# Effects of rate of rewetting dried soils on nutrient forms and concentrations in leachate

## Stapledon Memorial Trust Student Vacation Bursary

Final Report

**Dates of placement:** 05/07/2010 – 10/09/2010

**Name of student:** Alison Carswell

**Details of university and course:** University of Exeter, BSc Geography

**Location of placement:** Rothamsted Research, North Wyke

**Student supervisor:** Dr Martin Blackwell

**Title of project:**

Effects of rate of rewetting dried soils on nutrient forms and concentrations in leachate

## 1. Personal statement

The work placement at North Wyke Research has enabled me to gain insight into the workings of a research centre. Whilst working here I have been able to witness many projects being carried out by people with various specialities and interests. I was also involved with collecting samples from the Hoosefield acid strip at Rothamsted, Harpenden (shown in the photo below), where after spending the night in the manor house, I was given a tour of the long term experiments. This opportunity enabled me to see a research centre that operated on a much larger scale and over a longer period of time.

My involvement with a specific project into the forms and amounts of P leachate from soil after a period of drying and then different rewetting rates has allowed me to perform experiments, to undertake laboratory analysis, carry out field work and data analysis and present data to a group, as well as writing this report. Writing up this report and the pointers I have received from my

supervisor have been valuable in giving me a rough guide into how a scientific report is taken from its first draft and developed into something publishable, and how lengthy this process could be. All of the new skills I have gained will valuably contribute to my independent research project that I will carry out over the next 6 months as well as enhancing my C.V. with work experience relevant to my degree.

Working in the laboratory I was involved in some exploratory work. This involved me being trained on and using the manual spectrophotometry method to analyse my samples for low levels of MRP, the same samples were analysed on a plate reader and on the Aquakem 250. Previously when the MRP analysis was done on the Aquakem discrete photometric analyser it had produced very low or zero results. Using the manual method I was unable to get the AQC standards within their testing specification limits. These standards had been made up with ordinary deionised water, after they had been remade in ultra high quality deionised water their results were within the limits. Therefore we were able to work out that the deionised water was contaminated with low levels of phosphorus, now when analysing for low levels of phosphorus it is recognised that the ultra high quality deionised water must be used. This line of work was extremely interesting and gave me first hand knowledge of problem solving in the laboratory.

I have also had the opportunity to do the field work for my dissertation project which is based on the impacts of tillage erosion. For this I have borrowed equipment that I probably would not otherwise have had access to, I was also joined by my project supervisor who helped me collect my soil samples and gave me helpful advice on aspects of my project that I was struggling with. I have also been able to gain insight from other members of staff at North Wyke who always seemed happy to help you out.

During my time here I have felt welcomed and part of the team. There is a great working relationship between fellow students as well as other members of staff, which is enhanced by a daily game of volleyball or darts in the lunch hour. The facilities on offer to students are of a high standard. Although I mainly commuted in each day from Exeter I spent a couple of nights in the student cottages which were very comfortable, I also spent these evenings exploring parts of Dartmoor with the other students. During my time here I was able to take part in some of the activities from the Health and Safety week and took the opportunity to have a go at some tractor driving (as shown in the photo).

I feel as though I have particularly benefited through having an excellent supervisor that is well organised, has ensured that I have always had something to focus on and has allowed me to see a project through from start to end. I have been able to carry out laboratory work and data analysis independently, whilst in the knowledge that any help I should need is readily available, which has also been useful when considering my dissertation project.


I came into my placement with little knowledge of research institutes and so everything I have seen and learnt here has been insightful and broadened my experiences. I have arrived at the conclusion that working within a research institute, particularly one that focuses on environmental research, is an exciting and interesting area to be a part of.

I would like to thank the Stapledon Memorial Trust for providing me with this opportunity. After spending ten weeks working on a research project at North Wyke I have decided that I would like to go into research once completing my undergraduate degree, preferably in a position relating to the agricultural sector. If it was not for the opportunity given to me by the Stapledon Memorial Trust to experience working in a research centre I may not have been able to be so decisive about my future career, I am therefore very grateful to them for this.

## 2. List of skills/experiences gained during placement

Experiment Planning – Knowledge of how to plan, prepare and perform experiments.

Field Work – Coring, use of T bar and Dutch corers. Vegetation sampling, use of quadrat and shears. Sampling strategy, use of the W shaped random field sampling and sampling along a transect. Use of Trimble GPS to mark and relocate sample sites. Work vehicle training / familiarisation with various cars within the car pool and a tractor.

Laboratory Work – Training in basic laboratory techniques, using volumetrics, pipettes, making solutions and reagents etc. Trained to use UV spectrophotometer with automated sipper, water potential meter, Zinsser perifill dispenser. Laboratory health and safety training, including COSHH awareness.

Data Analysis – Use of Microsoft Excel and Sigma plot in data analysis and graph production

Data presentation – Presented work to the group I was assigned to at North Wyke in the form of a short presentation as well as preparing this report.

General – I have sat in on two bi-monthly meetings within the soil group and have therefore experienced how people communicate within a research centre and seen presentations of other people's projects within these meetings.

## 3. Report

### Introduction

Soils in the UK often experience drying and re-wetting (D/RW) cycles and with climate change projections of increasing summer temperatures and decreasing summer precipitation (UKCP09, 2010) these cycles are likely to be exacerbated. Many authors have reported soil mineral flushes after D/RW stress, such as Butterly et al. (2009), Gordon et al. (2008) and Fierer and Schimel (2002). Less is known about how the rate of re-wetting affects nutrient mobilisation. In a study by Blackwell et al. (2009a) they found that re-wetting rate significantly affected the concentrations of phosphorus (P) in leachate. The significance of these findings is great, particularly with relevance to eutrophication (with Conley et al. 2009 describing P and nitrogen (N) as the key limiting nutrients in aquatic environments), and decreasing worldwide P stocks (Cordell et al. 2009). This project looks to build on the experiments of Blackwell et al. (2009a) by looking at the effects of re-wetting rate at a higher temporal resolution in order to look in more detail at how different re-wetting rates affect forms and concentrations of P and N in soil leachate. Two different soils are investigated so that the impact of soil type can be taken into consideration.

### Objectives

The objectives of this study are:

1. To test the hypothesis that rate of re-wetting a dried soil affects the forms and concentrations of N and P in the leachate and that this will differ among varying soil types.
2. To measure the effects of re-wetting a dried soil and a moist control soil over time periods of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 24hours on total phosphorus (TP), molybdate reactive phosphorus (MRP), bicarbonate ($NaHCO_3$) extractable MRP, total oxidised nitrogen (TON) and ammonium ($NH_4$) concentrations in leachate.

### Materials and methods

*Soil sampling and preparation*

The soils were sampled in July 2010 from the Rothamsted Research Station at North Wyke, Southwest England. Two soils were sampled; the Rowden soil is of the Hallsworth series and the De Bathe soil is of the Crediton series as described by Harold and Hogan (2008) (table 1). Table 2 describes the land use history and fertiliser or slurry treatments applied to the soils. The soils were collected to a depth of 10cm using a soil corer. The samples were collected randomly across the field using the W shape system (Soil Microbial Biomass Research Group 2009). Both soils were prepared by removing as many stones, roots and earthworms as possible in 1 hour, according to the alternative soil preparation technique described by Blackwell et al. (2009b). Approximately 3kg of each soil was passed through a 2mm sieve, it was then divided into 2 equal parts, one of which was moistened with ultra high quality deionised (UHQ) water to be used as a control and the other was oven dried at 35˚C. The soil was kept at 4˚C until required for analysis.

