanism of the increase is not always clear because of the interactions between different biochar types, soil, and climate

(*Biederman and Harpole*, 2012). The high surface area of biochar means space for the nutrient to attach, protection of microorganisms in the micro- and macropores increased water holding capacity, etc. which makes it potentially suitable for the sandy soils of Omani plains (*Lehmann et al.*, 2011).

1.7 Research area

The field study from which subsequently samples were taken and analyzed in the laboratory for this Ph.D. thesis was carried out on a private farm in the Al-Batinah plain near the city of Sohar in northern Oman (22°36'N, 58°10'E). The Batinah region occupies almost 60% of Oman's total agricultural land area (FAO, 2008). The farm areas are typically flood-irrigated despite its poor water use efficiency.

The experimental site has a hot and arid climate with an average annual air temperature of 27°C, the relative humidity of 80 %, and total precipitation of 102 mm (WMO, 2009). The soils are derived from wadi deposits that contain gravel-rich subsoils. The surface soil contains a mixture of 82% sand, 16% silt, and 2% clay (*Siegfried et al.*, 2011) and has a pH of 8.8, 5.3% $CaCO_3$, and a bulk density of 1.7 g cm^{-3}.

1.8 Experimental setup

^{15}N labeled and unlabeled Rhodes grass (*Chloris gayana* Kunth) was produced and fed to male Omani Batinah goats (*Capra aegagrus hircus*) to produce labeled and unlabeled goat manure. In the cropping season 2013-2014, a field experiment with five manure treatments (soil amendments) and two different water regimes (100 % and 80% required irrigation) was set up with four replications in a randomized plot design (5×2×4=40). The dimension of the plots was 3×3 $m^{2,}$ and the treatments were: (1) Goat manure, (2) Goat manure and charcoal (10% w/w), (3) Compost, (4) Compost and charcoal (10% w/w) and (5) Mineral fertilizers. N, P, K content in manure applied fields were balanced by applying mineral fertilizer when necessary. The goat manure plots and the goat manure and charcoal applied plots both received labeled manure in the first season.

In the second cropping season (2014-2015), the 100% irrigated goat manure plots were split into half by inserting PVC sheets to 40 cm soil depth and treated with either labeled or unlabeled manure (Fig. 1.3). Eight new plots were created by the side of the field, four of which were unfertilized-control and four received labeled manure during the second cropping season. Cabbage (*Brassica oleracea* L. var.*capitata*) was followed by basil (*Ocimum basilicum*) in both seasons. Soil samples were collected after each cropping season for lab-

Figure 1.3 Plots split in the second year by inserting PVC sheet to 40 cm soil depth

incubation experiments (first and second study). Fresh and dry plant biomass was measured after each crop, and plant samples were collected for subsequent laboratory analysis at the University of Kassel, Germany. Goat manure treated plots with and without biochar, and unfertilized control treatments with 100% irrigation are only discussed in the dissertation.

Two separate incubation experiments were carried out with the soil collected before the 2014-2015 cropping season (Experiment 1) and at the end of the 2014-2015 cropping season (Experiment 2). The first experiment was carried out to investigate ^{15}N recovery in NH_3 and N_2O emissions and the second one to investigate the effects of repeated manure applications on N_2O and CO_2 emissions from the soil.

To calculate the nitrogen derived from manure in samples, the atom percent excess (ape) in the samples was calculated by substracting atom% of control samples (background or ambient atom%) from atom% of labeled samples. Nitrogen derived from manure (Ndfm%) was calculated by dividing ape of the sample by ape of labeled manure multiplied by 100. And finally, the total quantity of N derived from labeled samples was calculated by $Ndfm_{sample}$% multiplied by N in the sample divided by 100. The difference between differently labeled samples was used to determine the contribution of manure from different application years (Fig. 1.4).

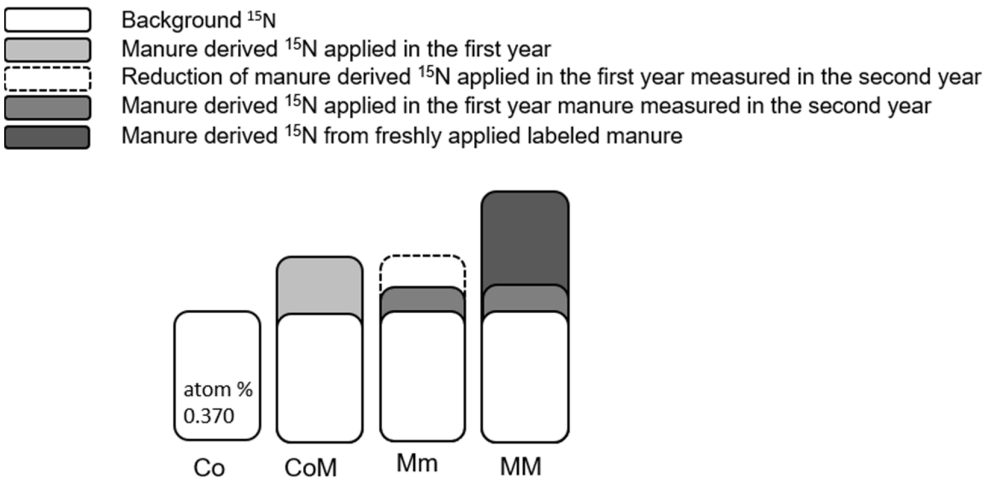

Figure 1.4 Isotopic N (15 N) increment in samples after application of labeled or unlabeled manure application. Where Co=control with background level 15 N, CoM = lebeled manure applied only in one cropping year, MM = labeled manure applied in the first year and second cropping year and Mm= labeled manure applied in the first cropping year and unlabeled manure applied in the second cropping year.

1.9 Objectives and hypotheses

To address the understanding gap, the research work presented here used goat manure as the source of nutrient to investigate N movement into different soil N pool, i.e., manure N, active microbial N, soil N, biomass N and gaseous N (NH_3, N_2O) (Fig. 1.2). The Investigation utilized ^{15}N to trace the movement of N into the different pools. Incubation studies were set up for the direct measurement of volatilized gases, but since methodologies to directly measuring ^{15}N in the NH_3 are lacking, a modified acid traps method (*Brooks et al.,* 1989) was tested. This study also investigated if the addition of biochar (in this case, activate-charcoal) together with ^{15}N labeled goat manure affects nitrogen uptake and yield. The overall objective of this doctoral project was to understand the soil N turnover of goat manure the incorporation into a typical soil of northern Oman using ^{15}N.

Specific objectives were to:

- determine ^{15}N in volatilized NH_3 from the air in incubation vessels with acidified glass fibre filters
- investigate if ^{15}N from low labeled manure can be reliably traced in N_2O and NH_3
- investigate the contribution of consecutive manure application to different soil and gaseous N pools
- investigate the effect of charcoal and manure application on N-uptake and yield by tracing ^{15}N in soil and plant.

Specific hypotheses were tested:

- The portion of N derived from manure N in the soil N pools and N_2O-N after repeated application of manure declines with time after application.
- Residual goat manure N from the first cropping season is resilient for at least another season.
- Charcoal increase biomass yield by increasing N-uptake from manure

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2. Chapter

NH_3 volatilization, N_2O emission and microbial biomass turnover from ^{15}N-labeled manure under laboratory conditions

This chapter has been published in Communications in Soil Science and Plant Analysis. *Ingold, M., Khanal, G., Dyckmans, J., Wachendorf, C., Buerkert, A.* (2018): NH_3 Volatilization, N_2O Emission and Microbial Biomass Turnover from ^{15}N-Labeled Manure Under Laboratory Conditions. *Commun. Soil Sci. Plant Anal*. 49, 537–551.

Gunadhish Khanal was involved in the production of the labeled manure and soil sample, generating the ideas about testing the volatilized ^{15}N, planning and preparation of the incubation experiments, measuring and preparing the samples (gas samples, soil samples, microbial samples) for isotopic analysis, preparation and the discussion of results.

2.1 Abstract

On irrigated agricultural soils from semi-arid and arid regions, ammonia (NH_3) volatilization and nitrous oxide (N_2O) emission can be a considerable source of N losses. This study was designed to test the capture of ^{15}N loss as NH_3 and N_2O from previous and recent manure application using a sandy, calcareous soil from Oman amended one or two times with ^{15}N labeled manure to elucidate microbial turnover processes under laboratory conditions. The system allowed to detect ^{15}N enrichments in evolved N_2O-N and NH_3-N of up to 17% and 9%, respectively, and total N, K_2SO_4 extractable N and microbial N pools from previous and recent ^{15}N labeled manure applications of up to 7%, 8%, and 15%. One time manured soil had higher cumulative N_2O-N emissions (141 µg kg^{-1}) than repeatedly manured soil with 43 µg kg^{-1} of which only 22% derived from recent manure application indicating a priming effect.

2.2 Introduction

For millennia, oasis agriculture and related nutrient cycling in Oman have been based on the continuous application of high rates of goat and sheep manure combined with flood irrigation (*Wichern et al.,* 2004; *Buerkert* et al., 2005; *Schlecht* et al. 2011). In this context, major knowledge gaps surged about the long-term effect of goat manure application on soil nitrogen (N) pools and causes of large N volatilization losses reported (*Buerkert et al.,* 2010; *Siegfried et al.,* 2011). The microbial activity was found to be high after application of goat manure to a sandy soil in Northern Oman, decomposing 50% of applied goat manure within 4 to 6 weeks followed by a low decomposition rate and nutrient release (*Ingold et al.,* 2017). It has been shown that despite high N losses the repeated application of goat manure (335 kg N ha^{-1} $year^{-1}$) over a two-year period increased soil carbon (C) and N stocks by up to 21% and 48%, respectively *(Ingold et al.,* 2014). In addition, the microbial biomass C and N significantly increased compared to mineral fertilizer application (Sradnick et al. 2014). However, how the application of manure affects the mineralization from previous applications has rarely been examined. To quantify manure effects on soil N pools, it is necessary to distinguish between N derived from the added substrate and the soil pool. This is possible with the use of isotopic tracing techniques, using ^{15}N labeled substrates (*Barraclough* 1995; *Wachendorf et al.,* 2008; *Wachendorf and Joergensen* 2011). Isotopic tracing techniques also allow to identify positive or negative priming effects of substrate application on the soil organic matter pool. Thereby priming effects are defined as short-term changes in the turnover of soil organic matter triggered by the application of C and/or N sources (*Kuzyakov et al.,* 2000). To obtain a labeling of organic N in manure, feeding of ^{15}N labeled hay is of advantage compared with feeding or spiking ^{15}N-enriched mineral N sources (*Powell et al.* 2002; *Wachendorf et al.,* 2008; *Lee et al.,* 2011).

The simultaneous sampling of N_2O and NH_3 and the analysis of their ^{15}N concentration enables to follow the pathway of gaseous N-losses from an isotopically labeled N source. On semi-arid and arid soils with high pH exposed to high temperatures, NH_3 can contribute considerably to N losses via gaseous emissions (*Yamulki, Harrison, and Goulding* 1996; *Bouwman, Boumans, and Batjes* 2002). For instance, on a frequently flood irrigated sandy soil in northern Oman fertilized with incorporated ammonium sulphate or buffalo manure, N losses via NH_3 were two- to four times higher than losses via N_2O (*Siegfried et al.,* 2011) and might be even underestimated as NH_3 analyzer bears several technical challenges caused by its high water solubility polarity (*Rom and Zhang*, 2010), and cross interferences in the used infrared photoacoustic gas analyzer (*Zhao et al.,* 2012). The sampling and analysis of NH_3 at low concentrations and in small air volumes, such as in laboratory incubation experiments remain difficult. Trapping NH_3 in an acidic solution and analyzing it with an Isotope Mass Spectrometer allows to distinguish the contribution of different N sources to NH_3 volatilization during short-term storage of manure and urine or after long-term application of urea to soil (*Lee et al.,* 2011; *Zhao et al*., 2016). In this context the diffusion method as described by *Brooks et al.,* (1989) is often used for the determination of ^{15}N in NH_4^+ and NO_3^- of aqueous solutions even at low concentrations. Thereby NH_3 is volatilized from aqueous samples by the addition of MgO and trapped in an acidified glass fiber filter. In our study this method was adapted to trap NH_3 volatilized from soil within a closed soil incubation system.

To close existing knowledge gaps in the turnover processes of soil applied manure the objectives of this methodological study were to (i) determine the recovery of NH_3 from air in incubation vessels with acidified glass fibre filters (Study 1), (ii) examine whether the simultaneous sampling of NH_3 within a closed incubation system affects the microbial biomass in the soil (Study 2) and (iii) investigate if ^{15}N from low labeled manure can be reliably traced in N_2O and NH_3, and how a single or double application of manure affects these emissions (Study 3).

2.3 Materials and methods

2.3.1 Experimental set-up and validation of the ammonia trap method (Study 1)

The basic set-up of the ammonia trap resembled that described by *Brooks et al*. (1989), with the difference that their method was used to transfer NH_3 from liquid solutions ranging from 50-200 µg N in filter traps. Acidified glass fiber filters (Whatman GF/D) were cut in pieces of 0.4 cm² and heated up to 550°C for two hours to remove possible N contamination. To check N contamination of glass fiber filters two pre-treatments including or excluding washing with 0.1 M HCl prior to heating at 550°C for two hours were compared (n = 8). Subsequently, the filter paper pieces were hung on a piece of stainless steel wire affixed to a septum. Flasks

with 120 mL volume were sealed with the prepared septa, evacuated, and filled with a standard NH_3 gas of 1.7 mmol m^{-3} NH_3 (at ambient air pressure and temperature, Basi Schöberl GmbH, Rastatt, Germany) at 1200 hPa whereby excessive amounts were released by a small syringe perforating the septum to reach ambient air pressure (1016 hPa) within the flasks. Taking the volume of the flasks and the density of the sample gas at ambient air pressure at 20°C into account, the amount of NH_3-N in the flasks was estimated at 4.6 µg N. This low concentration was chosen to mimic the amount of NH_3-N typically evolving from tropical soils low in organic matter.

The trapping process was started after the application of an acid on the filter trap by a microsyringe: 10 µL of a 2.5 M $KHSO_4$ solution and 50 µL of a 0.5 M $KHSO_4$ solution with a capacity to trap up to 350 µg N, and 50 µL of a 1 M H_2SO_4 solution with a trapping capacity of 1400 µg N (*Brooks et al.,* 1989). Subsequently, flasks were kept for five days in the dark and opened thereafter to remove the filter traps from the wires. All filters were dried for three days on Teflon tape in a desiccator containing a small beaker with concentrated H_2SO_4 to avoid contamination from NH_3 in ambient air.

