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Environmental legacy of water vole (*Arvicola amphibious*): An ecosystem engineering species affecting spatio-temporal patterns of nitrogen in a nitrogen poor environment

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Summary

Ecosystem engineers are species that directly or indirectly alter their immediate, and wider, habitats through physical disturbance modulating the availability of resources. Studies related to burrowing ecosystem engineers have predominantly focused on species in arid systems and tend to represent snapshots in time. In addition, in such studies the mechanism through which engineering was accomplished was through excavation of soil. The potential for ecosystem engineers to have subtler influences on their environment through altering the nitrogen cycle has been previously hinted to, but not fully explored as well as how such engineering effects arise and alter over time. There is therefore a knowledge gap as to how burrowing ecosystem engineers, in water saturated soils, may alter their environment through mechanisms that do not include excavation, and how this influence alters over time. Here it is shown that water voles (*Arvicola amphibious*) influence their environment through altering the nitrogen cycle, and that this influence alters dynamically over time of patch occupation. Such results highlight the importance of seeing ecosystem engineering as a dynamic response that varies with time, rather than the binary response that studies have tended to report. It is anticipated that this study will promote more considered approaches to ecosystem engineer studies, that will take into account the variation of engineering effects over time.

***Key-Words**:* Arvicola amphibious*, ecosystem engineers, environmental legacy, extinction debt, metapopulation, nitrogen, nitrogen cycle*

INTRODUCTION

1.0 ECOSYSTEM ENGINEERS

Ecosystem engineers are species that directly or indirectly alter their immediate, and wider, habitats through physical disturbance modulating the availability of resources (Jones *et al.*, 1994). Habitat heterogeneity is an important by-product of this ecosystem engineering (Jones, 1994; Bryce, 2006). Traditionally this was exemplified by species such as beavers (*Castor canadiensis*), with their iconic dam-building behaviours that can have wide scale effects on the landscape (Naiman, 1988). Through dam building, beavers create new habitats such as flooded meadows, that in turn create suitable conditions for other species (Naiman, 1988), which would otherwise not be able to make use of that area. This legacy of beaver ecosystem engineering has also been shown to vary in both time and beaver occupancy, resulting in responses to ecosystem engineering that are highly dynamic (Wright, 2009). While some of the most charismatic ecosystem engineers may be active above ground, many, if not most, ecosystem engineers spend part of their lives underground, often in burrows (Jones *et al.,* 1994).

Animal activity underground can create extensive burrow networks which will often outlive the lifespan of a single individual engineer (Bryce, 2006; Bryce *et al.,* 2013). The propensity of these burrow networks to be long-lived allows for ever larger, more complex, burrow systems to form over the lifespan of a population. With many burrowing species, the method of burrowing involves expulsion of soil out of burrow entrances (Reichman & Seabloom, 2002). For instance, Reichman & Jarvis (1989) found that mole rats burrowing activities (shunting soil out of burrow entrances) can result in 25% of the ground surface being covered in excavated dirt. Overwhelmingly studies seeking to address the effects of burrowing animals have focused on the movement of soil to the surface. A few exceptions have attempted to explain engineering effects as a result of the burrows themselves, rather than the movement of soil (Bryce, 2006).

Similarly, studies have tended to focus on ecosystems with arid soils (Bragg *et al.*, 2005; Desmet & Cowling, 1999; Kinlaw, 1999; Whitford & Kay, 1999), and a lack of understanding how engineering affects nitrogen cycle mechanisms in water saturated soils is evident (see Fig. 1 1 for nitrogen cycle summary).

2.0 NITROGEN CYCLE

Plant growth is limited by nitrogen in many terrestrial systems (Vitousek 1982; Aerts & Chapin III 2000). Herbivores are able to influence nutrient cycles either through accelerating microbial activity, increasing microbial abundance (Bryce, 2006; Holland & Detling, 1990; McNaughton *et al.,* 1997; Frank & Groffman, 1998) or decreasing the cycling rate (Pastor *et al.*, 1993; Ritchie *et al.,* 1998). In addition, herbivore density can specifically influence nitrogen mineralisation rates (Bakker, 2003; Shariff *et al.,* 1994; Kiehl *et al.,* 2001) and also microbial productivity (Hik & Jefferies, 1990). This becomes relevant for rodent ecosystem engineers. For instance field vole (*Microtus agrestis)* density has been identified as a cause for increased mineralization rates during peak population size in autumn (Bakker, 2003). These increased mineralization rates have been attributed to the burrows of rodents causing increased aeration, drying and water infiltration (Bryce, 2006). Since most nitrogen is locked within soil organic matter with a slow turnover rate (Knops *et al.,* 2002) it stands to reason that ecosystem engineers that are able to increase rates of nitrogen cycling, may have an ecosystem level impact on community structure.

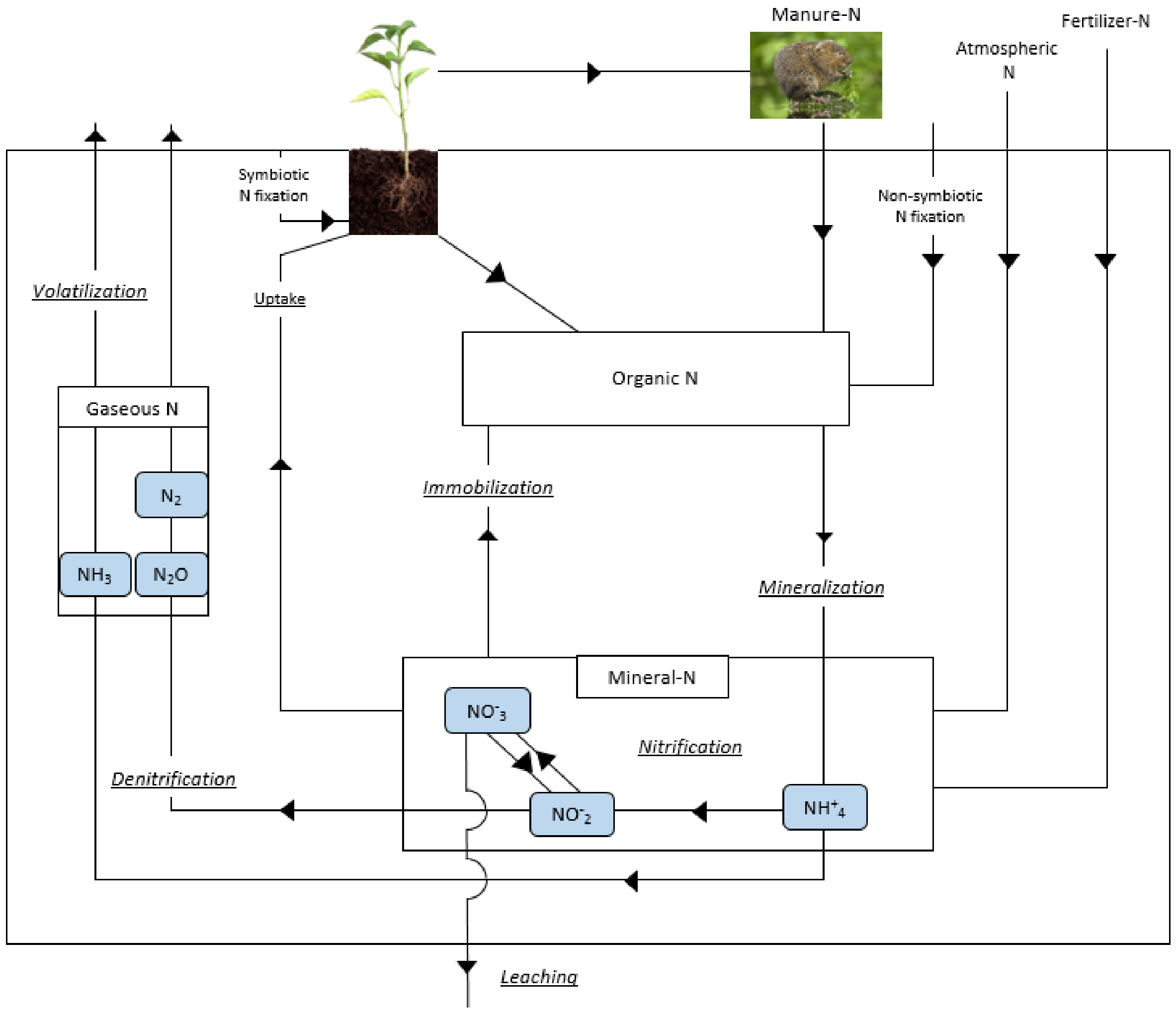

*Figure 1: Nitrogen cycle inputs and mechanisms. Potential water vole (*Arvicola amphibious*) influences on both cycle mechanisms and direct input include manure input, vegetation input, and aeration affecting mineralisation and microbial activity. Burrows may also alter leaching of NO^-_3. Adapted from Rowel (1994).*

Plants uptake N in the form of NO_3^- (Fig. 1). Therefore, in N limited systems, it will be the amount of Organic N that is converted to NO3- that will be the limiting factor for plant uptake (Rowell, 1994; Harrison *et al.,* 2007). While in the vast majority of systems this will be the case, the universality of this statement has been challenged (Harrison *et al.,* 2007). It has been noted that in many N limited areas, such as arctic and tundra systems, plants are able to bypass traditional microbial processing of N and uptake N directly in forms such as amino acids (Kielland 1994; Schimel & Chapin, 1996). This suggests that in severely N limited areas, mechanisms of N conversion play a less important role, relative to total N input. While this is not applicable to the vast majority of systems throughout the world, there may be ecosystems, severely lacking in N, where plants are able to bypass the traditional route and make use of more unusual forms of nitrogen. Whether this is the case in the study area seems unlikely, but should never-the-less be a consideration when studying the influence of ecosystem engineers on the nitrogen cycle in nitrogen limited areas, such as the artic and tundra.

3.0 RELEVANT NITROGEN CYCLE MECHANISMS

There is a good understanding of the fundamental processes, including the pathway whereby micro-organisms contribute to the N cycle. However, while several interactions could mediate the impact of a fossorial ecosystem engineer on such processes, there are gaps in our knowledge on the relevance and contribution of the different pathways involved. In particular, in ecosystems that are anaerobic, such as in water saturated riparian areas, there is a severe knowledge gap in how ecosystem engineers may alter nitrogen cycle mechanisms.

3.1 Aeration

In water saturated soils, anaerobic conditions can result in limited mineralization by soil microbes (Torbert & Wood, 1992). Mineralization is the conversion of organic nitrogen to NH_4^+ by soil microbes (Rowell, 1994). Burrowing ecosystem engineers in water saturated systems are able to alter mineralization rates through increasing aeration (i.e. oxygen availability, temperature and/or drying) (Bakker, 2003). Following this mineralization, nitrification is the conversion of the ammonium (NH_4^+) to nitrite (NO_2^-) finally to nitrate (NO_3^-) (Rowell, 1994), nitrate being the useable form of nitrogen available to plants. Again this may be enhanced by underground ecosystem engineers, if they create optimal conditions for soil microbe activity in the vicinity of their inhabited structures.

3.2 Direct Input

Organic nitrogen is normally input from the surface in the form of faecal deposits. This is not necessarily the case for burrowing engineers; nitrogen input can be introduced directly belowground through faeces, food storage, and nest building. These inputs can all increase nitrogen, in an organic form, that can then be converted to NH_4^+ and NO_3^- (Fig. 2), and is then available for plant uptake. This introduction of nitrogen belowground allows more immediate access for microbes. As a result, below ground engineers may have a disproportionately large effect on the nitrogen cycle, as they represent a unique form of nitrogen injection into systems.

