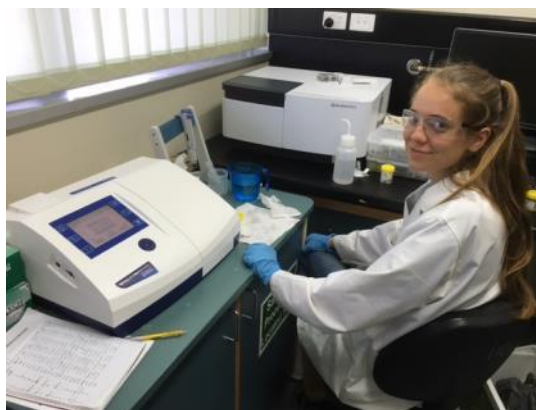


Project Report
CRDC Summer Scholarship 2015-16

**How wet and dry cycles affect mineral nitrogen supply from
nitrogen fertiliser**

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Executive Summary

Wet-dry cycles under rain and irrigation affect soil nitrogen mineralisation rates. Understanding the variable effects of wet- dry cycles on soil nitrogen mineralisation will help match fertiliser recommendations with cotton crop requirements and help constrain nitrogen supply and greenhouse gas emission models. A 31-day aerobic pot incubation study was conducted to measure urea fertiliser and soil mineral nitrogen supply under variable wet and dry soil conditions. The study successfully simulated one wet-dry cycle in a Black Vertosol but a second wet-dry cycle was not achieved due to excess water being available using the capillary rise method of wetting. Fertiliser treatment effects were evident for all forms of measured nitrogen (KCl-extractable nitrate-N, KCl-extractable ammonium-N and water extractable total dissolved N) when compared with an unfertilised control treatment, with the exception of absorbance at 224nm. The incubation trial allowed manipulation of soil moisture content to study soil nitrogen dynamics.

1.0 Introduction

This project aimed to contribute knowledge to the optimum use of fertiliser and irrigation in order to meet the crop demand for soil mineral nitrogen. Current methods used to approximate soil mineralisation rates over a crop growth cycle are usually based on assumed seasonal conditions. However, microbial activity and resulting net mineralisation rates are highly variable and dynamic in response to wet-dry cycles (Blackwell *et al.*, 2013; Fierer and Schimel, 2002). Repeated wet-dry cycles have been found to reduce cumulative nitrogen mineralisation rates compared with constantly wet soil (Mikha *et al.*, 2005). To evaluate the effect of rain and irrigation on soil mineral N supply after application of a range of types of nitrogen fertilisers, methods to experimentally test the effects of soil wetting and drying cycles on soil mineral nitrogen supply are required. A laboratory incubation trial was conducted to simulate soil fertilisation and the wet and dry cycles of irrigation patterns. Two soil moisture treatments were studied; a constantly wet treatment, in which soil was maintained at close to saturation to maximise microbial activity, and a wet-dry-wet-dry treatment, in which soil was wet, dried, then re-wetted and dried again, to simulate irrigation events. The soil nitrogen was measured at several different times throughout the process in order to identify trends in the nitrification of the urea fertiliser after periods of wet and dry. Initially it was planned to compare conventional nitrogen fertilisers with enhanced efficiency fertilisers. A lack of resources prevented this however and instead only conventional urea fertiliser was tested.

2.0 Method

2.1 - Initial setup

For the project approximately 15kg of a black vertosol was collected from a nearby cotton farm. The site “Yargullen” had a mid-season cotton crop growing in a spray irrigated field. The soil was collected from plots which had no nitrogen fertiliser applied to the crop. The soil was then transported back to the laboratory and spread out in two containers to air dry for approximately 8 days. Two containers of soil were put aside both weighing approximately 50g each. These were placed in the oven at 40°C to slowly oven dry. These were kept in the oven for twenty-four hours and weighed to determine the moisture after air drying. In the meantime the remainder of the soil was sifted. First through a 5 mm sieve and then a 2.3 mm sieve. The sieved soil was then placed back into the open containers to air dry overnight.