To investigate the applicability of the ammonia traps for soil incubation experiments, in a second experiment filter traps were fixed with stainless steel wires to the lids of 1.6 L incubation jars and acidified with 15 µL 2.5 M $KHSO_4$. To imitate NH_3 diffusion from soil placed at the bottom of the incubation jars, 20 mL of NH_4Cl solution was filled in the jars. To trigger volatilization of NH_3 the solution was alkalized with 2 mL of 0.025 M NaOH to pH 11. Three different concentrations of NH_4Cl were used to provide 5, 50, and 500 µg N in each jar (n = 5) which were closed immediately after alkalization, swirled gently and kept at room temperature for two days. After opening the jars, the NH_4Cl solution was acidified with 0.025 M H_2SO_4 to pH 3.3 to determine the ammonium left in the solution by ion chromatography (IC 850, Metrohm AG, Herisau, Switzerland). Total recovery of volatilized NH_3-N was calculated based on the amount of volatilized N and N trapped in the filters. Filter samples were dried in a similar way as in the first above described experiment.

2.3.2 Analysis of filters

Ammonia trap filters were wrapped carefully in tin capsules (IVA Analysetechnik GmbH & Co. KG, Meerbusch, Germany) and analysed by a µEA elemental analyzer (CE Instruments, Rodano, Milano, Italy) coupled to a Delta Plus Isotope Ratio Mass Spectrometer through a Conflo III interface (Thermo Finnigan MAT GmbH, Bremen, Germany) at the Centre for Stable Isotope Research and Analysis of Georg-August-Universität Göttingen (Langel and Dyckmans 2014). Ammonia traps acidified with H_2SO_4 were wrapped in silver capsules to avoid corrosion of the capsules and analyzed in a similar way.

2.3.3 Manure production and soil from the field experiment (Study 2 and 3)

For the production of uniformly labeled manure, ^{15}N labeled Rhodes grass hay (*Chloris gayana* Kunth) was produced at the Agricultural Experiment Station of Sultan Qaboos University in Al Khoud, Muscat, Northern Oman (22°36'N, 58°10'E, 50 m asl). The Rhodes grass was harvested repeatedly in 35 day cycles. Isotopically labeled hay was produced during one cutting cycle by early morning spraying of leaves at 6 kg ha^{-1} ^{15}N urea with a ^{15}N abundance of 10 at% on days 21 and 25 after the previous cut. Unlabeled mineral fertilizer was given at day 8, equivalent to 50 kg N ha^{-1}. Prior to foliar application, the urea solution was mixed with 1.2 g L^{-1} of the urease inhibitor N-(n-butyl)-thiophosphoric triamide (NBTT) and 450 mg L^{-1} of a surfactant containing lecithin and propionic acid (Li 700; Loveland Products, High River, AB, Canada) to improve N absorption through the leaves. The Rhodes grass was harvested after 35 days, air dried and stored as hay bundles until usage as fodder.

Subsequently, isotopically labeled (0.675 at% ^{15}N ± 0.002 SD) and unlabeled hay (0.369 at% ^{15}N ± 0.000 SD) was used to feed male goats of the Batinah breed (*Capra aegagrus hircus*) in combination with crushed barley (*Hordeum vulgare* L.) at a ratio of 60:40. Faeces from goats were collected after an adaptation phase of 7 days with specially constructed fabric bags attached to the goat back (*Schlecht et al.,* 2011), air dried and stored until usage. Air dried faeces of labeled and unlabeled manure had a ^{15}N concentration of 0.526 at% (± 0.003 SD) and 0.369 at% (± 0.000 SD), respectively.

The soil for the laboratory incubation experiment was sampled at the end of the first year of a field experiment in October in Sohar, Oman. The field experiment was conducted on a sandy, calcareous hyperthermic Typic Torrifluvent (Soil Survey Staff 2014) from October 2013 to April 2015, with a cabbage (*Brassica oleracea* L.) and basil (*Ocimum basilicum* L.) crop rotation. Each crop was amended with the above mentioned ^{15}N-labeled manure in two separate applications amounting to 120 and 90 respectively, on four replicate plots (3 m × 3 m) and flood irrigated with 17 to 22 mm per irrigation event depending on soil water status monitored by soil water sensors. Soil for the incubation experiment was sampled from the upper 15 cm from four randomly selected spots per plot as bulked samples (M = soil manured in the field), air dried and sieved to 2 mm. Composite soil samples from each of five plots outside the experimental field, which were not fertilized or cultivated, served as a control (C).

2.3.4 Set-up of incubation experiments (Study 2 and 3)

The 2 mm-sieved soil was filled in air-tight 1.6 L incubation jars (Fig. 2.1), compacted to 1.8 g cm^{-3} bulk density (field condition), and rewetted to 50% of the water holding capacity. During the incubation deionized water was added, when water content reached 40% of the water holding capacity. The soil was pre-incubated at 25°C for 10 days in a dark incubation

cabinet. Subsequently, crushed goat manure (< 5mm) was mixed into the soil at a rate of 35 mg N kg^{-1}, corresponding to 100 kg N ha^{-1}. Water was added to reach 50% water holding capacity of the manure-soil mix and a sample of 10 g was collected and stored at 4°C until analysis. Bulk density was readjusted to 1.8 g cm^{-3} by compacting the soil and the soil was incubated for 18 days at 25°C.

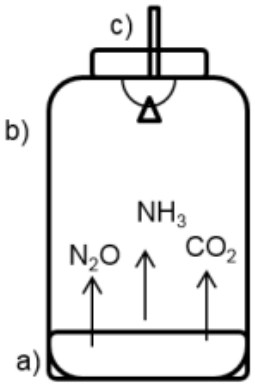

Figure 2.1 Air-tight 1.6 L-incubation jar containing a) wetted soil mixed with manure, b) ammonia filter trap affixed to the lid with a stainless-steel wire and c) a valve for gas sampling

During the entire incubation period soil emissions of CO_2 and N_2O were monitored in sampling intervals of one to four days. At the beginning of each sampling interval, jars were flushed with ambient air, closed and gas samples were taken to determine the basic gas concentrations in jars for calculation of emission rates. At the end of each sampling interval two gas samples were taken with a gas tight syringe and concentration of CO_2 and N_2O measured by gas chromatography (GC-14B Analysis System TCD/FID and ECD, Shimadzu Corp., Kyoto, Japan). The second gas sample was transferred in evacuated vacutainers and their ^{15}N concentration determined with an Isotope Mass Spectrometer Delta XP and GC interface coupled with a PreCon (Thermo Electron Corp., Bremen, Germany). After each gas sampling, jars were opened and flushed with ambient air, ammonia trap filters affixed on stainless steel wires at the lid were exchanged and a new sampling interval started. Sample filters were carefully removed using tweezers cleaned with 0.1 M HCl. Subsequently, the wire was carefully wiped with a fresh small piece of filter paper to collect acid droplets attached to the wire and added to the sample for drying. The wire was cleaned thereafter with 0.1 M HCl before installing a fresh filter for the following sampling period. Filter traps were acidified with 15 µL of 2.5 M $KHSO_4$ in jars containing soil and 50 µL 0.5 M $KHSO_4$ in blank jars, to keep the pre-dried filters moist during the entire sampling period. At the end of the incubation period, soil samples were collected and stored at 4°C until extraction of microbial biomass and determination of total C and N. For the determination of microbial biomass C and N, the chloroform-fumigation extraction (CFE) described by Brookes et al. (1985) was modified by

using 0.05 M K_2SO_4 for preparation of extracts for isotopic analysis. Organic C and total N in the extracts were measured using an automatic analyser (Multi N/C 2100, Analytik Jena GmbH, Germany). Total C and N in soil were analyzed in dried and grinded samples by a CN analyser (VarioMax® CHN, Elementar Analysesysteme GmbH, Hanau, Germany).

^{15}N analysis of soil and CFE samples was conducted with an Isotope Mass Spectrometer Delta V Advantage and a Conflo III interface (Thermo Electron Corp., Bremen, Germany) coupled with an Elemental Analyzer Flash 2000 (Thermo Fisher Scientific Inc., Cambridge, UK). The pH of the soil was determined in H_2O (1:2.5 w/w).

2.3.5 Testing the effect of ammonia traps on soil microbial activity (Study 2)

Air-dry soil from unmanured control plots (C) was used for an incubation experiment to test if the simultaneous trapping of NH_3 in the incubation jars affected soil microbial activity. Soils from five field replicates and blanks were incubated with and without ammonia traps. All samples were pre-incubated and thereafter mixed with crushed manure sieved to 5 mm at a rate of 35 mg N kg^{-1} soil DM to examine the effect of filters on C and N concentrations in the K_2SO_4 extractable and the microbial biomass C and N as well as cumulative CO_2 and N_2O emissions from the incubated soils (n=5).

2.3.6 Tracing of manure derived N in microbial biomass, N_2O and NH_3 (Study 3)

To test the effect of repeated manuring on different N pools a soil manured previously in the field with ^{15}N labeled manure (M) was compared with a soil which was not manured before (C). After a pre-incubation period of 10 days both soils were applied with unlabeled (m) or ^{15}N labeled manure (M) at a rate of 35 mg N kg^{-1}, resulting in treatments Mm, MM and CM respectively, and incubated for further 21 days (Table 2.1). Control soil without manure addition (C0) was incubated similarly to the other treatments to determine the basal microbial activity of the soil and the natural abundance of ^{15}N in total N, K_2SO_4 extractable N, microbial biomass N, N_2O and NH_3. To cope with the low N contents in ammonia filter samples, the soil and manure weight were increased 1.6-fold compared to Study 2 and the trapping periods for NH_3 were extended to intervals of 4 to 13 days.

Table 2.1 Fertilization treatments of control (C) and previously field-manured soil (M) applied with unlabeled (m) and ^{15}N labeled manure (M) and ^{15}N concentration of soil and added manure before incubation (Study 3).

	Previous manure amendment in the field	Recent manure amendment during incubation	Soil at%	Soil SD	Manure at%	Manure SD
C0	Unfertilized soil (C)	No (0)	0.370	0.000		
CM	Unfertilized soil (C)	^{15}N-labeled (M)	0.370	0.000	0.526	0.003
MM	^{15}N-labeled manure (M)	^{15}N-labeled (M)	0.378	0.001	0.526	0.003
Mm	^{15}N-labeled manure (M)	Unlabeled (m)	0.378	0.001	0.369	0.000

2.3.7 Calculations

Microbial biomass C was calculated according to J*oergensen* (1996) as E_C/k_{EC}, with E_C = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and k_{EC} = 0.45. Accordingly, microbial biomass N was calculated as E_N/k_{EN}, with k_{EN} = 0.54 (*Brookes et al*., 1985; *Joergensen and Mueller* 1996).

N_2O emissions were calculated using the following equation modified after *Siegfried et al*. (2011):

$$N_2O - N\ [mg\ h^{-1}\ kg_{-1}) = \left(\left(\frac{C_2}{(273{,}15+T_2)}\right) - \left(\frac{C_1}{(273{,}15+T_1)}\right)\right) \times \frac{\left(V \times \frac{3600}{t} \times 298{,}15\right)}{\left(\frac{8.3143 \times 298{,}15}{101325}\right) \times 1000\ 000 \times W} \times 28,$$

whereby C_1 and C_2 stand for the measured N_2O concentration (ppb), T_1 and T_2 for the temperature measured at the sampling events (°C), V for the volume of the incubation jars, t for the time between the two sampling events (sec) and W for the DM weight of the soil (kg). 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.

The atom% excess (^{15}N ape) of total N, K_2SO_4 extractable N, N_{mic}, N_2O-N and NH_3-N was calculated subtracting the natural ^{15}N abundance of the control treatment (C0) from the measured ^{15}N data obtained. The natural abundance of total N and K_2SO_4 extractable N was 0.369 (± 0.0004 SD), of N_{mic} was 0.374 (± 0.009 SD) and of N_2O-N was 0.364 (± 0.012 SD). In NH_3-N the natural abundance increased from 0.365 (± 0.007 SD) during pre-incubation to 0.394 (± 0.009 SD) during main incubation. For the ^{15}N ape of the microbial biomass N the following equation was used (*Wachendorf et al.,* 2011):

$$atom\ \%\ excess\ N_{mic} = \frac{N_f\ \times ape_f\ -\ N_{nf}\ \times ape_{nf}}{(N_f - N_{nf})} \times 100,$$

where N_f and ape_f stand for the N concentration and the atom% excess in fumigated extracts and N_{nf} and ape_{nf} for the N concentration and the atom% excess in non-fumigated extracts. To calculate the amount of N derived from manure (Ndfm) following equation was used (*Barraclough*, 1995):

$$Ndfm\ \% = \frac{ape\ sample}{ape\ manure} \times 100$$

At all sampling dates, for total, extractable and microbial N no significant differences in the N concentrations between labeled and unlabeled manure amended treatments were observed.

To estimate the amount of N derived from labeled manure, Ndfm % was multiplied by the respective N concentrations in the sample. The soil derived N in the treatment CM was calculated as the difference between total N concentration and manure derived N concentration. The freshly amended manure derived N concentration in MM was calculated as the difference of the $Ndfm\%_{MM}$ and $Ndfm\%_{Mm}$.

2.3.8 Statistics

Study 1: Prior to statistical data analysis, assumptions of normal distribution of residuals and homogeneity of variances for ANOVA and t-test were tested by examination of residuals by QQ-plots and histograms, and use of the Levene's test. The recovery of NH_3 in filter traps acidified with different acids and concentrations was compared by an ANOVA. The effects of washing in filter preparation were tested against non-washing by pair-wise comparison of means with a t-test.

Study 2: Pair-wise comparison of means with a paired t-test between blanks with and without filters and incubated soils with and without filters was conducted for microbial biomass C and N, K_2SO_4-extractable C and N, and cumulative CO_2-C and N_2O-N emissions. Residuals of data were normally distributed and homogeneity of variances given for all parameters.

