The Effect of Soil Moisture Content and a Nitrification Inhibitor on Nitrous Oxide Emissions from North Queensland Banana Farm Soil

Thesis submitted by:

Emily Sperling

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For Bachelor of Science (Environmental and Marine Geosciences)

Department of Earth and Oceans

In the College of Science Technology and Engineering

James Cook University, Cairns, Queensland

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Abstract

Banana are one of the most demanding crops in North Queensland, with high inputs of nitrogen fertiliser required throughout the year. As agricultural practices intensify and yearly demands on soils increase, there is a push towards best practice management over conventional practices. Best practice strategies aim to enhance soil organic carbon and reduce nitrous oxide (N_2O) emissions, which is particularly important for horticulturally intense crops such as banana. N_2O emissions are of particular importance in North Queensland as the warm and wet conditions are ideal for peak emissions. As there is very little research on mitigating N_2O emissions in tropical climates, it was important for this study to measure the effects of water content and a nitrification inhibitor (DMPP) on soils from a commercial banana farm. N_2O emissions have high temporal and spatial variability. Recognizing this issue, this project was done in the laboratory with undisturbed soil cores collected from the field, with each core treated separately, rather than being homogenized. This laboratory based study aimed to reduce the effects of unwanted variables, and to get a better indication of the individual and combined effects of the different treatments, which would be more difficult in the field. Multiple chemical and physical soil characteristics were measured, including mineral nitrogen, total nitrogen and carbon, pH, water-filled pore space (WFPS) and bulk density. The effect of these characteristics on N_2O and CO_2 emissions, as well as on $\delta^{13}C$ was measured. To determine the impact of management practices on N_2O emissions, two fertilisers (urea and urea with a nitrification inhibitor) and two rates (best practice management rate and the farmer's conventional rate) were used across a range of water contents (40, 60 and 80 % WFPS), and the effect on emission measured. Water content had a significant positive effect on N_2O emissions, which supports the current literature. The use of a nitrification inhibitor did not significantly reduce N_2O emissions. While there was evidence of the nitrification inhibitor working, there was no soil characteristic measured that could explain why the effects were not significant, implying that there were other unmeasured factors affecting emissions. Despite the insignificant results, the nitrification inhibitor did increase the concentration of ammonium (NH_4^+) and reduce the concentration of nitrate (NO_3^-). This is beneficial and expected to reduce leaching as NO_3^- is more mobile in the environment than NH_4^+, and is more readily lost, particularly in wet climates. These results suggest that more research is needed into the effect of a nitrification inhibitor in the tropics, to determine what other factors are affecting their performance.

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1. Introduction

1.1 Significance

Anthropogenic activities have significantly altered the natural nitrogen cycle. Large inputs of nitrogen fertilisers into the biosphere from agricultural practices is one of the main causes of increased nitrous oxide (N_2O) in the atmosphere (Del Grosso and Parton, 2012). N_2O has a global warming potential 300 times that of CO_2, and is a potent greenhouse gas, with an estimated 3 - 5 % of added nitrogen fertilisers lost as N_2O (Scheer et al., 2014b) . The strongest drivers of N_2O emissions are soil moisture and temperature, with increased levels enhancing emission rates (Wang and Cai, 2008). Establishing the optimum percentage of water-filled pore space (WFPS) of agricultural soils will enable lower nitrogen fertiliser applications, resulting in less cost to the farmer and the environment (Signor and Cerri, 2013). Many variables can add to the moisture budget of the soil, and while variables such as climate cannot be controlled, practices such as irrigation can. Controlling soil moisture might help to minimise N_2O loss from soil.

N_2O has both natural and anthropogenic sources. Natural sources include the microbial processes nitrification and denitrification, and anthropogenic sources include fossil fuel combustion, industrial processes and the application of nitrogen fertilisers. N_2O emissions from agricultural activities are predicted to increase around 50 % by the year 2020, due to intensified nitrogen fertiliser and animal manure application (Scheer et al., 2014b). 3, 4-dimethylpyrazole phosphate (DMPP) is a nitrification inhibitor which has been shown to slow down the conversion of ammonium to nitrate. As most nitrogen fertilisers are applied as ammonium, it has had various levels of success at reducing N_2O emissions in the field. The levels of success largely dependent on temperature and percentage of WFPS (Qiao et al., 2015, Scheer et al., 2014b).

Currently, one of the most intensive horticultural crops in North Queensland are banana, with high nitrogen demands (Prasertsak et al., 2001). Tropical regions are especially at risk of nitrogen loss through high N_2O emissions due to a higher percentage of WFPS in the soil, and a higher average temperature (Wang and Cai, 2008). It has been widely accepted that N_2O emissions are closely correlated with the percentage of WFPS in the soil; this is because soil water content effects the metabolic activity of microorganisms. A WFPS of 60 % has previously been established as the threshold between aeration limited and water limited microbial processes in most soils (Bouwman, 1998). Although this percentage is broadly accepted as the average, it has been measured at 70 % and 80 % in other studies, as many variables can contribute to the results (Wang and Cai, 2008). N_2O emissions are affected by soil texture and management, drainage, fertiliser application rates, soil organic carbon content and pH, as well as by WFPS (Signor and Cerri, 2013,

Bouwman, 1998). Plants differ in their nitrogen form uptake and preference. If all nitrogen forms are available, plants will generally prefer to take up NH_4^+ over NO_3^-, as it requires less energy to consume (Song et al., 2015). The main nitrogen source for banana is urea, which is often applied as a fertiliser. Once applied to the soil urea is often quickly converted by enzymes to NH_4^+, which can then be oxidised by microorganisms to form NO_3^- (Zhu et al., 2015).

1.2 Nitrous oxide production, processes and technology

The production of nitrous oxide emissions is associated with three common pathways including the oxidation of NH_4^+, oxidation of organic N and NO_3^- reduction (Zhang et al., 2015a). Emissions are produced mainly through the biological processes of nitrification and denitrification, but also through processes including the reduction of nitrite (Lan et al., 2014), dissimilatory nitrate reduction to ammonia (DNRA) and nitrifier denitrification by ammonia oxidisers (Vilain et al., 2014) *(Figure 1.)*. Nitrification occurs through the oxidation of NH_4^+ to NO_2^- and NO_3^-, whereby N_2O is then produced when the supply of O_2 is limited. The denitrification process involves the reduction of NO_3^- to NO_2^-, then N_2O and N_2, with N_2O as the final product if oxygen conditions are low (Vilain et al., 2014). The nitrification process in soils is thought to include two pathways, following either heterotrophic nitrification or autotrophic nitrification. As heterotrophic nitrifiers can use both inorganic and organic substrates, the N_2O production by heterotrophic nitrifiers is much larger than that of autotrophic nitrifiers (Zhang et al., 2015a). The ratio of N_2O to N_2 is largely affected by the activity of the N_2O reductase, an enzyme which catalyses the final step in denitrification. Activity of this enzyme is controlled by soil pH, O_2 partial pressure and NO_3^-

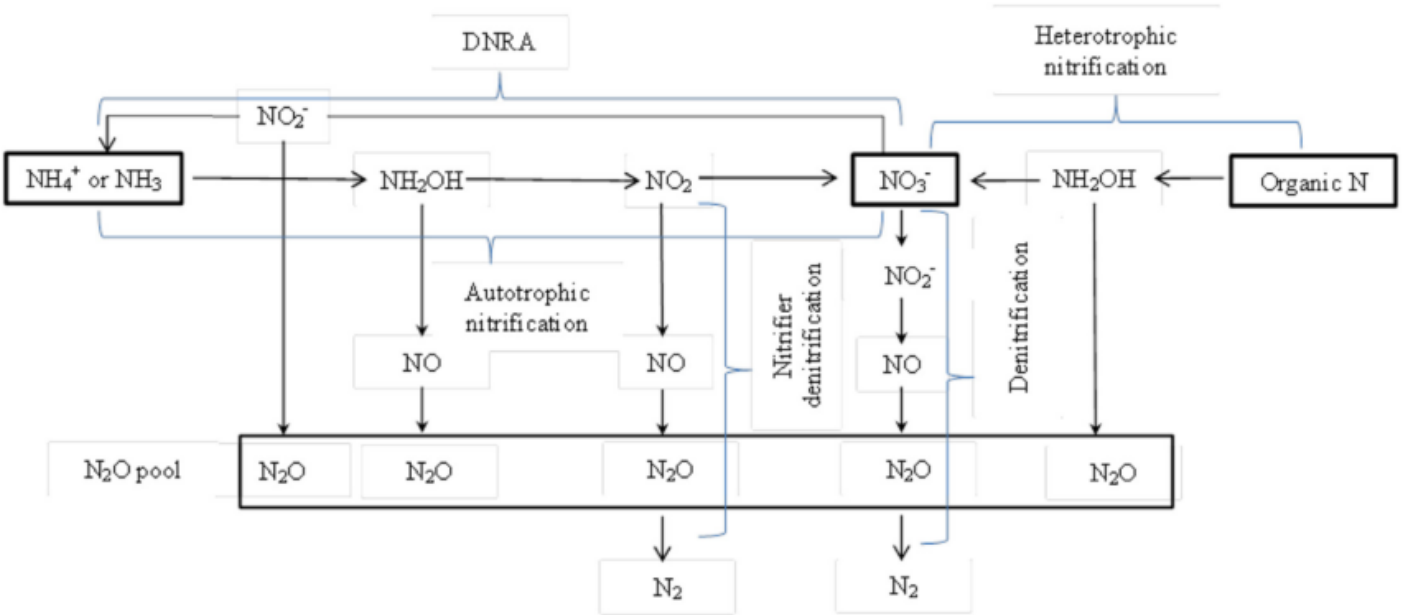


Figure 1. Nitrous oxide processes in soils (Zhang et al., 2015a)

concentration, and also by the number of bacteria in the soil genetically capable of reducing the N_2O to N_2 (Guo et al., 2014). The contributions of nitrification and denitrification to the total N_2O emissions can vary with climate, soil conditions, management, and the availability of O_2 (Vilain et al., 2014)

There is a general consensus in the literature concerning the main drivers affecting N_2O emissions, although the details vary in many studies. Climate plays a large part in controlling N_2O emissions, affecting both temperature and soil moisture content, which have been established as the main drivers of N_2O emissions (Wang and Cai, 2008). In a study by Wang and Cai (2008), it was found that at a high soil moisture content (above 80 % WFPS), the addition of NH_4^+ promoted the production of N_2O, while the addition of NO_3^- decreased the overall N_2O production. There are suggestions in the literature that soil moisture and temperature may play both a synergistic and antagonistic role in influencing the status of other emission drivers (Luo et al., 2013). Results show a substantially varied relationship between soil moisture and temperature across the seasons, with soil moisture showing a larger correlation with N_2O emissions in all seasons except for autumn. This suggests that soil moisture and temperature do not always play equally important roles when other environmental conditions may vary (Huang et al., 2012). The optimum pH to produce N_2O emissions differs between denitrifiying and nitrifiying bacteria, but is generally between a pH of 7 - 8. Despite this, the reduction of N_2O to N_2 is far more reactive to acidic conditions than the reduction of NO_3^- to N_2O, causing a rapid increase in N_2 at a decreasing soil pH (Lesschen et al., 2011, Zhang et al., 2015a, Van Zwieten et al., 2014, Lan et al., 2014). Carbon availability is also thought to be a major driver effecting the production of N_2O emissions. Qin et al. (2014) found that low amounts of organic matter and nitrate in the soil led to high numbers of N_2O reductase enzyme carrying bacteria, thereby completing denitrification as N_2 rather than as N_2O. Van Zwieten et al. (2014) also found that N_2O emissions were low in soils with a high clay content, as a higher clay content has limited available carbon to support denitrification. Although there are large amounts of information available about N_2O production processes, much about the system is still unknown.

Much of the literature places an emphasis on focusing future research into creating better N_2O emission prediction models (Zhang et al., 2015a, HÉNault et al., 2012, Baggs, 2011). Zhang et al. (2015a) highlight the importance of developing new isotope tracing methods to better determine the specific processes and rates in the nitrogen cycle that are directly associated with producing N_2O. They also urge that future research be directed toward better clarifying the connections between nitrogen cycle dynamics and microbial processes, with all of these studies working toward developing an improved understanding of how nitrogen processes operate across a diverse range of soils at different spatial and temporal levels (Zhang et al., 2015a). HÉNault et al. (2012) also have similar suggestions, advising that the spatial and temporal resolution of N_2O fluxes be further researched through the development of faster analysers based on infrared

spectrometry with quantum cascade laser. While many studies believe the focus should be on better analysis and tracing methods, Baggs (2011) describes the potential existence of entirely new N_2O producing soil microbial processes, and urges that focus be put on identifying them. The production of N_2O emissions from soil is a varied process involving many different factors. It is likely that if the variables involved in affecting the emission fluxes are properly identified and accounted for, it could lead to a better understanding of the entire nitrogen cycle (Zhang et al., 2015a).

1.3 The effect of soil moisture on nitrous oxide emissions

There is a consensus in the literature that regardless of soil type and environmental conditions, increased soil moisture content causes a significant increase in N_2O emissions (Kim et al., 2014, Huang et al., 2012) *(Figure 2.)*. As a general rule, a soil moisture content at 60 % WPFS appears to be the limit between aeration-limited and water-limited microbial processes in a wide range of soils (Menéndez et al., 2012), with levels around 70 - 80 % WFPS causing the greatest emissions due to the anoxic environment (Hayashi et al., 2015). While the correlation between N_2O emissions and soil moisture is well documented, the relative contributions of nitrification and denitrification to the emissions are debated (Lan et al., 2014, Toma

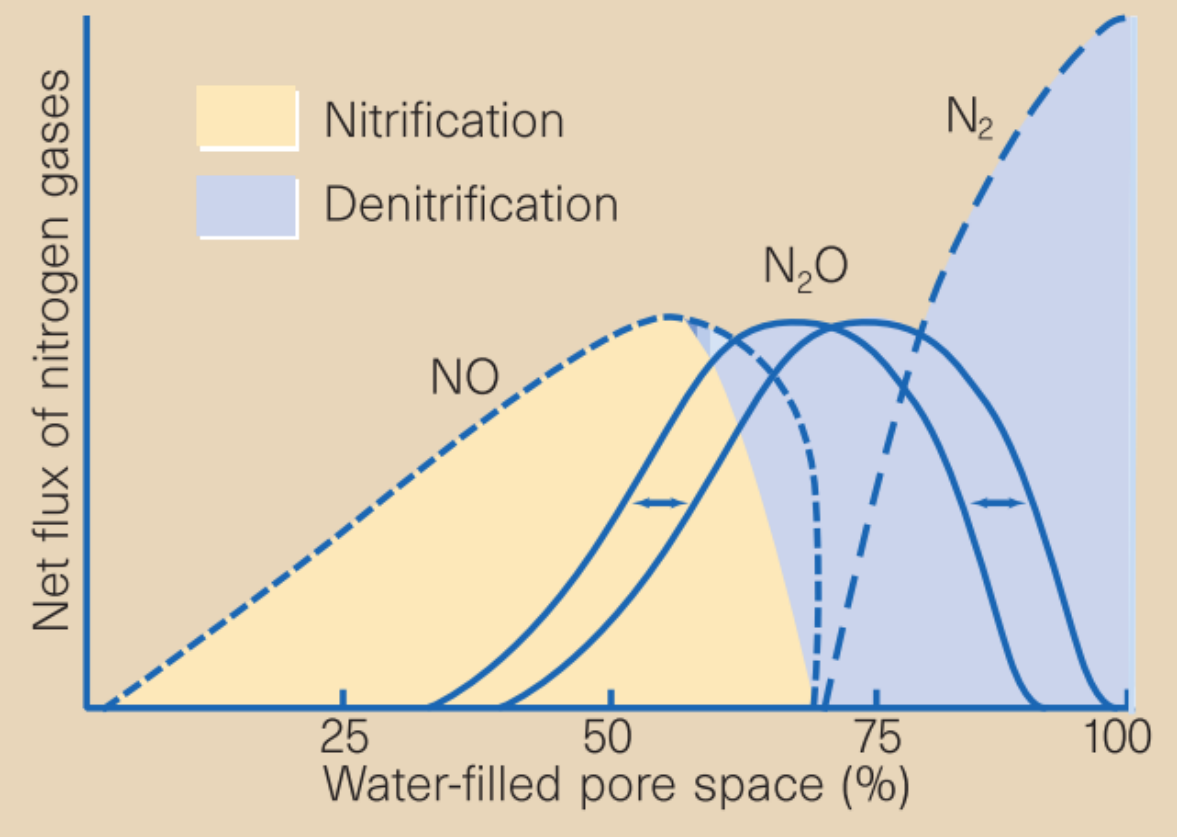


Figure 488. The relationship between water-filled pore space (WFPS) and the flux of nitrogen gases (Bouwman, 1998).

et al., 2011, Wang and Cai, 2008). While some reports indicate that denitrification was the main source of the N_2O emissions (Toma et al., 2011), others state that nitrification contributed more (Lan et al., 2014). These differences in opinion can mostly be explained by the soil moisture content, with aerobic soils (less than 60 % WFPS) promoting nitrification and anaerobic soils promoting denitrification. Despite this, Lan et al. (2014) found that denitrification can frequently occur under overall aerobic conditions. This is thought to be due to the existence of anaerobic microsites in the soil, which could be created either by water saturation in soil aggregates or by microbial growth (Lan et al., 2014). While it is a general rule that denitrification is dominant in anaerobic environments and nitrification dominant in aerobic environments, some studies have found them both operating at conditions well outside their presumed water content optimum.