In preparation for the pots, 24 plastic container lids approximately 75 mm in diameter and 20 mm in depth were upturned. Using a small saw blade, four evenly spaced slits were cut into the lid rims with all approximately 8 mm in depth and 5 mm in width. Two 2 mm diameter holes were also drilled into the lid to release air pockets once in use. These lids were then placed rim down into 24 600 mL plastic pots. The lids of the plastic pots had three holes drilled through them approximately 50 mm apart using a 5 mm drill bit. One hole on each lid had a 5 mm plastic drinking straw inserted. These lids were set aside. To act as the wick 24 pieces of hydrophilic fabric were cut into 75 mm diameter circles with six 20 mm overhangs to act as a fringe. When placed inside the pot, the fabric covered the top of the lid and the fringe pieces dropped down the sides of the lid and touched the bottom of the container. This way water could be wicked from the bottom of the reservoir. To hold the soil, 24 pieces of hydrophilic fabric were cut in approximately 18 cm X 18 cm squares. Before placing both sets of fabric in the pots they were soaked in deionised water for ten minutes. The wicking piece was then lightly squeezed to eliminate excess water and placed in the pot followed by the larger piece which was also lightly squeezed. The pots were then sealed with the lids. The straw was pushed down the side of the pot to ensure it almost touched the bottom and could successfully act as a way to directly supply water to a water reservoir beneath the upturned lid without directly

wetting the fabric or soil. The top 60 mm of the straw was cut off to reduce excess. The remainder of the straw top was folded and sealed with a paper clip. This process was completed on all 24 pots which were then all labelled from 1-24.

Each completely setup pot was weighed both with and without the lid. The weights were recorded. In each pot, 5 mL of deionised water was squirted onto the fabric followed by 200 g of air dry soil. For the urea treatment pots (U, n=12), 0.13 g of urea fertiliser was mixed into each 200 g measurement of soil before being added to the pot. This fertiliser addition was equivalent to 600 kg/ha of nitrogen. No fertiliser was added to the control treatment pots (C, n=12). Markings of 5 mm were made around the base of each pot in order to indicate the maximum water level for the reservoir. This water level was equivalent to 8 mm of suction and represented a modification of the tension table method of capillary wetting (Method 2C1) of Rayment and Lyons (2011). Then 125 mL of deionised water was added to each pot through the straw making sure the paperclip was placed back to seal the straw once wetted. This amount was previously calculated as being 62% of the dried soil weight which is approaching pore space saturation of a Black Vertosol such as this. Six Control pots and six Urea pots were then allocated to a constantly wet treatment (W) and randomly placed on the lower of two shelves in a fan-forced incubator set at 25°C. The remaining six Control and six Urea treatment pots were used to simulate a wet-dry-wet-dry cycle (WDWD) and randomly placed on the lower of the two incubator shelves.

2.2 - Sample Collection

After 24 hours in the incubator, the pots were removed. Firstly, the whole pot including the lid was weighed and recorded. Using a steel spatula, 3.07 g of soil was taken from each pot and placed into individual, pre-labelled 50 mL plastic vials for chemical analysis. The weight of soil was calculated as the equivalent of 2 g of oven-dried soil, assuming a constant water content of 62.5% w/w. A precision balance was used (0.001 g) and it was ensured that all weights were ± 0.005 g to reduce the overall margin of error.

To remove soil from the pot, a small scoop of soil was sampled from three different areas of the surface of the pot. This was to increase the representativeness of the samples and by taking small scoops, the chance of picking up a urea granule was low. The sampling technique also prevented the soil from being disturbed or mixed into a paste, which would otherwise have affected the aeration and associated nitrogen cycling. The spatula was thoroughly cleaned with deionised water and paper towel between each pot. It was also ensured that the control pots were sampled first to minimise cross contamination of urea into the control pot samples. Approximately 10 g of soil was then taken from each pot and placed into a pre-weighed ceramic crucible for soil moisture analysis. The exact weight of soil was recorded. The pots had the lids replaced and the reservoirs in the base of the constantly wet treatment (W) pots were refilled up to the 5 mm (8 mm suction) level where necessary. The volume of water supplied to each pot was also recorded. The pots were placed back into the incubator in the same configuration. Pots were checked every 2-3 days and on the afternoon prior to each sampling and water added to the W treatment where necessary to ensure the reservoir was full to 5 mm depth (8 mm suction). Pots were sampled for soil on Days 0, 3, 7, 11, 14, 19 and 31 of incubation. Rewetting of the WDWD treatment occurred on day 10.

2.3 - Sample Processing

Water-extracts of incubated soil samples were prepared according to methods of Pittaway and Eberhard (2014). On the same day as sampling, 40 mL of deionised water was added to each 50 mL vial using an auto-dispenser. The samples were then placed on a clock-faced shaker for one hour.

After shaking, the samples were centrifuged at 2000 rpm for 5 minutes. Using a syringe, the water from each vial was extracted and filtered through a 0.45 µm pore-size glass fibre filter into a labelled plastic 70 mL container. When extracting the substrate from the vials it was ensured that as much liquid was extracted without disturbing the soil at the base. The filtered samples were then transported in a cooler to another laboratory ready for UV Vis Spectrophotometer testing. The residual soil ('washed soil') was retained and refrigerated (4 °C) for further processing and analysis.