Study 3: Results were statistically analyzed by mixed-model ANOVA with treatment as fixed factor and sampling time as repeated measure variable. The relatedness of the four replicate plots in treatments C0 and CM, and MM and Mm was accounted for in the model structure. The assumptions for conducting an ANOVA were met for all tested parameters except for K_2SO_4 extractable C. The data of ^{15}N at% was analyzed for each sampling event separately by a mixed-model ANOVA with treatment as fixed factor. Comparison of treatments was conducted by pairwise comparison of means using the Bonferroni correction. Changes in N derived from soil and manure during main incubation were statistically evaluated by paired t-test.

2.3 Results

2.3.1 Validation of the ammonia trap method (Study 1)

Recovery of N from ammonia standard gas

The recovery of N from the NH_3 standard gas in acidified filter traps ranged between 81 and 87% (Table 2.2), and was not significantly affected by the treatments ($F(4, 32) = 0.21$, $p = 0.93$). Washing of filters did not significantly reduce background contamination of 0.04-0.06 µg N mg^{-1} filter paper ($t(4) = -1.01$, $p > 0.05$) and reduced recovery numerically by 5%.

Table 2.2 Mean amount of N trapped in acid filters, standard error (SE) and recoveries of ammonia standard gas-N for differently pre-treated and acidified filter traps in a ^{15}N laboratory trapping experiment (Study 1).

Treatments	Acid		Recovery		
Filter	Concentration (mol L^{-1})	Volume (µL)	(µg N $filter^{-1}$)	SE	(%)
unwashed	1.0 M H_2SO_4	50	4.0	0.28	87
unwashed	0.5 M $KHSO_4$	50	4.0	0.27	86
unwashed	2.5 M $KHSO_4$	10	3.9	0.26	84
Washed	0.5 M $KHSO_4$	50	3.7	0.40	81

Recovery of N from NH_4Cl solution

During the two-day incubation period 4, 42, and 237 µg NH_4-N volatilized from the ammonium solution, of which 2.6, 32.5 and 208.7 µg were trapped in ammonia traps (Fig. 2.2). The N recovery in ammonia traps was not significantly affected by the amount of N in the incubation system and averaged between 63 and 78%.

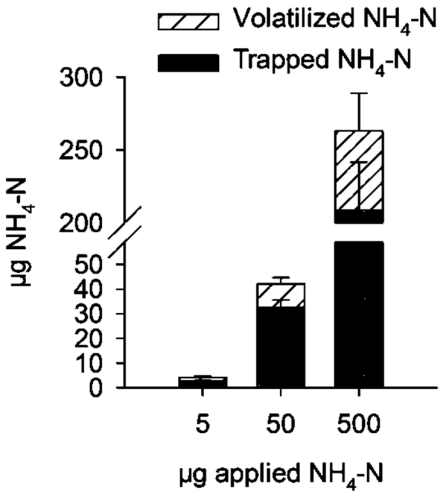

Figure 2.2 Average volatilized and trapped NH_4-N of the three NH_4-N application rates and N recovery (%) in filter traps (Study 1). Whiskers indicate ± one standard error of the mean.

2.3.2 Effect of ammonia traps on soil microbial activity (study 2)

The K_2SO_4 extractable C and N, the microbial C and N concentrations and the C_{mic}/N_{mic} ratio did not differ significantly between treatments with and without filters (Table 2.3). Similarly, no treatment effects were observed for CO_2 and N_2O emission in blanks with and without ammonia filter traps as well as from soils incubated with and without ammonia filter traps. NH_3 volatilization values were too low to detect treatment effects (data not shown).

Table 2.3 Mean K_2SO_4 extractable C, K_2SO_4 extractable N, microbial biomass C (C_{mic}) and N (N_{mic}) concentrations, C_{mic}/N_{mic} ratio and cumulative CO_2-C and N_2O-N emission with standard errors (in parentheses) at the end of incubation experiment (Study 2).

		With filter trap	Without filter trap	*P-value*
K_2SO_4 extr. C	µg g^{-1}	27.5 (2.74)	25.3 (1.93)	*0.579*
K_2SO_4 extr. N	µg g^{-1}	13.4 (1.61)	13.9 (1.52)	*0.870*
C_{mic}	µg g^{-1}	52.4 (7.85)	47.8 (7.28)	*0.246*
N_{mic}	µg g^{-1}	8.9 (2.18)	7.9 (0.65)	*0.878*
C_{mic}/N_{mic}		5.9 (0.65)	6.0 (0.95)	*0.851*
Cumulative CO_2	mg CO_2-C kg $soil^{-1}$	265.3 (11.63)	272.5 (16.77)	*0.762*
Cumulative N_2O	mg N_2O-N kg $soil^{-1}$	44.0 (10.43)	34.3 (5.01)	*0.341*

Table 2.4 Mean and standard deviation (in parentheses) of pH, total, K_2SO_4 extractable and microbial C and N concentrations in 0-15 cm soil depths of the control (C0 and CM) and ^{15}N amended manure plots (MM and Mm) from a field experiment in Sohar, Oman.

		C0 and CM	MM and Mm	*P-values*
pH (H2O)		8.5 (0.00)	8.4 (0.05)	*0.211*
Total C	mg g^{-1}	10.0 (0.03)	13.2 (0.04)	*0.000*
Total N	mg g^{-1}	0.4 (0.01)	0.6 (0.00)	*0.023*
K_2SO_4 extr. C	µg g^{-1}	70.9 (5.01)	140.3 (6.36)	*0.000*
K_2SO_4 extr. N	µg g^{-1}	8.9 (0.27)	18.4 (1.31)	*0.002*
C_{mic}	µg g^{-1}	65.0 (8.54)	81.4 (4.60)	*0.126*
N_{mic}	µg g^{-1}	10.0 (1.18)	13.2 (0.54)	*0.042*
C_{mic}/N_{mic}		6.8 (1.57)	6.2 (0.49)	*0.706*

2.3.4 Manure derived N in microbial biomass, N_2O, and NH_3 (Study 3)

N derived from the previous and the recent manuring was determined by comparing the MM treatment with the treatment (Mm), in which the previously manured soil was applied with unlabeled manure (Mm) and incubated. The amendment of unlabeled (m) and labeled (M) manure did not lead to significant differences between Mm and MM in total, extractable and microbial biomass C and N pools. The control and the manured soils were initially similar in their pH, C_{mic} and C_{mic}/N_{mic} ratio but differed in their C and N concentrations of total and K_2SO_4 extractable pools as well as N_{mic},with 10 mg g^{-1} and 13 mg g^{-1} total C, 0.4 mg g^{-1} and 0.6 mg

g^{-1} total N, 71 µg g^{-1} and 140 µg g^{-1} K_2SO_4 extractable C, 9 µg g^{-1} and 18 µg g^{-1} K_2SO_4 extractable N, and 10 µg g^{-1} and 13 µg g^{-1} N_{mic}, respectively (Table 2.4). Directly after recent manure amendment during the incubation experiment, C and N concentrations in the total, K_2SO_4 extractable and microbial biomass pools of CM soils were 20–55% higher compared with unmanured control treatment (C0), whereas the C_{mic}/N_{mic} ratio remained unaltered (Table 2.5). This difference remained similar during the main incubation period, except for extractable C and N concentrations, which reached similar levels of about 126 µg g^{-1} K_2SO_4 extractable C and 25 µg g^{-1} K_2SO_4 extractable N in C0 and CM. Microbial biomass C and N were constant during the main incubation in C0, whereas in CM concentrations doubled to 129 µg g^{-1} C_{mic} and 21 µg g^{-1} N_{mic} until the end of the experiment, though the treatment effect was only significant for microbial biomass C. The comparison of means by planned contrast revealed significantly higher total and extractable C and N concentrations in MM compared with CM (by 10–95%) 0 and 21 days after manure amendment, but there were no differences between Mm and MM.

For the total, K_2SO_4 extractable, and microbial N pools, manure treatments significantly altered ^{15}N concentration ($p < 0.05$), whereas sampling time was only significant for K_2SO_4 extractable N (Fig. 2.3a). In CM, the recent amendment of labeled manure significantly increased the ^{15}N concentration of total N by 4%, of K_2SO_4 extractable N by 6%, and of microbial biomass N by 9% compared with C0. After 21 days, ^{15}N concentrations in total, K_2SO_4 extractable and microbial N pools were with increases of 2, 2, and 9%, respectively, significantly higher in soils with double application of labeled manure (MM) compared with the previously labeled soil amended with unlabeled manure (Mm). The previous field application of manure in the MM treatment resulted in significantly higher ^{15}N concentrations in K_2SO_4 extractable N (1.5% to 2.7%) compared with CM. During the pre-incubation, the N_2O-^{15}N concentrations were with 0.38 to 0.40 at% significantly higher in field manured soil compared with those in control soil with 0.36 at% (Fig. 2.3b). After recent manure application, ^{15}N in N_2O in all manure treatments was by 6% to 9% higher (averaged across all sampling dates) than in C0 irrespective of single or double application of manure (CM versus MM) and irrespective of the application time of labeled manure (CM versus Mm). Furthermore, the recent application of unlabeled manure resulted in 5% lower ^{15}N enrichment of N_2O than the repeated application of labeled manure (Mm versus MM). The ^{15}N concentration during the pre-incubation was with a 6% increase significantly higher in NH_3 volatilized from field manured soil compared with control soil (Table 2.6). Within four days after manure application, the NH_3-^{15}N concentration was significantly higher by 9% in the treatments with labeled manure (CM and MM) compared with C0 and Mm, but did not differ thereafter.

Table 2.5 Mean total, K_2SO_4 extractable and microbial C and N concentrations and C_{mic}/N_{mic} ratio of manure amended soils after 0 and 21 days of incubation (Study 3) with statistical results of a mixed model ANOVA with treatment (see Table 1) as fixed factor and sampling time as repeated measure variable. Letters indicated significant differences between treatments.

Treatment	Total C	Total N	K_2SO_4 extractable C	K_2SO_4 extractable N	C_{mic}	N_{mic}	C_{mic}/N_{mic}
	---------mg g^{-1}-------		----------µg g^{-1}----------				
0 days after manure amendment							
C0	9.45 a	0.31 a	87.01 a	27.44 a	43.46 a	5.85	7.3
CM	11.21 b	0.40 b	111.12 b	37.48 a	67.28 b	9.45	8.6
MM	15.84 d	0.66 c	167.16 c	48.67 b	149.49 c	18.65	8.1
Mm	14.52 c	0.62 c	147.50 c	51.44 b	122.36 c	14.13	8.3
CV (%)	*6.2*	*11.8*	*12.7*	*8.5*	*31.2*	*58.7*	*32.3*
21 days after manure amendment							
C0	9.16 a	0.35 a	123.48 a	26.94 a	45.34 a	6.19	6.9
CM	10.32 b	0.40 b	128.09 b	23.14 a	129.46 b	20.90	6.3
MM	14.50 d	0.52 c	141.73 c	45.27 b	159.60 c	17.63	9.4
Mm	13.91 c	0.49 c	147.77 c	42.30 b	174.94 c	21.85	8.3
CV (%)	*6.7*	*5.1*	*7.2*	*10.6*	*26.2*	*50.3*	*33.7*
P-values							
Time Ti	*0.018*	*< 0.001*	*0.049*	*< 0.001*	*0.004*	*0.109*	*0.780*
Treatment Tr	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.045*	*0.718*
Ti x Tr	*0.515*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.334*	*0.689*

CV (%) = coefficient of variation

C0 = Control, CM = recent labeled manure application on unfertilized soil, MM = double labeled manure application, Mm = previous labeled and recent unlabeled manure application

During pre-incubation, the total NH_3-N trapped in ammonia traps amounted to 18.8 and 41.4 µg N kg^{-1} for C0 and MM, respectively. Results for main incubation are not reported as trapped NH_3-N was partly below blank filters traps from ambient air or below the detection limit.

Table 2.6 ^{15}N at% of NH_3-N during the incubation experiment with standard errors in parentheses (Study 3). Letters indicate significant differences between treatments.

Treatment	Pre-incubation (at%)	Day 0 to 4 (at%)	Day 5 to 8 (at%)
C0	0.365 (0.007) a	0.386 (0.014) a	0.398 (0.010)
CM		0.420 (0.016) b	0.395 (0.011)
MM	0.386 (0.003) b	0.419 (0.005) b	0.398 (0.056)
Mm		0.384 (0.005) a	0.435 (0.005)
P-value	*0.000*	*0.001*	*0.216*

C0 = Control, CM = recent labeled manure application on unfertilized soil, MM = double labeled manure application, Mm = previous labeled and recent unlabeled manure application

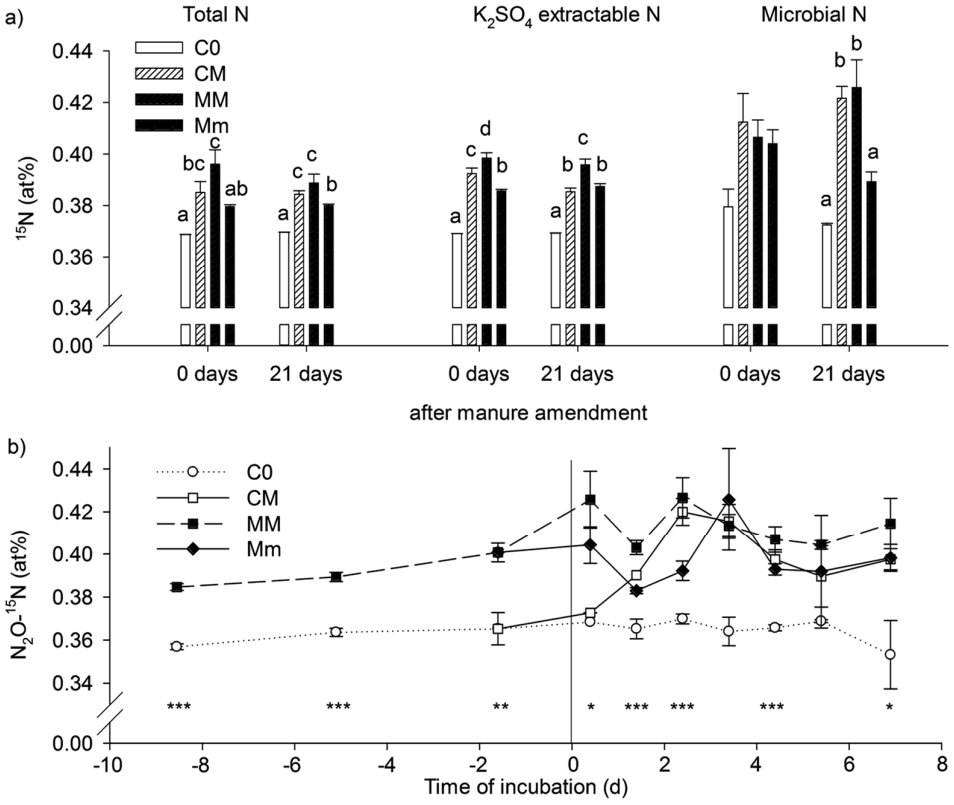

Figure 2.3 ^{15}N atom % of (a) total, K_2SO_4 extractable, and microbial biomass N in a soil-manure mix prior to recent manure amendment (0 days) and at the end of incubation (21 days), and (b) N_2O-N emissions prior to recent manure amendment indicated with a line (pre-incubation −10–0 days) and the main incubation after manure amendment (0–21 days, data is only shown for the first 8 days). Whiskers indicate +/- one standard error of the mean. Letters stand for significant differences between treatments, stars indicate significance levels of mean comparisons by mixed-model ANOVA * < 0.05, ** < 0.01, *** < 0.001.