Figure 2: Overwinter burrow activity of water voles. Image is of a now exposed, previous snow burrows of water voles. Deposition of both faecal and vegetation matter are both illustrated and may constitute a significant component of organic nitrogen introduced in a nitrogen limited environment with low animal diversity. Image courtesy of X. Lambin.

3.3 Leaching

The movement of nitrates from the surface, deeper into the soil profile, occurs through leaching, where nitrates bind with water and are pulled deeper into the soil. Burrowing ecosystem engineers may alter leaching in various ways. From the direct input of organic nitrogen below ground within burrows, ecosystem engineers may increase the ultimate amount of nitrate (following conversion to ammonium to nitrite to nitrate) leached into deeper soils (Bryce, 2006; Rowell, 1994). Additionally, water voles may water proof their burrow (Gemma Reid, unpublished honours project, pers. comm., 2015) resulting in decreased leaching, as burrows may form barriers to nitrogen movement. These two possible forms of interaction between burrowers and nitrogen cycle provide possible routes of engineering that may take place, but these have been overlooked within the literature, especially in regards to water saturated soils.

4.0 INFLUENCES ON PLANT COMMUNITY

Ecosystem engineers alter their environment. In many instances this affects the plant community (Bryce *et al.,* 2013). A cause and effect relationship is often implied, but often these will miss intermediary steps involved. While ecosystem engineers may influence their plant community, the causal relationship may often involve altering the nitrogen cycle, and in turn, promoting species diversity. This intermediary step of influencing the nitrogen cycle is lacking within the literature. While it has been demonstrated that engineers may alter the nitrogen cycle, the link between this effect on the nitrogen cycle and on plant communities is not made.

5.0 METAPOPULATIONS

Engineer's impact may alter the N cycle (Bakker, 2003; Gervais *et al.,* 2010; Bryce, 2006), but how these changes vary over time is unknown. Specifically, given that the structures of ecosystem engineers may outlive the engineer's themselves. In addition, it is not known whether the impacts of engineers operate in relation to the changes in abundance of the engineers. One type of population dynamic is whereby populations of organisms are ephemeral, and the result of an extinction and recolonization process. Such metapopulations are defined as “a population of unstable populations, inhabiting discrete habitat patches” (Levins 1969). Within the metapopulation, various populations may experience extinctions and subsequent recolonization by neighbouring dispersers (Telfer *et al.,* 2001).

Using these patch extinction/recolonization events allows for the construction of a chronosequence of occupation. A chronosequence is defined as the years in which a patch is either occupied or unoccupied resulting in a myriad of potential variations. In some instances belowground ecosystem engineers demonstrate metapopulation dynamics, such as the water vole (*Arvicola amphibious*). These instances may help exemplify the effects of an ecosystem engineer’s influence on aspects of their environment, such as nitrogen cycling, over varying lengths of occupation, to establish a timeline of effects. Specifically, using the chronosequence of a metapopulation species allows for the presence and/or absence of ecosystem engineers to be accounted for at varying lengths of occupation. In doing so, it is possible to address how the inception of engineering effects varies over time.

6.0 EXTINCTION DEBT

Animals are a crucial player in the movement of nutrients (Doughty *et al.*, 2015). Large animal in particular can have a significant role in this movement of nutrients. The number of large animals have declined or gone extinct (Doughty *et al.*, 2015), and the subsequent effect on nutrient cycles have been numerous. With large animals no longer able to provide this ecosystem service, the role with inevitably fall onto smaller animals. The question is thus, can small animals play a role in offsetting the loss of large animals?

In particular to ecosystem engineers, the length of time for ecosystem engineering effects to diminish has been investigated. Wright *et al.* (2004) used beaver engineering influences on their environments to study the decay of those same effects. While this allowed the time to be estimated for the extinction debt, it missed out on the time required for those effects to be established.

In this study, I used metapopulation induced chronosequences of occupation of a belowground ecosystem engineer, to simulate the influences of spatial and temporal trends of nitrogen (NH_4^+ and NO_3^-) of 1 to 6 years of continuous occupancy. In essence, I used metapopulation dynamics to simulate a 6 year occupancy study, within a single sampling season. Using this I was able to demonstrate the temporal variation of ecosystem engineering on the nitrogen cycle, from initial colonization, and track these effects for each incremental year of occupancy for 6 years total.

It was not the aim of this study to document individual pathways or mechanisms, but rather to focus on the overall impact that burrowing may have on the nitrogen cycle as a whole. When possible, however, key processes have been investigated through using variation in patch occupancy, using a carefully designed sampling scheme.

7.0 AIMS

1. To investigate if water vole length of occupation within a patch was a determining factor for NH_4^+ and $NO_{2/3}^-$ concentration. I predicted that increasing length of occupation would result in corresponding increases in both NH_4^+ and $NO_{2/3}^-$.
2. To investigate whether or not water vole ecosystem engineering occurred dynamically over time, from inception to culmination, over a six year time span.

3. To investigate what nitrogen cycle mechanisms may be causal for any observed changes in the nitrogen cycle as a result of water vole ecosystem engineering.

MATERIALS AND METHODS

This study aimed to investigate the effect that occupancy of a metapopulation species, water vole, had on nitrogen (NH_4^+ and $NO_{2/3}^-$) in a nitrogen limited environment.

The methodology will include; a description of the study species and their population dynamics, an explanation of field work, laboratory work, and finally the statistical analysis that was carried out, including the model selection process used to evaluate different hypotheses and processes.

1.0 STUDY SPECIES AND PATCHES OF OCCUPATION

Water voles (Fig. 3) are small mammals weighing between 200-300g in Britain (Lambin *et al.*, 2012). Water voles are listed on Schedule 5 of the UK Wildlife & Countryside Act 1981. This legal protection extends primarily to water vole burrow networks, but also discourages interference with the animal. Within these burrow networks water voles may form colonies of between 1-5 individuals (Sutherland *et al.*, 2012).

*Figure 3: Young adult water vole (*Arvicola amphibious*) with distinctive black colouration, typical of the Highland population.*

Sections of riparian habitat occupied by water voles (referred to as a vole patches hereafter) are characterised by a vegetation cover dominated by graminoid and herb plants separated by one, or a combination of, unsuitable habitats; heather dominated sections, bare ground, peat and/or rock (Lawton & Woodroffe, 1991; Telfer *et al.*, 2001; Aars *et al.,* 2001; Bryce *et al.,* 2013) (Fig. 5 a). These patches are restricted to sections of waterways and the accompanying riparian strips (up to 2m away from waterway are used by water voles, however affected vegetation may extend beyond this). This patchwork of suitable habitats results in fragmented patches which display regular vole metapopulation activity (colonization, stable or extinction). While it has been shown that water voles do influence the plants in patches (Bryce *et al.,* 2013), it is unclear how this occurs, and constitutes part of this study.

Figure 4: Images from Assynt study site. Image (a) is of one of the longest consistently occupied patches in Assynt, with arrows pointing to the water vole patch. Image (b) illustrates stochastic events that may lead to water vole extinctions. Bank collapses such as these (these chunks of earth were up to 5m in length, while the gorge itself was ~3m deep) have been posited to be the result of water vole burrowing activity undermining soil integrity (Bryce, 2006), although this particular waterway had no history of water vole occupation.

Water voles show metapopulation dynamics. That is, water voles have populations within populations, which have frequent stc (b) tic extinction events (Fig. 5 b) potentially followed by recolonization events. This extinction/recolonization dynamic results in patches that have varied occupation histories, with some being source population patches (i.e. long sustained occupation that exports juvenile voles into neighbouring patches) and others being sink populations (i.e. short lived patches that have frequent extinctions, which act as a drain on the overall population size) (Lambin *et al.*, 2012).

Water voles have been studied in the Assynt region of Sutherland, north-west Highlands of Scotland (Fig. 4) for almost 20 years. During those 20 years, 98 patches have been surveyed each summer between July and August. Trapping occurs following surveying. Given water vole metapopulation dynamics, this provides an opportunity to test whether extinction and recolonization events can have an ecosystem wide effect.

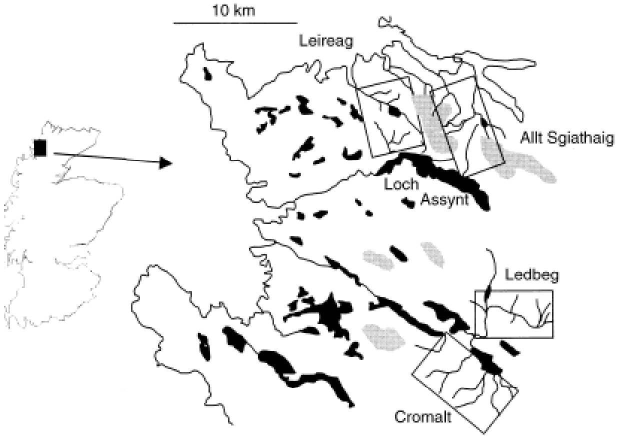

Figure 5: Map of study sites from the north west of the Scottish Highlands. Samples were collected from all Leireag, Sgiathaig, Ledbeg and Cromalt, but predominately came from Sgiathaig. From Aars et al.*, 2001.*

2.0 FIELD DATA COLLECTION

The intention of sample collection was to retrieve soil samples at set positions in relation to vole burrows in patches that were currently occupied, with known past yearly occupancies. Using a nested, stratified survey design samples were collected from 12 patches.

2.1 Site Selection and Sample Collection

The work took place as part of an annual water vole survey. This study capitalised on data of vole occupancy in each patch. Survey data was collected as follows: all 98 patches were surveyed over one week during mid-July. Each patch was surveyed twice to determine whether they were occupied, taking into account <100% detectability of signs. Signs of water vole include (in order of accuracy); latrines, plant clippings in runs and burrows, fresh plant cropping near to burrow entrances (fresh being little to no oxidation of the remaining plant), and fresh burrow entrances (fresh being a well-rounded burrow entrance, implying frequent use, with no obstructions). If the weather was optimal, only latrines were considered as they reflected most accurate recent activity, but if flooding occurred, and latrines were washed away, other signs were used (in order of accuracy). Following the initial survey, trapping occurs. At the end of the trapping session, if time allowed, a final round of surveying occurred in order to detect dispersal events that might have taken place over the summer.

2.2 Patch selection criteria

Patches used for soil measurements were selected amongst patches with known current vole occupancy status and history (chronosequence) for this study. The field protocol for establishing current occupancy status was through surveying patches known to have previously harboured water voles. Given the often cryptic nature of water vole signs, surveying can lead to false positives or negatives. It is possible, for instance, to identify a latrine of a disperser transiently visiting a patch without settling and therefore, mistakenly, designating a patch as being occupied (Sutherland *et al.*, 2013). To overcome such issues, patches were only designated as occupied if at least two surveys within the six week period had identified a patch as having signs. When a patch had only been surveyed twice because of time restrictions, they were considered even if only a single survey had found signs of occupation, but for the purposes of this study a strong preference was given to patches with at least two positive surveys. Using this method patches were identified as possible candidates in the study.

2.3 Length of occupation using chronosequence

In order to determine over what time scale vole occupancy might affect soil processes, patches of different past occupancy status (chronosequence) were considered for sampling. Using the survey database and applying the patch selection criteria, patches with differing lengths of continuous occupancy since the last extinction event were identified. These lengths of continuous occupancy ranged from one to six years. The length of continuous occupation was calculated as the number of years a patch had been continuously occupied since the last extinction event (the six year old patches had no extinction event in the time frame). For instance a patch that had had an extinction event in 2013, but had since had continuous occupation, would be classified as having a two year continuous occupancy (assuming the 2015 survey identified the patch as being currently occupied).