The crucibles with 10 g of soil were placed into the oven set at 105 °C for a total of 48 hrs. After this period they were weighed to determine the gravimetric moisture content of the soil in each pot when the sample was taken.

Within 3 weeks of soil sampling on Days 11, 14, 19 and 31, the washed soil was used to produce a Potassium Chloride (KCl) extraction. In each vial deionised water was added with a dropper until the vial read 10 mL. Then 4 M KCl was added until the level read 20 mL in each vial. The final soil:KCl ratio was equivalent to 1:10 of 2M KCl as per the mineral N extraction method of Rayment and Lyons (2011). Pre-washing was previously recommended for clay soils by Catchpoole and Weier (1980). The vials of soil were then placed on the clock-faced shaker for one hour. The samples were then centrifuged at 2000 rpm for 5 minutes. Then each sample was filtered (Whatman No.1, 90 mm diameter) into separate, labelled containers. Pot reservoir water was sampled at the conclusion of the incubation trial (day 32). These extracted samples and reservoir samples were stored at 4 °C and sent to a specialist laboratory (Agricultural Chemistry Pty Ltd, Ipswich) to be tested for ammonium and nitrate within 6 weeks of sampling (Method 7C2, Rayment and Lyons, 2011).

2.4 - Data Collection

Ultraviolet light absorbance was measured on the same day as sampling and extraction by pouring the water extract filtrate into a plastic cuvette and measuring absorbance (6705 UV/Vis spectrophotometer, Jenway) at 200 nm, 224 nm, 254 nm, 280 nm, 365 nm, 465 nm, 500 nm and 665 nm. Using a pipette one mL of sample was put in the cuvette along with two mL of deionised water using a different pipette tip. The sample was then run in the spectrometer and the data was both saved on a memory card and written down. In between each sample the cuvette was rinsed thoroughly with deionised water and wiped down on the sides with lint free tissue paper.

Total dissolved nitrogen (TDN) was measured using an oxidative combustion-infrared analyser (TOC-V CSH with total N unit, Shimadzu, Japan). This required the sample to be poured into a vial and placed into the testing rack. Urea treatment samples for days 1, 3 and 7 were diluted 1:50 prior to analysis. Blanks and quality control checks were prepared and included every 40 samples. The instrument and analysis was run by Dr Friederike Eberhard, USQ. Dissolved organic N was calculated as the difference between TDN and KCl-extractable mineral N (nitrate-N plus ammonium-N).

3.0 Results

3.1 – Soil moisture content

A clear and intended difference in soil moisture content occurred up until day 11 with the WDWD pots drying out compared with the W pots (Figure 1). As intended, the WDWD pot moisture content increased to levels similar to the W pots by day 11 due to rewetting on day 10. Rather than drying out from day 11 until day 19 the WDWD treatment stabilised, presumably because there was enough water in each pot's reservoir to keep the pots at a constant soil moisture content. According to the data the pots from both the W and WDWD treatments then dried out between day 19 and day 31. It was expected that the constantly wet (W) treatment would maintain a higher moisture content than the WDWD treatment because these pots were being rewetted regularly. The accuracy of the scales used on day 31 was therefore questioned. Accurate scales could explain the

unexpected drop in soil moisture in both treatments despite observations of near-saturated soil and reservoir water supply on day 31.