Soil drying and rewetting is a common occurrence in many climates, with these processes inducing changes in the normal N_2O flux for those environments. Deviations from the normal patterns of N_2O emissions may be explained by the activities of the N_2O reductase in the soil (Guo et al., 2014, Toma et al., 2011). Toma et al. (2011) reported that even at high soil moisture contents (above 80 % WFPS), N_2O emissions were not substantially reduced, suggesting that the N_2O reductase in the soil may still be inactive even at these high soil moisture contents. Yet in a similar study, Guo et al. (2014) reported that the N_2O reductase can increase in activity after prolonged periods of high soil water content, leading to the conclusion that both moisture content and the duration of wetness are factors driving N_2O emissions. During the soil drying phase, N_2O emissions were found to decrease, with a greater degree of drying before re-wetting associated with larger populations of microbial pools of carbon in the soil. This leads to the conclusion that substrate availability can play a major role in the enhancement of N_2O emissions, once dry soils have been re-wet (Guo et al., 2014).

While the climatic zone soil is subject to can't be changed, there are agricultural management practices that may aid in the reduction of N_2O emissions caused by particular climatic conditions. Variations in soil water content across fields can be limited through either irrigation, the reduction of soil bulk density, or another soil aeration technique (Guo et al., 2014). Wang and Cai (2008) make other suggestions involving the better establishment and quantification of the soil moisture contents that nitrification and denitrification can operate in. They also urge that future research be directed towards testing soils using the ^{15}N isotopic technique, an improvement of the acetylene inhibiting technique, and developing an improved method to identify substrates that are regulating the N_2O producing processes. The moisture content in soil can also be substantially affected by other factors such as soil characteristics, crop type and the incorporation of cover crops or residues into the soil (Hayashi et al., 2015). Better understanding the relationships between soil moisture content and N_2O emissions may allow researchers to manipulate factors other than moisture

content to reduce emissions at range of moisture contents, which can be achieved through looking at the interactions of moisture content with other factors. Further research into how these variables can work to reduce or increase soil moisture content is needed, in an attempt to lessen the effect of harsh climates on the soil and subsequent N_2O emissions.

1.4 The effect of cover type/crops on nitrous oxide emissions

Literature surrounding the use of cover crops to reduce N_2O emissions is conflicted, with the effects depending on temperature, soil characteristics, moisture, and the length of time the cover crop has been established (Basche et al., 2014, Abdalla et al., 2013, Lesschen et al., 2011). In general, it has been demonstrated that the application of residues or cover crops with a low C:N ratio can increase N_2O emissions, while materials high in lignin or polyphenols decrease nitrogen availability and hence N_2O emissions (Hayashi et al., 2015, Lesschen et al., 2011). Hayashi et al. (2015) and Lesschen et al. (2011) also both found that the microbial responses to residue applications may have a direct effect on N_2O emissions, with respiration increasing with O_2 consumption, hence promoting anoxia and denitrification. Conventional tillage has also been found to result in greater N_2O emissions (Wang and Dalal, 2015), with emission fluxes increasing immediately after addition of plant material (Scheer et al., 2014a). The actual incorporation of crop residues into the soil has been found to increase N_2O emissions, as does a cover crop which has begun to decompose (Basche et al., 2014). The literature shows contrasting results over the short term, with small fluxes changing depending on soil characteristics, cover crop type and tillage methods. Measurement of N_2O emissions over a wider spatial and temporal range is needed to better establish the effect of cover type on emissions (Basche et al., 2014, Abdalla et al., 2013).

The current consensus surrounding the effect of crop residues and cover crops on N_2O emissions, is that they can enhance emissions to a similar extent as nitrogen fertilisers, although this is subject to much variation (Hayashi et al., 2015). While that is the general understanding expressed in the literature, many studies report that when emissions are measured over a longer period of time (one year) they are around zero. This indicates that there is a balance between periods when cover crops and residues reduce N_2O emissions and when they enhance them, reinforcing the idea that these studies need to be performed over at least a year to establish the net effect of the cover material (Basche et al., 2014). N_2O emissions over short periods (less than a year) are diverse and contrasting amongst the literature, with a huge range of mostly unknown variables thought to cause the differences (Abdalla et al., 2013). Abdalla et al. (2013) discusses the importance of farmers modifying cover crop and residue practices for their particular environmental conditions and soil type. Adopting context specific management systems will help the residues and cover crops reduce N_2O emissions, while enhancing soil health (Hayashi et al., 2015, Abdalla

et al., 2013). Hayashi et al. (2015) recommends a multidisciplinary approach to future research, involving both plant physiology and soil, as there are still many unknown variables effecting N_2O emissions occurring in the rhizosphere. Adopting cover type and crop residue practices can have mixed implications for N_2O emissions, hence why a variety of management practices should be adopted. Future research into understanding the factors causing conflicting results and if other methods can be incorporated into the processes is vital to aid in the success of cover crops and residues at reducing N_2O emissions (Hayashi et al., 2015, Basche et al., 2014, Abdalla et al., 2013).

1.5 The effect of fertiliser rate and type on nitrous oxide emissions

The application of nitrogen fertiliser has long been one of the most effective agricultural management practices to help sustain crop yields (Wang and Dalal, 2015). Nitrogen fertiliser application is believed to be linearly related to N_2O emissions (Kelliher et al., 2014, Tokuda and Hayatsu, 2004), with soil characteristics, seasonal variation, application rates and nitrogen fertiliser type all contributing to differences in emissions (Sakata et al., 2014, Crill et al., 2000). The default values used by the IPCC calculate N_2O emissions as a linear relationship with nitrogen fertiliser application, assuming a constant rate which is not always the case in reality. There are two main types of nitrogen fertiliser used; ammonium- (high in NH_4^+) or nitrate- (high in NO_3^-) based. Urea is generally classed as an ammonium fertiliser as the rapid hydrolysis of urea in most soils leads to the formation of ammonium (Lesschen et al., 2011). Crill et al. (2000) found that of the total N_2O emissions measured in their study, 10 - 40 % could be attributed to the application of fertiliser nitrogen and 60 - 90 % from soil nitrogen. They also found that using nitrogen fertilisers with high NO_3^- concentrations caused higher N_2O emissions than fertilisers with high NH_4^+ concentrations, their findings were later supported by McTaggart and Tsuruta (2003). Despite these findings, multiple studies (Parkin and Hatfield, 2014, Lesschen et al., 2011, Sakata et al., 2014) have found no significant effect of nitrogen fertiliser type on cumulative N_2O emissions, with large differences between ammonium and nitrate fertilisers only occurring during laboratory incubation studies with altered water contents. While the addition of nitrogen fertilisers can dramatically increase N_2O emissions, the results of Toma et al. (2011) suggest that the emission induced by the applied nitrogen fertiliser is small when compared with other forms of nitrogen in the soil, such as organic nitrogen. Therefore the control of N_2O emissions through the use of proper fertiliser practices may be of limited value in regions with high concentrations of soil organic nitrogen, and a high overall microbial available nitrogen pool (Parkin and Hatfield, 2014, Crill et al., 2000).

Due to their effectiveness and relatively low cost, nitrogen fertilisers are used in many agricultural practices, often leading to over use. Iqbal et al. (2015) found in their field test that when nitrogen fertiliser was applied

at a higher rate (225 kg N ha^{-1}) than the recommended (135 kg N ha^{-1}), it resulted in a 16 % increase in mean N_2O emissions. This supports an ongoing trend in the literature stressing the need for site- and crop-specific nitrogen management practices, along with optimized nitrogen use efficiency to help minimise N_2O emissions while maximizing crop yield (Iqbal et al., 2015, Scheer et al., 2014b, Crill et al., 2000, Wang and Dalal, 2015). Wang and Dalal (2015) highlight the need for a reduction in the reliance on synthetic nitrogen fertilisers, stating that future technology needs to be directed at developing optimized nitrogen fertiliser application rates and types which can enhance both soil fertility and economic benefits. Changing the method by which nitrogen fertiliser is applied is also a common theme amongst the literature. Crill et al. (2000) emphasizes the importance of applying granulated forms of nitrogen fertiliser rather than liquid, as granulated fertilisers lead to lower N_2O emissions. Granulated forms coated with nitrification inhibitors are considered the best option for N_2O emission reduction due to their low NO_3^- concentration and also the potential reduction in the number of applications needed (Crill et al., 2000). Correct irrigation practices are also needed to replenish crops when water content is low, but not to the point of saturation which can dramatically increase N_2O emissions (Scheer et al., 2014b). The NO_3^- concentration in the soil following fertiliser addition is a key determinant of N_2O emissions, and reducing these concentrations through the use of nitrification inhibitors has the potential to reduce these emissions. The future of nitrogen fertiliser management needs to focus on evaluating both the economic and environmental cost and benefits of alternate practices to help farmers embrace a management strategy to help sustain soil fertility and reduce the effect they have on the climate system (Scheer et al., 2014a).

1.6 The effect of DMPP on nitrous oxide emissions

The chemical DMPP is one of the most successful nitrification inhibitors used to reduce N_2O emissions from soils. It works by inhibiting the ammonia monooxygenase enzyme involved in the oxidation of NH_4^+ to NO_3^- in soils. This inhibition directly reduces the nitrification rate and hence the NO_3^- concentration which is a vital step in denitrification (Ruser and Schulz, 2015). A global meta-analysis has suggested that nitrification inhibitors such as DMPP are capable of reducing N_2O emissions by between 31 - 44 % in agricultural systems (Lam et al., 2015, Qiao et al., 2015), with Lam et al. (2015) finding a reduction of 37 - 44 % in their study. Scheer et al. (2014b) also found a large reduction in N_2O emissions, with a 75 % reduction, much larger than the 50 % reduction that standard practice advocates. A study by Soares et al. (2015) even found reductions in N_2O emission greater than 90 % when nitrification inhibitors were added to urea. While other methods of emission reduction have been tried, no other method has so consistently reduced N_2O emissions as nitrification inhibitors have, in particular DMPP (Ruser and Schulz, 2015). It has been found that as well as inhibiting nitrification, DMPP increases the NH_4^+ concentration in the soil,

thereby reducing the concentration of NO_3^-. This change in dissolved nitrogen composition drastically reduces nitrogen loss via leaching, as NO_3^- is more readily lost than NH_4^+ (Qiao et al., 2015, Zhang et al., 2015a).

While there are many nitrification inhibitors on the market, DMPP remains the most widely used due to its desirable properties and positive effect on soil characteristics (Soares et al., 2015, Lam et al., 2015). DMPP has been shown more efficient than a rival nitrification inhibitor DCD, as DCD is more mobile in the environment, hence more readily lost, as well as degrading faster over time (Soares et al., 2015). Differences in the effectiveness of DMPP across multiple studies is thought to be attributed to different environmental conditions such as soil temperature and moisture content, which may influence the microbial population in the soil (Lam et al., 2015). Soil water content has been found to change the persistence of the DMPP molecule in the soil, causing the molecule to degrade slower in high water content environments. Changes in soil temperature is also thought to influence the effectiveness of DMPP, with studies showing a decrease in efficiency at increasing soil temperatures above 20 ˚C where the molecule can become unstable (Menéndez et al., 2012, Kleineidam et al., 2011, Subbarao et al., 2006). Nitrification inhibitors can also have other effects on soil characteristics such as increasing soil pH, which can be used to help combat soil acidification (Qiao et al., 2015). Long term studies have established that the continuous use of nitrification inhibitors does not impair the ability of the inhibitor to reduce N_2O emissions (Soares et al., 2015), with many studies reporting that the use of nitrification inhibitors actually increased the overall crop yield (Zhang et al., 2015b, Lam et al., 2015). Although nitrification inhibitors have the potential to considerably reduce N_2O emissions, more studies are required to define exactly how the molecules behave in the ecosystem and their long term effects on the overall environment (Ruser and Schulz, 2015).

DMPP has been one of the most intensively studied nitrification inhibitors on the market, with research mostly focused on its effect on emission reduction, plant available nitrogen and crop yields, in which very few negative effects have been found. Despite all of the research on DMPP, there is very little information on the long term effect the molecule may have on the biological populations in the soil (Ruser and Schulz, 2015). This is a topic beginning to appear in recent literature, with most concerns surrounding the possibility of tolerant microbial populations caused by frequent applications of nitrification inhibitors (Scheer et al., 2014b, Wakelin et al., 2013). While a study by Wakelin et al. (2013) found that short interval (57 days) applications of the nitrification inhibitor DCD did not initiate any form of resistance, they urged the need for long term studies. Ruser and Schulz (2015) also stress the need for long term studies, as they also raise the issue of the molecules having a potentially negative effect on non-target organisms in soils with a long history of nitrification inhibitor application. As it has recently been determined that nitrification inhibitors, in particular DMPP, can have a negative impact on some fungal populations, this further increases the need

for more research (Ruser and Schulz, 2015, Maienza et al., 2014). There is also a notable lack of research into the N_2O emission reduction potential in warm, humid areas with frequent rainfall events, as well as regions which experience intense frost/thaw cycles (Ruser and Schulz, 2015, Scheer et al., 2014b, Cavigelli et al., 2012). Although DMPP and many other nitrification inhibitors have been established to be safe and effective in the short term, more research needs to investigate their long term environmental effects, and if new soil and fertiliser management practices and technologies could help to mitigate future potential problems.

1.7 Research Objectives

This project intends to provide insight into the complex nature of the N_2O producing processes on soils from a North Queensland banana farm. It is the aim of this project to provide information and conclusions as to the effect of varied fertiliser treatments and water contents on the studied soil. It also aims to provide nitrogen management practices to minimise nitrogen costs to the environment and the farmer.

The two main aims of this project were to establish, on soils from a North Queensland banana farm;

- The effect of soil moisture content on N_2O emissions
- The efficiency of DMPP at reducing N_2O emissions

2. Methods

2.1 Field site and sampling

The soil cores used in this study were collected from a field trial located in East Palmerston, Queensland, Australia (17.59 ˚S, 145.83 ˚E). This trial was established in November of 2014 for a project entitled "Validation of greenhouse gas reduction methods in tropical perennial cropping systems across Queensland, Northern Territory and Western Australia", funded by the Carbon Farming Initiative. The soil has been classified as a Pin Gin soil association (Murtha et al., 1996). The climate in this region has been defined as tropical by the Bureau of Meteorology, with mean maximum and minimum temperatures of 31.2 and 15.1 ˚C. Annual rainfall averages around 3325 mm with March as the wettest month (http://www.bom.gov.au/climate/averages/tables/cw_032037.shtml).

The field site was described by Neil Enderlin of the Department of Natural Resources and Mines in July of 2014. The landform pattern is comprised of low hills with highly permeable and well drained basalt lithology. Slope ranges from 1 to 8%, with the average soil texture a light to medium clay, with granular weak 2-5mm structure. The soil pH ranges from 5.7 to 4.7 at 0 to 0.1 meters, decreasing to a pH of 4.5 at 0.9 to 1.5 meters below the soil surface.