In the soil amended with labeled manure one year before sampling (MM and Mm), 6% of total N, 10% of K_2SO_4 extractable N and 14% of microbial N originated from manure previously applied in the field. The amount of total N and extractable N derived from recently amended labeled manure in treatment CM significantly decreased by 9% and 57%, respectively, during the incubation, whereas the microbial N numerically increased by 296% (Table 2.7). The amount of N derived from the previous field applied manure (MM manure) significantly reduced by 26% in the total N pool during the incubation experiment. Nevertheless, similarly to CM recently amended manure N in previously manured soil (MM manure) decreased by 41% during the incubation in the extractable N pools, whereas the microbial N pool tended to increase.

Table 2.7 N derived from soil and manure (previous field and recent incubation application) in total, K_2SO_4 extractable, and microbial N pools of initial soil, 0 and 21 days after manure amendment with standard deviations across sampling times. Results in parentheses are based on calculations with non-significant ^{15}N enrichments between CM and MM, and MM and Mm, respectively. * indicate significant changes during main incubation: * < 0.05, ** < 0.01, *** < 0.001.

		C0	CM		MM		
		soil	soil	$manure_{rec}$	soil	$manure_{prev}$	$manure_{rec}$
Total N ($mg\ kg^{-1}$)	Initial	403.5	403.5	0.0	552.4	31.0	0.0
	0 days	305.7	360.3	40.5	526.7	44.5	67.5
	21 days	354.1*	359.0*	37.0*	441.3*	33.0*	28.4
	SD	36.7	45.2	14.0	59.4	9.1	38.5
K_2SO_4 extr. N ($mg\ kg^{-1}$)	Initial	8.9	8.9	0.0	18.4	1.8	0.0
	0 days	27.4	31.9	5.6	40.7	5.3	4.1
	21 days	25.9	20.7*	2.4**	36.4**	5.0	2.4**
	SD	2.4	6.7	1.9	3.2	0.3	1.6
N_{mic} ($mg\ kg^{-1}$)	Initial	10.0	10.0	0.0	13.2	1.8	0.0
	0 days	5.8	7.2	2.3	13.7	(2.2)	(1.2)
	21 days	6.2	14.1	6.8	13.3	(2.1)	(4.3)
	SD	3.3	4.6	3.8	5.8	0.7	1.9

C0 = Control, CM = recent labeled manure application on unfertilized soil, MM = double labeled manure application, Mm = previous labeled and recent unlabeled manure application

During the pre-incubation of treatments (C0 and CM, and Mm and MM), the CO_2-C emission peaked instantly after adjusting the water content to 50% WHC, while soil derived N_2O-N emission increased more slowly (Fig. 2.4 a-c). After fresh manure amendment, the N_2O emissions derived from soil increased instantaneously in CM, while MM soil responded with a threefold smaller peak. The recent application of manure to an unmanured soil led to a strong increase of the cumulative N_2O-N emissions of 141 $\mu g\ kg^{-1}$ from the soil and manure pool (22%), exceeding the N_2O-N released from unmanured control soil by 62 times (Fig. 2.4d). The cumulative emitted N_2O-N derived from the previously field applied manure was each 5 $\mu g\ kg^{-1}$ during the pre- and main incubation periods, (Fig. 2.4e) and was in a similar range as

the N_2O-N derived from recently amended manure (6 µg kg^{-1}, 14% of total emissions). Ammonia-N derived from previously applied manure amounted to 6 µg kg^{-1} (15% of total volatilization from MM) during pre-incubation and was not calculated for main incubation due to very low volatilization rates.

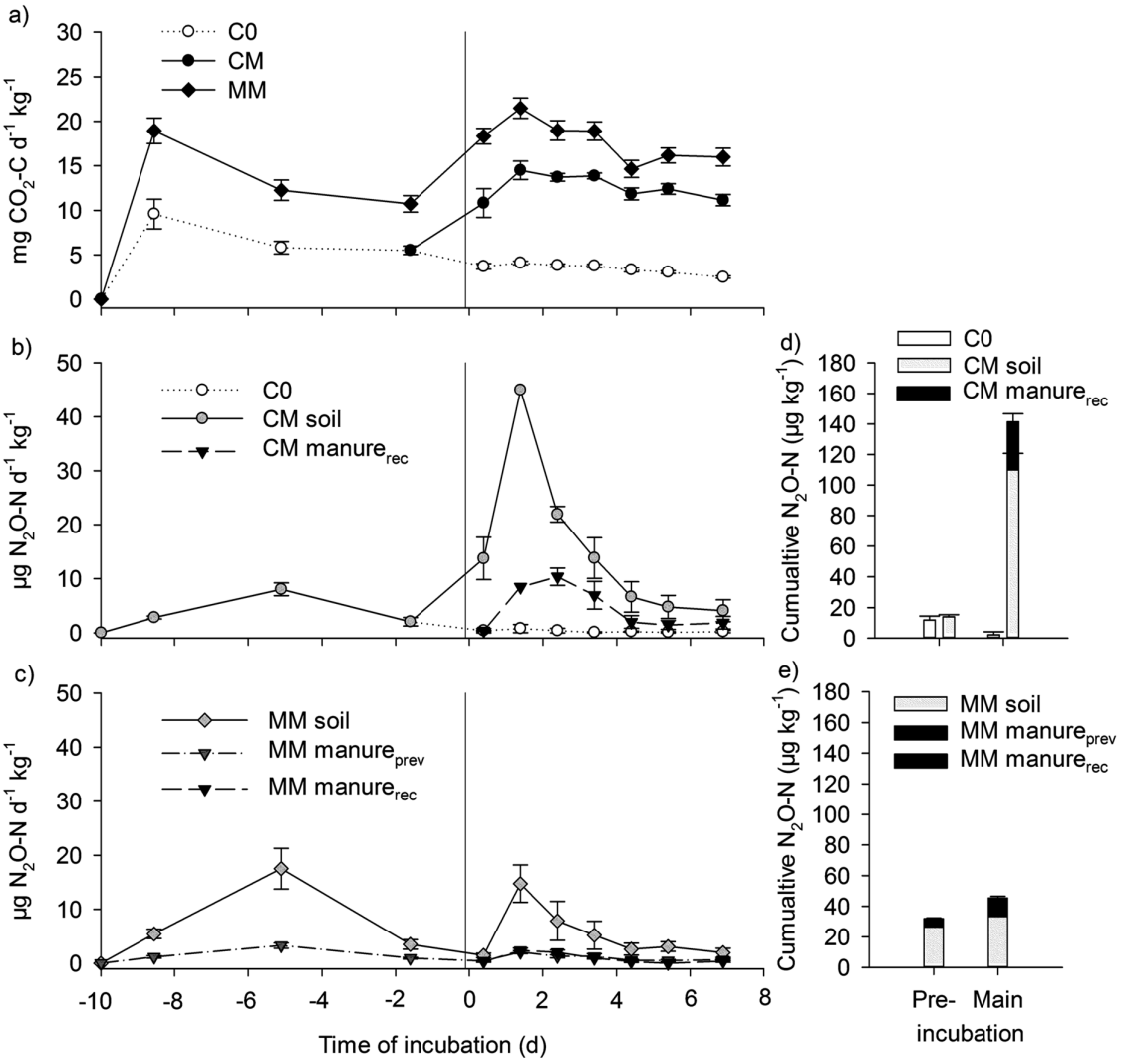

Figure 2.4 Daily emission rates with standard errors indicated by whiskers of CO_2-C (a) and total N_2O-N from soils C0 and CM (b), and MM (c), and calculated cumulative N_2O-N emissions derived from soil, previously field-applied manure and manure recently applied during the incubation experiment (d, e). Manure was amended after 10 days (indicated with a line) of a pre-incubation period (−10–0 days) followed by the main incubation (0–21 days, data is only shown for the first 8 days). There is no standard error for the initial peak after manure application as N_2O emissions of three samples exceeded the measurement range of the gas chromatograph.

2.4 Discussion

2.4.1 Validation of the ammonia trap method (Study 1 and 2)

The underlying ammonia trap method originally described by *Brooks et al*. (1989) for liquid N samples is suitable for sample masses of 50-200 µg N, where reported recoveries reach

100%. In our study N recovery from the standard gas reached 81 to 87% at N contents of 4.6 µg N in the used vessels. This lower N recovery may have resulted from a leakage of NH_3 from the vessels, adsorption to the inner surface of the vessel or premature drying of the acidified filter in the sample air (*Lory and Ruselle* 1994). On the other hand increasing the humidity in the vessel may cause NH_3 absorption (*Rooth et al.,* 1990). Recoveries obtained with other NH_3 trapping approaches reported by *Lee et al.* (2011) were higher than in our study, but these methods are not applicable to our experimental setup as our sample air volume and NH_3 concentration were low. The comparison between 0.5 M H_2SO_4 and 2.5 M $KHSO_4$ for the acidification of the filter paper did not yield significant differences in N recovery, which is in agreement with *Brooks et al.* (1989). $KHSO_4$ has the advantage that it does not absorb as much water as H_2SO_4 and does not corrode the tin capsules (*Brooks et al.,* 1989). The recovery of NH_3-N contained in ammonia traps volatilized from NH_4-solution reached 78%. This is slightly lower than recoveries obtained with NH_3 from standard gas and may result from disturbances caused by humidity. High humidity and the formation of condense water is a general problem for NH_3 sampling, regardless of the sampling and analytical method (*Rooth et al.,* 1990). As expected, the installation of the acid traps within an incubation system did not significantly affect the soil microbial biomass nor the CO_2 and N_2O emissions during incubation for 28 days and can be used to analyze NH_3, N_2O and CO_2 simultaneously within this system.

2.4.2 Tracing ^{15}N labeled manure in different soil N pools (Study 3)

Due to dilution of the ^{15}N during hay production and subsequent manure production, the abundance of ^{15}N in labeled manure was with 0.526 at% relatively low. While it is well known that denitrification and even more ammonia volatilization are processes affected by fractionation (*Högberg* 1997; *Robinson* 2001; *Hobbie and Ouimette* 2009) the ^{15}N of N_2O emitted from C0 did not significantly change in the course of the experiment. In contrast, the NH_3-N emitted from C0 increased ($P < 0.01$) from 0.365 at% to 0.398 at% and may indicate fractionation processes. However, the amount of N in ammonia filter traps during the main incubation was very low leading to values partly below the detection limit. Because of the surprisingly low NH_3 volatilization after manure amendment, manure derived N in NH_3 was not calculated for the main incubation. The amount of N in ammonia traps were partly lower than in traps incubated with ambient air (blanks), indicating other sinks within the system. This was also observed for the diffusion method by *Lory and Ruselle* (1994). Despite the regular flushing of water drops with an air stream to the soil surface, it is likely that small condense water drops attached to the inner glass surface absorbed some NH_3. Under the given conditions, the NH_3 trap method needs to be modified to increase recovery rates of ^{15}N in NH_3 by excluding other NH_3 sinks within the system.

The ^{15}N at% in total, K_2SO_4 extractable and N_2O-N differed between treatments and could thus be used to estimate the amount of N derived from soil, manure previously amended in the field and manure recently amended during the laboratory incubation. During the main incubation of MM, K_2SO_4 extractable N derived from recently applied manure significantly decreased by 40%, respectively. At the same time, the proportion of N_{mic} originated from recently applied manure increased numerically from 7 to 22% indicating an immobilization of mineralized N, which was also observed for goat manure by *Azeez and Van Averbeke* (2010). In addition, part of the K_2SO_4 extractable N from recently applied manure might have been lost via N_2O-N emissions, which increased 12-fold during main incubation. These changes were not observed for previously applied manure, indicating a preferential utilization of recently applied manure by microorganisms. In a field study conducted in the same location, 40% of goat manure applied in a litter bag experiment was estimated as relatively stable compartment, which was not decomposed within one cropping season of up to 20 weeks (*Ingold et al.,* 2017). On the previously unfertilized soil, N_2O emission rate from CM even increased by 25-fold after manure amendment of which 16% originated from recently applied manure. This coincided with an increase of microbial biomass N derived not only from the applied manure but also from soil by 96% indicating a priming effect. However, the increased microbial N uptake from the soil N pool may not result from an accelerated mineralization of soil organic N (real priming effect), but may indicate exchange reactions between mineralized extractable N derived from soil and manure (apparent priming; *Jenkinson et al.,* 1985; *Kuzyakov et al.,* 2000). This is, however, difficult to discriminate, as they can occur at the same time (*Jenkinson et al.,* 1985). The stronger impact of manure amendment on soil derived N_2O emission in CM compared with MM contradicts findings by *Hart et al.* (1986), whereby priming effects were larger in soils with higher C and N contents. Interestingly at the same time, CO_2 emissions were 28% lower from treatment CM compared with MM, leading to cumulative CO_2-C emissions per soil C of about 2% in both treatments, respectively. Thus, the CO_2 emission were governed mainly by the available C in the soil-manure mix, whereas N_2O emissions increased due to a higher mobilization of soil derived N in the CM soil, seen by an increase of labile organic matter (K_2SO_4 extractable) after manure amendment.