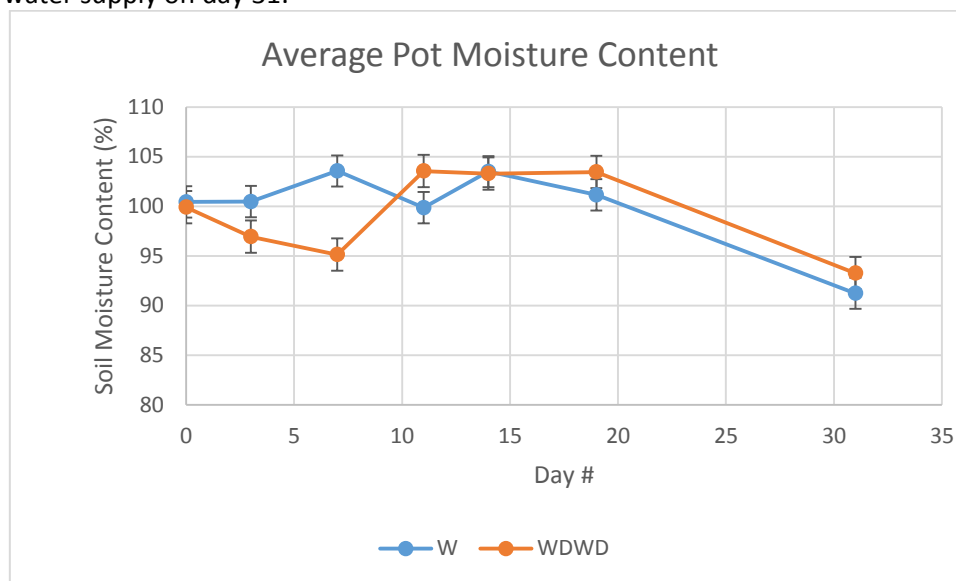


Figure 1. Mean soil moisture content (% w/w $\pm$ s.e.m.) measured on each day of sampling for the constantly wet (W, n=12) and wet-dry-wet-dry (WDWD, n=12) treatments

3.2 - Total Dissolved Nitrogen

The pots treated with urea had a higher average content of dissolved nitrogen than the control treatment on each day of sampling, as expected (Figure 2). There appeared to be no consistent difference between soil moisture treatments, however. The data for day 0 for the Urea – W (WU) and Urea – WDWD (WDWD U) treatments highlights the variability in sampled nitrogen with large standard errors evident and likely to reveal no significant difference in treatment means, as expected before drying would have commenced.

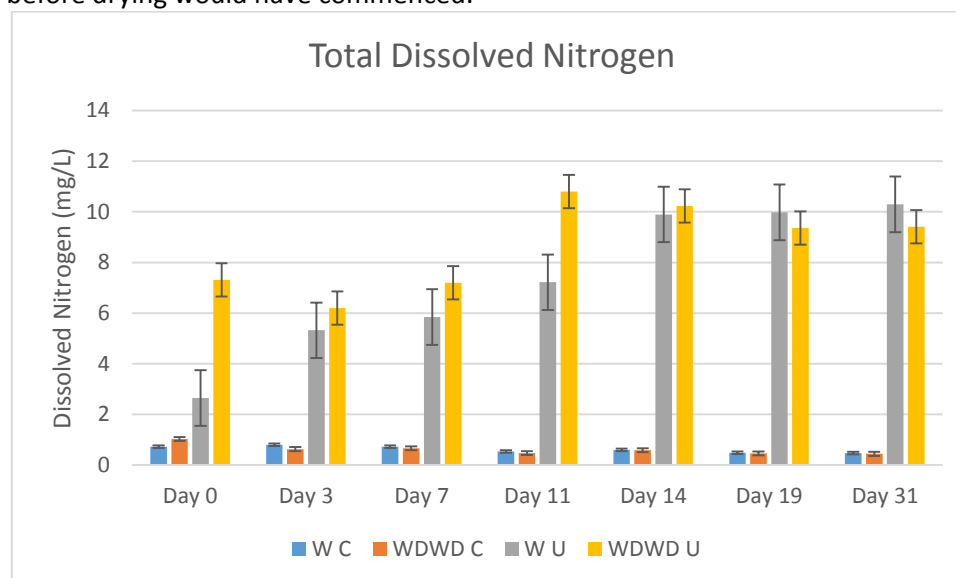


Figure 2. Mean total dissolved nitrogen (mg/L $\pm$ s.e.m.) measured on each day of sampling for the constantly wet, urea (W U, n=6) and control (W C, n=6) and for the wet-dry-wet-dry urea (WDWD U, n=6) and control (WDWD C, n=6) treatments.

3.3 – UV Absorbance at 224 nm

Absorbance at 224nm was measured to explore responses in absorbance 224 nm, which is a wavelength within the nitrate absorbance spectrum, to the incubation treatments. There appeared no consistent response in absorbance at 224nm between either soil moisture treatment or fertiliser treatment throughout the sampled period (Figure 3).

