



Preliminary study of impacts of a large roosting bird congregation on a pest exclusion fenced area of Rotopiko wetland ecosystem

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Abstract: This pilot study aims to quantify the impacts of roosting birds on the pest exclusion fenced area of Rotopiko wetland ecosystem in the Waikato region of New Zealand. Water samples were collected from five monitoring stations, encompassing four stations within the fenced area (three along a drain running through the fenced area into the lake, and one from the fenced lake itself), and one from a nearby unfenced lake, not affected by roosting. Soil samples were collected from three monitoring stations: two inside the fenced area, with varied roosting density, and one outside. Sampling was conducted once in each season, during winter (August 2021), spring (November 2021) and summer (February 2022). For each sampling time, a triplicate sample was taken at each station. Within the fenced area, increased nutrient loading in water and soil, and microbial growth in water, were observed in areas with the highest roosting pressure. Water tested where the drain enters the lake showed an up to 44-fold increase of total nitrogen, a 750-fold increase in faecal coliforms, and a 915-fold increase in *Escherichia coli* compared to water collected at the point where the drain enters the fenced area. Comparison between the lakes showed that the total nitrogen concentration in the fenced lake was 16 times higher than in the non-fenced lake, while the number of faecal coliforms and *E. coli* were over 30 times higher at the fenced lake. This study, although limited in sample size and statistical power, sheds light on the impacts of roosting birds on the Rotopiko ecosystem.

Keywords: microbiological analysis; New Zealand wetlands; nutrient analysis; pest-proof fence; roosting bird impacts

Introduction

The Rotopiko wetland, located in the Waikato region of New Zealand, comprises three interconnected peat lakes (Rotopiko-East, -South and -North) situated approximately 5 km south of the township of Ōhaupō. The lakes are a high priority ecosystem for ensuring the conservation of New Zealand's indigenous biodiversity, as there are only a few remaining peat lakes in the Waikato that support a healthy native macrophyte community (Wu et al. 2013).

The National Wetland Trust (NWT), supported by local government agencies, iwi, and community volunteers, has led a restoration project at Rotopiko, enhancing water quality, native flora and fauna, and proposing a Wetland Discovery Centre. As part of the restoration, NWT constructed a 1400-metre pest exclusion fence around the 11-hectare site, which includes the 10 000-year-old Rotopiko-East peat lake (fenced lake), a 400-year-old kahikatea forest, and various restoration plantings. Since 2013, the area has been mammalian pest-free, with the exception of mice (see Fig. 1 for site location map).

The eradication of mammalian pests has created an unexpected sanctuary for communal roosting birds such as starlings (*Sturnus vulgaris*) and sparrows (*Passer domesticus*) within the fenced area. An informal estimate provided by NWT

personnel suggested that the population of birds roosting at Rotopiko was in the range of around half a million during the peak winter season (Karen Denyer, National Wetland Trust, pers. comm.).

It is therefore expected that, within the fenced area, a high concentration of nutrients associated with the large amount of bird faeces produced each day have been deposited in the soil underneath roosting sites and eventually transported to the fenced lake via surface run-off and groundwater. In addition, direct nutrient loading to surface water occurs when birds roost in overhanging branches. Previous work assessing the birds' congregation at the site monitored them using (1) gridded plates for gathering guano and (2) the acoustic energy of the roost cacophony (Sandoval et al. 2023). Both methods showed a strong correlation and demonstrated a seasonal change in the bird community, with a reduction in bird numbers when moving from winter to summer, which in turn can be used as a reference when studying the seasonal changes in nutrient levels in the wetland. Roosting congregations are generally higher in winter, when birds group together for warmth, reduced predation risk, and increased foraging efficiency under colder and resource-scarce conditions (Wang & Chu 2021).

The aim of this study was to perform an initial quantification of the effects of the large exotic roost community on water and

soil at the pest exclusion fenced area of Rotopiko wetland, which could be used as a reference for future roost management efforts.

Methods

Study site

Rotopiko complex is surrounded by agricultural land, dominated by intense pastoral farming (mainly dairy farming and maize production) with some residential land use. Rotopiko-East, the fenced lake, is the smallest of the three peat lakes within the Rotopiko wetland. The area was fenced off to create a mammalian pest-free sanctuary, which comprises a lake and two kahikatea forest stands of different ages, referred to as mature (c. 400 years old) and young (c. 35 years old). A drain receiving outflow from adjacent land and the surrounding catchment crosses the forest for approximately 200 m before discharging into the lake. During the winter and spring seasons (June until November) the drain usually fills with water, but it dries off during the summer until late autumn.

Study design

To perform an initial quantification of the effects of the roosting birds on Rotopiko's nutrient levels, water and soil samples were collected and tested on three sampling dates in mid-August 2021, mid-November 2021 and at the end of February 2022. These specific dates were chosen to capture the expected differences in bird numbers inside the fenced area as the season transitioned from winter to summer.