In order to ensure that patches demonstrated effects of engineering over varying lengths of continued occupancy, patches used for sampling had to have chronosequences that provided continuous, unbroken spells of occupation, and were occupied during the 2015 surveying season. The list of potential patches were compared to the 2015 survey data (the first weeks survey, once completed) and patches that were currently occupied were then considered for sampling. Only patches recolonized in 2015 (i.e. occupied for one year) were limited to a single patch.

Patches selected for sampling were predominantly limited by the number of patches available for each continuous occupancy years (i.e. 1 year old occupied patches were limited to one patch). However, where multiple patches had the same length of occupation, ease of access (distance from road) was the determining factor, given the weight of the sampling equipment being carried out on foot.

2.4 GIS fine detail selection

While survey data provided information about which patches were occupied in a given year, it did not provide information where in that patch vole activity was concentrated. Since patches can be 2-3km long, more fine scale selection was needed prior to sampling. Using the survey database, once suitable patches were identified survey data was entered into GIS (QGIS v2.11) for each selected patch. Using GIS, a circle 10m in diameter was used to select sections of the patch that had the greatest concentration of signs from all relevant chronosequence years. Two sampling sites were identified from each patch to allow for a nested sampling design. These selected sites were placed as far apart as the data would allow, in order to avoid pseudoreplication. Grid references for these were noted, and followed to the relevant burrow networks.

2.5 Burrow density

The magnitude of the impact of vole activity on soil processes could depend not only on vole presence or absence, but also on the amount of burrow excavation that has taken place. Accordingly, to quantify vole activity, other than chronosequence, burrow entrance density was counted in a 3x2m square area, with the square centred on the targeted sample burrow within a burrow entrance network. It is important to note that burrow density may also be a proxy for vole abundance within a confined area.

2.6 Sample extraction

In order to quantify the impact of vole burrow structure on soil nitrogen cycling, soil samples were collected in the field in set locations relative to that of burrows. Using a Soiltax Soil Corer the burrow was tracked from entrance as far as possible (in practice this was normally to ~30cm from the entrance before the burrow was lost). Tracking involved using the soil core to probe for a burrow connecting to the identified burrow entrance. Probing would begin at the burrow entrance, and the burrow was considered identified if a gap in the extracted core was roughly 5cm apart, and in a direction that was congruent with the previous core. Once a burrow was identified, bamboo canes were inserted into the cored holes to allow the general direction to be kept consistent, allowing for more accurate tracking. If the burrow could not be tracked, or only tracked for a short distance e.g. 15cm, a new burrow entrance would be selected from the 3x2m square and the process repeated. Burrows were typically tracked to at least 30cm from the burrow entrance.

Once the burrow had been tracked, a pit was dug (under license of SNH) to expose the burrow. Samples were gathered at ranges of 0-5cm, 5-10cm, 10-15cm, 15-30cm, 30-60cm, 60-90cm from soil below the burrow, going directly down. The increase in range size was to allow a wider scope of soil profile to be considered at greater depths. Soil samples were collected sideways from the burrow at ranges of 0-5cm, 5-10cm, 10-15cm, 15-30cm at a constant depth, level to the burrow. Soil samples from above the burrow were collected in ranges of 0-5cm, 5-10cm, 10-15cm, 15-20cm although these were limited by the depth of the burrow. Most burrows were 15cm deep, while others were 20cm and others only 5cm deep. See figure 6 for diagrammatic example. Sample spatial ranges were based on Gervais *et al*. (2010). Once sampling was completed, burrows were refilled with soil and the vegetation replaced as best as able.

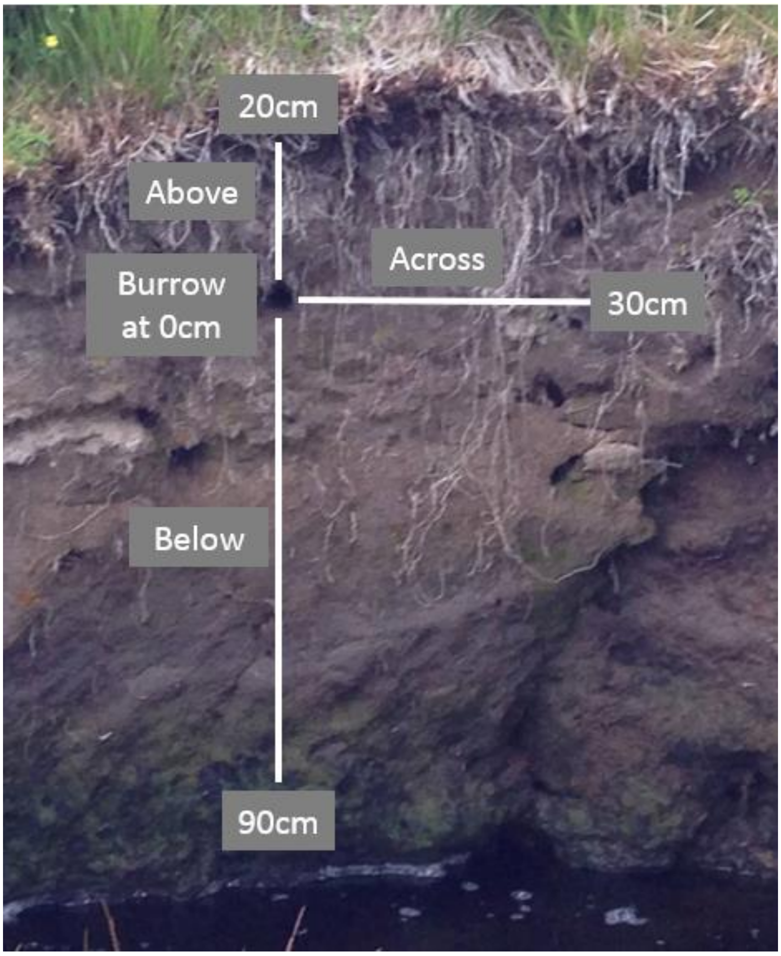

Figure 6 Illustration of positions sampled in relation to burrow, as well as the distance samples were taken from. The depth of burrows from the surface varied between patches, and as a result "Above" measurements varied between 0-5cm maximum, to 15-20cm maximum "Above". Similarly "Below" samples were limited to 90cm due to access restrictions (some pits were 1.1m deep, the limit at which samples could be extracted by hand) and substrate restrictions. Image is from a collapsed bank exposing an old burrow network.

All soil samples were gathered as 5x5xY cubes (with Y varying between different ranges, i.e. a 30-60cm range would be extracted as a 5x5x30cm cube). See figure 7 for schematic.

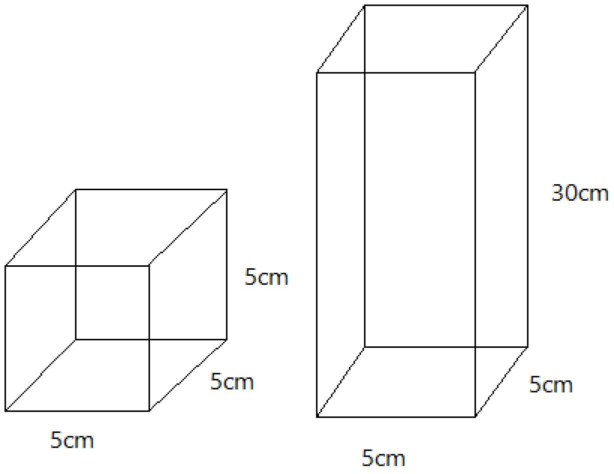

Figure 7: Example schematic of samples taken. The two examples are of a 5x5x5cm (e.g. 0-5cm "Below") sample and a 5x5x30cm (e.g. 30-60cm "Below") sample.

Given the volume of samples extracted, it was not possible to store the samples in a cold room, to prevent microbe activity. The best possible compromise was to store the samples in a darkened cupboard at ambient temperature for approximately 2-3 weeks until they could be moved into the lab where they were then kept at 1-2 °C.

3.0 LABORATORY WORK

3.1 Drying

Following a trial experiment earlier in 2015, it was decided to dry the samples to allow easier sieving. This was deemed necessary given that samples were wet following extraction. Samples were placed in an air dry oven because of the amount of space the samples required, but with no intention of turning on the oven. The samples were checked after one day with little to no change to soil wetness, then again at three days, again, with little to no difference. Following this, it was decided to leave the samples for an additional four days before beginning further work. Unfortunately, during this four day period, the oven was inadvertently turned on by another user of the laboratory. The oven was estimated to have reached 35-45°C and likely remained on for three to four days. Once the samples were removed, it became obvious that sieving was no longer an option given how hard the samples had turned. Thus an unplanned additional sample preparation step had to be implemented.

3.2 Ball milling

After various methods were trialled to break down the hardened soil, samples were ground in a ball mill using a Retsch PM 100 Ball Mill. This involved breaking the soil chunks with a mallet into manageably sized pieces (approximately 1x1cm) before placing them in the grinding chamber along with six steel ball bearings. The mill was set to 250rpm and was run for two minutes at a time. If the sample failed to break down the process would be repeated until it did so. Most samples broke down after only one cycle, while others required up to three cycles. The instruments used during this were washed in warm water before being cleaned using ethanol between each sample.

3.3 Nitrogen extraction

Once milled, the samples were prepared for NH_4^+ and $NO_{2/3}^-$ extraction. An initial 70 samples were given priority during milling to test if the samples were still viable. These samples were weighed to 10g (accurate to 0.001g), then mixed with 100ml of 1M KCl, and shaken for 30 minutes in an orbital shaker. The samples then had NH_4^+ and $NO_{2/3}^-$ extracted using a FIASTAR 5000. Following successful extraction from the trial samples, the remaining samples were prepared, but weighed to 2.5g (accurate to 0.001g) and mixed with 25ml of 1M KCl instead. The reduction in sample weight was purely to prevent waste since only a small fraction of the KCl mixture was used for the nitrogen extraction. Results were given, in all cases, as mg NH_4^+ or $NO_{2/3}^-$ in 1L of KCl.

3.4 Moisture content

Since the samples were not dried to a consistent standard, knowledge of moisture content was required to correct the NH_4^+ and $NO_{2/3}^-$ extracted concentrations, since this could affect the weight of sample used.

Moisture content was calculated for all samples following milling. "Wet weight" was measured to roughly 10g, but accurate to 3 decimal places. Vessel weight was also measured. The samples were then placed in an oven at 105 °C overnight for 18 hours. "Dry weights" were then measured. Moisture content was then calculated using the following formula:

$$\%\,Moisture\ Content = \frac{Weight\ of\ wet\ soil\ sample - weight\ of\ dry\ soil\ sample}{Weight\ of\ wet\ soil\ sample} * 100$$

3.5 Conversion

To allow statistical analysis, units were converted from mg/L to mg of NH_4^+ and $NO_{2/3}^-$ in 1g of soil (mg/g). Sample weights were recalculated to account for moisture factor. NH_4^+ and $NO_{2/3}^-$ extracts were converted initially to mg/25ml. These were then entered into the following equation to get mg/g:

$$N\ mg\ per\ soil\ g = \left(\frac{1}{Sample\ weight}\right) * N\ mg\ per\ 25\ ml\ KCl$$

This provided a comparable measure of nitrogen (NH_4^+ and $NO_{2/3}^-$) in collected samples.