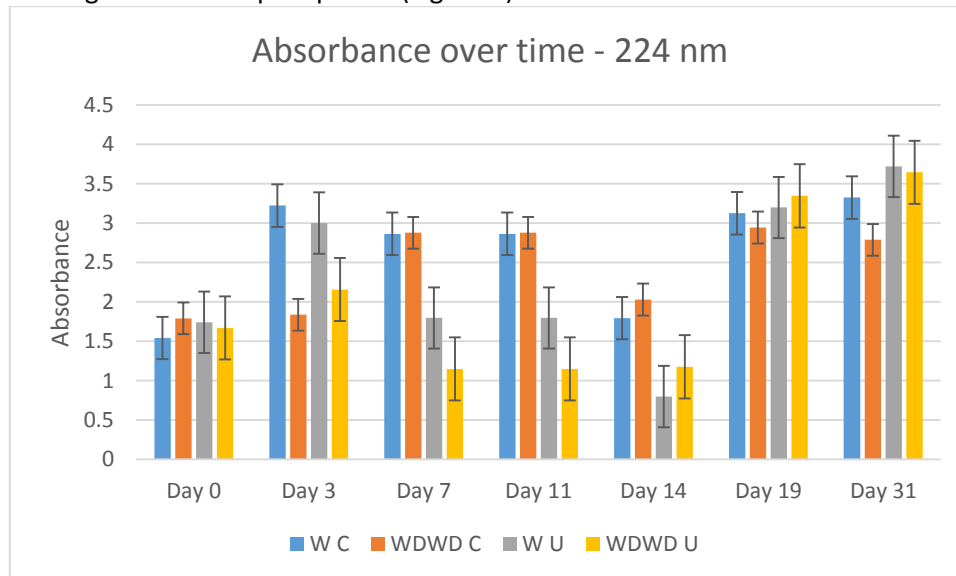


Figure 3. Mean UV absorbance at 224 nm (abs ± s.e.m.) measured on each day of sampling for the constantly wet, urea (W U, n=6) and control (W C, n=6) and for the wet-dry-wet-dry urea (WDWD U, n=6) and control (WDWD C, n=6) treatments.

3.4 –KCl-extractable Nitrate-N (NO_3^- -N)

Only samples from after the day 10 re-wetting event were analysed for KCl-extractable nitrate-N due to resource limitations. On average, there was a higher concentration of nitrate-N in the urea-treatment KCl extracts throughout the last four test days compared with control treatment, as expected (Figure 4). No consistent difference between soil moisture treatments was observed, however, there was a spike in nitrate content on Day 14 in the WDWD treatment three days after being rewetted. The spike was consistent with activation of nitrifying bacteria.

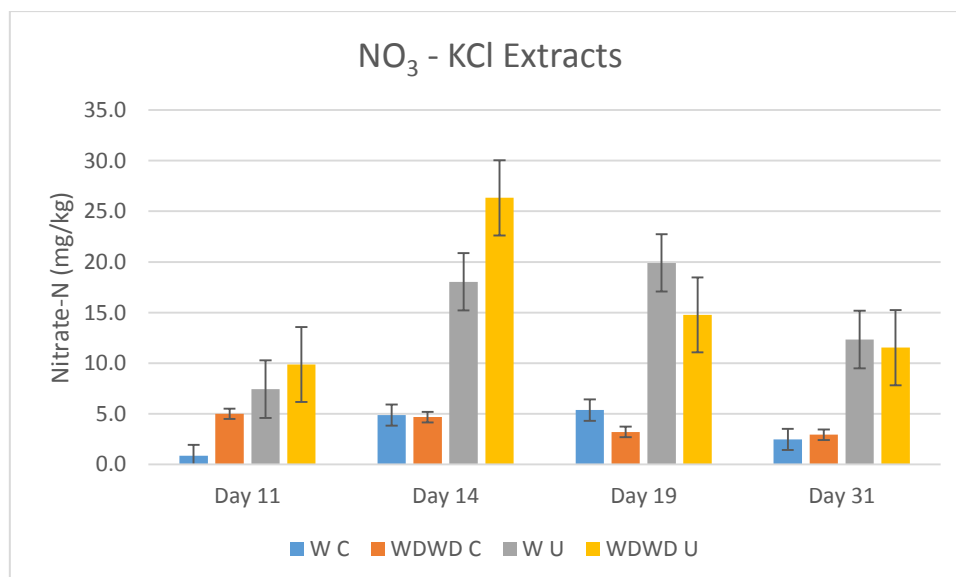


Figure 4. Mean KCl-extractable nitrate-N content (mg/kg ± s.e.m.) measured on each day of sampling after re-wetting on day 10, for the constantly wet, urea (W U, n=3) and control (W C, n=3) and for the wet-dry-wet-dry urea (WDWD U, n=3) and control (WDWD C, n=3) treatments.

3.5 – KCl-extractable Ammonium-N ($\text{NH}_4^+\text{-N}$)

Throughout Days 11 to 19 the constantly wet, urea treatment (W U) had much higher levels of ammonium than the other treatments (Figure 5). This may be due to the wet soil driving hydrolysis of urea into ammonia, and delayed nitrification converting that ammonia to nitrate. Because there was little difference in soil moisture content between the urea treatments over this period (Figure 1), the lower ammonia concentrations of the WDWD urea treatment was possibly due to a lower rate of urea hydrolysis (and therefore residual ammonium supply) prior to re-wetting, when the soil was drier than the constantly wet treatment.