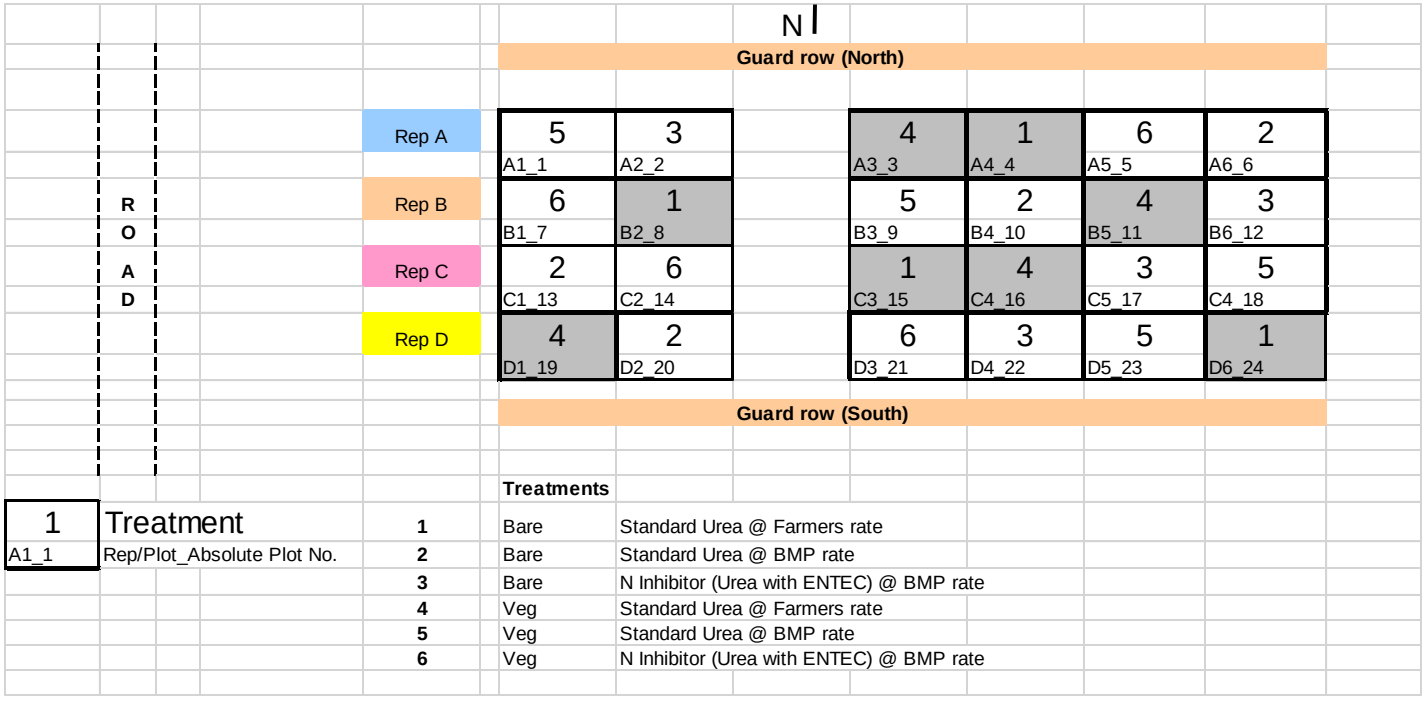


Figure 489. Trial plan for the East Palmerston site. Soil cores were taken from plots having treatments 1 and 4, highlighted in grey. There are approximately 10-13 Cavendish plants in each row and each row is a replicate. Total plot area is approximately 10-13 plants by 6 rows.

The site has single rows of Cavendish banana plants with 6 treatments *(Figure 3.).* Plant guard rows were grown to the north and south of the site and were not sampled. The trial has a factorial design, with two factors: (bare or vegetated) and fertiliser (standard farmer's rate, standard urea at the best practice management rate, and urea with ENTEC at best management practice rate).

2.2 Soil collection from field site

Undisturbed soil cores were taken from the field site on three separate occasions; the 27th of April and the 1st and 29th of July. Soil was always taken from the site at the end of month just before the area was sprayed or fertilised. The method for soil collection was the same for all sampling days.

Soil cores were taken from into two different zones, the inter-row and row. The inter-row samples were taken from the center of the space between the rows, and the row samples were taken from on top of the mounds along which the banana plants grew from *(Figure 4).* Soil cores for Experiments one and two (the first soil collection day) were evenly collected from treatments one and four, and from the inter-row near these treatments. Bulk density cores were also taken on this day, with three from each of the vegetated treatment row, bare treatment row and from the inter-rows between them. Soil cores used in Experiments three and four (the second and third soil collection days) were also collected from treatments one and four, but inter-row samples were not taken.

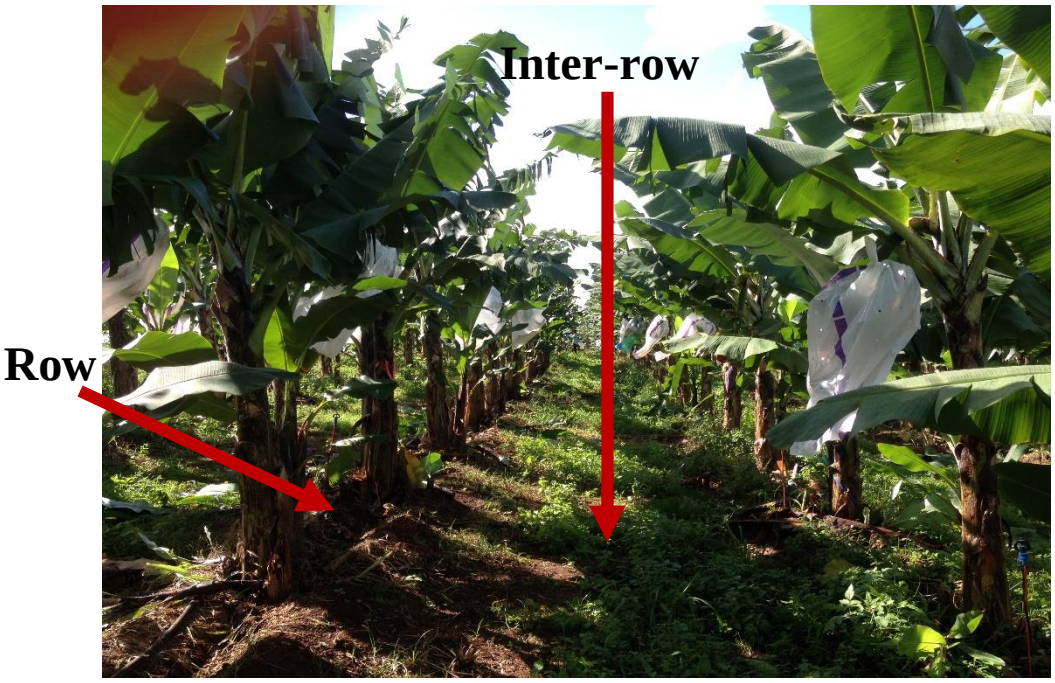


Figure 876. The row and inter-row sections of the field site.

The cores were collected by pushing PVC tubes *(Figure 5.)* 100 mm into the ground before twisting and removing. After excess roots were trimmed with scissors, an acrylic plate was duct-taped to the bottom. When necessary, the top of the tube was capped with a PVC cap housing a septum.

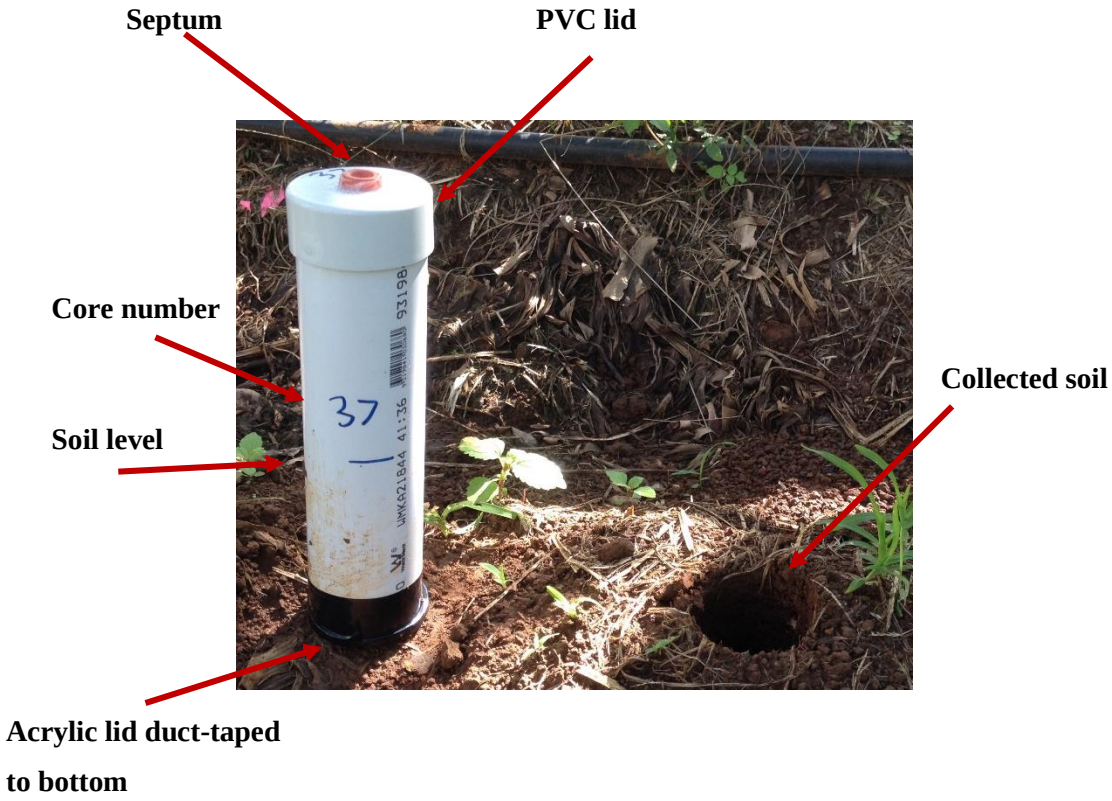


Figure 1326. PVC chamber after collecting soil. 200 mm tall by 50 mm wide.

2.3 Soil characterisation

Once back at James Cook University (Cairns campus), each chamber was weighed to estimate the field water content and the lids were removed. The chambers were then left to air dry until the water contents reached an appropriate level to which the various treatments could be applied.

2.3.1 Bulk density

During the first sampling trip, cores were taken using 100 mm diameter x 50 mm deep steel rings, for determination of bulk density and total porosity. Two rings were hammered into each sample area to obtain cores from 0-50 and 50-100 mm depth. Cores were taken from three areas under each cover type (vegetated, bare and inter-row), resulting in a total of 18 cores. In the laboratory the 18 cores were

weighed, then dried in an oven at 105 ˚C for 48 hours, and re-weighed. The bulk density (BD) was calculated by the formula

BD (g/cm^3) = Dry soil mass (g) / soil volume (cm^3) *Equation 1.*

2.3.2 Water filled pore space (WFPS)

The gravimetric water content (θ_g) of the soil cores was determined by the formula

θ_g (%) = ((wet soil mass (g) - dry soil mass (g)) / dry soil mass (g)) *100 *Equation 2*

Total porosity (φ_t) was calculated, assuming a particle density of 2.65 g/cm^3 and BD was in the same units, by the formula

φ_t (%) = (1 - (BD / 2.65)) *100 *Equation 3*

Volumetric water content (θ_v) of the soil was determined by the formula

$\theta_v = BD * \theta_g$

Air-filled porosity (Φ_a) of the soil was determined by the formula

$\Phi_a = \varphi_t - \theta_v$ *Equation 4*

Water-filled pore space (WFPS) of the soil was calculated by the formula

WFPS (%) = (θ_v / φ_t) * 100 *Equation 5*

2.4 Experimental design

2.4.1 Experiment one: Time course of N_2O emission

The aim of Experiment one was to determine the best durations for the incubation and for gas sampling times (to determine gas emission rates) for the subsequent Experiments. Nine soil chambers taken on soil collection day one were used in this Experiment, with three from each cover type; vegetated, bare and inter-row. A urea fertiliser solution was applied at a rate of 0.0687 g of urea per core dissolved in 10 mL of deionized water. This amount was equivalent to the farmer's standard fertiliser application rate of 360 kg N/ha/yr. The water content of each chamber was brought to a water-filled pore space (WFPS) of 80 % by pipetting distilled water over the soil surface until the desired total weight (hence water content) was

reached. In this and subsequent Experiments the water content was changed after the urea application to wash the urea into the soil.

Gas emission rates were measured at 0, 3, 24, 48 and 144 hours after treatment addition (water and fertiliser). Starting at each of those times, emissions were measured over 0-5, 5-10, 10-30 and 30-60 minute-periods, opening the chamber briefly to vent between each period. Between measurement periods the chamber lids were left off.

2.4.2 Experiment two: Variability of N_20 emission

The aim of Experiment two was to determine the variability of the N_2O emission rate among soil cores, to establish the number of replicates for the subsequent Experiments. Fifty one soil chambers from the first soil collection day were used, consisting of 17 from each cover type; vegetated, bare and inter-row. Urea and water were added as described for Experiment one.

Based on the results of Experiment one, it was decided that gas emission would be measured during a 10-minute period 24 hours after treatment additions. At the end of the Experiment 10 of the cores (representing the full range of N_2O emission rates) were air-dried, crushed and sieved to <2 mm and sent to the DSITIA laboratory in Brisbane for the analysis of mineral nitrogen.

2.4.3 Experiment three: Effect of water content and fertiliser rate

The aim of Experiment three was to determine the effects of water content, fertiliser rate and their interaction on N_2O emission. The Experiment was set up in a factorial design, with 3 levels of water content (40, 60, 80 % WFPS) and two levels of urea fertiliser rate (best practice at 0.0289 g per core and farmers rate at 0.0687 g per core). Water and urea were added as described for Experiment one. The urea was added as a 20 g/L solution with 1.445 mL applied to the best practice treatments and 3.435 mL applied to the farmer's rate treatments. Ten replicate chambers were used, based on the results of Experiment two, resulting in a total of 60 experimental units (cores).

It was hypothesised that the treatments might change the time course of N_2O emissions, so gas emissions (over a 10-minute period) were measured at two times, at 24 and 48 hours after addition of urea and water. The gas emission measurements were made in the same way as in Experiment two. At the end of the Experiment each core was thoroughly homogenized and subsamples were analysed for water contents, pH and total N and C contents. Other subsamples were frozen for later analysis of mineral N content.

2.4.4 Experiment four: Effect of water content and nitrification inhibitor

The aim of Experiment four was to determine the effects of water content, fertiliser type and their interaction on N_2O emission. The Experiment was set up in a factorial design similar to Experiment three, with 3 levels of water content (40, 60, 80 % WFPS) and two levels of nitrification inhibitor (with and without). The nitrification inhibitor used in this Experiment was 3, 4-dimethylpyrazole phosphate (DMPP), known commercially as ENTEC®. Urea (either standard urea or ENTEC®-urea) was added to all cores as 5 mL of a 5.74 g/L solution (i.e. the best practice rate of 0.0287 g per core). Water content was then changed as per the previous treatments.

Gaseous emissions were measured over a 10-minute period at 24 and 48 hours after the urea and water were added. At the end of the Experiment each core was thoroughly homogenized and subsamples were analysed for water contents, pH and total N and C content. Other subsamples were frozen for later analysis of mineral N content.

2.5 Analytical methods

2.5.1 Gas emission measurements

To measure gas emission rates, a closed headspace was created above the soil core by placing a cap on the PVC tube, and taking one gas sample immediately and a second after a period of closure. The same type of equipment was used in all Experiments. Syringes used to withdraw the samples were 20 mL NIPRO syringes with 25G 0.5x16mm BD PrecisionGlide needles. Syringes and needles were both replaced after each Experiment, with the needles often also replaced mid Experiment due to breakages. The butyl rubber septa used in the chamber lids were sealed onto the lid with Selleys roof silicone to prevent gas leakage. The septa were replaced after Experiment one. The inside edge of the chamber lids was coated with RS silicone grease to minimise gas leakage and increase ease of lid removal. Throughout each Experiment the soil chambers were left in the laboratory at a relatively constant temperature and the exact temperature during the gas sampling period was measured.