The monitoring stations were selected based on the known bird population numbers (Sandoval et al. 2023),

and their hypothesised impact on nutrient levels, as well as site accessibility. Water samples were collected from five monitoring stations, numbered 1 to 5 (Fig. 1). The drain flowing through the young forest stand within the fenced area most affected by roosting was tested in three distinct locations: as it enters the fenced area (Station 3), a halfway point (Station 2), and just before the lake inlet (Station 1). Water samples were also taken from the lake within the fenced area (fenced lake, Rotopiko-East; Station 4), and from the non-fenced lake (Rotopiko-South; Station 5). Soil samples were collected from three monitoring stations, A to C (Fig. 1). Station A was located within the fenced area in the mature forest stand, which is less populated by roosting birds than the young forest stand, possibly due to the lower density of the canopy. Station B was located in the young forest stand within the fenced area, which has the highest roosting congregation estimates. Station C was located in a very similar young forest stand, located about 20 metres outside of the fenced area, which is not affected by roosting birds (Karen Denyer, National Wetland Trust, pers. comm.)

Sampling and testing

Water samples were collected from surface water (10 cm depth) at each monitoring station in triplicate, taken about 2 m apart along the drain or lake shoreline. For microbiological testing, 250 mL sterile containers were used. All samples were transported directly to Hill Laboratories, Hamilton, for testing.

Water samples were tested for nutrients, in the form of nitrates and nitrites, ammonia nitrogen, organic nitrogenous compounds (total Kjeldahl nitrogen [TKN]), total nitrogen (Total N), and total phosphorus (Total P). Samples were also tested for microbiological data, including total coliforms and, from November onwards, specifically for faecal coliforms and

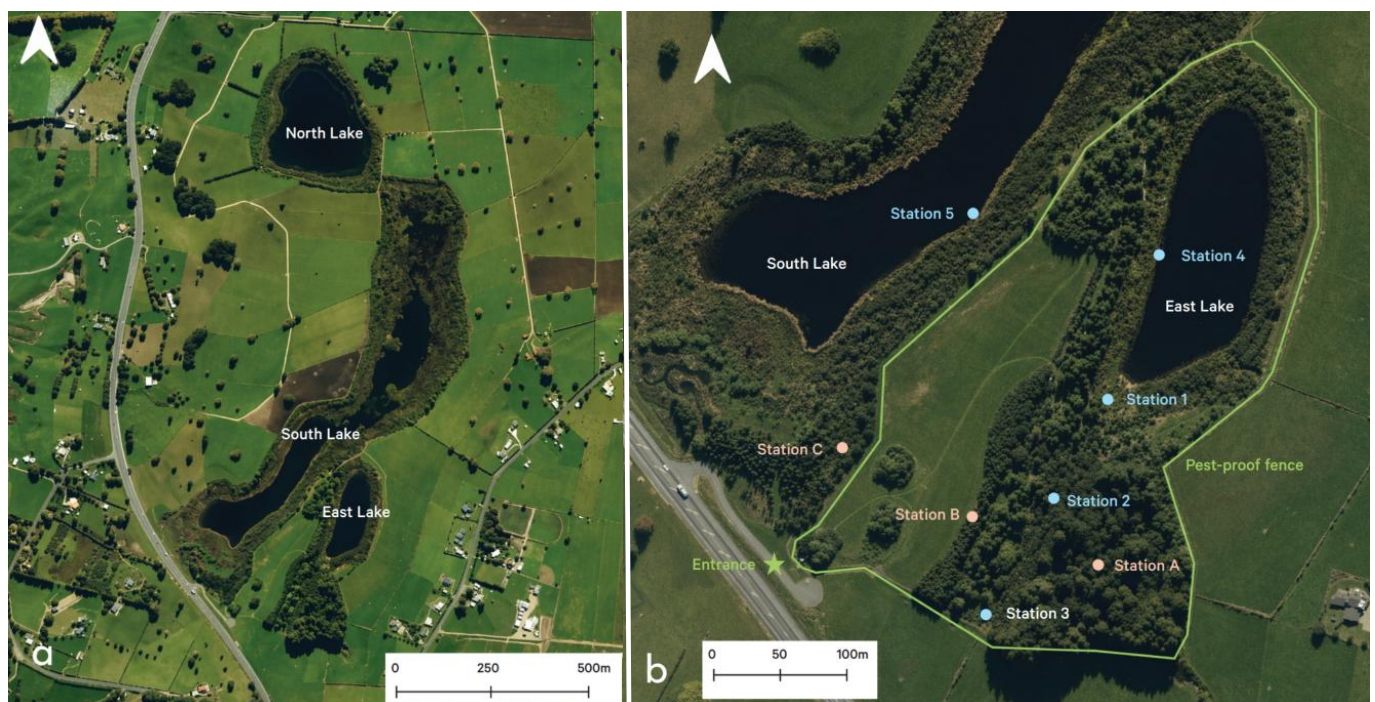


Figure 1. (a). Site plan of Rotopiko Wetland, Waikato, New Zealand, showing three interconnected lakes and surrounding areas. (b) Close-up map of East Lake with the boundaries of the pest-proof fence, and an indication of water and soil testing stations. Water testing: Station 1 – drain at the fenced lake inlet, Station 2 – drain halfway through the fenced area, Station 3 – drain at the entry to the fenced area, Station 4 – fenced lake, Station 5 – non-fenced lake. Soil Testing: Station A – mature kahikatea region within fenced area, Station B – young kahikatea region within fenced area, Station C – young kahikatea region outside of fenced area.