***Table 1.*** *Different descriptions given to the soils used in the drying and re-wetting experiments, taken from Harrod and Hogan (2008).*

| **Soil Series** | **Soil Type** | **Avery (1980)** | **USDA** |
|---|---|---|---|
| **Hallsworth** | Rowden | Pelo-stagnogley soils | Typic haplaquept |
| **Crediton** | De Bathe | Typical brown earths | Dystric eutrochrept |

***Table 2.****Land use history and information on slurry/fertiliser applications and concentrations. Rowden data from C. Hodgson (02Sep2010 personnel communication). De Bathe data from M. Brint (03Sep2010 personnel communication).*

| **Soil Type** | **Fertiliser/Slurry Applications Rates** | **N and P content of Fertiliser/Slurry** | **Land Use History** |
|---|---|---|---|
| **Rowden** | • May 2008<br>• September 2008<br>• June 2009<br>• October 2009<br>• June 2010 | June 2010 analysis:<br>• Total N 3.3 kg/t<br>• Total P205 1.25kg/t | • Undrained plot<br>• Grazed 9 Jul – 3 Sep 2010<br>• Last grazed in summer 2008<br>• Stocking density 2-6 cattle |
| **De Bathe** | • March 2010<br>• No fertiliser from 2005 to March 2010, field run under organic principles | • 450kg of 34.5% ammonium nitrate | • 2005-present day field used predominantly for grazing sheep |

*<u>Rewetting method</u>*

The rewetting experiments were carried out separately for the 0-4hr Rowden samples, the 0-4hr De Bathe samples and the 24hr Rowden and De Bathe samples. The method used for this experiment was the same as that used by Blackwell et al. (2009a).

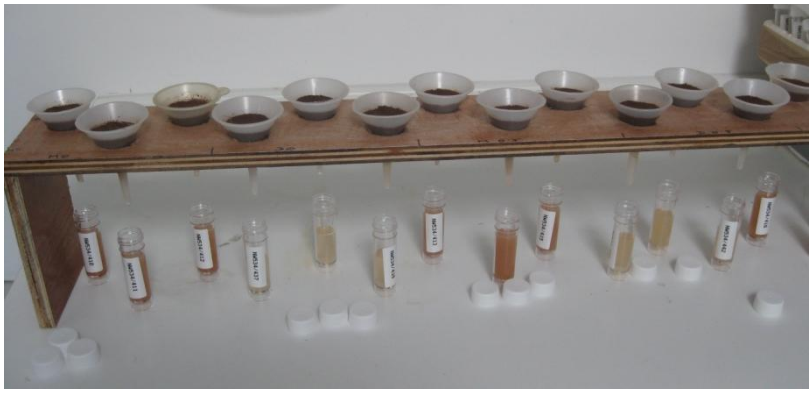

**Photo 1.** The re-wetting experiment for the De Bathe soil.

Moisture content was determined by drying at 105˚C to a constant weight three sub-samples of the moist and dried soil samples. The weight of soil required for 21g dry weight equivalent (DWE) was then determined. For each sub-sample (Rowden dry and moist, De Bathe dry and moist) 21g DWE of soil was weighed into 50ml plastic, conical funnels, which were previously plugged with 0.3g of glass wool. For each re-wetting period 3 replicates of each sub-sample and 3 blanks were prepared. The samples were covered with Parafilm and incubated at 18˚C for 24hrs prior to the experiment. The rewetting periods were 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 24hrs, thus making a total of 30 replicates for each soil sub-sample. During the re-wetting periods the samples were irrigated with 3mls of UHQ water at regular intervals, totalling 30mls throughout the rewetting period. The leachate was collected in 30ml polycarbonate vials which had been labelled and weighed. Fifteen minutes after the final irrigation the soil in the funnel was covered with Parafilm and the vial was capped and weighed to determine the volume of leachate collected. Half the leachate was filtered through a Whatman 0.45µm cellulose nitrate membrane filter to produce samples for determining dissolved nutrient concentrations, the other half was analysed for total nutrient concentrations. Particulate concentrations of nutrients were determined by subtracting the concentrations measured in the filtered samples from corresponding values measured in the unfiltered samples. Table 3 shows the analysis techniques. In the case of the Rowden dried samples not enough leachate was generated after the 30ml irrigation, so the leachates for these sub-samples were given a 3 x dilution with UHQ water before filtering. A 3 x dilution was made from both the filtered and unfiltered samples by taking 3mls of leachate and adding 6mls of UHQ water. All samples were stored at 4˚C prior to analysis.

*Bicarbonate extractable molybdate reactive phosphorus*

Analysis of $NaHCO_3$ extractable MRP was performed based on the method by Olsen et al. (1954). Extractions were carried out on the soil used in the rewetting experiments as soon as possible following completion of the rewetting and on 21g DWE of the original moist and dried soils, along with 3 blanks. The soil was weighed into 500ml plastic extraction bottles and 420mls of $NaHCO_3$ extractant was dispensed into each bottle, giving a soil to extractant ratio of 1:20. The bottles were then put on a reciprocal shaker at 90rpm for 1 hour before being filtered through a pre-pleated Whatman 42 filter paper in a 50ml conical funnel. The filtrate was collected into 30ml polycarbonate vials and stored at 4˚C prior to analysis.

*Water extractable P*

Water extractions were carried out in triplicate on the initial moist and dried sub-samples of the Rowden and De Bathe soil along with 3 blanks. 20g DWE of soil were weighed into 400ml centrifuge tubes and 80mls of UHQ water was dispensed into each bottle, giving a soil to extract ratio of 1:4. The bottles were shaken on a reciprocating shaker at 150rpm for 1 hour, following which the samples were then centrifuged at 10000rpm for 10mins. The supernatant was filtered through a pre-pleated Whatman 42 filter paper in a 50ml conical funnel and the filtrate was collected in a 30ml polycarbonate vial and stored at 4˚C prior to analysis.

*Microbial biomass P and carbon (C)*

The microbial biomass P was measured in the dried and moist sub-samples of the Rowden and De Bathe soil. Each sub-sample was analysed in triplicate. The microbial biomass P was measured using the $CHCl_3$ fumigation and 0.5M $NaHCO_3$ extraction method detailed by Brookes et al. (1982). Microbial biomass C was measured in the dried and moist sub-samples of the Rowden and De Bathe soils using the method detailed by Vance et al. (1987). Each sub-sample was analysed in triplicate along with 3 blanks.

*Potassium chloride (KCl) extractions*

The KCl extractions were performed referring to Bremner and Keeney (1966). The extractions were carried out singularly on the initial dried and moist sub-samples of the Rowden and De Bathe soil along with 3 blanks. 25g of soil was weighed into 500ml plastic extraction bottles and 50mls of 2M KCl solution was added to the bottle. The bottles were shaken on a reciprocating shaker for 1 hour at 150rpm, after which the samples were filtered through Whatman 2V filter papers. The filtrate was collected in 30ml polycarbonate vials and stored at 4˚C until analysed.

*Laboratory analyses*

***Table 3.*** *All the laboratory analysis information is shown in table 3. Included are the nutrient form tested for, the treatment of the soil which the leachate or extractant were taken from, whether the leachate was filtered or unfiltered and the equipment/method used for the analysis.*

| Nutrient tested for | Soil condition | Analysis performed on | Methods used | Laboratory equipment used |
|---|---|---|---|---|
| **MRP** | Re-wetted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 & 24 hours | Filtered and unfiltered leachate | Aquakem method PHOS, issue 2, 01/01/06. Murphy & Riley (1962) | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **TON** | Re-wetted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 & 24 hours | Filtered and unfiltered leachate | Aquakem method TON, issue 2, 01/01/06. Kamphake et al. (1967) and Kempers & Luft (1988) | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **$NH_4$** | Re-wetted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 & 24 hours | Filtered and unfiltered leachate | Methods for Examination of Waters and Associated Materials. Krom (1980), Searle (1984) | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **TP** | Re-wetted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 & 24 hours | Filtered and unfiltered leachate | Environment Agency National Laboratory Service Method of Analysis (2008) and Murphy & Riley (1962) | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **Particulate P** | Re-wetted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 & 24 hours | Filtered and unfiltered leachate | Calculated by subtracting dissolved TP from particulate TP | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **Organic P** | Re-wetted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 & 24 hours | Filtered and unfiltered leachate | Calculated by subtracting MRP results from TP results | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **$NaHCO_3$ extractable P** | Re-wetted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 & 24 hours and initial dried and moist soils | Filtered extractant | MAFF reference book 427, Olsen et al. (1954) and Murphy & Riley (1962) | Thermo Fischer Aqua Kem 250, discrete photometric analyser |

| Nutrient tested for | Soil condition | Analysis performed on | Methods used | Laboratory equipment used |
|---|---|---|---|---|
| **Water extractable TP & MRP** | Initial dried and moist soil | Filtered extractant | See MRP and TP rows | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **KCl extractable TON** | Initial dried and moist soils | Filtered extractant | Skalar methods, analysis: Nitrate + Nitrite, range0.1-5ppm N (1-50ppm), sample surface water/soil extract | Skalar SAN$^{PLUS}$ segmented flow analysis |
| **KCl extractable $NH_4$** | Initial dried and moist soils | Filtered extractant | Skalar methods, analysis: Ammonia, range0.5-5ppm N (0.5-50ppm), sample surface water/soil extract | Skalar SAN$^{PLUS}$ segmented flow analysis |
| **Microbial biomass P** | Initial dried and moist soils | Filtered extractant | MAFF reference book 427, Olsen et al. (1954) and Murphy & Riley (1962) | Thermo Fischer Aqua Kem 250, discrete photometric analyser |
| **Microbial biomass C** | Initial dried and moist soils | Filtered extractant | Samples sent to Harpenden | Samples sent to Harpenden |
| **Soil moisture content** | Initial dried and moist soils | Initial soil | n/a | n/a |
| **Soil Water Holding Capacity** | Initial field moist soil | Initial field moist soil | n/a | n/a |