3.6 Treatment effects

Since samples had to undergo unorthodox preparations for NH_4^+ and $NO_{2/3}^-$ extraction, where possible preparation effects were tested. Where samples were found that could still be sieved, they were split into halves, one half getting milled, and the other sieved (2 mm sieve). Such samples were uncommon (11 in total), as the soil type had to be sufficiently sandy that drying did not cause them to harden to the point of being unmalleable. Additionally, drying and not drying were tested to an extent. Some samples failed to dry (even given the prolonged time in the oven), but these provided an opportunity to test the effect of drying on extracted nitrogen components. Samples that were sufficiently wet (up to 79% moisture content) were particularly rare, with only five samples found that could test the effect of drying. The "wet" samples were cut in halve. Given all but one of the samples were from samples 15cm or longer, it was possible to cut them "vertically" and preserve any variation due to N leaching. The one 5cm long sample was cut into quarters, each opposite corners were combined in an attempt to avoid selecting only the top or bottom and biasing the test. After splitting the samples into halves, one half were placed back into the oven and remained in for three days. Once the "dried" samples were dried, all samples, both wet and dry were milled.

All treatment effect samples were extracted as explained earlier.

4.0 STATISTICAL ANALYSIS

4.1 Treatment effects

In order to test whether treatment methods had any effects on the data one-way ANOVAs were carried out for both treatments, dried/not dried and milled/sieved, and for both nitrogen forms, NH_4^+ and $NO_{2/3}^-$. Minitab 17 was used for ANOVA statistical analysis of treatment effects.

4.2 General Linear Models

General linear models, with a Gaussian error were constructed to test if water voles had a spatio-temporal effect on NH_4^+ and $NO_{2/3}^-$. Both dependent variables were log transformed to achieve best possible normalised distribution. Models were fitted and AICc terms used to inform on the best fitting model. AICc was used as opposed to AIC due to the small sample size in this study. The statistical programme R (version 3.1.3) and the R programming suite Tinn-R (version 4) were used to construct all models and related graphs (Crawley, 2013). The AICc package used in R was AICcmodavg.

Variables used for modelling, along with a brief description of each is included within table 1.

Table 1: Variables used in models with a brief description of each.

Variable	Description
logNH_4^+	Amount of NH_4^+ extracted from sample measured in mg/g. Transformed by log(NH_4^+)
log$NO_{2/3}^-$	Amount of $NO_{2/3}^-$ extracted from sample measured in mg/g. Transformed by log($NO_{2/3}^-$)
Chronosequence	Years occupied since last extinction event
GrChrono	Years occupied grouped as "New" and "Old". New being patches occupied for 2 years and less, old being patches occupied for 3 to 6 years. 2 years was set as a benchmark following as it was the median vole occupancy length as well as time for plant community shifts to occur Bryce *et al.* (2013).
GrChrono2	Years occupied grouped into 2 years, i.e. 1-2 years, 3-4 years & 5-6 years, as per Bryce *et al.* (2013)
GrChrono3	Years occupied grouped into 3 years i.e 1-3 years & 4-6 years, was used to account for any possibility of a time lag in the findings of Bryce *et al.* (2013)
BurrowCount	Number of burrows counted in a 2x3m area centred on target burrow, this was used as a proxy for vole density
Direction	Direction that sample was taken from in relation to burrow, i.e. "Below", "Above" and "Across"
Distance	Sample distance, i.e. 0-5cm, away from burrow
Below	Samples only taken from below the burrow, used as a proxy for leaching from burrow
Above	Samples only taken from above the burrow, used as a proxy for leaching from the surface
Across	Samples only taken sideways away from the burrow, used as a proxy for aeration
F*Variable	Various variables were changed to factors and were donated as such with a prefix of "F"

RESULTS

To understand the effects of water vole ecosystem engineering on the local environment, this study focused on soil NH_4^+ and $NO_{2/3}^-$ in spatial patterns around burrows over differing chronosequence lengths. The data was explored graphically, then by statistical modelling in General Linear Models (GLM) produced in R. Models tested biologically sensible factors as additive effects, but also as interactions where it was applicable and AICc was used to determine the most parsimonious models.

Table 2: Descriptors of data showing mean with standard error as well as range

Variable	*Mean (+/- se)*	*Range*
NH_4^+ (mg/g)	0.121 +/- 0.009	0.005-1.023
$NO_{2/3}^-$ (mg/g)	0.012 +/- 0.001	0.0001-0.093
Length of occupancy (years)	3.945 +/- 0.101	1-6
Burrow Density (per 3mx2m area)	7.524 +/- 0.268	1-16

Various descriptions for selected parameters can be found in the appendix.

Burrow density and continuous occupancy had the potential to be correlated, since biologically they would presumably be related, i.e. the longer a patch has been occupied, the greater the number of burrows. However, they were not found to be correlated and were therefore suitable to be included within the same model (Pearson correlation = -0.269, P-value = <0.005).

1.0 TREATMENT EFFECT

The two preparation methods used to prepare samples for nitrogen extraction had a significant effect on $NO_{2/3}^-$, both increasing it with drying by 0.40 mg/g +/- 0.09 mg/g (df=9, F=64.19, P<0.001), and decreasing $NO_{2/3}^-$ following milling by 0.11 mg/g +/- 0.05 mg/g (df=21, F=4.33, P=0.050). The increase of $NO_{2/3}^-$ is believed to be the result of improved mixing with KCl, since the wet samples mixed poorly, and would often result in clumps remaining after shaking. The decrease with grinding is possibly explained by loss of volatile $NO_{2/3}^-$.

Neither preparation effects had any significant effect on NH_4^+ (Drying; df=9, F=1.65, P=0.234, milling; df=21, F=0.16, P=0.691).

All samples used for analysis hereafter were both dried and milled to the same standard and were therefore all impacted equally.

2.0 EXPLORING THE DATA

Both NH_4^+ and $NO_{2/3}^-$ were log transformed to achieve better distribution before analysis began (Figs. 8 & 9).

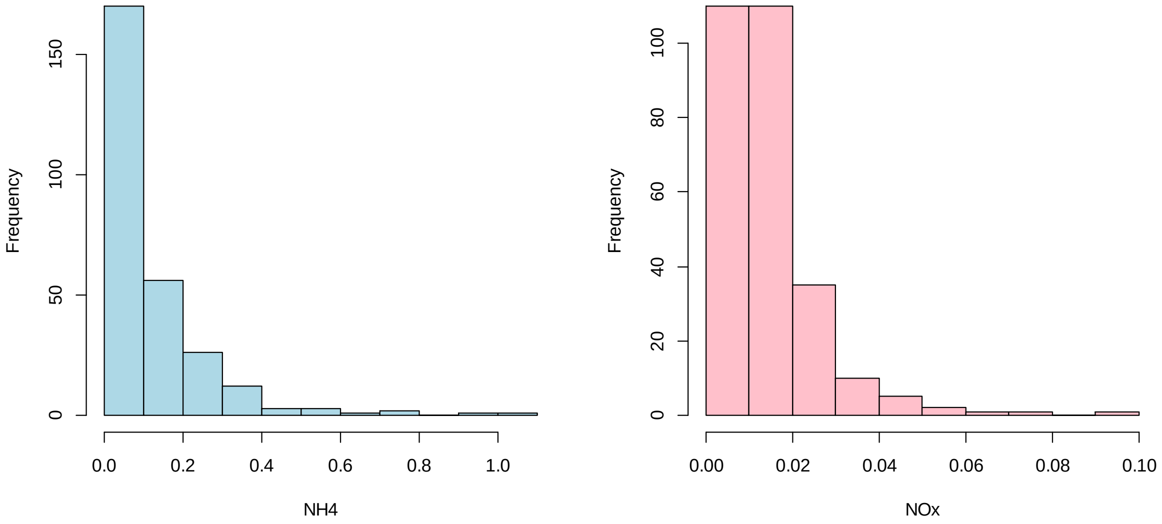

Figure 8: Frequency distribution of untransformed NH_4^+ (blue) and $NO_{2/3}^-$ (pink). Both had a skewed distribution and it was preferred to transform them to allow better distribution.

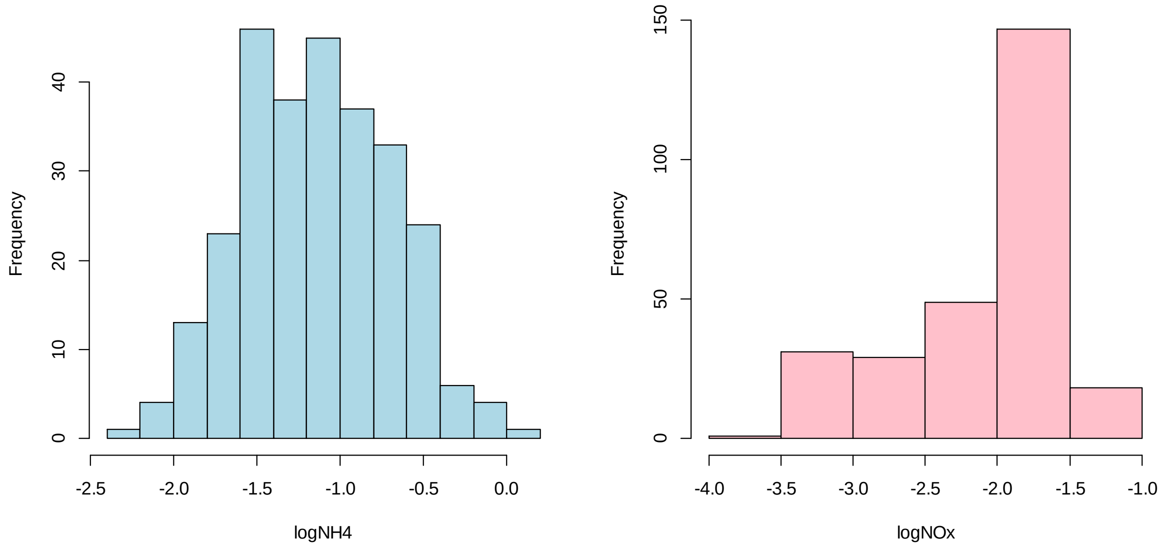

Figure 9: Frequency distribution of log transformed NH_4^+ *(blue) and* $NO_{2/3}^-$ *(pink).* NH_4^+ *had a normal distribution, while* $NO_{2/3}^-$ *had the closest fit to normal distribution that any attempted transformation could achieve.*

Throughout analysis sample outliers were identified, but these were retained within the dataset. Despite some of these outlying samples having ~10x the amount of NH_4^+ and/or $NO_{2/3}^-$, they were still low biologically, and were believed to potentially represent localised vole influences.

2.1 Nitrogen Variation by Occupancy Length

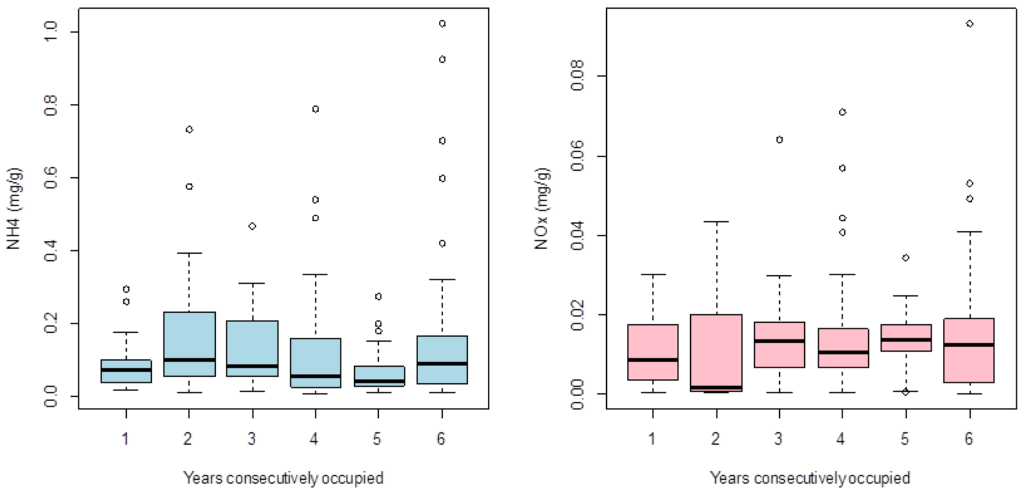

Figure 10: Nitrogen variation by length of patch occupation. Years a patch has been continously occupied plotted against NH_4^+ *(blue) and* $NO_{2/3}^-$ *(pink). No clear trends were apparent with either, but this was not unexpected. Given*

Bryce et al. (2013), it was believed that grouping patches around two years would be more suitable.