E. coli. Since the drain running through the fenced area dries off during the summer period, there were no measurements taken at Stations 1, 2, and 3 in February.

Soil samples were collected through pit sampling at each monitoring station in triplicate, along a transect line, with sample points spaced 2 m apart, at a consistent depth of 20 cm. This depth was selected to capture the mixed layer of topsoil, where immobile nutrients such as phosphorus and potassium predominantly accumulate. All samples were transported directly to Hill Laboratories, Hamilton, for testing.

Soil samples were prepared and tested for nutrients including total nitrogen (Total N), phosphorus (Total P), and potassium (Total K). Potassium was included in the soil testing due to its typically high concentrations in bird droppings (Hilde et al. 2005), and its relatively limited mobility in soil compared to nitrogen compounds such as NO_3^- (da Silva et al. 2018). Consequently, potassium was not analysed in water samples.

The summary of testing methods performed by Hill Laboratories for water and soil analysis is presented in Table 1.

Data analysis

The results of this study are primarily descriptive and are presented as bar graphs with standard deviation error bars to illustrate the variation among triplicate samples. Due to small sample sizes and limited data, inferential statistical analyses such as hypothesis testing were not conducted. The reduced statistical power restricted the ability to detect significant differences or patterns with confidence. Therefore, the findings should be interpreted as indicative trends rather than definitive conclusions.

Results

Water analysis

Water analysis showed a clear contrast between the fenced lake and non-fenced lake for all tested parameters (Total N, nitrate/nitrite, TKN), as presented in Fig. 2. While fenced lake (Station 4) readings for total nitrogen and nitrite/nitrate were measured at 9.7 and 7.0 g m^{-3} respectively in August 2021, they reached their highest levels in November 2021 at 17.5 and 14.4 g m^{-3} , before sharply declining to 3.2 and 1.0 g m^{-3} in February 2022. The corresponding values in the non-fenced lake (Station 5) remained relatively constant at around 1 and 0 g m^{-3} during the investigation period.

In the drain, the concentration of all nitrogen indicators steadily increased towards the lake inlet point, as seen in Fig. 2. For example, in August 2021, the concentration of total nitrogen went from 1.9 g m^{-3} at the fenced area entry point (Station 3) to 38.3 g m^{-3} at the halfway point (Station 2), before reaching the highest concentration of 53.7 g m^{-3} at the lake inlet (Station 1). Total nitrogen showed the same patterns during November 2021 but with lower concentrations of 0.7 g m^{-3} , 16.6 g m^{-3} , and 30.7 g m^{-3} respectively.

Total phosphorus measurements (Fig. 3), show that the phosphorus concentration in the fenced lake (Station 4) increased from 0.04 g m^{-3} in August 2021 to 0.15 g m^{-3} in November 2021 and then decreased back to 0.03 g m^{-3} in February 2022. The non-fenced lake (Station 5) showed less variation, with levels at 0.02 to 0.04 g m^{-3} across seasons.

Within the fenced area, in the drain flowing through the young forest stand towards the lake, the phosphorus at the

Table 1. Summary of testing methods performed by Hill Laboratories for water and soil samples analysis.

Water testing	
Parameter	Analytical method
Nitrates and nitrites (g m^{-3})	Total oxidised nitrogen. Automated cadmium reduction, flow injection analyser. APHA 4500- NO_3^- I (modified) 23 rd ed. 2017 (Rice et al. 2017a)
Total Kjeldahl nitrogen (TKN) (g m^{-3})	Total Kjeldahl digestion, phenol/hypochlorite colorimetry Discrete analyser. APHA 4500-Norg D (modified) 4500 NH3 F (modified) 23 rd ed. 2017 (Rice et al. 2017b)
Total N (g m^{-3})	Calculated as a sum of TKN + nitrates and nitrites. APHA 4500-NH ₃ F 23 rd ed. 2017 (Rice et al. 2017c)
Total P (g m^{-3})	Total phosphorus digestion, automated ascorbic acid colorimetry. Flow injection analyser. APHA 4500-P H 23 rd ed. 2017 (Rice et al. 2017d)
Total coliforms (MPN 100 mL^{-1})	MPN count using Colilert 18 (incubated at 35 °C for 18 hours) and 97 wells. APHA 9223 B 23 rd ed. 2017 (Rice et al. 2017e)
Faecal coliforms (cfu 100 mL^{-1})	Membrane filtration, count on mFC agar, incubated at 44.5 °C for 22 hours, confirmation. APHA 9222 D 23 rd ed. 2017 (Rice et al. 2017f)
<i>E. coli</i> (cfu 100 mL^{-1})	Membrane filtration, count on mFC agar, incubated at 44.5°C for 22 hours, MUG confirmation. APHA 9222 I 23 rd ed. 2017. (Rice et al. 2017g)
Soil testing	
Parameter	Analytical method
Soil preparation	Air dried at 35–40 °C overnight (residual moisture typically 4%) and crushed to pass through a 2 mm screen
Total N ([%, recalculated to ppm)	Determined by NIR, calibration based on total N by Dumas combustion
Total P (ppm)	Nitric/hydrochloric digestion based on US EPA 200.2 (Martin et al. 1994) followed by ICP-OES
Total K (ppm)	Nitric/hydrochloric digestion based on US EPA 200.2 (Martin et al. 1994) followed by ICP-OES