*Data analyses and statistics*

Significant differences between the mean values of individual experiment replicates were calculated using a two-sample T-test using the software Genstat v. 10.1 (VSN International, Hemel Hempstead, UK) and were described as significant at the $p<0.05$ level, in accordance with Cochran and Cox (1950). Statistical analysis has only been carried out on re-wetting periods of relevance, such as 0.5 and 24hrs and 0.5 and 4hrs, because of time constraints.

## Results

*Initial results*

***Table 3*** *The results below are from the analysis of the initial dried and moist soils. Microbial biomass C results are not included because the results had not yet been returned.*

| Analysis Type | Rowden Soil | | De Bathe Soil | |
|---|---|---|---|---|
| | Moist | Dried | Moist | Dried |
| Moisture Content (%) | 39.8 | 2.1 | 17.3 | 1.1 |
| Water Holding capacity | 67.55 | n/a | 31.52 | n/a |
| Bicarbonate MRP (mg P / kg | 9.78 | 17.51 | 17.36 | 24.37 |
| Water Extractable TP (mg P/kg dry soil) | 0.87 | 7.73 | 2.11 | 38.25 |
| Water Extractable MRP (mg P/kg dry soil) | 0.22 | 1.20 | 1.30 | 2.03 |

| Analysis Type | Rowden Soil | | De Bathe Soil | |
|---|---|---|---|---|
| | Moist | Dried | Moist | Dried |
| Ph | 5.86 | 5.84 | 7.11 | 6.96 |
| Microbial Biomass P (mg P/kg dry soil) | 455.51 | 13.39 | 54.86 | 6.22 |
| Microbial Biomass C | n/a | n/a | n/a | n/a |
| Soil Moisture Potential (Mpa) | -0.03 | -116.55 | -0.14 | -91.12 |
| KCl Extract $NH_4$ (mg/litre) | 0.12 | 11.70 | 0.12 | 2.32 |
| KCl Extract TON (mg/litre) | 11.69 | 3.28 | 8.35 | 8.05 |

The results shown in **table 3** indicate the differences between the moist and dried soils before the re-wetting experiment had taken place. The moisture content of the moist Rowden soil at 39.8% is higher than that of the moist De Bathe soil at 17.3%. This is because of the different properties of the two soils the Rowden soil is clayey and the De Bathe soil is sandy this means the Rowden soil can hold more water per unit of soil than the De Bathe (Buckman and Brady 1969), reflected by the higher water holding capacity of the Rowden soil (67.55%) than the De Bathe soil (31.52%).

The results show that drying resulted in significantly higher concentrations of bicarbonate extractable MRP in comparison to the moist soil for both the Rowden soil ($P$ = <0.001) and for the De Bathe soil ($p$ = 0.025). The concentration of water extractable P in the dried samples for both soil types relative to the moist soil were also significantly higher (De Bathe water extractable TP $p$ = 0.012 and MRP $p$ = 0.001 and the Rowden water extractable TP $p$ = 0.001 and MRP $p$ = 0.001).

The microbial biomass P is more than 8 x higher in the moist De Bathe soil than in the dried, while in the Rowden moist soil the microbial biomass P was 34 x more than in the dried soil. These results are consistent with decreases in microbial biomass C after drying that were found by Blackwell et al. (2009a) and Van Gestel et al. (1993).

The impact of drying on the Rowden soil relative to the moist soil led to a 98 x higher concentration of $NH_4$ and a 3.5 x lower concentration of TON in the extract. These figures are less extreme for the De Bathe soil which had more than 19 x the concentration of $NH_4$ in the dried soil extract than in the moist and there was only 0.2mg/litre less TON in the dried soil extract than in the moist.

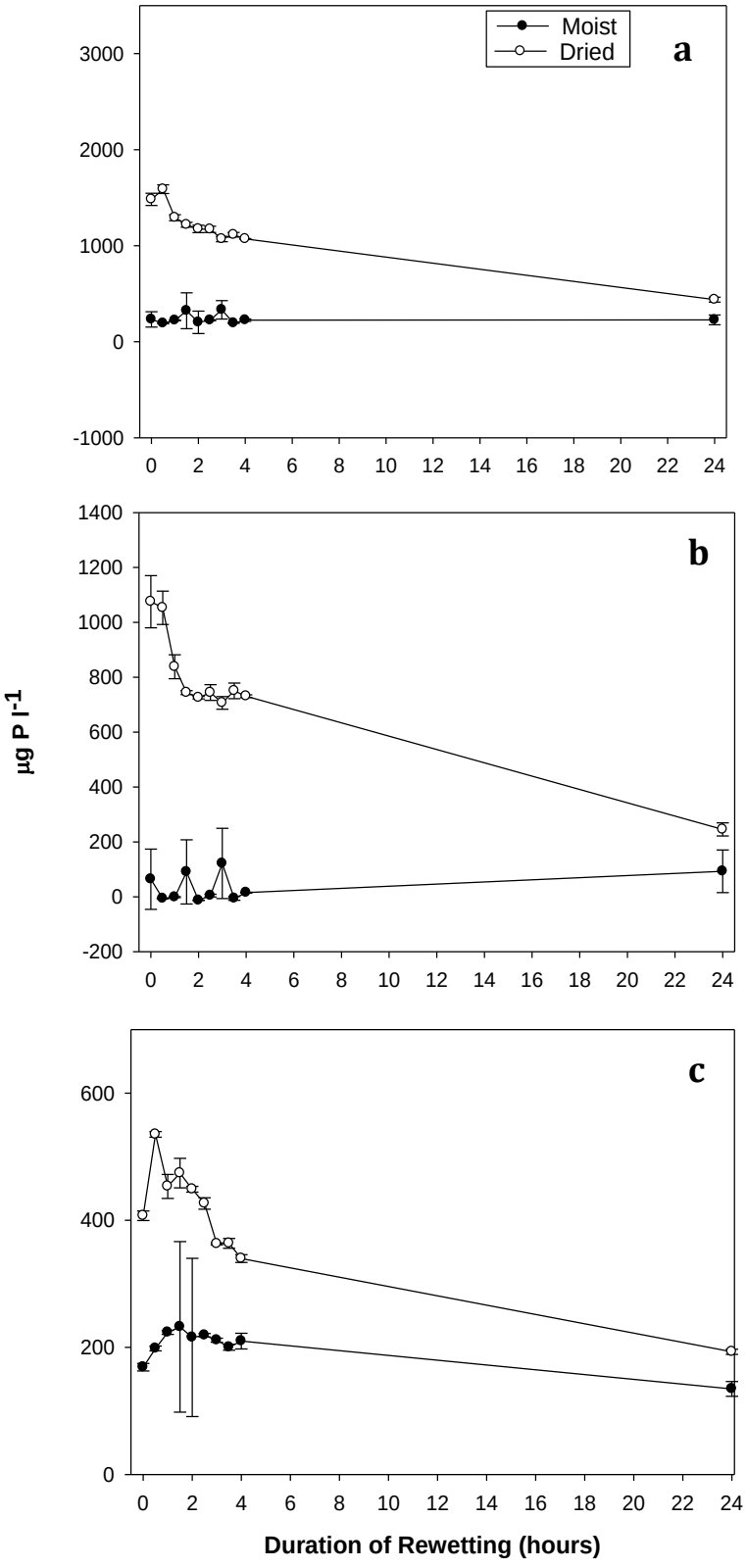


**Figure 1.** Dissolved P fractions in leachate from De Bathe soil (**a**. is TP, **b**. is unreactive P and **c**. is MRP). The error bars show standard error which is calculated from three replicates for each time