To ensure that the air inside the cores was not pressurized while the lids were closed, a syringe needle was inserted through the septum during closure. Before taking a headspace gas sample, the empty syringe was inserted and plunged three times to promote mixing of air in the headspace above the soil. In Experiment one, 20 mL of the headspace gas was withdrawn and reinjected each time. However, this procedure caused considerable changes in headspace pressure, which would change the concentration gradients in the core,

so in the subsequent Experiments only 3 mL was withdrawn and reinjected. Following this headspace mixing, a 20 mL sample of gas was drawn out of the headspace and into the syringe, and then, the syringe was depressed to 15 mL and the remaining 15 mL of sample was immediately injected into a labelled 12 mL, pre-evacuated glass Labco Exetainer vial with Teflon septum. The gas samples were analysed for CO_2 and N_2O on a Shimadzu GC2010 gas chromatograph, and emissions were calculated based on increase over time in the amount of gas in the headspace.

2.5.2 $\delta^{13}C$ of emitted CO_2

The remaining gas from Experiment three and four was run through a Picarro cavity ring-down spectrometer for the analysis of $\delta^{13}C$. The $\delta^{13}C$ values (in ‰ relative to Vienna Pee Dee Belemnite) results were then plotted against the inverse of the CO_2 concentrations (measured using the gas chromatograph) in Keeling plots. $CaCO_3$ and $NaHCO_3$ standards with $\delta^{13}C$ values of -35.27 and -6.02 respectively, were used to calibrate the data. The intercept values from each treatment, which were taken to be the $\delta^{13}C$value of the CO_2 emitted from the soil, were then compared. More information on the sampling and analysis procedure is given by Munksgaard et al. (2013).

2.5.3 pH

Soil pH was measured using a 1:5 ratio of soil to distilled water. After the addition of water, the samples were shaken by hand for approximately one minute before a glass pH electrode was suspended into the solution.

2.5.4 Mineral nitrogen

Sub samples taken from Experiment three and four were analysed for mineral nitrogen content using the method detailed in Rayment and Lyons (2011). 2 g dry soil weight was placed into centrifuge tubes with 20 mL of 2M KCl solution. The tubes were then turned end over end for one hour before being centrifuged at 3000 rpm for 5 minutes. 10 mL aliquots of extract were then refrigerated and sent to TropWater in Townsville for analysis of nitrate, ammonium and urea.

2.5.5 Total nitrogen and carbon

Total N and C contents were measured on a vario MAX cube Elementar using the Dumas combustion method. Due to time constraints and lack of materials not all samples were able to be analysed. A similar

proportion of samples from each treatment was however analysed, so the results are shown in subsequent sections.

2.6 Statistical analysis

Statistical analysis was completed using S-PLUS 8.0 and Microsoft Excel 2013. Treatment effects were analysed using three-way ANOVAs and relationships between measured variables were analysed using Pearson correlation. $\delta^{13}C$ results were analysed using the data analysis regression function on Excel.

3. Results

3.1 Experiment 1

Experiment 1 was carried out to determine the most effective incubation times for measuring N_2O and CO_2 emissions to help design the subsequent Experiments. There was no clear difference in N_2O emissions between the vegetated and bare cover types, and emissions were most variable in the inter-row *(Figure 6.)*. However, there were insufficient replicates to determine whether or not the differences were significant. Emissions were low at 0 and 3 hours, peaked at 24 or 48 hours, and lowered at 144 hours. CO_2 emissions increased more rapidly in the vegetated cover type than the bare cover, rapidly increasing from 0 and 3 hours, before reaching a peak at 24 hours *(Figure 7.)*. CO_2 emissions then decreased to a stable rate, close to the intial rate at 48 to 144 hours.

The emission rates (i.e. the change in concentrations over the period of chamber closure) showed little change between the 0-5 minute closure time and the 30-60 minute closure time, although they did tend to decline as closure time increased *(Figure 8.)*. Emission rates were sufficiently high to detect using a 0-5 minute closure time. From this Experiment it was established that there was no need to extend the incubation time longer than 48 hours after water and urea were added, nor to extend the emission measurement time (i.e. the chamber closure time) longer than 10 minutes.

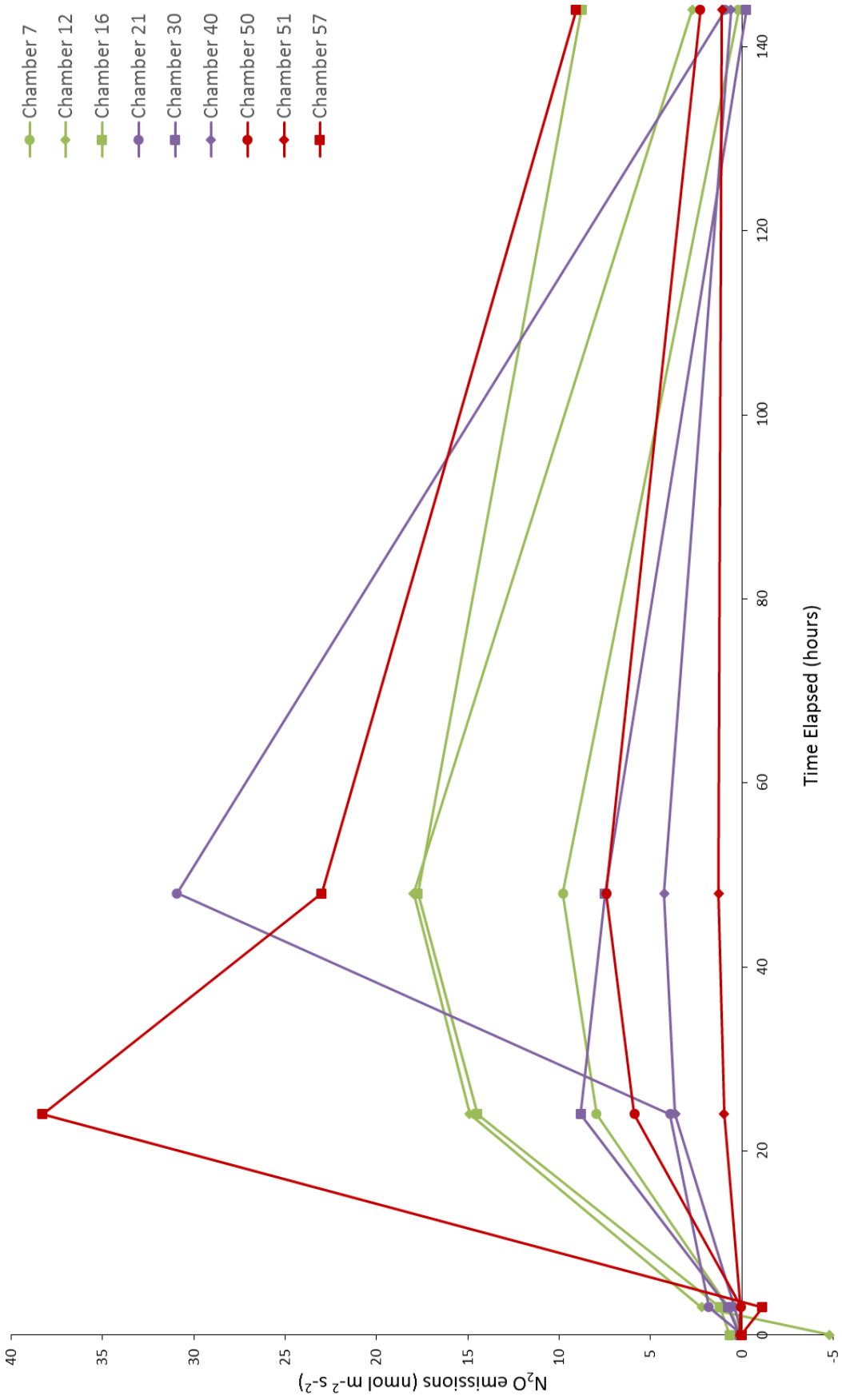


Figure 1454. N_2O emissions from 3 different cover types calculated from the first 5-minute closure period at each measurement time. Green is vegetated, purple is bare and red is inter-row.

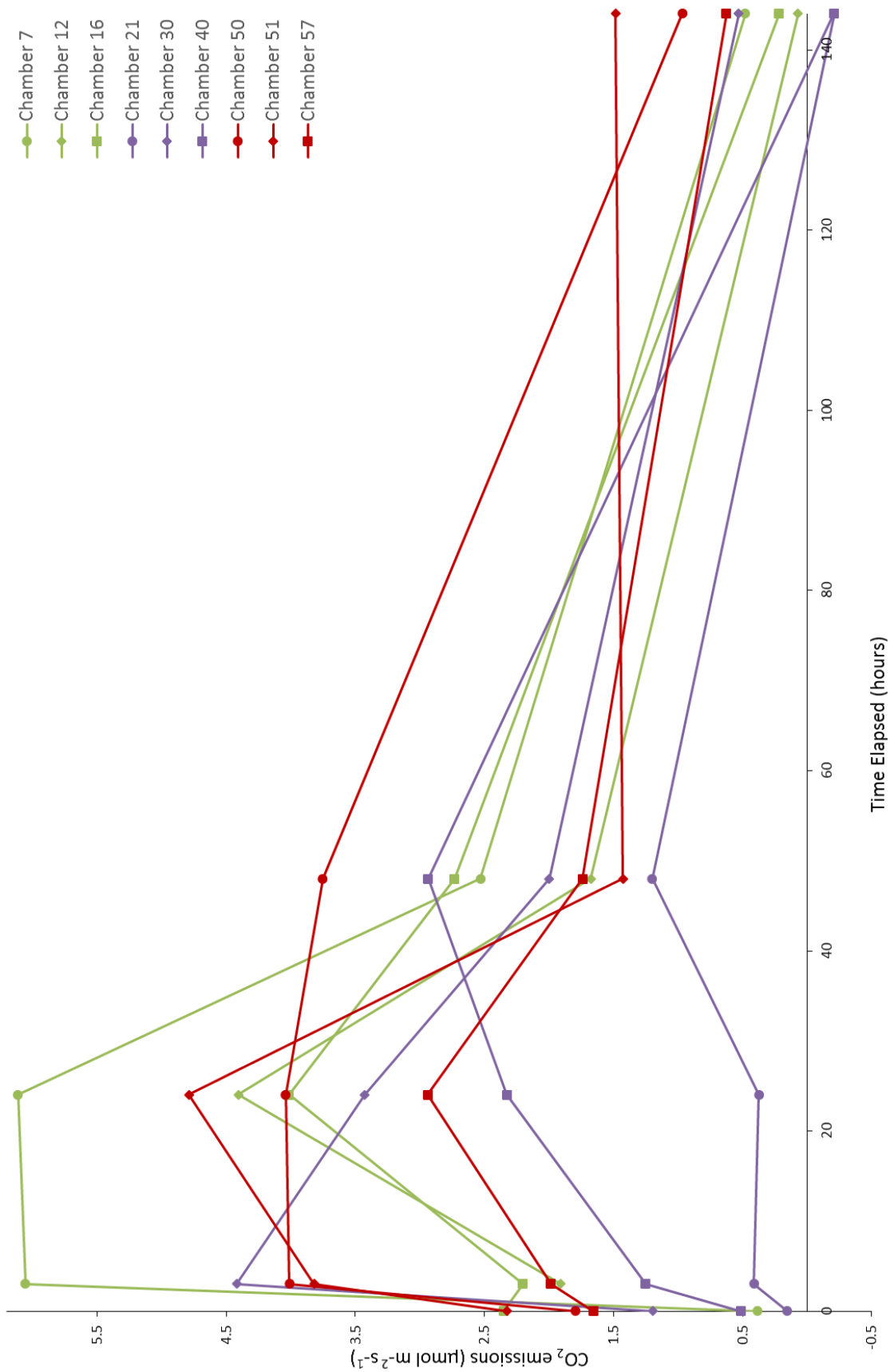
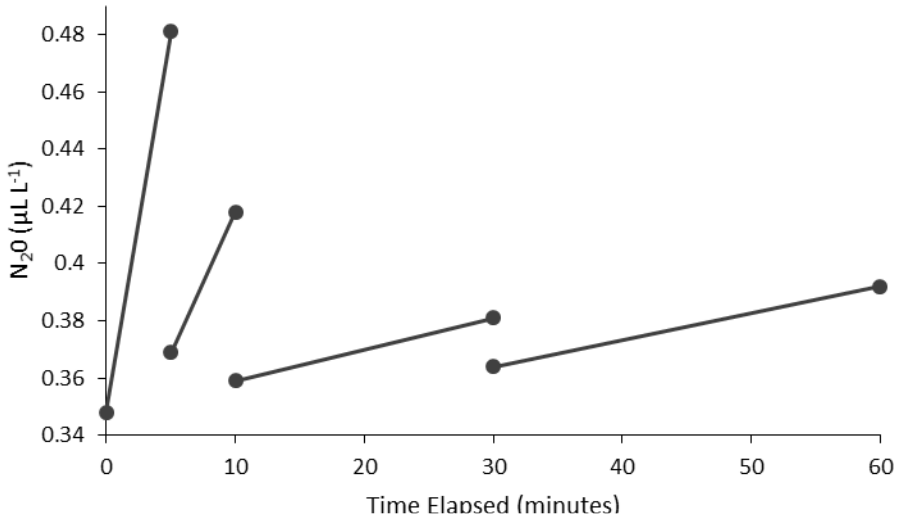


Figure 7. CO_2 emissions from 3 different cover types calculated from the first 5-minute closure period at each measurement time. Green is vegetated, purple is bare and red is inter-row.



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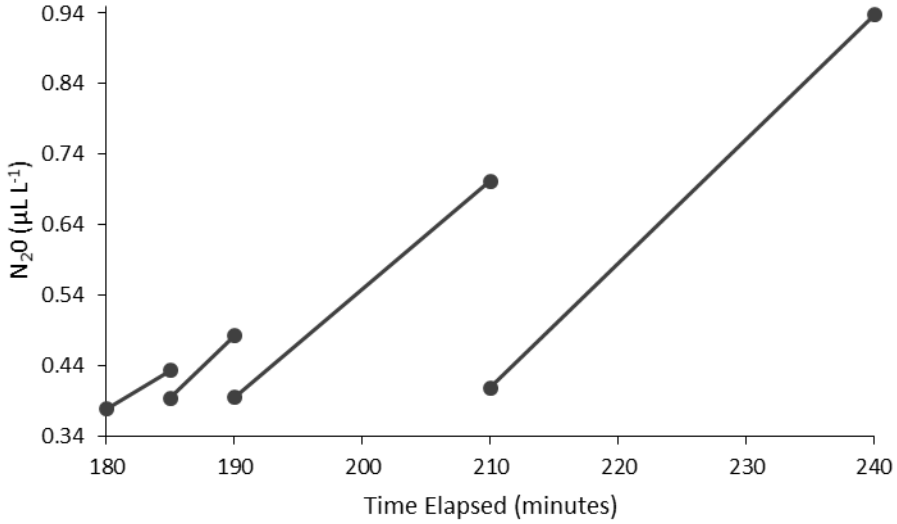

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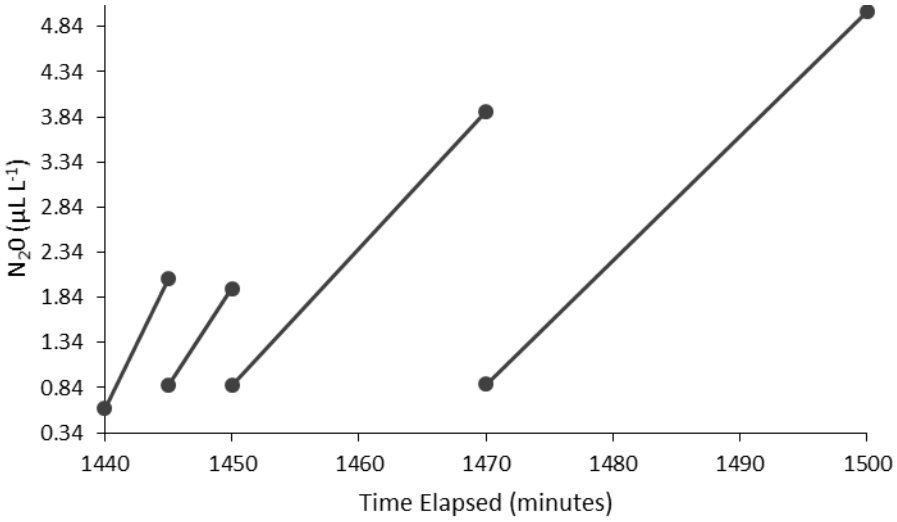