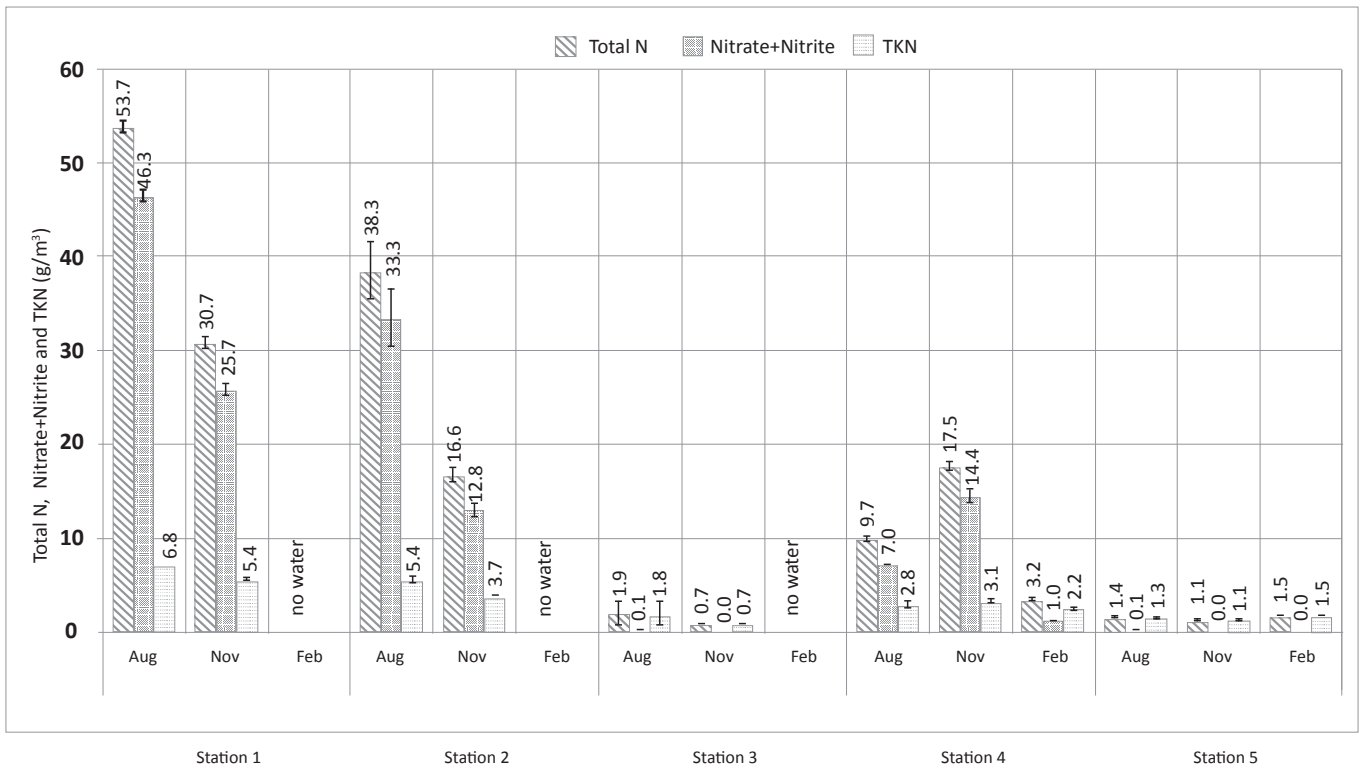


Figure 2. Chemical water analysis of nitrogen levels at Rotopiko Wetland, Waikato, New Zealand from August 2021 to February 2022. Levels of total nitrogen, nitrate and nitrite and TKN (total Kjeldahl nitrogen) (± 1 SD, $n = 3$) were measured in the drain flowing through the fenced area at the drain entry point (Station 3), halfway point (Station 2), and at the fenced lake inlet (Station 1), and in the fenced lake (Station 4) and the non-fenced lake (Station 5). The distance from Station 1 to Station 3 is approximately 200 m.