*Rewetting experiment results*

**Figure 1** shows the dissolved P fractions from the De Bathe soil leachate. **Figure 1a** shows that the concentration of TP in the leachate of the dried soil from the 0-4hr re-wetting periods was always higher than in the leachate from the moist soil. The highest concentration of 1588µg P $l^{-1}$ occurred in the 0.5hr sample, after which the concentration decreased to a minimum of 439µg P $l^{-1}$ in the 24hr sample. The difference between the TP concentration in the leachate for the dried and moist 24hr samples (211µg P $l^{-1}$) is much reduced in comparison to the 4hr re-wetting rates (846µg P $l^{-1}$). The difference in TP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant with *p* =0.007 and *p* =<0.001 respectively. **Figure 1b** shows the data for unreactive P. The concentration in leachate is highest at all re-wetting rates from the dried soil with the maximum concentration of 1075µg P $l^{-1}$ occurring in the 0hr sample, after which the concentration of unreactive P decreases and is reduced by more than 75% to 246µg P $l^{-1}$ in the 24hr sample. The difference in unreactive P concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting rates are significant (*p* =0.002 and *p* =<0.001 respectively). For the moist soil the unreactive P concentration increases with the slower re-wetting rate, from 64µg P $l^{-1}$ at 0hrs to 93µg P $l^{-1}$ at 24hrs. The MRP leachate concentrations in **figure 1c** are lower than in 1a indicating that the majority of the P is in an unreactive form. However the pattern of concentration is similar, with higher concentrations in the leachate from the dried soils than that in the leachate from the moist soils for all re-wetting

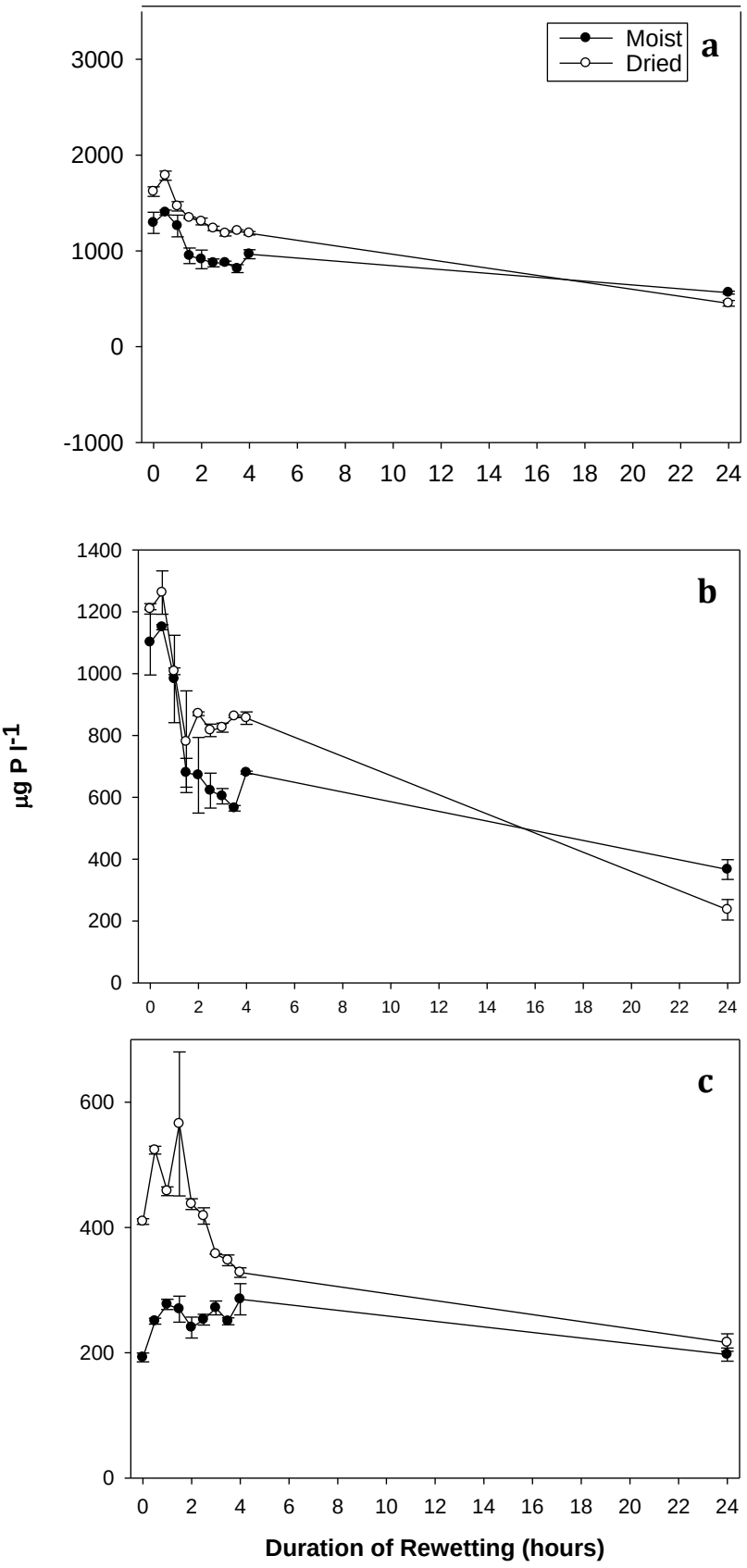


**Figure 2.** Particulate P fractions in leachate from De Bathe soil (**a**. is TP, **b**. is unreactive P and **c**. is MRP). The error bars show standard error which is calculated from three replicates for each time period.

rates. The concentration peaks at 535µg P $l^{-1}$ at 0.5hrs and is reduced by more than half to 193µg P $l^{-1}$ in the 24hr sample. The difference in MRP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ =<0.001 and $p$ =<0.001 respectively).

**Figure 2** shows the particulate P fractions in leachate from the De Bathe soil. **Figure 2a** shows the TP fraction in the leachate and displays a similar pattern to that of the dried soil dissolved TP in figure 1a, with the maximum TP concentration of 1785µg P $l^{-1}$ in the 0.5hr sample, decreasing to 453µg P $l^{-1}$.in the 24hr sample. The difference in TP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = <0.001 and $p$ = <0.001 respectively). **Figure 2b** shows the data for the unreactive P. The concentration in the leachate is highest at all re-wetting rates from the dried soil with maximum concentration of 1150µg P $l^{-1}$ in the 0.5hr sample and the minimum concentration of 236µg P $l^{-1}$ in the 24hr sample. The difference in unreactive P concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = 0.002 and $p$ = <0.001 respectively). **Figure 2c** shows the data for particulate MRP. The maximum concentration of 565µg P $l^{-1}$ occurs in the 1.5hr sample, after which it decreases rapidly to

357µg P $l^{-1}$ in the 3hr sample, the decline is then reduced and reaches a minimum of 216µg P $l^{-1}$ in the 24hr sample. The difference in MRP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = 0.002 and $p$ = <0.001 respectively)