2.5 Conclusions

The recovery of NH_3 with the ammonia acid trap method with $KHSO_4$ was satisfactory for standard gas, but needs adaptation for volatilization from NH_4Cl solution and from incubated soil. Condensation water within a sampling system is a general problem for NH_3 sampling, regardless of the utilized analytical method, and needs to be avoided or integrated into the sample for acceptable results. Including ammonia acid traps within an incubation system for simultaneous measurement of CO_2 and N_2O emissions, and NH_3 volatilization is generally

possible, if the recovery of NH_3 trapped in the acidified filters can be increased. In a short-term laboratory incubation higher total N_2O emissions and higher N_2O emissions derived from soil gave evidence for higher priming effect after a single manure application. Nevertheless, the smaller effect of repeated manure applications on N-losses has to be proven under field conditions.

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3. Chapter

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

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3.1 Abstract

Under the hot and moist conditions of irrigated agriculture in the arid subtropics, turnover of organic manure is high, leading 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 derived from previously field-applied manure. While the application of manure during incubation increased microbial biomass N by 225% and 410% in control soil and previously field-manured soil, respectively, N_2O emissions were more affected on 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 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.

3.2 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 well 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 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 improvement of soil organic C stocks but also contributes to fertilizing crops. In animal

manure considerable 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 mineralization 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 extend the cultivated areas under arid and semi-arid conditions in the future. To distinguish between C and N derived from different sources isotope labeling 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 fodder grass 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).

The objective of this study therefore was to investigate the contribution of consecutive manure applications 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.

3.3 Materials and methods

3.3.1 Production of ^{15}N labeled manure and soil

To obtain uniformly labeled manure, ^{15}N-labeled Rhodes grass (*Chloris gayana* Kunth) hay was produced in a 35-day harvesting cycle 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). For isotope labeling two foliar applications of ^{15}N labeled urea with 10 at% ^{15}N (6.67 g L^{-1} i.e, 0.6% of H_2O) were used on 21st and 25th days after the previous harvest. To

facilitate optimal uptake of N through the stomata and leaf surface, the aqueous solution was mixed with the following additives: a) 6.67 mg L^{-1} N-urease inhibitor NBTT (N-(n-butyl)-thiophosphoric triamide, 0.1% of N) and, b) 2.5 mL L^{-1} surfactant Li 700- Soy (lecithine and propionic acid, Loveland Products, High Rivers, AB, Canada). 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 Omani Batinah goats (*Capra aegagrus hircus*) ranging from 20-27 kg live body weight 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 carbon (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, respectively.

Subsequently, the labeled (M) and unlabeled manure (m) were used in 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). It contained 80% sand, 12% silt and 3% clay in the upper 15 cm, a pH of 8.8, $CaCO_3$ content of 5.3% and bulk density of 1.7 g cm^{-3}. In the first year, four 3 × 3 m^2 irrigated plots were amended with labeled manure (M). A total of 210 kg N ha^{-1} (120 + 90, equivalent to 77.8 mg kg^{-1} N) was applied to cabbage (*Brassica oleracea* L.) followed by basil (*Ocimum basilicum* L.) in split applications during one year, 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 (referred to as 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 cropping season (April 2015). Samples were air-dried, sieved to 2 mm mesh and stored at room temperature until analysis and use for laboratory incubation experiments.

3.3.2 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), had been 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 (Table 3.1).

Table 3.1 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 (Cmic and Nmic).

		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}	403.5		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	

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

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 a thermostat chamber at 25°C for 31 days. The soil was adjusted to a bulk density of 1.7 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. To estimate soil gas fluxes incubation jars were flushed with fresh air and sealed with a gas-tight lid for an accumulation period of one to three days. Gas samples were collected at the beginning and at the end of each accumulation period with pre-evacuated gas flask (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 analyzed by an Isotope Mass Spectrometer Delta XP and GC interface coupled with a PreCon (Thermo Electron (Bremen) GmbH, Bremen, Germany), whereas in experiment 2 samples from two accumulation periods during pre-incubation and three accumulation periods during main incubation were measured. 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.

For the analysis of soil microbial biomass, 10 g of fumigated (24 hr with $CHCl_3$ at 25°C) and non-fumigated soil were extracted with 40 ml 0.05 M K_2SO_4 solution for 30 minutes in a horizontal shaker at 200 rev min^{-1} according to the chloroform fumigation-extraction method by *Brookes* et al. (1985). Soil extracts were filtered (hw3, Sartorius Stedim Biotech GmbH, Göttingen, Germany) and organic C and total N were measured using an automatic analyzer (multi N/C® 2100, Analytic Jena GmbH, Germany). 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 Analyzer Flash 2000 (Thermo Fisher Scientific Inc., Cambridge, UK) for the isotopic composition of N. Microbial biomass C and N were calculated using the equations $C_{mic}= E_C/K_{EC}$ and $N_{mic}=E_N/K_{EN}$ with E_C= organic C extracted from fumigated soil-organic C extracted from non-fumigated soil and K_{EC}= 0.45 (*Joergensen* et al., 2011) and E_N= Total N extracted from fumigated soil-total N extracted from non-fumigated soil and K_{EN}= 0.54 (*Brookes* et al., 1985).

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):

$$at\ \%\ excess\ _{Nmic} = (N_f \times ape_f - N_{nf} \times ape_{nf})/(N_f - N_{nf})$$

N_2O and CO_2 emissions were calculated by using the following equation (*Siegfried* et al., 2011; *Ingold* et al., 2018):

$$Gas_{N_2O\ or\ CO_2}\ [mg\ h^{-1}\ kg_{-1}) = \left(\left(\frac{C_2}{(273{,}15+T_2)}\right) - \left(\frac{C_1}{(273{,}15+T_1)}\right)\right) \times \frac{\left(V \times \frac{3600}{t} \times 298{,}15\right)}{\left(\frac{8.3143 \times 298{,}15}{101325}\right) \times 1000\ 000 \times W} \times m_{Cor\ N}$$

whereby C_1 and C_2 represents the measured N_2O or CO_2 concentration (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 (sec), 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):

$$Ndfm_{treatment}\ \% = {}^{15}N\ ape_{\text{sample (gas, soil, microorganism)}} / {}^{15}N\ ape_{\text{goat manure}} \times 100$$

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.

$$Ndf\ L_{CoM} = \frac{Ndfm_{CoM}\ \% \times N_{CoM}}{100}$$

$$Ndf\ Soil\ Co = N_{CoM} - N_{dfm}\ L$$

$$Ndf\ F1_{MM} = \frac{Ndfm_{Mm}\ \% \times \bar{x}N_{Mm+MM}}{100}$$

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

$$Ndf\ Soil\ M = \bar{x}N_{Mm+MM} - Ndf\ F1_{MM} - Ndf\ L_{MM}$$

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

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

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

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

$$Ndf\ Soil\ MM = \bar{x}N_{Mmm+MMm+MMM} - Ndf\ F2 - Ndf\ F1 - Ndf\ L$$

3.3.3 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 $P < 0.05$.

3.4 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, Table 3.2) 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, cumulative CO_2-C emissions were twice as high in field-manured soil (Mm and MM) compared with control soil and increased two- to four-fold after fresh manure application, while emission from unmanured 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 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 main incubation compared to the pre-incubation.

Table 3.2 Mean and standard deviation 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.

		SOC		K_2SO_4 extractable C		C_{mic}		Cumulative CO_2-C	
								Pre-Incubation	Main-Incubation
Sampling time (d)		g kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
		0	21	0	21	0	21	-10 to 0	0 to 21
Experiment 1#	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)
Experiment 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#	Tr	0.001		<0.001		<0.001		0.008	<0.001
	Ti	0.018		0.049		0.004			
	Tr x 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 x Ti	0.553		0.055		0.576			

Results for K_2SO_4 extractable C and C_{mic} of experiment 1 were published in *Ingold* et al. (2018)

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. 3.1), 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 significantly 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. 3.1. 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 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 Table 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.

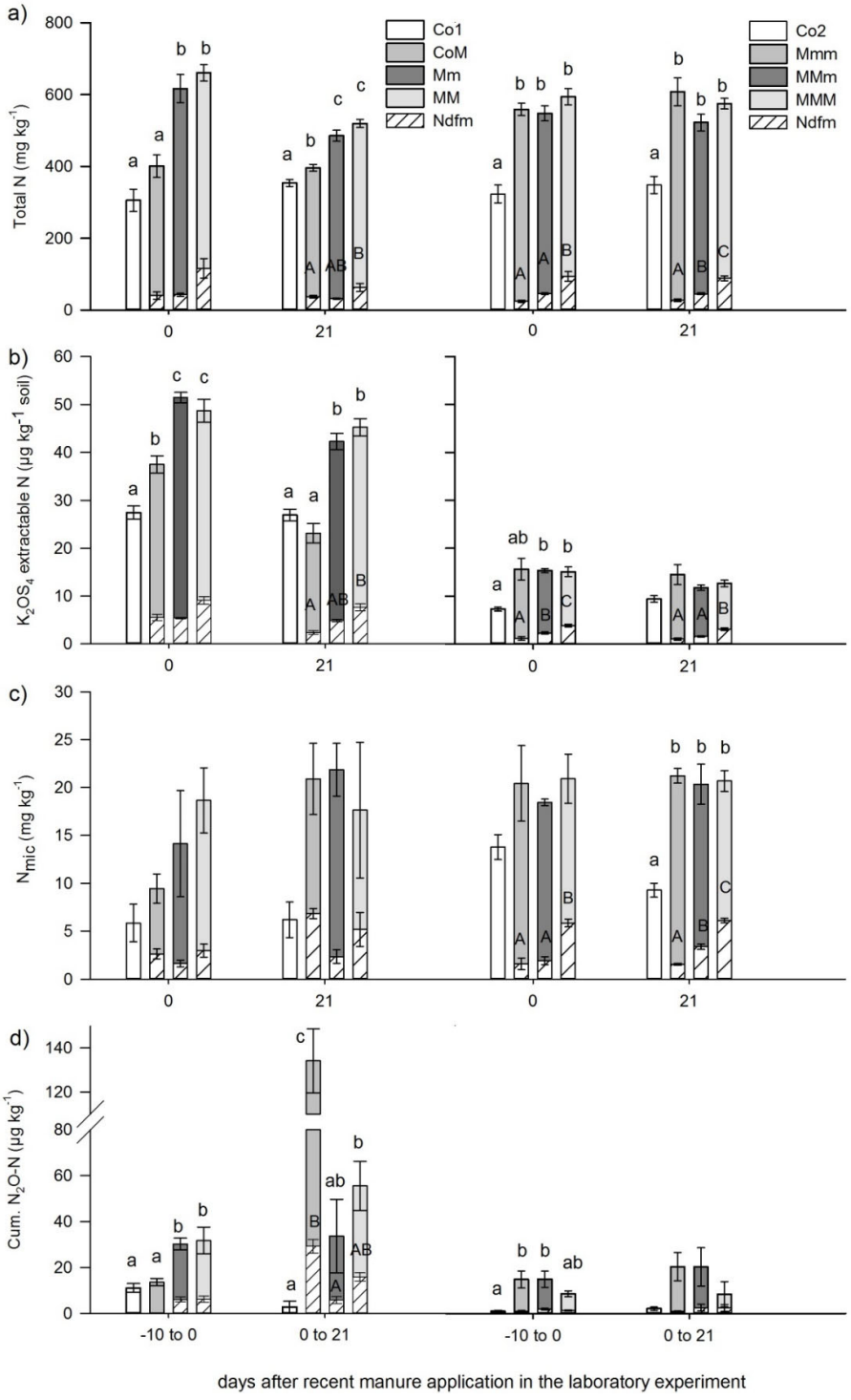

Figure 3.1 Mean total N (a), K_2SO_4 extractable N (b), and microbial biomass N (N_{mic}; c) at the beginning (0 days) and at the end (21 days) of the main incubation and cumulative N_2O-N emissions (d) during pre- (-10 to 0 days) and main-incubation (0 to 21 days) of two laboratory incubation experiments 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 $p < 0.05$.

Table 3.3 Means and standard errors 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 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.

		Total N		K_2SO_4 extractable N		N_{mic}		Daily N_2O-N emission rate	
								Pre-Incubation	Main-Incubation
		g kg^{-1}		mg kg^{-1}		mg kg^{-1}		µg kg^{-1} d^{-1}	
Sampling time (d)		0	21	0	21	0	21	-10 to 0	0 to 21
Experiment 1#	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)

Results for total, K_2SO_4 extractable N and N_{mic} of experiment 1 were 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

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%.

3.5 Discussion

The 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} a^{-1} and 134-350 kg N ha^{-1} a^{-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 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 78 mg kg^{-1}, and 30% after a further year. This is in a similar range as in a litterbag experiment conducted in a comparable soil in Northern Oman, where 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) and in ^{15}N recovery studies conducted with ^{15}N labeled crop residues under (*Smith* and *Chalk*, 2018). 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 expectable as the soil N pool mainly comprises of a large humus N pool with slow mineralization rates (*Beauchamp* et al. 2011). In experiment 1, previously field applied manure ($F1_{MM}$) contributed 7% to 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 proportion 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, 1.7 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, which 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 and emphasised 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 contradictour 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. 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 unfertilized control soil.

3.6 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 manure applications in irrigated agriculture of the arid Subtropics.

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4. Chapter

Effects of charcoal and ^{15}N labeled goat manure on nitrogen uptake by plants in an irrigated subtropical soil of northern Oman

Part of the work has been presented in ‘Regulation of soil organic matter and nutrient turnover in agriculture workshop,’ Witzenhausen, Germany.

Khanal, G., Buerkert, A. (2015): Effect of irrigation and fertilization on biomass yield of cabbage (*Brassica oleracea* L. var. *capitata*) and basil (*Ocimum basilicum*) in Oman. Regulation of soil organic matter and nutrient turnover in agriculture workshop in Witzenhausen, Germany.

4.1 Introduction

Losses of organic C and N from agricultural fields are of global concern because of their economic and environmental impacts. In irrigated agricultural soils of hot arid and semi-arid regions, such losses can be major but depend on management practices such as tillage and irrigation frequencies (*Austin et al.*, 2004; *Conant et al.*, 2011; *Fiedler et al.*, 2016). Maintaining or restoring soil C and N in these regions is essential for the sustainability of agriculture, which requires regular incorporation of manure and other sources of organic matter into the soil.