No clear trend appears with either NH_4^+ or $NO_{2/3}^-$ with increasing lengths of occupancy since last extinction event (Fig. 10). To discover if trends were more apparent in longer time spans, groupings of length of occupation were constructed for entry as parameters. Length of occupancies were grouped as; new/old (new being one to two years and old being three to six) (Fig. 11, a & b), two year incremental groupings (Fig. 11, c & d), and three year incremental groupings (Fig. 11, e & f). These were compared to see if any showed clear trends (Fig. 11). These demarcations came from Bryce *et al.* (2013), where it was found that vole patches were occupied for a median of two years, and plant community responses to vole absence was delayed by two years. In addition to this Bryce *et al.* (2013) found that plant species was most consistently explained by both time since last occupation and burrow density. This implied that two years continuous occupancy was a reasonable grouping.

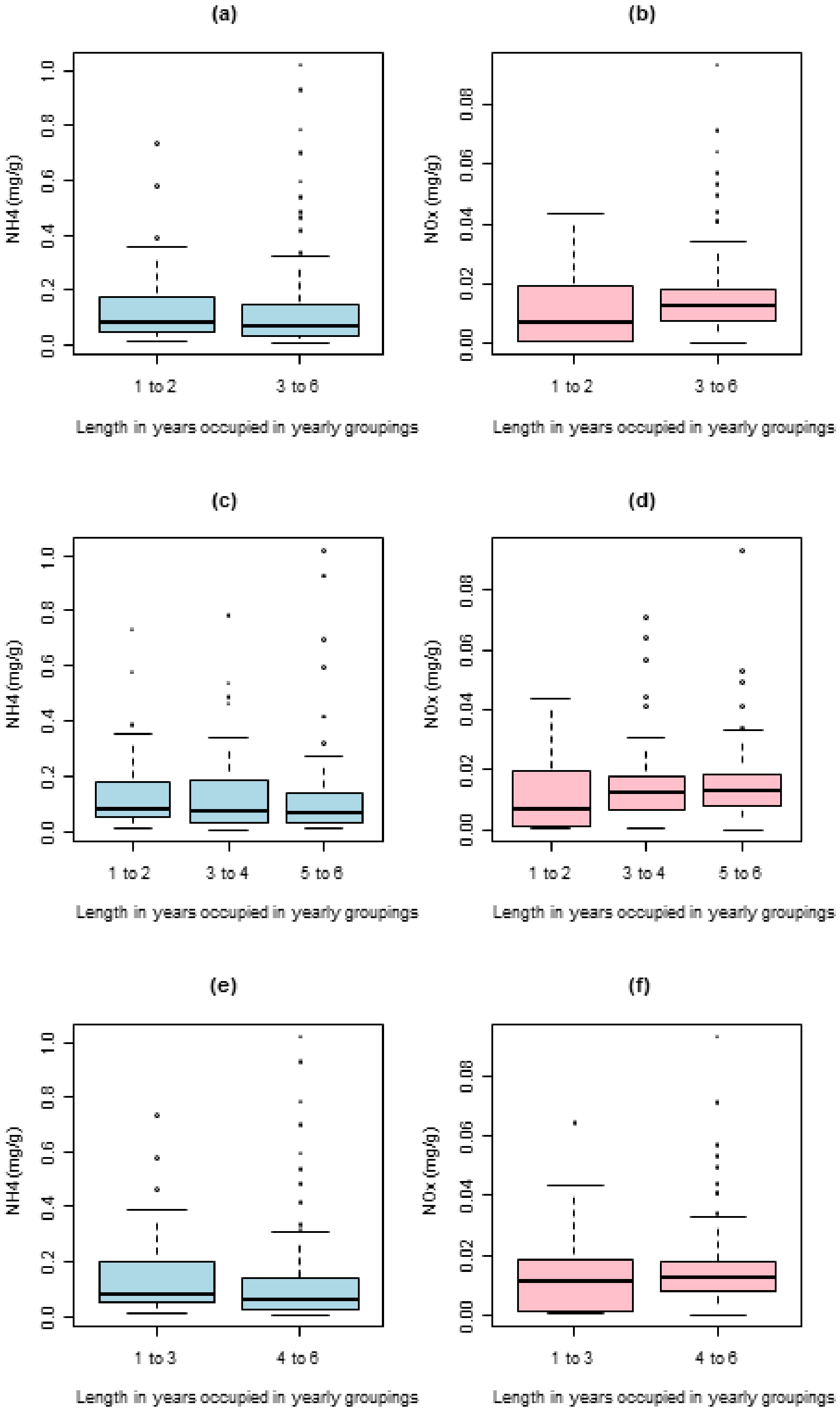

Figure 11: Chronosequence groupings plotted against NH_4^+ *(blue) and* $NO_{2/3}^-$ *(pink). All show subtle trends of decreases for* NH_4^+*, and increases with* $NO_{2/3}^-$*. As trends can be identified, all were considered in model selection.*

3.0 MODELLING

General linear models were constructed for logNH_4^+ and log$NO_{2/3}^-$ to initially test if water voles had an influence on nitrogen in a 3D sphere around their burrows. Parameters considered were length of occupation (of each grouping), direction ("Above", "Across" and "Below"), distance (centimetre away from the burrow) and burrow count. Whenever direction and distance were both included in a model, a two way interaction was specified, since the two were intrinsically linked.

Model selection, using AICc (R package AICcmodavg), was performed. Parameters were sequentially removed, resulting in multiple combinations of models being tested. P values were ignored in favour of selecting models using AICc as P value null hypothesis testing is not well suited for model selection (Anderson, Burnham & Thompson, 2000). Normality was scrutinised, but no stringent requirements were placed on it since GLMs are relatively robust to non-normal distribution (Zuur *et al.,* 2010).

3.1 What determines the concentration of LogNH_4^+ in water vole patches?

LogNH_4^+ model selection:

Table 3: All logNH_4^+ models considered in model selection. The numbers of parameters used, as well as the ΔAICc scores, are included for each model. Interaction was denoted as "" in all models and includes the main effects of the interaction.*

logNH_4^+ Model	**# Parameters**	**ΔAICc**
GrChrono3 + Distance * Direction + BurrowCount	5	0
GrChrono3 + Distance * Direction	4	0.91
FGrChrono3 + Distance * Direction	4	0.91
GrChrono2 + Distance * Direction	4	4.92
GrChrono2 + Distance * Direction + BurrowCount	5	5.10
GrChrono2 + Distance + BurrowCount	3	5.63
FGrChrono2 + Distance * Direction	4	6.93
GrChrono + Distance * Direction	4	7.38
Chronosequence + Distance * Direction	4	7.98
Direction	1	8.36
Direction + BurrowCount	2	10.16
Distance * Direction + BurrowCount	4	10.40

Distance * Direction	3	11.64
GrChrono2 + Distance	2	41.01
GrChrono2	1	41.04
GrChrono2 + Distance + BurrowCount	3	42.30
GrChrono2 + BurrowCount	2	42.41
Distance	1	42.42
BurrowCount	1	44.36
Distance + BurrowCount	2	44.37

Table 4: Selected LogNH_4^+ models. Models 2, 3, and 4 were the most parsimonious, while model 1 was the initial model used to determine ΔAICc. Interaction was denoted as "" in all models and includes the main effects of the interaction.*

Model	Variables	ΔAICc
1	logNH4 ~ GrChrono2 + Distance ∗ Direction + BurrowCount	5.10
2	logNH4 ~ GrChrono3 + Distance ∗ Direction + BurrowCount	0
3	logNH4 ~ GrChrono3 + Distance ∗ Direction	0.91
4	logNH4 ~ FGrChrono3 + Distance ∗ Direction	0.91

The initial and most complex logNH_4^+ model (model 1) had a ΔAICc score of 5.10, proposed that logNH_4^+ was a result of occupation grouped into 2 year increments, distance away from burrow, direction from burrow (as an interaction with distance), and burrow density. Parsimony was improved through parameter exclusion and exchange until the model with the lowest AICc score was achieved.

Model 2 was deemed to be the most parsimonious, although two other models (models 3 and 4) showed similar parsimony, both with ΔAICc scores of 0.91 (Table 4).

Table 5: Coefficients for LogNH_4^+ model 2. Estimates are on the log scale.

	Estimate	Std. Error	t value	P value
(Intercept)	-1.06	0.09	-12.08	<0.001
GrChrono3	-0.04	0.01	-3.53	<0.001
BurrowCount	-0.01	0.01	-1.73	0.09
Distance:DirectionBelow	-0.01	0.12	0.96	0.34
Distance:DirectionAbove	0.03	0.01	2.55	0.01
Distance:DirectionAcross	0.004	0.01	0.70	0.49

Model 2 showed that variation in logNH_4^+ was best explained by length of occupation grouped into 3 year groupings (1-3, 4-6 years), and distance and direction as an interaction (Table 5). The coefficients (Table 5) showed logNH_4^+ decreased slightly between years 1-3, to years 4-6, while interactions between distance and direction had varying responses. Below the burrow NH_4^+ decreased slightly with distance per distance away from the burrow. Conversely, samples from above the burrow increased per distance away from the burrow. And finally soil samples taken across the burrow increased marginally per distance away from burrow. With burrow density, logNH_4^+ was found to decrease marginally.

Overall, model 2 shows that burrows have an effect on the nitrogen cycle, which decreases logNH_4^+ in longer occupied patches. This effect was dependent on the direction and distance away from the burrow. Similarly burrow density had an influence in determining the logNH_4^+ concentration in the soil surrounding burrows. This is consistent with aeration of the soil, and indicate burrows as being responsible.

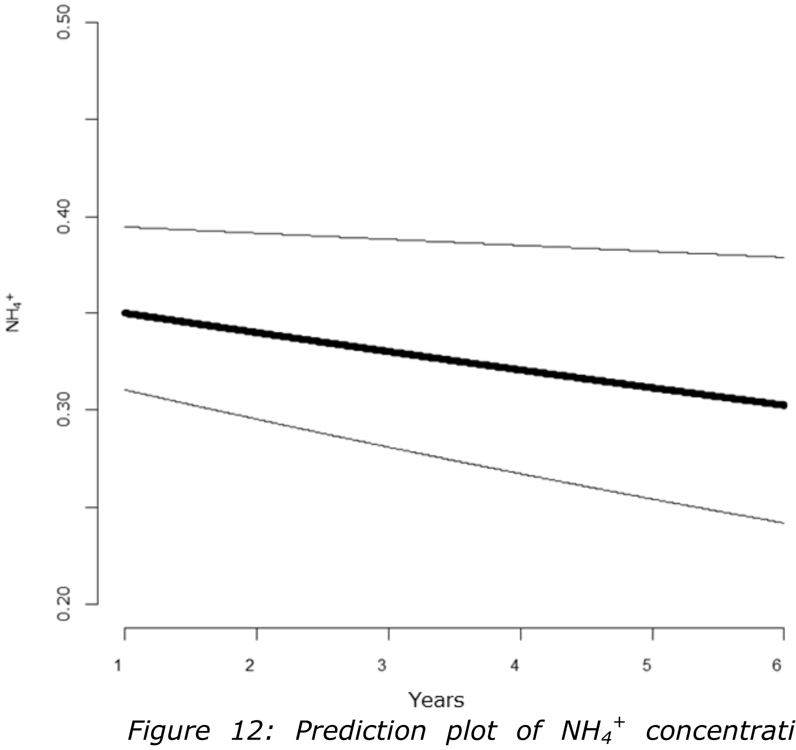

Figure 12: Prediction plot of NH_4^+ concentration (mg/g) over 6 years of occupancy. The decline in ammonium in the system over 6 years is apparent, and is likely explained by conversion to $NO_{2/3}^-$ increasing following aeration. 95% CI are represented by the grey lines.