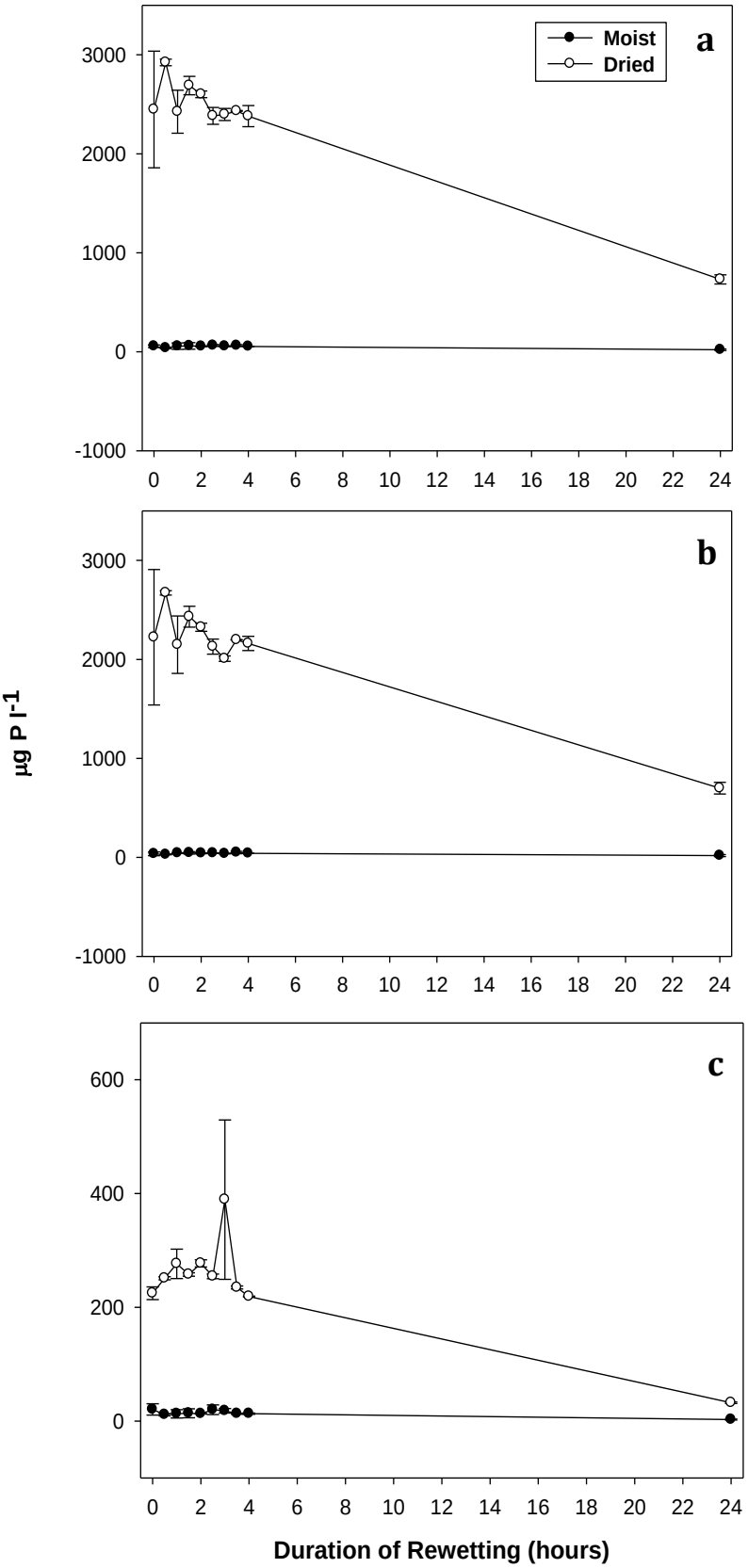


**Figure 3.** Dissolved P fractions in leachate from Rowden soil (**a** is TP, **b** is unreactive P and **c** is MRP). The error bars show standard error which is calculated from three replicates for each time period.

**Figure 3** shows the dissolved P fractions for the re-wetting experiment on the Rowden soil. **Figure 3a** shows that the concentration of TP in the leachate of the moist soil was always lower than in the leachate from the dried soil. The highest TP concentration occurred in the 0.5hr sample (2689µg P $l^{-1}$), following this the TP concentration declined to a minimum of 731µg P $l^{-1}$ in the 24hr sample. This variation in TP concentration is not visible in the moist soil. The difference in TP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = 0.008 and $p$ = <0.001 respectively). **Figure 3b** shows a very similar pattern to that of Figure 3a with the unreactive P concentration in the dried soil leachate peaking at 2672µg P $l^{-1}$ in the 0.5hr sample. The concentration minimum is reached in the 24hr sample at 699µg P $l^{-1}$. The difference in unreactive P concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = 0.010 and $p$ = <0.001 respectively). There is very little variation in the leachate concentration of unreactive P in the moist soil over all of the re-wetting periods. **Figure 3c** shows the MRP concentrations in the leachate. The MRP concentrations in the dried soil leachate are highest in the dried soil in the 0-4hr re-wetting rates peaking

at 389µg P $l^{-1}$ in the 3hr sample. However the large standard error bar suggests this value could be an anomaly. The difference in MRP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = <0.001 and $p$ = <0.001 respectively).

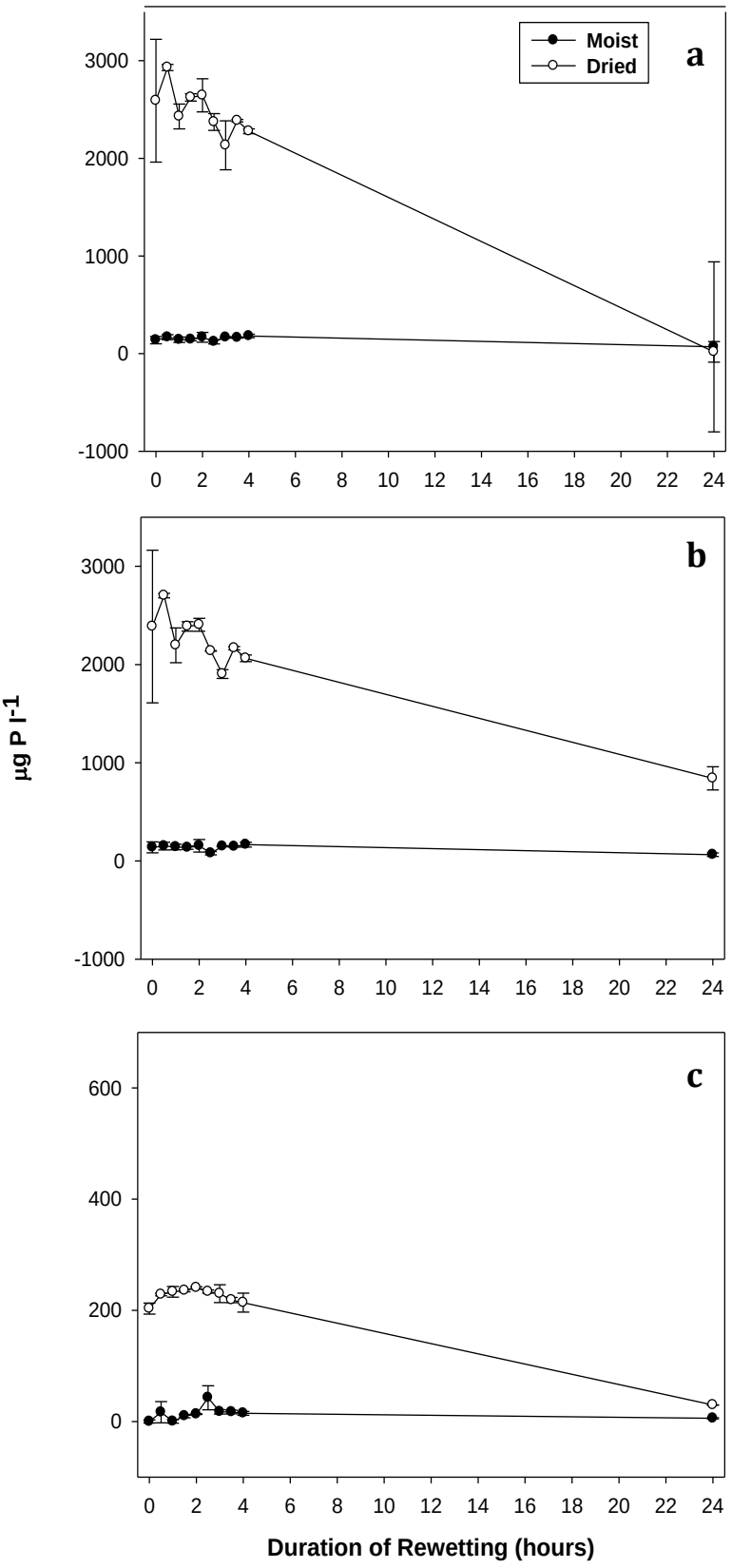


**Figure 4.** Particulate P fractions in leachate from Rowden soil (**a** is TP, **b** is unreactive P and **c** is MRP). The error bars show standard error which is calculated from three replicates for each time period.