The millennia-old cultivation of human-made terraces with high SOC (23-32 g kg^{-1}) in Omani mountain oases bears vivid testimony that the application of animal manure may allow sustainable agricultural cultivation under irrigation even in hot and arid regions (*Nagieb et al.*, 2004; *Wichern et al.*, 2004). The annual organic C turnover in these terraces was found to range from 2.4 to 8.5 Mg C ha^{-1} (*Wichern et al.*, 2004), which may require several applications of manure per year at individual rates of 18 Mg dry matter ha^{-1} into the soil (*Buerkert et al.*, 2010).

In a newly established agricultural farm in the Al-Bathinah plain of northern Oman, with SOC of 13 g kg^{-1}, gaseous losses up to 10.6 t C ha^{-1} and 55 kg N ha^{-1} were reported after the application of different buffalo manures treatments (*Siegfried et al*., 2011). Turnover of nutrients in the soil is partly governed by the quality of incorporated manure, such as its C/N ratio, which mostly depends upon animal feeding. Goat manure, which is physically compact, is likely to decompose slower than cattle manure (*Powell et al.*, 1994). In a litter bag experiment with goat manure placed at 10 cm under optimal decomposition condition, 60-70% of manure-N and 60% of manure-C showed recalcitrance to decomposition after 4-8 weeks (*Ingold et al.*, 2017). The authors also reported that the application of goat manure was able to maintain or increase C and N balance in the soil despite the disappearance of C and N from the topsoil during the fallow period (*Ingold et al.*, 2015b). A better understanding of the fate of applied manure N in the extreme climate of northern Oman is needed.

The application of biochar, in combination with fertilizers, has resulted in increased crop productivity and soil fertility (*Biederman and Harpole*, 2012). Biochar application may be particularly effective on sandy and degraded soils because the properties of biochar can help to improve water holding capacity, soil structure, cation exchange capacity and facilitate overall microbial turnover in the soil (*Lehmann et al.*, 2011). Biochar has been reported to interact with the N cycle as it has decreased N leaching, N_2O emissions, and NH_3 volatilization losses (*Singh et al.*, 2010; *Taghizadeh-Toosi et al.*, 2011; *Cayuela*, 2014). While the processes are often not clear for particular biochar types, some common properties like high surface area, increased water holding capacity, increased cation exchange capacities, etc. can be important

in sandy soil like Oman for maintaining SOC and increasing productivity and utilizing water effectively. In this study, we investigated the effect of charcoal (similar properties to biochar) on N-uptake and biomass yield by tracing ^{15}N in the repeatedly applied manure in soil and plants. We hypothesize that charcoal increases biomass yield by increasing N-uptake and residual goat manure N from the first cropping season is resilient for at least another season.

4.2 Methodology

4.2.1 Study site

The study was carried out for two -years in a field experiment with two cropping seasons from October 2013 to May 2014 and October 2014 to May 2015 on a private farm of the coastal Al-Batinah Plain near the city of Sohar in northern Oman (24°14'59.1"N 56°47'31.8"E). The soil originated from fluvial wadi deposits and was classified as a hyperthermic Typic Torrifluvent (US Taxonomy; Al-Farsi, 2001) with 82% sand, 16% silt, and 2% clay, in the upper 15 cm (*Siegfried et al.*, 2011). The soil had a C content of 1.2%, N content of 0.05%, pH of 8.8, $CaCO_3$ content of 5.3%, and a bulk density of 1.7 g cm^{-3}.

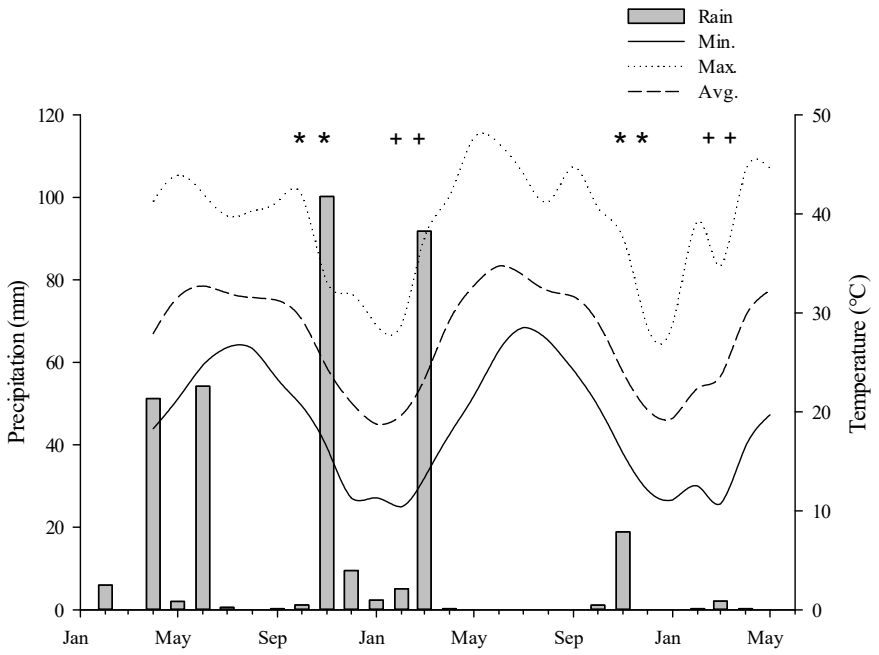

Figure 4.1 Monthly sum of precipitation (mm), daily minimum temperature (Min.), daily maximum temperature (Max.) and average temperature (Avg.) of the field site in northern Oman from February 2013 to May 2015. The * and + symbolizes the manure application dates in two splits for cabbage (75:25) and basil plants (70:30), respectively.

The minimum, maximum, and average air temperatures during the first and second cropping season were 15.3, 36.4, and 24.8 and 14.9, 37.3, and 25.1°C, respectively. Rainfall of 210 mm

and 23 mm was recorded during the first and second cropping season, respectively (Fig. 4.1). The precipitation was minimal during the second cropping season, but the crops were irrigated throughout the cropping season.

4.2.2 Experimental setup

The study was part of a larger field experiment with 3×3 m^2 plots receiving five different manure treatments (goat manure, goat manure and charcoal (10% w/w), compost, compost and charcoal (10% w/w) and mineral fertilizers.) and two different water regimes (100 % and 80% required irrigation) replicated four times in a randomized block design. Crop water requirements were estimated based on readings from WatchDog Watermark® soil moisture sensors (Spectrum Technologies Inc, Aurora, IL, USA) and field observations on day to day basis. Prior to the experiment, the excess N in the soil was harvested using cotton as a catch crop. After the cotton roots were removed, the soil was leached with ~20 cm of water twice approximately two months prior to the experiment. In an annual crop rotation, cabbage (*Brassica oleracea* L.) was followed by basil (*Ocimum basilicum* L.). Cabbage plants were grown in a nursery for 4 weeks and basil for 6 weeks. Cabbage and basil seedlings were transplanted on October 9th, 2013, and January 28th, 2014, respectively, and on October 27th, 2015 and Feb 17th, 2015 respectively. Plants were harvested after 80 (cabbage) and 60 (basil) days after transplantation. In this chapter, the following treatments were only compared: 100% irrigated plots treated with labeled or unlabeled goat manure with or without charcoal and mineral fertilizer (Table 4.1). Plots applied with labeled goat manure in the first season were split into half by inserting PVC plastic sheets up to 40 cm deep into the soil at the beginning of the second season. One half of the split-plot received a second application of labeled goat manure in the second season, whereas the other half received unlabeled goat manure.

Table 4.1 Treatments used in the charcoal & manure experiment on a private farm at Sohar, northern Oman. L treatments were split into half and treated with labeled or unlabeled manure in the second year.

Treatment	First cropping season manure/ mineral fertilizer	Second cropping season manure/mineral fertilizer
L	Labeled	
L+CH	Labeled + Charcoal	
LL	Labeled	Labeled
LU+CH	Labeled + Charcoal	Unlabeled + Charcoal
Mn	Mineral	Mineral

Plots receiving goat manure plus charcoal (activated charcoal) were not split but received labeled manure in the first year (L+CH) and unlabeled manure in the second year (LU+CH; Table 4.1). Charcoal at the rate of 10% by weight of manure (equivalent to ~1.5 Mg $ha^{-1}year^{-1}$) was mixed with manure after sprinkling some water in a bucket so that the charcoal powder got stuck on the surface of goat manure pellets, before soil application. The charcoal was manufactured from coconut shells heated at 780°C – 3780°C for 18-40 hours, followed by steam activation (AquaSorb® CP1; Jacobi Carbons Service GmbH, Premnitz, Germany). It was a finely powdered (44µm) product with a surface area of 1050 $m^2 g^{-1}$, a tamped density of 510 kg $m^{-3,}$ and a total pore volume of 0.62 $cm^3 g^{-1}$ (*Ingold et al.*, 2015b). The concentrations of nutrients in labeled and unlabeled goat manure and charcoal are given in Table 4.2.

In order to produce the ^{15}N labeled and unlabeled manure, labeled or unlabeled Rhodes grass (*Chloris gayana* Kunth) was fed to male Omani Batinah goats (*Capra aegagrus hircus*) that were equipped with specially constructed fabric bags (*Schlecht et al.*, 2011) to collect manure as described by *Ingold et al.* (2018). Before soil application, all manure was air-dried and stored. Also, gypsum ($CaSO_4$) was applied at the rate of 300 kg ha^{-1} at the beginning of each year. NPK fertilizer was applied at the rate of 120:60:80 for cabbage and 90:90:90 for basil crops. For Mn treatment, ammonium sulfate $(NH_4)_2SO_4$, triple-superphosphate $Ca(PO_4)_2$, and potassium sulfate (K_2SO_4) were used. Any shortage of P and K in manure treated plots was compensated by mineral fertilizers. The fertilizers were split applied for both cabbage (75:25) and basil crops (70:30), second fertilization was done thirty days after the first application.

Table 4.2 Properties of activated charcoal and goat manure applied on the private farm of northern Oman. SD=standard deviation, *n*=3

	C	N	P	K	Na	pH	^{15}N Atom% ± SD
	g kg^{-1} dry matter						
Charcoal	921	1.3	0.3	8.1	1.3	9.1	
Goat manure unlabeled	452.9	17.6	6.69	1.20			0.369±0.000
Goat manure labeled	456.5	18.4	5.55	1.04			0.526 ±0.003

4.2.3 Sampling of shoot biomass and soils

For leaf samples and dry matter determination of biomass, the inner 16 plants surrounded by a row of border plants were used. For ^{15}N analysis of cabbage plants and dry matter determination, 1-2 fully expanded leaves near the tip were collected from each plant. For the analysis of ^{15}N in basil plants, 10-15 fully developed leaves from every 16 plants from the center were collected a day before harvesting, 4-5 random basil plants were sampled for dry

matter determination. The samples were dried in a well ventilated ~2.2×2.2 m storage room heated with 3-4 heaters up to 50°C until the constant weight of the samples. Tissue samples for ^{15}N analysis were grinded and packed until further analysis. For soil sampling, at least 5-6 samples were collected from the top 15 cm from each experimental plot with the help of an auger, air-dried, and packed at the beginning of the experiment and few days after harvesting of the crops.

4.2.4 Statistical analysis

Normality and homogeneity of variance were tested by the Shapiro-Wilk test and Levene's test, respectively. Data that didn't pass the normality test and homogeneous variance were log-transformed. The effect of treatments in each crop was tested using one-way ANOVA in SPSS statistical software version 20 (SPSS Inc., Chicago, USA). Pair-wise comparisons between the treatments were made using Tukey's-test.

4.3 Results and discussion

4.3.1 Crop yield and nitrogen uptake

In the first cropping season, cabbage crops yielded poorly for manure applied plots compared to mineral applied plots. The average dry matter yields for L and L+CH were 2.9 and 1.6 Mg ha^{-1}, which was significantly lower than 7.2 Mg ha^{-1} of Mn (Fig. 4.2). The cabbage plants in the manure plots were stunted and produced only 12.2 to 14.5 heads per 36 plants compared to 29.8 in Mn. Manure was applied 10 days prior to the transplantation of cabbage seedlings to allow time for the sun-dried goat manure pellets to release nutrients while the mineral fertilizers were applied only 2 days prior to the transplantation. All other manure and mineral fertilization for cabbage and basil plants were done 1-2 days prior to the seedling transplantation. Losses of N through volatilization after manure incorporation, leaching of N in the early irrigation events could have reduced available N during the crucial early developmental stage of cabbage plants compared to mineral plots affecting biomass yield (*Hara and Sonoda*, 1978). The large single-day rain event (56.5 mm) on 17th Nov. and overall high precipitation (100 mm) within the month could have leached nutrients from the soil profile, but the yield difference for Mn plots was not different in the second cropping season when large rainfall events were absent.

Even though the cabbage yield increased in the manure applied plots LL, LU, and LU+CH by 79%,70%, and 167% respectively in the second cropping season, it was still significantly less than Mn that had a similar yield to the earlier season. Immobilization of N after manure application could have partially affected plants during the early developmental stage resulting in the lower yield from manure applied plots. After manure incorporation in the soil microbes-

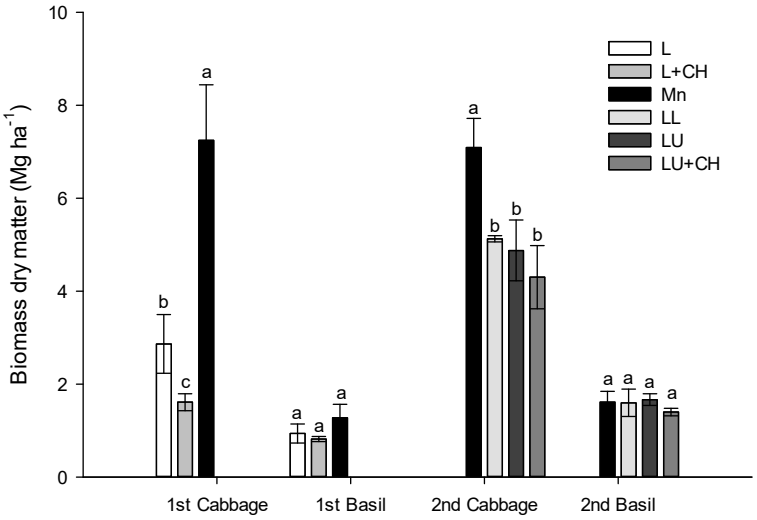

Figure 4.2 Biomass dry matter of each crop in the first and second season on a private farm near Sohar, northern Oman. Different letters indicate significantly different treatments ($p<0.05$), and the whiskers represent standard deviation from the mean (SD), n=4.

start to rapidly utilize labile C and available N, and if mineralization is not fast enough, N can be immobilized and will be unavailable to plants (*Jensen and Magid*, 2002). The manure incorporated in the experiment had C/N ratios between 24.8 to 25.7, which is close to or higher than the critical C/N ratio of 25, above which net immobilization occurs (*Jensen and Magid*, 2002).