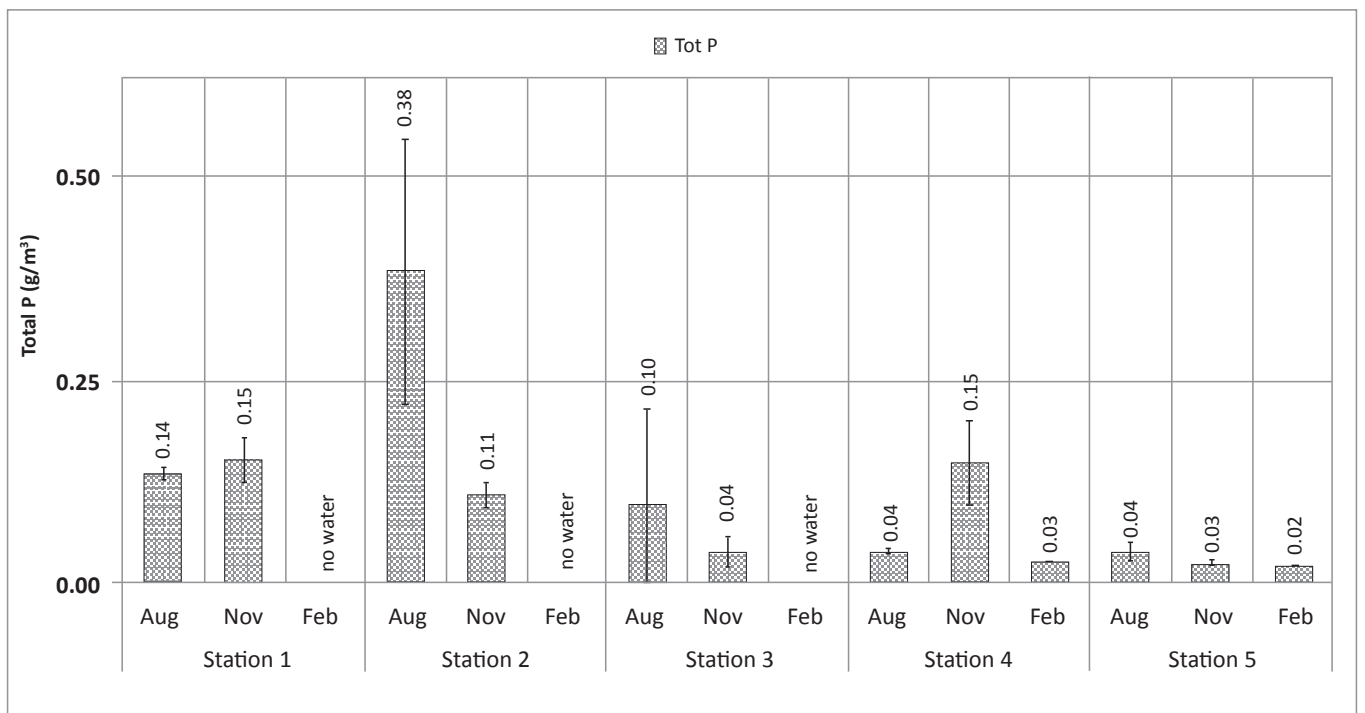


Figure 3. Chemical water analysis of phosphorus levels at Rotopiko Wetland, Waikato, New Zealand from August 2021 to February 2022. Levels of total phosphorous (± 1 SD, $n = 3$) were measured in the drain flowing through the fenced area at the drain entry point (Station 3), halfway point (Station 2), and at the fenced lake inlet (Station 1), and in the fenced lake (Station 4) and the non-fenced lake (Station 5). The distance from Station 1 to Station 3 is approximately 200 m.

lake inlet (Station 1) was measured at 0.14 g m^{-3} in August 2021 and 0.15 g m^{-3} in November 2021. At the halfway point (Station 2) these measurements were 0.38 and 0.11 g m^{-3} respectively and, finally, at the fenced area entry point (Station 3) the phosphorus measurements were 0.10 and 0.04 g m^{-3} (Fig. 3).

Soil analysis

The soil analysis (Fig. 4) shows results for the tested parameters at two locations inside the fenced area (Stations A and B) and one location outside the fenced area (Station C). For nitrogen, the concentration was consistently high at stations within the fenced area throughout the measurement period, ranging between 9400 to 11 800 ppm, as compared to 3030 to 3430 ppm outside of the fenced area (Station C).

The concentration of phosphorous within the fenced area was within a range of 1316 to 2150 ppm at the mature forest stand (Station A), and 5777 to 7197 at the young forest stand (Station B). Readings outside the fenced area (Station C) remained low and relatively unchanged across seasons at between 360 to 381 ppm.

For potassium, the concentrations at Stations A and C varied between 408 to 503 ppm, with only one higher reading of 719 ppm at Station A in August 2021. The young forest stand within the fenced area (Station B) recorded the highest levels of potassium, at 1337 ppm in August 2021, 963 ppm in November 2021, and 1205 ppm in February 2022.

Microbiological analysis

Within the fenced area, the initial microbiological testing of

water included only the number of total coliforms; however, these readings exceeded the upper detection limit of $24200 \text{ MPN } 100 \text{ mL}^{-1}$ in the drain water at Stations 1 and 2 (data not shown). For this reason, further testing targeted specifically faecal coliforms and *E. coli* counts, as presented in Fig. 5. It was observed that in November 2021 the numbers of faecal coliforms and *E. coli* were very low at the start of the drain (Station 3), with 63 and $40 \text{ cfu } 100 \text{ mL}^{-1}$ respectively. At the halfway point (Station 2) these numbers increased respectively to 36 667 and $28\,000 \text{ cfu } 100 \text{ mL}^{-1}$, and then to 47 333 and $36\,667 \text{ cfu } 100 \text{ mL}^{-1}$ at the lake inlet (Station 1). The levels of faecal coliforms and *E. coli* in the fenced lake (Station 4) were higher than in the non-fenced lake (Station 5), with corresponding readings of 1933 and $1733 \text{ cfu } 100 \text{ mL}^{-1}$ versus only $53 \text{ cfu } 100 \text{ mL}^{-1}$ for both factors. During the summer, the levels of coliforms and *E. coli* in both lakes were relatively similar, with the non-fenced lake having slightly higher readings.