Hour 24

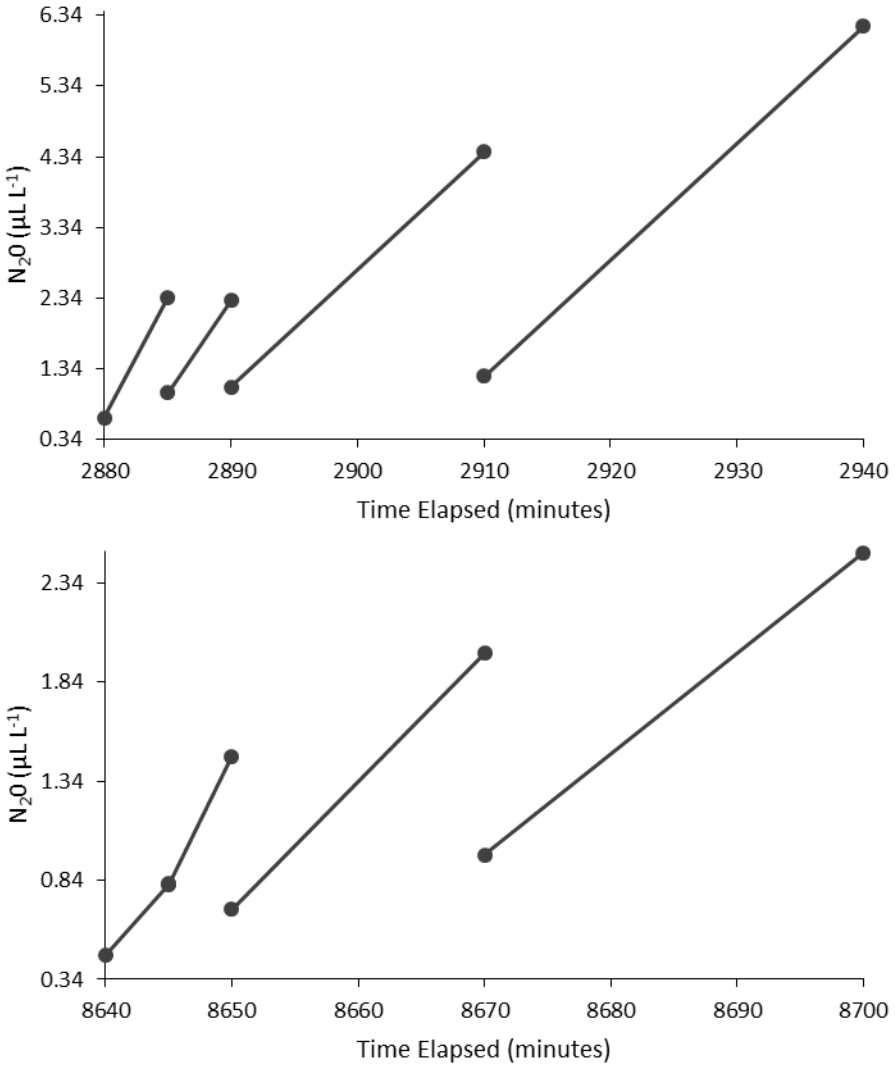


Figure 8. Chamber headspace N_2O concentration at each sampling hour over 144 hours in chamber 16. Chamber 16 was chosen as it best represented the overall trend of all of the chambers sampled in experiment one.

3.2 Experiment 2

Experiment 2 was carried out to identify the number of replicates needed for the subsequent Experiments. A large proportion of the chambers had emission rates on the lower end of the scale, with very few toward the higher end *(Figure 9.)*. Only two chambers had N_2O emission rates above 166 nmol m^{-2} s^{-1}. The highest frequency of chambers was in the 16 - 30 nmol m^{-2} s^{-1} category. From this Experiment it was established that the vegetated and bare cover types would be pooled, as there was no significant difference between them (with a p-value of 0.095), and that the inter-row would not be sampled in Experiment 3. Experiment 2 indicated the N_2O emissions were highly variable ranging from 3.62 to 312.07 nmol m^{-2} s^{-1} of N_2O, but with 86 % of the emissions occurring between the < 15 and 76 - 90 categories. Using the equation (Garcia et al., 2001) (*Equation 6.)* it was established that 10 replicate chambers were required in Experiment 3 and 4 to yield a precision of 0.05 µmol m^{-2} s^{-2} of N_2O or less.

No. of replicates = 2 * (1.96 + 0.84) * (σ/0.05) ^2 *Equation 6.*

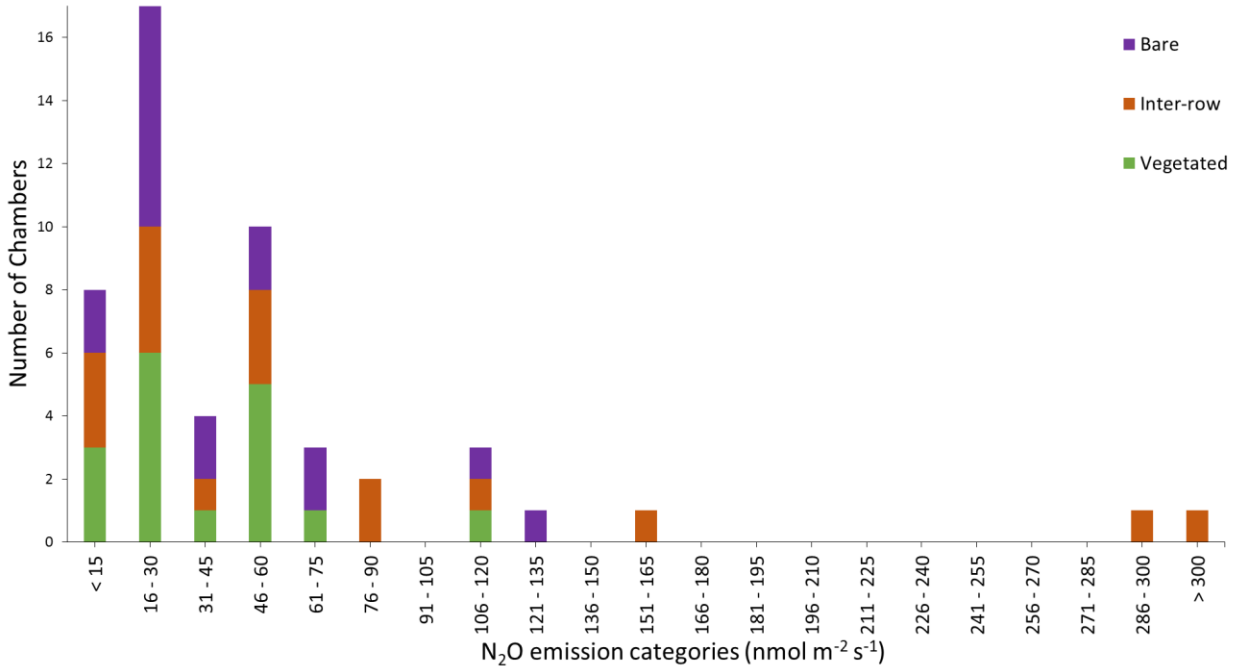


Figure 9. Number of chambers per N_2O emission category.

The 10 samples chosen for NH_4^+ and NO_3^- analysis displayed a wide variety of values *(Table 1.)*. Neither the N_2O emission nor NH_4^+ and NO_3^- values had any significant relationship (p = 0.31) to each other.

Table 1. Mineral nitrogen concentrations and N_2O emission rates of the subsample of 10 cores.

Chamber	NH_4^+ (N/mg/kg)	NO_3^- (N/mg/kg)	N_2O emission ($nmol/m^2/s$)
17	91	280	9.5
8	17	181	10.5
22	37	149	13.9
45	45	91	43.0
41	18	148	54.2
44	67	74	78.6
31	40	123	110.3
24	25	184	132.4
42	16	104	225.7
47	24	118	312.1

3.3 Experiment 3

The N_2O emissions were significantly influenced by water content and fertiliser rate treatments only *(Table 2.)*. Water content and fertiliser rate both had highly significant positive effects on N_2O emissions *(Table 2.)*, and none of the treatment effects were significant. The CO_2 emissions were significantly influenced by all 4 treatment variables, with time and water content highly significant *(Table 2.)*. The CO_2 emissions were highest with the vegetated cover type, high N rate and lower water contents and at 24 hours rather than 48.

Table 2. Experiment 3 significance table displaying p values for the effects of the main treatment effects on N_2O and CO_2 emissions, using a 4-way ANOVA. Significant values (p=<0.05) are shown in bold. None of the interactions between treatments were significant.

Treatment	N_2O	CO_2
Water content	**0.00**	**0.00**
Fertiliser rate	**0.00**	**0.02**
Cover type	0.51	**0.00**
Time	0.57	**0.00**

Table 3. Table of means for main effects of the treatments on soil emissions. Significant relationships are in bold.

Treatment	N_2O (nmol m^{-2} s^{-1})	CO_2 (nmol m^{-2} s^{-1})
Water content		
40	**0.66 ± 0.12**	**1597.30 ± 220.89**
60	**4.12 ± 0.61**	**1526.81 ± 176.43**
80	**4.56 ± 0.70**	**762.51 ± 133.44**
Fertiliser rate		
Best practice	2.20 ± 0.33	**1079.06 ± 150.56**
Farmers	4.03 ± 0.59	**1512.02 ± 153.79**
Cover type		
Vegetated	3.53 ± 0.48	**1647.46 ± 163.98**
Bare	2.70 ± 0.50	**943.62 ± 129.69**
Time		
24	2.94 ± 0.51	**1783.02 ± 173.91**
48	3.29 ± 0.48	**808.06 ± 98.01**

Table 4. Experiment 3 significance table displaying p values for the effects of the main treatment on soil characteristics, using a 4-way ANOVA. Significant values (p=<0.05) are shown in bold. Total N and C data was incomplete, but with at least 3 replicates from each treatment represented. WC is water content, FeR is fertiliser rate and CT is cover type.None of the treatment interactions were significant.

Treatment	pH	Total N	Total C	Urea	Nitrate	Ammonium
WC	0.34	0.34	0.63	0.62	0.86	**0.03**
FeR	**0.01**	0.33	0.72	**0.00**	0.80	**0.04**
CT	0.95	0.08	0.13	0.10	0.24	0.50

Of all the treatments, fertiliser rate had the most significant effect on soil characteristics *(Table 4.)*. Fertiliser rate had a significant positive effect on ammonium content and negative effect on pH and urea content *(Table 4.)*. Water content had a significant effect on ammonium content, which decreased substantially from 40 to 60 % *(Table 4.)*. Cover type did not have a significant effect on any of the soil characteristics.

Table 5. Table of means for treatments and soil characteristics. Significant relationships are in bold.

Treatment	pH	Urea (mg/kg)	Ammonium (mg/kg)
Water content			
40	6.25 ± 0.08	39.60 ± 2.22	**1063.10 ± 192.87**
60	6.40 ± 0.07	42.80 ± 3.14	**398.00 ± 121.88**
80	6.35 ± 0.06	39.45 ± 3.06	**600.25 ± 183.67**
Fertiliser rate			
Best practice	**6.44 ± 0.06**	**45.47 ± 2.33**	**480.57 ± 104.04**
Farmers	**6.23 ± 0.05**	**35.77 ± 1.91**	**893.67 ± 170.44**

The emission rates of N_2O and CO_2 were not highly correlated with any soil characteristic, neither were many soil characteristics highly correlated with each other *(Table 6.).* The only relationship is between total N and total C, which were highly correlated.

There was variation between nitrogen partitioning across the treatments *(Figure 10.).* The best practice fertiliser rate had more added N left than the farmer's fertiliser rate, with a majority of the N as NH_4^+. More N_2O was produced by both fertiliser rates at the higher WFPS of 60 and 80 %.

Table 6. Experiment 3 correlation table displaying r values. The 48 hour N_2O and CO_2 emission values were used in this table. Total N and C data was incomplete, but there were at least 3 reps from each treatment.

	N_2O	CO_2	pH	Total N	Total C	Urea	Nitrate	Ammonium
N_2O	1.0							
CO_2	0.27	1.0						
pH	0.13	0.06	1.0					
Total N	0.13	0.23	0.09	1.0				
Total C	0.15	0.22	0.11	0.95	1.0			
Urea	0.11	0.19	0.10	0.08	0.10	1.0		
Nitrate	-0.16	-0.05	-0.01	0.28	0.27	0.18	1.0	
Ammonium	-0.11	0.24	-0.30	0.14	0.04	0.06	0.21	1.0

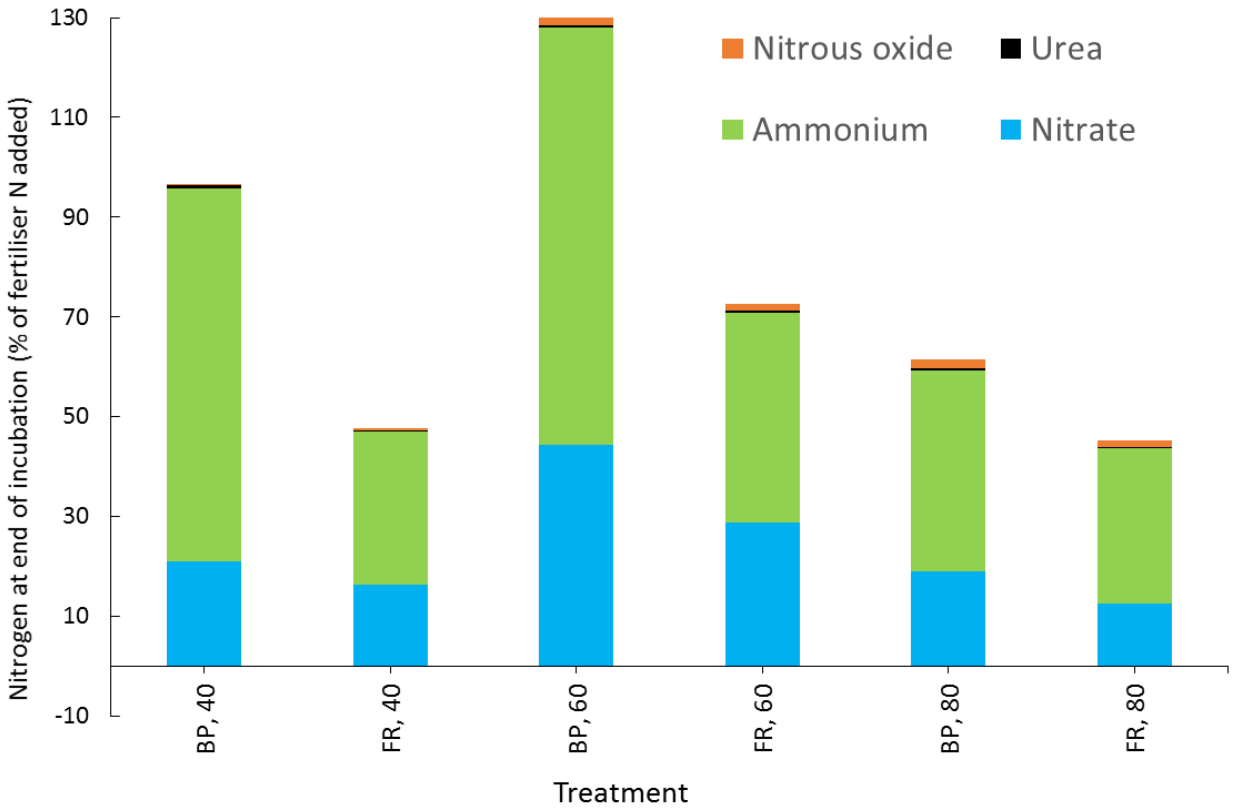


Figure 10. The form of nitrogen at the end of Experiment 3, showing mean values from Table 7 Treatments over 100 % had more nitrogen remaining than was added, and treatments under 100 % used up nitrogen in another form to what was measured.

