4. Laboratory experiments- N management

4.1 Introduction

In all laboratory experiments, fresh litter was used as the sole food source for earthworms (*E. andrei*) in a batch flow vermiculture system. Previous research has considered it difficult to use high N concentration poultry manures in vermiculture (Edwards et al. 1985). High mortality of *E. fetida* has also been observed when using fresh cattle manure as a substrate (Gunadi and Edwards 2003), probably due to the same negative effects of N that generally make poultry wastes unsuitable. As a result when vermiculture experiments have been conducted using animal manures, the manure was either partially composted or allowed to stabilise before introducing earthworms (Garg et al. 2005). This is what is generally referred to as 'cooling down' manure before use.

Ultimately the vermiculture system was designed for the on-site utilisation of litter, and had to accommodate for bio-security concerns of integrators. Integrators requested that the system should be self-sustaining from one cleanout of litter to the next, so as to avoid materials being brought on-site. This eliminated the option of mixing other low nutrient organic wastes with litter and improving its suitability as an earthworm food, which is a common vermiculture management technique (Garg et al. 2006). Integrators have strict regulations on the storage of litter on a grower's farm; hence the vermiculture system also had to be capable of utilising all litter immediately after shed clean out. Therefore, this research focused on using fresh litter as the sole food source for earthworms *(E. andrei)* in a batch flow system.

An inverted batch flow vermiculture system was adopted, where the earthworms and their bedding were placed above (capped) the litter. This provided a mechanism whereby soluble nutrients could be leached down away from the earthworms. Literature has suggested that earthworms are especially sensitive to N, most importantly NH_4^+ (Edwards 1995). This approach attempted to use large water applications to flush soluble nutrients away from earthworm bedding, including N compounds. This method also ensured that all litter was covered, thereby minimising odour and the need to continually surface apply and expose un-treated litter, avoiding on-site storage of litter. The use of capping is in contrast to traditional vermiculture feeding methods, where food is surface applied to an earthworm substrate (Standards Australia International 2003). This new approach was investigated in the following three experiments.

The first two experiments were conducted together to establish if litter could be vermi-processed in experimental containers. This involved developing measurements of earthworm behaviour that could determine earthworm stress. Two measurements of earthworm behaviour were recorded including a dispersion score and retraction rate, which were good indicators of system health or efficiency. In experiment 1, the application volume and timing of water was varied, primarily in an attempt to improve litter conversion and reduce earthworm mortality. Whilst experiment 2 investigated the effect ceasing water at day 8 and day 13 had on the system.

The third experiment investigated if increases in TN and salinity and variation in pH of earthworm bedding were responsible for a slower rate of vermicast production. The leachate from the system was analysed for NH_4^+, TN and EC to investigate if their removal from the system changed under different water regimes and if this was related to earthworms experiencing different stress levels.

4.2 Methods

4.2.1 Establishment of mini-vermiculture systems

Small replicated vermiculture systems were designed in an attempt to utilise fresh litter by using 4 l ice-cream containers. Nine small holes (3 mm) were punched in a cluster into the bottom of the containers, to allow drainage. A wick was then inserted into one of these holes to encourage fluid to drip from the one location (Figure 4.1). Each container was raised to allow leachate collection into 500 ml receptacles located beneath the containers (Figure 4.2). The litter was collected at shed cleanout (~40 days old) chilled and transported to the laboratory. Without removing the feathers the litter was homogenised and a 900 g sample taken using a soil splitter before it was weighed ready for loading.

To establish the earthworm substrate, 900 g of litter was added to the container followed by 170 g of bedding (coconut husk) placed on top (Figure 4.1). Coconut husk was chosen as bedding as it is the preferred substrate among vermiculturalists (Selden et al. 2005). The litter to earthworm (*E. andrei*) ratio was 5.3:1, based on wet weight of earthworms to fresh litter. Water (700 ml) was then added the day before earthworms were placed in the container to allow the bedding to become moist throughout, without any flow of leachate from the base. For descriptive purposes, during the process containers were divided into two distinct zones, earthworm bedding and litter. As the experiment continued a mixing effect by the earthworms led to the whole medium being called substrate (approximately 40 days). At completion the medium was then called vermicast, distinctive due to its lack of odour.

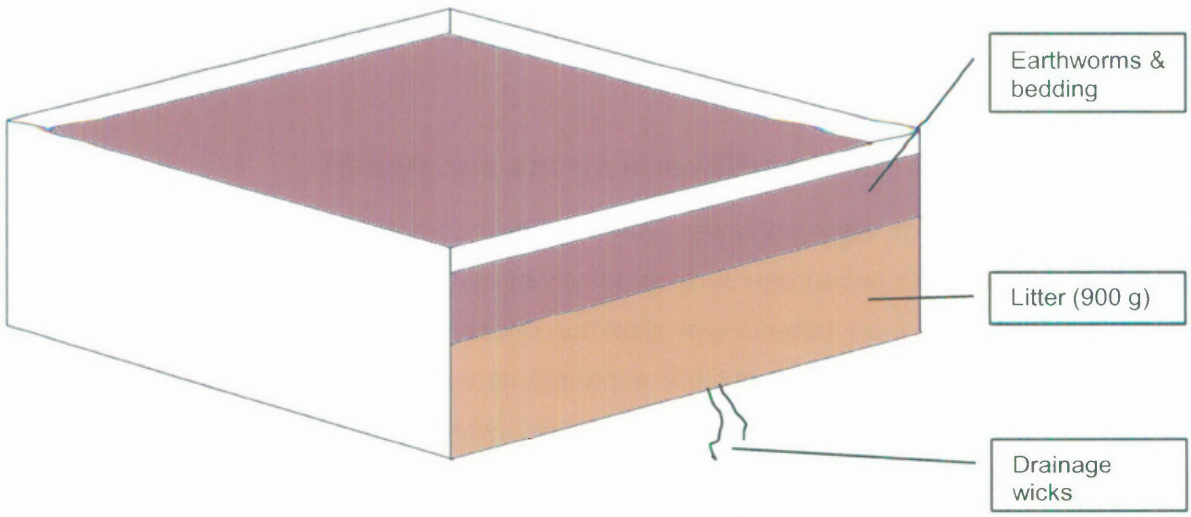


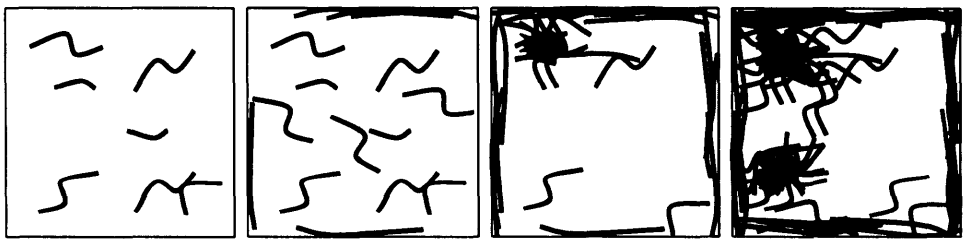
Figure 4.1 Loading procedure for small vermiculture experimental containers

A mix of different size earthworms were supplied in mature vermicast from a commercial supplier. From a large mass of earthworms, 170 g were weighed and loaded into the top of each experimental container and watered in with 200 ml of tap water. All containers were then covered with a dense black polypropylene lid to reduce light, leaving a small gap (10 cm^2) to allow gas transfer. A previous pilot study indicated the temperature of the litter, bedding and substrate was similar to ambient air temperatures throughout the conversion process; therefore temperature data were not collected in these experiments. The experiments were run in summer and winter with average laboratory temperatures ranging from 22 to 16°C, respectively. From experiment 3 onwards a nail was inserted into the drainage holes daily to promote leachate removal, especially during the first 10-15 days. Care was taken when clearing the drainage holes so as not to dislodge the drainage wicks, otherwise leachate would not be guided into the receptacles (Figure 4.1 & Figure 4.2).

Figure 4.2 Vermiculture containers with leachate receptacles

4.2.2 Measurements of earthworm stress and health

The polypropylene lid on each container was moved to completely exclude light for 5 minutes before sampling so as to encourage surface activity of earthworms. A dispersion score from 1 to 4 was assigned based on behavioural patterns observed in the pilot studies. The same observer assigned scores throughout the experiments by the use of a chart (Figure 4.3). A score of 1 represented a few highly dispersed earthworms on the surface with the majority deeper in the substrate. While a score of 4 indicated that the majority of earthworms preferred to bunch together, avoiding the deeper regions of the bedding.