**Figure 4** shows the particulate P fractions within the Rowden soil Leachate. **Figure 4a** shows TP concentrations in the leachate. The TP concentration in the dried soil is at a maximum in the 0.5hr sample at 2930µg P $l^{-1}$, after which the concentration declines to 2277µg P $l^{-1}$ in the 4hr sample and carries on decreasing to 871µg P $l^{-1}$in the 24hr sample. There is little variation (minimum of 70µg P $l^{-1}$ and a maximum of 180µg P $l^{-1}$) in the concentration of TP in the moist soil samples. The difference in TP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = <0.001 and $p$ = <0.001 respectively). **Figure 4b** illustrates the unreactive P concentrations in the leachate. Rapid re-wetting of the dried soil leads to high concentrations of unreactive P with a maximum value of 2702µg P $l^{-1}$ in the 0.5hr sample, after which the concentration declines to a minimum of 841µg P $l^{-1}$in the 24hr sample. The difference in unreactive P concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = <0.001 and $p$ = <0.001 respectively). **Figure 4c** shows the data for MRP concentrations in the leachate. The dried samples in the first 4hrs have higher concentrations of MRP than the moist samples (MRP concentration is at a maximum in the 2hr sample at 241µg P $l^{-1}$, the moist 2hr sample concentration

is 13µg P $l^{-1}$), the concentration of the dried samples decreases to a minimum of 30µg P $l^{-1}$. The difference in MRP concentrations between the 0.5 and 4hour, and the 0.5 and 24hour re-wetting periods are significant ($p$ = <0.001 and $p$ = <0.001 respectively).

**Figure 5** shows the particulate and dissolved TON concentrations (figure 5a and 5b respectively) of the leachate from the De Bathe soil. The concentration of particulate TON in the dried soil increases from 22mg TON $l^{-1}$ in the 0hr sample to a maximum of 25mg TON $l^{-1}$ in the 4hr sample and then declines to a minimum of 15mg TON $l^{-1}$ in the 24hr sample. The opposite occurs between the 4 and 24hr sample for the moist soil where the TON concentration increases from 19mg TON $l^{-1}$ in the 4hr sample to 25mg TON $l^{-1}$ in the 24hr sample. The concentrations of dissolved TON in the leachate are the same. The rate of re-wetting has led to significantly different concentrations in the leachate between the 0.5 and 4hour and the 0.5 and 24hour re-wetting periods for the dissolved TON $p$ = 0.045 and $p$ = <0.001 respectively and for the particulate TON ($p$ = 0.045)

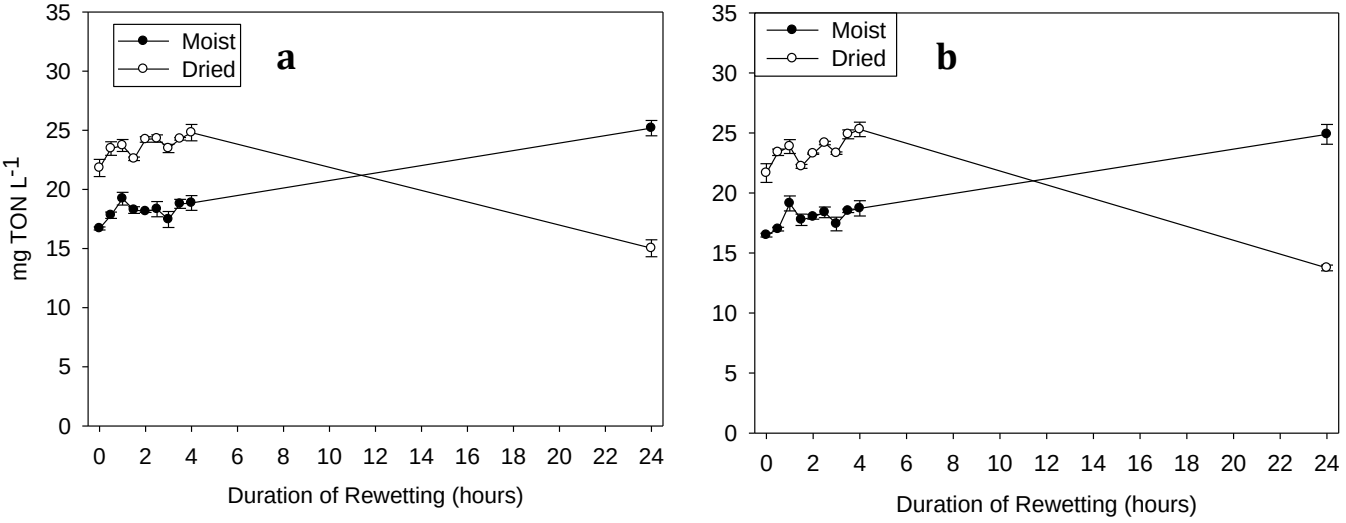


**Figure 5.** Total oxidised nitrogen (TON) in leachate from De Bathe soil (**a** is particulate TON and **b** is dissolved TON). The error bars show standard error which is calculated from the three replicates for each time period.

**Figure 6** shows the $NH_4$ concentration of the leachate from the De Bathe soil in both particulate and dissolved forms. There is little difference between the figures 6a and 6b, therefore implying that the majority of $NH_4$ is in a dissolved form. Only in the leachate of the dried soil is $NH_4$ present. The concentration is at a maximum in the 1.5hr sample at 0.9mg $NH_4$ $l^{-1}$ and decreases to 0.5mg $NH_4$ $l^{-1}$ in the 24hr sample. The $NH_4$ concentration in the leachate of the 0.5hr sample is significantly higher than that of the 24hr sample in both the particulate $NH_4$ and the dissolved $NH_4$ ($p$ =<0.001 for both).

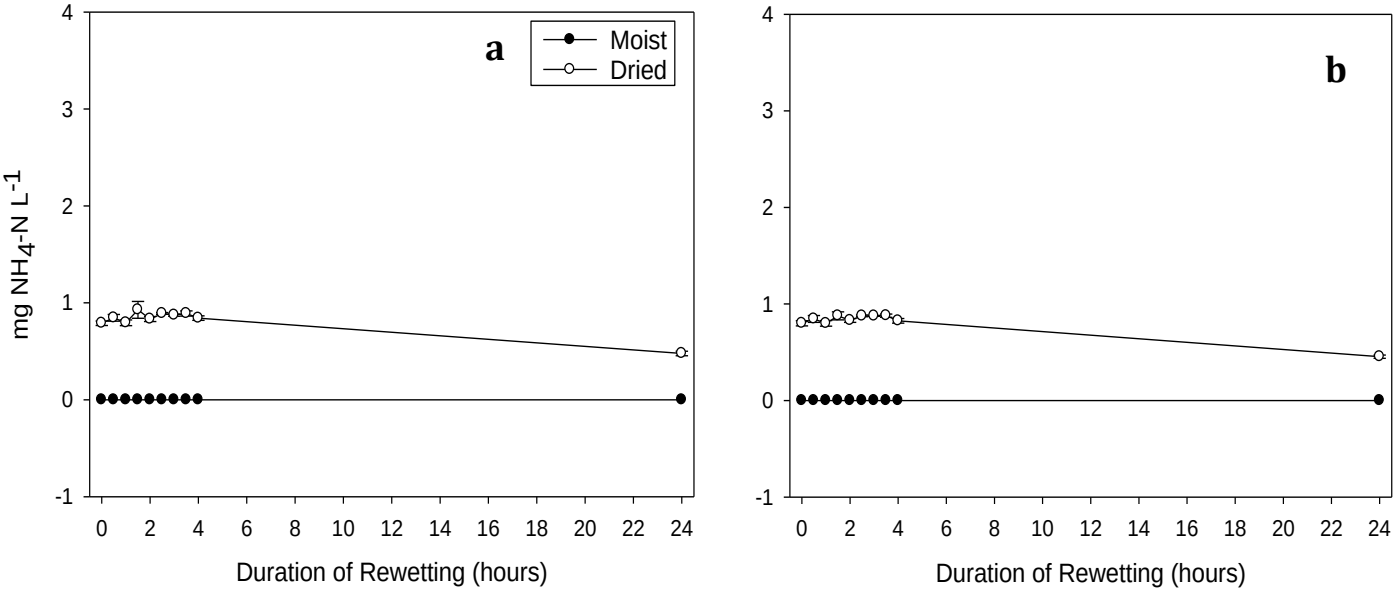


**Figure 6.** Ammonium ($NH_4$) in leachate from De Bathe soil (**a** is unfiltered leachate and **b** is filtered leachate). The error bars show standard error which is calculated from the three replicates for each time period.