Table 7. Table of means (± SE) for nitrogen partitioning expressed as a proportion of the fertiliser nitrogen added, for the significant treatment effects. Values in bold have remaining nitrogen exceeding 100 % of fertiliser nitrogen that was added

Treatment		Urea	NO_3^- (%)	NH_4^+ (%)	N_2O (%)
WC	**FeR**				
40	BP	0.7 ± 0.3	20.9 ± 3.0	74.8 ± 19	0.1 ± 0.1
40	FR	0.3 ± 0.0	16.3 ± 3.0	30.7 ± 3.6	0.4 ± 0.2
60	**BP**	**0.4 ± 0.1**	**44.4 ± 10.3**	**83.6 ± 20.4**	**1.7 ± 0.4**
60	FR	0.3 ± 0.0	28.8 ± 7.5	42.1 ± 8.6	1.5 ± 0.3
80	BP	0.4 ± 0.1	18.9 ± 2.7	40.3 ± 11.8	2.0 ± 0.4
80	FR	0.4 ± 0.1	12.5 ± 4.2	31.1 ± 2.8	1.2 ± 0.4

The N_2O emissions varied across the different treatments *(Figure 11).* The farmer's rate treatment produced higher emissions than the best practice rate treatment over all three water contents. From 60 to 80 % WFPS emissions trended downwards for the farmer's rate treatment but upwards for the best practice rate

treatment. N_2O emissions were similar between the bare and vegetated treatments, especially at 40-60 % WFPS. Above 60 % WFPS, emissions increased in cores from vegetated cover type and decreased in cores from the bare cover type.

CO_2 emissions were higher in the farmers rate treatment than in the best management practice rate, but only at 40 and 60 % WFPS *(Figure 12.)*. Cover type had a significant effect on CO_2 emission, with the vegetated cover at a higher emission rate than the bare, over all water contents *(Figure 12.)*. The relationship between CO_2 emission and water content followed a similar trend across both cover types and fertiliser rates, with emissions similar at 40 and 60 % WFPS, before reducing by half at 80 % WFPS.

There was a significant effect of fertiliser treatment rates on $\delta^{13}C$ values *(Figure 13)*. The difference between fertiliser rates was significant at all 3 water contents at the 24 hour sampling time, with the best practice rate having higher $\delta^{13}C$ values. At the 48 hour sampling time the difference between fertiliser rates (higher with best practice rate) was significant at 40 and 80 % WFPS but not 60 % WFPS.

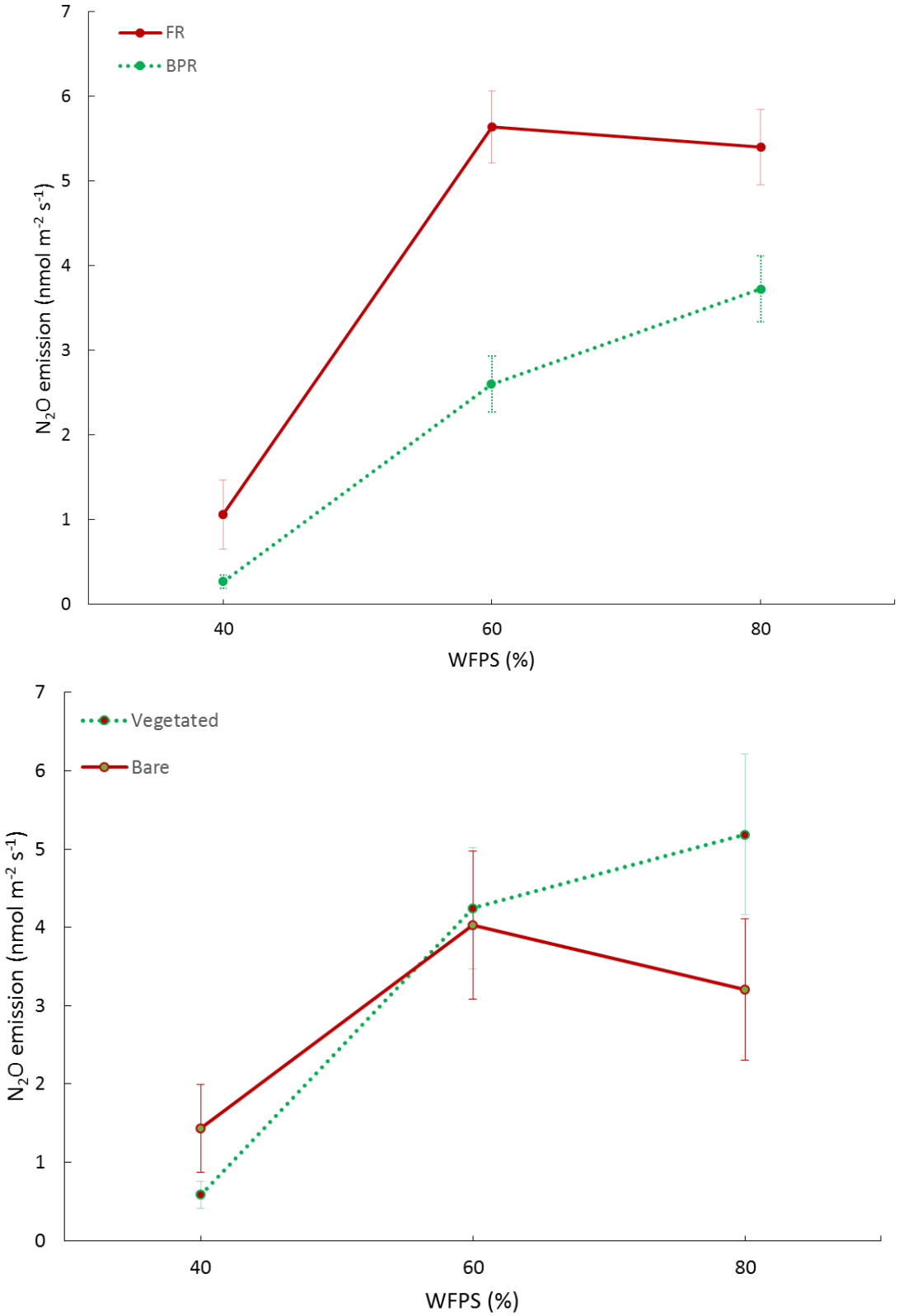
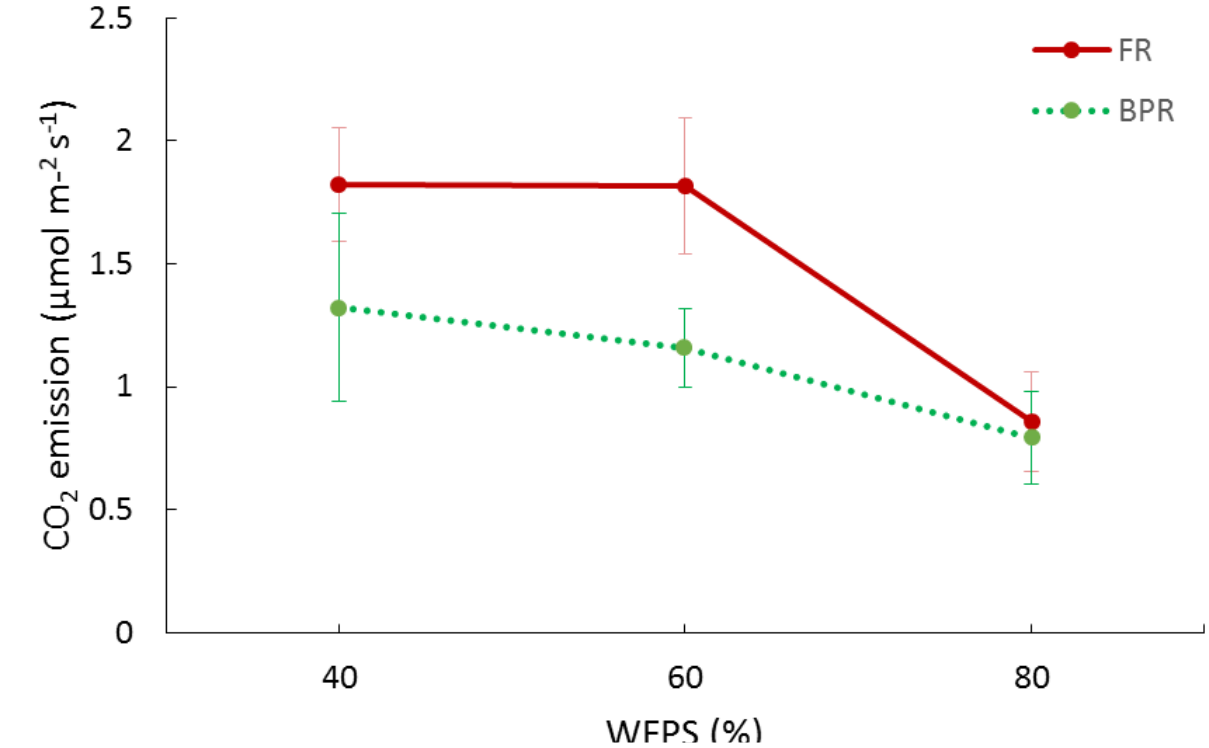


Figure 11.Mean N_2O emission over three water-filled pore space (WFPS) contents, averaged over the two sampling times. Upper plot shows BPR (best practice rate) and FR (farmers rate), and the lower plot shows bare and vegetated cover types. Error bars show standard error of the mean.



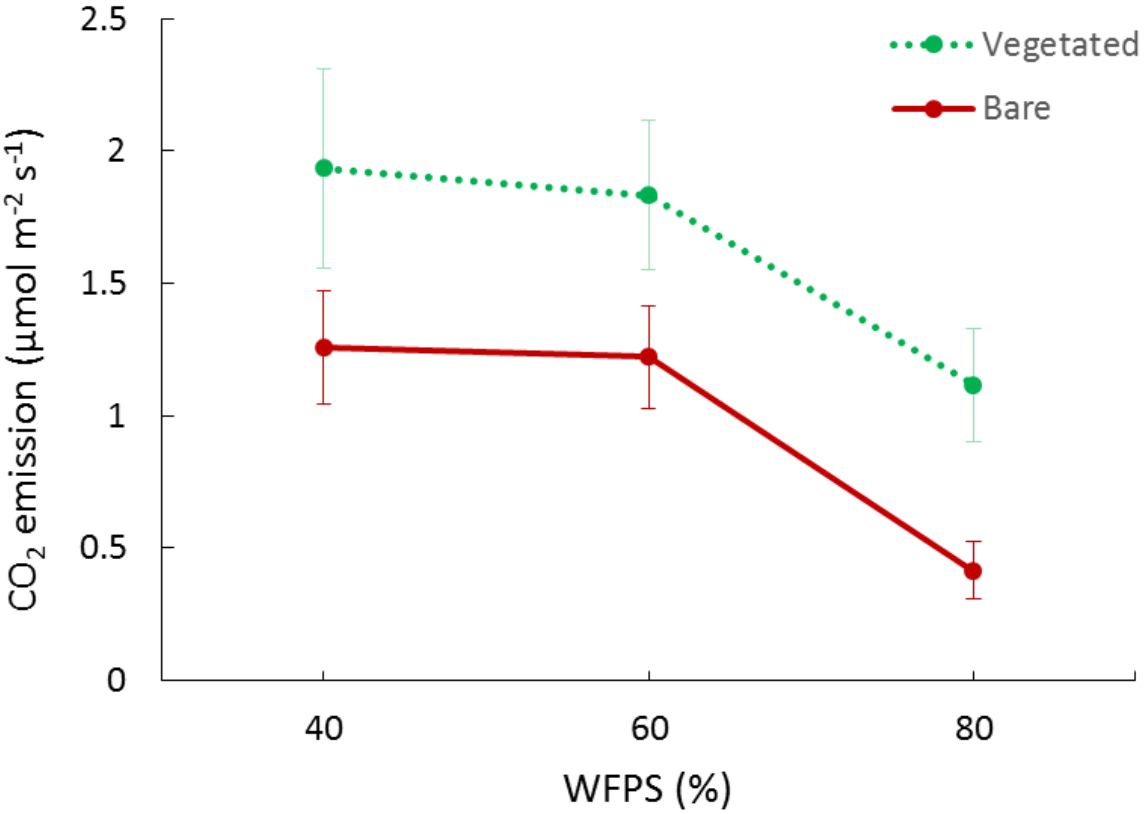


Figure 12.Mean CO_2 emission over the three water-filled pore space (WFPS) contents, averaged over two sampling times. Upper plot shows BPR (best practice rate) and FR (farmers rate), and the lower plot shows bare and vegetated cover types. Error bars show standard error of the mean

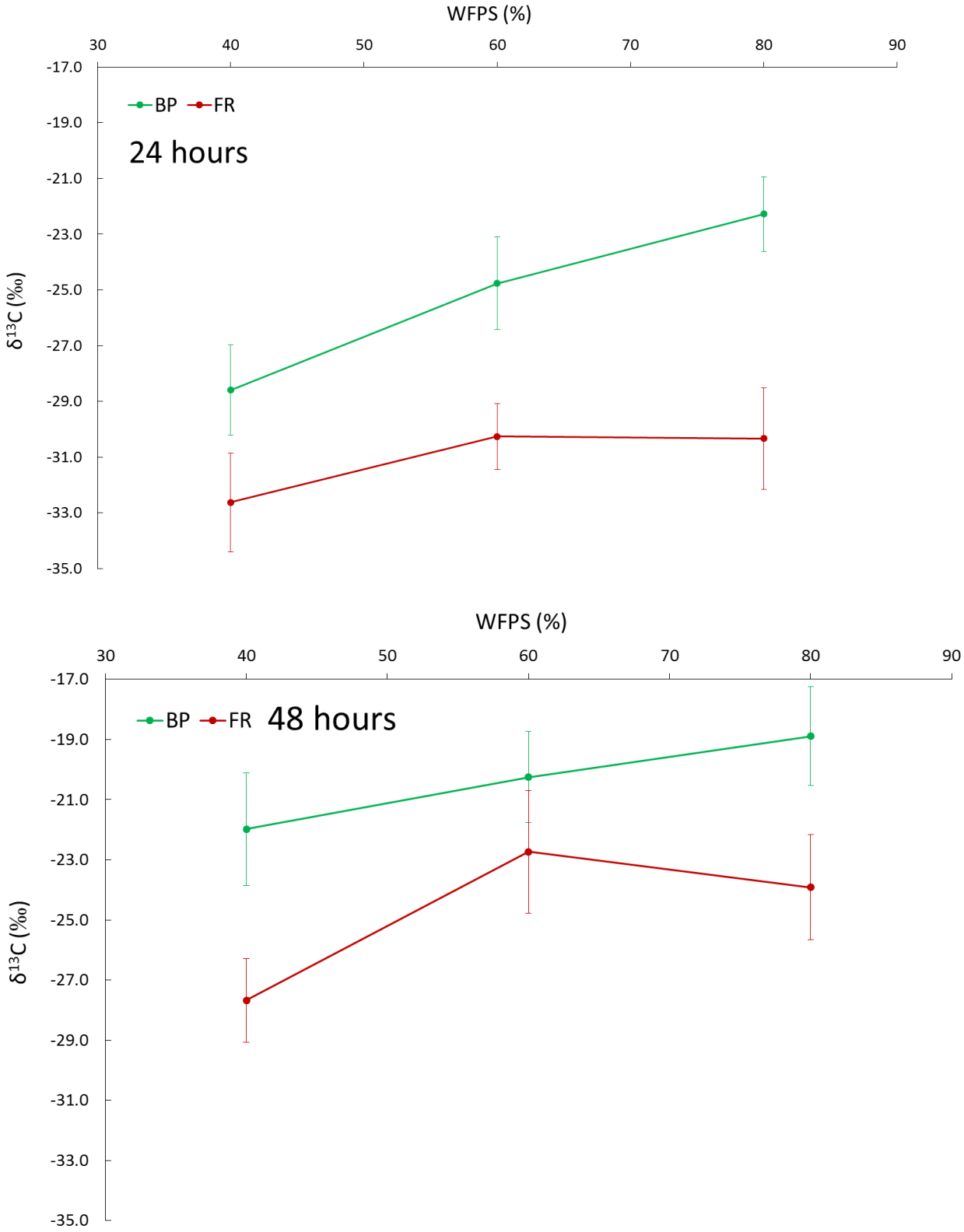


*Figure 13. **$\delta^{13}C$** values over the three water-filled pore space (WFPS) contents with the upper plot showing the results at 24 hours and the bottom plot showing 48 hours incubation time. BP is the best practice fertiliser rate and FR is the farmer's fertiliser rate. Error bars show the standard error of the mean.*

3.4 Experiment 4

In Experiment 4, water content had a significant positive effect on N_2O emission, as in the previous experiment *(Table 8)*. N_2O emissions were not significantly influenced by fertiliser type, cover type or time. Water content and time also had a significant effect on CO_2 emissions, which decreased with increasing water content and time *(Table 8.)*.