(1) (2) (3) (4)

Figure 4.3 Plan view of earthworm dispersion (score of 1-4) after lid removed from ice-cream containers

A second measure was taken from the moment the lid was removed. The rate at which the earthworms retracted into the medium away from the light was measured with a stopwatch. A desk lamp (60 W incandescent bulb) was also positioned 30 cm above the container so that the light stimulus for the earthworms was consistent on all occasions. Full retraction, in seconds (s), was achieved when all healthy earthworms had disappeared. Immobile and dead earthworms were excluded from this measure. A retraction time of greater than 60 s was assessed as indicating that earthworms were not behaving normally, choosing to avoid the bedding. After earthworm retraction had occurred the dead earthworms left behind were counted, then very lightly covered with moist coconut husk (<0.25 g) which avoided these earthworms from being counted again at a later assessment.

Treatments were terminated when 75% of the earthworms had died, which equated to approximately 100 dead earthworms (~100 g). When earthworms would not retract into bedding (i.e. >60 s) daily mortality could still be determined as dead earthworms were pushed into the open areas of bedding by the bunching (stressed) survivors. Dead earthworms also have a distinctive colour and could be easily differentiated from sessile living earthworms, and if the observer was in doubt then a gentle prod would invoke a response if an earthworm was alive.

4.2.3 Conversion measurements

The decline in odour from the leachate in each receptacle indicated the system was nearing bio-stabilisation or conversion. Containers could then be gently inverted on their propylene lids and the container removed, which left a mound of vermicast the shape of the ice-cream container (Figure 4.4 and Figure 4.5). It was apparent if vermi-processing had not finished at this time, as wet offensive smelling litter was persistent at the bottom of the container. The container could then be replaced over the mound and inverted again, restoring the contents without changing the profile for later assessment.

For experiments that were not run to completion, the contents of the ice cream containers were inverted, leaving the contents intact on a bench (Figure 4.4). It was then possible to measure the depth that the earthworms had penetrated the litter to the nearest 0.5 cm. A colour distinction between the dark vermicast and the untouched pale litter made this a simple but accurate method to determine conversion depth. Due to earthworm's physically redistributing the rice hulls to the bottom of the container, a lighter layer of persistent hulls were evident in containers that were taken through to completion, and were not confused with pale untouched litter (Figure 4.5).

Figure 4.4 Measuring conversion depth after inversion, note the unprocessed litter

Figure 4.5 Fully processed litter with a layer of persistent rice hulls

4.2.4 Methods for experiments 1 and 2 (application, timing and ceasing water)

Considering that N is often water soluble (Nahm 2003), and that earthworms are very sensitive to NH_4^+ (Edwards 1995, Garg et al. 2005), water use was considered the most important factor affecting the system's design. It was evident that by placing the earthworms and their bedding above the litter food source, vertical water movement could potentially move N down and away from the bedding. This approach would require regular watering so that the effects of capillarity would not result in water and N moving up into bedding as the surface layers dried out. Also, the volume of water applied at each watering event was expected to be influential in removing N away from the bedding layer. Importantly, by placing earthworms above their food resulted in this vermiculture approach adopting a batch flow design.

The aim of experiment 1 was to determine the best watering regime to alleviate earthworm stress and avoid mortality, and maximise litter conversion. Experiment 2 was run in conjunction with experiment 1, and shared two treatments. In total, both experiments used 85 containers split into 17 treatments with five replicates (Table 4.1 & Table 4.2). All containers were loaded

and sampled as described in section 4.2. All data were statistically analysed using a two-way AOV in Statgraphics Plus 5.1™.

Water at 100 and 200 ml doses were applied every 0.5,1,2,3,4 and 5 days. Another treatment had 50 ml applied twice daily as an attempt to generate a stress response with a frequent small water volume application (Table 4.1). Four treatments were also maintained daily up to day 8 and 13 for both volumes, when watering was then ceased (Table 4.2). Earthworm dispersion, retraction and mortality parameters were recorded daily. After 23 days all treatments were inverted to determine conversion depth to the nearest 0.5 cm, after which the experiment was concluded.

Table 4.1 Volume of water and watering interval for experiment 1 treatments

Treatment	Volume of water (ml)	Watering interval
1	50	0.5*
2	100	0.5*
3	100	1
6	100	2
7	100	3
8	100	4
9	100	5
10	200	0.5*
11	200	1
14	200	2
15	200	3
16	200	4
17	200	5

*Twice daily

Table 4.2 Volume of water and day watering was stopped for experiment 2 treatments

Treatment	Volume (ml)	Day water stopped
3	100	23
4	100	13
5	100	8
11	200	23
12	200	13
13	200	8

4.2.5 Methods for experiment 3 (earthworm stress and N)

The high mortality of earthworms encountered during experiment 1 and 2 led to a need to identify the agent/s responsible so as to improve survival rates. By sampling bedding during the first five days when the system was most unstable, it was envisaged that the concentration of N and salts may increase. For example, bedding in a system with earthworms exhibiting stress may have higher concentrations of both N and salts (EC) than non-stressed systems. Also by comparing the concentrations of NH_4^+, TN and salts in leachate after the first five days it might be possible to show how successfully these compounds were being removed.

Experiment 3 used four treatments with five replicates to promote different "stress" levels in *E. andrei* populations by using four different water application rates. The hypothesis was that the concentration of N, salts and H^+ (pH) in leachate would not vary between treatments or with time. Secondly, the concentration of N, NH_4^+ and salts in leachate collected over the first five days would be similar. All containers were loaded and sampled as described in section 4.2.1. Statistically, repeated measure AOV using Statistix 8.0™ was used for TN, EC and pH over the five days of sampling, while all other variables were analyses in Statgraphics Plus 5.1™using a one-way AOV.

Water was applied to containers using a step-down approach which allowed for larger water volumes to be applied initially, as recommended by commercial vermiculturalists. This approach was chosen due to the early earthworm losses encountered in experiment 1 and 2 when only using 100 or 200 ml volumes. Water stress was expected to be exacerbated in treatments 2-4 by extending watering past daily applications (Table 4.3).

Table 4.3 Water volume, timing, and rate over the first five days

	Volume of water (ml)					
Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Total water
S1	1400	500	500	400	400	3200
S2	1400	400	200	200	0	2200
S3	1400	300	0	100	0	1800
S4	1400	300	0	0	0	1700

Approximately 20 g of bedding was collected from all treatments on days 3, 5 and 6. It was important not to remove more bedding than was required for analysis so as to avoid earthworm stress. This material was oven dried at 50°C and ground using a mortar and pestle before being analysed for TN, EC, and pH. At this stage in the research NH_4^+ analysis was not undertaken due to the 2.5 g dry wt of bedding sample required (Keeney and Nelson 1982). Total N was determined using Kjeldahl nitrogen digests (TKN) and spectrum absorption. The EC and pH of bedding was determined using a 1:10 suspension after agitation for 1 h. A 1:5 suspension for EC and pH was not possible due to the high water holding capacity of the coconut husk. This approach has been adopted for other vermiculture substrates with high water holding capacity (Ndegwa and Thompson 2000, Garg et al. 2005).

The leachate from each replicate was transferred daily into 1 l plastic containers and frozen. At the completion of the experiment on day 5 the container was thawed, agitated and a sample taken for analysis of TN, NH_4^+ as well as EC and pH (Keeney and Nelson 1982, Rayment and Higginson 1992). Healthy treatments were run until complete conversion of litter had occurred using 100 ml/day of water for 10 days after the initial experiment and 100 ml/4 days thereafter until completion.

4.3 Results

4.3.1 Experiment 1 (application and timing)

Sample error (SE) for all tables is located in Appendix C, while detailed statistical analysis for all figures and tables are in Appendix D.

The hypothesis for this experiment was that by decreasing watering interval and increasing water volume the litter conversion rate would increase and earthworm mortality would be reduced. The conversion of litter was effected by the experimental factors (watering interval and volume, Figure 4.6). However there was a treatment effect whereby 100 ml/0.5d achieved greater litter conversion ($P<0.001$) than 100 ml/1d, this relationship did not hold for the 200 ml/0.5d and 200 ml/1d. The 50 ml twice a day treatment indicates that less litter will be converted over 23 days when compared to both 100 and 200 ml applied once and twice a day (Figure 4.6).