Residuals vs fitted, scale-location, normal Q-Q and residuals vs leverage were scrutinised for all models. The plots for model 2 can be found in the appendix.

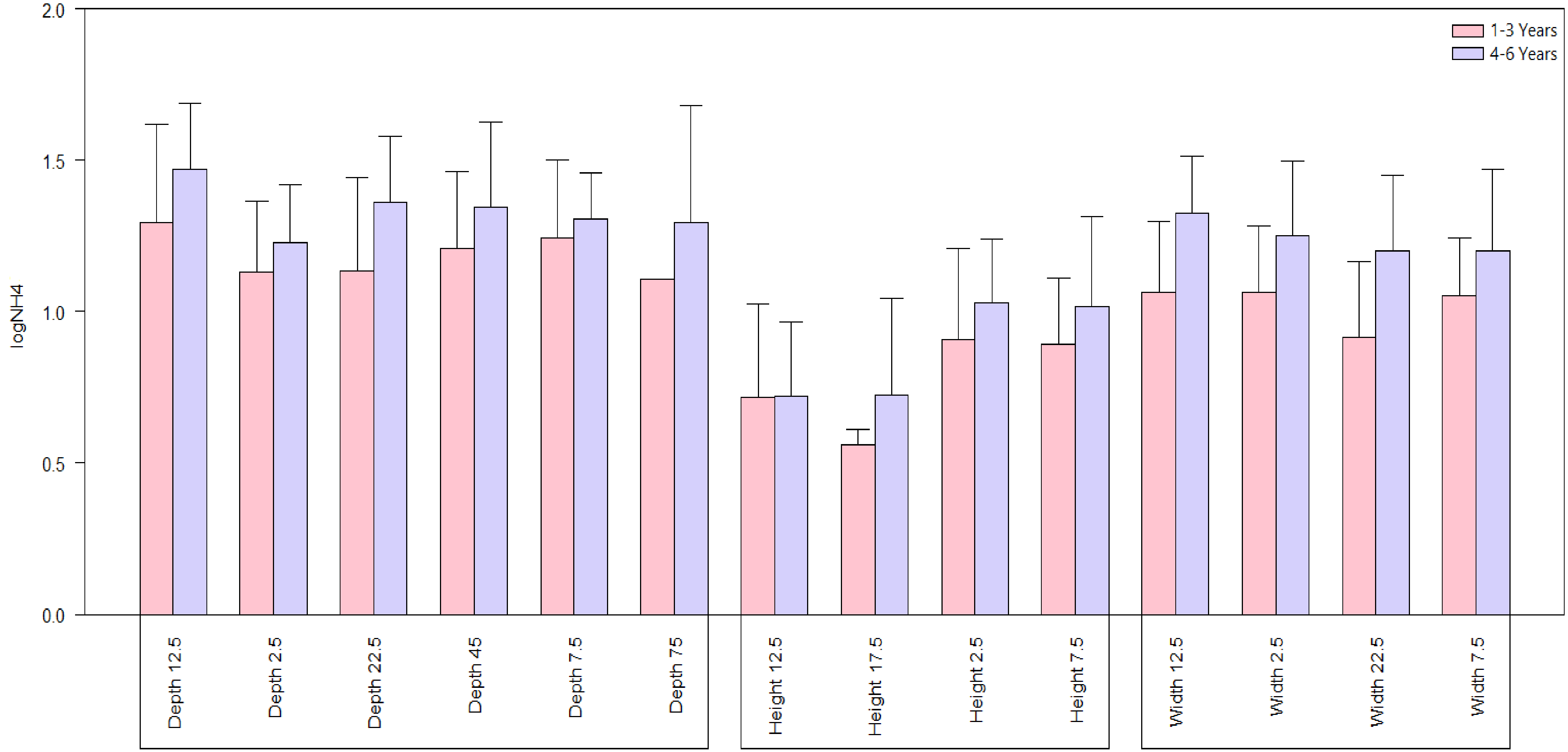

*Figure 13: Log NH4 plotted against distance and direction from burrow, grouped into length of continuous occupancy of 3 year increments (GrChrono3). LogNH4 values converted to positive values to allow plotting (square root(logNH4^2)). LogNH4 is measured in mg per g of soil. Distance is measured in cm from burrow. See figure 6 for direction illustration. In all directions*distances logNH4 was greater in patches with vole occupancy between 4 to 6 years, compared to 1 to 3 years. Temporal variation is apparent.*

3.2 What determines the concentration of Log$NO_{2/3}^-$ in water vole patches?

Table 6: All log$NO_{2/3}^-$ models used in model selection. The numbers of parameters used, as well as the ΔAICc scores, are included for each model.

log$NO_{2/3}^-$ Model	**# Parameters**	**ΔAICc**
GrChrono + FDistance	2	0
GrChrono + FDistance + BurrowCount	3	1.56
GrChrono + Distance	2	1.80
GrChrono2 + Distance	2	7.00
GrChrono * FDistance	3	7.10
GrChrono2 + Distance + BurrowCount	3	8.50
GrChrono3 + Distance	2	9.74
GrChrono2 + Distance * Direction + BurrowCount	5	10.84
GrChrono2 + Distance * Direction	4	11.46
Chronosequence + Distance	2	11.60
Distance + BurrowCount	2	13.95
GrChrono2	1	14.10
Distance	1	14.37
GrChrono2 + BurrowCount	2	15.43
Distance * Direction + BurrowCount	4	17.72
Distance * Direction	3	18.42
GrChrono2 + Direction + BurrowCount	3	19.55
Direction + BurrowCount	2	24.41
Direction	1	25.07

Table 7: Selected Log$NO_{2/3}^-$ models. Models 2, 3, and 4 were the most parsimonious, while model 1 was the initial model used to determine ΔAICc.

Model	**Variables**	**ΔAICc**
1	$\text{logNOx} \sim \text{GrChrono2} + \text{Distance} * \text{Direction} + \text{BurrowCount}$	10.84
2	$\text{logNOx} \sim \text{GrChrono} + \text{FDistance}$	0
3	$\text{logNOx} \sim \text{GrChrono} + \text{FDistance} + \text{BurrowCount}$	1.56
4	$\text{logNOx} \sim \text{GrChrono} + \text{Distance}$	1.80

The starting, most complex log$NO_{2/3}^-$ model (model 1), proposed that log$NO_{2/3}^-$ was a function of; length of occupation grouped into 2 year increments, distance away from burrow interacting with direction, and burrow density. Model 1 had a ΔAICc score of 10.84. The most parsimonious model was selected through parameter exclusion and exchange until the model with the lowest ΔAICc score was achieved.

Table 8: Coefficients for LogNO$_{2/3}^{-}$ model 2. Estimates are on the log scale.

	Estimate	Std. Error	t value	P value
(Intercept)	-2.36	0.08	-28.45	<0.001
GrChronoOld	0.29	0.07	4.02	<0.001
FDistance 5-10cm	-0.10	0.09	-1.09	0.28
FDistance 10-15cm	-0.06	0.09	-0.71	0.48
FDistance 15-20cm	0.38	0.24	1.57	0.12
FDistance 15-30cm	0.25	0.10	2.52	0.01
FDistance 30-60cm	0.30	0.14	2.14	0.03
FDistance 60-90cm	0.14	0.24	0.56	0.57

Model 2 was deemed to be the most parsimonious, although two other models show similar parsimony (Table 7). This model stated that logNO$_{2/3}^{-}$ was best explained by length of occupation grouped into new (1-2 years occupancy) and old (3 years and older), with logNO$_{2/3}^{-}$ increasing in old patches (Table 8). Distance as a parameter showed that logNO$_{2/3}^{-}$ decreased 5cm away from the burrow, but began increasing 15cm away, with a peak at 15-20cm away. It is important to note that these represent only "Above" samples, and likely give a skewed impression, since there are no "Below", or "Across" samples in this measurement. "Below" and "Across" samples included 15-30cm, rather than 15-20cm and are not suited to comparison. Overall, model 2 shows that burrows have an effect on the nitrogen cycle, which increases logNO$_{2/3}^{-}$ in longer occupied patches, and this effect has a 3 dimensional effect, not limited to any direction in particular. This is consistent with aeration of the soil, as a result of burrows being responsible.

Residuals vs fitted, scale-location, normal Q-Q and residuals vs leverage were scrutinised for all models. The plots for model 2 can be found in the appendix.

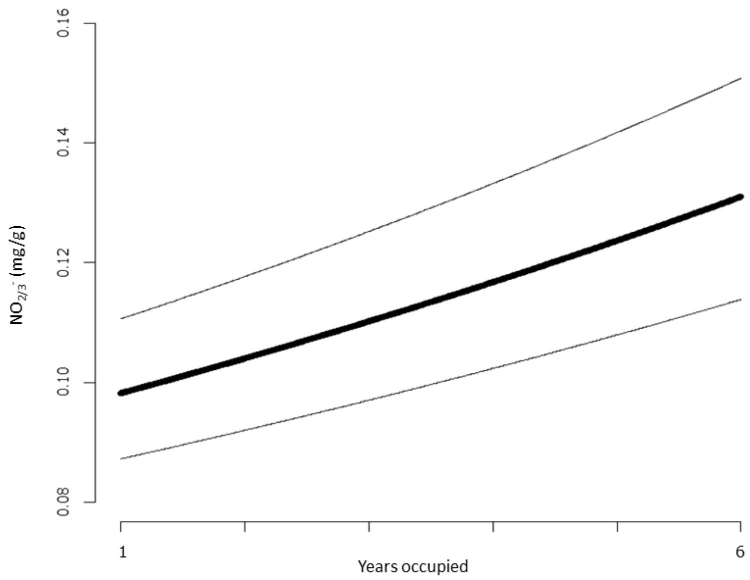

Figure 14: Prediction plots for years occupied against $NO_{2/3}^-$*. The plot shows an increase of* $NO_{2/3}^-$ *over the 6 year time frame, and likely represents increased aeration as a result of water vole burrows. 95% CI are represented by the grey lines.*

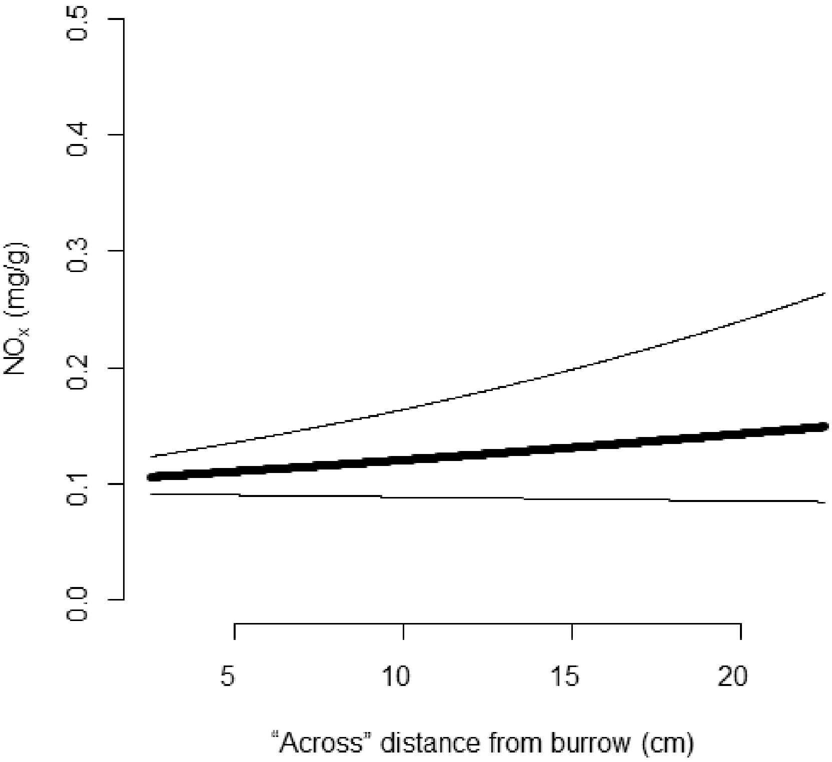

Figure 15: Prediction plot for distance "Across" burrow against $NO_{2/3}$*. The plot shows little change over the distances, other than an increase in the 95% CI towards at greater distances. The lack of a drop off suggests an interaction with neighbouring burrows. 95% CI are represented by the grey lines.*

DISCUSSION

The above results demonstrated that water voles act as underground ecosystem engineers with a strong influence on belowground nitrogen cycle processes (Figs. 12, 13, 14). Water vole burrows have a larger spatio-temporal influence on the nitrogen cycle than was previously expected. These influences on the nitrogen cycle certainly have the potential to contribute to the plant community shifts around water vole burrow networks seen in Bryce *et al*. (2013).