Discussion

Water analysis trends

Despite the limited understanding of groundwater transport occurring in the Rotopiko catchment area, it is believed that shallow groundwater movements and surface run-off are major sources of nitrogen in the lake (Song et al. 2022; Liu et al. 2024). The amount of total nitrogen shows a steady increase in the concentration along the drain running through the fenced area (Fig. 2): the lake inlet (Station 1) had a 28-fold higher level of total nitrogen (1.9 vs. 53.7 g m^{-3}) than the

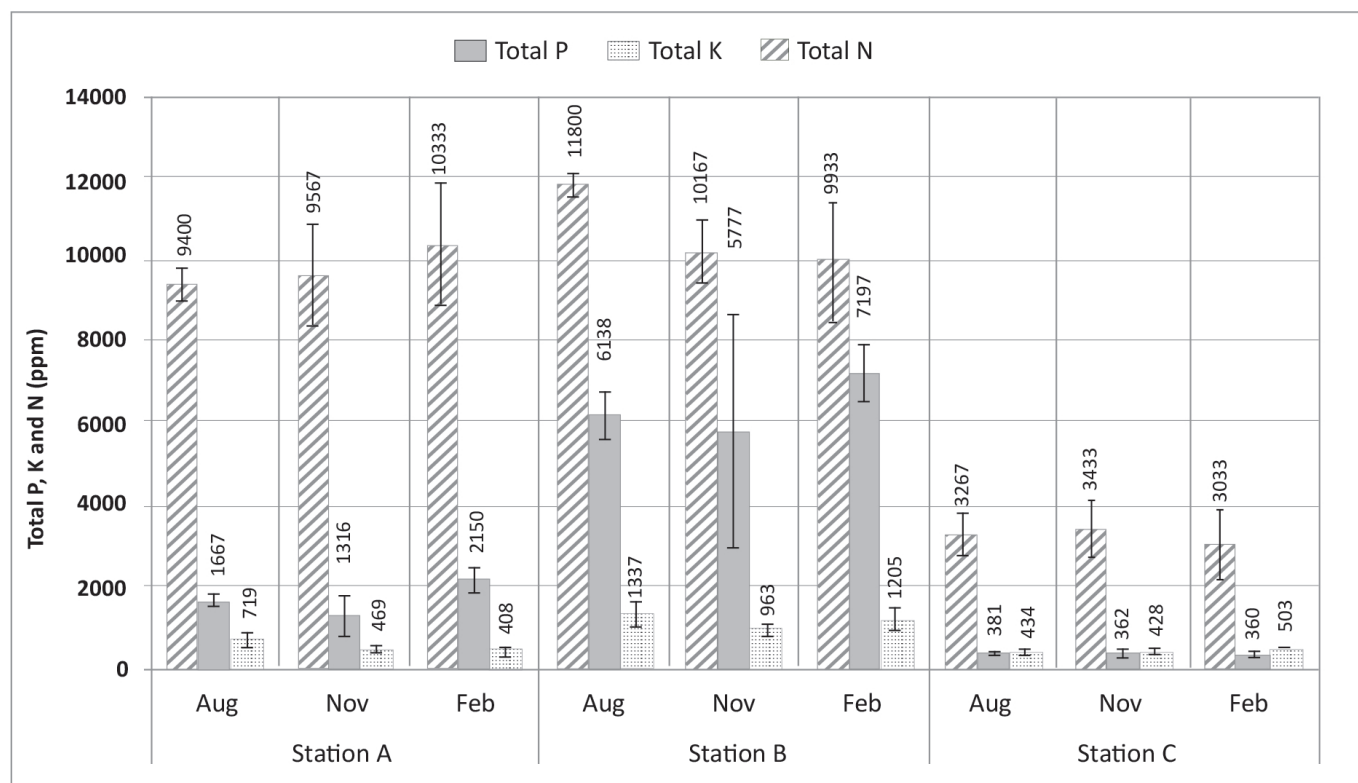


Figure 4. Chemical soil analysis at Rotopiko Wetland, Waikato, New Zealand from August 2021 to February 2022. Levels of total phosphorous (P), total potassium (K) and total nitrogen (N) ($\pm 1 \text{ SD}$, $n = 3$) were measured from subsurface samples (c. 20 cm) at the mature forest stand inside the fenced area (Station A), young forest stand inside the fenced area (Station B), and young forest stand outside the fenced area (Station C).