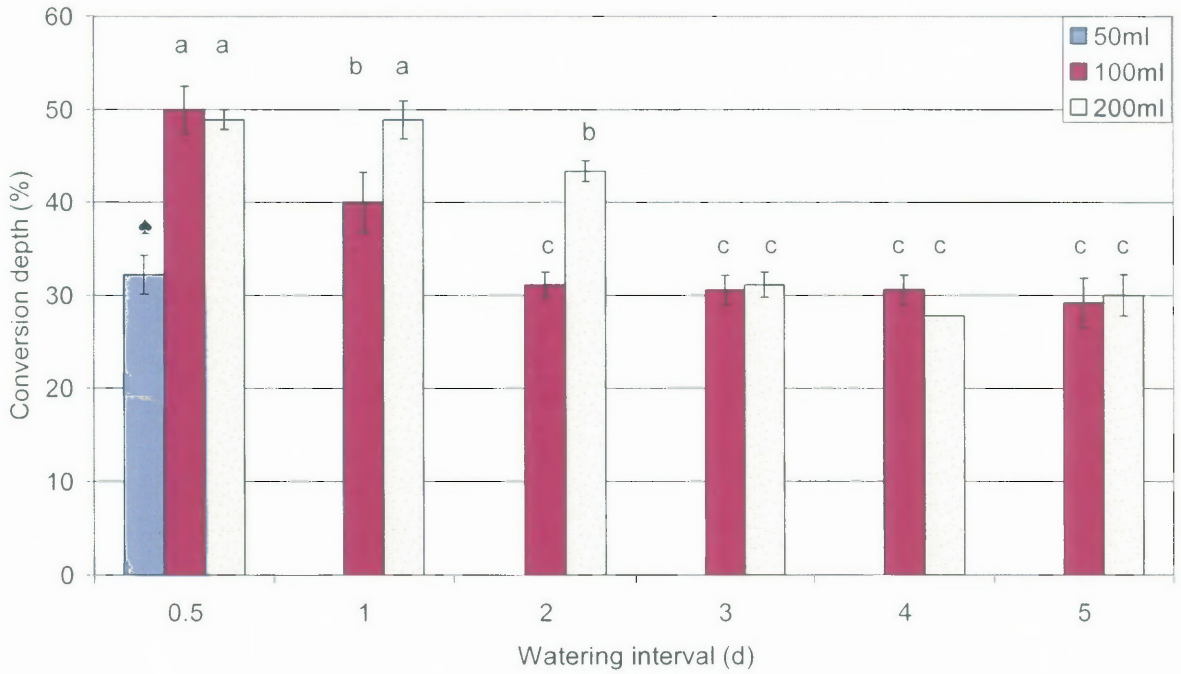


Figure 4.6 Depth of converted litter as a response to watering interval and volume (±SE)

♠ 50 ml applied twice daily representing a low volume/small interval treatment

Different superscripts show significant differences between treatments ($\alpha=0.05$)

Both experimental factors had an effect on percentage earthworm mortality, and a treatment effect was apparent, where 100 ml/3d had higher mortality ($P<0.0001$) than 200 ml/3d. In comparison there was a trend indicating that 100 ml/0.5d would result in lower mortality than 200 ml/0.5d (Figure 4.7). The 50 ml/0.5d treatment has similar mean total mortality over 23 days

as 100 and 200 ml/0.5d treatments (Figure 4.7). With decreasing watering intervals mean dispersion scores and retraction rates were reduced ($P<0.001$), however water volume had no affect (Table 4.4).

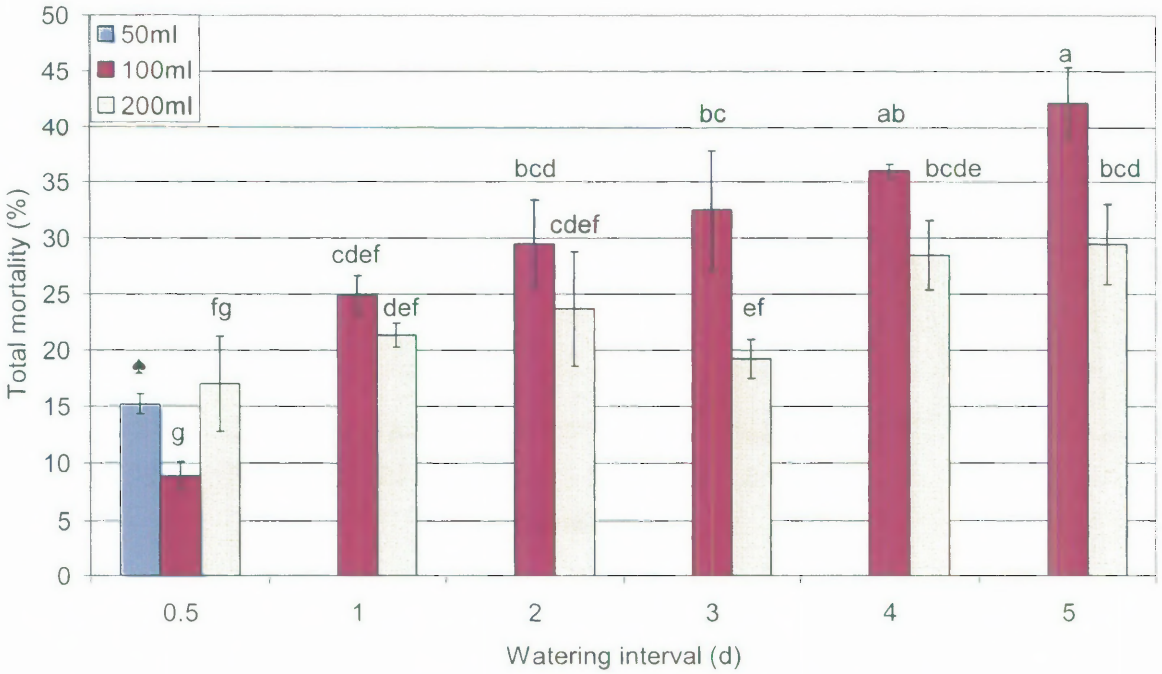


Figure 4.7 Mean total mortality as a response to watering interval and volume (±SE)

♠ 50 ml applied twice daily representing a low volume/small interval treatment

Different superscripts show significant differences between treatments ($\alpha=0.05$)

Table 4.4 The effect of watering interval and volume on earthworm dispersion and retraction

	Watering interval (Int.) (days)						P-value		
	0.5	1	2	3	4	5	Int.	Vol.	Int. x Vol.
Dispersion (score)^	1.33 ^{d}	1.35 ^{d}	1.53 ^{c}	1.60 ^{bc}	1.69 ^{a}	1.77 ^{ab}	0.0000	NS	NS
Retraction (s)«	7.9 ^{c}	9.6 ^{c}	15.6 ^{b}	16.5 ^{b}	23.4 ^{a}	23.8 ^{a}	0.0000	NS	NS

Different superscripts show significant differences between treatments ($\alpha=0.05$)

^Chi-square transformation «square root transformation

Daily mortality, dispersion scores and retraction rates were higher at the start of the experiment for both 100 and 200 ml watering volumes. Also, as daily mortality, dispersion score and retraction rates increased, the variation between replicates was greater (Figure 4.8, Figure 4.9 & Figure 4.10). The 100 ml applications on day 1 had significantly higher mortality on day 2 (Figure 4.8) compared with 200 ml applications.

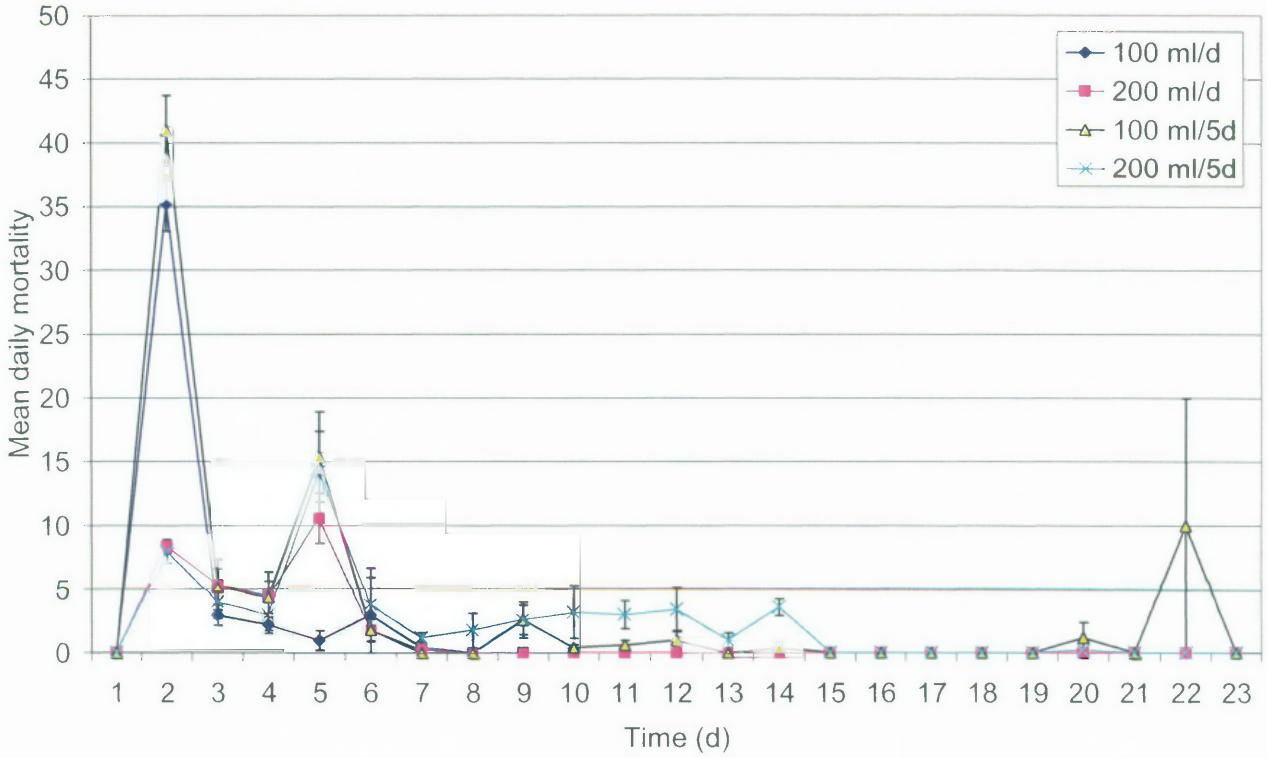


Figure 4.8 The mean daily mortality for treatments receiving 100 and 200 ml volumes when comparing 1 and 5 day watering intervals (±SE)

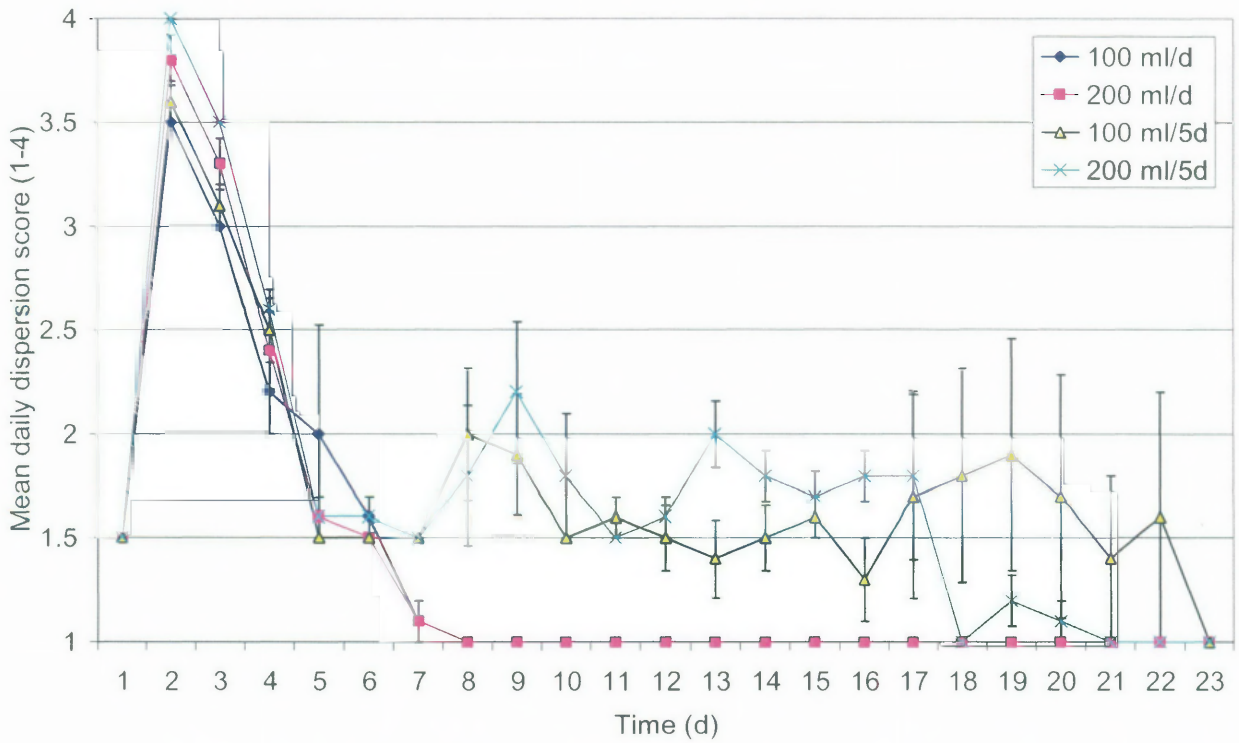


Figure 4.9 The mean daily dispersion score for treatments receiving 100 and 200 ml volumes when comparing 1 and 5 day watering intervals (±SE)

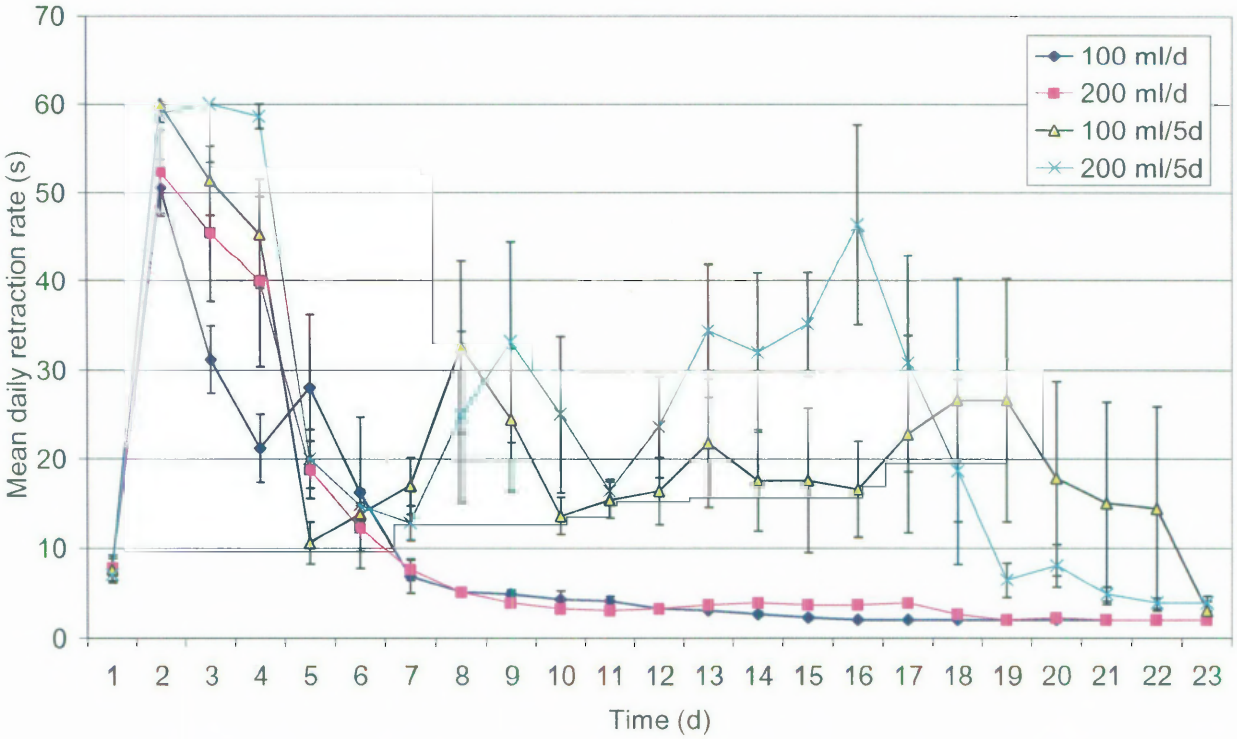


Figure 4.10 The mean daily retraction rate for treatments receiving 100 and 200 ml volumes when comparing 1 and 5 day watering intervals (±SE)

4.3.2 Experiment 2 (ceasing water)

Eliminating water at day 8 and 13 would result in elevated earthworm mortality and a reduction in litter conversion, was the hypothesis for this experiment. Ceasing watering on day 8 resulted in less litter being converted ($P<0.01$), greater dispersion score ($P<0.01$) and greater retraction rates ($P<0.01$) when compared to ceasing watering on either day 13 or 23. Water volume had no effect on dispersion or retraction (Table 4.5) however the conversion of litter was also affected by volume ($P<0.05$). In contrast, increasing water volume from 100 to 200 ml decreased earthworm mortality ($P<0.001$), while ceasing watering on either day 8, 13, or 23 had no effect on mortality (Table 4.6).