Table 8. Experiment 4 significance table displaying p values of treatments to N_2O and CO_2 emissions. Statistics were run on S-PLUS using a 4-way ANOVA with significant values (p=<0.05) in bold. None of the treatment interactions were significant.

Treatment	N_2O	CO_2
Water content	**0.00**	**0.00**
Fertiliser type	0.17	0.63
Cover type	0.15	0.19
Time	0.15	**0.00**

Table 9. Table of means for main effects of the treatments on soil emissions. Significant relationships are in bold.

Treatment	N_2O (nmol $m^{-2}s^{-1}$)	CO_2 (nmol $m^{-2}s^{-1}$)
Water content		
40	**0.81 ± 0.30**	**453.33 ± 107.53**
60	**3.22 ± 0.59**	**847.15 ± 71.23**
80	**4.26 ± 0.65**	**626.71 ± 69.81**
Time		
24	3.22 ± 0.54	**850.35 ± 65.20**
48	2.12 ± 0.40	**430.77 ± 67.85**

Cover type had the most significant effect on soil characteristics *(Table 10.)*, affecting total nitrogen and carbon contents significantly. Fertiliser type had a significant effect on urea content, but no other treatments or interactions had a significant impact on soil characteristics.

As with Experiment 3, the only significant correlation between soil characteristics was between total nitrogen and carbon contents *(Table 12.)*. Urea and CO_2 emission had a slight relationship at -0.48. N_2O and CO_2 were also not significantly correlated, as in Experiment 3.

Table 10. Experiment 3 significance table displaying p values for the effects of the main treatments on soil characteristics, using a 4-way ANOVA. Significant values (p=<0.05) are shown in bold. Total N and C data was incomplete, but with at least 3 replicates from each treatment represented. WC is water content, FeT is fertiliser type and CT is cover type. None of the treatment interactions were significant

Treatment	pH	Total N	Total C	Urea	Nitrate	Ammonium
WC	0.48	0.75	0.72	0.89	0.87	0.79
FeT	0.42	0.38	0.68	**0.03**	0.06	0.18
CT	0.56	**0.01**	**0.02**	0.95	0.13	0.51

Table 11. Table of means for treatments and soil characteristics. Significant relationships are in bold.

Treatment	Total N (%)	Total C (%)	Urea (N/mg/kg)
Fertiliser type			
Urea	0.19 ± 0.01	1.94 ± 0.07	**80.40 ± 12.62**
ENTEC	0.18 ± 0.01	1.90 ± 0.09	**118.23 ± 11.07**
Cover type			
Vegetated	**0.17 ± 0.00**	**1.80 ± 0.06**	99.83 ± 11.62
Bare	**0.20 ± 0.01**	**2.05 ± 0.08**	98.80 ± 13.10

Table 12. Experiment 3 correlation table displaying r values. The 48 hour N_2O and CO_2 emission values were used in this table. Total N and C data was incomplete, but there were at least 3 reps from each treatment.

	N_2O	CO_2	pH	Total N	Total C	Urea	Nitrate	Ammonium
N_2O	1.0							
CO_2	0.17	1.0						
pH	0.05	0.10	1.0					
Total N	-0.21	-0.18	-0.12	1.0				
Total C	-0.22	-0.18	-0.12	0.97	1.0			
Urea	-0.16	-0.48	0.20	0.14	0.15	1.0		
Nitrate	0.30	0.17	0.10	-0.41	-0.34	-0.12	1.0	
Ammonium	-0.18	0.13	-0.06	-0.18	-0.14	0.09	-0.22	1.0

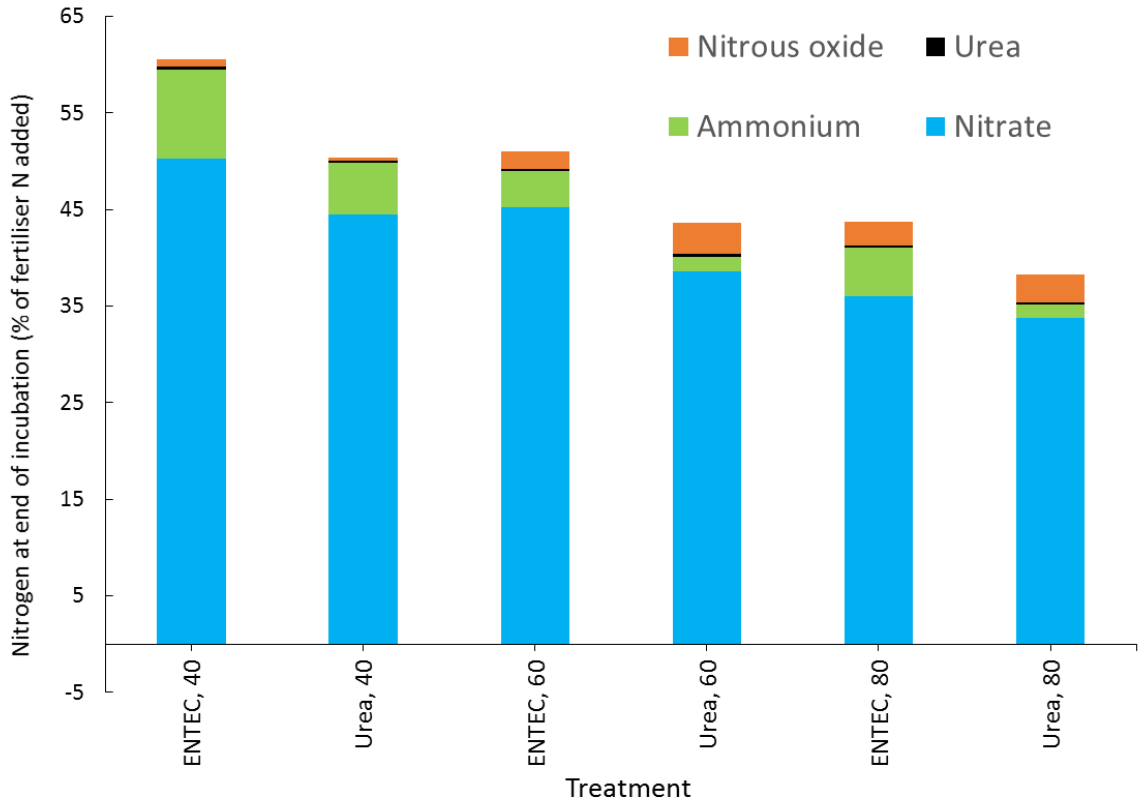


Figure 14. The form of nitrogen at the end of Experiment 4 showing mean values from Table 13. Treatments over 100 % had more nitrogen remaining than was added, and treatments under 100 % used up nitrogen in another form to what was measured.

The amount of nitrogen left at the end of incubation decreased with increasing water content *(Figure 14).* The ENTEC fertiliser treatments have a higher level of nitrogen remaining than the urea fertiliser treatment at each water content. The largest proportion of nitrogen left is nitrate, which is in contrast to Experiment 3 where ammonium was the largest contributor.

The urea treatment produced higher emissions at 60% WFPS than the ENTEC treatment, with both treatments having similar rates at the 40 and 80% WFPS *(Figure 15.)*. The urea treatment also decreased in emissions at 80% WFPS while the ENTEC treatment increased. The bare cover type had lower N_2O emissions than the vegetated cover type at 80 and 40 % WFPS. The bare treatment shows a downward trend of emissions at 80% WFPS while the vegetated treatment increased.

CO_2 emissions were similar between the fertiliser treatments. The ENTEC treatment produced higher CO_2 emissions than the urea treatment *(Figure 16.)*. Both cover types decreased at the 80% water content level. The bare cover type had lower CO_2 emissions than the vegetated treatment, similar to Experiment 3.

Table 13. Table of means (± SE) for nitrogen expressed as a proportion of the fertiliser nitrogen added for the significant treatment effects. Values in bold have remaining nitrogen exceeding 100 % of nitrogen fertiliser that was added

Treatment		**Urea**	**NO_3^- (%)**	**NH_4^+ (%)**	**N_2O (%)**
WC	**FeT**				
40	Urea	0.3 ± 0.0	44.5 ± 6.6	5.3 ± 1.5	0.3 ± 0.1
60	Urea	0.3 ± 0.0	38.6 ± 3.8	1.5 ± 0.1	3.2 ± 1.1
80	Urea	0.2 ± 0.0	33.8 ± 3.5	1.4 ± 0.1	2.9 ± 0.7
40	ENTEC	0.3 ± 0.0	50.3 ± 11.0	9.2 ± 2.4	0.7 ± 0.5
60	ENTEC	0.2 ± 0.0	45.2 ± 14.6	3.8 ± 2.2	1.8 ± 0.7
80	ENTEC	0.2 ± 0.0	36 ± 7.9	5.1 ± 2.1	2.9 ± 0.7

There was no significant effect of fertiliser type, time and water content on and $\delta^{13}C$ *(Figure 17.).* At 24 hours and 80 % WFPS, the different fertiliser treatments displayed opposite trends; with urea showing decreased $\delta^{13}C$ values and ENTEC showing increased $\delta^{13}C$ values. Both treatment types show similar patterns at the 48 hour sampling time, but with urea having higher $\delta^{13}C$ values on average.

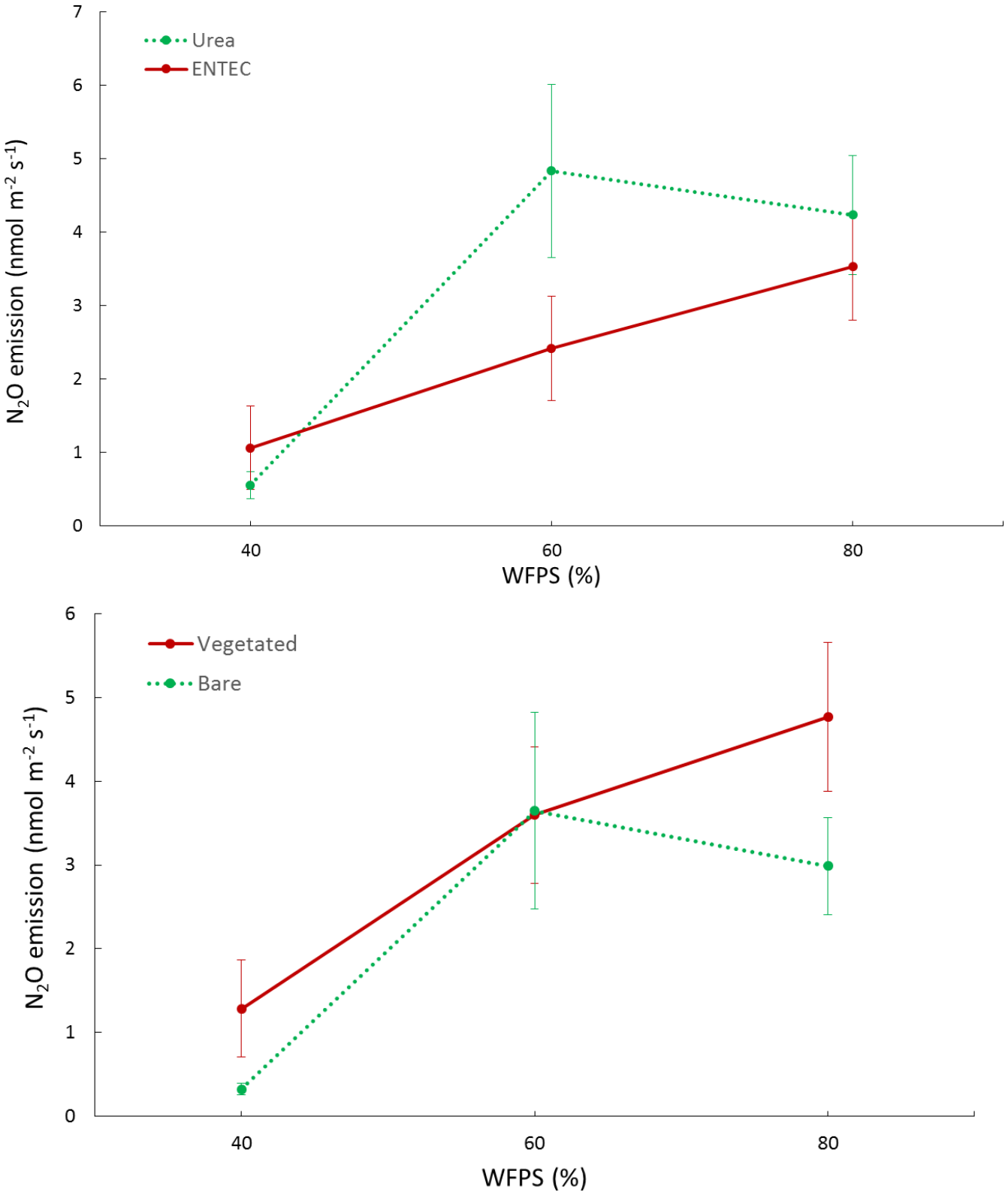


Figure 15.Mean N_2O emissions over the three water-filled pore space (WFPS) contents, averaged over the two sampling times. Upper plot shows urea fertiliser and urea fertiliser with DMPP (ENTEC), and the lower plot showing bare and vegetated cover types. Error bars show standard error of the mean

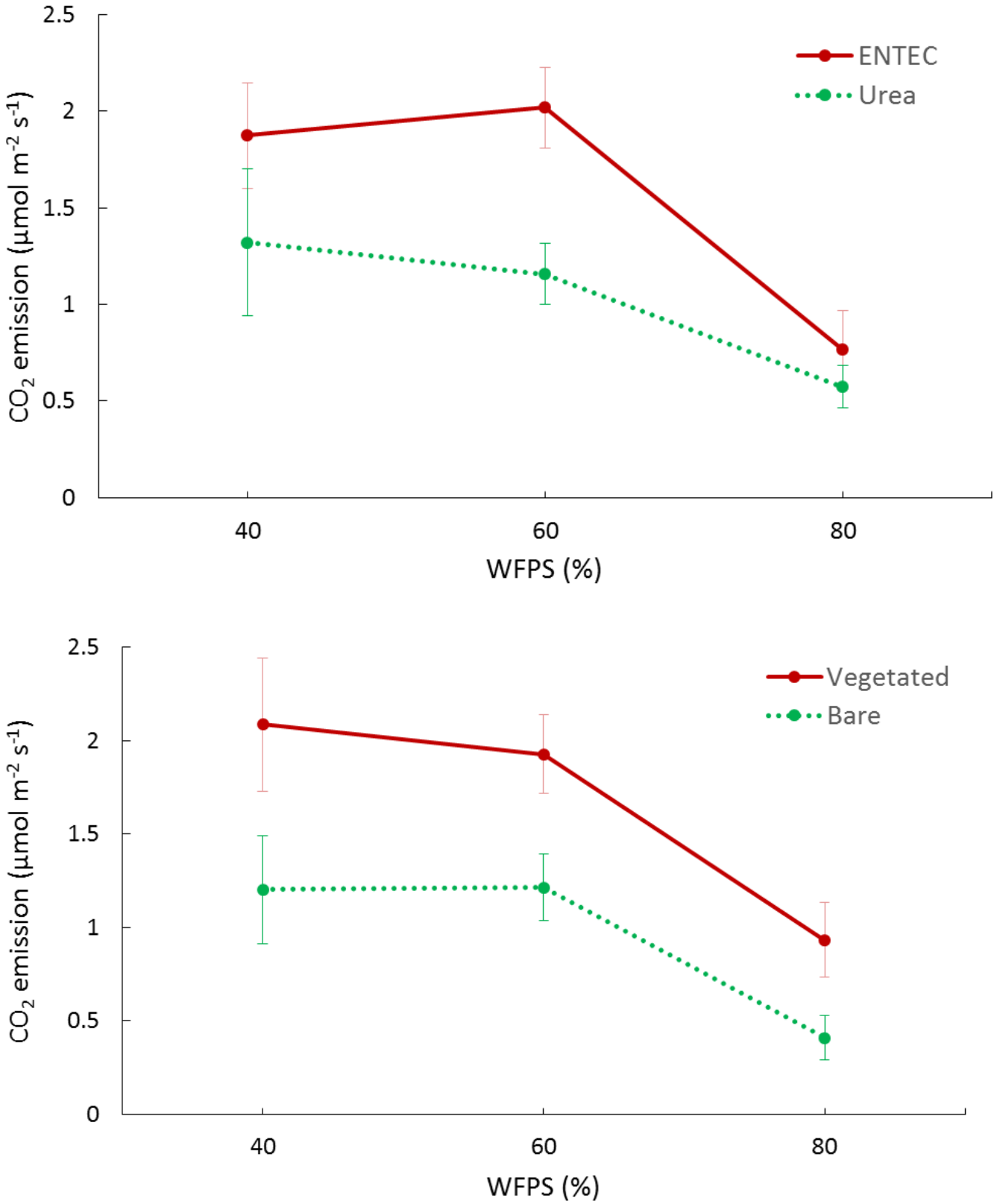


Figure 16.Mean CO_2 emission over the three water-filled pore space (WFPS) contents, averaged over the two sampling times. Upper plot shows urea fertiliser and urea fertiliser with DMPP (ENTEC), and the lower plot showing bare and covered cover types. Error bars show the standard error of the mean