The first-year basil also had a low dry matter yield of 0.9 Mg ha^{-1}, 0.8 Mg $ha^{-1,}$ and 1.3 Mg ha^{-1} for L, L+CH, and Mn, respectively. The frequent lower minimum temperature below 12°C was recorded in the month of February in the first year, dropping up to 9.8°C, which might have affected the overall growth of the crop as they are susceptible to lower temperatures. *Putievsky* (1983) reported basil plants performed poorly at day/night temperatures of 18/12°C, and the fresh weight of the plants increased as the temperature rose to 30/12 °C. The yield increased by 71% for LL and LU+C, and by 78% for Mn in the second year but was not significantly different from manured plots in both years (Fig. 4.2). *Rhodes and Chong* (2016) reported the application of 21 ppm, 48 ppm and 91 ppm N did not result in significantly different dry weight in a pot experiment (cv. Dark Opal) and the two higher fertilizer treatments resulted in similar leaf number and leaf weight. However, N in the plant tissue increased with increased N application. N uptake from Mn plots in this study was also significantly higher compared to the manured plots in the first year, further supporting the theory that immobilization or slower turnover of nutrients from manure limits nutrient supply to the crops (Fig. 4.3). However, there was no difference in N uptake in basil in the second cropping season.

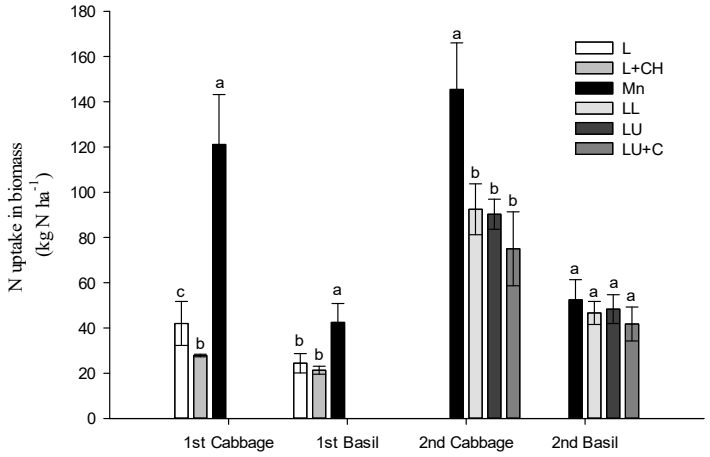

Figure 4.3 Nitrogen uptake (N) in cabbage and basil biomass in different treatments with standard deviation (*n*=4) in a field experiment conducted near Sohar, northern Oman. The values in first-year cabbage was log transformed for statistical analysis but not shown. Different letters above columns indicate significant differences at *P*<0.05.

The fresh yield of 33 to 68 Mg ha^{-1} of cabbage in the second year was higher than average production of 28 Mg ha^{-1} in 2014 and 20 Mg ha^{-1} in 2015 reported in Oman (*MoSPI*, 2016). But the average fresh weight per plant ranged from 0.9 kg to 1.2 kg in manured plots and 1.9 kg (SD ±0.2) in Mn plots were lower than 2.2 kg fresh weight reported in an irrigation study conducted in Oman (*Al-Rawahi et al.*, 2004). For basil, fresh biomass of 16 kg per 50 plants reported in a recent study by *Hanif et al.* (2011) was comparable to 14-20 kg per 50 plants (1.4-1.7 Mg ha^{-1}) harvested in our study.

Application of charcoal with manure did not have any effect on crop yield or N uptake except for the cabbage crops in the first year when the yield in L+CH was 44% lower than L although the application rate of 1.5 Mg ha^{-1} was less than many other field studies involving charcoal (*El-Nagger et al*., 2019). As the charcoal was high in pH (9.1), mixing it with manure might have expedited the volatilization losses through NH_3 from manure at its interface. On the contrary, biochar surface can also absorb NH_4^+ or NH_3 (*Kastner et al*., 2009; *Taghizadeh-Toosi et al.*, 2012) and the balance between the absorption and liming effect determines volatilization losses (*Liu et al*., 2009). However, similar charcoal used in this study did not affect N losses from the compost peat or N losses from the soil in laboratory experiments compared to soil amended with only compost (*Jordan et al*., 2015).

Although most of the charcoal is recalcitrant, some portion of charcoal is usually labile and available to the microorganism. The added charcoal with goat manure may have caused temporary immobilization of N affecting the crucial initial growth of the plants (*Rondon et al.*,

2007; *Steiner et al.*, 2008). Other studies have reported an increase in net N mineralization after biochar addition due to enhanced microbial activity (*Nelissen et al*., 2012). The same batch of charcoal used in this study had no effect or lowered biomass yield in an earlier study irrespective of how the charcoal was applied (ingested by animals or mixed with manure; *Ingold et al.*, 2015a). However, there was no noticeable difference in soil total N between the manured plots with or without charcoal at the end of the experiment. Although not significant, the p-value for the compared mean of LL+CH and Mn for total N was <0.052, and soil total C was significantly different from Mn at the end of the experiment (Table 4.3).

Since the biochar used in this study was prepared at high temperature (>700°C) and long residence time, it increases the possibility of polycyclic aromatic hydrocarbons (PAH) in the biochar (*Wang et al.*, 2017). Low molecular weight hydrocarbons such as ethane, ethylene, and PAH can inhibit nitrification (*Spokas et al.*, 2010). However, the bioavailability of PAH to suppress nitrification is not yet clear. Due to differences in biochar type, soil type, and climatic conditions and their interactions, the effects of biochar on N cycling are inconsistent, and the underlying mechanisms are not clear (*Cayuela*, 2014; *Liu et al.*, 2018).

Table 4.3 Total soil N and total soil C at the beginning and end of the field experiment. Different letters represent significant differences between manure treatments (p<0.05), and SD is the standard deviation (*n*=4).

	Total soil N µg g^{-1}					**Total soil C µg g^{-1}**				
	Beginning	**SD±**	**End**	**SD±**		**Beginning**	**SD±**	**End**	**SD±**	
Pooled		(n=2)		n=4			n=4		n=4	
sample	0.49	0.014				1.252	0.026			
LL			0.58	0.026	a			14.38	1.10	ab
LU			0.56	0.024	a			13.77	0.12	ab
LL+CH			0.63	0.120	a			15.48	1.43	a
Mn			0.49	0.023	a			12.71	0.77	b

4.3.2 Nitrogen derived from labeled manure (N_{dfm})

As basil was always the second crop, it had a higher chance of utilizing residual labeled N (Fig. 4.4). Application of unlabeled manure in the second cropping season in LU and LU+C resulted in a significant difference in the N_{dfm} in the cabbage plants. However, the same was not true for basil plants even though soil N_{dfm} in LL at the end of the experiment was significantly higher than in LU and LU+CH (Fig 4.5).

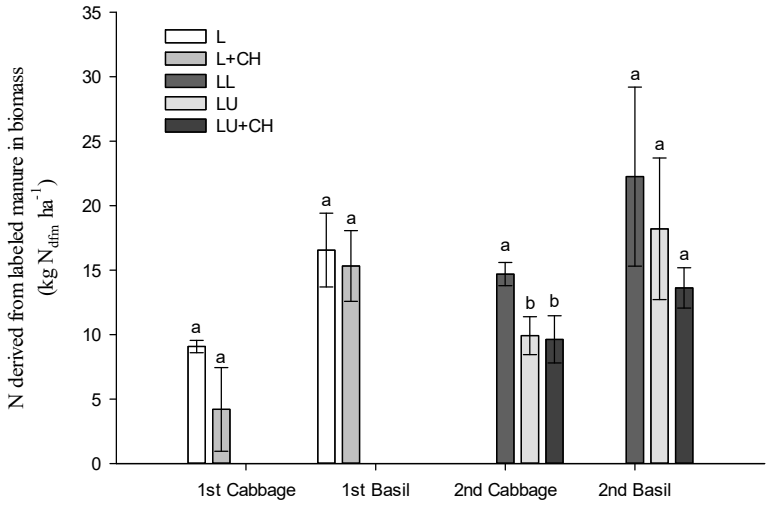

Figure 4.4 Nitrogen derived from labeled manure (N_{dfm}) in cabbage and basil biomass in different treatments with standard deviation (n=4) in a field experiment conducted near Sohar, northern Oman. The different letters above columns indicate significant differences between manure treatments at $P<0.05$.

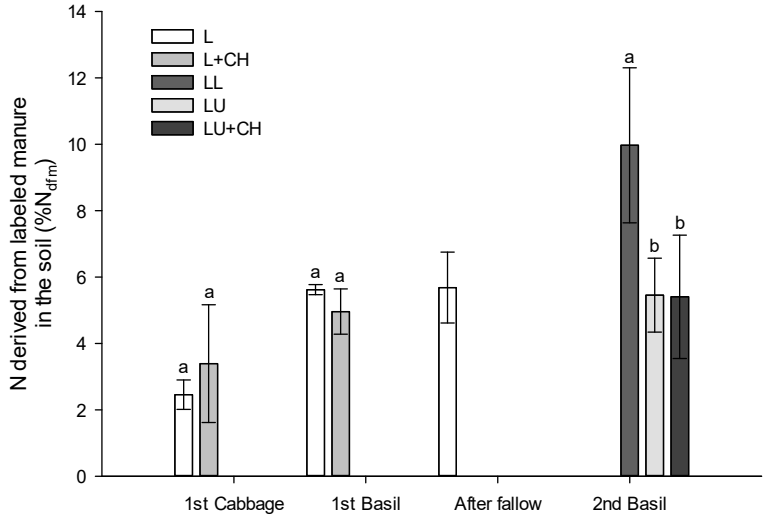

Figure 4.5 Percentage of nitrogen derived from labeled manure (%Ndfm) measured in the 15 cm soil depth after first-year cabbage, after first-year basil, after fallow and after second-year basil in a field experiment near Sohar, northern Oman. Whiskers show +/- one with standard deviation, n=3. For after fallow, n=6

N_{dfm} in the top 15 cm soil did not change during the fallow period (Fig. 4.5). This was in contrast to the disappearance of soil organic C and total N observed by *Ingold et al.* (2015b) at a nearby site. However, this study did not allow to compute N balances because of the incomplete bulk-density data. The increase and decrease in the N_{dfm} in the 15 cm soil depth were as expected, with LL being significantly higher than LU and LU+CH at the end of the experiment (Fig 4.5).

However, N_{dfm} in the soil in LU and LU+CH was 55% and 54% of LL, respectively, at the end of the second year, which is similar to that of L and L+CH at the end of the first year. This is in accordance with a litter bag experiment, where 60-70 % of N applied in the form of goat manure at 10 cm depth was recovered after 6-12 weeks in a similar soil in northern Oman (*Ingold et al.*, 2017). This suggests that a significant portion of applied goat manure was recalcitrant for at least two seasons.

4.4 Conclusion

Goat manure showed resilience even in the harsh climatic condition of Oman since a significant portion of N derived from labeled manure was still found in the soil at the end of the second cropping season. However, the biomass yield of cabbage was distinctly lower in manure applied plots compared to mineral fertilizer applied plots for two years, but it can be expected to change as the manure is repeatedly applied. And there was no difference in basil biomass because of the difference in fertilizer. This is important since stabilizing or increasing soil C is only feasible while economic returns are maintained.

The application of charcoal did not have any effect on yield, except it lowered the production of cabbage biomass in the first cropping season. However, at the end of the cropping seasons, soil total C (%) was significantly higher in charcoal applied plot compared to the mineral applied plots. The application of charcoal showed it could be important in the long run, even with the lower rate of application.

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5. Chapter

General Discussion

5.1 Soil labeling with ^{15}N labeled manure

About 700 kg (dry weight) Rhodes grass hay was produced at the beginning of the research by spraying 10 atom % ^{15}N urea over the leaves. Feeding the labeled hay to the animal to produce uniformly labeled goat manure is one of the most effective but expensive and time-consuming methods to produce ^{15}N marked manure (*Sørensen et al.*, 1994; *Powell et al.*, 2004; *Wachendorf and Joergensen*, 2011). The application of too much urea could burn the plants resulting in total failure of the investment and too little urea risks not labeling the plants enough to allow the detection of ^{15}N in a subsequently manured soil. In our study, isotope labeling reached only 0.675 at% ^{15}N ± 0.002 SD in the Rhodes grass leaf material. This low labeling level could have resulted because the plants utilized previously applied fertilizer N from the soil rather than from the labeled urea sprayed on the leaves. Nevertheless, the labeling of goat manure (0.526 atom% ± 0.003 SD) was satisfactory, although lower than the expected.

About 25 goats were fed labeled or unlabeled Rhodes grass hay and crushed barley (60:40) in isolated cages to collect manure twice a day in the morning and evening to produce enough manure for meeting the N demands of the field crops. The barley that was fed to the goats was not labeled. This has added to the heterogeneity in the labeling of the manure fractions. To obtain the highest possible ^{15}N labeling of the manure, all manure produced during the initial eight days of feeding was voided after which the ^{15}N atom% in the manure remained constant (*Sørensen et al.*, 1994). The collected goat manure was sun-dried at 30-40° C for 7-9 hours to conserve N losses during the storage. Subsequently, the labeled and unlabeled manure were applied in the field as N source for two cropping seasons. The successful labeling of the soil was reflected by the ^{15}N at% analysis of soil (sampled after first season cabbage: 0.374 ±0.002, sampled after first season basil: 0.3780 ±0.001; n=6) and plant samples (first season cabbage: 0.380±0.005; first season basil: 0.395±0.004; n=6) from the field experiment. However, low ^{15}N atom % in the samples meant difficulty during the calculations.

5.2 Tracing ^{15}N emissions (NH_3 and N_2O) in the laboratory

Sampling and measuring N_2O emission in the field is relatively easy, but measurement of NH_3 is tricky, as NH_3 is a highly reactive gas that sticks to surfaces, dissolves in condensed moisture and its volatilization is affected by a range of physical, chemical and environmental and management factors (*Sommer et al.,* 1991; *Misselbrook et al.,* 2000; *Rochette et al.,* 2013). The extremely volatile gas also rapidly diffuses in the air. Thus, it is complicated to reliably determine NH_3 under field conditions when the emissions are low in concentration. Other than

estimating NH_3 emissions by the N balance method, direct measurement of NH_3 volatilization is mostly done by drawing gas samples into acid solutions to convert NH_3 to NH_4 salts, which can be conveniently measured in the laboratory (*Yang et al.*, 2018). A portable photo-acoustic infrared multi-gas monitor, also used in the study, can also determine the gas fluxes (CO_2, N_2O, and NH_3). It is a robust way to estimate gas fluxes because of its portability, low maintenance, and ease of operation compared to gas chromatography, particularly in remote areas (*Iqbal et al.*, 2013). However, in recent years the consistency of the equipment under varying moisture and temperature conditions has been questioned (*Rosenstock et al.*, 2013).

Labeled and unlabeled soil samples were collected at the end of each cropping season to conduct laboratory experiments to trace manure applied N in NH_3 and N_2O gaseous emission, microbial N, and soil N. Since mass spectrometry is a destructive method that rapidly ionizes small samples to separate them according to their mass and charge, samples other than N_2O had to be prepared in a small solid and dry form. The N_2O gas can be directly sampled and analyzed for ^{15}N, but few articles were available on methodologies to directly measure ^{15}N in volatilized NH_3 (*Zhao et al.*, 2016). Thus, one of the main objectives of the methodological study was to test the accuracy of a modified method to directly measure ^{15}N in volatilized NH_3 and N_2O.

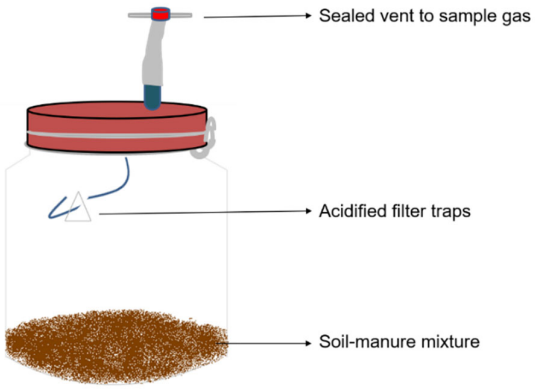

Figure 5.1 Glass jar with soil and manure mixture with the acidified filter hung on the top. The vent was used for gas sampling.

In the context of our study, a modification of a filter trap method was developed (Fig. 5.1) to determine ^{15}N in volatilized ammonia based on the diffusion method of *Brooks et al.* (1989), that is widely used to measure NH_4^+-^{15}N in soil extracts. The modified method allowed to recover 81% to 87% of N in the acid traps using a standard NH_3 gas, while the original method recovered 100%. However, the original method was described for liquid N samples that are suitable for sample masses of 50-200 µg N. However, the N content in the jar in this experiment was 4.6 µg N that represented more realistically the concentrations evolving from a tropical soil low in SOM incubated under laboratory conditions. The lower recovery of NH_3-N could

have resulted from leakage, adsorption to the inner surfaces of the glass jar, premature drying of the acidified filter papers, or dissolving of NH_3 in the moisture inside the jar. After volatilization from NH_4Cl solutions, where recovery reached only 63-78% of N, moisture could have been even more influential in the N recovery in the acid traps.

Nevertheless, in the soil incubation experiment, the volatilization of NH_3 was unexpectedly low after manure application. The amount of N in ammonia filter traps was partly lower than in the blank filter traps from jars with only ambient air indicating other sinks like moisture within the system. While the cause of the filter contamination was not clear in this study, a series of tests (not shown) have revealed that NH_3 leaked into the vessels from the atmosphere as the amount of NH_3 increased linearly in the blanks (personal communication, Mariko Ingold). However, the N_2O-^{15}N could be easily traced as the at% of N_2O-^{15}N was 0.38% to 0.40% compared to 0.36% of controls in the first incubation experiment during pre-incubation of the samples. In the same experiment, freshly applied labeled manure first the first time increased the N_2O-^{15}N by 6% to 9% against control. While N_2O emission picks were not observed after manure application in the second incubation experiment, ^{15}N fraction in N2O was traceable.

Our method performed better with the standard gas than in the incubation studies with soil and moisture in the system. The lack of emissions from the soil manured in the second incubation experiment was not expected, which could have helped to improve our understanding. Nevertheless, the approach may thus be useful in similar studies but needs to be modified to eliminate possible secondary sinks of NH_3 in the system and leakage of NH_3 to or from the system.

5.3 Tracing manure N movements in different soil pools

In the first laboratory incubation experiment (Chapter 2), when manure was applied for the first time on the soil, N_{mic} increased by 120%, and only 32.5% of N_{mic} was derived from manure. The K_2SO_4 extractable N significantly decreased from 31.9 to 20.7 mg kg^{-1} and from 5.6 to 2.4 mg kg^{-1} in both the manure pool and the soil pool, respectively. When manure was applied on previously manured soil in the first experiment, the contribution of soil N and previously applied manure N to N_{mic} was unchanged, but the contribution of freshly applied manure on N_{mic} increased by 7-22%, indicating N immobilization. In first-time manure applied to the soil, the cumulative N_2O-N was 141µg N $kg^{-1,}$ of which only 22% came from the manure pool. Thus, when the manure was first applied, the microorganisms were utilizing N mostly from the limited soil N pool, causing N immobilization. This effect was much smaller after the subsequent application, and in the second laboratory experiment (Chapter 3), the microbial N remained unchanged or decreased after manure application on the previously manured soil. While net mobilization and immobilization of N depend upon various factors, the high C/N ratio of the

goat manure used in the study should be noted, which was around the critical limit of 25 above which immobilization of N can occur (*Azeez and van,* 2010; *Buerkert et al.*, 2012).

The first-time manure applied to the soil, and repeatedly manure applied soil had a K_2SO_4 extractable C concentration of 111 mg kg^{-1} and 167 mg kg^{-1}, respectively, and a K_2SO_4 extractable N concentration of 37.5 mg kg^{-1} and 48.7 mg kg^{-1}, respectively in the first incubation experiment. This was much higher than ~50 mg kg^{-1} K_2SO_4 extractable C and ~15 mg kg^{-1} K_2SO_4 extractable N for the all the manured plots in the second incubation experiment. This difference most probably had arisen from the soil sampling time of the two samples. For the first incubation experiment, the soil was sampled after the five months fallow period before the beginning of the second cropping season. During this fallow period, some residual manure must have mineralized. Furthermore, for the second incubation experiment, the soil was sampled 10 days before the harvest of the crop, i.e., while the crops were still standing exhausting most of the labial portion of manure.

In the first incubation experiment, the cumulative N_2O-N emissions in the first-time manure applied soil were more than three times higher than for previously manure applied soils. Only 16% of the emission was derived from applied manure. As previously stated, this coincided with an increase in microbial N, of which 67.5% was derived from the soil. This indicates strong evidence of loss of native N after manure application referred to as 'priming effect' (*Jenkinson et al.*, 1985). A study conducted in the irrigated Omani mountain oasis agriculture also suggested soil-driven NH_3 volatilization instead of manure-based emissions (*Buerkert et al*., 2010).

5.4 Residual effect of manure and application of biochar

The more time it takes for manure to decompose in the soil, the slower the nutrient release. Typically, 70-95% of straw-based farmyard manure N, 50-60% poultry manure N and 30-60% slurry N is in organic form (*Bhogal et al., 2016*). The residual effect of manure is often seen in the succeeding crops (*Kihanda et al*., 2006) however, the untimely nutrient release can also lead to severe nutrient losses through leaching, runoff, volatilization losses, and erosion resulting in considerable economic and environmental damages.

The incubation studies (Chapter 3) showed that 57% of the leftover N in the top 15 cm soil was derived from manure applied during the first cropping season in the field. This share decreased to 30% in the second year. This was in line with the results of a litterbag experiment conducted in a comparable soil in Northern Oman, where 60-70% of the applied manure N remained in the manure placed in litterbags at 10 cm depth after 6-7 weeks and in ^{15}N recovery studies conducted with ^{15}N labeled crop residues (*Ingold et al.*, 2017; *Smith and Chalk*, 2018). Some

organic residues can also physically move to a lower soil horizon and thus become unavailable to plants or are unaccounted in the calculation. *Bosshard et al.* (2009) reported that 61% of applied sheep manure ^{15}N could not be recovered from the top 18 cm soil in 30 months with winter wheat, soybean, and maize crops.

In previous studies conducted near the site of the experiment, the difficulty of maintaining soil C and N stocks due to a rapid turnover of manure was highlighted (*Siegfried et al.*, 2011; *Ingold et al.*, 2015). Both studies suggested that soil C and N stocks could be maintained or even increased with repeated applications of animal manure at rates of 4-8 t ha^{-1} and 300-350 kg N ha^{-1} annually. Long term experiments conducted in semi-arid Kenya on sandy clay loam, goat manure was applied for 13 years at the rate of 5 or 10 t ha^{-1} yr^{-1} resulted in a steady increase of SOC for seven years after which a new dynamic equilibrium was reached irrespective of treatments (*Kihanda et al.*, 2006). For measurements regarding grain yield, the residual effect of 4 years of manure application lasted for about seven years when compared to unmanured control soil, while research on other semi-arid drylands has reported effects lasting only for 2-3 years (*Kihanda et al.*, 2005). In another study, a rather high amount of cattle farmyard manure of, 27, 55 and 110 t ha^{-1} yr^{-1} were applied for seven years in a highly calcareous silty clay-loam soil in semi-arid Iran that significantly increased SOC content from 0.5% to 1.6%, 2.3%, and 2.8%, respectively (*Hemmat et al.*, 2010).

In the study (chapter 4), the labeled manure derived N ($\%N_{dfm}$) in the soil at the end of the first cropping season was similar to the $\%N_{dfm}$ at the end of the second even though labeled manure were not applied in the second season. The $\%N_{dfm}$ in the soil was 54-55% of that of labeled manure applied soil, meaning around half of it came from the manure applied in the first year, and this portion did not change in the whole cropping season. This indicates that a significant portion of goat manure was re-calcitrant for at least two seasons, and this indicated the possibility to increase soil organic C and N under the climatic conditions of Oman.

The possible N shortage at the crucial early developmental stage of cabbage in the first cropping season in the manured plots could be the reason for the significantly lower biomass yield compared to Mn plots (*Hara and Sonoda*, 1978). Manure was applied 10 days prior to the transplantation of cabbage compared to 2 days prior to mineral plots because the goat manure pellets were dry. There could have been high losses of available N after the first manure application, as we saw in the first incubation experiment in chapter 2, where the single application of manure caused significantly higher N_2O emissions compared to the repeated manure applied plots. Only 16% of those emissions were manure derived. The manure was crushed in the lab for homogeneity of application, but they were not crushed in the field. The physical structure of the sun-dried goat manure with a high C/N ratio was rigid and might have

protected manure from microbial degradation. Even though the cabbage biomass increased in the manured plots in the second cropping season, it was still significantly different from mineral fertilizer applied plots. However, the fertilizer effect was not observed in basil biomass. As *Rhodes and Chong* (2016) discussed, basil plants might grow fine even below recommended doses.

The combination of manure and charcoal decreased the cabbage biomass yield compared to manure only plots significantly in the first cropping season. The highly alkaline biochar with high pH 9.1 could have increased the loss of N from the manure biochar interface through NH_3 depleting N in the soil for the cabbage crops even more. However, confirming this is not within the scope of the study as biochar can also absorb NH_4^+ and NH_3, and the volatilization losses is the balance between the two factors (*Taghizadeh-Toosi et al.*, 2012; *Liu et al*., 2018). But charcoal did not affect the biomass yields in any other crops. This was in contrast to many other studies, where increased yield after biochar application was reported (*Biederman and Harpole*, 2012; Crane-Droesche *et al.,* 2013). However, the effect of biochar in combination with organic matter on yield has more conflicting reports from positive, to neutral/minimal (*Borchard* et al., 2014; *Adekiya et al., 2019).*

The application of charcoal, however, significantly increased the total C% of the soil compared to Mn and, although not significant, the p-value for the compared mean for total N% was <0.052. This was impressive, considering the rate of charcoal applied in the field experiment was only 1.5 Mg ha^{-1} while the application rates in field studies are often much higher (*Biederman et al*., 2012). Most of the C in the charcoal is recalcitrant to microbial degradation and can be in the soil for years, thus improving the SOC stock. The beneficial effects of charcoal in the soil come from improved water holding capacity, better nutrient retention, protection to micro-organisms, and increased cation exchange capacity. While the finely powdered charcoal like the one used in our study may improve the soil properties, a lot of it can be lost in erosion and runoff or can simply move down vertically in the sandy soil.

5.5 Conclusions

The production of labeled Rhodes grass in the field needs proper planning as the investment might be at stake if not satisfactorily labeled. However, samples with lower ^{15}N labeling can still be used in experimental studies. Despite the low labeling of the samples tracing, the ^{15}N from different N pools were successfully done. The complexity of measuring ^{15}N in low concentration NH_3 gas directly in the incubation studies, however, still persists. Even after the promising results of the modified acid trap method with the standard gas, the leakage of NH_3 gas or it's absorption in the system in the presence of moisture was problematic. However, it can be improved with further testing.

Application of manure for the first time resulted in increased microbial biomass, and substantial N loss from the system through N_2O derived mostly from the soil N pool. This strongly suggests the existence of a substantial 'priming effect.' However, this did not happen after repeated manure application highlighting the importance of regular manure application to such soils. The results also showed that repeated goat manure application could potentially resist the rapid decomposition in irrigated agricultural soil as a significant portion of goat manure were recalcitrant. Furthermore, the addition of charcoal with manure could potentially increase C stock in the soil.

It is always important for farmers to see the economic side of changing farming practices. In the two-year field experiment, fertilizer treatments did not affect the biomass yields of basil crops, while for the cabbage crops, the yields were significantly high when mineral fertilizers were applied, although the yields increased in the second cropping season for manure applied plots compared to first cropping season. However, this could change in a few cropping cycles when the manure is repeatedly applied to the soil accumulating organic matter and nutrients.

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