**Figure 7** shows the particulate and dissolved TON concentrations in the leachate from the Rowden soil. As in figure 5, the particulate and dissolved concentration values are similar therefore it is likely that most of the TON is in a dissolved form. TON concentrations are less in the dried soil than in the moist soil, in the 3hr samples the concentrations are 13 and 15mg TON $l^{-1}$ for the dried and moist soil respectively, for the 24hr samples the concentrations are 5 and 33mg TON $l^{-1}$ respectively. The differences are slight in the 0-4hr samples (2mg TON $^{l-1}$ between the 3hr samples), however they are significantly greater (28mg TON $l^{-1}$) between the 24hrsamples. The difference in concentration between the 0.5 and 24hr dried samples are significant ($p$ = <0.001)

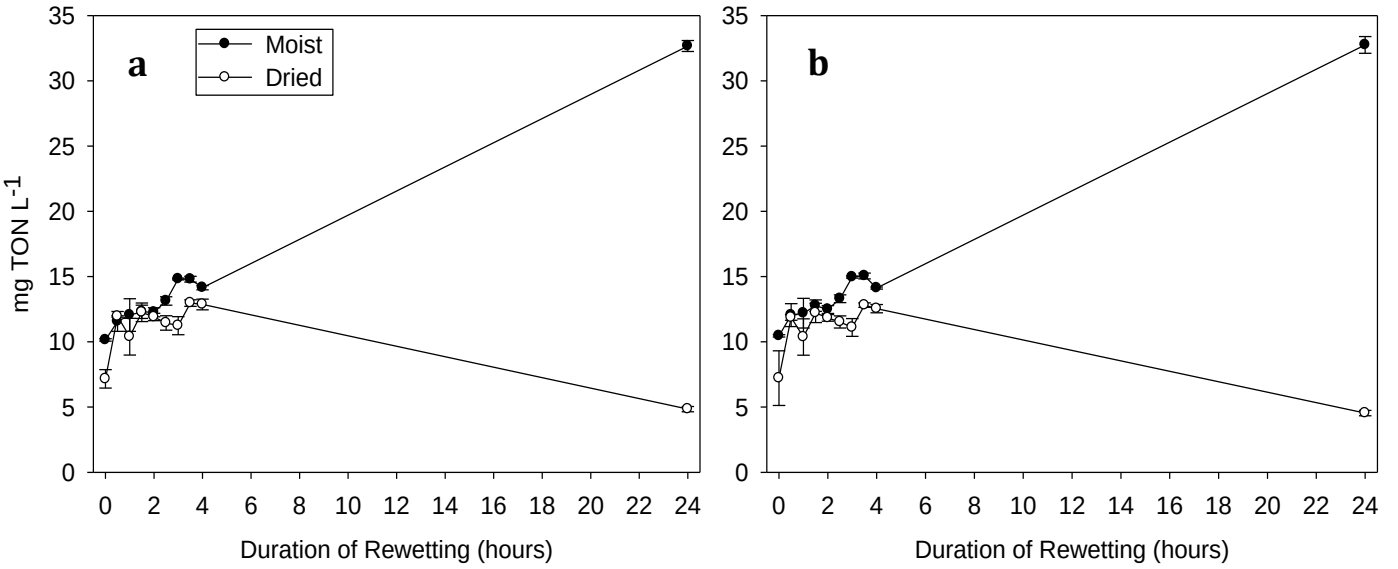


**Figure 7.** Total oxidised nitrogen (TON) in leachate from Rowden soil (**a** is unfiltered leachate and **b** is filtered leachate. The error bars show standard error which is calculated from the three replicates for each time period.

**Figure 8** shows the $NH_4$ concentration of the Rowden soil leachate for particulate and dissolved forms. Due to the small differences between the dissolved and particulate concentration levels it can be assumed that the majority of the $NH_4$ is in its dissolved form. The $NH_4$ concentration in the dried leachate peaks at 3mg $NH_4$ $l^{-1}$ in the 3.5hr sample, after which it declines by 50% to 1.5mg $NH_4$ $l^{-1}$ in the 24hr sample. The values in the moist control soil remain at zero throughout. The differences between the leachate concentration are significant between the samples for 0.5 and 4hrs ($p$ = 0.004 for both particulate and dissolved $NH_4$ at 95% confidence levels), and between the samples for 0.5 and 24hours (*p = <0.001)*.

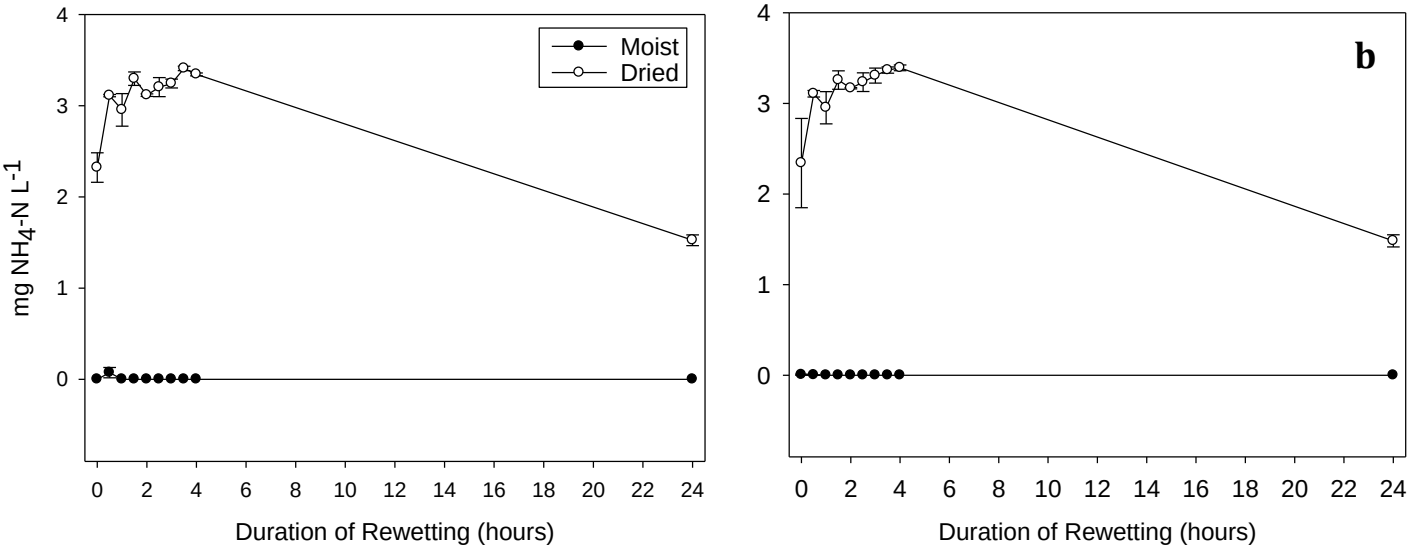


**Figure 8.** Ammonium ($NH_4$) in leachate from Rowden soil (**a** is unfiltered leachate and **b** is filtered leachate). The error bars show standard error which is calculated from the three replicates for each time period.