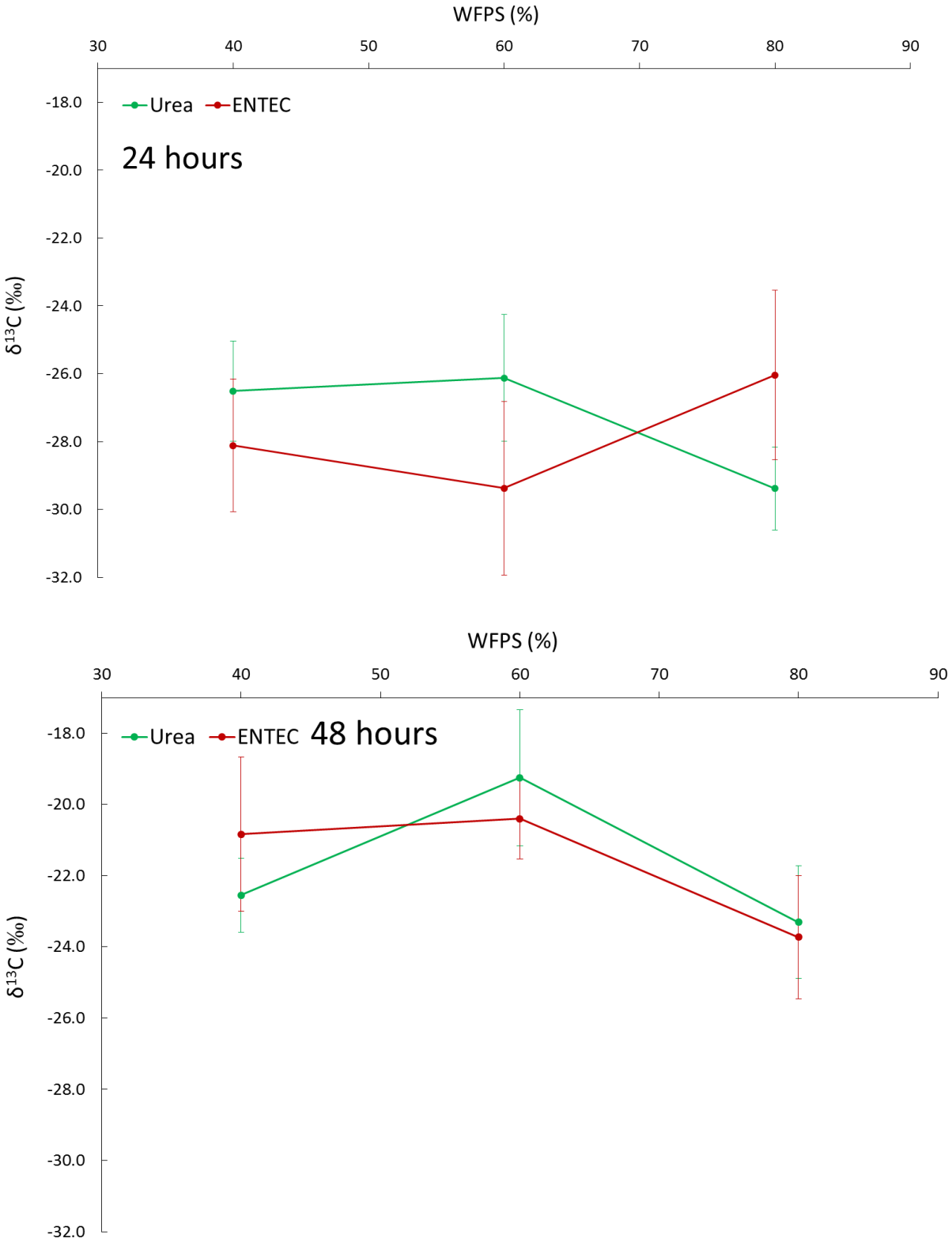


*Figure 17. **δ¹³C** values over the three water-filled pore space (WFPS) contents with the upper plot showing the results at 24 hours and the bottom plot showing 48 hours incubation time.*

4. Discussion

4.1 Soil moisture

Soil moisture content did have a significant positive relationship with N_2O emissions, which supports the current literature (Bouwman, 1998, Wang and Cai, 2008). The general consensus in the literature is that a soil water content of 60 % WFPS generates peak production of N_2O emissions (Ruser and Schulz, 2015). This is supported by Experiment 4, where the best practice urea treatment shows a peak of N_2O emissions at 60 % WFPS. This is not evident in Experiment 3 however, where the best practice urea treatment displays a steady increase of emissions past 80 % WFPS. A likely reason for this may be that there was a higher concentration of denitrifying bacteria in Experiment 3, prompting the N_2O emissions to peak at a higher WFPS than 60 %, as nitrifying bacteria require water contents below 60 % WFPS to function efficiently (Rich et al., 2003). It can be assumed that if there was a larger denitrifying community in Experiment 3, that this would result in a lower nitrate (NO_3^-) concentration when compared with Experiment 4, because NO_3^- is created during nitrification and consumed during denitrification. There was no significant difference between N_2O emissions at 60 % WFPS between the two best practice urea treatments, nor was there a significant difference between the NO_3^- concentrations. Experiment 3 did have a lower mean NO_3^- concentration than Experiment 4, which may have contributed to the differences in N_2O emission at 60 % WFPS. This leads to the assumption that Experiment 3 may have had either a larger community of denitrifying bacteria, or a smaller community of nitrifying bacteria than in Experiment 4.

Soil moisture content was also found to have an effect on $\delta^{13}C$, although none of the effects at the different WFPS contents were found to be significant. A similar finding has been observed by Phillips et al. (2010), but it was found that the differences they recorded was due to an incomplete diffusive equilibrium in the different sized containers used for soil incubation. In this experiment however, the same sized incubation chambers were used for all treatments. In order to establish if heterotrophic respiration is changing the enrichment level of $\delta^{13}C$, further studies would need to be done with more extreme water contents and an absence of other variables. It is unlikely that soil moisture alone is changing the enrichment level of $\delta^{13}C$ in the study, as the same urea best practice treatment in Experiment 3 and 4 show different patterns, but similar values; with Experiment 3 ranging between -28 - -18 ‰, and Experiment 4 between -29 - 19 ‰.

4.2 Fertiliser type and rate

The use of a nitrification inhibitor in the form of ENTEC did not significantly reduce N_2O emissions, which is in direct contrast with the literature (Lam et al., 2015, Menéndez et al., 2012, Scheer et al., 2014b). Nitrification inhibitors work by inhibiting the oxidation of ammonium (NH_4^+) to NO_3^-, which is the first step in nitrification. It can therefore be assumed that if the DMPP molecule was inhibiting this step, that there would be a higher NH_4^+ concentration in the ENTEC treated samples than in the urea treated samples. The ENTEC treated samples did have a higher mean NH_4^+ concentration, but with a wider spread of data than in the urea treated samples.

DMPP should be inhibiting nitrification, which should in turn also inhibit denitrification as nitrate is not being produced which denitrification needs to consume. This should result in a lower level of NO_3^- produced in the ENTEC treated samples than in the urea treated samples. This is difficult to measure over such a short time frame (48 hours), as once NO_3^- is produced it is likely to be soon consumed by denitrifying bacteria and converted to N_2O, rather than building up as it normally would over a longer period (more than a week). The difference between the NO_3^- content in the samples treated with and without DMPP were not significant, but with the DMPP treated samples having a larger variability amongst the data and a slightly higher mean.

As it has been shown that the DMPP molecule was working to a small extent, with slightly higher NH_4^+ concentrations, another factor must be reducing its potential. A reason as to why the ENTEC may not have reduced N_2O emissions to a significant extent may be due to the length of time over which the experiment was run. Most experiments found in the literature were run in the field over time courses from a few weeks, to a few seasons (Kleineidam et al., 2011, Menéndez et al., 2012, Scheer et al., 2014b, Zhu et al., 2015). Experiments that were done in the laboratory were done over weeks to months (Menéndez et al., 2012, Ruser and Schulz, 2015), so it is difficult to compare these results. To see if time had an effect on DMPP performance the 24 and 48 hour N_2O emissions were separated and compared, but there was no significant difference between them. Samples treated with ENTEC did have slightly higher means at both 24 and 48 hours, with urea having a wider spread of data. The insignificant effect of ENTEC may also be due to the low amount of nitrogen fertilisers applied to the soil, as Experiment 4 was run using the lower, best practice rate of fertiliser addition. The lack of support for this hypothesis is likely to be a combination of the limited time the N_2O emissions were measured over, the low amount of nitrogen fertiliser added and the huge spatial variability of N_2O emissions. The main known drivers of N_2O emissions were controlled and analysed in each soil core. However when the correlation between each of these parameters and N_2O

emissions was examined, there was no strong correlation. This implies that there must be other, unknown factors involved in the N_2O emission production process.

There was a higher proportion of the added fertiliser N left behind in Experiment 3 than in Experiment 4. There was almost half as much nitrogen remaining in Experiment 4 than there was for the same best practice urea rate in Experiment 3. The largest contributor to the remaining nitrogen was NO_3^- in Experiment 4 and NH_4^+ in Experiment 3. Experiment 4 would be expected to have a lower level of NO_3^- remaining in the ENTEC treated samples when compared with other N types, as the DMPP molecule should be inhibiting this chemical conversion. Experiment 3 had NH_4^+ as the largest contributor to the remaining nitrogen in the samples, this is expected, as the urea applied would be catalyzed and turned into NH_4^+. It is possible that the high proportion of NO_3^- remaining in Experiment 4 and the lack of NH_4^+ remaining when compared with Experiment 3, may be due to a larger portion of the NH_4^+ being lost as other forms of N gas including N_2, NO and NO_x. It is unknown why the amounts and proportions of remaining nitrogen are not similar in the best practice urea treatment across Experiment 3 and 4, but the factors involved may also be related to the insignificant effect of the ENTEC treatment on N_2O emissions.

The farmer's rate (higher fertiliser N rate) of urea produced significantly more N_2O emissions than the best management practice rate (lower fertiliser N rate). This supports the literature (Iqbal et al., 2015, Zhu et al., 2015), whereby higher rates of fertiliser N caused higher N_2O emissions. The largest differences between the two rates were the N_2O emissions at 60 % WFPS, which is likely because both nitrification and denitrification were operating. There was a larger proportion of the added fertiliser N left behind in the best practice treatments than in the farmer's rate treatments. As all of the farmer's rate treated samples had less than 100 % of the added fertiliser N accounted for, it can be assumed that the remainder was lost as another form of N gas including N_2, NO and NO_x.

There was no significant effect of CO_2 on N_2O emissions. In Experiment 3 the only significant difference between fertiliser N rates and CO_2 emissions were observed at 60 % WFPS. This significant difference was also evident in Experiment 4 between the fertiliser types, also at 60 % WFPS. The farmer's rate of fertiliser and ENTEC fertiliser produced higher CO_2 emissions in their respective experiments. There is little research on the effect of nitrogen fertiliser rate and type on CO_2 emissions, with the work that has been done finding no significant relationship (Sakata et al., 2014).

The use of two different cover types (vegetated and bare) throughout Experiment 3 and 4 did not have a significant effect on N_2O emissions. The concentration and pattern of CO_2 emissions were very similar across both cover types in both Experiment 3 and 4. It has been reported that increased C availability in soil triggered by elevated levels of CO_2 can increase N_2O emissions. The mainly occurs because the N_2O

producing microorganisms have more C substrate to consume, and therefore have the ability to increase in activity and produce higher levels of N_2O (Li et al., 2013). This may not have had an effect of the N_2O emissions in this study, as the study site had been established for less than a year when soil samples were taken. As the site had not been established for a long period of time, it is likely that the different cover types were not in place long enough to have made a significant impact on the soil characteristics; hence the amount of C available in the soil.

Nitrogen fertiliser rate and type was not found to have an overall significant effect on $\delta^{13}C$ enrichment. In Experiment 3 both fertiliser rates show similar patterns of $\delta^{13}C$, with enrichment decreasing with increasing water content at both the 24 and 48 hour sampling times. While the best practice fertiliser rate is significantly higher than the farmer's rate at 24 hours, it is insignificantly different at 48 hours. None of the $\delta^{13}C$ values are significantly different between the urea and ENTEC treatments, and with both sampling times showing different patterns. The same urea best practice treatment shows a different pattern between Experiments 3 and 4. In Experiment 3 the $\delta^{13}C$ enrichment decreases with increasing water content, while in Experiment 4 it increases with increasing water content. The ENTEC treatment in Experiment 4 also shows opposite trends between the 24 and 48 hour sampling times. The effect on the $\delta^{13}C$ values may also be attributed to the same factor causing discrepancies with the N_2O emissions, as the unexpected N_2O emissions also occur when the $\delta^{13}C$ values do not behave as expected.

Many of the unexpected results in this study could not be explained by the variables and soil characteristics that were measured, implying that other factors must be interacting with the soil. It has been suggested that other N_2O producing pathways may exist, and that the processes and controls of N_2O emissions are not well understood (Baggs, 2011, Butterbach-Bahl et al., 2013). As there have been very few N_2O studies done in tropical climates, and none of a similar topic in Far North Queensland, it is likely to be the case that there is another unknown factor interacting with N_2O emissions and causing the results found in this study.

5. Conclusion

The intensification of agriculture has led to high inputs of nitrogen fertilisers into crops. This is especially the case for bananas, which have a high nitrogen demand throughout the year. The mitigation of N_2O emissions is particularly important in high demand crops, as they generally have the largest reduction potential. This project has established that soil moisture content has a significant relationship with N_2O emissions, and that a nitrification inhibitor (DMPP) has an insignificant effect on N_2O emissions on soil from a North Queensland banana farm. The soil moisture content results have implications on the farm management practices. As the field site is irrigated as well as relying on rainfall events, it is likely that the area frequently experiences soil water contents around 60 % WFPS. In order to keep the soil moisture below 60 % WFPS to minimise N_2O emissions, it would be beneficial for the site to be well drained and for irrigation schedules to be closely monitored in relation to rainfall events. Reducing the nitrogen fertiliser rates to the best practice rate, established in previous trials and based on maximizing productivity, also reduces N_2O emissions when compared to the farmer's usual application rate. The nitrification inhibitor results also have implications on farming practices. While significant results were not seen in this project, there was evidence of the DMPP molecule blocking the nitrification process, and although it didn't significantly reduce N_2O emissions, it might be expected to reduce leaching, as NO_3^- is more mobile than NH_4^+. It may be beneficial, in terms of the reduction of N_2O emissions, if the farmer were to add a nitrification inhibitor to their crop as it is likely that reduction results would be seen over the long term. Further research would be needed to establish the long term effect of ENTEC on plant health and crop yield, and the overall cost of adding the inhibitor, in order for it to be a viable change. This project has provided some insight into the complex nature of the N_2O producing processes, which urges further investigation into this largely under researched area of science.

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