As well as having demonstrated that water voles act as ecosystem engineers, this study also illustrated that that engineering has a temporal component (Figs. 12, 13, 14). Since water voles in the uplands of the Highlands of Scotland show classic metapopulation dynamics (Sutherland *et al*., 2012; Telfer *et al*., 2001; Lambin *et al*., 2004; Lambin *et al*., 2012) this means that water vole patches do not have fixed, binary effects of ecosystem engineering, but rather a dynamic one dependant on length of occupancy. The fact that NH_4^+ and $NO_{2/3}^-$ do not follow similar patterns of distribution over time (Figs. 12, 14) or space only serves to underscore that the ecosystem engineering effects on the nitrogen cycle act in a complex way, not a simple binary one.

1. TIMELINE FOLLOWING RECOLONIZATION

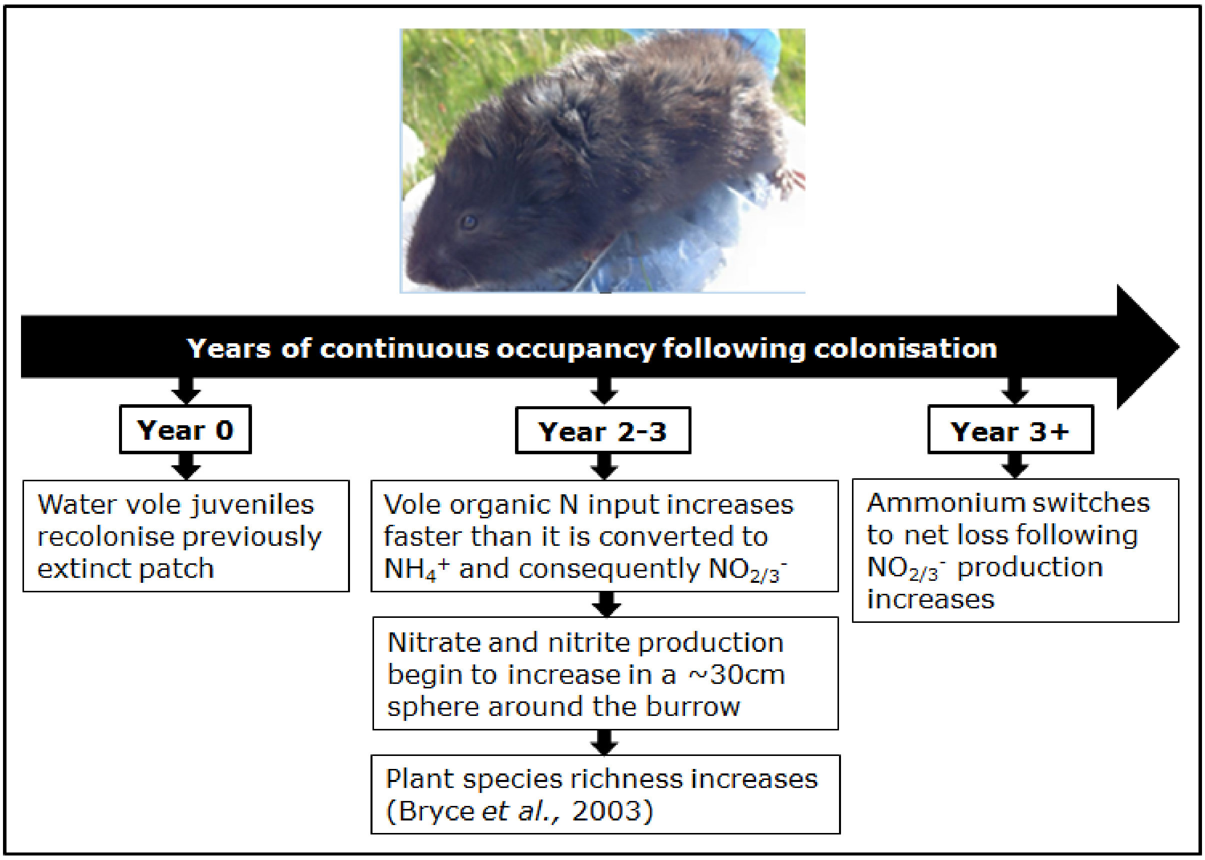

Figure 16: Hypothetical timeline of predicted shifts in the nitrogen cycle as a result of water vole length of occupation based on findings in this thesis.

2.0 TEMPORAL VARIATION IN THE NITROGEN CYCLE IN A NITROGEN LIMITED HABITAT

The inclusion of chronosequence, and ultimately length of vole patch occupancy, in both models of NH_4^+ and $NO_{2/3}^-$, highlights its importance in explaining variation in both decreased NH_4^+ and increased $NO_{2/3}^-$ (Figs. 12, 14). The time lag between decreased NH_4^+ and increased $NO_{2/3}^-$ suggests there is a similarly connected time lag within the microbial community in the study site, between increased substrate (food) and eventual output. The modelling suggests that this time lag is one year, following colonization, between increased NH_4^+ and the subsequent increase in $NO_{2/3}^-$. The modelling also suggests that it is not necessarily the direct input of voles that cause increased nitrogen in the habitat, but rather through burrows increasing mechanistic shifts to promote mineralization and nitrification, as a result of increased aeration. The rationale for invoking this sequence of events is the delay for NH_4^+ to begin to decrease following vole introduction (Fig. 12). If direct inputs were responsible, it would be assumed that NH_4^+ would increase throughout all years.

The delay in time before equilibrium (where both NH_4^+ and $NO_{2/3}^-$ no longer increase nor decrease within the study time frame) is reached within water vole patches brings into question whether or not vole patches remain occupied for long enough to achieve this point. Bryce *et al.* (2013) noted that median length of occupancy was two years across the Assynt study area. This suggests that the majority of water vole patches never persist long enough for water vole ecosystem engineering effects on the nitrogen cycle to reach equilibrium within such patches. The minority that do remain occupied past this two year median are therefore particularly important for maintaining habitat heterogeneity.

3.0 IMPACT OF WATER VOLE BURROWS ON NITROGEN CYCLE

3.1 Aeration

Preliminary results based on a small sample size suggest that water voles alter the nitrogen cycles in their habitats through increased aeration (Bryce, 2006). From the results of my study, I contend that it is through the aeration of soil that water voles have the largest impact on their environment. Based on four pits Bryce (2006) found no evidence that the aeration effect extended beyond burrow walls. Here I demonstrated that the effect reaches further away, up to the limit of the "Across" samples (30cm) (Fig. 15). Bryce noted that, as a result of burrow presence, soils were dried up to 25cm away. The disparity between drying effects and aeration ranges noted by Bryce (2006) and those of this study may represent sampling differences between the two (as well, Bryce sampled during a particularly dry year, compared to the wet one experienced during sampling for this study). In her study, Bryce (2006) used isolated burrows with no neighbouring burrows. This design allowed her to examine the effects of a single burrow in isolation but provided no evidence on the interactions within a burrow network. No attempts to use isolated burrows were made in this study; indeed, burrows were selected that were central within burrow networks (see burrow density method), where presumably interactions with neighbouring burrows would be greatest. While digging the "Across" samples, neighbouring burrows were often encountered out with sampling range. This suggests that burrows are often roughly 30-40cm apart (pers. obs.) (to gain access to the 30cm sample, the sample pit was dug to approximately 35cm). This is similar to findings in pocket gophers, which were found to maintain consistent and optimal spacing of burrows (Reichman, Whitman & Ruffner, 1982). The close range of neighbouring burrows may mean that the aeration effects of multiple burrows may overlap between neighbouring burrows creating a "matt" of soil with increased mineralization, as a result of increased aeration. This observation suggests that if water vole burrow networks establish a matt of increased microbial activity, then it may, in turn, result in a wider area affected by burrows, not limited to areas directly above burrow walls.

3.2 Leaching

Given the high precipitation within the area (125-250mm average July rainfall 1981-2010, >250mm during January (MetOffice, 2016)), leaching was expected to be a significant determinant of nitrate concentration spread in soil. No evidence of nitrate or nitrite leaching was found to occur, as $\log NO_{2/3}^-$ showed no trend across all directions from the burrows. It was expected that nitrate concentrations would be higher within the rhizome layer of the soil, as this is where the majority of nitrification occurs, due to the increased amount of inorganic nutrients and organic matter (Lynch & Panting, 1980). Some evidence of this was found in "Above" 15-20cm samples (only "Above" samples had 15-20cm samples) which showed the greatest relative increase in $\log NO_{2/3}^{-}$ for any direction. Ammonium followed the expected trend of being concentrated at the surface, but nitrites and nitrates not following this trend was unexpected. This may have been due to multiple reasons.

The first may have been that, in a nitrogen-limited system, such as the study area, any nitrification of ammonium to nitrates were absorbed by plants as quickly as it was produced, leading to homogenous nitrate concentration across all sampled depths. Any nitrates produced would therefore be removed before leaching could take place.

Similarly, the second possibility explaining the apparent lack of a leaching effect is that leaching was occurring, but did not appear in the samples due to it being hidden by other mechanisms. Nitrate is absorbed by plants close to the surface; this would have decreased the small amount of nitrate recovered in samples closer to the surface. Simultaneously nitrate could have leached further into deep soil. The result of both the uptake by plants and leaching of small concentrations of nitrates may be a viable explanation for the constant concentrations across directions. To account for this, if samples were taken deeper into the soil, the drop-off point for leaching might have been determined.

A faint possibility remains that aeration is still a factor at 90cm below the burrow. If aeration was still occurring 90cm below, then the input of nitrates may result in the homogenous concentrations found. Certainly, Bryce (2006) hinted that microbial activity may be increased below the burrows, although this was almost certainly limited to a few cm. While this may occur a short distance from the burrow (as Bryce was alluding to), it is highly unlikely that this could explain the $NO_{2/3}^{-}$ amounts recovered from 90cm in this study, as any aeration from burrow is undoubtedly not a factor so far from the burrow.