*<u>Bicarbonate extractable MRP results</u>*

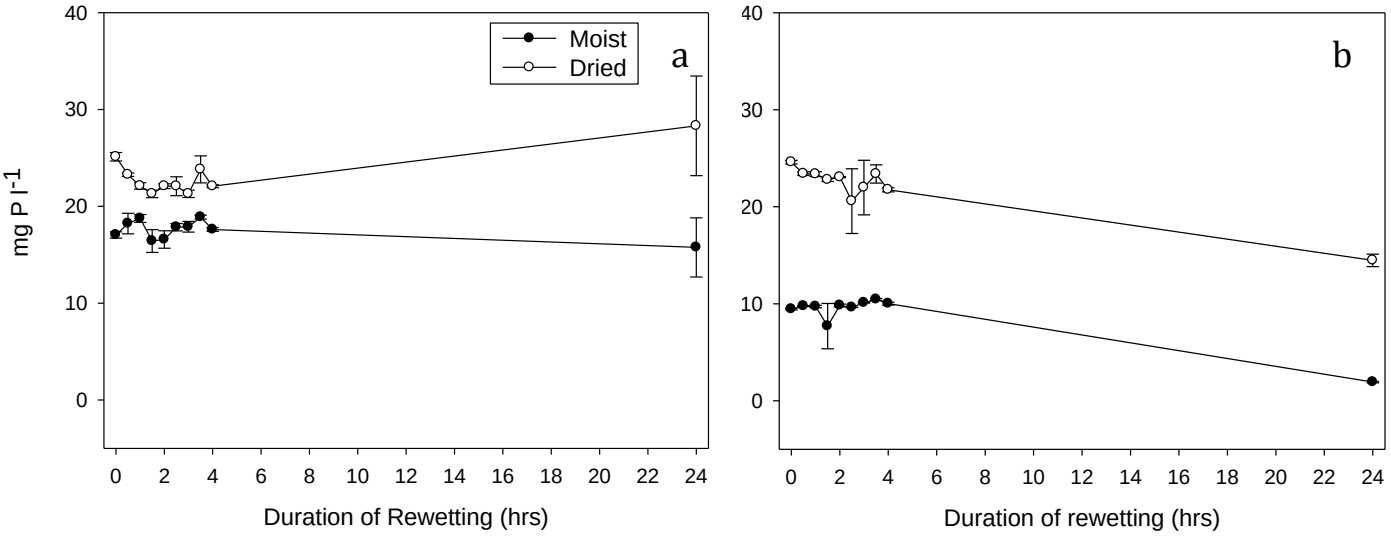


**Figure 9.** $NaHCO_3$ extractable MRP in leachate from **a** De Bathe soil and **b** Rowden soil. The error bars show standard error which is calculated from the three replicates for each time period.

**Figure 9a** shows the results from the analyses of the $NaHCO_3$ extracts for the De Bathe soil. The MRP concentration in the De Bathe soil are variable in both the moist and dried soil with concentration maximums of 28mg P $l^{-1}$ and 19mg P $l^{-1}$ in the 24hr dried and the 3.5hr moist samples respectively. The minimum values of 21mg P $l^{-1}$ and 16mg P $l^{-1}$ occur in the dried 3hr sample and the moist 24hr sample respectively. The difference between the amount of MRP leached at the 0.5 and the 4hour re-wetting rates is significant ($p$ = 0.007). The 24hr sample shows the maximum concentration, however as is shown on the graph this point has large error bars associated with it and may be an anomaly.

**Figure 9b** shows the $NaHCO_3$ extract results for the Rowden soil. The rapid re-wetting rates correlate with higher concentrations of MRP in the leachate with maximum concentrations of 25mg P $l^{-1}$ and 10mg P $l^{-1}$ in the dried 0hr and the moist 3.5hr samples respectively. The concentration of MRP in the leachate decreases from these samples to minimum values of 14mg P $l^{-1}$ and 2mg P $l^{-1}$ in the 24hr dried and moist samples respectively. The difference between the amount of MRP leached at the 0.5 and 4hour dried samples is significant ($p$ = 0.004). The difference between 0.5 and 24hr dried sample concentration is significant ($p$ = <0.001).

**Discussion**

The graphs in figures 1-4 show that both the dissolved and particulate TP, unreactive P and MRP concentrations in the leachate of the dried soil are elevated greatly beyond that of the moist soil when rapidly re-wetted. In the Rowden dissolved and particulate samples and the De Bathe dissolved and particulate samples the TP maximum concentrations (1588, 1785, 2689 and 2930µg P $l^{-1}$ respectively) occur in the 0.5hr samples. This TP pulse is much reduced in the 24hour samples where minimum TP concentrations occur. In the results for the unreactive P maximum concentrations in the leachate occur at either 0 or 0.5hrs in both the dissolved and particulate forms for both soils, as found in the TP results the minimum concentrations of unreactive P from the dried soil is in the 24hr sample. The close correlation between the TP and unreactive P for the separate soils suggests that the majority of the TP in the leachate is unreactive (or organic) P. This pulse of available P after re-wetting was also observed by Butterly et al. (2009) in their Australian study where re-wetting a dried soil led to a P availability increase of 35-40%. Turner et al. (2002) also found that air-drying soil increased the amount of water extractable unreactive P from 0.45µg P $g^{-1}$ to up to 1.63µg P $g^{-1}$. The unreactive P is potentially sourced from the breakdown of aggregates upon drying and re-wetting which exposes previously protected organic compounds to solubilization (Powlson and Jenkinson 1976).

It seems likely that at least part of the source of the P pulse must come from the microbial biomass. It is thought that cell lysis in a D/RW cycle occurs due to the osmotic shock of rapidly re-wetting desiccated cells, rather than during the drying process (Salema et al. 1982). This could also help to explain the elevated concentrations of TON in the dried soil leachate samples from between the 0 and 4hr re-wetting periods and it would have been interesting to have tested for microbial biomass N to see if N concentrations were affected by drying and re-wetting the soil. Turner et al. (2003) found in their study of two Australian pasture soils that potentially 88-95% of water extractable P released after rapid re-wetting came from lysed bacterial cells. The results in

table 3 show that the moist Rowden soil contained a microbial biomass P pool more than 8 x greater than that of the De Bathe soil. This larger microbial biomass P pool in the Rowden soil may partly be the source of the larger P pulses experienced in the Rowden soil, in comparison to the De Bathe soil (figures 1, 2, 3 and 4) after rapid re-wetting.

The $NH_4$ concentration in the leachate decreases substantially over the 24hour re-wetting period and this could be due to nitrification. Mian et al. (2008) suggest that accumulation of $NH_4$ is prevented by oxidation of $NH_4$ to $NO_3$ and that the initial N flush from re-wetting a soil is sourced from lysed cells or $NO_3$ residuals which are stored in the soil as it dries out. That the N flush is sourced from lysed microbial cells and mineral N is a theory supported by Van Gestel et al. (1993).

**Conclusions**

- Rate of re-wetting does significantly impact concentrations and forms of P in leachate, with rapid re-wetting (particularly 0.5hours) leading to higher concentrations than slower re-wetting
- In soil that has experienced D/RW cycle the microbial biomass P is much reduced in comparison to moist soil, this could partly account for the source of the P flush after re-wetting
- TON concentrations in leachate from the moist soils increase with slower rates of re-wetting, possibly due to nitrification, whilst the opposite is true for the dried and re-wetted soils

This work could be enhanced by carrying out more experiments to see how other forms of N are affected by rate of re-wetting, such as $NO_2^-$ and $NO_3^-$. It would also be of interest to investigate other soil types to see how mineral solubilization and mobilization are affected in them with different irrigation intensities. Work of this kind is essential in order to determine the most efficient ways to use our current stocks of fertiliser and to ensure that waterways are not affected by nutrient leaching from agricultural land.

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## 4. Comments by supervisor

Alison has been an excellent student and a pleasure to work with, integrating well with the community here at North Wyke and contributing to a lively research atmosphere over the summer. She has shown a keen interest in the project and was involved with all aspects, from experimental design, through sample collection and sample analysis to data analysis and report writing. She was quick to learn new techniques, especially in the laboratory, and never shied away from some of the more difficult or arduous tasks, always working conscientiously and methodically. She has carried out some interesting data analysis, and produced a detailed, extensive and comprehensive report of the work. We are still awaiting the analysis of some samples by our colleagues at the Rothamsted Research who agreed to process samples for biomass C content. When this data is available, I fully anticipate that it will be easily integrated into her report, which will be transformed into a scientific paper and published in a peer reviewed scientific journal such as Soil Biology and Biochemistry. Alison has accomplished a great deal in the time she has been here and I hope will stay involved with the work up to its publication. She has demonstrated that she has the ability and qualities to pursue a career in Environmental Research, which I hope she does. Overall, I think the bursary has been a great success and worthwhile exercise for all involved. I would like to thank the Stapledon Memorial Trust for supporting this initiative and providing this opportunity for Alison, and I hope this first award of the Stapledon Vacation Bursary has demonstrated to the Trust that it is a worthwhile scheme.

## Signatures:

**Student:** **Date:**

**Supervisor:** **Date:**