Table 4.5 The effect of stopping watering at day 8 and 13 on conversion depth, earthworm dispersion and retraction

	Day water stopped						P-value		
	23		13		8		Day	Vol.	Day x Vol.
Conversion depth (%)	44.4	a	42.2	a	36.7	b	0.0027	0.0141	NS
Dispersion (score)^^	1.4	b	1.4	b	1.5	a	0.0068	NS	NS
Retraction (sec)^^	9.6	b	9.0	b	14.7	a	0.0081	NS	NS

Different superscripts show significant differences between treatments ($\alpha=0.05$)

^^Rank transformation

Table 4.6 The effect of water volume on earthworm mortality

	Volume (ml)				Significance		
	100		200		Day	Vol.	Day x Vol.
Conversion depth (%)	38.9	b	43.3	a	0.0027	0.0141	NS
Mortality (%)~	26.1	a	20.5	b	NS	0.0023	NS

Different superscripts show significant differences between treatments (α=0.05)

~Log transformation

Cessation of water provision at day 8 for 100 and 200 ml volumes resulted in only a slight increase in daily mortality (Figure 4.11). While ceasing both water volumes on day 8 increased dispersion scores and retraction rates, and the variation between replicates (Figure 4.12 & Figure 4.13). Ceasing water on day 13 had no effect on mortality, dispersion or retraction for either water volumes.

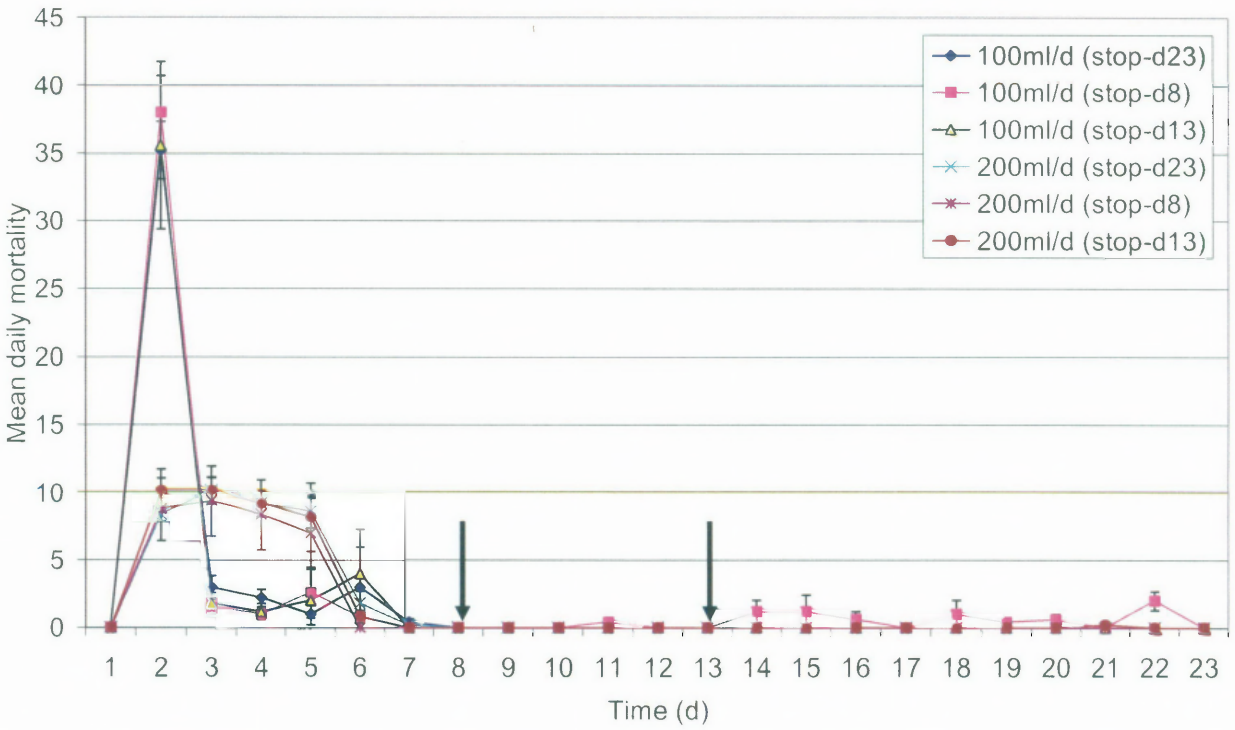


Figure 4.11 The mean daily mortality for treatments receiving 100 and 200 ml volumes daily where watering was stopped on day 8 and 13 (±SE)

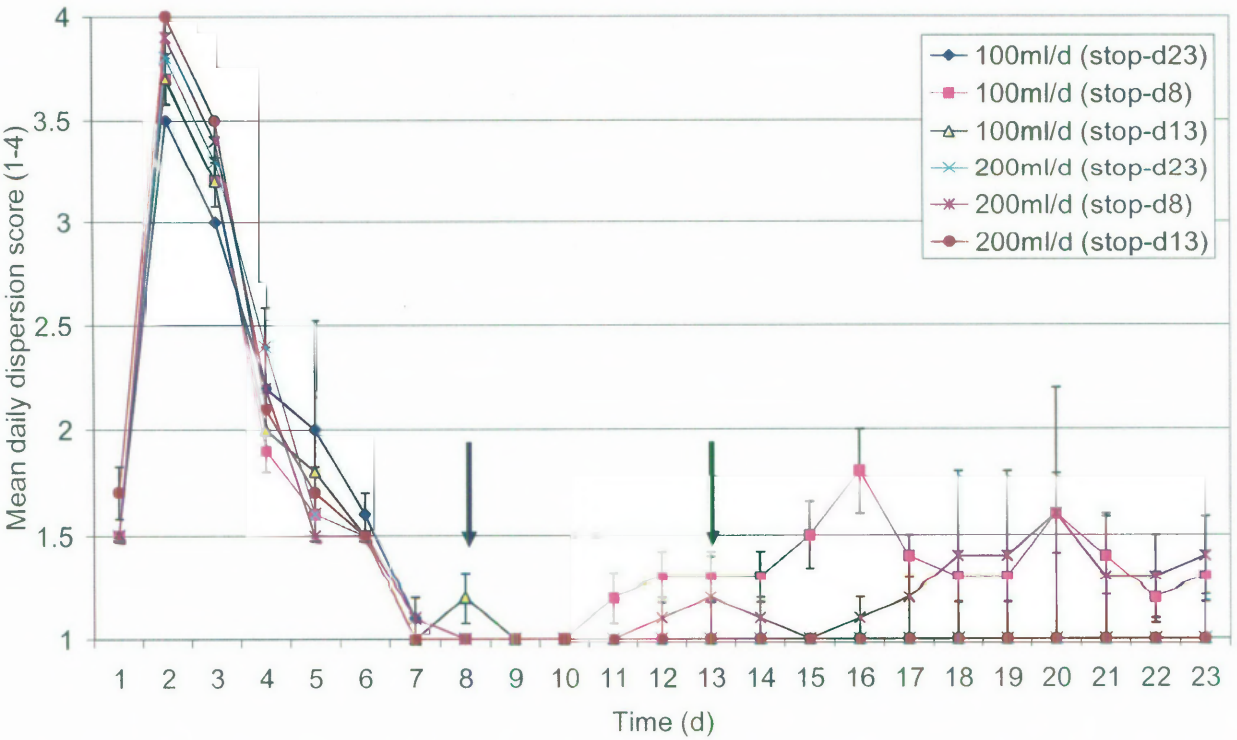


Figure 4.12 The mean daily dispersion scores for treatments receiving 100 and 200 ml volumes daily where watering was stopped on day 8 and 13 (±SE)

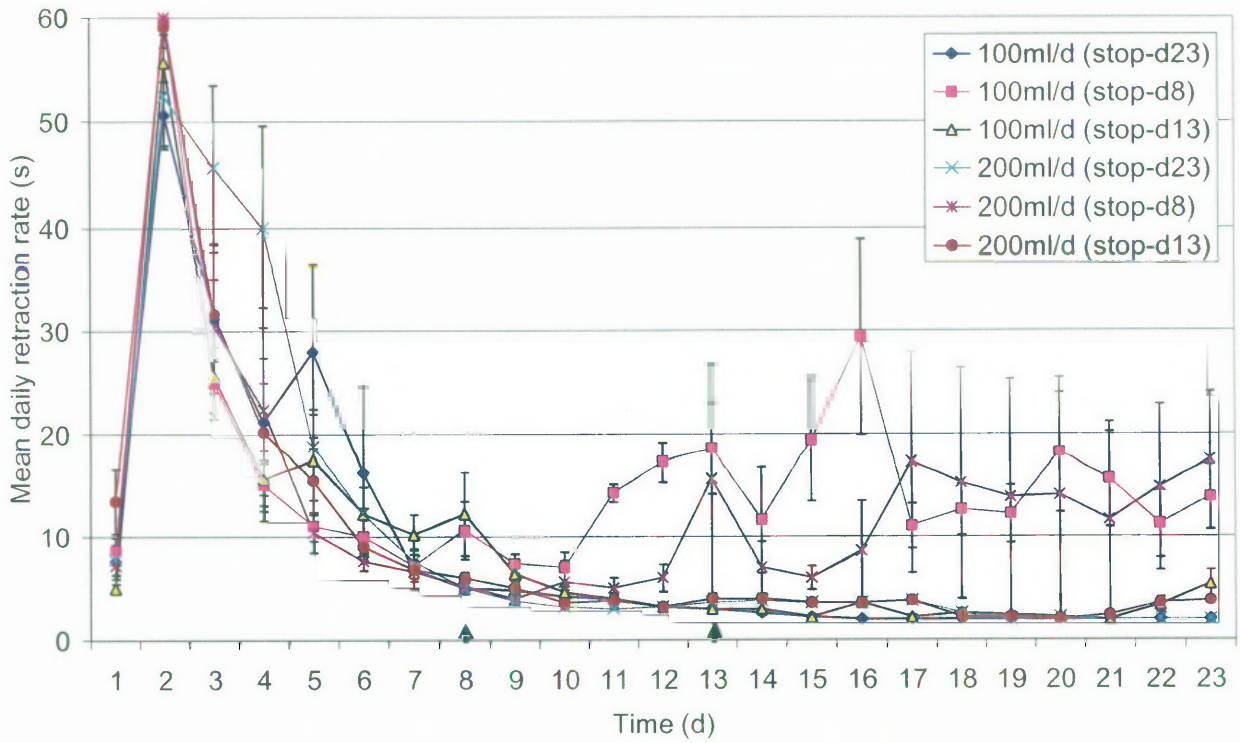


Figure 4.13 The mean daily retraction rate for treatments receiving 100 and 200 ml volumes daily where watering was stopped on day 8 and 13 (±SE)

4.3.3 Experiment 3 (earthworm stress and N)

The first hypothesis for this experiment was that earthworm stress was related to concentrations of salts in earthworm bedding. Second hypothesis was that the concentration of salts in earthworm bedding would increase over the first five days. The final hypothesis was that greater concentrations in TN and NH_4^+ would be lost via leachate from systems receiving larger water applications.

Only the least stressed treatment (S1) completely converted the litter into vermicast in 35 days (Figure 4.5). Earthworm mortality ($P<0.001$), dispersion scores ($P<0.001$) and retraction rates ($P<0.001$) were significantly lower for S1 (Table 4.7). The majority of earthworm mortality for S2-S4 occurred between day 4 and 5 (Figure 4.14).

Table 4.7 The effect stress level on mortality, dispersion and retraction of earthworms

Health parameters	Water regime induced stress levels				P-value
	Stress 1 (S1)	Stress 2 (S2)	Stress 3 (S3)	Stress 4 (S4)	
Mortality (%)^	2.2 b	76.3 a	77.9 a	76.6 a	0.0000
Dispersion (score)^	2.7 c	3.6 b	3.7 ab	3.8 a	0.0000
Retraction (s)	35.5 d	47.8 c	50.8 b	54.0 a	0.0000

Different superscripts show significant differences between treatments ($\alpha=0.05$)
^Chi-square transformation

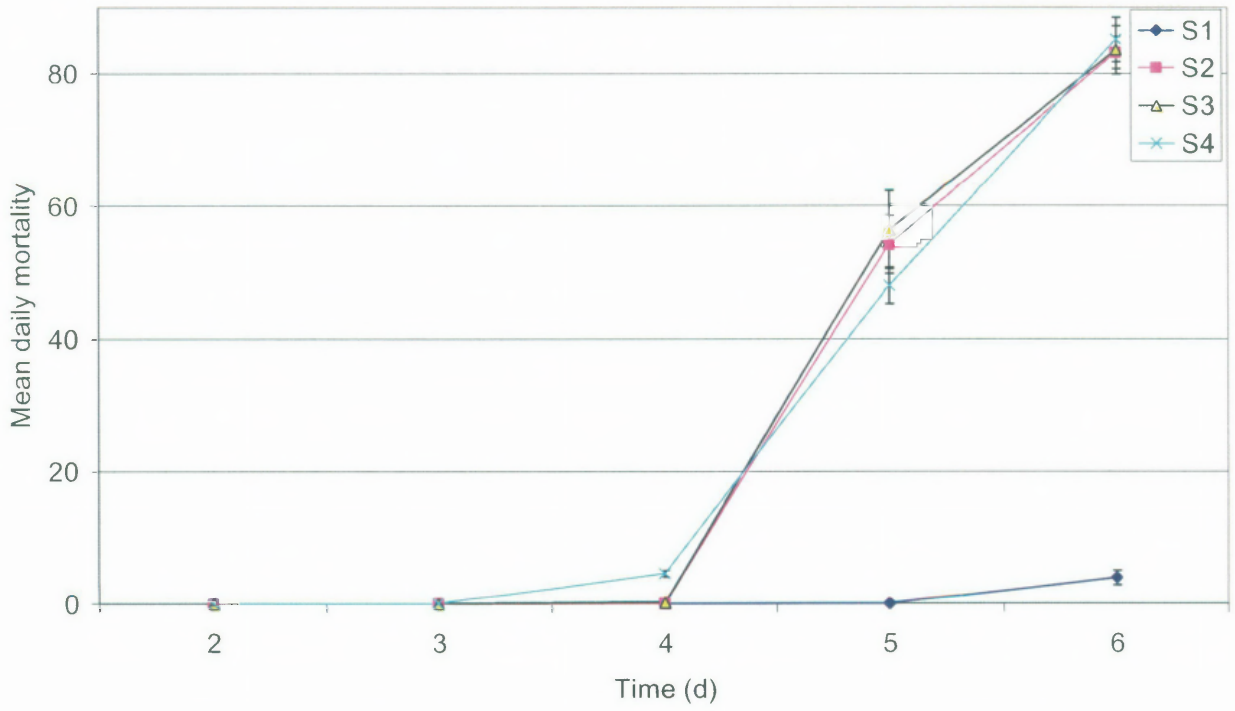


Figure 4.14 Mean daily mortality for four water regime induced stress treatments (±SE)

Day sampled affected TN concentrations in earthworm bedding ($P<0.001$) however stress levels had no effect. There is possibly a treatment effect where on day 5 S1 had similar ($P\sim0.05$) concentrations of TN in earthworm bedding as S3 and S4. From day 4 to day 5 the TN in bedding from S1 and S2 did not change, while S2 and S3 increased (Figure 4.15). Both day sampled and stress level affected the EC, however a treatment affect resulted in S1 containing less salts ($P<0.001$) than S2, S3 and S4 (Figure 4.16). Both experimental factors (day sampled and stress level) had an effect on pH and a treatment affect was apparent ($P<0.01$, Figure 4.17). The pH for all treatments remained between 5.5 and 8.5 for the duration of the experiment, within the acceptable range for *E. andrei* (Standards Australia International 2003).

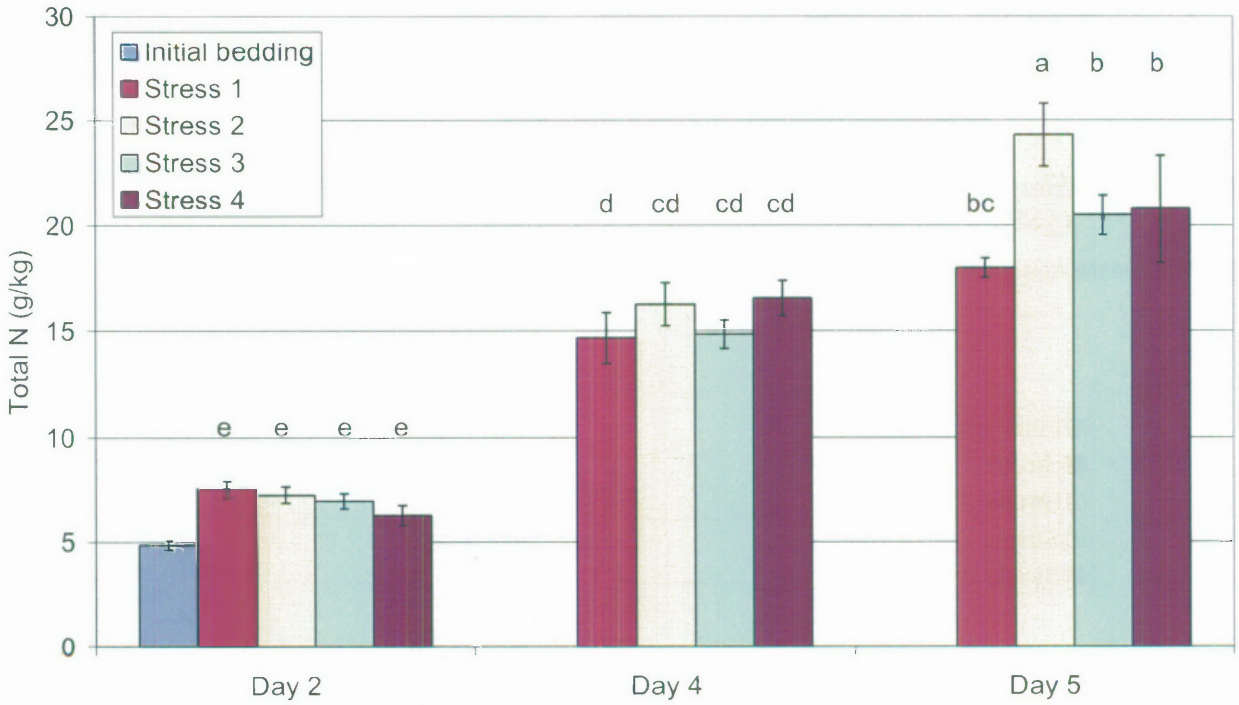


Figure 4.15 Total N in earthworm bedding on day 2, 4 and 5 prior to watering (±SE)

Different superscripts show significant differences between treatments ($\alpha=0.05$)

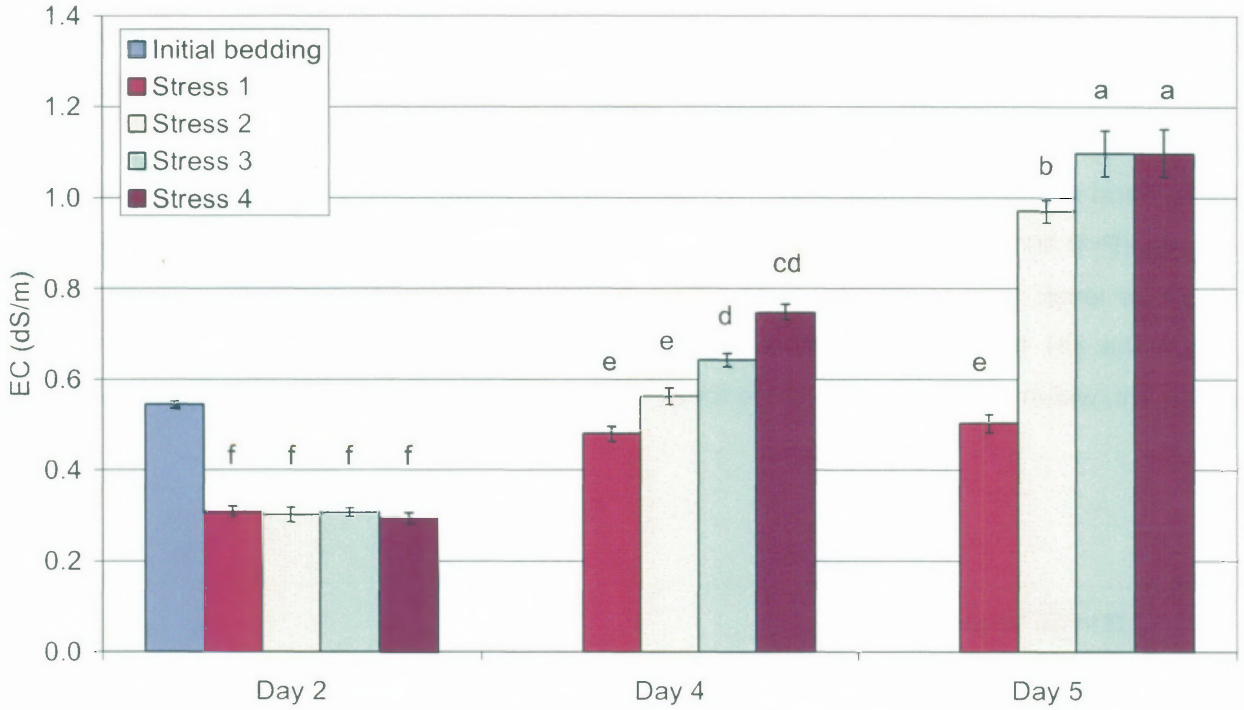


Figure 4.16 Electrical conductivity in earthworm bedding on day 2, 4 and 5 prior to watering (±SE)

Different superscripts show significant differences between treatments (α=0.05)

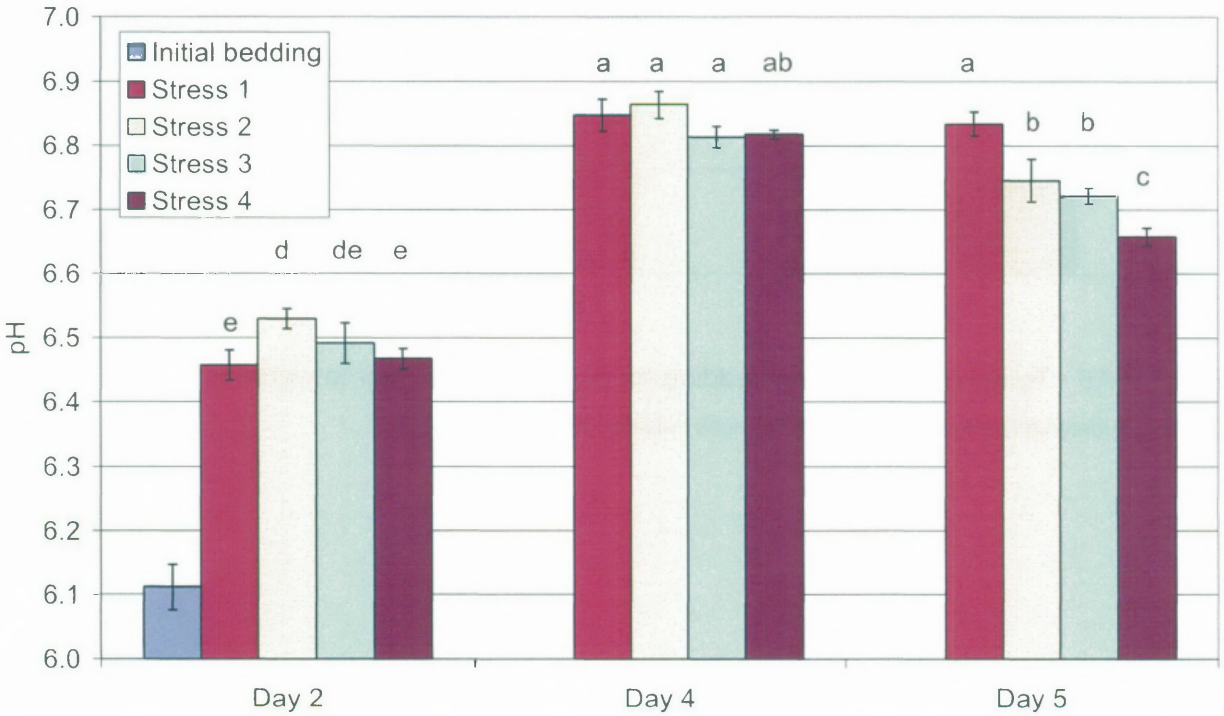


Figure 4.17 pH in earthworm bedding on day 2, 4 and 5 prior to watering (±SE)

Different superscripts show significant differences between treatments (α=0.05)

Both the amount leached from each treatment ($P<0.001$) and the concentration in ammonium NH_4^+ ($P<0.05$) in leachate from each treatment were different (Table 4.8). NH_4^+ was more concentrated in S1 leachate compared to treatments that had lesser volumes of liquids leached. Concentrations of TN and the EC in leachate did not vary significantly between stress levels (Table 4.8).

Table 4.8 The effect of stress level on volume leached, the concentration of NH_4^+, TN and EC of leachate

Parameters	Water regime induced stress levels								P-value
	S1		S2		S3		S4		
	Mean		Mean		Mean		Mean		
Volume leached (l)	1.62	a	0.85	b	0.46	c	0.38	d	0.0000
NH_4^+ (g/kg)	2.28	a	2.06	a	1.94	ab	1.68	b	0.0274
TN (g/kg)	4.01		4.45		4.21		4.51		NS
EC (dS/m)	28.94		32.24		29.78		31.14		NS

Different superscripts show significant differences between treatments (α=0.05)

4.4 Discussion

4.4.1 Experiment 1 (application and timing)

Decreasing water interval and increasing water volume led to faster litter conversion and less earthworm mortality, supporting the hypothesis. The use of a specially developed batch flow vermiculture technique enabled up to 50% conversion of hot litter into vermicast in 23 days. To achieve this conversion rate regular 100 and 200 ml water applications were required. With increasing watering intervals (d) there was a decrease in the percentage of litter converted into vermicast (Figure 4.6). Water volume had no effect on litter conversion when applied at 0.5, 3, 4 and 5 day intervals; however at 1 and 2 day intervals the larger volume led to greater litter conversion.

It was apparent that when watering was more regular the benefits of using a larger volume were diminished. For the 0.5 day interval water volume was not a causal factor as water was probably in excess due to its regular application. In contrast, for the longer watering intervals (3, 4 and 5 days) the system was so unstable due to the lack of regular watering, that it overshadowed the effects of water volume. Only the 1 and 2 day intervals benefited from an increase in water volume. Interestingly, 50 ml/0.5d resulted in relatively low conversion, indicating that water volume is still important when trying to maximise litter conversion (Figure 4.6).

A regular watering interval resulted in lower mean earthworm mortality, especially when combined with the larger water volume. The 200 ml/3d and 5d applications resulted in lower

mortality than the 100 ml/3 and 5d, suggesting that if watering events were extended beyond daily watering then larger volumes would be required to minimise mortality (Figure 4.7). For 200 ml/0.5d there was a trend where the higher water volume resulted in higher mortality, this could have been due to insufficient drainage from the containers (Figure 4.7). Litter is high in N and salts (Turnell et al. 2006), and it was possible that they dissolved in leachate and thereby were negatively affecting the earthworm's ability to achieve homeostasis (Wallwork 1983). For the 50 ml/0.5d earthworm mortality was relatively low (Figure 4.7) which suggested that the system was progressing well, however poor litter conversion had occurred (Figure 4.6). These results suggested that either too little or too much water could negatively affect this vermiculture system.

Earthworm health was not only measured by mortality but also by behavioural characteristics, including the dispersion of earthworms on the bedding surface and the rate earthworms retracted from light. Mean earthworm dispersion scores and mean retraction rates were lower for shorter watering intervals, indicating lower stress on earthworms in the litter substrate (Table 4.4). Mean daily mortality was greatest over the first 6 of 23 days while the earthworms became accustomed to the system. It was evident that 100 ml volumes had higher mortality on day 2 compared to 200 ml, this could have been due to less water removing fewer salts from their bedding. However, on day 5 200 ml/1d suffered higher mortality than 100 ml/1d, this could have been due to poor drainage distressing the earthworms as they could not escape from the leachate.

Poor drainage led to future experiments requiring daily insertion of a small nail into the drainage holes to encourage leachate removal. Poor drainage and excessive water applications could become a problem in the field (Murphy 2005). That being said, excessive applications would increase water costs for this system, and the leachate produced would need to be utilised through on-site vermi-filtration (section 6.2.4.1), increasing costs.

The mortality of earthworms did not continue to be elevated for the duration of the experiment for 100 and 200 ml treatments for all watering intervals (Figure 4.8). Suggesting, that once the system stabilised infrequent watering would only lead to earthworm stress (higher dispersion scores and retraction rates) and not necessarily deaths. This was observed from day 6 onwards for 100 and 200 ml/5d (Figure 4.9 & Figure 4.10) and could be linked to why these treatments converted the least amount of litter (Figure 4.6). At this point a question was raised as to whether watering was as important after initial stabilisation, which formed the basis of experiment 2.

4.4.2 Experiment 2 (ceasing water)

The hypothesis that stopping watering on day 8 and 13 would lead to higher mortality was invalid, and ceasing water on day 13 did not lead to less litter conversion over 23 days, again not supporting the hypothesis. However, ceasing water on day 8 did support the hypothesis with less litter converted over 23 days. By stopping the water on days 8 and 13 it was expected that conversion rates of litter and earthworm health would suffer, however the response was much less than expected. The most critical time for the process was in the first 8 days. Ceasing watering on day 13 resulted in no less conversion or increase in dispersion or retraction, compared to treatments continuing to receive daily water until completion on day 23 (Table 4.5).

Interestingly, earthworm mortality was not affected by the day water was stopped but was highly affected by the water volume (Table 4.6). This result could be explained by the higher mortality experienced by 100 ml treatments on day 2, where the majority of earthworm deaths occurred, which overshadowed the mortality for the rest of the experiment (Figure 4.11). Furthermore, if salts caused earthworm stress then watering early in the process would be most beneficial. This use of water was probably why other vermiculture experiments allowed time for stabilisation of the substrate before adding earthworms (Garg et al. 2005), as some of these irritants would have had time to leach out.

Stopping watering on day 8 did not affect mortality however both retraction rates and dispersion scores were affected (Figure 4.12 & Figure 4.13). The elevated stress on the earthworms indicated that the vermiculture process was being inhibited, as indicated by the reduced conversion rates (Table 4.5). This observation reiterates that earthworm behavioural characteristics (dispersion and retraction) were good preliminary indicators of reduced vermi-processing potential. Understanding earthworm stress and identifying the most likely causes were investigated in the third experiment.

4.4.3 Experiment 3 (earthworm stress and N)

Hypothesis 1 for this experiment was supported, with treatments with higher salt concentrations in bedding showing greater earthworm stress and higher mortality. Hypothesis 2 was supported with the concentration of N and salts increasing in bedding over the first five days. The third hypothesis was also supported with greater concentrations of TN and NH_4^+ lost via leachate from systems receiving larger water applications.

The results from experiment 1 suggested that the agents responsible for the stress and mortality of earthworms in a litter substrate were critical to the rate at which litter could be converted into vermicast. Identification of these agents was the basis of experiment 3, which focused on

earthworm health and chemical analysis of both earthworm bedding and leachate. To do this different stress levels were imparted on the earthworms by varying the amount and timing of water application. In the previous experiment, the highest mortality occurred within the first 8 days, but the most critical times were days 3-5 (Figure 4.8). In this experiment the earthworm health data showed that the least stressed treatment (S1) had lower mortality, dispersion and retraction (Table 4.7) than the other treatments over the first five days. The high stress level treatments (S2, S3 and S4) were so severe that they were terminated on day 6, whereas S1 fully vermi-processed the litter by day 35 (Figure 4.5).

Nitrogen compounds and salts, and possibly pH are considered the most likely causes of earthworm stress in vermiculture systems (Edwards 1995). It has also been mentioned that the success of a vermiculture system would to some extent depend on the rate at which nitrifying bacteria could convert NH_4^+ to NO_3^-, a form of N which is more favourable to earthworms (Tereshchenko and Naplekova 2002). Total N in earthworm bedding increased from day 2 to 5 ($P<0.001$) for all treatments (Figure 4.15). Since on day 5 there was no difference in TN concentration between S1, S3 and S4, TN could not be a factor in high earthworm mortality, as otherwise these treatments should have all experienced the same mortality levels (Figure 4.14). This result could be explained by the experimental design, where S1 received daily watering, and this regular flush could have allowed a reprieve of TN concentrations within the bedding layer. Furthermore, it was possible that proportionally more NH_4^+ was in S2, S3 and S4 bedding, which TN data does not show. This was important as earthworms are most sensitive to N in the NH_4^+ form (Edwards 1995) and suggests that further investigation is required. This experiment did not analyse NH_4^+ concentrations in earthworm bedding due to the size of the sample required. Unfortunately, the experimental design did not accommodate for the quantity of bedding required, as it was likely that earthworms would be excessively disturbed.

Apart from N this experiment also quantified the EC and pH of the bedding. In comparison to TN the EC of S1 on day 5 was lower than the other stress treatments, to the extent that it was lower than the EC of the initial bedding (Figure 4.16). Wallwork (1983) concluded that maintaining homeostasis of earthworms was fundamental in the vermiculture process. The results of experiment 3 suggested that excess salts may have contributed to earthworm stress and the high levels of mortality experienced on day 5. Although the pH did vary slightly from day 2-5, it could not explain the earthworm stress and high mortality as it remained in the neutral range (6-7) and within the tolerance range for earthworms (Edwards et al. 1985).

Water was identified as the vector for the movement of N and salts in the system, therefore the volume and chemical composition of leachate was measured. The potential for N and salts to be leached out of the system was greatest for S1 as it had the highest volume of water added

(Table 4.8). It was expected that the concentration of NH_4^+ for each treatment would increase with decreasing leachate volume due to a concentration affect, however the reverse occurred. Probably due to more complete saturation of the litter for S1, resulting in greater transport of NH_4^+ via leaching. This result is important as less NH_4^+ would then have been available to move into the earthworm bedding for S1. Furthermore this supports the hypothesis that proportionally more NH_4^+ was concentrated in the bedding when earthworms were stressed. Interestingly, the TN and salts showed no variation in concentration between the treatments; therefore the affect of NH_4^+ was probably more important as an indicator of suitable earthworm bedding (Table 4.8).

In conclusion, the three hypotheses for this experiment were supported; however, future experiments should not only investigate TN and EC of the bedding, but also NH_4^+. To enable this, a larger experimental unit would be required compared to the containers used in this study. Using vermicast earthworm bedding as opposed to coconut husk was examined in experiments 4 and 5.