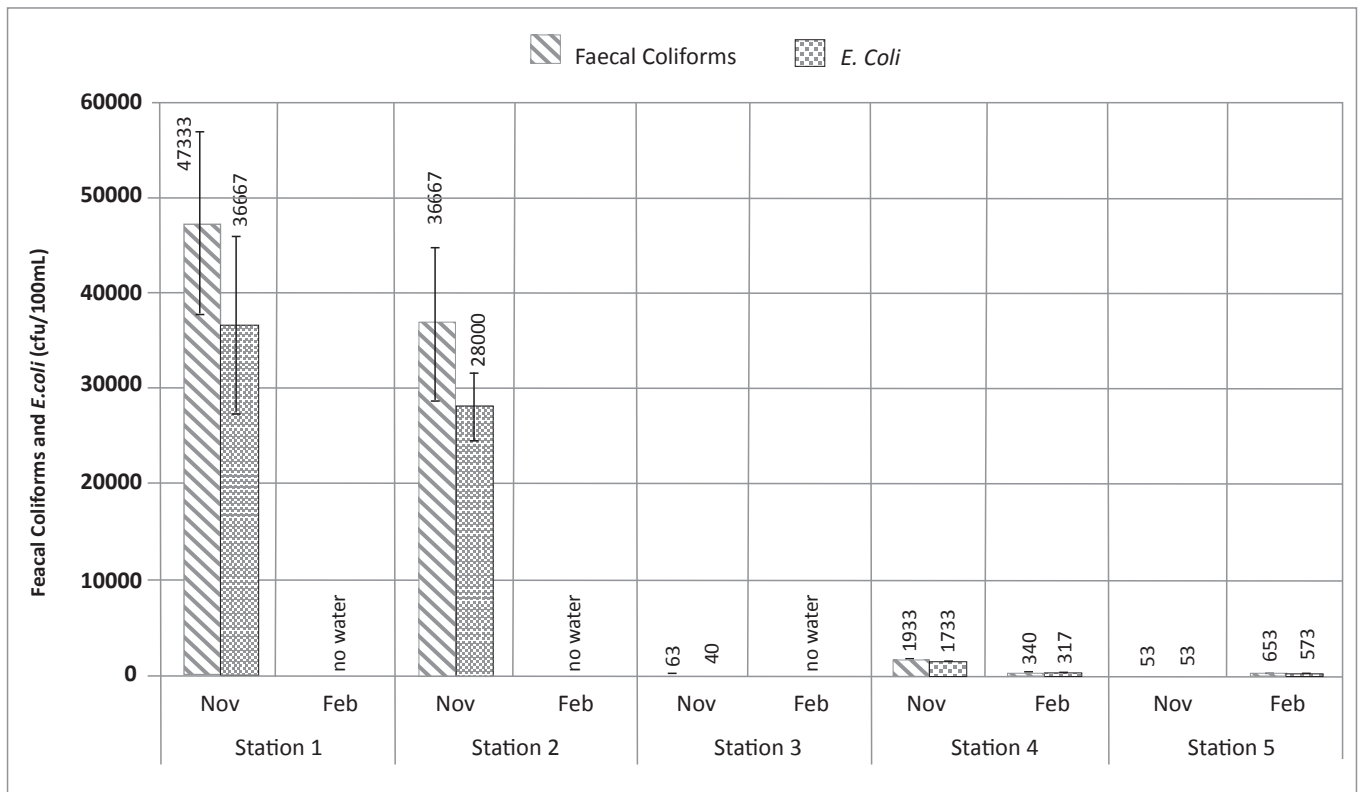


Figure 5. Microbiological water analysis at Rotopiko Wetland, Waikato, New Zealand from November 2021 to February 2022. Levels of faecal coliforms and *Escherichia coli* (± 1 SD, $n = 3$) were measured in the drain flowing through the fenced area at the drain entry point (Station 3), halfway point (Station 2), and at the fenced lake inlet (Station 1), and in the fenced lake (Station 4) and the non-fenced lake (Station 5). The length of the drain, from Station 1 to Station 3, is approximately 200 m.

entry point (Station 3) in August 2021, and a 44-fold higher level (0.7 vs. 30.7 g m^{-3}) in November 2021. This clearly indicates that water entering the fenced area has lower levels of nutrients, and most of the total nitrogen likely comes as run-off from the fenced area surrounding the drain and direct input of guano from the trees above the drain. The total nitrogen carried through the drain also has a direct influence on its concentration in the lake inside of the fenced area. In August 2021, the total nitrogen in the fenced lake was seven times higher than the total nitrogen in the non-fenced lake (9.7 vs. 1.4 g m^{-3}) (Fig. 2). Interestingly, the data recorded in November 2021 shows an even higher difference between the lakes, with a 16 times higher reading for the fenced lake (17.5 vs. 1.1 g m^{-3}). This is despite the levels of total nitrogen in the drain having approximately halved compared with August 2021 readings. This suggests that the total nitrogen had likely accumulated in the fenced lake during the wet seasons, before declining to similar levels to the non-fenced lake levels over the summer. Both lakes exceed the National Policy Statement for Freshwater Management bottom-line threshold (total N > 0.8 g m^{-3}) for ecosystem health in lakes (Ministry for the Environment 2020). These findings suggest that the whole wetland complex is likely affected by agricultural activities in the surrounding catchment, while the fenced lake is subject to additional impact from bird roosting.

Further analysis of nitrogen showed that at the drain entry point (Station 3) the low amount of total nitrogen in water was present mostly in the form of ammonium cations (TKN). The same was observed in the non-fenced lake, across all seasons.