Water vole burrows have been shown to be water proofed to a degree (Gemma Reid, unpublished honours project, pers. comm., 2015). This provides another explanation. When leaching occurs, water saturated with nitrates enter a burrow but may be obstructed from continuing directly down. Instead, the water may flow deeper down into the burrow network before pooling at the lowest depression. Once settled, the nitrate laden water would be concentrated in a confined area of soil. No burrows sampled were at the deepest point. Leading from this, burrows tend to be at their deepest point where they link with the waterway (Lambin, pers. comm., 2015). If, as is likely, this were to occur, burrows would therefore represent a loss of nitrates from burrows, similar to peat pipes (Holden *et al.,* 2012). This may best explain why no leaching effect was found, as leaching could become highly localised at the deepest points of a burrow network.

Finally, the lack of a leaching mechanism may reflect the sample preparation for the 1M KCl nitrogen extraction. As stated previously, preparation methods significantly increased (drying) 0.40 mg/g (+/- 0.09 mg/g) and decreased (milling) 0.11 mg/g (+/- 0.05 mg/g) the amount of $NO_{2/3}^-$ mg/g retrieved. While it was not possible to test if samples from different directions or distances had different responses to preparation methods, the possibility remains.

In all, the lack of demonstration of a leaching mechanism suggests that water vole burrows may have a negligible effect on leaching in the nitrogen-limited area. Conversely, if water-proofing results in highly concentrated regions of $NO_{2/3}^-$, burrows may have a strongly significant effect in concentrating leaching effects in discrete, highly localised areas or export from the system.

3.3 Landscape changes

Vole presence has been shown to alter the plant community in their environment (Questad & Foster, 2007; Bryce *et al.,* 2013). However, the mechanism with which the ecosystem engineer's caused this response was unknown. Through this study, it has been shown that the manner in which this plant community shift is altered, is through influencing the nitrogen cycles as a result of burrows effecting microbial activity. The specific mechanism can only be inferred. However, it seems likely that increased aeration of soil is the cause, as a result of combining the results from this study, as well as Bryce (2006). The finding in Bryce (2006) that soil dryness increased up to 25cm away, and from this study, where no drop-off point was found for $NO_{2/3}^-$ (30cm away from the burrow) is highly suggestive that the mechanism which causes the plant community shifts was increased aeration.

Other mechanisms could also play a role although evidence for this was not clear from this study. Direct input, leaching and nitrification may all prove to increase or decrease nitrogen available for plant uptake. Further research would be required to answer which mechanisms are significantly responsible for influencing available nitrogen.

Undoubtedly, this research has shown that below-ground ecosystem engineers can play a significant role in habitat heterogeneity within nitrogen limited environments, such as the study site. This finding is likely transferrable to other similar environments where below-ground ecosystem engineers are found (Questad & Foster, 2007), and highlights the need to study and conserve such species, to protect vulnerable areas around the world.

4.0 EXTINCTION DEBT

Water voles in Scotland are under pressure currently from American mink (Aars *et al.,* 2001). Combined with the fragmented nature of the metapopulations of water voles, extinction at the local level is a high probability. This brings into question the extinction debt that may be faced if water voles were to go extinct, either locally or across the United Kingdom. Extinction of single species has previously been shown to have habitat wide level impacts (Branch *et al.,* 1996). The modelling conducted within, in combination with work by Bryce *et al.* (2013) shows that the effect of water vole extinction would be to lose soil nitrogen productivity, and as a consequence loss of habitat plant heterogeneity (Questad & Foster, 2007; Bryce *et al.,* 2013).

The extinction debt of losing water voles is difficult to gauge. Concerning habitat heterogeneity, the truly relevant entities are the water vole burrows, not the animals themselves. As such, the rate at which burrows degrade in an occupied site will dictate the extinction time lag for water voles. 23% of burrows degrade each year a patch is unoccupied (Bryce et a, 2013). In combination with this, is the suggestion that mats of increased aeration form when burrows are in close proximity to each other. This would imply that the loss of each successive burrow has a greater effect, as the interaction between neighbouring burrows is also broken.

In addition, soil biota have been identified as facing greater extinction risk than previously thought (Veresoglou *et al,* 2015). The intimate role that water voles have on the soil microbe community identified in this paper and others (Bryce, 2006; Bakker, 2003; Gervais *et al,* 2010) suggests that if water vole were to go extinct, then this will likely increase the extinction risk of soil biota in the upland heathlands of North West Scotland.

5.0 ECOSYSTEM ENGINEERING RESPONSE

One important finding from this work is that ecosystem engineering is not suited to be thought of as a binary response as is often portrayed (Raynaud *et al*., 2013). Instead, ecosystem engineering is best thought of as a spectrum of responses that vary temporally as well as spatially. This spatio-temporal spectrum of responses that may only be identified years after colonization, suggests that many potentially important effects of ecosystem engineering, which may have subtle yet significant effects on their habitats, are being missed due to the cryptic delay in response.

CONCLUSION

This study is the only one known that explores the impact of burrowing ecosystem engineers within a water saturated system. Traditionally, work that has been carried out on burrowing ecosystem engineers has focused on species in arid conditions whose engineering effects amount to the movement of soil from belowground to the surface (Bragg *et al.*, 2005; Desmet & Cowling, 1999; Kinlaw, 1999; Whitford & Kay, 1999). The study shows that ecosystem engineering effects can be much more subtle, and involve influencing the nitrogen cycle around the burrow networks and is unconnected to the movement of soil to the surface.

The most likely mechanism that water vole engineering influences the nitrogen cycle is through the aeration of soil increasing soil microbe activity, and as a result, increasing the available nitrates for plants. Further potential to affect leaching, resulting in highly concentrated, localised areas of nitrates, or alternatively expulsion from the system, is an exciting possibility but requires further research to answer. Certainly the influence on the nitrogen cycle is responsible for plant heterogeneity found in the nitrogen-limited environment identified by Bryce *et al*. (2013).

The timeline following colonization, reaching equilibrium at year four, suggests that the majority of water vole patches fail to reach this point since most patches have a median occupancy length of 2 years before an extinction event (Bryce *et al*., 2013). This puts an even greater value on long term occupied stable patches, which are therefore responsible for increased habitat heterogeneity, as well as being source populations for dispersal events (Lambin *et al*., 2012).

Not only has been it been demonstrated that water voles act as ecosystem engineers, but it has also been shown that the engineering effect has a temporal component. Since water voles in the Highlands of Scotland show classic metapopulation dynamics (Sutherland *et al*., 2012;

Telfer *et al*., 2001; Lambin *et al*., 2004; Lambin *et al*., 2012) this means that water vole patches of occupation do not have fixed, binary effects of ecosystem engineering, but rather dynamic effects dependant on length of occupancy.

The interaction between soil microbes, neighbouring burrow proximity and time lags in nitrogen components suggests that the time required for extinction debt to be reached may be substantial. Above all, this highlights the need to protect the species, but also that small mammals may play an increasingly important role in nutrient cycling within environments with limited nutrients.

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APPENDIX

Descriptors of parameters:

Distance	n
Below	111
Above	68
Across	96

$NH_4^{\pm}$

Min	1st Qu.	Median	Mean	3rd Qu.	Max
0.004964	0.03423	0.0723	0.1214	0.1565	1.023

log $NH_4^{\pm}$

Min	1st Qu.	Median	Mean	3rd Qu.	Max
-2.304	-1.466	-1.141	-1.137	-0.8055	0.009869

$NO_{2/3}^{-}$

Min	1st Qu.	Median	Mean	3rd Qu.	Max
0.0001136	0.004852	0.01204	0.01362	0.01822	0.09314

log$NO_{2/3}^{-}$

Min	1st Qu.	Median	Mean	3rd Qu.	Max
-3.945	-2.314	-1.92	-2.103	-1.739	-1.031

Burrow Count

Min	1st Qu.	Median	Mean	3rd Qu.	Max
1	4	6	7.524	11	16

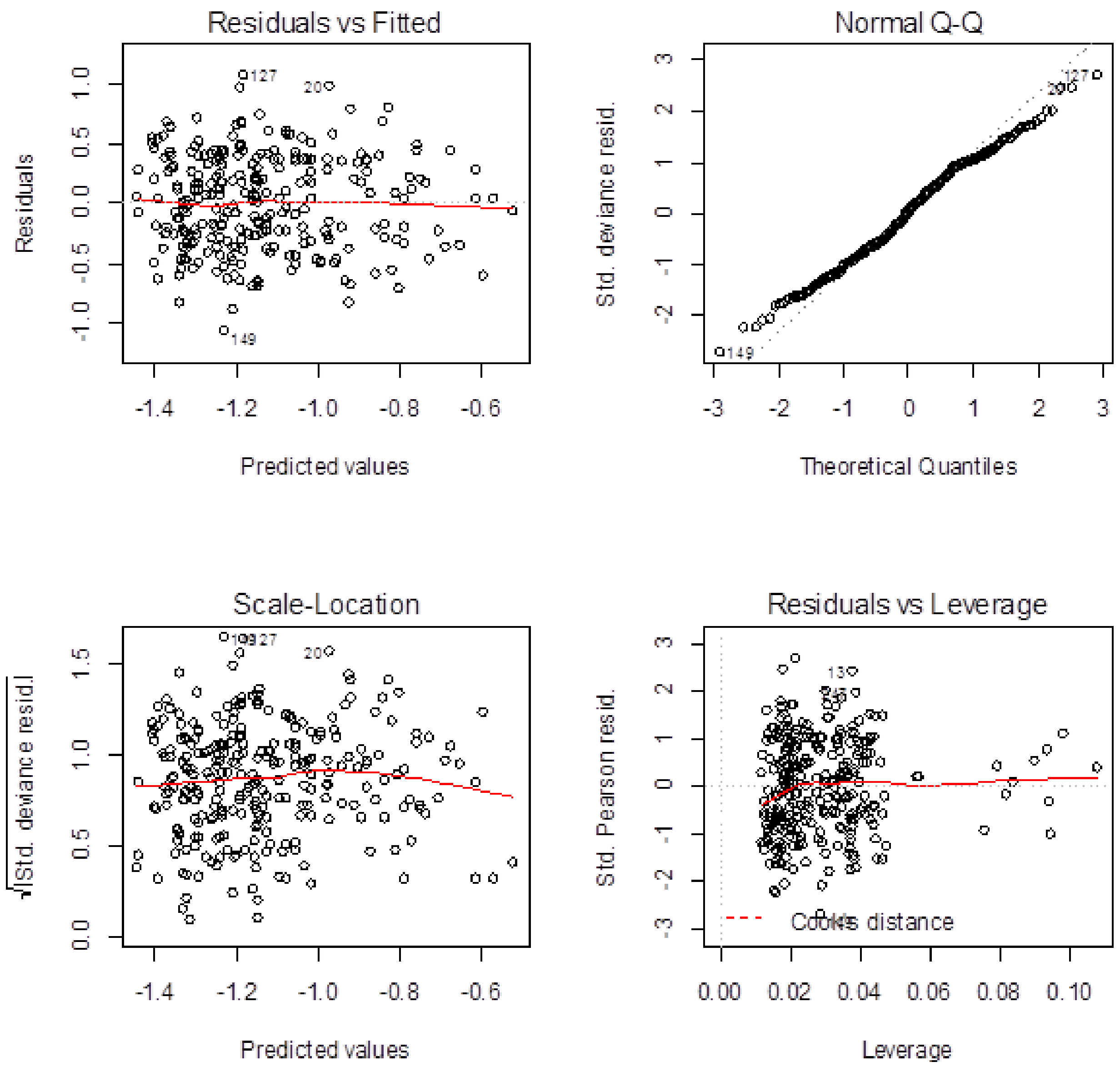
Distance

Min	1st Qu.	Median	Mean	3rd Qu.	Max
2.5	2.5	7.5	13.52	17.50	75

Chronosequence

Min	1st Qu.	Median	Mean	3rd Qu.	Max
1	3	4	3.945	6	6

Plots for NH_4^+ model 2:



Plots for $NO_{2/3}^-$ model 2: