THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Effect of soil amendments on greenhouse gas emissions from subtropical soils

Maren Westermann

Master of Science

Bachelor of Science

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2017

School of Agriculture and Food Sciences

Abstract

The concentration of the potent greenhouse gas nitrous oxide (N_2O) in the Earth's atmosphere is increasing. The main reason is agricultural activity, especially the application of nitrogenous fertilisers and animal manures to soils. In tropical and subtropical climates, N_2O emissions from fertilised soils can be particularly high, however, there is considerable uncertainty in N_2O estimates as data coverage is poor. The research presented here aimed to close this knowledge gap and investigate strategies for abating N_2O emissions from agricultural soils. This thesis focused on animal manures because (i) intensive livestock production is expanding in tropical and subtropical regions, and (ii) manures from intensive animal production are increasingly considered as alternative nutrient sources for crops. N_2O mitigation strategies were evaluated by amending soils with geological or plant-derived materials that have shown potential to decrease N_2O emissions from agricultural soil in previous research but have not been studied extensively. Experiments across different spatial scales (microcosm to field) have been conducted to investigate the transferability of observed effects.

In the first study (Chapter 3), manures were applied to the soil surface as a simulation of no-till farming practice using microcosm systems. It was hypothesised that adding bentonite, a clay with ion adsorption capacity, would decrease N_2O emissions from manures. Blends of bentonite with beef, pig or poultry layer manure were applied to three different soils from South East Queensland and N_2O fluxes were quantified over three weeks. Contrary to expectations, blending bentonite with manures tended to increase N_2O emissions. This observation was interpreted as the combined effect of increased moisture content at the soil surface caused by the strong water binding capacity of bentonite and decreased oxygen concentrations in the soil due to restricted gas exchange. In the following studies, bentonite was therefore incorporated into the soil rather than applied to the surface.

The second study (Chapter 4) examined the effect of bentonite, biochar or green waste compost additions on N_2O emissions from poultry litter at a commercial sugarcane farm. Sugarcane was chosen because it is a major crop in Queensland and globally and N_2O emissions from sugarcane soils can be high. Poultry litter and blends of poultry litter were applied subsurface along sugarcane rows. Over ten months, fluxes of N_2O, and also CO_2 and CH_4 were quantified with static chambers. The early phase of the field experiment was simulated in a parallel laboratory experiment. Biochar addition to poultry litter decreased N_2O emission in the field (-36%) and in the laboratory (-18%) compared to poultry litter only application. Bentonite addition decreased N_2O emissions from poultry litter in the field (-16%) but increased N_2O emissions in the laboratory (+8%). Differences in soil aeration caused by bentonite's swelling-shrinking characteristics are the likely cause for

these opposite effects in field and laboratory experimentation. Blending compost and poultry litter increased N_2O emissions in the field (+34%) and the laboratory (+286%) compared to poultry litter only application. Compost addition increased nitrate levels in soil thus promoting N_2O emissions from denitrification. Furthermore, the fixed soil moisture of 60% water filled pore space (WFPS) in the laboratory experiment would have created conditions more favourable for N_2O production than variable WFPS in field soil. The laboratory experiment was a useful indicator for treatment effects but patterns of N_2O fluxes differed from those in the field, with explanations including the variability of field soil moisture, amongst other differences. Emission factors of N_2O calculated from the field experiment ranged from 3.36% to 8.02%, which exceed the default emission factor of 1% for managed soils as given by the International Panel for Climate Change (IPCC) and 1.25% for sugarcane cropping as given by the Australian Department of Environment.

The third study (Chapter 5) examined the effect of bentonite and biochar on soil N dynamics to investigate the underlying mechanisms of the N_2O decreases observed in the field experiment and in the scientific literature. A dose-response experiment was performed in microcosm systems in which urea and poultry litter were blended with increasing rates of bentonite and biochar, respectively, and applied to soil that had been sampled from the sugarcane field in Chapter 4. By using the minimally invasive microdialysis technique, soil inorganic N fluxes were measured simultaneously to N_2O fluxes. Combining these two analyses had not been done before. Overall, N_2O emissions from urea and poultry litter decreased with both amendments, especially at high application rates. Exchangeably-bound ammonium ions in the presence of both amendments reduced the flux rates of ammonium and nitrate in soil and were a likely reason for the lower N_2O emissions.

In summary, this thesis has generated empirical data and produced new insights into soil N turnover processes. The results obtained (i) contribute to the revision of emission factors from cropping, (ii) may improve N_2O emission models that take variations in climate, soil and nutrient scenarios into consideration, and (iii) provide foundations for abating reactive nitrogen losses from farming systems using amendments.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer-reviewed papers:

Pratt, C., M. Redding, J. Hill, S. R. Mudge, M. Westermann, C. Paungfoo-Lonhienne and S. Schmidt (2014). Assessing refrigerating and freezing effects on the biological/chemical composition of two livestock manures. Agriculture, Ecosystems & Environment 197: 288-292.

Pratt, C., M. Redding, J. Hill, G. Brown and M. Westermann (2016). Clays Can Decrease Gaseous Nutrient Losses from Soil-Applied Livestock Manures. Journal of Environmental Quality 45: 638-645.

Conference abstracts:

Robinson, N., Brackin, R., Paungfoo-Lonhienne, C., Lonhienne, T., Westermann, M., Salazar, M., Yeoh, Y. K ., Hugenholtz, P., Ragan, M. A., Redding, M., Pratt, C., Wang, W. J., Royle, A., DiBella, L., Lakshmanan, P. and Schmidt, S. (2016). Addressing the nitrogen problem in sugarcane production to reduce pollution of the Great Barrier Reef. In: 7th International Nitrogen Initiative Conference (INI 2016): Solutions to improve nitrogen use efficiency for the world. International Nitrogen Initiative Conference, Melbourne, Australia. 4-8 December 2016.

Reports:

Pratt, C., Redding, M., Hill, J., Brown, G., Duncan, T., Westermann, M. and Schmidt, S. (2015). Chapter 3: Laboratory sorber trials. In: Advancing Livestock Waste as Low Emission-High Efficiency Fertilisers. Department of Agriculture and Fisheries, Queensland Government.

Publications included in this thesis

No publications included.

Contributions by others to the thesis

Guidance with the design of experiments, interpretation of results and editing was provided by my supervisory team Prof Dr Susanne Schmidt, Dr Ryosuke Fujinuma and Dr Weijin Wang. Additionally, the following people have contributed to this thesis as stated below.

Chapter 3:

Dr Stephen Mudge provided the concept for the experimental design. Dr Matthew Redding and Dr Christopher Pratt provided editorial assistance.

Chapter 4:

The field experiment in Chapter 4 was part of a joint experiment with Monica Elizabeth Salazar Cajas. Dr Richard Brackin provided assistance with the experimental design, planning and set-up. Monica Elizabeth Salazar Cajas provided assistance with planning and set-up of the experiment as well as with data collection and laboratory analyses. Taleta Bailey and João Carlos de Freitas Junior assisted with data collection and laboratory analyses. The laboratory experiment was maintained by Dr Christopher Pratt and Dr Jaye Hill. Dr Matthew Redding processed and provided the data of the laboratory experiment. Editorial assistance was provided by Dr Richard Brackin and Dr Matthew Redding.

Chapter 5:

Dr Richard Brackin and Scott Buckley provided assistance with the experimental design and gave advice on the usage of the microdialysis equipment. Editorial assistance was provided by Dr Richard Brackin, Dr Christopher Pratt and Dr Matthew Redding.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Acknowledgements

I wish to thank Sugar Research Australia (SRA; formerly Sugar Research and Development Corporation (SRDC)), the Department of Agriculture and Fisheries (DAF) of the Queensland Government and the Department of Science, Information Technology and Innovation (DSITI) of the Queensland Government for funding this thesis. Furthermore, the field experiment in Chapter 4 would not have been possible without the kind provision of (i) the field site by Troy Apps, (ii) poultry litter by Guy Holcroft (Darwalla Milling Group), (iii) bentonite by Dougal Scott (AMCOL Australia), (iv) biochar by Dr Jitka Kochanek and (v) green waste compost by Johannes Biala (Queensland University of Technology).

I would like to give special thanks to my supervisory team Prof Dr Susanne Schmidt, Dr Ryosuke Fujinuma and Dr Weijin Wang. I am very grateful for their professional guidance, mentorship, support, open-door policies, encouragement, trust and the donation of their time. I couldn't have done this thesis without them.

I am thankful to Dr Matthew Redding, Dr Christopher Pratt and Dr Jaye Hill from DAF for their guidance and cooperation and for the time they have invested in my research.

I am lucky to have been part of a friendly and supportive research group. I experienced a great working atmosphere in which people help each other and listen to each other's problems to make everyone's project a success. I am very thankful to everyone in the group for helping me in various ways.

I am grateful to Dr Richard Brackin for the time and energy he has put into my research and for inspiring discussions. I learned a lot from him and his assistance in the field and in the laboratory was invaluable for me.

I am also grateful to Dr Nicole Robinson for assistance with organisational matters and set-up of the field experiment.

I would like to thank Dr Diane Allen for giving advice on experiment outlines, especially regarding the field experiment.

Many thanks go to Monica Elizabeth Salazar Cajas for accompanying me during the field experiment. During the initial phase of the experiment we were working together almost every day from dawn till dusk and her positive and strong nature were a great support in this time.

I would like to thank Lisa Xian for sharing an office with me during my whole PhD time. I much enjoyed the little tea parties in our office and I will remember the time with joy.

I am also grateful to Taleta Bailey and João Carlos de Freitas Junior for helping out with the field experiment. Their hard work and positive attitudes were a great support.

I also would like to acknowledge my housemates for personal support and for taking the time to correct some of the grammatical mistakes.

My heartfelt thanks go to my family and friends for personally supporting me. I can always count on them which gave me the strength to not only do a PhD but to do it in a country almost as far away from home as possible.

Last but not least I would like to thank my partner Nikolai Weh for personal support, for encouraging me to learn programming, for moving to Australia because of me and for doing all the household duties during the times I was very busy with my PhD.

Keywords

Greenhouse gas, nitrous oxide, nitrogen cycle, subtropics, sugarcane, animal manure, bentonite, biochar, compost

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 050304, Soil Chemistry (excl. Carbon Sequestration Science), 50%
ANZSRC code: 040104, Climate Change Processes, 30%
ANZSRC code: 070306, Crop and Pasture Nutrition, 20%

Fields of Research (FoR) Classification

FoR code: 0503, Soil Sciences, 60%
FoR code: 0703, Crop and Pasture Production, 20%
FoR code: 0701, Agriculture, Land and Farm Management, 20%

Table of Contents

List of Figures

List of Tables

List of Abbreviations

°C	Degree Celsius
%	Percent
β	Standardized regression coefficient
µl	Microlitre
µS	Microsiemens
ANOVA	Analysis of variance
C	Carbon
Ca	Calcium
CEC	Cation-exchange capacity
CH_4	Methane
$cmol_c$	Centimoles of charge
CO_2	Carbon dioxide
cm	Centimetre
d	Day
EC	Electric conductivity
EF	Emission factor
g	Gram
GWP	Global warming potential
h	Hour
ha	Hectare
IPCC	Intergovernmental Panel on Climate Change
K	Potassium
KCl	Potassium chloride
KOH	Potassium hydroxide
kg	Kilogram
lm	Lumen
L	Litre
LSD	Least significant difference
log	natural logarithm
M	Molar concentration
MΩ	Megaohm
m	Metre
mg	Milligram
ml	Millilitre
Mg	Magnesium
min	Minute
mm	Millimetre
mS	Millisiemens
n	Sample size
N	Nitrogen
N_2	Dinitrogen
Na	Sodium

N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Dioxygen
P	Phosphorus
P	p-value
R^2	Coefficient of determination
rcf	relative centrifugal force
rpm	Revolutions per minute
SD	Standard deviation
sec	Second
SEM	Standard error of the mean
t	Ton
Tg	Teragram
WFPS	Water-filled pore space
WHC	Water holding capacity

Chapter 1 – Introduction

Greenhouse gases are natural components of the Earth's atmosphere. These molecules, the primary ones being water vapour (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and ozone (O_3), are the drivers of the greenhouse effect (IPCC, 2007) which shields the Earth from extreme temperature fluctuations. The greenhouse effect is based on a sensitive equilibrium between thermal infrared radiation temporarily captured by greenhouse gases and thermal infrared radiation leaving the atmosphere towards space. Since the Industrial Revolution human activities are changing this equilibrium by increasing atmospheric concentrations of greenhouse gases (Denman et al., 2007; Fowler et al., 2009) leading to the so-called 'enhanced greenhouse effect' and thus to global warming (IPCC, 2007; Mosier 1998). The three major anthropogenic greenhouse gases are CO_2, CH_4 and N_2O. While burning of fossil fuel is the main reason for production of CO_2, CH_4 and N_2O mainly originate from biogenic processes (Fowler et al., 2009; Tian et al., 2016).

Nitrous oxide has a particularly strong impact on the enhanced greenhouse effect as its GWP is 298 times that of CO_2 on a 100 year time scale (Myhre et al., 2013). The largest contribution to anthropogenic N_2O emissions is connected to agricultural activities (Table 1.1; Syakila and Kroeze, 2011; Tian et al., 2016) with soils being a major source due to application of organic and inorganic N (Denman et al., 2007; Fowler et al., 2009; Mosier et al., 1998). Underlying causes for the production of human-induced N_2O emissions from soils are a frequent mismatch between soil N application rates and plant N uptake rates as well as soil disturbance (Matson et al., 1997). Tropical soils are notably a major source of N_2O emissions because of low crop N use efficiency, warm temperatures and high precipitation (Denman el al., 2007; Granli and Bøckman, 1995). However, there is a low confidence in reported estimates of N_2O emissions from tropical and subtropical climates as there is a lack of coverage of data (Fowler et al., 2009; Tian et al., 2016).

Table 1.1. Estimation of global agricultural N_2O emissions in 2011.[1]

Source	N_2O (Tg N $year^{-1}$)
Direct N_2O emissions	
Synthetic fertilizer	0.9
Animal waste	0.4
Biological N_2 fixation	0.1
Crop residue	0.3
Cultivated histosol	0.1
Total	1.8
Animal production	
Animal waste management system	2.3
Indirect N_2O emissions	
Atmospheric deposition	0.4
Nitrogen leaching and runoff	0.6
Human sewage	0.3
Total	1.3
Total	5.3

[1] Syakila and Kroeze, 2011

1.1 Nitrogen turnover in soil and N_2O fluxes

In soils, N_2O is mainly produced biologically by microorganisms (Granli and Bøckman, 1995). The production of N_2O takes place in the turnover process of NH_3 (applied to soil as NH_4^+ or mineralised/ hydrolysed from organic N compounds) to N_2 (Figure 1.1). This process was traditionally separated into nitrification, the conversion of NH_3 to NO_3^-, and denitrification, the conversion of NO_3^- to N_2 (Davidson, 1991). Although this principle is still valid today, ongoing research has revealed that N turnover in soil and the connected N_2O production is actually far more complex (e.g. Wrage et al., 2001; Zhu et al., 2013). The pathways through which N_2O is formed are identical worldwide across different climates (Granli and Bøckman, 1995; Mosier et al., 2004). However, soil microbial community compositions and responses to alterations in environmental parameters are potentially different between climates (Mosier et al., 2004).

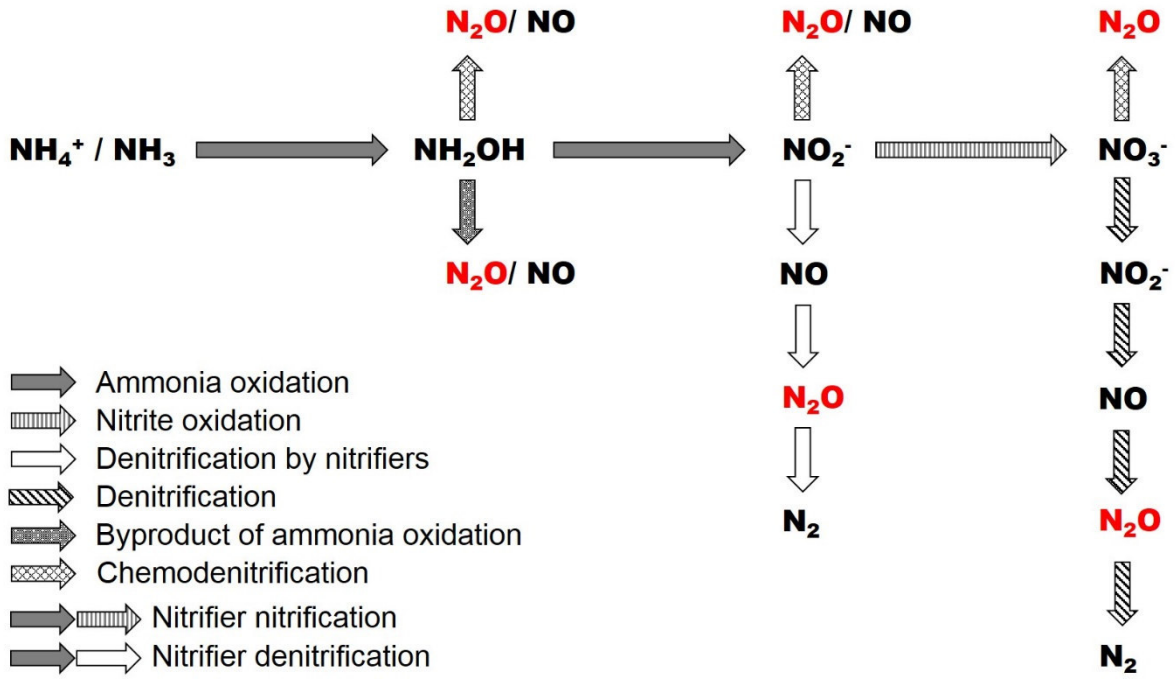


Figure 1.1. Main soil N turnover pathways in agricultural soils connected to N_2O production. Adapted from Dalal et al. (2003), Wrage et al. (2001) and Zhu et al. (2013).

1.1.1 Autotrophic nitrification

Autotrophic nitrification is an aerobic pathway carried out by nitrifying bacteria (Bremner and Blackmer, 1981). This process consists of ammonia oxidation, the conversion of NH_3 via NH_2OH (hydroxylamine) to NO_2^- and nitrite oxidation, the conversion of NO_2^- to NO_3^- (Figure 1.1; Firestone and Davidson, 1989). The first step is carried out by NH_3-oxidizers (primary nitrifiers, e.g. *Nitrosomonas europaea*) and the second step is carried out by NO_2^--oxidizers (secondary nitrifiers, e.g. *Nitrobacter winogradskyi*; Bremner and Blackmer, 1981; Wrage et al., 2001). Species of the genera *Nitrosomonas*, *Nitrospira* (NH_3-oxidizer) and *Nitrobacter* (NO_2^--oxidizer) are assumed to play a major role in autotrophic nitrification in soil (Mosier et al., 2004; Schmidt, 1982). Nitrifying organisms have high substrate turnover rates which make them relevant contributors to soil N processes (Wrage et al., 2001).

1.1.2 Heterotrophic nitrification

Heterotrophic nitrification is an aerobic process which is assumed to be performed predominantly by fungi (Granli and Bøckman, 1994). The metabolic steps of the heterotrophic

nitrification process are identical to those of the autotrophic nitrification process, however, the enzymes responsible are not the same (Wrage et al., 2001). It has been discovered that these organisms are also capable of denitrifying under aerobic conditions (Robertson et al., 1989). The relevance of heterotrophic denitrification in terms of N_2O emissions is believed to be low but could gain importance at low pH, conditions of good aeration and organic nutrient supply (Anderson et al., 1993; Papen et al., 1989; Wrage et al., 2001).

1.1.3 Denitrification

Denitrification is an anaerobic pathway of N turnover that is carried out by facultative anaerobic, heterotrophic denitrifiers (Firestone, 1982; Firestone and Davidson, 1989). Under hypoxic or anaerobic soil conditions these organisms use NO_3^- instead of O_2 as an electron acceptor (Firestone, 1982; Granli and Bøckman, 1994). In this process NO_3^- is transformed to N_2 via NO_2^-, NO and N_2O (Figure 1.1, Firestone, 1982). However, N_2 is not inevitably the end product (Fowler et al, 2009) creating potential for N_2O emission. The capability for denitrification has been found in several bacterial genera (Firestone, 1982). Common genera of denitrifiers are *Pseudomonas* and *Alcaligenes* (Firestone and Davidson, 1989; Tiedje, 1988). Optimum soil conditions for denitrification are presence of organic C which serves as an electron donor, low concentrations of O_2 and the presence of N oxides which serve as electron acceptors (Firestone and Davidson, 1989; Mosier et al., 2004).

1.1.4 Nitrifier denitrification

Nitrifier denitrification is performed by bacterial autotrophic ammonia oxidisers. In this pathway NH_3 is converted to NO_2^- and subsequently, without the production of NO_3^-, is transformed via NO and N_2O to N_2 (Wrage et al., 2001). Enzymes involved in the stepwise reduction of NO_2^- are similar to enzymes in denitrifiers or are even the same, but regulation mechanisms of these processes seem to differ (Kool et al., 2011; Wrage et al., 2001). It has been shown that nitrifier denitrification can become an important contributor to total N_2O emissions from soil under sub-ambient O_2 conditions and at soil moistures below 90% WFPS (Kool et al., 2011; Venterea, 2007; Zhu et al., 2013). Although the importance of this pathway has been demonstrated in recent years, knowledge about this mechanism is still limited.

1.1.5 Chemodenitrification

Chemodenitrification includes all abiological transformations that lead to NO, NO_2 and N_2O production (Davidson and Schimel, 1995). These reaction take place under acidic soil conditions (pH < 5; Chalk and Smith, 1983). It is known that metallic cations, particularly Fe^{2+}, and organic matter influence chemodenitrification by reacting with NO_2^- (Van Cleemput and Samater, 1996). It has been assumed that these mechanisms generally play a minor role in soil (Van Cleemput and Samater, 1996), however, Venterea (2007) showed that N_2O formation from chemodenitrification can be substantial.

1.1.6 Discussion

The soil N turnover pathways described above refer predominantly to bacteria. However, it has been discovered that also fungi do not only have the ability to nitrify as described in section 1.1.2, but are also capable of denitrification (Shoun et al., 1992). Additionally, denitrifying fungi have been found to produce hybrid N_2 and N_2O from NO_2^- and another N-containing molecule like an amino acid, azide or salicylhydroxamic acid which is called 'co-denitrification' (Shoun et al., 1992; Tanimoto et al., 1992). Moreover, nitrification and denitrification mechanisms are also present in archaea (Leininger et al., 2006; Philippot, 2002). Furthermore, other bacterial N turnover processes exist which are not described above. These are the dissimilatory nitrate reduction to ammonium (DNRA), aerobic denitrification and the anaerobic ammonium oxidation ('anammox') with the latter process not contributing to N_2O formation (Mulder et al., 1995; Patureau et al., 2000; Tiedje, 1988).

The mechanisms briefly mentioned in this section emphasize the complexity of N-related soil processes. However, contribution of these processes to total N_2O production in agricultural soil is still a matter of debate which is why they are not described in more detail here. A review of bacterial, fungal and archaeal nitrification and denitrification pathways as well as of the anammox process can be found in Hayatsu et al. (2008). Information about the DNRA process is given in Tiedje (1988).

1.2 Environmental factors controlling N_2O fluxes

Substrate availability, especially the availability of organic and inorganic N compounds as well as organic C compounds and CO_2, is a prerequisite for microbial activity and thus N_2O

formation. Apart from substrate availability there are several environmental factors that influence N_2O production in soil which are outlined below.

1.2.1 Factors affecting diffusion (water, aeration, texture)

Soil moisture is a key factor for emission of N_2O because it influences gas diffusion, aeration status and microbial activity (Granli and Bøckman, 1995; Linn and Doran, 1984). Nitrification takes place at a soil moisture range of roughly 30 to 80% WFPS with activity reaching a peak around 60% WFPS (Linn and Doran, 1984). Denitrification occurs above 60% WFPS and increases with increasing soil moisture (Linn and Doran, 1984). While N_2O emissions from nitrification generally correlate with nitrification activity, emissions of N_2O from denitrification tend to decrease above 80% WFPS as increasingly restricted aeration prevents the diffusion of N_2O to the atmosphere causing increased reduction of N_2O to N_2 in the soil (Davidson, 1991; Granli and Bøckman, 1994). According to this classical concept N_2O emissions from soil are highest between 50 and 70% WFPS (Davidson, 1991). However, this model has been challenged in recent years. It has been shown that peak N_2O emissions occur at different %WFPS for different types of soil and for soils under different types of land-use (Butterbach-Bahl et al., 2013; Redding et al., 2016; Schaufler et al., 2010).

Moisture conditions of soils, and consequently N_2O emissions, are connected to soil texture. For example, soils rich in clay can take up and retain more water than sandy soils and gases can diffuse more easily in coarse-textured soils (Granli and Bøckman, 1995). Moreover, soil structure, which is influenced by management practices, also plays an important role. Tillage can increase soil aeration and thus decrease soil N_2O emissions, but this effect seems to be limited to poorly aerated, clay-rich soils (Rochette, 2008). On the other hand, compaction of soil usually results in increased N_2O emissions, probably due to decreased aeration and consequently increased denitrification rates (Granli and Bøckman, 1995; Gregorich et al., 2014; Hansen et al., 1993).

1.2.2 Temperature

Microbial activity increases with increasing temperature until an optimum is reached. For nitrification this optimal temperature lies between 25 to 35°C (Granli and Bøckman, 1995). The ideal temperature for denitrification seems to be higher but has not been identified as biological

and chemical processes appear to overlap at high temperatures (Keeney et al., 1979). However, it has been shown that denitrification is more temperature sensitive than nitrification (Castaldi et al., 2000). This is because on the one hand increasing temperatures lead to increasing soil respiration and thus to a decrease in soil O_2 concentration which in turn enhances N_2O emissions. On the other hand increasing temperatures also increase mineralisation and nitrification processes resulting in increased substrate availability for denitrification (Butterbach-Bahl et al., 2013). Temperature optima for microbiological processes can vary between different climatic regions (Granli and Bøckman, 1995).

1.2.3 pH

The influence of pH on N_2O emissions is inconsistent (Granli and Bøckman, 1994). For example, N_2O emission from an acidic forest soil (pH 4.0) decreased with increasing pH but N_2O emissions from an alkaline agricultural soil (pH 7.8) showed an increased when the pH was lowered to 6.5 followed by a decrease when the pH was reduced further (Nägele and Conrad, 1990). For nitrification an increase in N_2O emissions has been reported with increasing pH in the range of 5.9 to 8.3 (Bremner and Blackmer, 1981). For denitrification, highest N_2O emissions have been reported between a pH of 4 and 5.5 (Weier and Gilliam, 1986). This observation can be explained with the inhibitory effect of acidic soil conditions on the nitrous oxide reductase in the denitrification process (Knowles, 1982; Wrage et al., 2001). Heterotrophic nitrification can also become important at acidic soil conditions (Papen et al., 1989; Wrage et al., 2001). At alkaline soil conditions it is possible that significant amounts of N_2O are emitted as a result of NO_2^- accumulation (Granli and Bøckman, 1995; Van Cleemput and Samater, 1996).

1.3 N_2O emissions from manure application to soils

Future food production will increasingly depend on the recycling of animal waste as fertiliser because synthetic fertilisers are expensive for farmers and because modern agriculture is reliant on mineral resources which are dwindling (Sommer, 2013). However, application of animal manures to soil can result in substantial N_2O emission. In 2014, direct and indirect N_2O emissions from animal manure application to soils comprised around 25% of the total global direct and indirect N_2O emissions from N applied to soil (FAO, 2017). Cattle (dairy and non-dairy), pig and chicken (broiler and layer) excreta contributed 80% to the total global N_2O

emissions from soil applied manure (FAO, 2017). Among the top producers of these animals are China, India, Brazil and Indonesia which all have tropical or subtropical climates. It is known that N_2O emissions from soils in warm climates tend to be higher compared to temperate climates (Granli and Bøckman, 1995; Denman et al., 2007), however, realistic quantifications cannot be made as data are insufficient (Fowler et al., 2009; Tian et al., 2016). The number of studies investigating the impact of manure application on N_2O emissions from agricultural soils in warm climates is notably low. Given that manure production and N_2O emissions from soil are both highest in countries with tropical or subtropical climates and given that information about N_2O emissions from manure application to soils in these climates is limited, there is an urgent need to study N_2O emissions from manure application in tropical and subtropical climates to improve assessments of the effects of climate change.

1.4 Sugarcane farming

Sugarcane is an important crop grown in tropical and subtropical climates. It is mainly used for food and bioethanol production. In 2014, 27 million hectares of arable land were used for sugarcane cropping globally and 1884 million tons of sugarcane were produced, the three main producers being Brazil, India and China, in this order (FAO, 2017). In Australia, around 375,000 ha are currently under sugarcane cultivation resulting in an output of around 30 million tons per year (Canegrowers, 2010; FAO, 2017). Within Australia, 95% of sugarcane is grown in Queensland and 5% in northern New South Wales (Canegrowers, 2010).

Sugarcane cropping systems are prone to high losses of N_2O from soil. The default EF for sugarcane cropping in Australia is 1.25% (Department of Environment, 2014) and the default EF for managed soils in general is 1% (IPCC, 2006). However, N_2O losses from sugarcane have been shown to reach EF of up to 6.7% for non-burning practices (Allen et al., 2010) and up to 21% for burning practices (Denmead et al., 2010). The global extent of sugarcane cropping as well as the potential for high N_2O losses make sugarcane production systems an important contributor to global N_2O emissions from agricultural soils and highlight the need for N_2O mitigation strategies.