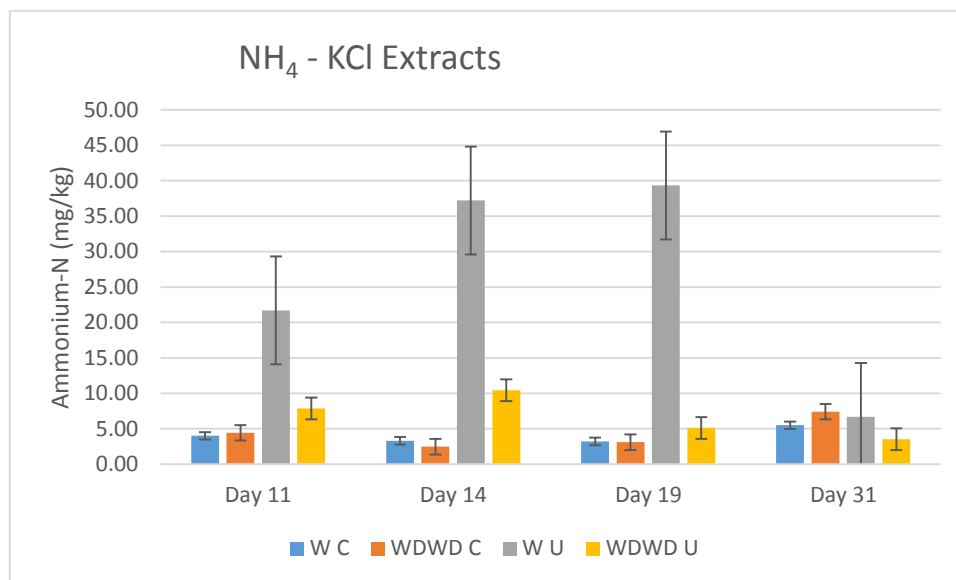


Figure 5. Mean KCl-extractable ammonium-N content (mg/kg $\pm$ s.e.m.) measured on each day of sampling after re-wetting on day 10, for the constantly wet, urea (W U, n=3) and control (W C, n=3) and for the wet-dry-wet-dry urea (WDWD U, n=3) and control (WDWD C, n=3) treatments.

3.6 – KCl $\text{NO}_3^-\text{-N}$ vs. UV 224nm

UV absorbance technology is widely used to measure total oxidisable nitrogen concentrations in water samples (Ferree and Shannon, 2001; Melland *et al.* 2012). Absorbance at 224 nm was measured to explore simple relationships between absorbance in water extracts at 224 nm and nitrate concentrations, and therefore for the potential for absorbance at 224 nm to be used as a surrogate for nitrate analysis in soil water extracts. With an R^2 value of 0.0885 there was little to no correlation between UV_{224} absorbance and nitrate content. Second derivative analysis of the nitrate UV spectrum (Crumpton *et al.* 1992) would unlikely improve the relationship given the negative correlation.

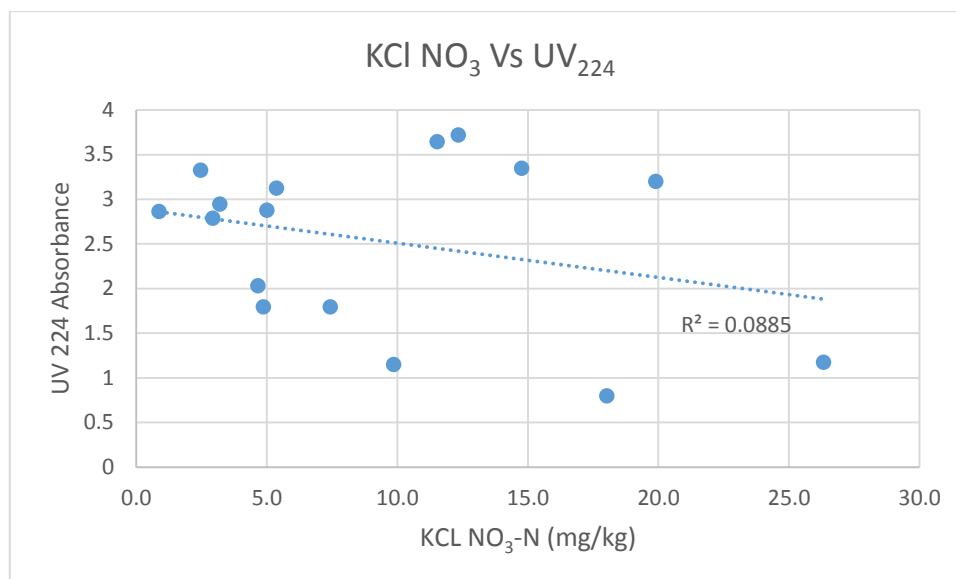


Figure 6. Pre-washed KCl-extractable nitrate-N (mg/kg) vs absorbance at 224nm of filtered (<0.45 μ m) water-extracts

3.7 – Dissolved Organic Nitrogen – Days 11 to 31

One to two-hundred times more dissolved organic nitrogen (i.e. TDN-NO₃N-NH₄N) was measured in both the urea treatments compared with the control treatment for all occasions measured (Figure 7). Despite this, there were no consistent differences between soil moisture treatments. Therefore the higher levels of dissolved organic nitrogen can likely be attributed to the presence of the organic urea.

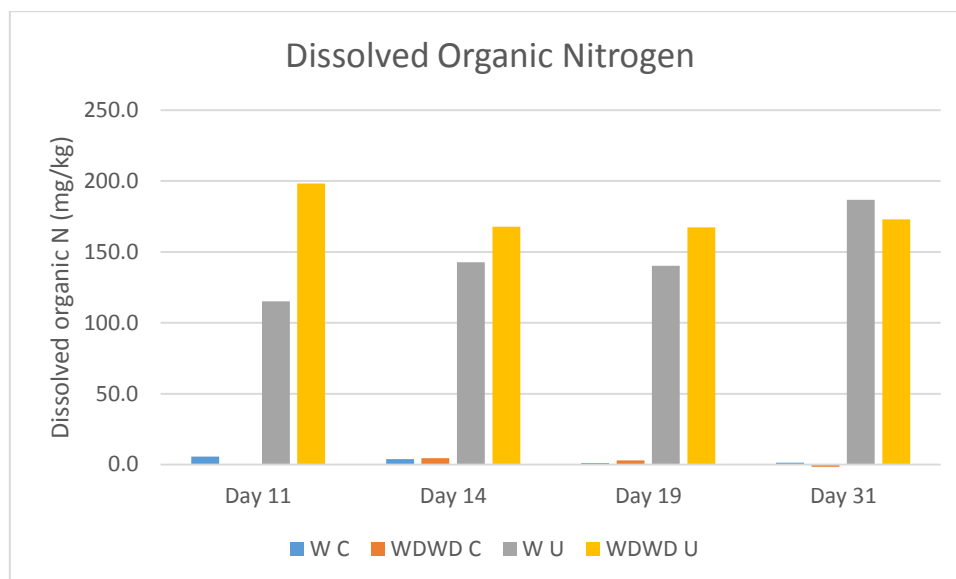


Figure 7. Mean dissolved organic N content (mg/kg) measured on each day of sampling after re-wetting on day 10, for the constantly wet, urea (W U, n=3) and control (W C, n=3) and for the wet-dry-wet-dry urea (WDWD U, n=3) and control (WDWD C, n=3) treatments.

3.8 – Average Nitrate (NO₃⁻-N) in Drainage Water

The drainage water from pots treated with urea had a nitrate content on average 83 times higher than the control pots (Figure 8). This indicates that some of the urea in the soil had percolated down into the drainage water. There was no clear difference between soil moisture content treatments. This was attributed to the presence of drainage water in the WDWD despite the intention being that the reservoir water would have been depleted in that treatment by day 31. Depletion did not occur, however, because when being rewetted on day 10, an excess of water was added such that water remained over the reservoir line (i.e. < 8 mm suction) for several days.

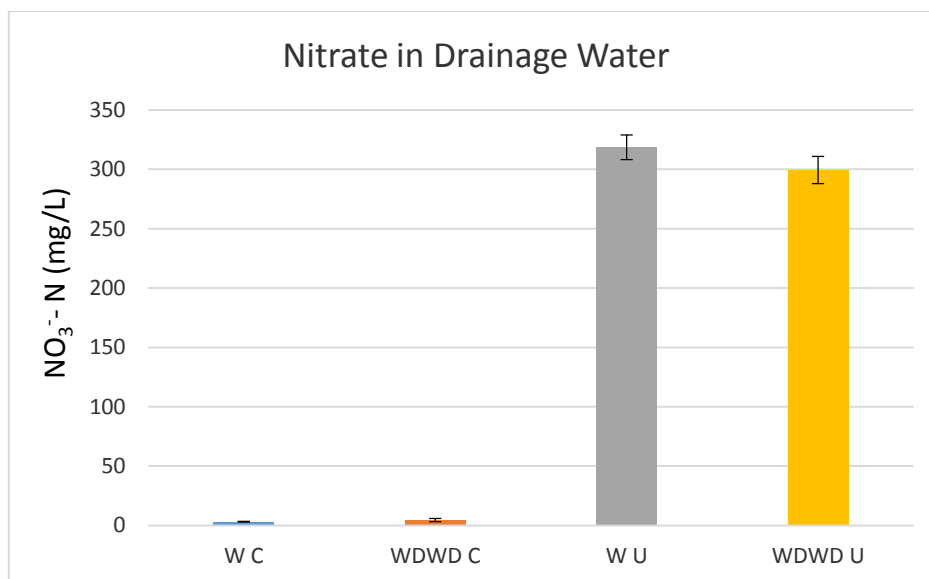


Figure 8. Mean nitrate-N (mg/L) concentration of pot reservoir water, sampled at the conclusion of the incubation trial (day 32) for the constantly wet control (W C), wet-dry-wet-dry control (WDWD C), constantly wet urea (W U) and wet-dry-wet-dry urea (WDWD U) treatments. Error bars indicate ± 1 s.e.m.

4.0 Discussion

Evaluation of wet and dry cycle simulation

The method was able to simulate the wet and dry cycles to some extent. It can be seen that both treatments began at the same water content after the initial wetting and the WDWD pots began to dry out while the wet treatment continued at a high level of moisture, as intended (Figure 1). As rewetting of the WDWD treatment occurred on day 10, it is likely that the moisture continued to drop in the WDWD pots between day 7 and day 10. A measurement of soil moisture immediately before re-wetting would have confirmed the full extent of drying. After rewetting the WDWD treatment pots, the moisture content rose very quickly and stabilised, rather than dried, over the following days as there was enough water in each reservoir to sustain the level of moisture. There was a sudden drop in measured moisture content by day 31 for both treatments despite there still being water in the reservoirs of each pot and despite visual observations that the soil in both treatments was still equally wet to previous sampling occasions. It is therefore unclear as to why the measured moisture content reduced. The scales used to weigh the oven dried samples were possibly inaccurate on day 31, causing the dramatic drop in the soil moisture for all data on that day of sampling.

The first cycle therefore simulated the wet and dry cycles as hoped, although to simulate more extreme drying, either a longer period of drying, a higher rate of evaporative demand (e.g. by creating more holes in the pot lids) or a higher incubation temperature, would be required. After rewetting the WDWD pot with too much water and with only having limited time to complete the incubation study, the second wet and dry cycle was not simulated as intended.

Recommended modifications to the method for future wet-dry-wet-dry experimental protocols

There are several modifications that could improve the overall reliability and consistency of all stages of the wetting, drying and sampling of the pots.

If time allowed it would be ideal if moisture content could be measured both before and after rewetting days so the full extent of the drying could be noted. Calibration checks of balances should also be conducted prior to each set of measurements.

An incubator that is not fan forced may better simulate the real soil conditions in the field.

If greater resources and time was available more data could have been collected for each sample day for a more comprehensive data set. The data collected could also be more reliable if all soil analyses could be conducted on the same day as sampling rather than storing the sample for days to weeks in the fridge before being analysed.

There could also be improvement in terms of sample collection technique. Taking samples from three different places around the surface of the pot meant that the sample was a better representation of the soil than if sampled from one spot only. Despite this there is a risk of taking a urea granule during collection, and surface samples may differ from the soil beneath. Perhaps a more consistent and representative technique could be implemented for this process to reduce large variations in results.

Differences in soil nitrogen fractions between the soil moisture treatments

There were no clear differences in soil nitrogen fractions throughout the sets of data, with the exception of ammonium. Ammonium was higher in the constantly wet treatment after re-wetting of the WDWD treatment possibly owing to more urea hydrolysis occurring in the constantly wet treatment during the first 10 days. Larger differences in soil moisture regime are likely to be needed in order to more fully investigate differences in nitrogen mineralisation rates due to wetting and drying cycles in the field.

Differences in soil nitrogen fractions between the Control and fertiliser treatments

There were consistent differences in the soil nitrogen fractions between the control and fertiliser treatments. Throughout the results higher levels of nitrate, dissolved organic nitrogen, and total dissolved nitrogen in the soil, and nitrate in the drainage water were found in the fertiliser treatment pots. This is likely due to the presence of organic nitrogen from the urea fertiliser and inorganic by-products after hydrolysis and nitrification processes were mediated by active soil microbes.

5.0 Conclusion

The 31-day aerobic pot incubation study successfully simulated a wet-dry cycle in a Black Vertosol but a second wet-dry cycle was not achieved due to excess water being supplied. Careful consideration of initial soil moisture conditions and period of incubation should be made in developing future wet-dry-wet-dry experimental protocols that utilise the capillary rise method of wetting. Fertiliser treatment effects were evident for all forms of measured nitrogen when compared with the unfertilised control treatment, with the exception of absorbance at 224nm. Absorbance at 224nm did not offer a simple surrogate analysis for nitrate in this experiment.

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