1.5 Mitigation options for N_2O emissions from agricultural soil

There are no relevant terrestrial sinks for N_2O, therefore mitigation of N_2O emissions from agricultural soil needs to focus on the prevention of N_2O formation (Paustian et al., 2016). Today, a variety of mitigation options for N_2O emissions from soil are known. Soil physical management practices like the control of soil moisture by drainage or careful use of irrigation (e.g. Allen et al., 2010) and responsible tillage (e,g. Rochette, 2008) provide options for decreasing soil N_2O emissions (Dalal et al., 2003; Davidson et al., 2012). Another possibility of attenuating soil N_2O emissions are ecological control mechanisms. These include crop rotations, growing cover crops during bare fallow periods to reduce excess soil N, growing perennial plants and breeding plant cultivars with enhanced N use efficiency (Dalal et al., 2003; Davidson et al., 2012; Paustian et al., 2016; Robinson et al., 2011). Alterations to fertiliser/ manure application practices can also mitigate soil N_2O emissions, for example by improving the time and rate of application as well as the placement of fertiliser/ manure (Allen et al., 2010; Davidson et al., 2012; Paustian et al., 2016). Moreover, the use of enhanced efficiency fertilisers, like polymer coated urea or nitrification inhibitor coated urea (e.g. Wang et al., 2016b) has been shown to decrease N_2O emissions from soils. When soils are fertilised with manure, changes in animal diets can also be an effective mitigation strategy (e.g. Velthof et al., 2005).

In recent years, there has been an increasing interest in abating soil N_2O emissions through the amendment of soils with different materials, for example geological materials, such as zeolite or bentonite, and plant based materials, such as biochar or compost. There is evidence in the literature that through the application of these materials to soil (from here on referred to as "soil amendments") N_2O emissions can be decreased (e.g. Cayuela et al., 2014; Dalal et al., 2010; Pratt et al., 2016; Zaman et al., 2007). However, underlying mechanisms have not been identified unambiguously and studies focusing on the effect of soil amendments on N_2O emissions at field scale are limited.

Chapter 2 – Objectives

The central aspect of this thesis is to investigate mitigation options for N_2O emissions from subtropical agricultural soils with a particular focus on fertilisation with animal manures. Research presented in this thesis aims to improve understanding of abating N_2O emissions with soil amendments. Furthermore, it intends to make a contribution to closing the knowledge gap of N_2O emission rates from agricultural soils in warm climates and to provide data that can be fed into climate models to predict future climatic scenarios. This thesis provides an interface between understanding of soil N turnover processes and N_2O mitigation options at field scale.

Chapter 3 addresses whether bentonite, a type of clay, can decrease N_2O losses from manure applied to soil in no-till systems. For this purpose, N_2O emissions from three contrasting subtropical Australian soils from South East Queensland were studied in the laboratory. Fluxes of N_2O were measured after application of different animal manures (beef, pig, and poultry layer manure) and blends of these manures with bentonite, respectively to the three different soils.

In Chapter 4, three contrasting soil amendments (bentonite, biochar and compost) were investigated for their potential to decrease N_2O emissions from animal excreta application to agricultural soil at field scale. Poultry litter and poultry litter blends, respectively were applied to sugarcane at a commercial sugarcane farm. Fluxes of N_2O, and also CO_2 and CH_4 in order to assess the total climatic impact of treatments, were measured over a period of 10 months. The experiment was repeated without plants in the laboratory to investigate treatment effects in a more controlled environment and across spatial scales.

Chapter 5 focuses on identifying the mechanisms by which bentonite and biochar can decrease N_2O emissions from N fertilised soil. Samples of the soil studied in Chapter 4 were amended with urea, poultry litter, bentonite blends of these N sources or biochar blends of these N sources. Soil N dynamics were investigated by simultaneous measurements of N_2O fluxes and soil N fluxes with the minimally invasive microdialysis technique.

Chapter 3 – Effect of bentonite on N_2O emissions from soil-applied manures in no-till systems

3.1 Introduction

Nitrous oxide is a greenhouse gas that has 298 times the GWP of CO_2 on a 100-year time scale (Myhre, 2013) and plays an important role in regulating the temperature on Earth. However, since the industrial revolution human activities have increased the concentration of N_2O in the atmosphere leading to global warming. The majority of human-induced N_2O emissions can be attributed to agricultural activities with N application to soils being one of the main causes (Mosier et al., 1998; Syakila and Kroeze, 2011).

A way of abating N_2O emissions from N fertilised soils is the application of geological materials such as zeolite, vermiculite or bentonite. These materials are strongly negatively charged and therefore have a high CEC. This characteristic enables exchangeable binding of NH_4^+ ions from fertilisers which can potentially lead to reduced N_2O losses from soils. The first study in which the potential of geological materials of high CEC to mitigate N_2O emissions from N fertilised soil was examined was Zaman et al. (2007). The authors studied the effect of zeolite on N_2O emissions from soils fertilised with either urea or urine and found significant decreases in N_2O emissions when zeolite was applied. Similarly, significantly lower N_2O emissions from manure application (beef, pig, broiler, and poultry layer manure) to soil have been reported when the respective manures were blended with vermiculite (Hill et al., 2016; Pratt et al., 2016) or bentonite (Pratt et al. 2016). These studies show that it is possible to decrease N_2O emissions from N application to soils with geological materials that have high CEC. However, in all studies that tested the application of geological materials to soil for the purpose of N_2O abatement, soil amendments and N sources have been incorporated into the soil. It has not been tested yet if geological materials of high CEC have the potential to mitigate N_2O emissions from no-till systems. Ploughing agricultural soil is a major cause for land degradation motivating farmers to adopt no-till practices (Huggins and Reganold, 2008). In Australia, about 9 million hectares of arable land are under no-till practice, which in total is the fifth largest area per country in the world (Huggins and Reganold, 2008).

The objective of this study was to quantify the potential decrease of N_2O emissions from no-till systems with surface application of the geological material bentonite. Three contrasting Australian agricultural soils (Ferrosol, Vertosol and Sodosol) were selected as well as three

different manures (beef, pig and poultry layer manure) to cover a range of soil types and N sources. The chosen manure types account for 68% of N_2O emitted from manure applied to soils in Australia and 48% of N_2O emitted from manure applied to soils worldwide (FAO, 2017). Here, an incubation experiment was carried out under aerobic conditions (55% WHC) in which manures and blends of manures with bentonite were applied to soil surfaces to simulate no-till farming practice.

3.2 Materials and Methods

3.2.1 Collection of soil, manure and bentonite

Soil and manure samples were collected four weeks prior to the experiment from the Darling Downs region in Queensland, Australia. Soil samples were taken from the top 10 cm of a Ferrosol in Toowoomba that was under pasture, of a Black Vertosol near Dalby used for cereal cultivation, and of the sandy A horizon of a Grey Sodosol near Warwick that was under pasture. Classification of the soils was done according to Isbell (2002). Soils were stored at room temperature in containers impenetrable to light and opened every few days to allow gas exchange. Poultry layer manure was collected from a commercial layer farm geared to egg production and did not contain bedding material. Pig manure was collected from a commercial piggery and consisted of faeces and straw, while beef manure was collected off the ground of a feedlot and consisted of faeces and a small proportion (<5%) of dry soil and straw. Manures were stored in the dark in sealed containers at 4°C for preservation until use (Pratt et al., 2014). Bentonite, a type of clay, was obtained from AMCOL Australia (North Geelong, Australia) and was classified as sodium bentonite.

Before commencement of the experiment, the main physical and chemical properties of the soils, manures and bentonite were determined. Soil pH, EC and CEC were analysed as described by Rayment and Lyons (2011) following method 4A1 for analysis of pH, method 3A1 for analysis of EC and method 15F1 for analysis of CEC. Total C and N contents were determined by combustion following methods 6B2 and 7A5, respectively of Rayment and Lyons (2011). Concentrations of NH_4^+ and NO_3^- were analysed by KCl extraction (1.5 M, 1:2 soil: solution ratio) and subsequent colorimetric analyses (Kandeler and Gerber, 1988; Miranda et al., 2001). Furthermore, the soil samples were analysed for microbial activity and microbial biomass N. Microbial activity was determined by fluorescein diacetate (FDA) hydrolysis

described by Adam and Duncan (2001) and microbial biomass N was determined by chloroform fumigation-extraction following the method of Joergensen and Brookes (2005). Results of the analyses are presented in Tables 3.1 and 3.2.

Table 3.1. Main physical, chemical and biological properties of the three soils used in this study.

Property	Ferrosol	Vertosol	Sodosol
Soil texture[2]			
Sand (%)	24.7 ± 0.6	47.0 ± 1.0	93.3 ± 0.6
Silt (%)	22.0 ± 1.5	14.0 ± 0.0	3.0 ± 0.0
Clay (%)	53.0 ± 1.0	38.7 ± 0.6	6.0 ± 0.0
pH	6.44 ± 0.05	7.65 ± 0.13	6.58 ± 0.26
EC (µS cm^{-1})	98.3 ± 8.5	219.7 ± 11.3	18.9 ± 0.5
CEC ($cmol_c$ kg^{-1})	17.7 ± 0.2	35.6 ± 0.6	1.2 ± 0.1
Total C (%)	3.87 ± 0.10	1.50 ± 0.02	0.18 ± 0.04
Total N (%)	0.35 ± 0.00	0.21 ± 0.01	0.06 ± 0.01
NH_4^+-N (mg kg^{-1})	2.15 ± 0.41	4.69 ± 1.48	Not detected
NO_3^--N (mg kg^{-1})	3.8 ± 0.4	46.4 ± 6.0	2.8 ± 0.3
Microbial activity (mg kg^{-1} fluorescein)	83.0 ± 6.9	22.2 ± 2.7	26.9 ± 2.5
Microbial biomass N (mg kg^{-1})	0.73 ± 0.30	0.28 ± 0.10	0.31 ± 0.06

[2] Soil texture data obtained from parallel research. Ferrosol: Hill et al. (2016), Vertosol and Sodosol: Redding et al. (2016)

Table 3.2. Physical and chemical properties of the manures and bentonite.

Property	Poultry layer manure	Pig manure	Beef manure	Bentonite
Dry matter (%)	46.6	36.1	57.7	N/A
pH	9.08 ± 0.03	5.32 ± 0.33	7.09 ± 0.13	9.21 ± 0.02
CEC ($cmol_c$ kg^{-1})	Not determined	Not determined	Not determined	78.3 ± 2.3
Total C (%)	30.3 ± 0.8	42.3 ± 1.7	40.3 ± 0.6	0.07 ± 0.03
Total N (%)	6.22 ± 0.27	3.25 ± 0.69	3.00 ± 0.06	0.01 ± 0.00
C/N ratio	4.9	13.0	13.4	N/A
NH_4^+-N (mg kg^{-1})	8026 ± 563	8074 ± 478	2352 ± 374	6.30 ± 0.75
NO_3^--N (mg kg^{-1})	10.5 ± 4.5	Not detected	9.7 ± 9.2	Not detected

3.2.2 Experimental design

Soil WHC was determined separately for each soil following the method of Wilke (2005). Unsieved subsamples of the collected soils (45 g dry weight) were loosely filled into microcosm

systems that were constructed as described in Inselsbacher et al. (2009) and soil moistures were brought to 55% WHC. Microcosms were then centrifuged for 1 min at a low speed (60 rcf; Hettich Rotina 420R, Andreas Hettich GmbH & Co. KG, Germany) in order to mimic mild compaction in the field. The resulting bulk densities were 1.00 g cm^{-3} for the Ferrosol, 1.01 g cm^{-3} for the Vertosol and 1.40 g cm^{-3} for the Sodosol and the resulting WFPS for these soils were 43%, 44% and 51%, respectively. Microcosms were pre-incubated for 2 days for acclimatisation to experiment conditions. The incubator (Percival E75-L1, Percival Scientific, Perry IA, USA) was programmed to perform a 14/10 h day/night cycle (light intensity: 4800 lm) with temperatures of 30/22 °C, respectively. Humidity was fixed at 80%. Incubation settings represented climatic conditions that are typically prevalent during summer months in South East Queensland.

One day prior to the experiment, two sub-samples of each manure were homogenously mixed with bentonite at a rate of either 50% or 150% in terms of fresh manure mass. The manure and manure plus bentonite blends were spread on top of the soils within the microcosms. Application rates were equivalent to 160 kg N ha^{-1} (203 mg N kg^{-1} dry soil). The final number of treatments was 30: 3 soils (Ferrosol, Vertosol, Sodosol) to which 3 different manures (poultry layer, pig, beef) were added at 3 application rates of bentonite (0%, i.e. manure only; 50%; 150%) plus 1 control treatment (i.e. soil only) for each soil. Each treatment was replicated 3 times resulting in a total number of 90 microcosms. In the incubator microcosms were positioned randomly and were rearranged on a daily basis to account for possible differences in light, humidity and aeration. After 18 days of experiment running time N_2O emissions ceased and the experiment was terminated. During the experiment deionised water was added from the top with a syringe to compensate for water loss through evaporation.

3.2.3 Measurements

Samples of greenhouse gases (N_2O as well as CO_2 and CH_4) were collected on 10 occasions during the incubation period at 12 h, 24 h, 2 d, 3 d, 5 d, 7 d, 9 d, 12 d, 15 d and 18 d after starting the experiment[3]. Gases were sampled manually by covering the microcosm tubes air tight for 1 h with 50 ml plastic tubes which were cut off at the bottom and sealed with a rubber lid at the top. Headspace volumes in the microcosms ranged between 59 and 65 ml for the Ferrosol, between 61 and 66 ml for the Vertosol and between 74 and 78 ml for the Sodosol. After 1 h of

[3] Results of the CO_2 and CH_4 measurements can be found in the appendix (Figures A1, A2).

sealing time, samples (20 ml) of the headspace gases were taken with a syringe, transferred into pre-evacuated Exetainer vials (Labco Limited, Lampeter, UK) and analysed by gas chromatography (GC-2010, Shimadzu, Kyoto, Japan). Cumulative N_2O losses were calculated by linear interpolation between sampling events. The percentage of applied N that was lost as N_2O from the microcosm systems was calculated by the equation

(cumulative N_2O-$N_{treatment}$ – cumulative N_2O-$N_{control}$)/ total N application x 100

as described in (Granli and Bøckman, 1995). At the end of the experiment microcosms were analysed for mineral N (NH_4^+ and NO_3^-) concentrations, microbial activity and microbial biomass N following the procedures described above.

3.2.4 Statistical analyses

Analyses of data were carried out using R, version 3.3.1 (R Core Team, 2016). A Three-way-ANOVA of cumulative N_2O emissions was done after subtracting the mean N_2O fluxes of the control treatments in order to obtain a balanced dataset. Additionally, correlation analyses of cumulative N_2O emissions per microcosm with net changes in NH_4^+, NO_3^-, microbial activity and microbial biomass per microcosm between start and end of the experiment were performed using a linear model. The distribution of the residuals was checked prior to statistical analyses.

3.3 Results

3.3.1 Cumulative N_2O emissions

In control treatments N_2O fluxes were stable around 0 (± <0.01) mg N_2O-N kg^{-1} h^{-1} at all times of measurement and resulted on average in a small positive cumulative N_2O flux (N_2O emission) in the Ferrosol (<0.01 mg N_2O-N kg^{-1}) and the Vertosol (0.01 mg N_2O-N kg^{-1}) and a small negative cumulative N_2O flux (N_2O uptake) in the Sodosol (-0.01 mg N_2O-N kg^{-1}).

Application of poultry layer manure led to N_2O emissions from all three soils with highest values recorded from the Vertosol (0.27 mg N_2O-N kg^{-1}, equivalent to 0.13% of N applied; Figure 3.1a). Application of pig manure also led to emissions of N_2O from all soils with highest emissions measured from the Sodosol (0.26 mg N_2O-N kg^{-1}, equivalent to 0.13% of N applied; Figure 3.1b). When beef manure was applied to the Ferrosol and the Vertosol, N_2O fluxes were

around 0 (± <0.01) mg N_2O-N kg^{-1} h^{-1} at all times of measurement and on average resulted in small positive cumulative N_2O fluxes (Ferrosol: 0.01 mg N_2O-N kg^{-1}, equivalent to 0.01% of N applied; Vertosol: <0.01 mg N_2O-N kg^{-1}, equivalent to <0.01% of N applied; Figure 3.1c). Application of beef manure to the Sodosol caused cumulative N_2O emissions of 0.39 mg N_2O-N kg^{-1} (equivalent to 0.19% of N applied).

Mixtures of poultry layer manure and bentonite increased N_2O emissions from all soils compared to poultry layer manure only application (Figure 3.1a). However, increases in bentonite from 50% to 150% manure mass were not accompanied by increases in N_2O emissions except for the Vertosol. In this soil the highest addition rate of bentonite resulted in highest N_2O losses from poultry layer manure application across soils (0.77 mg N_2O-N kg^{-1}; equivalent to 0.38% of N applied). Blending pig manure with bentonite led to higher N_2O losses compared to pig manure only application from the Vertosol and the Sodosol (Figure 3.1b). The effect was especially strong in the Sodosol. While in the Vertosol N_2O emissions of up to 0.26 mg N_2O-N kg^{-1} (equivalent to 0.12% of N applied) were measured (150% bentonite treatment), cumulative N_2O emissions from the Sodosol reached on average 3.08 mg N_2O-N kg^{-1} (equivalent to 1.51% of N applied) at 50% bentonite and 4.91 mg N_2O-N kg^{-1} (equivalent to 2.41% of N applied) at 150% bentonite. Contrary to this, blending pig manure with bentonite decreased N_2O emissions compared to pig manure only application from the Ferrosol (50% bentonite: 0.01 mg N_2O-N kg^{-1}, equivalent to 0.01% of N applied; 150% bentonite: 0.02 mg N_2O-N kg^{-1}, equivalent to 0.01% of N applied). Bentonite plus beef manure blends showed mixed results compared to beef manure only application across soils (Figure 3.1c). In the Ferrosol the application of bentonite led to an increase in N_2O emissions compared to beef manure only application but the effect was stronger at 50% bentonite (0.12 mg N_2O-N kg^{-1}, equivalent to 0.06% of N applied) than at 150% bentonite (0.07 mg N_2O-N kg^{-1}, equivalent to 0.03% of N applied). In the Vertosol the application of bentonite resulted in higher N_2O losses compared to beef manure only application with the strongest effect observed in the 150% bentonite treatment (50% bentonite: 0.36 mg N_2O-N kg^{-1}, equivalent to 0.18% of N applied; 150% bentonite: 0.96 mg N_2O-N kg^{-1}, equivalent to 0.47% of N applied). In the Sodosol blending beef manure with 50% bentonite led to a decrease in N_2O emissions compared to beef manure only application (0.32 mg N_2O-N kg^{-1}, equivalent to 0.16% of N applied) whereas the manure plus 150% bentonite blend resulted in increased N_2O emission (0.46 mg N_2O-N kg^{-1}, equivalent to 0.23% of N applied).

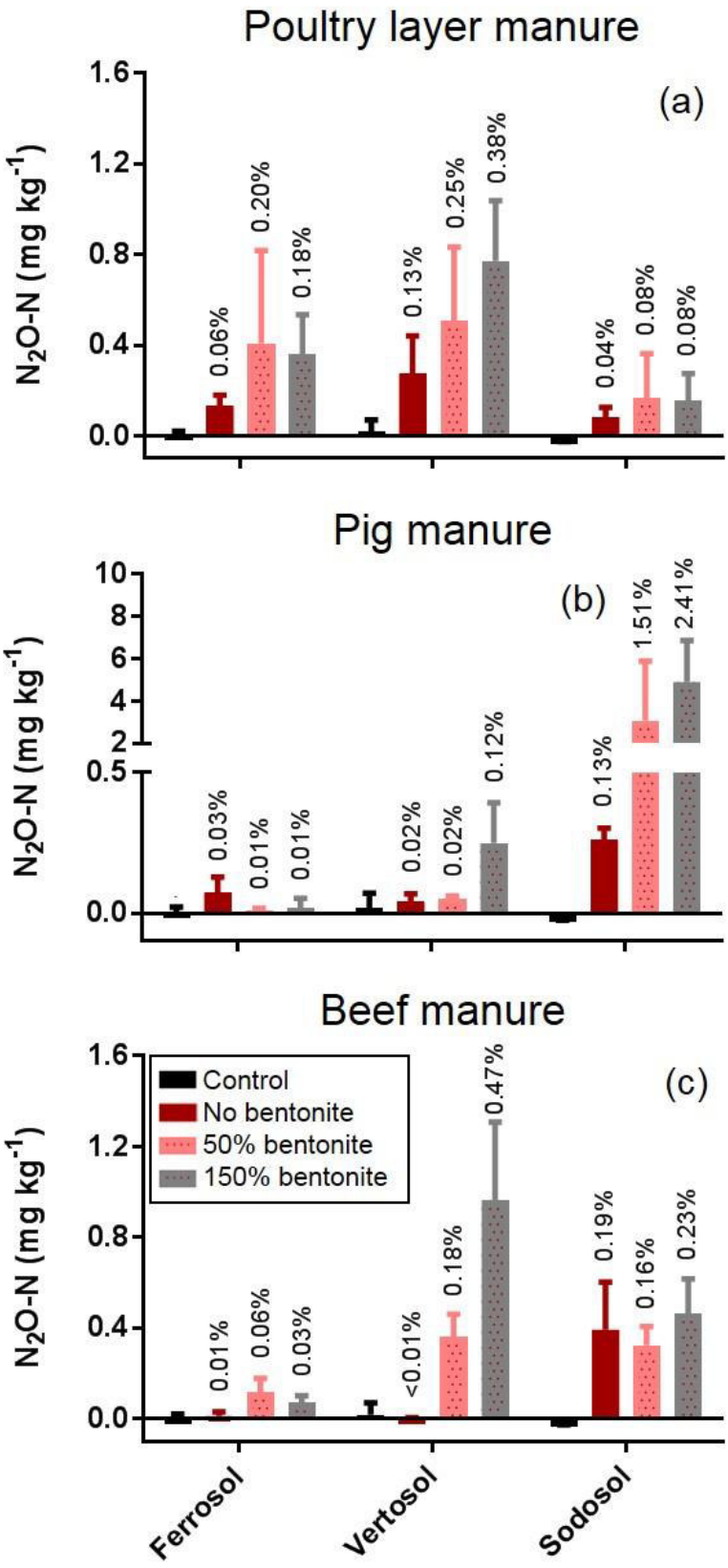


Figure 3.1. Cumulative N_2O fluxes (mean ± SD) over 18 days after application of a) poultry layer manure, b) pig manure and c) beef manure. Application rates of bentonite are given in percentages of fresh manure mass. Numbers in panels refer to N_2O losses expressed in % of applied N. Results of statistical analysis are given in Table 3.3.

Effects of treatments on cumulative N_2O emissions were statistically analysed by a three-way ANOVA (Table 3.3). The analysis revealed significant effects for the main factors (soil, manure and bentonite), for all three first order interactions (soil × manure, soil × bentonite and manure × bentonite) as well as for the second order interaction that analyses the interaction of all three main factors.

Table 3.3. Three-way ANOVA of cumulative N_2O-N emissions. Analysis was carried out after subtraction of control treatments in order to create a balanced dataset. Significant effects are given at levels of $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Source of variation	Degrees of freedom	Sum of Squares	Mean square	Variance ratio	*P*
Main factors					
Soil	2	14.20	7.10	15.24	***
Manure	2	7.74	3.87	8.31	***
Bentonite	2	7.52	3.76	8.08	***
1st order interactions					
Soil × manure	4	30.80	7.70	16.54	***
Soil × bentonite	4	5.61	1.40	3.01	*
Manure × bentonite	4	5.09	1.27	2.73	*
2nd order interaction					
Soil × manure × bentonite	8	16.78	2.10	4.50	***
Residual	54	25.15	0.47		

3.3.2 Inorganic N pools

At the end of the experiment, concentrations of NO_3^- in microcosms were most strongly influenced by soil type (Figure 3.2). Across treatments NO_3^- concentrations were highest in the Vertosol (54.9 - 79.3 mg NO_3^--N kg^{-1}; Figure 3.2d-f) and lowest in the Sodosol (1.1- 5.4 mg NO_3^--N kg^{-1}; Figure 3.2g-i) with NO_3^- concentrations in the Ferrosol ranging in between (17.0 - 40.2 NO_3^--N kg^{-1}; Figure 3.2a-c). In all soils application of manure, regardless of type, increased NO_3^- concentrations compared to the respective control treatments by end of the experiment. When poultry layer and beef manure were blended with bentonite this led to a decrease in NO_3^- concentrations by the end of the experiment across soils compared to manure only application. This effect was also observed when pig manure was applied to the Vertosol. However, in the Ferrosol and the Sodosol the application of bentonite blended pig manure led

to an increase in NO_3^- concentrations by the end of the experiment compared to pig manure only application.

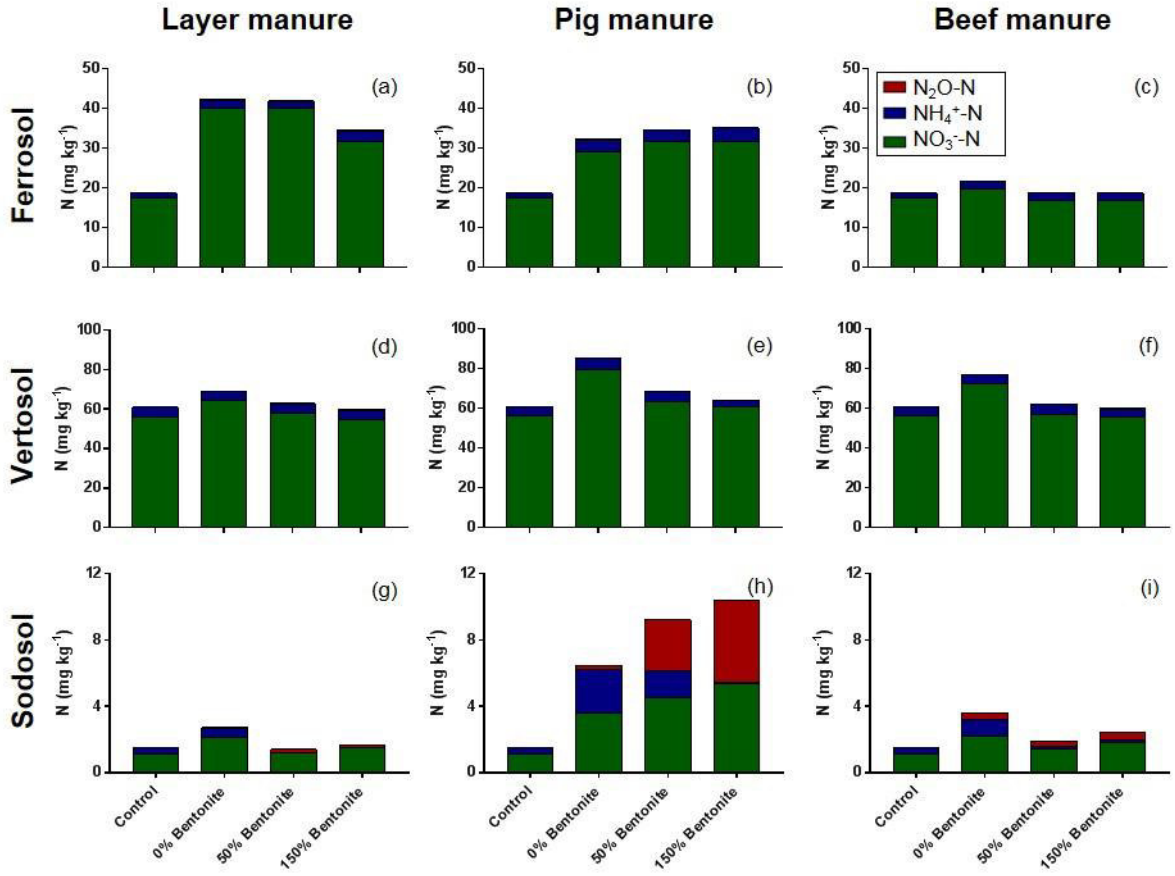


Figure 3.2. Mean inorganic N pools (NH_4^+, NO_3^- and N_2O) on day 18 at the termination of the experiment. Nitrous oxide pools are cumulative N_2O fluxes calculated from N_2O fluxes that were measured on a per day basis. Nitrogen application rate was 203 mg N per kg dry soil (equivalent to 160 kg ha^{-1}). Application rates of bentonite are given in percentages of manure mass.

In the Ferrosol and in the Vertosol NH_4^+ concentrations at the end of the experiment were similar across treatments with values ranging between 1.2 – 3.6 mg NH_4^+-N kg^{-1} in the Ferrosol (Figure 3.2a-c) and between 3.3 and 6.1 mg NH_4^+-N kg^{-1} in the Vertosol (Figure 3.2d-f). A clear effect of bentonite on soil NH_4^+ concentrations was not discernible in these two soils. In the Sodosol however, application of bentonite decreased soil NH_4^+ concentrations by the end of the experiment across types of manures (Figure 3.2g-i). When poultry layer manure was applied, adding bentonite to the manure resulted in no detectable NH_4^+ at the end of the experiment (Figure 3.2g). When pig manure was applied, soil NH_4^+ concentrations at the end of the experiment were gradually decreased with increasing rates of bentonite (Figure 3.2h). At an

application rate of 150% bentonite only negligible amounts of NH_4^+ were found (on average 0.07 mg NH_4^+-N kg^{-1}). Decreases in soil NH_4^+ concentrations in the treatments that received bentonite compared to manure only application were accompanied by higher N_2O losses when poultry layer manure and pig manure were applied.

3.3.3 Linear regression analyses

Negative and significant correlations were found between cumulative N_2O fluxes per microcosm and the net changes in NH_4^+ per microcosm (r = -0.243, $P < 0.05$, n = 90) as well as between cumulative N_2O fluxes and the net changes in NO_3^- per microcosm (r = -0.214, $P < 0.05$, n = 90; Table 3.4). Correlations between cumulative N_2O fluxes per microcosm and net changes in microbial parameters were also negative (microbial activity: r = -0.310, n = 90; microbial biomass: r = -0.036, n = 90). The correlation between cumulative N_2O fluxes and microbial activity was significant at $P < 0.01$ whereas the correlation between cumulative N_2O fluxes and microbial biomass was not significant.

Table 3.4. Correlation analyses of cumulative N_2O-N emissions per microcosm with net changes in NH_4^+-N, NO_3^--N, microbial activity and microbial biomass per microcosm over 18 days. Net change in microbial activity was calculated from net changes in fluorescein (mg) as measured by fluorescein diacetate (FDA) hydrolysis. Significant effects are given at levels of $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Variable	r, n = 90
Δ NH_4^+-N (mg)	-0.243*
Δ NO_3^--N (mg)	-0.214*
Δ microbial activity	-0.310**
Δ microbial biomass (mg)	-0.036

3.4 Discussion

3.4.1 Effect of bentonite on N_2O fluxes

Under most of the soil and manure combinations tested here, the application of bentonite led to an increase in N_2O emissions. Bentonite has a strong water binding characteristic and thus it is possible that the application of bentonite increased the soil moisture in the top few mm of the

soil, thereby promoting denitrification (Granli and Bøckman, 1994; Linn and Doran, 1984). Furthermore, increasing rates of bentonite could have resulted in an increasingly strong surface sealing effect leading to decreasing O_2 concentrations in the soils and by this also promoting denitrification (Firestone and Davidson, 1989). These effects show that the potential beneficial effect of bentonite to decrease N_2O emissions through exchangeable binding of NH_4^+ ions is probably overridden by alterations in soil moisture and soil aeration across soil types when bentonite is applied to the soil surface. It is possible that N turnover rates in the soils were actually decreased by the application of bentonite. This could have reduced N_2O losses from the nitrification pathway (Pratt et al., 2016). The likely increase in soil moistures and the likely decrease in soil O_2 concentrations through the application of bentonite however, seem to be the cause for denitrification happening in the soils leading to in total higher N_2O losses, in spite of possible lower N turnover rates.

3.4.2 Effect of soil type and manure type on N_2O fluxes

The effect of bentonite on N_2O emissions was especially strong in the Sodosol when this soil was fertilised with pig manure. This was a combined effect of soil, manure and bentonite as shown by the three-way-ANOVA.

A major soil related influence was probably soil structure as soil structure is known to play an important role in N_2O emissions (Skiba and Ball, 2002). The Ferrosol and the Vertosol were well aggregated, whereas the sandy A horizon of the Sodosol used in this study was poor in its structure with small and homogenous aggregate sizes. This characteristic led to a higher bulk density and thus to a higher %WFPS in the Sodosol (Linn and Doran, 1984) compared to the other two soils. This in turn might have caused a decrease in gas exchange between soil and atmosphere and consequently a decrease in O_2 concentration in the soil (Gregorich et al., 2014; Lapen et al., 2004; Sweeney et al., 2006) resulting in increased N_2O emissions from denitrification (Firestone and Davidson, 1989; Gregorich et al., 2014).

The main influences on N_2O emissions attributable to the manures are likely the differences in water content, mineral N content and different mineralisation rates from the manures. Pig manure had the highest water content of all manures (36.1% dry matter) thereby possibly most strongly enhancing denitrification (Granli and Bøckman, 1994; Linn and Doran, 1984). Furthermore, pig manure had the highest concentration of NH_4^+ (8074 mg NH_4^+-N kg^{-1}), but NH_4^+ concentration in the poultry layer manure was similarly high (8026 mg NH_4^+-N kg^{-1}).

Given the different composition and characteristics of the manures it is likely that differences in N mineralisation occurred. For example, it could be possible that N mineralisation was highest from the pig manure, thereby providing more substrate for nitrification and ensuing denitrification. However, soil mineral N concentrations were not monitored during the experiment.

Soil NH_4^+ concentrations in the Ferrosol measured at the end of the experiment did only marginally differ between treatments and were almost identical to the concentrations measured before the start of the experiment. The same phenomenon was observed in the Vertosol. Contrary to this, no NH_4^+ was detected in the Sodosol before the experiment and the application of bentonite led to the disappearance of NH_4^+ at the end of the experiment across manure types. It is possible that in the Ferrosol and in the Vertosol NH_4^+ applied through the manures was either completely or almost completely nitrified and that thus the NH_4^+ that was measured at the end of the experiment in these two soils was the NH_4^+ that was already in the soil before the experiment. This theory is consistent with Redding et al. (2016) who studied N_2O emissions from the same Vertosol used here under a range of N application rates soil and moisture conditions in a laboratory trial for 37 days and found that nitrification was almost complete at the end of the experiment. In the Sodosol, nitrification may have been inhibited without bentonite addition. The Sodosol was low in CEC (1.2 $cmol_c\ kg^{-1}$) and therefore fertilisation of this soil with manure might have led to NH_4^+/ NH_3 accumulation in the soil solution, especially in the top few mm of the microcosm. This in turn could have had an inhibitory effect on NO_2^- oxidising bacteria (Anthonisen et al., 1976) resulting in N_2O losses from the accumulated NO_2^- (Van Cleemput and Samater, 1996). Venterea et al. (2015) observed this mechanism in a soil of low CEC. When bentonite was added, the NH_4^+ from the manures was likely exchangeably bound because of bentonite's high CEC which could have prevented the accumulation of NH_4^+/ NH_3 in the soil (Venterea et al., 2015). Consequently, there was maybe either no or only a small inhibition of NO_2^- oxidising bacteria and nitrification could be completed. However, with the addition of bentonite, high soil moisture and low O_2 concentration conditions were likely created in the top few mm of the soil leading to N_2O production from denitrification, especially when pig manure was applied because of its high moisture content (Firestone and Davidson, 1989; Linn and Doran, 1984).

The results of this study match the results of Redding et al. (2016) who investigated N_2O fluxes from the same samples of Vertosol and Sodosol that were used in this study across a wide range of soil N concentrations and soil moistures. The authors showed that N_2O fluxes from the

Vertosol are minor up to 85% WFPS but increase strongly above this value and form a sharp peak between 85% and 93% WFPS. Soil moisture conditions in the study presented here were much lower than 85% WFPS. However, increasing application rates of bentonite led to increasing N_2O losses from the Vertosol across manures indicating that soil moisture conditions at the surface of this soil might have approached the identified range in which N_2O production is highest. It is also possible that the likely sealing effect caused by bentonite simulated the low soil O_2 conditions at around 90% WFPS. Furthermore, Redding et al. (2016) measured low nitrification rates in the Sodosol compared to the Vertosol and found N_2O production in this soil to peak around 60% WFPS at low mineral N levels. As hypothesized in the present study, the authors discussed suppressed nitrification by NH_3 accumulation as a possible reason for their finding. A striking result from Redding et al. (2016) is the high abundance of bacterial denitrifiers found in the Sodosol. Especially the genera *Streptomyces*, *Alicyclobacillus* and *Bacillus* were common in this soil. This result links to the high N_2O losses observed here from the Sodosol after applying blends of pig manure and bentonite and supports the theory that these losses were mainly caused by denitrification.

3.4.3 Experiment design

Soil mineral N (NH_4^+ and NO_3^-) concentrations and soil biological characteristics (microbial activity and microbial biomass N) were measured before the start of the experiment and at the end of the experiment. Monitoring of these properties by destructive harvesting of additional replicates of microcosms during the experiment was not done due to restricted incubator capacity. Analysing soil properties only before the start and at the end of the experiment reveals very limited information about soil N and soil microbiological dynamics and this circumstance might have led to the weak correlations found by the linear regression analyses. Measurements of soil NH_4^+ concentrations during the experiment for example could have revealed if accumulation of NH_4^+ was actually happening in the Sodosol when it was fertilised with manure. However, because these data were not recorded, the occurrence of this mechanism remains a matter of speculation. Additionally, monitoring of water loss from microcosms through evaporation would have been helpful for elucidating the effect of bentonite on soil moisture.

An important point to mention is that soil moisture in this experiment was determined based on WHC. As it has been pointed out by Linn and Doran (1984) methods for determination of WHC vary and %WFPS is a more accurate expression of soil moisture and soil aeration. Therefore

and because the expression of soil moisture as %WFPS has been widely adopted by scientists it is better to use %WFPS as an index for soil moisture. Furthermore, the WHC in this study was determined before centrifugation of the microcosms resulting in different %WFPS across the soils.

Differences in pH and C content across soils and especially across manures probably influenced the results presented here, thus making a direct comparison of results between treatments difficult. However, these parameters seem to have played a secondary role in this study.

3.4.4 Conclusion

Under most soil plus manure combinations tested here the application of bentonite resulted in increased N_2O losses. The surface application of bentonite probably increased the soil moisture in the top few mm of the soils and decreased O_2 concentrations in the soils thereby creating conditions that are favourable for denitrification and consequently enhancing N_2O emissions. Thus, it can be concluded that bentonite has potential to decrease N_2O losses from N fertilised soil when it is incorporated into the soil (Pratt et al., 2016). However, based on the results of this study, applying bentonite to the soil surface as it would be the practice in no-till systems cannot be recommended.

Chapter 4 – Effect of bentonite, biochar and compost on N_2O emissions from spent poultry litter applied to field-grown sugarcane

4.1 Introduction

Animal manures are a valuable source of plant nutrients (Jensen, 2013) and therefore are widely used as fertiliser in crop production systems. However, application of manure to soil can result in substantial emission of N_2O to the atmosphere (e.g. Akiyama and Tsuruta, 2003; Rochette et al., 2008; Velthof et al., 2003). Thus, it is critical to develop effective mitigation techniques that reduce environmental damage from manure application to agricultural soils.

In recent years, researchers have started to address N_2O losses from manure application to soil by blending manures with soil amendments. Emissions of N_2O were significantly decreased across different manures (beef manure, pig litter, poultry litter and egg manure) when these were mixed with the clays vermiculite or bentonite (Pratt et al., 2016). The effect was attributed to the high CEC of these clays enabling exchangeable binding of NH_4^+-ions present in the manures. Furthermore, results of a meta-analysis, carried out for investigating the influence of biochar on N_2O emissions from organic or mineral soil-applied N fertilisers revealed that N_2O emissions tend to be lower in the presence of biochar (Cayuela et al., 2014). However, underlying mechanisms are still unclear; potential contributing factors are CEC, NH_4^+ ions caught in the pores of biochar, improved soil aeration, labile C input shifting denitrification more towards N_2 production, increase of soil pH, or N immobilisation (Clough et al., 2013). Compost addition to soil can also decrease N_2O emissions from manure application (Dalal et al., 2009; Dalal et al., 2010). The authors of both studies reported strong decreases of N_2O emissions from feedlot manure when it was applied together with green waste compost and suspected N immobilisation as well as reduced organic matter decomposition as underlying causes. Taken together, these studies illustrate the potential of soil amendments in reducing N_2O losses from soil-applied manure. However, co-application of animal manures with soil amendments for tackling N_2O emissions from crop production has not received substantial study. Existing knowledge is mainly laboratory-based and more investigations into whether observed effects of decreased N losses are transferable to field scale are needed. Therefore, the aim of this study was to test a range of soil amendments for their potential to decrease N_2O emissions from animal manure application to agricultural soil at field scale.

We compared three materials (bentonite, biochar and compost) in a commercial field under sugarcane cultivation for their potential to attenuate N_2O emissions from fertilisation with poultry litter. With a total production of around 30 million tonnes per year, sugarcane is the most produced commodity in Australia being worth in total around 2 billion USD (Canegrowers, 2010; FAO, 2017). Considerable N_2O losses have been reported from sugarcane soils fertilised with synthetic urea (e.g. Allen et al., 2010; Denmead et al., 2010; Wang et al., 2012) making the industry to a major contributor to Australia's annual agricultural greenhouse gas production. Sugarcane growers are interested in recycling organic wastes to partially or fully substitute synthetic/mineral fertilisers but the environmental effects are unknown. For addressing this knowledge gap, poultry litter was chosen because poultry businesses are located in the sugarcane production area studied here. Aiming for a circular nutrient economy, the application of poultry litter could provide multiple benefits by (1) recycling animal waste, (2) reducing fertiliser costs for farmers, (3) decreasing the environmental footprint inherent to the production of synthetic N fertilisers, and (4) improving soil health.

We applied spent poultry litter alone or in combination with bentonite, biochar or green waste compost, respectively in a field experiment on a commercial sugarcane farm. Additionally, urea was applied as a reference treatment to represent standard fertiliser application practice in Australian sugarcane farming. Fluxes of N_2O were quantified over a period of 10 months representing an annual sugarcane crop cycle. Fluxes of CO_2 and CH_4 were also quantified during the experiment for assessing the overall climatic impact of treatments. Furthermore, the materials used in the field experiment were also tested in a laboratory experiment for their potential to decrease N_2O emissions from soil to ascertain if field-observed effects are reproducible in a tightly controlled environment. The objectives of this study were (1) to examine the effects of blends of intensive livestock manure with soil amendments on N_2O emissions from soil, (2) to identify key drivers of soil N_2O emissions, and (3) to investigate the effects of manure formulations on sugarcane growth.

4.2 Materials and methods

4.2.1 Field experiment

4.2.1.1 Description of the experimental site

The field experiment was conducted on a commercial sugarcane farm at Maroochy River, Queensland, Australia (26°34'S, 153°00'E). The climate is subtropical with a mean daily maximum temperature of 29.0 °C in summer, a mean daily minimum temperature of 9.6 °C in winter and a mean annual rainfall of 1467 mm (Bureau of Meteorology, 2017; Site 040861). The site had been under sugarcane cultivation for about 80 years. The soil is a sandy light clay and was classified as an Oxyaquic Hydrosol (Isbell, 2002).

4.2.1.2 Sample collection and characterisation

Spent poultry litter consisted of faeces, feathers and eucalypt sawdust and was collected from an industrial shed that holds 60,000 broilers. Collection took place a few hours after birds had been removed from the shed. The litter was stored for eight weeks in light-impermeable containers at 4 °C to maintain chemical properties (Pratt et al., 2014). The bentonite was a sodium bentonite and originated from a mine near Miles, Australia (AMCOL Australia, North Geelong, Australia). Biochar was produced from sugarcane harvesting residues, green waste, shredded wood, and cardboard at heating temperatures between 400 and 600 °C. The material was stored in light-impermeable containers at room temperature. Compost was generated from the public local green waste bin and was stored outdoors in a windrow.

Soil texture was analysed using the hydrometer method (Gee and Or, 2002). Bulk density was determined separately for rows and inter-rows at 10 cm depth by the core method (Wilke, 2005). Measurements of pH and EC were done according to methods 4A1 and 3A1, respectively in Rayment and Lyons (2011). For analysis of CEC the unbuffered salt extraction method was used (Sumner and Miller, 1996). Total C and N contents were determined by combustion following methods 6B2 and 7A5, respectively of Rayment and Lyons (2011). Total P and K contents were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Concentrations of NH_4^+ and NO_3^- were measured by a standard 1M KCl extraction (1:2

soil: solution ratio) with subsequent colorimetric analyses (Kandeler and Gerber, 1988; Miranda et al., 2001). Microbial biomass N was determined using the chloroform fumigation-extraction method (Joergensen and Brookes, 2005).

Table 4.1. Main soil properties (mean ± SD) of the top 10 cm at the experimental field site at Maroochy River in December 2014 before the start of the trial.

Soil properties	
Texture	
Sand (%)	50
Silt (%)	27.50
Clay (%)	22.50
Bulk density row (g cm^{-3})	1.00
Bulk density inter-row (g cm^{-3})	1.07
pH	5.27 ± 0.39
EC (µS cm^{-1})	13.08 ± 3.75
CEC ($cmol_c$ kg^{-1})	36.81 ± 1.22
Total C (%)	2.04 ± 0.08
Total N (%)	0.19 ± 0.01
Total P (%)	0.03 ± 0.00
Total K (%)	0.41 ± 0.03
NH_4^+-N (mg kg^{-1})	6.80 ± 6.79
NO_3^--N (mg kg^{-1})	0.92 ± 0.86
Microbial biomass N (mg kg^{-1})	0.56 ± 0.83

Table 4.2. Physical and chemical properties of manure and amendments used in the field trial. Chemical data are presented as mean ± SD.

	Poultry litter	Bentonite	Biochar	Compost
Dry matter (%)	79.40	N/A	N/A	66.90
pH	8.39 ± 0.06	8.85 ± 0.01	8.78 ± 0.08	6.96 ± 0.08
EC (mS cm^{-1})	N/A	1.66 ± 0.04	0.26 ± 0.09	0.57 ± 0.08
CEC ($cmol_c$ kg^{-1})	74.68 ± 7.19	76.81 ± 2.27	39.04 ± 3.10	52.02 ± 2.19
Total C (%)	37.70 ± 1.69	0.66 ± 0.04	25.89 ± 4.89	17.72 ± 0.39
Total N (%)	3.05 ± 0.10	0.04 ± 0.01	0.75 ± 0.18	1.19 ± 0.01
C/N ratio	12.35	N/A	34.43	14.87
Total P (%)	0.77 ± 0.15	0.03 ± 0.00	0.01 ± 0.00	0.20 ± 0.01
Total K(%)	0.94 ± 0.07	0.39 ± 0.21	0.10 ± 0.01	0.50 ± 0.04
NH_4^+-N (mg kg^{-1})	2836 ± 48	7.98 ± 5.44	Not detected	9.23 ± 7.37
NO_3^--N (mg kg^{-1})	Not detected	Not detected	13.20 ± 3.27	350.6 ± 103.9

4.2.1.3 Experimental design

The experiment was conducted for 280 days from 17 December 2014 to 23 September 2015 over an annual sugarcane production cycle. Sugarcane plants were in the 6th ratoon cycle when measurements commenced. The distance between rows was 1.65 m and the ground had been covered by a sugarcane trash blanket from the previous harvest which was equivalent to 13.7 t ha^{-1} (dry weight basis). Six treatments replicated three times were arranged in a randomised block design in sections of the field that had received no fertiliser or other nutrient inputs in the year preceding the experiment. Treatments were as follows,

'C' = Zero N, i.e. control (no nutrient input);

'UA' = Urea;

'PL' = Poultry litter;

'PL+BE' = Poultry litter + bentonite;

'PL+BC' = Poultry litter + biochar;

'PL+CO' = Poultry litter + compost.

Each experimental plot was 6.6 m in width covering four rows. All plots were 5 m in length except for the plots that received urea which were 10 m in length due to this treatment being shared with a parallel experiment. Buffer strips between plots were 5 m long. Application rates of urea and poultry litter were equivalent to 160 kg N ha^{-1} following N fertiliser guidelines for sugarcane in this region (Sugar Research Australia, 2013). The application rate of amendments was equivalent to 5 t ha^{-1} (dry weight basis). Amendments were thoroughly pre-mixed with manure by hand using shovels and applied within an hour. Urea, poultry litter and poultry litter blends were applied to 10 cm deep trenches that were dug into one side of the shoulders of sugarcane rows and covered first with soil and subsequently with sugarcane trash. Where necessary, phosphorus and potassium stocks were topped up with industrial fertiliser to meet the recommended application rates of 40 kg P ha^{-1} and 100 kg K ha^{-1}, respectively for sugarcane cultivation (Sugar Research Australia, 2013). Air temperature and humidity were logged every 30 minutes by a weather station situated in the experimental field site (ICT International, Armidale, Australia) and daily rainfall data were accessed from a nearby public weather station (Dunethin Rock, in 400m distance to the experiment).

4.2.1.4 Gas sampling and analysis

Fluxes of the greenhouse gases N_2O, CO_2 and CH_4 were measured manually with static chambers over a period of 275 days after the start of the experiment. The measurement system consisted of square metallic chamber bases covering an area of 0.25 m^2 and cubic chamber tops holding a total volume of 140 L. Two chamber bases were installed in each plot close to the centre, one in the shoulder of the cane row on the site that received treatment and one in the inter-rows. Bases were inserted about 5 cm deep into the soil. Junctures between chamber bases and tops were sealed with air tight door seals. Chamber tops were equipped with vent tubes connected to valves. Twenty-eight millilitres of gas were taken by connecting a syringe to the valve and transferring gas samples into pre-evacuated Exetainer vials (Labco Limited, Lampeter, UK). Gas sampling was carried out between 9:00 am and 12:00 pm according to Allen et al. (2010) and Reeves et al. (2016) for best representation of daily mean fluxes. Chamber closing duration ranged between 60 and 90 minutes. Spot checks of greenhouse gas accumulation within the chambers revealed a linear increase in gases over time for at least two hours. Sampling frequency was adapted to rainfall events and gradually decreased from twice a week in the first eight weeks of the experiment to every three weeks in the last two months of the experiment. Gas samples were analysed by a gas chromatograph equipped with a flame ionization detector (FID) and an electron capture detector (ECD) (GC-2010, Shimadzu, Kyoto, Japan). Daily greenhouse gas fluxes were estimated by multiplying hourly fluxes with the factor 24. Cumulative emissions were calculated using trapezoidal integration between sampling events as described by Allen et al. (2010). Emission factors were calculated using the IPCC (2006) Tier 1 method:

$(N_2O\text{-}N_{treatment} - N_2O\text{-}N_{control})$/ total N application x 100,

where $N_2O\text{-}N_{treatment}$ refers to the cumulative N_2O emissions from fertilised plots, and $N_2O\text{-}N_{control}$ refers to the cumulative N_2O emissions from unfertilised plots.

4.2.1.5 Soil sampling and analysis

Each gas sampling event was accompanied by recording the soil temperature of the top 10 cm with a hand-held thermometer and by a soil sampling event. At each event two separate soil samples of 10 cm depth were taken from each plot, from the side of the row shoulder that was subjected to treatment and from the inter-row. Within 48 h of collection a sub-sample of each

collected soil sample was extracted with a 1M KCl solution (1:2 soil: solution ratio) and extracts were frozen at -80 °C until analysis of extractable NH_4^+ and NO_3^- concentrations, and microbial biomass N following the methods described above. Another set of sub-samples was used for determination of gravimetric soil moisture by drying samples at 60 °C for four days.

4.2.1.6 Sugarcane biomass

The two inner rows of the experimental plots were hand harvested at the end of the experiment on 23 September 2015. In total, 10 m of sugarcane (2 x 5 m) were cut close to the ground from each plot. Immediately after cutting, the total biomass of the harvested sugarcane plants was quantified with scale.

4.2.2 Laboratory experiment

4.2.2.1 Experimental set-up

For the laboratory experiment, fresh soil was taken from the upper 10 cm of an unfertilised area in the field experiment. Treatments of this experiment corresponded with the treatments of the field experiment and were replicated four times. However, the urea treatment ('UA') was not included as the agronomic aspect of the field experiment did not apply to the laboratory experiment. Fresh spent poultry litter was acquired from the same business that supplied the poultry litter for the field experiment. The amendments used in the laboratory were sub-samples of the batches used in the field experiment. Properties of soil, poultry litter and compost for the laboratory experiment are shown in Table B2 (Appendix).

Of the field moist soil 668 g (equivalent to 508 g of dry soil) were weighed for each experimental unit. Poultry litter and pre-mixed poultry litter + amendment blends, respectively were then homogeneously incorporated. Soil in the control treatment was also mixed thoroughly to simulate the disturbance effect in the other treatments. Manure and soil amendments were applied at the same rates as in the field corresponding to application rates of 0.25 g N per kg dry soil for manure and 7.73 g per kg dry soil for amendments (dry basis) in the laboratory. Treated soil samples were placed into glass vessels with a surface area of 78.5 cm^2 and a height of 25 cm and carefully compacted by hand to reproduce the soil bulk density

of sugarcane rows the field (1.00 g cm^{-3}; Table 4.1). The filling height of soil was 10 cm to represent an incorporation depth comparable to the field experiment. Glass vessels were sealed air tight with a rubber ring and a fitted plastic lid. The soil WFPS was set at 60% (gravimetric soil moisture: 0.31 g_{water}/g_{soil}) and was maintained throughout the experiment by compensating evaporation with regular addition of deionised water shortly after gas sampling events. Ambient temperature was constant at 25 °C.

4.2.2.2 N_2O flux measurement

In the laboratory, N_2O was measured using an automated system described in detail by Redding et al. (2016). Briefly, a mass flow controller created a vacuum by which ambient air was moved first into a mixing drum to correct for potential deviations from mean gas background concentrations and then through the vessels that were connected to the drum. After passing through the vessels the gas was then drawn to an N_2O analyser (N_2O1A-23e-EP, Los Gatos, San Jose, CA, USA) and a CO_2 and CH_4 analyser (GGA-30r-EP, Los Gatos, San Jose, CA, USA). Both instruments logged concentrations of N_2O continually every 20 seconds. One sweep cycle lasted for 10 min and the flow rate was 3 L min^{-1}. After each gas sampling event the system was cleaned by sweeping ambient air through an empty vessel for 10 min. Headspace gas of each vessel was sampled every 10.5 hours. Between sampling events vessels were sealed by closing inlet and outlet valves. After two weeks of experiment running time N_2O emissions went back to background level and the experiment was terminated thereafter.

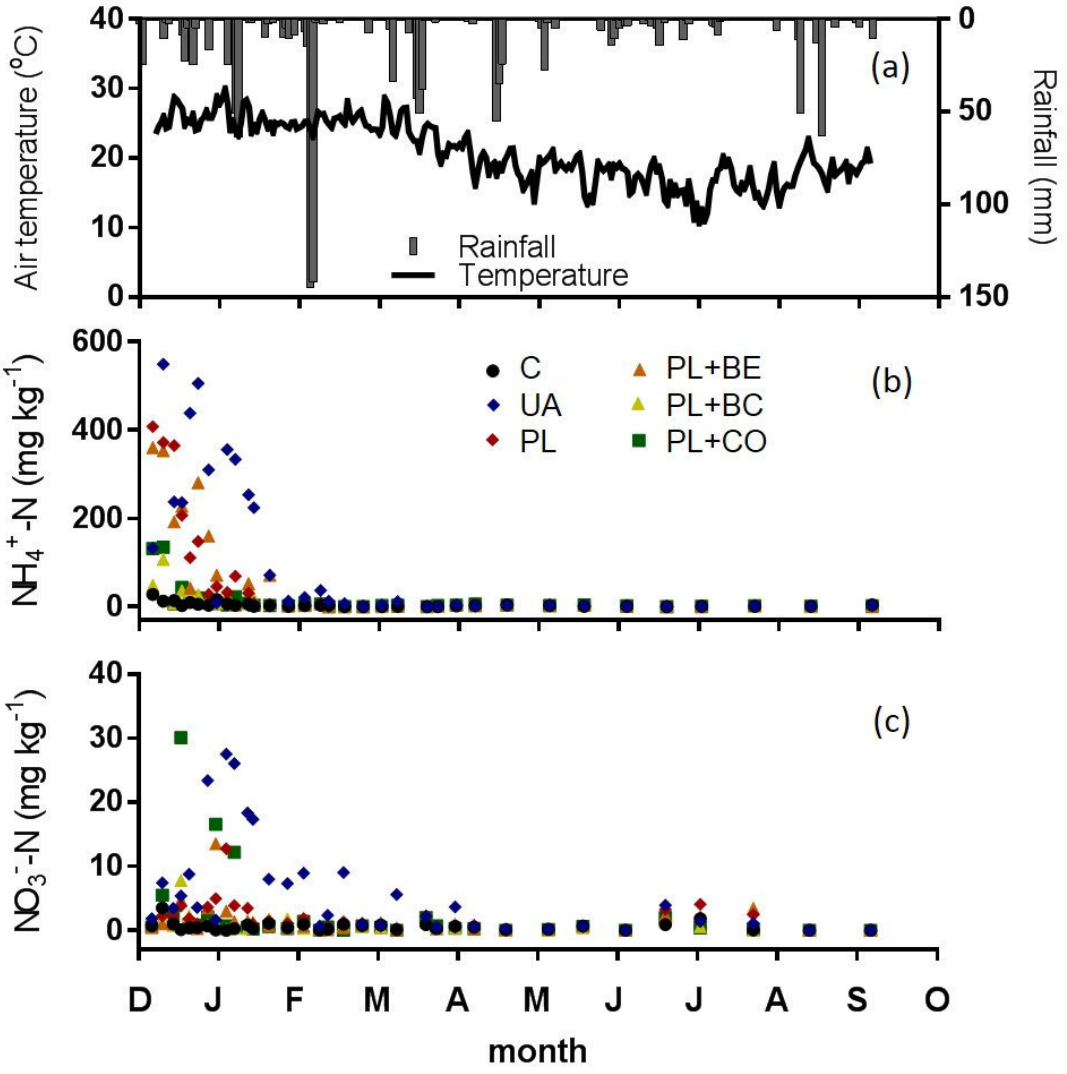
4.2.3 Data analyses

Statistical analyses were performed using R, version 3.3.1 (R Core Team, 2016). Differences between treatments were analysed using One-Way ANOVA and Fisher's LSD post-hoc test at $P < 0.05$ significance level. Correlation analyses and a standardized multiple regression analysis were performed for investigating the influence of soil variables on N_2O emissions. Data were log-transformed with natural logarithm in case residuals were not normally distributed. For details of the analyses see Appendix B.

4.3 Results

4.3.1 Environmental variables of the field experiment

Air temperature and rainfall varied during the time the field experiment was conducted (December 2014 to September 2015), following the typical climatic pattern of South East Queensland, Australia (Figure 4.1a). Recorded temperature and rainfall events were highest during summer from December to February and lowest during winter from June to July. Temperature measurements ranged between a minimum of 1.6 °C at night in winter and a maximum of 38.6 °C at day during summer. The total precipitation over the duration of the field experiment was 1292 mm.



▲ Figure 4.1. Environmental variables of the sugarcane field experiment measured from December 2014 to September 2015. (a) Daily mean air temperatures (°C) and daily rainfall (mm), (b) mean soil NH_4^+-N concentrations (mg kg^{-1}; n = 3) in sugarcane rows, (c) mean soil NO_3^--N concentrations (mg kg^{-1}; n = 3) in sugarcane rows. C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost. Error bars in sections b and c were omitted because of high variances of data within treatments. For statistical analyses of data see Table 4.3.

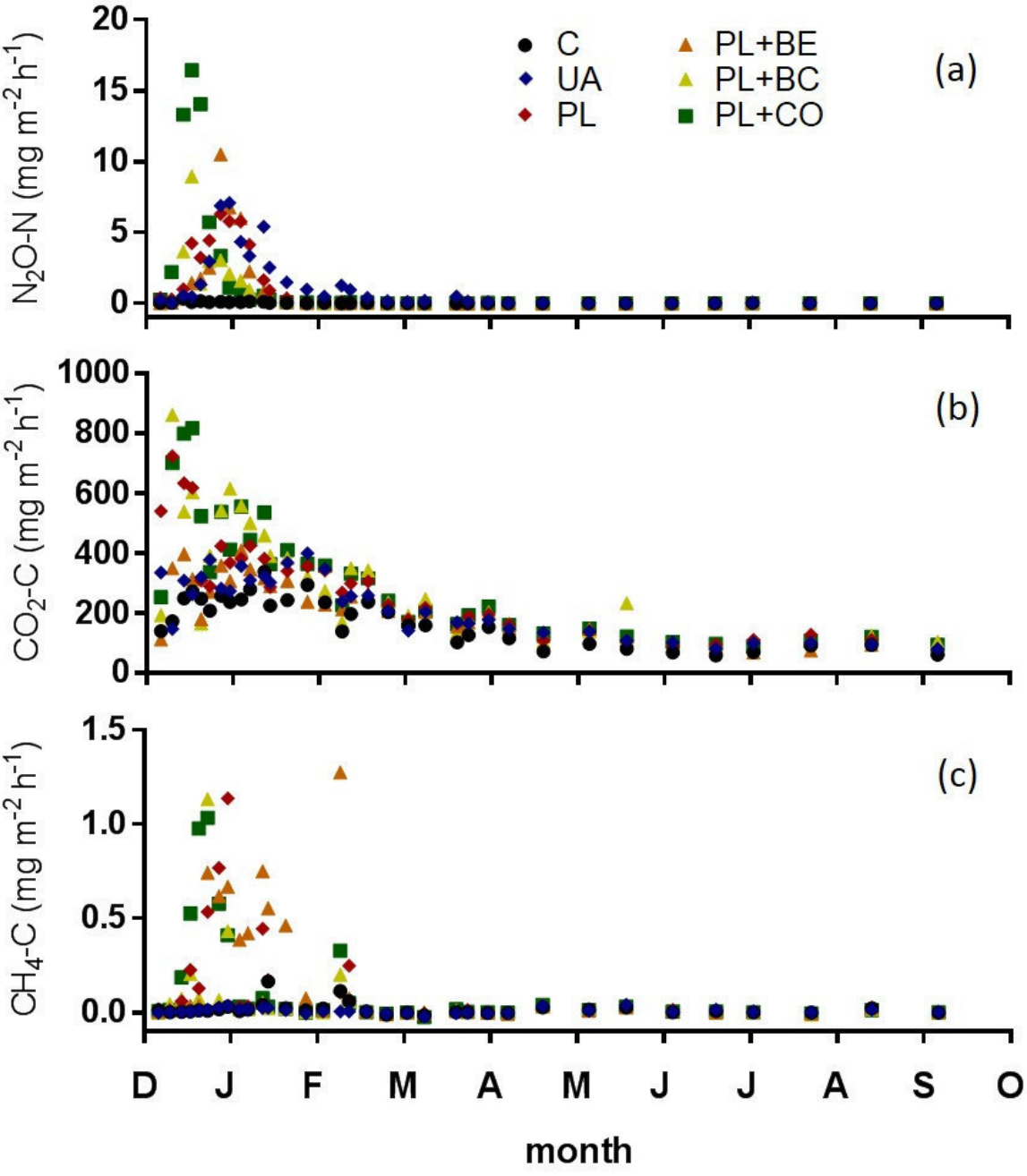
Extractable soil mineral N concentrations in inter-rows were negligible compared to rows throughout the experiment (Figure B1, Appendix). The highest soil NH_4^+-N concentration measured from sugarcane rows was 861 mg kg^{-1} (in the poultry litter treatment). In general, NH_4^+ concentrations peaked in the second and third week after starting the experiment and decreased within two months (Figure 4.1b). The highest detected soil NO_3^--N concentration was 47 mg kg^{-1} and was measured in urea treatment (Figure 4.1c). In general, NO_3^- concentrations peaked in the third and fourth week of the experiment. Concentrations of NO_3^- decreased within 45 days in all poultry litter treatments and within four months (130 days) in the urea treatment. Analysis of mean soil mineral N concentrations per treatment of sugarcane rows over the first 130 days showed that soil NH_4^+ concentrations were significantly highest in the urea treatment (Table 4.3). Compared to the poultry litter only treatment, soil NH_4^+ concentrations of the poultry litter + bentonite treatment were in the same range but were significantly lower when biochar or compost was applied. Mean soil NO_3^- concentrations were significantly highest in the urea treatment, followed by the poultry litter treatment (Table 4.3). Concentrations of soil NO_3^- from poultry litter application were significantly reduced by all types of amendment.

Table 4.3. Mean concentrations (± SD; n = 75) of soil NH_4^+ and NO_3^- (mg N kg^{-1}) per treatment of sugarcane rows of the first 130 days of the experiment. Statistical analyses: repeated measures one-way ANOVA with Fisher's LSD post-hoc test after log-transformation of data. Differences between treatments at $P < 0.05$ significance level are indicated with different letters within columns.

Treatment	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
Control	4.98 ± 7.78^{d}	0.62 ± 0.83^{c}
Urea	150.7 ± 216.5^{a}	7.80 ± 10.10^{a}
Poultry litter	74.0 ± 152.8^{b}	2.15 ± 3.71^{b}
Poultry litter + bentonite	75.2 ± 151.8^{b}	1.63 ± 4.92^{c}
Poultry litter + biochar	14.1 ± 26.9^{c}	1.07 ± 2.59^{c}
Poultry litter + compost	17.4 ± 52.8^{c}	3.18 ± 8.86^{c}

4.3.2 Greenhouse gas fluxes in the field experiment

Fluxes of N_2O remained at background level (-0.01 to 0.23 mg N_2O-N m^{-2} h^{-1}) in inter-rows throughout the experiment (Figure B2a, Appendix). In contrast, N_2O-N fluxes between -0.02 and 27.05 mg m^{-2} h^{-1} were detected in sugarcane rows (both fluxes were measured in the poultry litter + compost treatment). In general, fluxes of N_2O were highest from the second to the fifth week of the experiment and went back to background level within two months in the poultry litter treatments and within four months in the urea treatment (Figure 4.2a).



▲ Figure 4.2. Greenhouse gas fluxes from sugarcane rows at the experimental field site from December 2014 to September 2015. (a) Mean N_2O-N fluxes (mg m^{-2} h^{-1}; n = 3), (b) mean CO_2-C fluxes (mg m^{-2} h^{-1}; n = 3), (c) mean CH_4-C fluxes (mg m^{-2} h^{-1}; n = 3). C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost. Error bars were omitted because of high variances of data within treatments. For statistical analyses see Table 4.4 and Table B1 in the Appendix.

Fluxes of CO_2 ranged from 27 mg CO_2-C m^{-2} h^{-1} (measured in control treatment) to 1232 mg CO_2-C m^{-2} h^{-1} (measured in poultry litter + compost treatment). Overall, the highest fluxes of CO_2 occurred in the first two months of the experiment (Figure 4.2b). During this time, elevated CO_2 emissions were detected in poultry litter, poultry litter + biochar, and poultry litter + compost treatments compared to control, urea, and poultry litter + bentonite treatments. Fluxes of CO_2 from inter-rows followed the same pattern as CO_2 fluxes from sugarcane rows with values ranging between 1.28 mg CO_2-C m^{-2} h^{-1} (measured in poultry litter + compost treatment) and 425 mg CO_2-C m^{-2} h^{-1} (measured in poultry litter treatment). No treatment specific variations in flux patterns were observed in inter-rows.

Fluxes of CH_4 were neutral around 0 mg CH_4-C m^{-2} h^{-1} in sugarcane rows as well as inter-rows throughout the experiment with slightly elevated values in the first three months in rows from treatments that received organic C input (poultry litter, poultry litter + bentonite, poultry litter + biochar, poultry litter + compost; Figure 4.2c). Methane fluxes of inter-rows were in the range of -0.03 mg CH_4-C m^{-2} h^{-1} (measured in poultry litter + compost treatment) to 0.63 mg CH_4-C m^{-2} h^{-1} (measured in poultry litter treatment) and CH_4 fluxes of rows were in the range of -0.04 mg CH_4-C m^{-2} h^{-1} (measured in control treatment) to 3.69 mg CH_4-C m^{-2} h^{-1} (measured in poultry litter + bentonite treatment).

4.3.3 Cumulative greenhouse gases in the field experiment

Cumulative N_2O-N emissions were 0.84 kg ha^{-1} from the control treatment, 13.67 kg ha^{-1} from the urea treatment, 11.67 kg ha^{-1} from the poultry litter treatment, 9.74 kg ha^{-1} from the poultry litter + bentonite treatment, 7.48 kg ha^{-1} from the poultry litter + biochar treatment, and 15.65 kg ha^{-1} from the poultry litter + compost treatment (Figure 4.3, Table 4.4). Total N_2O emissions were significantly lower from the control treatment than other treatments, but there were no significant differences between the treatments receiving N as urea or poultry litter.

Table 4.4. Nitrogen application rates, total N_2O emissions, N_2O emissions factors and total greenhouse gas (N_2O, CO_2 and CH_4) emissions expressed in CO_2-equivalents.Values of gas emissions are given in mean ± SD. Calculation of CO_2-equivalents was done based on a 100 year timescale according to Myhre et al. (2013) with GWP for N_2O given as 298 and for CH_4 given as 34. Statistical analyses (one-way ANOVA with Fisher's LSD post-hoc test) were carried out after log-transformation of data. Differences between treatments at $P < 0.05$ significance level are indicated with different letters.

Treatment	N applied through fertiliser (kg ha^{-1})	N applied through amendment (kg ha^{-1})	Total N applied (kg ha^{-1})	Total N_2O-N emissions (kg ha^{-1})	Emission factor (%)	CO_2-equivalents of cum. N_2O, CO_2 and CH_4 emissions (t ha^{-1})
Control	N/A	N/A	0	0.84 ± 0.30^{b}	N/A	19.92 ± 0.43^{b}
Urea	160	N/A	160	13.67 ± 6.82^{a}	8.02 ± 4.26	30.10 ± 7.24^{a}
Poultry litter	160	N/A	160	11.67 ± 6.75^{a}	6.77 ± 4.22	33.23 ± 5.90^{a}
Poultry litter + bentonite	160	1.83	161.83	9.74 ± 5.97^{a}	5.50 ± 3.69	29.10 ± 4.42^{a}
Poultry litter + biochar	160	37.60	197.60	7.48 ± 3.73^{a}	3.36 ± 1.89	29.35 ± 6.14^{a}
Poultry litter + compost	160	59.58	219.58	15.65 ± 10.21^{a}	6.75 ± 4.65	33.69 ± 6.47^{a}

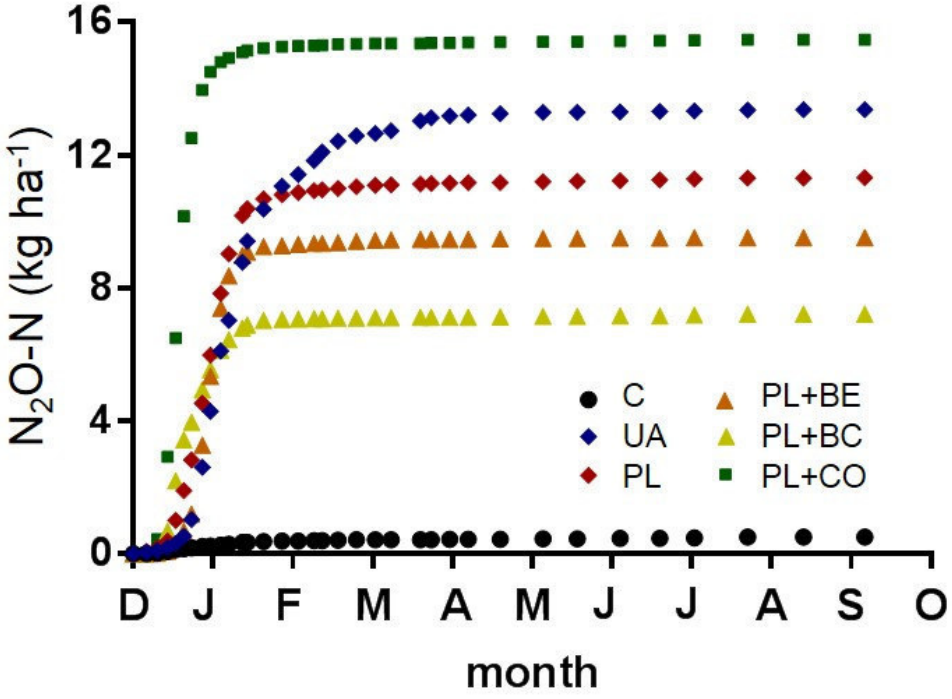


Figure 4.3. Mean cumulative N_2O fluxes (kg ha^{-1}; n = 3) at the experimental field site from December 2014 to September 2015. C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost. LSD at $P < 0.05$ is 59.5.

Based on the calculation of emission factors that expresses N_2O emissions in relation to N application rates (IPCC, 2006), N_2O emissions were highest in the urea treatment (EF = 8.02%), followed by the poultry litter treatment (EF = 6.77%) and the poultry litter + compost treatment (EF = 6.75%; Table 4.4). Bentonite and biochar addition reduced the EF from poultry litter application to 5.50% and 3.36%, respectively.

Treatments that received N application did not differ significantly from each other in terms of their CO_2-equivalents but were significantly higher compared to the control treatment (19.92 t ha^{-1}; Table 4.4). The CO_2-equivalents of treatments that received N input were in the order: poultry litter + compost (33.69 t ha^{-1}) > poultry litter only (33.23 t ha^{-1}) > urea only (30.10 t ha^{-1}) > poultry litter + biochar (29.35 t ha^{-1}) > poultry litter + bentonite (29.10 t ha^{-1}).

4.3.4 Cumulative greenhouse gases in the laboratory experiment

The laboratory experiment showed similar trends in cumulative N_2O emissions as the field experiment (Figure 4.4; Table 4.5). Similarly to the field experiment, total N_2O emissions from the laboratory incubation were significantly lowest in the control treatment (<0.01 mg N_2O-N kg^{-1}). In the poultry litter treatment, cumulative N_2O emissions reached 0.40 mg N_2O-N kg^{-1} at the end of the experiment. Addition of bentonite to poultry litter resulted in an 8% increase in total N_2O emission (0.43 mg N_2O-N kg^{-1}), addition of biochar to poultry litter resulted in an 18% decrease of total N_2O emission (0.33 mg N_2O-N kg^{-1}), but differences were not significant. The addition of compost to poultry litter showed an almost threefold and significant increase in cumulative N_2O emission compared to poultry litter only application (1.16 mg N_2O-N kg^{-1}). Cumulative CO_2 emissions of treatments that received poultry litter were significantly higher than the control treatment, but did not differ significantly among each other (Table B3, Appendix). Cumulative CH_4 emissions of treatments that received poultry litter also showed no significant differences, but were significantly higher than from the control treatment (Table B3, Appendix).

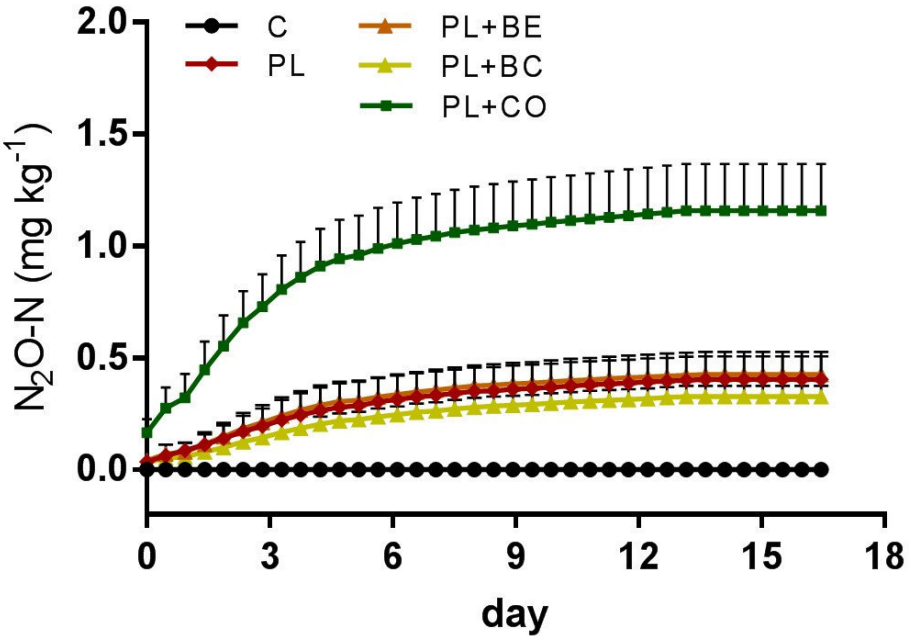


Figure 4.4. Mean cumulative N_2O fluxes (± SD; n = 4) of the laboratory experiment per kg dry soil. C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost.

Table 4.5. Cumulative N_2O fluxes (mean ± SD; n = 4) of the laboratory experiment per kg dry soil. Statistical analyses: one-way ANOVA with Fisher's LSD post-hoc test. Differences between treatments at $P < 0.05$ significance level are marked by different letters.

Treatment	cumulative N_2O-N emissions (mg kg^{-1})
Control	$<0.01 \pm 0.00^{c}$
Poultry litter	0.40 ± 0.10^{b}
Poultry litter + bentonite	0.43 ± 0.10^{b}
Poultry litter + biochar	0.33 ± 0.05^{b}
Poultry litter + compost	1.16 ± 0.21^{a}

4.3.5 Sugarcane biomass

Sugarcane biomass at the end of the field experiment was lowest in the control treatment and significantly higher in the urea, poultry litter, poultry litter + bentonite and poultry litter + compost treatments with no significant differences among these (Figure 4.5). The poultry litter + biochar treatment did neither differ significantly from the remaining treatments nor from the control.

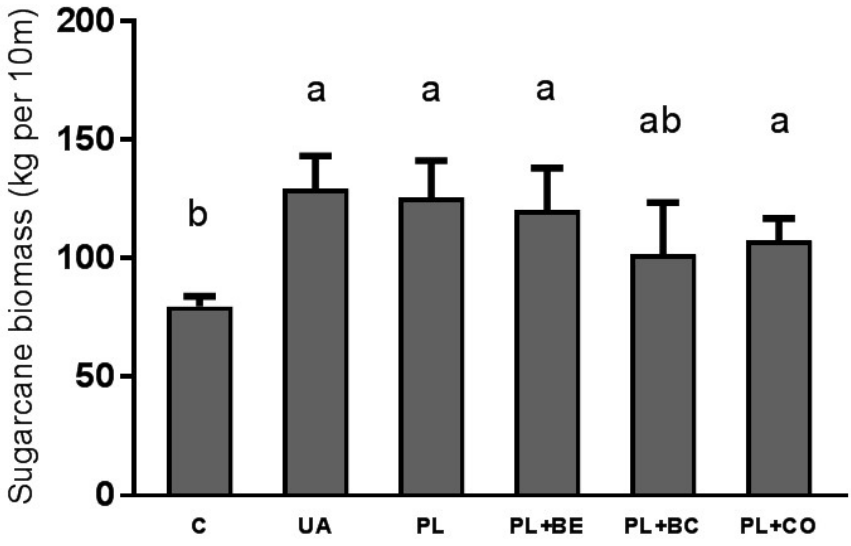


Figure 4.5. Sugarcane biomass per treatment (n = 3) recorded on the final day of the field experiment on 23 September 2015. C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost. Performed statistical analyses: one-way ANOVA plus Fisher‘s LSD post-hoc test. Different letters indicate treatment differences at $P < 0.05$ level.

4.3.6 Effects of soil properties on soil N_2O fluxes

The relationships between soil N_2O flux and soil physical, chemical, and biological variables were analysed using data from the field experiment, but only from sugarcane rows as only rows received applications of fertiliser and amendments. Fluxes of N_2O-N were most strongly correlated with soil temperature ($r = 0.619$, $P < 0.001$), soil NH_4^+-N concentrations ($r = 0.540$, $P < 0.001$), and soil NO_3^--N concentrations ($r = 0.436$, $P < 0.001$; Table 4.6). Correlation between soil N_2O-N flux and microbial biomass N was weak but significant ($r = 0.148$, $P < 0.01$). No correlation was detected between soil N_2O-N flux and gravimetric soil moisture ($r = 0.068$, $P > 0.05$). A standardised multiple linear regression analysis of soil N_2O-N flux on soil temperature, soil NH_4^+-N and soil NO_3^--N confirmed that soil temperature was the strongest predictor of N_2O-N flux ($\beta = 0.526$, $P < 0.001$), followed by soil NH_4^+-N ($\beta = 0.237$, $P < 0.001$), and soil NO_3^--N ($\beta = 0.219$, $P < 0.001$; Table 4.7).

Table 4.6. Pearson's product-moment correlation coefficients for log-transformed N_2O-N flux data versus soil physical, chemical and biological variables. Results refer to data from sugarcane rows of the field experiment. Significance is indicated at levels of $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Variable	*r*	*n*
Soil temperature	0.619***	575
Gravimetric soil moisture	0.068	575
log NH_4^+-N	0.540***	575
log NO_3^--N	0.436***	575
log Microbial biomass N	0.148**	462

Table 4.7. Standardised regression coefficients of log-transformed N_2O-N flux data on the three strongest predictor variables of the correlation analysis shown in Table 4.6. Results refer to data from sugarcane rows of the field experiment. $R^2 = 0.526$, $n = 576$. Stars indicate significance at $P < 0.001$ level.

Variable	β
Soil temperature	0.462***
log NH_4^+-N	0.265***
log NO_3^--N	0.242***

Correlation analyses of N_2O-N flux with soil NH_4^+-N concentration subdivided by treatment showed that correlation was strongest for the poultry litter treatment ($r = 0.620$, $P < 0.001$) and the urea treatment ($r = 0.594$, $P < 0.001$; Table 4.8). Correlations for treatments that received amendments were reduced ($r = 0.465$ at $P < 0.001$ for poultry litter + bentonite, $r = 0.541$ at $P < 0.001$ for poultry litter + biochar, and $r = 0.493$ at $P < 0.001$ for poultry litter + compost). The lowest correlation between N_2O-N flux and soil NH_4^+-N was found in the control treatment ($r = 0.324$, $P < 0.01$). When subdividing the correlation analysis of N_2O-N flux with NO_3^--N by treatment, the highest correlation was found in the urea treatment ($r = 0.594$, $P < 0.001$; Table 4.8). The correlation was reduced in the poultry litter treatment ($r = 0.432$, $P < 0.001$) and the poultry litter + compost ($r = 0.480$, $P < 0.001$) treatments and was again lower in the poultry litter + bentonite ($r = 0.230$, $P < 0.01$) and the poultry litter + biochar ($r = 0.227$, $P < 0.05$) treatments. No significant correlation between N_2O-N flux and soil NO_3^--N concentration was detected in the control treatment ($r = 0.190$, $P > 0.05$).

Table 4.8. Pearson's product-moment correlation coefficient for log-transformed N_2O-N flux data versus soil NH_4^+-N and NO_3^--N concentrations from sugarcane rows subdivided by treatment. Significance is indicated at levels of $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Treatment	log NH_4^+-N		log NO_3^--N	
	r	*n*	*r*	*n*
Control	0.324**	91	0.190	91
Urea	0.594***	98	0.594***	98
Poultry litter	0.620***	99	0.432***	99
Poultry litter + bentonite	0.465***	92	0.230**	92
Poultry litter + biochar	0.541***	93	0.227*	93
Poultry litter + compost	0.493***	97	0.480***	97

4.4 Discussion

4.4.1 Cumulative greenhouse gas emissions

In the field experiment, the emission factors of treatments ranged between 3.36% (poultry litter + biochar) and 8.02% (urea). These values are substantially higher than the default EF of 1% of the IPCC (2006) for managed soils and the EF of 1.25% for sugarcane cropping in Australia

as given by the Department of Environment (2014). However, this range broadly overlaps with the N_2O emission factor of 3.87 ± 1.16% for sugarcane cropping calculated by Lisboa et al. (2011) and the N_2O emission factors from other sugarcane field studies in which synthetic N fertilisers were used as the main source of nitrogen and in which green cane harvesting was practiced (EF: 0.04% to 6.7%; Allen et al., 2010; Carmo et al., 2012; Soares et al., 2014; Soares et al., 2016; Wang et al., 2012; Wang et al., 2016). Only Denmead et al. (2010) measured much higher cumulative N_2O emissions (EF: 21%) from a sugarcane field experiment in which sugarcane was grown on an acid sulfate soil and was burnt before harvest. Contrary to this, cumulative N_2O losses from manure application in field experiments conducted in comparable climates (subtropical and tropical) are lower than in the study presented here. Dalal et al. (2010) applied feedlot manure (FLM) to a sorghum crop in the same climatic region this study was conducted in (South East Queensland) and found EF of N_2O from FLM application to be 0.61% (for 10 t ha^{-1}) and 0.38% (for 20 t ha^{-1}). However, annual rainfall in their study was much lower (~700 mm) compared to the study presented here (1292 mm). Khalil et al. (2002) reported cumulative N_2O emissions of 0.83% per total N applied (400 kg N ha^{-1}) from chicken manure application in Malaysia (annual rainfall: 2293 mm), but the manure was applied in combination with inorganic N in this study.

While biochar and compost showed the same trends in cumulative N_2O emissions in the field and the laboratory experiment, bentonite generated contrasting results. Bentonite addition to poultry litter decreased N_2O emissions by 16% in the field but increased emissions by 8% in the laboratory experiment (effects were not significant). This difference may be explained by the high swelling capacity of bentonite when in contact with water. The effect of decreased N_2O emissions observed in the field may have been overridden in the laboratory by constant swelling of bentonite (soil moisture was constant at 60%), possibly leading to a decrease in gas exchange and thus to oxygen deficiency in soil microsites and consequently to an increase in denitrification (Firestone and Davidson, 1989; Granli and Bøckman, 1994).

Another noticeable difference between the field and the laboratory experiment is that the application of compost to poultry litter resulted in an increase of 34% of N_2O emissions in the field and in an increase of 286% of N_2O emissions in the laboratory compared to poultry litter only. The reason for this strong difference in N_2O emissions between field and laboratory experiment cannot be determined unambiguously but it is assumed that the constant WFPS of 60% in the laboratory allowed nitrification and denitrification to happen simultaneously. Poultry litter contained substantial NH_4^+ while compost contained relatively large amounts of

NO_3^-. This combination together with the constant WFPS of 60% might have led to the almost threefold increase in N_2O emissions in the laboratory when compost was added to poultry litter.

Carbon dioxide equivalents of the greenhouse gases quantified here (N_2O, CO_2, CH_4) are one to two orders of magnitude higher than CO_2-equivalents reported from other sugarcane field experiments (Carmo et al., 2012; Denmead et al., 2010). Carbon dioxide equivalents of combined N_2O, CO_2, and CH_4 fluxes from manure application to soil in non-flooded fields in tropical or subtropical climates are not available. The comparatively high CO_2-equivalents measured here are likely due to the timing of N application at the beginning of the wet summer season. Coinciding N and C application as well as warm temperatures and high rainfall during the initial two months of the experiment seem to have provided ideal conditions for high microbial activity and thus high N_2O and CO_2 losses from the soil.

4.4.2 Influence of soil physical variables on N_2O fluxes in the field

In the field experiment, the strongest predictor of N_2O emissions as identified by the standardised multiple linear regression analysis was soil temperature. Application of N, as mentioned above, coincided with the start of summer and N_2O emissions are known to increase with increasing temperatures (Granli and Bøckman, 1994). As production of N_2O in soils mainly occurs through the biological processes of nitrification and denitrification (Granli and Bøckman, 1994), higher temperatures may have increased microbial activity. This theory is supported by measurements of soil respiration (CO_2 emission) which was highest during summer months (December to February).

No significant correlation between soil N_2O flux and gravimetric soil moisture was detected. There are many studies that show the dependency of N_2O emissions on soil moisture (e.g. Linn and Doran, 1984; Redding et al., 2016; Schaufler et al., 2010), therefore it can be concluded that, at least during the first four month of the field experiment, when mineral N was abundant, soil moisture was not a limiting factor for N_2O production. In combination with high temperatures at the start of the experiment, this circumstance likely contributed to the high N_2O losses measured in the field.

4.4.3 Soil mineral N and effects on N_2O fluxes and sugarcane growth

In the field, soil NH_4^+ and NO_3^- concentrations were significantly greatest in the urea treatment and concentrations of NO_3^- remained elevated for a longer time period in this treatment compared to the treatments that received poultry litter as N input. These circumstances were probably the cause for the highest cumulative N_2O emissions measured from the urea treatment in terms of emission factor, although the differences to the other treatments were not significant. Nitrification as well as denitrification seem to have been the sources of N_2O emission in the urea treatment. This suggestion is supported by results from correlation analyses (Table 4.8). While in the urea treatment N_2O flux was equally well correlated to soil NH_4^+ and NO_3^- concentrations, soil N_2O flux was better correlated to soil NH_4^+ concentrations than soil NO_3^- concentrations in the poultry litter only treatment. These results imply that nitrification may have played a stronger role in N_2O emissions in the poultry litter only treatment, but the experimental design of this study does not allow a validation of this presumption. Nitrification as well as denitrification have been proposed as main pathways of N_2O production from poultry based manures applied to soil (Akiyama et al., 2004; Khalil et al., 2002; Khalil et al., 2002b; Velthof et al., 2003). Significantly lower NH_4^+ and NO_3^- concentrations in the poultry litter only compared to the urea treatment did not result in significant differences in sugarcane biomass, indicating that soil mineral N concentrations in the poultry litter only treatment did not limit sugarcane growth.

The addition of bentonite to poultry litter did not reduce soil NH_4^+ concentrations. This result, together with the sugarcane biomass that was not significantly decreased by the addition of bentonite, indicates that NH_4^+ ions were not fixed between the layers of bentonite but were dissolved in soil solution and/ or exchangeably bound. In contrast, the mean soil NO_3^- concentration was significantly decreased by the addition of bentonite. This result indicates that the addition of bentonite might have reduced the turnover rate of NH_4^+ to NO_3^- through the process of nitrification, which could be the cause of the observed decrease in N_2O emissions of 16% in the field. However, this decrease in N_2O emissions was not significant. The CEC of bentonite was high enough to bind all NH_4^+ ions in the poultry litter, however competition with other cations (e.g. Ca^{2+}, Mg^{2+}, K^+, Na^+) in the manure and the soil for exchange sites might have occurred as discussed by Pratt et al. (2016). The authors of this study effectively decreased N_2O emissions from pre-incubated blends of poultry litter with bentonite, applied to soil in a laboratory experiment. The authors tested four increasing rates of bentonite and found strong decreases of N_2O emission at the highest and second highest application rates (1: 3.02 and 1:

1.21 manure: bentonite dry mass ratio, respectively). In the study presented here, the manure: bentonite dry mass ratio was 1: 0.95. Therefore, although the application rate of bentonite was high enough to significantly decrease NO_3^- concentrations from poultry litter application, the rate might not have been high enough for achieving significant decreases in N_2O emissions. As indicated above, it is likely that bentonite changed soil physical properties and thus influenced soil N turnover dynamics. Bentonite swells strongly when in contact with water and shrinks when moisture decreases which results in the formation of cracks. Alternating soil moisture conditions in the field might have created small cracks in the soil and therefore improved aeration. Consequently, the observed non-significant decrease in N_2O emissions in the field was either due to a chemical effect (i.e. cation exchange) or a physical effect (i.e. improvement in gas permeability), or a combination of both. An increase in NH_3 volatilisation with bentonite as the result of an increased pH, and therefore reduced substrate availability for nitrifiers, was probably negligible because the pH of bentonite was close to the pH of poultry litter (bentonite: 8.85; poultry litter: 8.39). Furthermore, Redding (2013) showed that NH_3 volatilisation from poultry litter can be decreased when the manure is blended with bentonite.

In the field, mean soil NH_4^+ and NO_3^- concentrations were significantly reduced when biochar was added to poultry litter and N_2O emissions were decreased by 36% in the field and by 18% in the laboratory. These results imply that binding of NH_4^+ ions via at least one mechanism other than cation exchange was occurring and that consequently turnover rates of NH_4^+ to NO_3^- might have been reduced. The individual contributions of potential underlying mechanisms can't be clearly identified with the experimental design of this study. The biochar used here had a C/N ratio of 34.43 which might have caused N immobilisation. Similar to crop residues (e.g. Muhammad et al.. 2011), biochars with C/N ratios >30 have been found to decrease soil N_2O emissions (Cayulea et al., 2014). Thus, it is possible that N immobilisation was the underlying cause for the decreased NH_4^+ and NO_3^- concentrations and the decreased N_2O emissions measured here, but the relevance of this mechanism has been questioned (Cayuela et al., 2014). It is also possible that some of the available NH_4^+ ions were entrapped in the pores of the biochar, potentially making them unavailable for nitrifiers (Clough et al., 2013). Reduced substrate availability for nitrifiers because of increased N loss through NH_3 volatilisation probably did not play an important role as the pH of the biochar was similar to the pH of the poultry litter (biochar: 8.78; poultry litter: 8.39). Another mechanism that could explain the reduced amount of reactive N in soil when biochar was present, might have been the binding of NH_4^+ ions onto biochar through interaction with functional groups on the biochar's surface as discussed for adsorption of NH_3 (Spokas et al., 2012; Taghizadeh-Toosi et al., 2012). The

biochar used here had a very low density and therefore could have improved soil aeration and consequently decreased N_2O emissions. However, this mechanism seems to have played a minor role in this study as reduced soil mineral N concentrations seem to be the main cause for the decreased N_2O emissions observed here. Sugarcane predominantly uses NH_4^+ for N acquisition (Robinson et al., 2011) and reduced soil NH_4^+ concentrations likely resulted in reduced sugarcane growth.

The addition of compost significantly decreased the mean soil NH_4^+ concentration from poultry litter application but highest N_2O emissions have been measured in the field and in the laboratory from this treatment. The introduction of labile C to soil can cause NH_4^+ immobilisation (Recous et al., 1990) which could be the reason for the observed decrease in soil NH_4^+ concentrations. The compost used in this study contained substantial and variable amounts of nitrate which is a substrate for denitrification (Granli and Bøckman, 1994) and therefore this mechanism could be the cause for the comparatively high and variable N_2O emissions. However, this theory is inconsistent with the C/N ratio of the compost which was 14.87 and thus is generally associated with N mineralisation (Dalal et al., 2010; Janssen, 1996). Consequently, it is also possible that heterotrophic nitrification was enhanced by the application of organic C with the compost (Wrage et al., 2001) and that therefore N_2O production from this pathway was strongly promoted.

4.4.4. Relationship between soil microbes and N_2O flux

Across treatments, microbial biomass N showed a low but significant correlation with N_2O flux ($r = 0.052$). This result suggests that microbial biomass as a whole is not a good indicator for predicting N_2O fluxes. Only a fraction of all microbial species in the soil produces N_2O and therefore microbes would need to be separated into categories for the identification of biological predictors of N_2O emissions.

4.4.5 Conclusion

Compared to urea, poultry litter as an N source was associated with a trend of lower cumulative N_2O emissions. Moreover, blending poultry litter with bentonite or biochar further decreased N_2O emissions in the field and field plus laboratory, respectively. Therefore, poultry litter, and

especially poultry litter blended with biochar looks favourable over urea. In contrast, when considering the CO_2-equivalents of all treatments, urea appears favourable over poultry litter. However, the higher CO_2-equivalent of poultry litter application compared to urea application was offset by blending the manure with bentonite or biochar. Poultry litter application resulted in significantly lower soil mineral N concentrations compared to urea application without decreasing sugarcane growth. Therefore, fertilisation with poultry litter is favourable over fertilisation with urea in terms of diminishing soil N loss through leaching. When biochar was added to the poultry litter, soil mineral N concentrations seemed insufficient for full sugarcane growth. Thus, taking greenhouse gas emissions, soil mineral N concentrations and agronomic effects into account, amending poultry litter with bentonite seems to be the best choice for sugarcane cropping. However, it needs to be pointed out that differences in N_2O emissions and CO_2-equivalents were not significant among treatments in the field experiment due to high spatial variability and that therefore results presented here should be treated with caution.

Nitrous oxide emissions measured in the laboratory showed similar trends to the N_2O emissions measured in the field but the ratios of N_2O emissions varied between laboratory and field. Thus, it can be concluded that testing soil amendments for their effect on N_2O fluxes from soil in the laboratory is a useful step for testing treatment effects. However, climatic conditions in the field are far more complex and thus field experiments are needed to quantitatively assess the environmental impact of soil amendments.

This study highlights the challenge of modern agriculture to simultaneously maintain high crop yields and decrease the concomitant environmental damages of crop production. Here, emissions of the greenhouse gases N_2O, CO_2 and CH_4 as well as soil mineral N contents have been taken into account for assessing the environmental footprint of treatments. However, a whole life cycle analysis of the materials used here (urea, poultry litter, bentonite, biochar and green waste compost) is needed for calculating their total environmental impact.

This study demonstrates that the default N_2O emission factors of 1% for N additions to soil and 1.25% for sugarcane cropping as given by the IPCC (2006) and the Department of Environment (2014), respectively need to be adjusted in order to correct modelling of future climatic scenarios.

Chapter 5 – Influence of bentonite and biochar on soil N and N_2O fluxes from a sugarcane soil fertilised with urea or poultry litter

5.1 Introduction

Nitrous oxide is a naturally occurring molecule in the Earth's atmosphere that is classed as a greenhouse gas due to its thermal forcing effect. Currently, concentrations of N_2O are rising by about 0.3% per year (U.S. Department of Commerce, 2017) which contributes to global warming and causes decomposition of stratospheric ozone (Denman et al., 2007; Ravishankara et al., 2009). One of the main reasons for the increase in atmospheric N_2O is the application of nitrogenous fertilisers and animal manures to soils for the purpose of crop production (Mosier et al., 1998; Syakila and Kroeze, 2011). In soils, the majority of N_2O originates from the biological processes of nitrification and ensuing denitrification (Granli and Bøckman, 1994). In order to reduce losses of reactive N from soil, including N_2O emissions, nitrification inhibitors are commonly used. However, concerns about the use of these chemical products have arisen after traces of the nitrification inhibitor dicyandiamide (DCD) have been detected in milk (Shen et al., 2013). A potential alternative to nitrification inhibitors are soil amendments such as geological materials or biochar. The application of geological materials to soil can decrease N_2O emissions with the effect being predominantly based on binding of NH_4^+ ions, probably through CEC (Zaman et al., 2007; Pratt et al., 2016). Decreases in N_2O emissions from soil can also be achieved through the application of biochar (Cayuela et al., 2014). Comparable to geological materials, biochar can reduce NH_4^+ concentrations in the solution-phase of soil, presumably through CEC (Ding et al., 2010; Dempster et al., 2012). Thus, it is possible that the observed N_2O decreasing effect of biochar is due to exchangeable binding of soil NH_4^+, but whether this mechanism actually plays an important role is unknown.

In this study, the underlying causes of the N_2O decreasing effects of bentonite and biochar observed in Chapter 4 and in the literature were investigated. Soil fertilised with either urea or poultry litter and amended with increasing rates of bentonite or biochar was incubated in microcosm systems under aerobic conditions. Measurements of N_2O fluxes and soil N dynamics were carried out over four weeks. Soil N dynamics were studied by microdialysis, a minimally invasive technique that had first been used to study soil N by Inselsbacher et al. (2011). With this technique, diffusive fluxes of soil N compounds can be quantified based on the amount of particles that passively diffuse over the membrane surface of the probe per unit

of time, thus giving an indication of soil N turnover. It was hypothesised that N_2O emissions from N fertilised soil can be decreased by either amendment through exchangeable binding of NH_4^+ ions due to the amendments' high CEC. Accordingly, it was further hypothesised that diffusive fluxes of NH_4^+ ions in the solution-phase of the soil are gradually decreased with increasing application rates of amendments. Soil N analyses with microdialysis were compared to the conventional KCl extraction technique that is commonly used to quantify soluble N ions in soil. Furthermore, NO_2^- was analysed in this study with both analysis techniques because it has been revealed recently that CEC influences soil NO_2^- which is a precursor for N_2O emissions (Venterea et al., 2015).

5.2 Materials and methods

5.2.1 Collection of soil, poultry litter and amendments

The soil used in this study was taken from the field experiment in Chapter4. The experiment was located at the Sunshine Coast, Australia (26°34'S, 153°00'E) and the site had been used for commercials sugarcane cultivation for about 80 years. At the time of soil sampling, sugarcane plants were in the early growing stage of the 7th ratoon season. The soil at this site was a sandy light clay and has been classified as an Oxyaquic Hydrosol (Isbell, 2002). Soil was collected from the top 10 cm of sugarcane interrows in a part of the field that was not fertilised in the current and preceding cropping season. It was then homogenised and stored at 4°C for a few weeks. During storage, gravimetric soil moisture was around 0.35 g_{water}/ g_{soil}. Three subsamples were used to analyse the main physical and chemical soil properties (Table 5.1) prior to the experiments. Soil texture was determined following the hydrometer method of Gee and Or (2002). Soil pH and EC were analysed with a 1:5 soil to distilled water ratio (Rayment and Lyons, 2011). For determination of CEC the unbuffered salt extraction method was used (Sumner and Miller, 1996). Total soil C and N were analysed by combustions following methods 6B2 and 7A5, respectively of Rayment and Lyons (2011). Soil NH_4^+ and NO_3^- concentrations were determined by KCl extraction (1M, 1:2 soil:solution ratio) and subsequent colorimetric analyses (Kandeler and Gerber, 1988; Miranda et al., 2001).

Table 5.1. Main physical and chemical soil properties.

Characteristic	
Soil texture	
Sand (%)	50
Silt (%)	27.5
Clay (%)	22.5
pH	5.13 ± 0.06
EC (μS cm^{-1})	13.1 ± 3.75
CEC ($cmol_c$ kg^{-1})	36.8 ± 1.22
Total C (%)	2.77 ± 0.05
Total N (%)	0.36 ± 0.02
NH_4^+-N (mg kg^{-1})	5.14 ± 1.89
NO_3^--N (mg kg^{-1})	17.4 ± 1.63

Spent poultry litter was collected one week prior to experiment commencement from Mount Cotton, Brisbane from an industrial broiler shed in which 60,000 broilers were housed. Collection took place in an empty shed on the same day birds had been removed. The litter was a blend of bird faeces, feathers and eucalypt sawdust that was used as bedding material. It was stored in buckets covered with lids at 4°C in order to preserve chemical conditions (Pratt et al., 2014).

Table 5.2. Physical and chemical properties of poultry litter, bentonite and biochar.

Characteristic	Poultry litter	Bentonite	Biochar
Dry matter (%)	80.9	N/A	N/A
pH	8.26 ± 0.04	8.85 ± 0.01	7.99 ± 0.11
CEC ($cmol_c$ kg^{-1})	74.7 ± 7.19	76.8 ± 2.27	81.6 ± 1.86
Total C (%)	33.2 ± 1.19	0.66 ± 0.04	36.4 ± 1.10
Total N (%)	3.07 ± 0.07	0.04 ± 0.01	0.70 ± 0.03
C/N ratio	10.8	N/A	51.8
NH_4^+-N (mg kg^{-1})	3514 ± 72.0	7.98 ± 5.44	Not detected
NO_3^--N (mg kg^{-1})	92.1 ± 11.7	Not detected	6.71 ± 0.89

The bentonite used in this study was a sodium bentonite mined near Miles, Australia (AMCOL Australia, North Geelong, Australia). Feedstocks used for biochar production were sugarcane harvesting residues, green waste, shredded wood, and cardboard. Highest heating temperatures

of the pyrolysis ranged between 400 and 600 °C. Biochar was stored at room temperature in light-impermeable containers. The physical and chemical properties of poultry litter, bentonite and biochar were determined following the methods described above and are presented in Table 5.2.

5.2.2. Design of experiments

Two experiments were carried out in microcosm systems that are described in detail in Inselsbacher et al. (2009). In experiment 1 urea was the N source, in experiment 2 poultry litter was the N source. Each experiment consisted of eight treatments that included three increasing rates of bentonite and biochar. The treatments were (with % referring to percent of dry soil mass): (1) control (soil only), (2) soil + N source, (3) soil + N source + 1% bentonite, (4) soil + N source + 5% bentonite, (5) soil + N source + 10% bentonite, (6) soil + N source + 1% biochar, (7) soil + N source + 5% biochar, (8) soil + N source + 10% biochar. Each treatment was replicated four times and six identical experimental sets were constructed in order to allow for gradual destructive harvesting. Four days prior to each experiment, the soil was sieved with a 5.13 mm sieve and an equivalent of 30 g dry soil per microcosm was weighed and filled into the microcosm tubes. Soil WFPS was adjusted to 50% and microcosms were pre-incubated for acclimatisation to experiment conditions. Biochar was ground to a powder by hand with mortar and pestle in order to minimise differences in bulk density between treatments. One day prior to the start of each experiment bentonite and biochar subsamples were weighed, filled into sterile storage containers and mixed with the respective N source by manually shaking containers for 1 minute. Nitrogen application rates represented a fertilisation practice of 160 kg N ha^{-1} in the field and were equivalent to 0.31 g N per kg dry soil (microcosm surface area: 5.73 cm^2). On the day experiments commenced, pre-incubated soil samples together with an N source or an N source pre-mixed with either bentonite or biochar were placed into sterile containers and mixed thoroughly by hand to achieve a homogenous blend. Soil of the control treatment was also mixed to simulate the disturbance effect in the other treatments. Amended and unamended soil samples were filled into the respective microcosm tubes and were mildly compacted by carefully tapping microcosms on the laboratory bench. In both experiments, bulk densities ranged between 0.80 and 0.87 g cm^{-3} in treatments 1 to 6. Addition of biochar at medium (5%) and high (10%) rates decreased bulk densities because of biochar's high volume:weight ratio. Bulk densities varied between 0.74 to 0.78 g cm^{-3} and between 0.71 to

0.74 g cm^{-3} at 5 and 10% application rates, respectively. The WFPS was corrected to 50% by adding deionised water. Prior to the experiment the correct WFPS had been determined separately for every treatment. The expression of soil moisture as %WFPS was used in this study to account for the variation in bulk densities between treatments. Both experiments were run for 28 days. On days 2, 5, 7, 10, 14 and 28 gas sampling, soil N flux measurements with microdialysis, destructive harvesting for KCl extraction and pH measurements occurred. Experiment 2 was started 19 days after experiment 1 due to restricted availability of microdialysis equipment. Both experiments were fully randomised. Microcosm tubes were randomly assigned a position in the incubator (IM1000RG, Clayson Laboratory Apparatus Pty Ltd, Narangba, QLD, Australia) and were re-arranged randomly on a daily basis. The incubator was set at a constant temperature of 29°C and microcosms were incubated in the dark. Once a day water loss through evaporation was determined by weight and compensated for by addition of deionised water. Microcosms used for soil N sampling with the microdialysis technique were watered one hour before sampling commenced.

5.2.3 Soil N recovery and gas sampling

Soil N sampling by microdialysis and sampling of greenhouse gases occurred simultaneously. First, the microdialysis probes were inserted horizontally 1.5 cm below the soil surface through a hole in the microcosm tube and sealed air tight with adhesive rubber (Figure 5.1a). Then, the pump connected to the probes (CMA 4004 Syringe Pump; CMA Microdialysis AB, Kista, Sweden) was started and high-purity deionized water of 18 MΩ·cm (termed perfusate) was moved through the probes at a flow rate of 5 µl min^{-1} (Figure 5.1b). The outflow solution, termed dialysate was collected over 1 h in a refrigerated fraction collector (CMA 470; CMA Microdialysis AB, Kista, Sweden). Immediately after starting the pump, microcosms were air-sealed with modified 50 ml plastic centrifuge tubes. The bottom cones of these tubes were removed and rubber caps were fitted to the mouth (Figure 5.1c). Headspace volumes ranged between 70 and 73 ml in treatments 1 – 6. When biochar was applied at rates of 5% and 10%, headspace volumes ranged between 67 and 69 ml and between 65 and 67 ml, respectively. Sealing time was 1 h after which gas samples (20 ml) were transferred into pre-evacuated Exetainer vials by using a syringe (Labco Limited, Lampeter, UK). Analysis of N_2O, CO_2 and CH_4 was done by gas chromatography (GC-2010, Shimadzu, Kyoto, Japan).

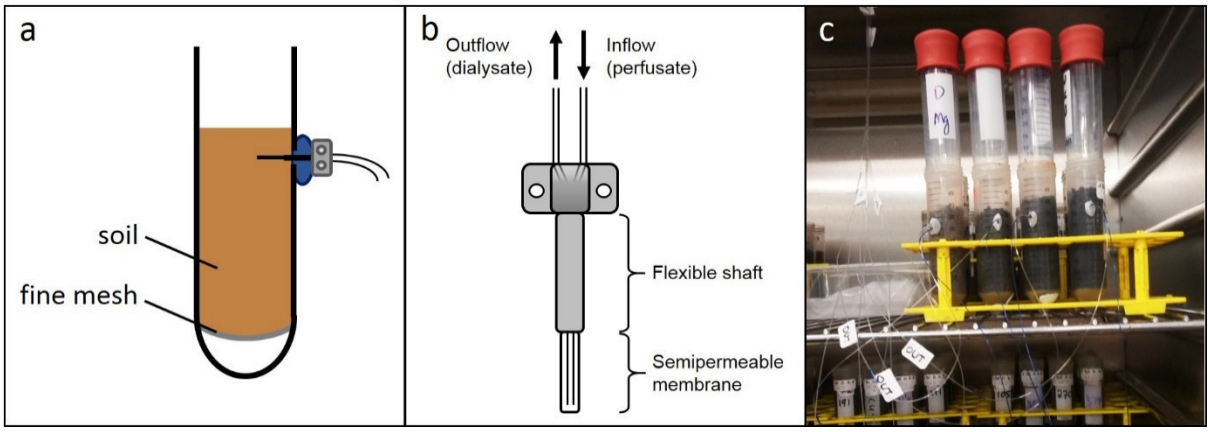


Figure 5.1. Soil N flux measurement with the microdialysis technique. (a) Schematic diagram of the microcosm system into which a microdialysis probe is inserted. Probes were inserted 1.5 cm below the soil surface through a hole in the microcosm tube and sealed air tight with adhesive rubber. (b) Detailed diagram of the microdialysis probe; image adapted from Inselsbacher et al. (2011). Surface area of the membrane is 15.9 mm^2. (c) Photograph of soil N sampling by microdialysis and concurrent gas sampling in the laboratory.

The KCl extraction procedure for analysis of soil NO_2^- and NO_3^- was done based on Stevens and Laughlin (1995). Ten grams of fresh soil were weighed and 20 ml of a 2 M KCl solution were added. Samples were shaken intensely by hand for 30 sec and subsequently placed onto an oscillating shaker for 5 min. Samples were then immediately centrifuged at 4000 rpm for 4 min, the supernatant was pipetted into 1.5 ml Eppendorf tubes and samples were refrigerated at 4°C. The pH of the 2 M KCl solution was adjusted to 13.1 with concentrated KOH pellets to ensure that during the extraction procedure the pH did not fall below 8.0 as acidity negatively affects recovery of NO_2^- (Stevens and Laughlin, 1995). For analysing soil NH_4^+ by KCl extraction 10 g of fresh soil were weighed and 20 ml of a 1 M KCl solution were added. Extraction was done by shaking soil-solution mixtures for 30 min on an oscillating shaker. Samples were centrifuged at 4000 rpm for 4 min and supernatants were frozen at -80°C until analysis.

5.2.4 Chemical analyses

Microdialysis dialysates were stored at 4°C until NO_2^- and NO_3^- analysis and frozen thereafter at -80°C until NH_4^+ analysis. Concentrations of soil NO_2^- and NO_3^- in dialysates and KCl extracts were determined following Miranda et al. (2001). The stability of NO_2^- in both types of samples was tested prior to the experiments by adding a 1 mM $NaNO_2$ standard solution to

treatments. The KCl extraction procedure was identical to the KCl extraction procedure for subsequent NO_2^- analysis described above. Nitrite was stable in refrigerated KCl extracts and non-pH-stabilised dialysate samples for at least 24 h as tested by a *t*-test with a significance level of $P \leq 0.05$. Concentrations of soil NH_4^+ in dialysates and KCl extracts were analysed as per Kandeler and Gerber (1988). Estimations of total soil N compounds sampled with microdialysis were done by calculating the rate of N compounds that diffused through the membrane surface within one hour as described by Inselsbacher and Näsholm (2012). The resulting unit is nmol cm^{-2} h^{-1}. Measurements of pH followed the procedure described above using 6 g of fresh soil and 30 ml of deionised water.

5.2.5 Greenhouse gas flux calculations

Spot checks for linearity of greenhouse gas accumulation in microcosm headspaces during one hour of sealing time revealed that accumulation declined over time. This was corrected for with the univariate quadratic function $f(x) = ax^2 + bx + c$. Calculation of cumulative greenhouse gas emissions was done by linear interpolation between sampling events. Total N lost as N_2O from the microcosm systems was calculated by the equation

(cumulative $N_2O\text{-}N_{treatment}$ – cumulative $N_2O\text{-}N_{control}$)/ total N application x 100

(Granli and Bøckman, 1995) and is expressed as percent of total N applied.

5.2.6 Data analyses

Statistical analyses were performed using R, version 3.3.1 (R Core Team, 2016). Cumulative N_2O emissions were analysed using One-Way ANOVA with Tukey's HSD post-hoc test at $P < 0.05$ significance level. Interaction and main effects of treatments were analysed by repeated measures ANOVA using the 'Linear Mixed-Effects Models' function (lme) in the 'Linear and Nonlinear Mixed Effects Models' (nlme) package (Pinheiro et al., 2016). Tukey's HSD post-hoc tests at $P < 0.05$ significance level were performed for the repeated measures ANOVAs using the 'General Linear Hypotheses' function (glht) in the 'multcomp' package (Hothorn et al., 2008). For correlation analyses, cumulative values of variables (as a measure of intensity) were calculated using linear interpolation between sampling events as described in Engel et al. (2010) and Venterea et al. (2015). Cumulative NH_4^+-N and NO_3^--N fluxes and concentrations were calculated using data measured on per day basis. Cumulative H^+ concentrations as a

measure of exposure to acidity during experiments were calculated using pH data measured on per day basis and by applying the formula $H^+ = 10^{-pH}$ (Venterea et al., 2015). Evaporation was measured as weight loss of microcosms in a 24 h time period. Weight loss was determined on three separate dates in each experiment and mean values were used for correlation analyses.

5.3 Results

5.3.1 Overview of daily data

In the controls treatments, levels of N_2O, NH_4^+ and NO_3^- were low compared to the other treatments throughout the incubation period, and pH values remained stable over time (5.2 ±0.3; Figure 5.2). Fluxes of NH_4^+ and NO_3^- measured with microdialysis showed stronger treatment differences than concentrations of NH_4^+ and NO_3^- in KCl extracts. Nitrous oxide fluxes reached higher levels with poultry litter (experiment 2) than with urea (experiment 1) although fluxes and concentrations of KCl extractable NH_4^+ and NO_3^- were generally lower when poultry litter was applied. Over the course of the experiments no NO_2^- was detected in any treatment.

5.3.1.1 N_2O fluxes

When urea was used as the N source, highest mean fluxes of N_2O were measured in the urea only treatment on day 7 (0.83 nmol g^{-1} h^{-1}; Figure 5.2a); with bentonite added at low concentration (1%) and biochar at medium concentration (5%), mean values also peaked on day 7 with values of 0.29 nmol g^{-1} h^{-1} and 0.37 nmol g^{-1} h^{-1}, respectively. Fluxes of N_2O in the remaining bentonite and biochar treatments remained at background level throughout the experiment.

The poultry litter treatments (experiment 2) showed largest N_2O fluxes on day 2; earlier N_2O fluxes were not recorded. Fluxes of N_2O declined quickly and reached background levels by day 14 (Figure 5.2g). Mean N_2O fluxes on day 2 with 1% or 5% bentonite added were 5.23 nmol g^{-1} h^{-1} and 4.28 nmol g^{-1} h^{-1}, respectively, exceeding the mean N_2O flux of the poultry litter only treatment (2.66 nmol g^{-1} h^{-1}). Peak emissions of the high bentonite (10%) treatment and all biochar treatments were below peak emissions of the poultry litter only treatment.

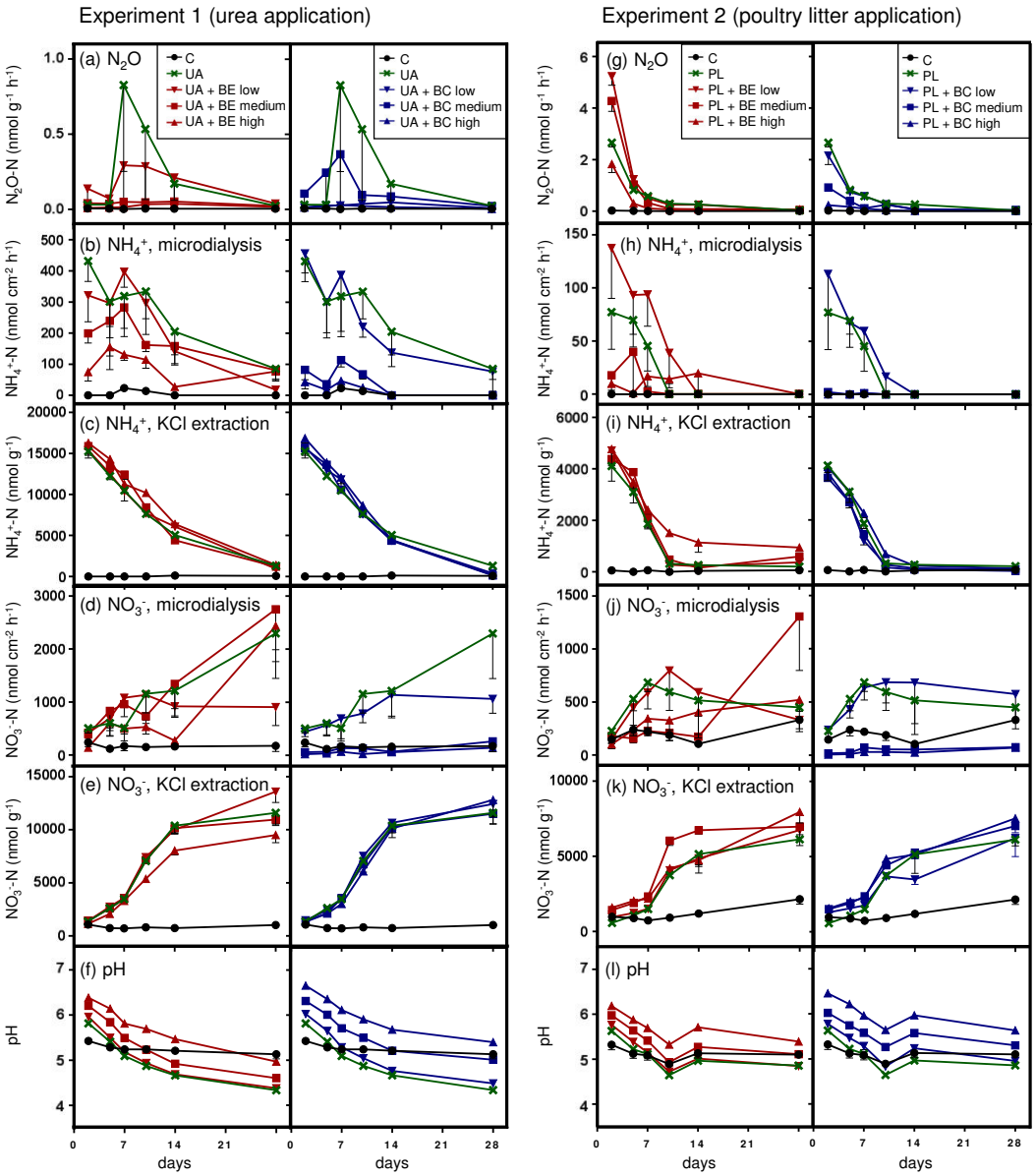


Figure 5.2. Overview of data of experiment 1 and 2 (mean plus SEM) collected on a daily basis. Fluxes of (a) and (g) N_2O-N, fluxes of (b) and (h) NH_4^+-N measured with microdialysis, concentrations of (c) and (i) NH_4^+-N of KCl extracts, fluxes of (d) and (j) NO_3^--N measured with microdialysis, concentrations of (e) and (k) NO_3^--N of KCl extracts, and measurements of (f) and (l) pH. C = control, UA = urea, PL = poultry litter, BE low = bentonite applied at 1% of dry soil mass, BE medium = bentonite applied at 5% of dry soil mass, BE high = bentonite applied at 10% of dry soil mass, BC low = biochar applied at 1% of dry soil mass, BC medium = biochar applied at 5% of dry soil mass, BC high = biochar applied at 10% of dry soil mass.

5.3.1.2 NH_4^+ and NO_3^- fluxes and concentrations

In both experiments, NH_4^+ fluxes decreased over the course of the experiment (Figure 5.2b, h). Addition of biochar at low application rate (1%) generally resulted in NH_4^+ fluxes similar to those of the urea only and poultry litter only treatments. Addition of bentonite at low application rate (1%) also resulted in similar NH_4^+ fluxes compared to the urea only treatment but compared to the poultry litter only treatment increased NH_4^+ fluxes were measured during the first 10 days (Figure 5.2h). Fluxes of NH_4^+ decreased with higher application rates of bentonite and biochar across both N sources, and these effects were most pronounced with biochar. Similar to NH_4^+ fluxes measured with microdialysis, NH_4^+ concentrations in KCl extracts decreased over time (Figure 5.2c, i), but treatment differences were less distinct.

Fluxes of NO_3^- increased over the duration of the experiment in the urea only treatment (Figure 5.2d) but in contrast peaked on day 7 in the poultry litter only treatment (Figure 5.2j). With poultry litter as the N source, fluxes of NO_3^- at low application rates (1%) of bentonite and biochar were similar to fluxes of the poultry litter only treatment over the course of the experiment. These effects were also observed when urea was the N source but only until day 14 after which fluxes remained stable. The application of medium (5%) and high (10%) rates of biochar reduced NO_3^- fluxes to background level when urea was the N source (Figure 5.2d) and to below background level when poultry litter was the N source (Figure 5.2j). Concentrations of NO_3^- in KCl extracts increased over time in both experiments but treatment differences were smaller compared to NO_3^- fluxes (Figure 5.2e, k).

5.3.1.3 Soil pH

The application of urea and poultry litter increased the soil pH and further increases were observed with increasing rates of bentonite and biochar (Figure 5.2f, l). However, the pH of all treatments decreased over the duration of the experiment, partly falling below the pH of the control treatment at the end of the experiment.

5.3.2 Cumulative N_2O emissions

With urea as N source, bentonite and biochar addition to the soil decreased N_2O emissions. The most pronounced effects were observed at the highest (10%) application rates (bentonite: -93%; biochar: -95%) but these effects were not statistically significant (Figure 5.3a, b; Table 5.3).

With poultry litter as N source, the addition of bentonite at a low application rate (1%) significantly increased N_2O emissions (Figure 5.3c; Table 5.3). Compared to poultry litter only, adding bentonite at 5% application rate elevated N_2O emissions slightly but not significantly, while 10% of bentonite resulted in a significant decrease of N_2O emissions. All biochar application rates led to a decrease of N_2O emissions compared to poultry litter only (Figure 5.3d; Table 5.3). Emissions of N_2O decreased with increasing rates of biochar and at 5% and 10% application rate differences to the poultry litter only treatment were significant.

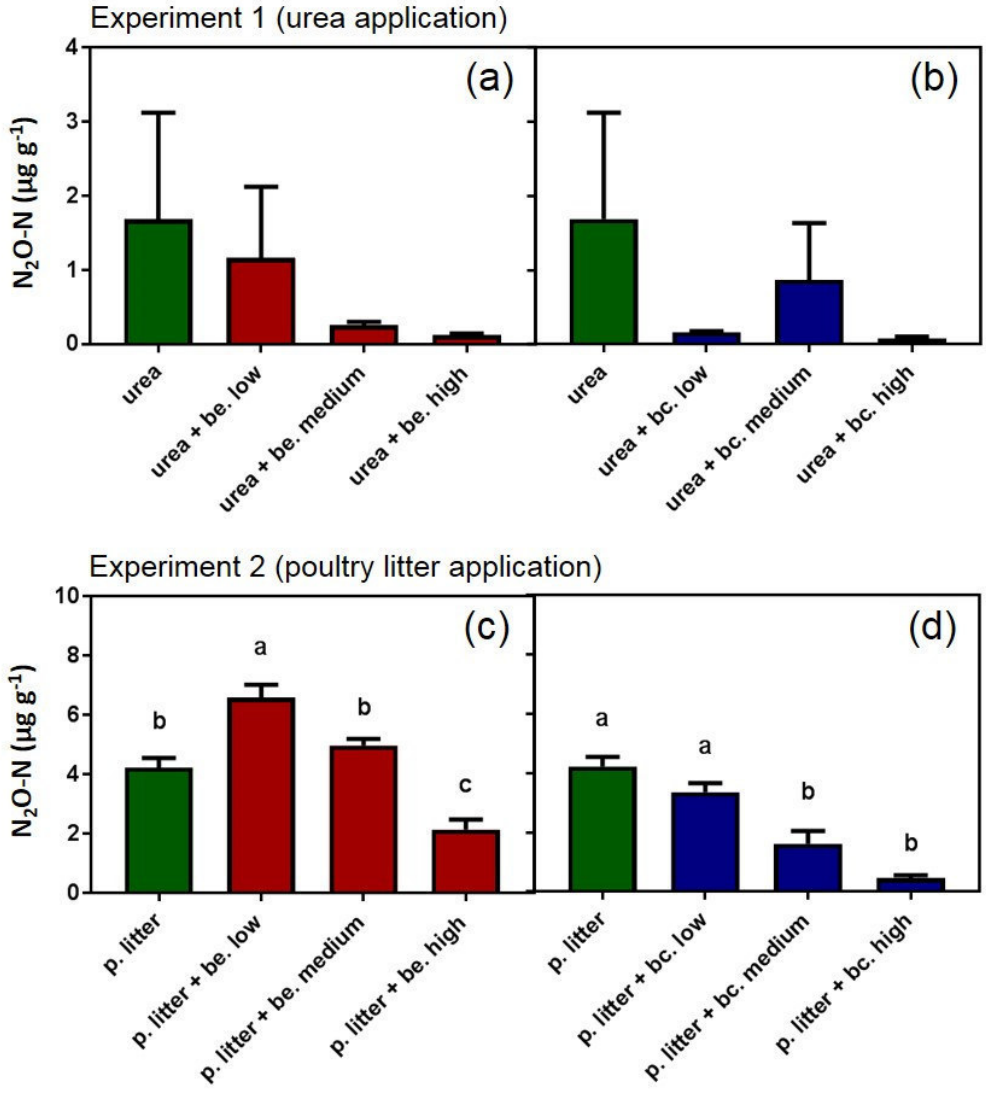


Figure 5.3. Cumulative N_2O-N fluxes (mean ± SEM) after subtraction of control treatments, expressed in µg per g dry soil. (a) Urea and bentonite application, (b) urea and biochar application, (c) urea and bentonite application, (d) urea and biochar application. Statistical analyses: One-way ANOVA plus Tukey's HSD post-hoc test. Different letters indicate significant differences at $P < 0.05$ level. Terms 'low', 'medium' and 'high' refer to 1%, 5%, and 10% of dry soil mass respectively.

Table 5.3. Nitrogen lost as N_2O (mean ± SD) from the microcosm systems, expressed in % N applied. Statistical analyses: one-way ANOVA plus Tukey's HSD post-hoc test. Different letters indicate significant differences at $P < 0.05$. Terms 'low', 'medium' and 'high' refer to 1%, 5%, and 10% of dry soil mass respectively.

Experiment 1		**Experiment 2**	
Treatment	N_2O (%)	Treatment	N_2O (%)
Urea	0.55 ± 0.94	Poultry manure	1.39 ± 0.21^{b}
Urea + bentonite low	0.38 ± 0.63	P. manure + bentonite low	2.16 ± 0.28^{a}
Urea + bentonite medium	0.08 ± 0.03	P. manure + bentonite medium	1.63 ± 0.14^{b}
Urea + bentonite high	0.04 ± 0.01	P. manure + bentonite high	0.70 ± 0.22^{c}
Urea + biochar low	0.05 ± 0.01	P. manure + biochar low	1.10 ± 0.20^{bc}
Urea + biochar medium	0.28 ± 0.50	P. manure + biochar medium	0.53 ± 0.29^{cd}
Urea + biochar high	0.03 ± 0.02	P. manure + biochar high	0.15 ± 0.07^{d}

5.3.3 Soil NH_4^+ and NO_3^-, interaction and main effects

With urea as N source, NH_4^+ fluxes showed a significant interaction effect ($P < 0.01$) between the type of amendment and application rate. In this experiment, increasing rates of bentonite and biochar led to decreasing fluxes of NH_4^+ ions but the effect was stronger with the application of biochar than with bentonite (Table 5.4). High application rates of bentonite and biochar (10%) resulted in the highest concentrations of KCl extractable NH_4^+ ions when urea was the N source, and the difference to the urea only treatment was significant with bentonite (Table 5.4). However, there was no significant ($P < 0.05$) interaction effect.

With poultry litter as N source, low application rates of bentonite and biochar (1%) increased fluxes of NH_4^+ ions compared to the poultry litter only treatment but the effect was not significant (Table 5.4). Higher application rates of bentonite and biochar (5% and 10%) led to significant decreases of NH_4^+ fluxes. There was no significant ($P < 0.05$) interaction effect between type of amendment and application rate but the application of biochar at medium (5%) and high (10%) concentrations caused stronger decreases in NH_4^+ fluxes. Comparable to urea treatments, KCl extractable NH_4^+ concentrations were highest at the highest application rates of bentonite and biochar (10%) and the difference to the poultry litter only treatment was significant with bentonite (Table 5.4). However, there was no significant ($P < 0.05$) interaction effect.

Table 5.4. Main effects of treatments. Daily NH_4^+-N and NO_3^--N fluxes and concentrations (mean ± SD) after subtraction of control values. Statistical analyses were done separately per experiment per type of amendment with One-way ANOVA with repeated measures design (linear mixed-effects models) and Tukey's HSD post-hoc test. Different letters indicate significant differences at $P < 0.05$. Terms 'low', 'medium' and 'high' refer to 1%, 5%, and 10% of dry soil mass respectively.

	Treatment	NH_4^+-N (nmol cm^{-2} h^{-1}), microdialysis	NH_4^+-N (nmol g^{-1}), KCl extraction	NO_3^--N (nmol cm^{-2} h^{-1}), microdialysis	NO_3^--N (nmol g^{-1}), KCl extraction
experiment 1, bentonite	urea	274 ± 194^{a}	8627 ± 4806^{b}	878 ± 1023	5232 ± 4084^{a}
	urea + bentonite low	240 ± 173^{a}	8796 ± 4919^{b}	689 ± 560	5607 ± 4472^{a}
	urea + bentonite medium	186 ± 122^{ab}	9266 ± 5365^{ab}	999 ± 1161	5114 ± 3819^{a}
	urea + bentonite high	92.5 ± 74.2^{b}	9936 ± 5163^{a}	578 ± 889	4056 ± 3183^{b}
experiment 1, biochar	urea	274 ± 194^{a}	8627 ± 4806	879 ± 1023^{a}	5232 ± 4084
	urea + biochar low	258 ± 214^{a}	8800 ± 5504	613 ± 567^{a}	5479 ± 4308
	urea + biochar medium	44.4 ± 49.6^{b}	8632 ± 5509	-48.8 ± 124^{b}	5093 ± 4145
	urea + biochar high	16.3 ± 26.2^{b}	9317 ± 5883	-111 ± 76.1^{b}	5059 ± 4495
experiment 2, bentonite	poultry litter	35.9 ± 50.3^{ab}	1605 ± 1651^{b}	295 ± 336	1883 ± 2057^{b}
	p. litter + bentonite low	60.6 ± 74.6^{a}	1760 ± 1791^{b}	280 ± 376	2085 ± 1872^{ab}
	p. litter + bentonite medium	10.0 ± 33.7^{b}	1903 ± 1733^{b}	169 ± 530	3089 ± 2237^{a}
	p. litter + bentonite high	10.2 ± 19.1^{b}	2337 ± 1509^{a}	118 ± 249	2638 ± 1941^{a}
experiment 2, biochar	poultry litter	35.9 ± 50.3^{a}	1605 ± 1651^{a}	295 ± 336^{a}	1883 ± 2057^{b}
	p. litter + biochar low	43.0 ± 53.7^{a}	1332 ± 1494^{ab}	336 ± 415^{a}	1860 ± 1639^{b}
	p. litter + biochar medium	0.55 ± 1.97^{b}	1304 ± 1449^{b}	-156 ± 72.0^{b}	2608 ± 1715^{a}
	p. litter + biochar high	0.20 ± 0.96^{b}	1699 ± 1547^{a}	-173 ± 58.6^{b}	2752 ± 1861^{a}

Application of the high rate of bentonite to soil (10%) resulted in lowest NO_3^- fluxes with both N sources, however, differences to the urea only and poultry litter only treatments, respectively were not significant (Table 5.4). When biochar was applied at medium (5%) and high (10%) application rates, NO_3^- fluxes were significantly reduced compared to the urea only and poultry litter only treatment, respectively. The interaction effects in relation to NO_3^- fluxes were significant at $P < 0.001$ (urea) and at $P < 0.01$ (poultry litter).

The highest application rates of bentonite and biochar (10%) resulted in the strongest reduction of KCl extractable NO_3^- from urea application and the difference was significant with bentonite (Table 5.4). There was a significant interaction effect at $P < 0.05$. With poultry litter, medium (5%) and high (10%) application rates of bentonite and biochar led to significant increases in KCl extractable NO_3^- concentrations compared to poultry litter only and there was no significant interaction effect.

5.3.4 Correlation analyses of N_2O emissions with soil chemical and physical variables

Results of correlation analyses varied in part strongly across the N sources and amendments for selected variables (Table 5.5). Cumulative NH_4^+ fluxes showed significant positive correlations with cumulative N_2O emissions when urea was the N source (bentonite: $r = 0.738$, $P < 0.001$; biochar: $r = 0.516$, $P < 0.05$). With poultry litter, cumulative NH_4^+ fluxes were also positively correlated to cumulative N_2O emissions with either bentonite or biochar but the correlation was only significant with biochar (bentonite: $r = 0.423$, $P > 0.05$, biochar: $r = 0.743$, $P < 0.05$). Cumulative KCl extractable NH_4^+ concentrations were positively and significantly correlated with N_2O emissions in urea treatments, irrespective of type of amendment (bentonite: $r = 0.700$, $P < 0.001$, biochar: $r = 0.634$, $P < 0.01$). In contrast, cumulative KCl extractable NH_4^+ concentrations in poultry litter treatments were significantly negatively correlated to cumulative N_2O emissions in the presence of bentonite ($r = -0.644$, $P < 0.01$) and weakly and not significantly correlated in the presence of biochar ($r = -0.011$, $P > 0.05$).

Table 5.5. Correlation analyses of cumulative N_2O-N emissions with soil chemical and physical variables. Cumulative N_2O-N data and independent variables were log-transformed before analysis. Significance is indicated at levels of $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Correlations of $r > 0.5$ are highlighted by underscores, correlation of $r > 0.8$ are given in bold.

	Urea, bentonite	Urea, biochar	Poultry litter, bentonite	Poultry litter, biochar
Variable	*r, n* = 20	*r, n* = 20	*r, n* = 15	*r, n* = 10
NH_4^+-N flux	0.738***	0.516*	0.423	0.743*
KCl extractable NH_4^+-N	0.700***	0.634**	-0.644**	-0.011
NO_3^--N flux	0.579**	0.342	0.180	**0.804****
KCl extractable NO_3^--N	0.718***	0.628**	-0.163	-0.566
H^+ (calculated from pH)	0.565**	0.340	0.748**	**0.965*****
Bulk density	0.132	0.090	0.068	**0.922*****
Water loss (evaporation)	0.010	-0.108	0.177	-0.120

Correlations between cumulative NO_3^- fluxes and cumulative N_2O emissions varied strongly across the two N sources and amendments. All correlations were positive but ranged from weak and non-significant in poultry litter plus bentonite combinations ($r = 0.180$, $P > 0.05$) to strong and significant in poultry litter plus biochar combinations ($r = 0.804$, $P < 0.01$). Cumulative concentrations of KCl extractable NO_3^- showed positive and significant correlations to cumulative N_2O emissions with urea as N source (bentonite: $r = 0.718$, $P < 0.001$, biochar: $r = 0.628$, $P < 0.01$) but negative and non-significant correlations with poultry litter as N source (bentonite: $r = -0.163$, $P > 0.05$, biochar: $r = -0.566$, $P > 0.05$).

Cumulative H^+ ions were positively correlated to cumulative N_2O emissions across both N sources and amendments. The strongest correlation was found with poultry litter plus biochar combinations ($r = 0.965$, $P < 0.001$) and the only non-significant correlation was observed with urea plus biochar combinations ($r = 0.340$, $P > 0.05$). Bulk density was weakly correlated to cumulative N_2O emissions, except for the combination of poultry litter and biochar which showed a strong and significant correlation ($r = 0.922$, $P < 0.001$). Evaporation of water was only weakly and non-significantly correlated across both N sources and amendments.

5.4 Discussion

This study investigated how the amendments bentonite and biochar affect the N turnover in soil in relation to N_2O emissions. For the first time, quantifications of N_2O fluxes were accompanied by simultaneous quantifications of soil N fluxes. Overall, both amendments decreased N_2O

fluxes from soil fertilised with either urea or poultry litter and decreasing trends in N_2O, NH_4^+ and NO_3^- fluxes with increasing application rates of amendments were discernible.

5.4.1 Soil NO_2^- and N_2O production

While it has been proposed that NO_2^- is a critical precursor for N_2O emissions (e.g. Venterea et al., 2007), no NO_2^- was detected in this study and thus this mechanism is not supported by the presented experiments. The results of the study presented here likely reflect a rapid conversion of any NO_2^- formed to other nitrogen species. This is supported by the accumulation of NO_3^- in treatments that received N input. Venterea et al. (2015) reported NO_2^- accumulation in a soil of low CEC (14 $cmol_c\ kg^{-1}$) after N fertilisation and linked this result to elevated N_2O production. It is possible that the CEC of the soil used here (36.8 $cmol_c\ kg^{-1}$) was sufficiently high to prevent NO_2^- accumulation and that thus nitrification could be completed. Other reasons could be that the concentration of N in the microcosms (equivalent to 305 mg N kg^{-1} dry soil) was not high enough to cause NO_2^- accumulation or that acidic soil conditions led to rapid decomposition of NO_2^- (Nelson and Bremner, 1969). However, van Cleemput and Samater (1996) measured low levels of NO_2^- at an application rate of 100 mg urea-N kg^{-1} soil under acidic soil conditions.

5.4.2 Urea versus *poultry litter application*

Overall, higher N_2O emissions were measured with poultry litter than with urea, although fluxes of mineral N and KCl extractable mineral N were generally higher with urea. Differences in pH were probably of minor importance as the pH values were in the same range in both experiments at all measurement times. A possible explanation for the differences in N_2O emission between the two N sources could be differences in soil O_2 concentration. The WFPS in both experiments was kept at 50% to maintain aerobic conditions and to limit N_2O production from denitrification. However, the organic C added with the poultry litter in experiment 2 likely increased microbial respiration and subsequently led to oxygen depletion in soil microsites. Thus, the creation of increasingly heterogenic microsites could have promoted N_2O production from both nitrification and denitrification (Granli and Bøckman, 1994). The higher CO_2 fluxes detected with poultry litter support this suggestion (Figure C1c,d). Akiyama and Tsuruta (2003) reported significantly higher N_2O emissions from poultry litter application than from urea application and explained their results with this theory. If aeration was not restricted too strongly, heterotrophic nitrification might also have played a role in poultry litter treatments as

these organisms use organic C as substrate (Papen et al., 1989; Anderson et al., 1993; Wrage et al., 2001).

A noticeable difference in N_2O emissions between both N sources is that emissions from poultry litter treatments peaked on day 2 whereas emissions from urea treatments peaked on day 7. However, in both experiments highest NH_4^+ fluxes and concentrations were measured on day 2. Further research is needed to explain this phenomenon.

5.4.3 Bentonite versus *biochar application*

Additions of bentonite and biochar to soil caused decreases in N_2O emissions from urea and poultry litter application. When poultry litter was the N source, decreases were significant at the high (10%) application rate of bentonite and the medium (5%) and high (10%) application rates of biochar. Decreases in N_2O emission can likely be attributed to lower diffusive fluxes of NH_4^+ and increased concentrations of exchangeable NH_4^+ across experiments and type of amendment as shown in Figure 5.2 and Table 5.4. This theory is supported by the positive correlations found between cumulative N_2O emissions and cumulative diffusive fluxes of NH_4^+ in both experiments. Thus, the effect of decreased NH_4^+ fluxes and decreased N_2O emissions was likely caused by exchangeable binding of NH_4^+ ions through the amendments' CEC. Regarding biochar, interaction of NH_4^+/ NH_3 with surface functional groups as discussed in Spokas et al. (2012) could also be a possible mechanism. The results of the NH_4^+ measurements presented here are compatible with other studies. Redding (2011) found decreasing soluble and increasing exchangeable NH_4^+ concentrations in blends of poultry litter and bentonite with increasing rates of bentonite. Ding et al. (2010) reported decreased leaching of NH_4^+ from soil columns when biochar was added and assigned the effect mainly to cation exchange.

With both N sources, positive and significant correlations between cumulative N_2O emissions and cumulative H^+ ions were found when bentonite was applied (urea: $r = 0.565$; poultry litter: $r = 0.748$). It is therefore possible that the change of soil pH with the application of bentonite has affected N_2O emissions. Fixation of N between the sheets of bentonite particles either did not take place or occurred with negligible amounts of NH_4^+ as increasing rates of bentonite application did not result in decreased KCl extractable NH_4^+. However, other physical effects of bentonite could have had an impact on N_2O emissions. For example, when poultry litter was the N source, bentonite significantly increased N_2O emissions in comparison to the poultry litter only treatment at a low application rate (1%). Bentonite is known for its strong swelling

characteristic that could have decreased gas exchange and thus created restricted O_2 levels in the microcosms and subsequently caused higher N_2O emissions via denitrification (Granli and Bøckman, 1994). At higher application rates, the effect of increased CEC might have outweighed this drawback. Furthermore, at medium (5%) and high (10%) application rates of bentonite, evaporation of water from the microcosms resulted in vertical cracks in the soil column through shrinking of bentonite. This effect might have improved soil aeration and thus decreased N_2O production.

With poultry litter, increasing rates of biochar application led to decreasing rates of N_2O emissions. This result is consistent with the result of a meta-analysis conducted by Cayuela et al. (2014). Apart from the two mechanisms mentioned above (CEC and interaction with surface functional groups) several other mechanisms that could play a role in mitigation of N_2O emissions through application of biochar have been suggested in the literature, such as N immobilisation, physical entrapment of NH_4^+ in pores, increase in soil pH or improved soil aeration (Clough et al., 2013). Physical capture of NH_4^+ as well as N immobilisation were probably minimal in this study because in both experiments the application of biochar did not decrease KCl extractable NH_4^+ ions. The application of biochar to soil increased the pH and reduced the bulk density. With poultry litter, positive and strong correlations were found between cumulative N_2O emissions and cumulative H^+ ions and also between cumulative N_2O emissions and bulk density (H^+ ions: $r = 0.965$; bulk density: $r = 0.922$). This indicates that at least in this experiment increases in pH and improved soil aeration affected soil N_2O emissions by attenuating these. The results are in accordance with Cayuela et al. (2014) who found that biochar is less effective in mitigating N_2O emissions from soil under acidic conditions.

Comparing both types of amendment, the application of biochar resulted in stronger decreases in N_2O emissions in experiment 2 in which poultry litter was the N source. Furthermore, in both experiments the application of biochar led to stronger decreases in diffusive fluxes of NH_4^+ (significant interaction effect with urea) and NO_3^- (significant interaction effect in both experiments). The exact reason for these differences in response could not be identified unambiguously. With both amendments, changes in soil N turnover seem to have mainly been caused by CEC. Regarding biochar, interaction of NH_4^+ / NH_3 with surface functional groups could also have played a role. Additionally, these mechanisms were likely influenced by changes in soil pH and aeration. A combination of these chemical and physical factors probably led to the changes in soil N dynamics measured here. The importance of each factor probably

varied between source of N and type of amendment as reflected in part by the strongly varying results of correlation analyses across experimental conditions.

5.4.4 Microdialysis vs. KCl extraction

General information about the advantages and disadvantages of the use of microdialysis for investigating soil N compared to the conventional KCl extraction technique can be found in Inselsbacher et al. (2011) and Brackin et al. (2015). Here, the features and advantages of microdialysis relative to conventional KCl extraction in regard to this study are discussed.

Compared to the classic KCl extraction technique, differences between treatments were more distinct with microdialysis. With this technique, diffusive fluxes are measured which likely better represent soil N dynamics than the total extractable N. This suggestion is supported by the positive correlations between cumulative N_2O emissions and cumulative NH_4^+ fluxes between experiments and types of amendments. However, a drawback of the experimental design used here is that NH_4^+ and NO_3^- ions were always sampled from the same spot in the microcosms (compare Figure 5.1a) and that the same microcosms were used for repeated measurements with microdialysis. Thus, the sampling of NH_4^+ and NO_3^- with microdialysis spatially and temporarily reduced or even depleted these concentrations. How quickly this supply regenerates still needs to be investigated. However, in experiment 1 for treatments 3 (soil + urea+ 1% bentonite) and 4 (soil + urea + 5% bentonite), highest fluxes of NH_4^+ have been measured on day 7, indicating that NH_4^+ had been replenished at least within two days. Another disadvantage is that with every microdialysis sampling procedure a certain amount of soil N was removed from the system. Future research is needed to assess the extent of N removal.

5.4.5 Conclusion

This study demonstrates that N_2O emissions from soil fertilised with urea or poultry litter can be decreased by the application of bentonite or biochar. The effect was attributed to decreases in soil NH_4^+ fluxes thought to be mainly caused by increases in soil CEC with increasing rates of amendments. Regarding biochar, interaction of NH_4^+ / NH_3 with surface functional groups could also be a potential mechanism. Changes in soil pH and aeration likely influenced these mechanisms and the importance of each factor probably varied between sources of N applied

and type of amendment. This highlights the complexity of soil and the entailing difficulty of investigating soil processes. This study contributes to the understanding of how soil amendments such as bentonite and biochar influence soil N processes and shows the potential of the microdialysis technique for investigating soil N dynamics.

Chapter 6 – General discussion

Research presented in this thesis confirms that soil amendments have the potential to decrease N_2O emissions from agricultural soils. However, an important finding of this thesis is that there is no universal approach and consequently the application of soil amendments needs to be carefully adjusted to specific circumstances. This is explained in more detail in the following sections.

For bentonite it has been shown that the type of application of this material plays an important role. In Chapter 4 and 5, N_2O emissions from N fertilised soil could be decreased by the application of bentonite when it was incorporated into the soil. Contrary to this, surface application of bentonite, as investigated in Chapter 3, seems to generally increase N_2O emissions from soil. Furthermore, the application rate of bentonite seems to be crucial. When poultry litter was used, low application rates of bentonite ($\leq$ 1% of dry soil mass) increased soil N_2O emissions in the laboratory (Chapter 4 and 5), while higher application rates resulted in decreases of N_2O emissions. This outcome is consistent with Pratt et al. (2016). However, this finding does not seem to be applicable to other N sources. In Chapter 5 for example, increasing application rates of bentonite resulted in a trend of decreased N_2O emissions when the soil was fertilised with urea. In contrast, an increasing trend in N_2O emissions with increasing rates of bentonite has also been observed when egg manure was used an N source (Pratt et al., 2016). The type of bentonite is also important. The clay bentonite is available in different types such as potassium bentonite, sodium bentonite, or calcium bentonite according to the element that is most prevalent in a batch. In this thesis, sodium bentonite was used, firstly because Na^+ ions are close to NH_4^+ ions in the lyotropic series (Troeh and Thompson, 1993) and can thus comparatively easily be replaced, and secondly because potassium bentonite was not available. In the field experiment (Chapter 4), the sodium concentration did not exceed the sodium tolerance level for sugarcane but a high sodium concentration can be detrimental to crops. Therefore, the use of calcium bentonite is favourable regarding plant and also soil health, but with this type of bentonite the rate of substitution of Ca^{2+} ions for NH_4^+ ions might be small as Ca^{2+} ions have a higher position in the lyotropic series than NH_4^+ ions (Troeh and Thompson, 1993). Another aspect that needs to be taken into consideration when using bentonite is its influence on soil aeration through its strong swelling / shrinking characteristic. In the field, a positive or negative influence on soil N_2O emissions depending on climate or even seasonal variations is therefore possible.

Out of the three amendments investigated in this thesis (bentonite, biochar and green waste compost), biochar was most effective in reducing N_2O emissions from soil. However, in the field experiment (Chapter 4), application of biochar tended to reduce sugarcane growth. Biochar can be very variable depending on feedstock and can thus result in different effects on soil N_2O emissions and crop growth (Biederman and Harpole, 2013; Cayuela et al., 2014). Meta-analyses have shown that biochar has an overall decreasing effect on soil N_2O emissions (Cayuela et al., 2014) and that biochar generally does not limit plant growth (Biederman and Harpole, 2013), but more studies are needed to identify optimum biochar types and application rates.

While it has been shown that green waste compost can be used to decrease N_2O emissions from soil fertilised with feedlot manure in a subtropical climate (Dalal et al., 2009; Dalal et al., 2010), research conducted as part of this thesis revealed that application of green waste compost to soil can also result in the opposite effect (Chapter 4). Comparable to biochar, compost can be very variable depending on its feedstock and future research needs to identify optimum types of compost, the optimum age of a certain type of compost, and also optimum application rates for agronomic practices. Furthermore, compost-plant interactions need to be studied in more detail. The compost used in the field experiment in Chapter 4 contained a substantial amount of NO_3^- but sugarcane has a comparatively low NO_3^- uptake efficiency (Robinson et al., 2011). If the same compost would have been applied to a crop that predominantly uses NO_3^- as an N source, N_2O losses from this treatment might have been lower.

In contrast to compost, geological materials and biochar degrade very slowly in soils and can persist for decades or even centuries. This aspect is a major advantage of these materials over compost, especially regarding geological materials as they are not an infinite resource. However, knowledge about long-term effects of geological materials and biochar on soil N_2O emissions is lacking and studies investigating this topic are needed (Clough et al., 2013; Pratt et al., 2016).

Knowledge gaps are still an obstacle for the development of best management practices for decreasing N_2O emissions from N fertilised soils. For example, in this thesis CEC has been identified as the likely main mechanism by which N_2O emissions were decreased from bentonite and biochar application, but the interaction with other factors is still unclear. Moreover, regarding biochar, results of the field experiment (Chapter 4) don't fully match the results obtained in the laboratory in Chapter 5. In the field, the application of biochar led to significant decreases in KCl-extractable soil mineral N, whereas in the laboratory no significant changes were observed. This conflict cannot be explained and needs further investigation.

Ongoing technological advances such as isotopic labelling or molecular analyses of whole microbial communities continue to improve our understanding of soil N turnover processes (Butterbach-Bahl et al., 2013). In this thesis, the microdialysis technique that previously had not been used for the investigation of soil N_2O fluxes, proved to be a useful tool to gain new insights into the effect of soil amendments on soil N dynamics and connected N_2O production. In combination with software-based life cycle analyses and modelling approaches, these technologies contribute to closing knowledge gaps and advancing the development of site-specific best management guidelines that are needed to effectively mitigate N_2O emissions from managed soils.

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Appendices

Appendix A - Supplementary information (Chapter 3)

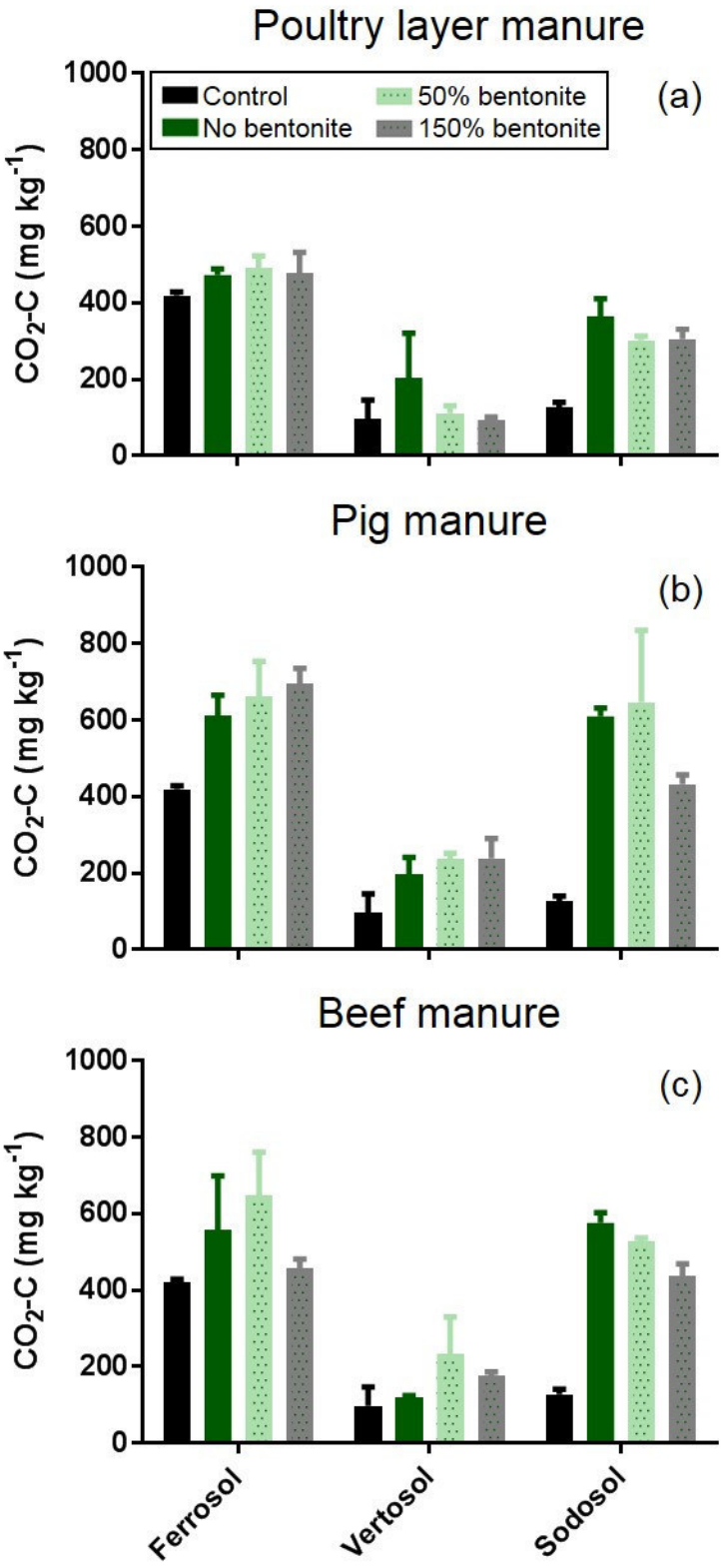

Figure A1.Cumulative CO_2 fluxes (mean ± SD) from three Australian soils over 18 days after application of a) poultry layer manure, b) pig manure and c) beef manure. Application rates of bentonite are given in percentages of fresh manure mass.

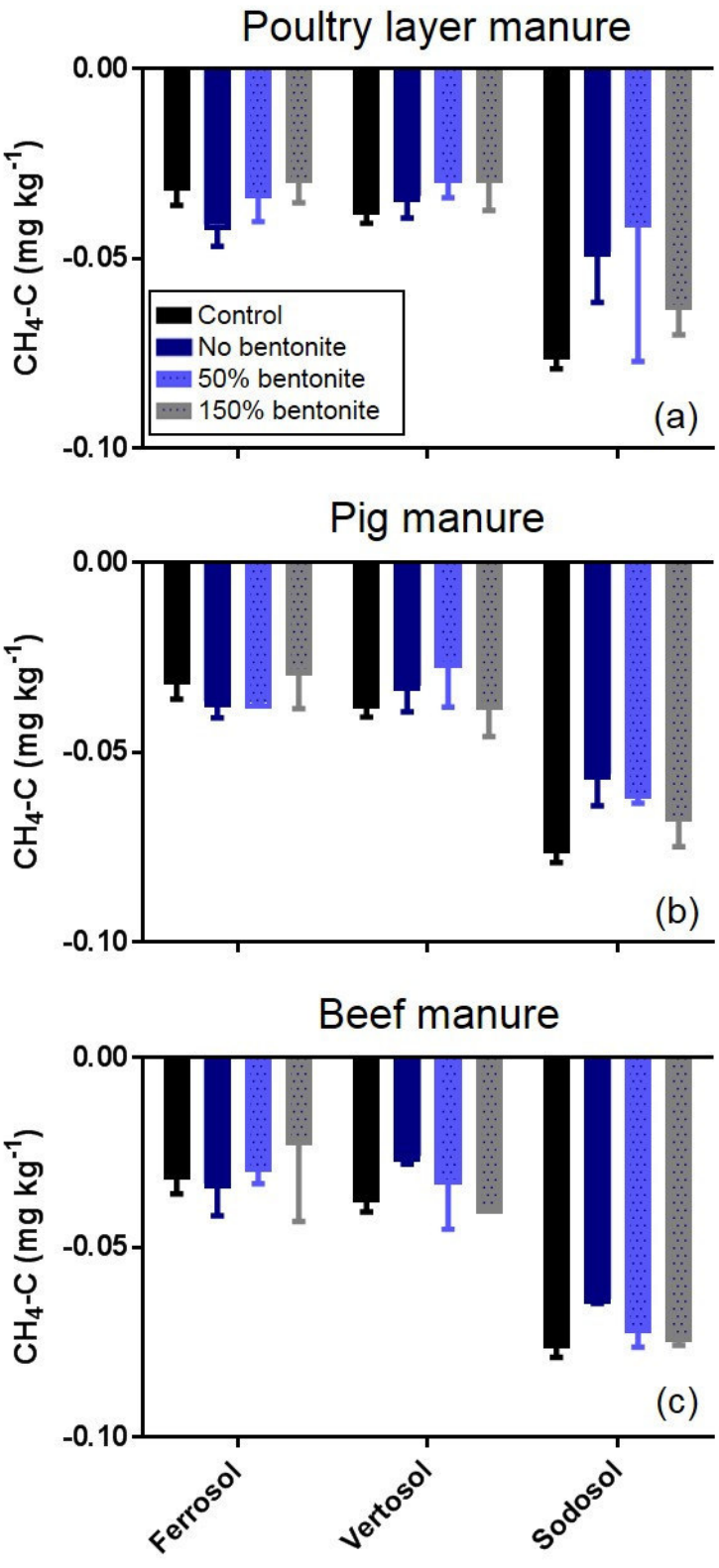

Figure A2.Cumulative CH_4 fluxes (mean ± SD) from three Australian soils over 18 days after application of a) poultry layer manure, b) pig manure and c) beef manure. Application rates of bentonite are given in percentages of fresh manure mass.

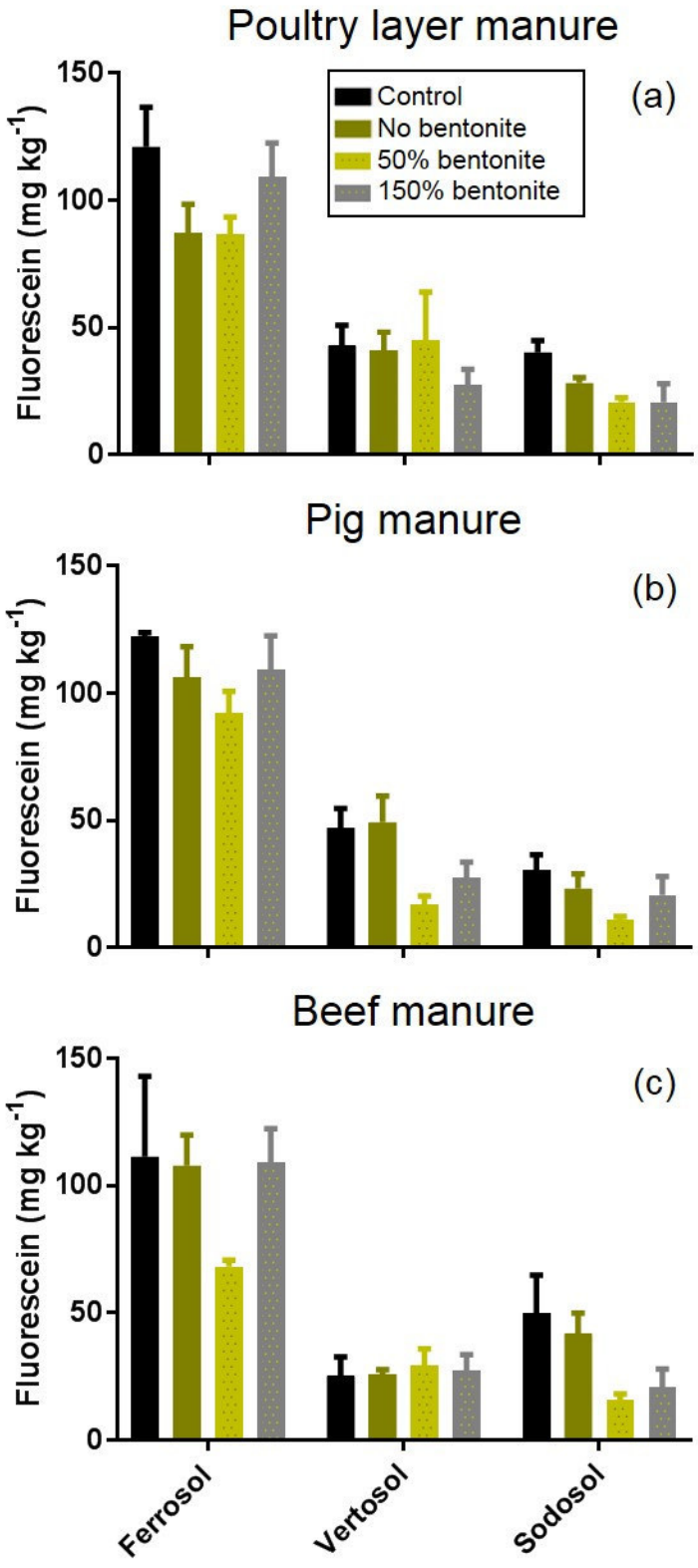

Figure A3. Microbial activity (mean ± SD) in three Australian soils at the end of the experiment on day 18 after application of a) poultry layer manure, b) pig manure and c) beef manure. Microbial activity was measured by fluorescein diacetate (FDA) hydrolysis (Adam and Duncan, 2001). Application rates of bentonite are given in percentages of fresh manure mass.

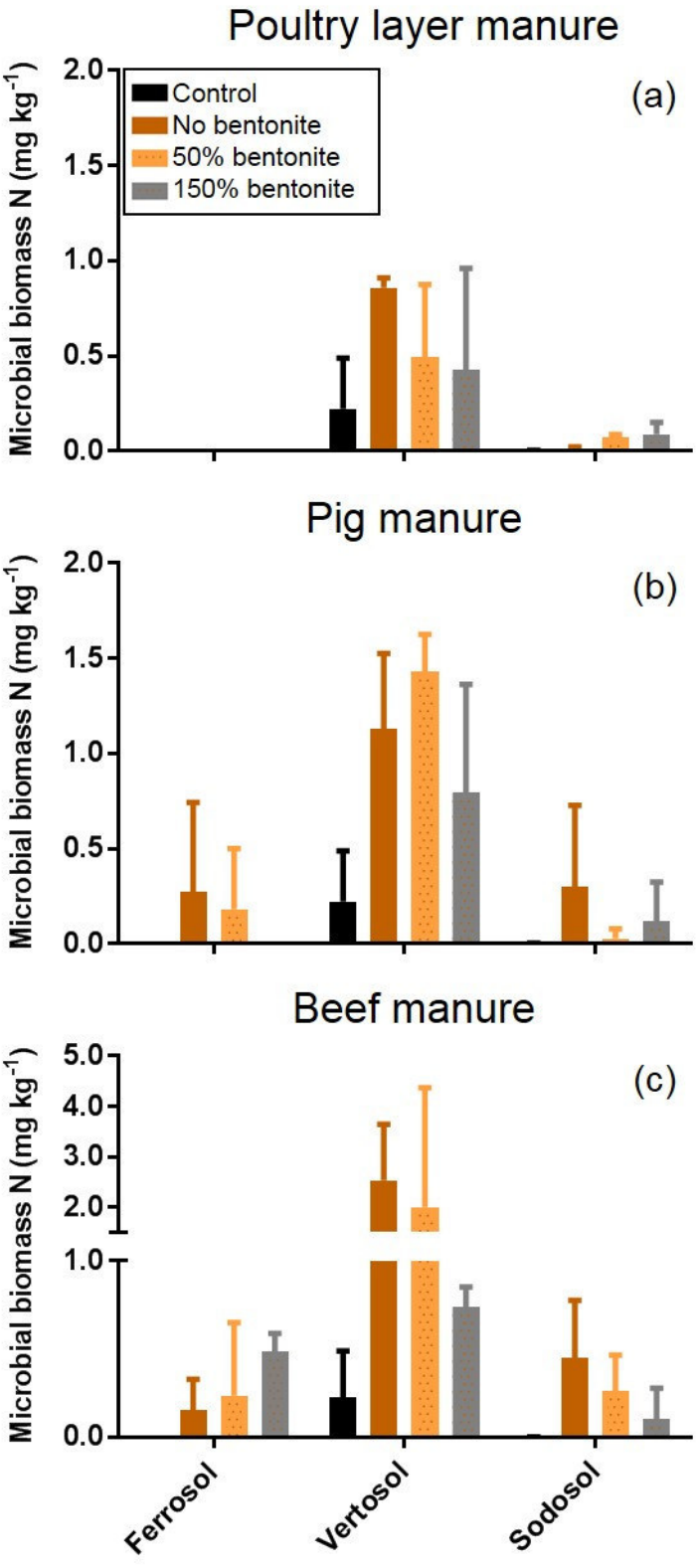

Figure A4. Microbial biomass N (mean ± SD) in three Australian soils at the end of the experiment on day 18 after application of a) poultry layer manure, b) pig manure and c) beef manure. Application rates of bentonite are given in percentages of fresh manure mass.

R code of the three-way-ANOVA presented in Table 3.3:

```
cum_data <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(1 and 2) DAFF manure/first experiment/results and
calculations/stats/cum_data_exp1_control_subtr.csv")

anova_N2O <- aov(N2O ~ soil*manure*bentonite, data = cum_data)
summary(anova_N2O)
```

R code of the correlation analyses presented in Table 3.4:

```
cum_data <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(1 and 2) DAFF manure/first experiment/results and
calculations/stats/cum_data_exp1.csv")

## N2O vs. NH4+
# linear model
NH4_d <- lm(cum_data$N2O_total ~ cum_data$NH4_d)
summary(NH4_d)
# correlation coefficient (r)
cor.test(cum_data$NH4_d, cum_data$N2O_total)



## N2O vs. NO3-
# linear model
NO3_d <- lm(cum_data$N2O_total ~ cum_data$NO3_d)
summary(NO3_d)
# correlation coefficient (r)
cor.test(cum_data$NO3_d, cum_data$N2O_total)

## N2O vs. microbial activity
# linear model
MA_d <- lm(cum_data$N2O_total ~ cum_data$MA_d)
summary(MA_d)
# correlation coefficient (r)
cor.test(cum_data$MA_d, cum_data$N2O_total)



## N2O vs. microbial biomass N
# linear model
MB_d <- lm(cum_data$N2O_total ~ cum_data$MB_d)
summary(MB_d)
# correlation coefficient (r)
cor.test(cum_data$MB_d, cum_data$N2O_total)
```

Appendix B - Supplementary information (Chapter 4)

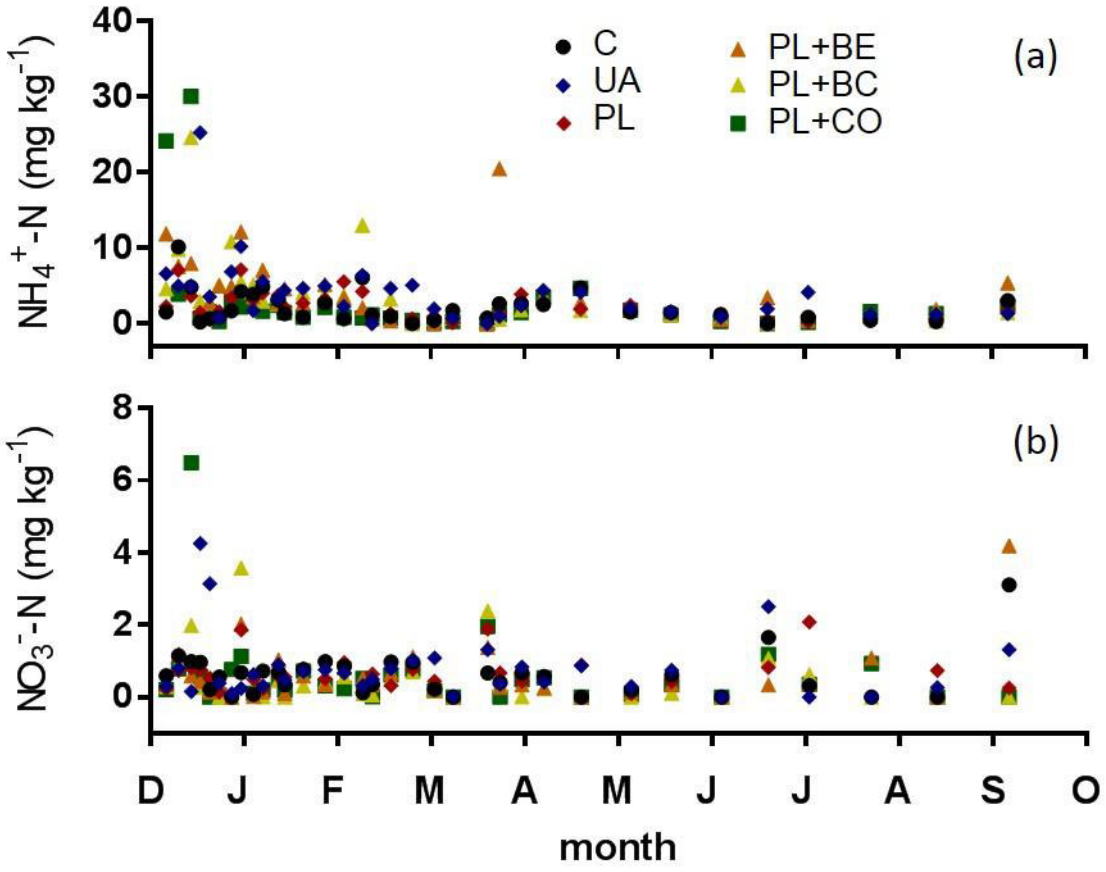

Figure B1. Soil mineral N measured in the field experiment from December 2014 to September 2015 in sugarcane inter-rows. (a) Mean soil NH_4^+-N concentrations (mg kg^{-1}; n = 3), (b) mean soil NO_3^--N concentrations (mg kg^{-1}; n = 3). C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost. Error bars were omitted because of high variances of data within treatments.

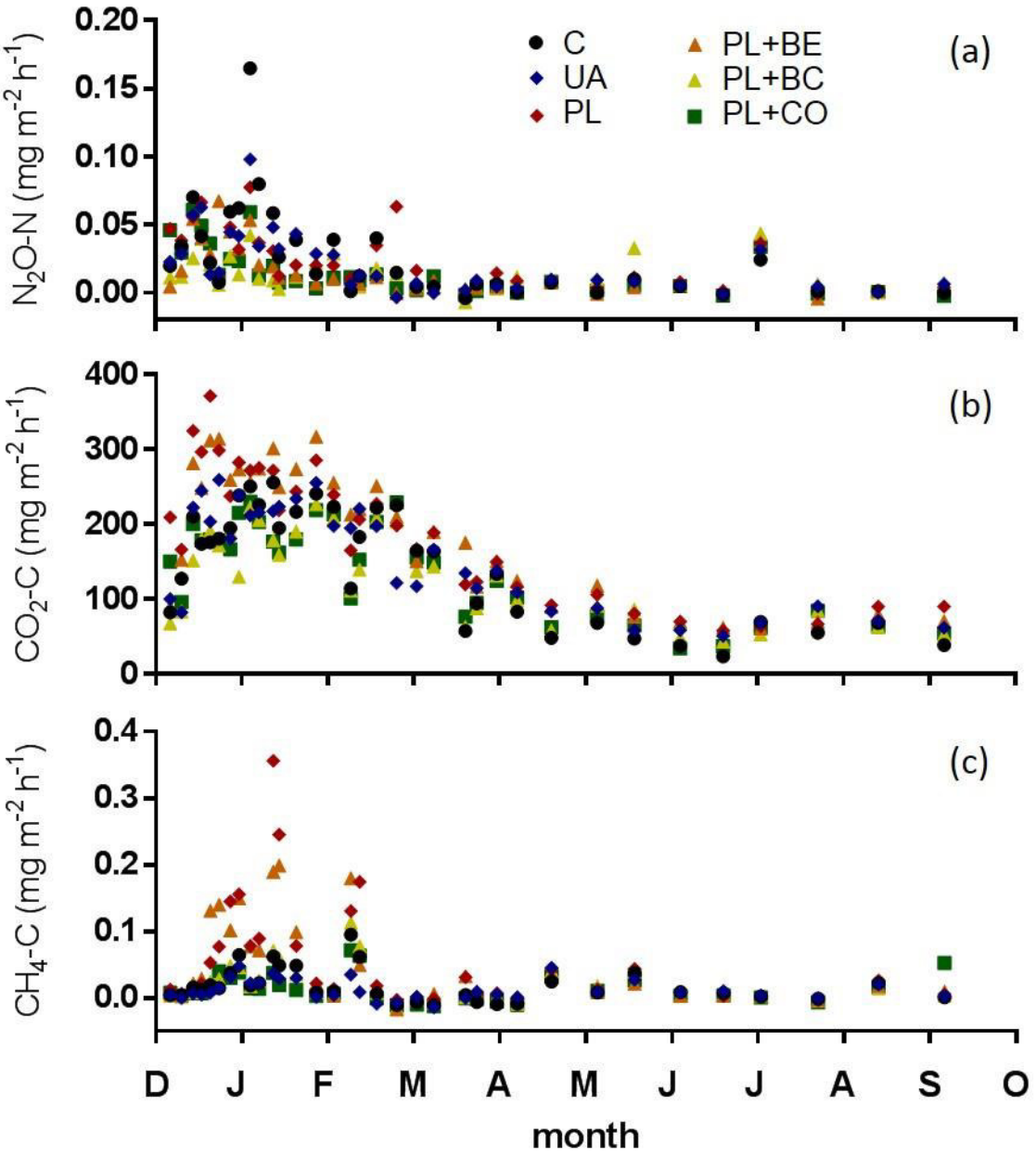

Figure B2. Greenhouse gas fluxes measured from sugarcane inter-rows at the experimental field site from December 2014 to September 2015. (a) Mean N_2O-N fluxes (mg m^{-2} h^{-1}; n = 3), (b) mean CO_2-C fluxes (mg m^{-2} h^{-1}; n = 3), (c) mean CH_4-C fluxes (mg m^{-2} h^{-1}; n = 3). C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost. Error bars were omitted because of high variances of data within treatments.

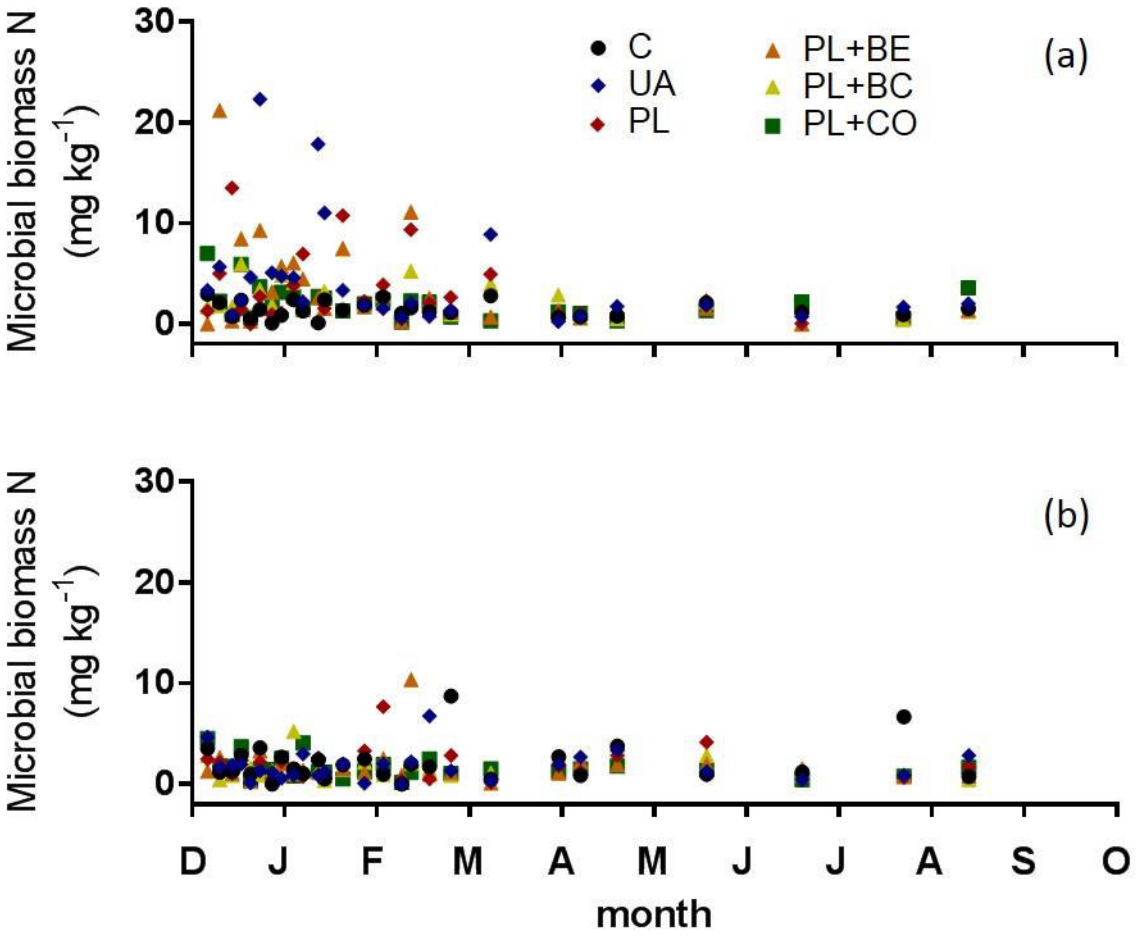

Figure B3. Mean soil microbial biomass N (mg kg^{-1}; n = 3) measured in the field experiment from December 2014 to September 2015. (a) Sugarcane rows, (b) sugarcane inter-rows. C = control, UA = urea, PL = poultry litter, PL+BE = poultry litter plus bentonite, PL+BC = poultry litter plus biochar, PL+CO = poultry litter plus compost. Error bars were omitted because of high variances of data within treatments.

Table B1. Cumulative CO_2 and CH_4 emissions (mean ± SD; n = 3) of the field experiment. Statistical analyses: one-way ANOVA with Fisher's LSD post hoc test after log-transformation of data. Differences between treatments at $P < 0.05$ significance level are indicated with different letters.

Treatment	Total CO_2-C emissions (kg ha^{-1})	Total CH_4-C emissions (kg ha^{-1})
Control	5321 ± 154^{b}	0.61 ± 0.11^{ab}
Urea	6463 ± 1348^{ab}	0.45 ± 0.03^{b}
Poultry litter	7554 ± 743^{a}	2.06 ± 0.96^{a}
Poultry litter + bentonite	6664 ± 554^{ab}	2.75 ± 3.12^{a}
Poultry litter + biochar	7040 ± 1360^{a}	1.19 ± 1.12^{ab}
Poultry litter + compost	7176 ± 1011^{a}	1.56 ± 1.66^{ab}

Table B2. Physical and chemical properties of soil, poultry litter and green waste compost used in the laboratory experiment.

Property	Soil	Poultry litter	Compost
Dry matter (%)	N/A	83	70
pH	5.20 ± 0.09	7.42 ± 0.03	6.98 ± 0.04
Total C (%)	2.04 ± 0.07	29.40 ± 0.71	13.93 ± 1.05
Total N (%)	0.27 ± 0.01	2.47 ± 0.19	1.10 ± 0.05
C/N ratio	N/A	11.90	12.66
NH_4^+-N (mg kg^{-1})	0.58 ± 0.28	1776 ± 374	1.34 ± 0.29
NO_3^--N (mg kg^{-1})	0.92 ± 1.59	Not detected	641 ± 128

Table B3. Cumulative CO_2 and CH_4 fluxes (mean ± SD; n =4) of the laboratory experiment. Data are presented on a dry soil basis. Statistical analyses: one-way ANOVA with Fisher's LSD post-hoc test. Differences between treatments at $P < 0.05$ significance level are indicated with different letters.

Treatment	cumulative CO_2-C emissions (mg kg^{-1})	cumulative CH_4-C emissions (mg kg^{-1})
Control	64.4 ± 35.2[b]	353.1 ± 37.4[b]
Poultry litter	563.1 ± 87.6[a]	853.1 ± 87.9[a]
Poultry litter + bentonite	572.9 ± 67.6[a]	862.7 ± 65.3[a]
Poultry litter + biochar	601.9 ± 63.4[a]	881.6 ± 60.6[a]
Poultry litter + compost	646.2 ± 52.3[a]	921.2 ± 51.6[a]

R code of the repeated measures one-way ANOVA presented in Table 4.3:

```
daily_row_final_130 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(3) field experiment/stats/daily
data/field_exp_daily_data_row_final_130d.csv")

## conversion of the samping day to a categorical variable
daily_row_final_130$sampling_day <- factor(daily_row_final_130$sampling_day)

##NH4+
aov_NH4_final <- aov(daily_row_final_130$NH4_ln ~ daily_row_final_130$treatment
+ Error(daily_row_final_130$sampling_day/daily_row_final_130$treatment))
summary(aov_NH4_final)
with(daily_row_final_130, pairwise.t.test(daily_row_final_130$NH4_ln,
daily_row_final_130$treatment, p.adjust = "none", paired = T))
describeBy(daily_row_final_130$NH4, daily_row_final_130$treatment)


##NO3-
aov_NO3_final <- aov(daily_row_final_130$NO3_ln ~ daily_row_final_130$treatment
+ Error(daily_row_final_130$sampling_day/daily_row_final_130$treatment))
summary(aov_NO3_final)
with(daily_row_final_130, pairwise.t.test(daily_row_final_130$NO3_ln,
daily_row_final_130$treatment, p.adjust = "none", paired = T))
describeBy(daily_row_final_130$NO3, daily_row_final_130$treatment)
```

R code for the analyses of the cumulative gas emissions of the field experiment (Tables 4.4 and B1):

```
total_gases <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(3) field experiment/stats/gases total/field_exp_gases_total.csv")


##N2O
anova_N2O <- aov(total_gases$N2O_ln ~ total_gases$treatment)
summary(anova_N2O)
pairwise.t.test(total_gases$N2O_ln, total_gases$treatment, p.adjust = "none")
describeBy(total_gases$N2O_kg_per_ha, total_gases$treatment)

##CO2
anova_CO2 <- aov(total_gases$CO2_ln ~ total_gases$treatment)
summary(anova_CO2)
pairwise.t.test(total_gases$CO2_ln, total_gases$treatment, p.adjust = "none")
describeBy(total_gases$CO2_kg_per_ha, total_gases$treatment)

##CH4
anova_CH4 <- aov(log(total_gases$CH4_kg_per_ha) ~ total_gases$treatment)
summary(anova_CH4)
pairwise.t.test(log(total_gases$CH4_kg_per_ha), total_gases$treatment, p.adjust =
"none")
describeBy(total_gases$CH4_kg_per_ha, total_gases$treatment)

##CO2-eq
anova_CO2_eq <- aov(total_gases$CO2_eq_total_t_per_ha_ln ~
total_gases$treatment)
summary(anova_CO2_eq)
pairwise.t.test(total_gases$CO2_eq_total_t_per_ha_ln, total_gases$treatment,
p.adjust = "none")
describeBy(total_gases$CO2_eq_total_t_per_ha, total_gases$treatment)
```

R code for the analyses of the cumulative gas emissions of the laboratory experiment (Tables 4.5 and B3):

```
gases_total <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(4) GHG monitoring experiment Toowoomba/gases_total.csv")

##N2O
anova_N2O_kg <- aov(gases_total$N2O_N_kg ~ gases_total$treatment)
summary(anova_N2O_kg)
pairwise.t.test(gases_total$N2O_N_kg, gases_total$treatment, p.adjust = "none")
describeBy(gases_total$N2O_N_kg, gases_total$treatment)

##CO2
anova_CO2_kg <- aov(gases_total$CO2_C_kg ~ gases_total$treatment)
summary(anova_CO2_kg)
pairwise.t.test(gases_total$CO2_C_kg, gases_total$treatment, p.adjust = "none")
describeBy(gases_total$CO2_C_kg, gases_total$treatment)

##CH4
anova_CH4_kg <- aov(gases_total$CH4_C_kg ~ gases_total$treatment)
summary(anova_CH4_kg)
pairwise.t.test(gases_total$CH4_C_kg, gases_total$treatment, p.adjust = "none")
describeBy(gases_total$CH4_C_kg, gases_total$treatment)
```

R code of the one-way ANOVA for analysis of sugarcane biomass presented in Figure 4.4:

```
biomass_end <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(3) field experiment/stats/biomass/biomass_final.csv")

anova_biomass <- aov(biomass_end$biomass ~ biomass_end$treatment)
summary(anova_biomass)
pairwise.t.test(log(biomass_end$biomass), biomass_end$treatment, p.adjust =
"none")
```

R code of the correlation analyses presented in Table 4.6:

```
daily_row <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(3) field experiment/stats/daily
data/field_exp_daily_data_row_final.csv")

cor.test(daily_row$soil_temperature, daily_row$N2O_ln)
cor.test(daily_row$soil_moisture, daily_row$N2O_ln)
cor.test(daily_row$NH4_ln, daily_row$N2O_ln)
cor.test(daily_row$NO3_ln, daily_row$N2O_ln)
cor.test(daily_row$microbial_biomass_ln, daily_row$N2O_ln)
```

R code of the standardised multiple linear regression analysis presented in Table 4.7:

```
daily_row <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(3) field experiment/stats/daily
data/field_exp_daily_data_row_final.csv")

# standardised multiple linear regression analysis
model_2 <- lm(scale(daily_row$N2O_ln) ~ scale(daily_row$NH4_ln) +
scale(daily_row$NO3_ln) + scale(daily_row$soil_temperature), na.action = na.omit)
summary(model_2)
```

R code of the correlation analyses presented in Table 4.8:

```
daily_row <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(3) field experiment/stats/daily
data/field_exp_daily_data_row_final.csv")

## separating data by treatment
urea <- subset(daily_row, daily_row$treatment == "T1")
control <- subset(daily_row, daily_row$treatment == "T5")
PM <- subset(daily_row, daily_row$treatment == "T6")
BE <- subset(daily_row, daily_row$treatment == "T7")
BC <- subset(daily_row, daily_row$treatment == "T8")
CO <- subset(daily_row, daily_row$treatment == "T9")

## correlation analyses, NH4+
cor.test(control$NH4_ln, control$N2O_ln)
cor.test(urea$NH4_ln, urea$N2O_ln)
cor.test(PM$NH4_ln, PM$N2O_ln)
cor.test(BE$NH4_ln, BE$N2O_ln)
cor.test(BC$NH4_ln, BC$N2O_ln)
cor.test(CO$NH4_ln, CO$N2O_ln)

## correlation analyses, NO3-
cor.test(control$NO3_ln, control$N2O_ln)
cor.test(urea$NO3_ln, urea$N2O_ln)
cor.test(PM$NO3_ln, PM$N2O_ln)
cor.test(BE$NO3_ln, BE$N2O_ln)
cor.test(BC$NO3_ln, BC$N2O_ln)
cor.test(CO$NO3_ln, CO$N2O_ln)
```

Appendix C - Supplementary information (Chapter 5)

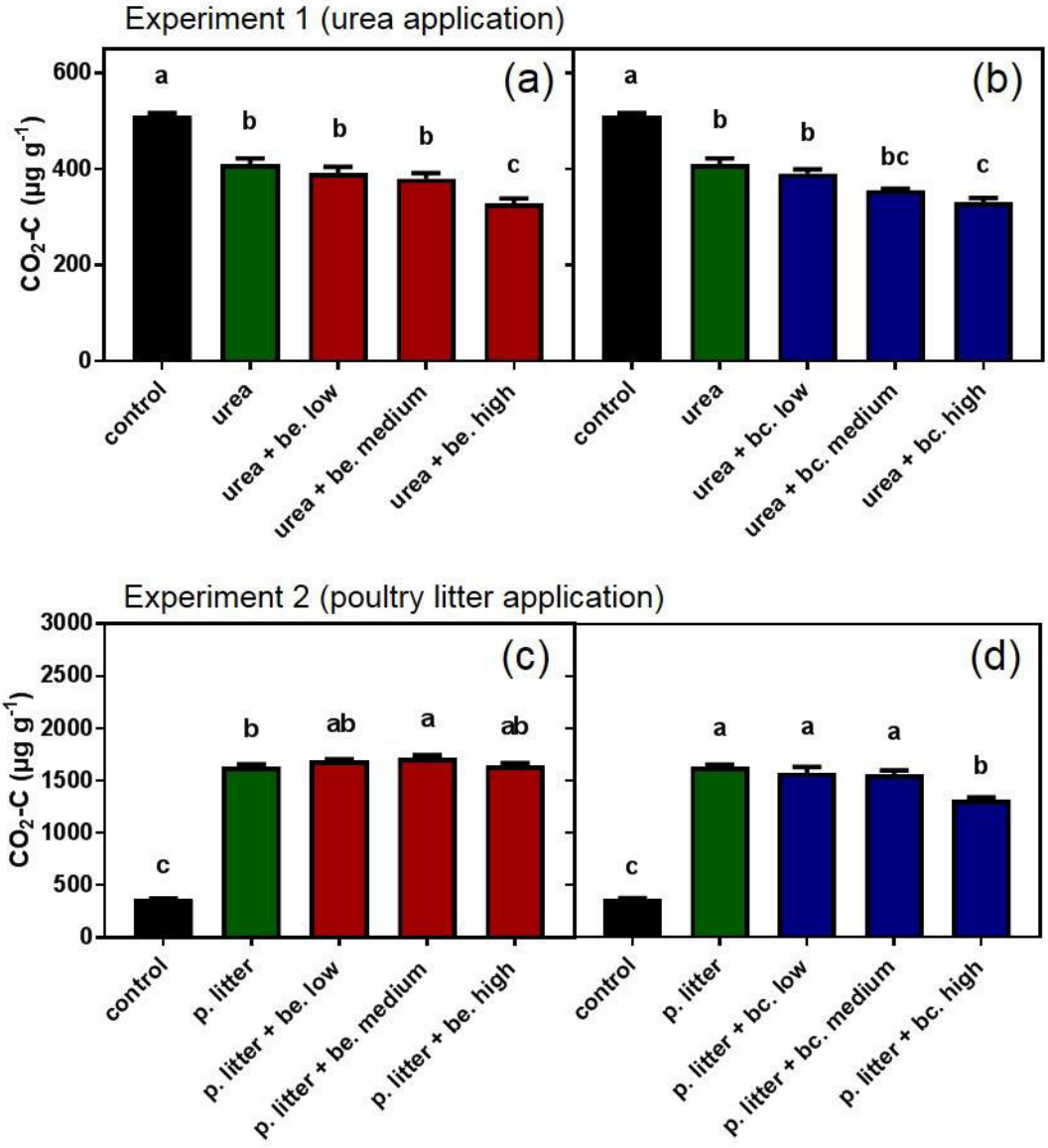

Figure C1. Cumulative CO_2-C fluxes (mean ± SEM), expressed in µg per g dry soil. (a) Urea and bentonite application, (b) urea and biochar application, (c) urea and bentonite application, (d) urea and biochar application. Statistical analyses: one-way ANOVA plus Tukey's HSD post-hoc test. Different letters indicate significant differences at $P < 0.05$ level. Terms 'low', 'medium' and 'high' refer to 1%, 5%, and 10% of dry soil mass respectively.

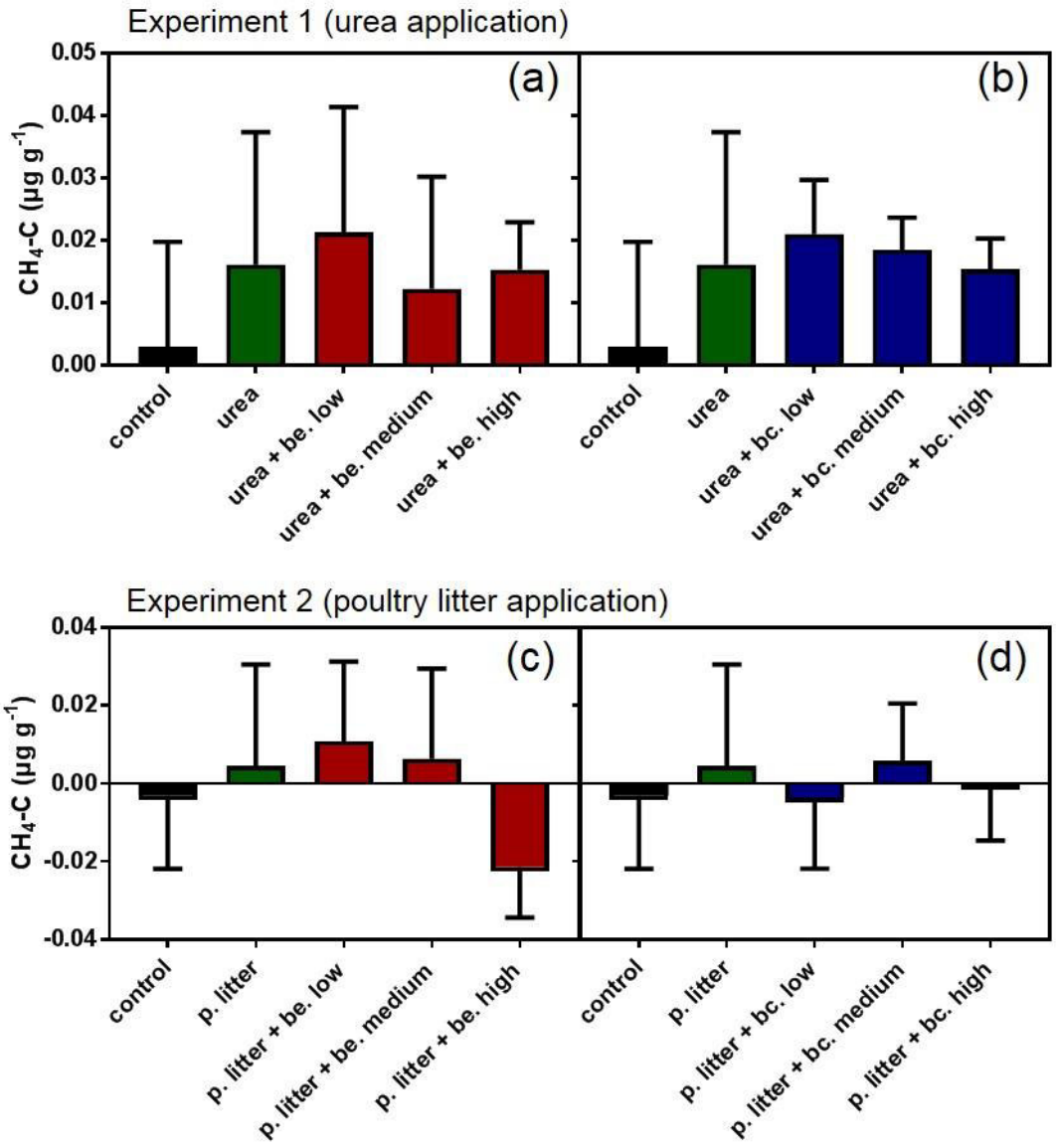

Figure C2. Cumulative CH_4-C fluxes (mean ± SEM), expressed in µg per g dry soil. (a) Urea and bentonite application, (b) urea and biochar application, (c) urea and bentonite application, (d) urea and biochar application. Statistical analyses: one-way ANOVA plus Tukey's HSD post-hoc test. No significant differences between treatments were detected. Terms 'low', 'medium' and 'high' refer to 1%, 5%, and 10% of dry soil mass respectively.

R code for the analysis of cumulative N_2O fluxes presented in Figure 5.3:

```
cum_data <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/cumulative data/exp6 cum
data control subtr.csv")

##separation by experiment and treatment
urea_be <- cum_data[1:16,]
urea_bc <- cum_data[c(1:4,17:28),]
pm_be <- cum_data[29:44,]
pm_bc <- cum_data[c(29:32,45:56),]

## urea, bentonite
urea_be_N2O <- aov(urea_be$N2O ~ urea_be$Treatment)
summary(urea_be_N2O)
TukeyHSD(urea_be_N2O)

## urea, biochar
urea_bc_N2O <- aov(urea_bc$N2O ~ urea_bc$Treatment)
summary(urea_bc_N2O)
TukeyHSD(urea_bc_N2O)

## poultry manure, bentonite
pm_be_N2O <- aov(pm_be$N2O ~ pm_be$Treatment)
summary(pm_be_N2O)
TukeyHSD(pm_be_N2O)

## poultry manure, biochar
pm_bc_N2O <- aov(pm_bc$N2O ~ pm_bc$Treatment)
summary(pm_bc_N2O)
TukeyHSD(pm_bc_N2O)
```

R code for the analysis of N lost as N_2O (in percent) presented in Table 5.3:

```
cum_data <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/cumulative data/N loss as
N2O.csv")

# separation by treatment
cum_data_1 <- subset(cum_data, cum_data$part =="1")
cum_data_2 <- subset(cum_data, cum_data$part =="2")

# part 1
N2O_1 <- aov(cum_data_1$N2O_loss_pc ~ cum_data_1$Treatment)
summary(N2O_1)
TukeyHSD(N2O_1)

# part 2
N2O_2 <- aov(cum_data_2$N2O_loss_pc ~ cum_data_2$Treatment)
summary(N2O_2)
TukeyHSD(N2O_2)
```

R code for the analysis of interaction effects. The code shown here is the code that was used for experiment 1 (urea application). The same code was used for experiment 2 (poultry litter application).

```
daily_data_1 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/daily
data/interaction_part1.csv")

daily_data_1$typeconc=interaction(daily_data_1$type,daily_data_1$conc)

## comment: when I used "typeconc" in the anova command I couldn't see in the
## output whether there is a significant interaction. The output just showed
## "Intercept" and "typeconc". However, the glht command did not accept the
## "type*conc" interaction of variables. Thus, I did a second lme model with   ##
"typeconc" in it.

## NH4+

# NH4+ microdialysis
lme_NH4_mi <- lme(NH4_mi ~ type*conc, random = ~1|day, data = daily_data_1)
summary(lme_NH4_mi)
anova(lme_NH4_mi)
lme_NH4_mi_2 <- lme(NH4_mi ~ typeconc, random = ~1|day, data = daily_data_1)
summary(glht(lme_NH4_mi_2, linfct=mcp(typeconc = "Tukey")))
describeBy(daily_data_1$NH4_mi, daily_data_1$typeconc)

# NH4+ KCl extraction
lme_NH4_ex <- lme(NH4_ex ~ type*conc, random = ~1|day, data = daily_data_1)
summary(lme_NH4_ex)
anova(lme_NH4_ex)
lme_NH4_ex_2 <- lme(NH4_ex ~ typeconc, random = ~1|day, data = daily_data_1)
summary(glht(lme_NH4_ex_2, linfct=mcp(typeconc = "Tukey")))
describeBy(daily_data_1$NH4_ex, daily_data_1$typeconc)

## NO3-

# NO3- microdialysis
lme_NO3_mi <- lme(NO3_mi ~ type*conc, random = ~1|day, data = daily_data_1)
summary(lme_NO3_mi)
anova(lme_NO3_mi)
lme_NO3_mi_2 <- lme(NO3_mi ~ typeconc, random = ~1|day, data = daily_data_1)
summary(glht(lme_NO3_mi_2, linfct=mcp(typeconc = "Tukey")))
describeBy(daily_data_1$NO3_mi, daily_data_1$typeconc)

# NO3- KCl extraction
lme_NO3_ex <- lme(NO3_ex ~ type*conc, random = ~1|day, data = daily_data_1)
summary(lme_NO3_ex)
anova(lme_NO3_ex)
lme_NO3_ex_2 <- lme(NO3_ex ~ typeconc, random = ~1|day, data = daily_data_1)
summary(glht(lme_NO3_ex_2, linfct=mcp(typeconc = "Tukey")))
describeBy(daily_data_1$NO3_ex, daily_data_1$typeconc)
```

R code for the analysis of main effects presented in Table 5.4. The code shown here is the code that was used for experiment 1 (urea application). The same code was used for experiment 2 (poultry litter application).

```
bentonite_1 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/daily
data/bentonite_1.csv")
biochar_1 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/daily data/biochar_1.csv")

## NH4+

# bentonite NH4+ microdialysis
lme_be_NH4_mi <- lme(NH4_mi ~ Treatment, random = ~1|day, data = bentonite_1)
anova(lme_be_NH4_mi)
summary(glht(lme_be_NH4_mi, linfct=mcp(Treatment = "Tukey")))

# bentonite NH4+ KCl extraction
lme_be_NH4_ex <- lme(NH4_ex ~ Treatment, random = ~1|day, data = bentonite_1)
anova(lme_be_NH4_ex)
summary(glht(lme_be_NH4_ex, linfct=mcp(Treatment = "Tukey")))

# biochar NH4+ microdialysis
lme_bc_NH4_mi <- lme(NH4_mi ~ Treatment, random = ~1|day, data = biochar_1)
anova(lme_bc_NH4_mi)
summary(glht(lme_bc_NH4_mi, linfct=mcp(Treatment = "Tukey")))

# biochar NH4+ KCl extraction
lme_bc_NH4_ex <- lme(NH4_ex ~ Treatment, random = ~1|day, data = biochar_1)
anova(lme_bc_NH4_ex)
summary(glht(lme_bc_NH4_ex, linfct=mcp(Treatment = "Tukey")))

## NO3-

# bentonite NO3- microdialysis
lme_be_NO3_mi <- lme(NO3_mi ~ Treatment, random = ~1|day, data = bentonite_1)
anova(lme_be_NO3_mi)
summary(glht(lme_be_NO3_mi, linfct=mcp(Treatment = "Tukey")))

# bentonite NO3- KCl extraction
lme_be_NO3_ex <- lme(NO3_ex ~ Treatment, random = ~1|day, data = bentonite_1)
anova(lme_be_NO3_ex)
summary(glht(lme_be_NO3_ex, linfct=mcp(Treatment = "Tukey")))

# biochar NO3- microdialysis
lme_bc_NO3_mi <- lme(NO3_mi ~ Treatment, random = ~1|day, data = biochar_1)
anova(lme_bc_NO3_mi)
summary(glht(lme_bc_NO3_mi, linfct=mcp(Treatment = "Tukey")))

# biochar NO3- KCl extraction
lme_bc_NO3_ex <- lme(NO3_ex ~ Treatment, random = ~1|day, data = biochar_1)
anova(lme_bc_NO3_ex)
summary(glht(lme_bc_NO3_ex, linfct=mcp(Treatment = "Tukey")))
```

R code of the correlation analyses presented in Table 5.5. The code shown here is the code that was used for bentonite addition in experiment 1 (urea application). The same code was used for biochar addition in experiment 1, bentonite addition in experiment 2 (poultry litter application) and biochar addition in experiment 2.

```
bentonite_1 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/cumulative
data/bentonite_1_cum.csv")

## N2O vs. NH4_mi
cor.test(log(bentonite_1$NH4_mi), log(bentonite_1$N2O))

## N2O vs. NH4_ex
cor.test(log(bentonite_1$NH4_ex), log(bentonite_1$N2O))

## N2O vs. NO3_mi
cor.test(log(bentonite_1$NO3_mi), log(bentonite_1$N2O))

## N2O vs. NO3_ex
cor.test(log(bentonite_1$NO3_ex), log(bentonite_1$N2O))

## N2O vs. H
cor.test(log(bentonite_1$H), log(bentonite_1$N2O))

## N2O vs. bulk_d
cor.test(log(bentonite_1$bulk_d), log(bentonite_1$N2O))

## N2O vs. evap
cor.test(log(bentonite_1$evap), log(bentonite_1$N2O))
```

R code for the analysis of cumulative CO_2 fluxes presented in Figure C1. The same code was used for the analysis of cumulative CH_4 fluxes presented in Figure C2.

```
bentonite_1 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/cumulative
data/bentonite_1_cum.csv")
biochar_1 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/cumulative
data/biochar_1_cum.csv")
bentonite_2 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/cumulative
data/bentonite_2_cum.csv")
biochar_2 <- read.csv("C:/Users/Maren/Dropbox/UQ PhD/PhD
work/experiments/(6) NH4+ sorption experiment/stats/cumulative
data/biochar_2_cum.csv")


##CO2

## urea, bentonite
urea_be_CO2 <- aov(bentonite_1$CO2 ~ bentonite_1$Treatment)
summary(urea_be_CO2)
TukeyHSD(urea_be_CO2)

## urea, biochar
urea_bc_CO2 <- aov(biochar_1$CO2 ~ biochar_1$Treatment)
summary(urea_bc_CO2)
TukeyHSD(urea_bc_CO2)

## poultry manure, bentonite
pm_be_CO2 <- aov(bentonite_2$CO2 ~ bentonite_2$Treatment)
summary(pm_be_CO2)
TukeyHSD(pm_be_CO2)

## poultry manure, biochar
pm_bc_CO2 <- aov(biochar_2$CO2 ~ biochar_2$Treatment)
summary(pm_bc_CO2)
TukeyHSD(pm_bc_CO2)
```