In contrast, the areas strongly affected by roosting (drain areas captured by Station 1 and 2, as well as the fenced lake at Station 5) had between 77 to 87% of total nitrogen in the form of nitrates and nitrites in August and November 2021. This ratio changed over the summer, with the fenced lake nitrate/nitrite percentage falling to 31% of the total nitrogen. This indicates that organic nitrogen was mineralised, forming ammonium cations. These cations underwent rapid nitrification, first converting them into nitrites and then into nitrates, likely due to oxidation or the presence of nitrifying bacteria in the sediments and water column itself. During the summer months, nitrate and nitrite concentrations in the fenced lake declined, likely due to denitrification, which converts nitrates into their gaseous form, and to microbial activities, with the algae and bacteria using excess nutrients in the water (Li et al. 2024). In addition, roosting activity during the summer is at its lowest level, and the dry conditions limit nitrogen run-off to the lake.

It has been reported that increases in phosphorus can also lead to higher nitrification rates (DeForest et al. 2020). The concentration of phosphorus in the water was higher at all sites inside the fenced area than outside of it, especially at Stations 1 and 2, although the patterns are not as clear as for nitrogen (Fig. 3). Unlike nitrogen, which is more immediately transported to the lake via groundwater, phosphorus can accumulate and remain stored in soils for decades and may be slowly released to the water and lake depending on sediment transport, peat soil processes (Geurts et al. 2010), and redox conditions in shallow groundwater. In peat catchment soils, like in Rotopiko, shallow groundwater (under oxic conditions) can be highly retentive of

phosphorus because of its naturally high iron content (Geurts et al. 2010). The high standard deviation between the samples might be caused by higher sediment content in some of the samples, which could influence the result, despite the filtration performed directly before testing. Inclusion of suspended solids or water clarity measurements would have provided valuable additional insights during this testing.

While the phosphorus in the non-fenced lake was consistently low, the fenced lake and its catchment area are affected by the phosphorus content from the guano input. The phosphorus stored in soil and sediments may be released to the lake under certain localised conditions (Geurts et al. 2010). Because the lake is so enriched in nitrate, exceeding nitrate toxicity criteria on some occasions (Ministry for the Environment 2020), any further enrichment of phosphorus may result in algal blooms and push the lake to a stratification shift, disrupting the natural balance of the lake.

Soil analysis trends

For soil analysis, it was expected that soil at Station B was exposed to more faeces than Station A, as young kahikatea trees accommodate more roosting birds, as compared to the mature forest stand, due to denser canopies (Sandoval et al. 2023). Station C, located in a young forest stand outside of the fenced area, is not affected by roosting. The average value for the total nitrogen during the measurement period was 9790 ppm at Station A, 10 630 ppm at Station B, and 3240 ppm at Station C (Fig. 4), thus confirming our expectations. Most nitrogen compounds are highly mobile in soil and can easily leach into the groundwater, hence both areas affected by roosting birds have high, but relatively similar readings, while total nitrogen at Station C is three times lower than within the fenced area.

For total phosphorous in soil, the highest concentrations over the sampling period were recorded at Station B, with an average of 6385.7 ppm, which is almost 4 times higher than at Station A (1711 ppm), and almost 20 times higher than at Station C (367.7) (Fig. 4). These findings show a strong compounding effect of phosphorous dependent on the bird population at each location. Phosphorus binds to soil and accumulates over time, showing a strong correlation with the investigated bird congregation within each location (Sandoval et al. 2023).

The average potassium concentration at Station B (1168.3 ppm) appears to be higher than the average concentration of potassium at Stations A (532 ppm) and C (455 ppm) (Fig. 4). This finding might indicate a correlation between the bird congregation and potassium concentration, though the dependence is not as well defined as for phosphorous. Peat soils have a low capacity to retain potassium due to low clay content, making it more susceptible to leaching (Krishnan et al. 2021).

Microbiological water analysis trends

The microbiological analysis shows a strong trend of an increasing number of faecal coliforms and *E. coli* along the drain (Fig. 5). The concentration of faecal coliforms at the lake inlet is 750 times higher than in the water entering the fenced area, while the *E. coli* number was 915 times higher. The number of faecal coliforms and *E. coli* in the fenced lake was over 30 times higher than in the non-fenced lake during November 2021 testing, while the numbers were similar during the February 2022 testing.

Conclusions

Our results show that elevated concentrations of nutrients and bacteria are present in areas affected by roosting bird guano, highlighting the potential ecological impacts of such inputs. However, the limited statistical power reduces confidence in detecting definitive patterns or significant differences, so the results should be viewed as preliminary trends rather than conclusive evidence.

A range of mitigation measures were put in place in the Rotopiko pest exclusion fenced area since this research was completed, which are currently being assessed, with promising initial observations. We recommend that the potential effects of roosting bird population increases are considered before introducing pest exclusion fenced areas within farming regions, particularly in areas with dense canopy cover. The pest exclusion fences in such locations may bring unintended consequences which can be detrimental to the overall ecosystem.

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Additional information and declarations

Ethics: Ethics approval was not required for this research.

Data and code availability: The data from this article is available by request. There is no code associated with this article.

Author contributions: AM: conceptualisation, methodology, data analysis, fieldwork, writing. NS: conceptualisation, methodology, data analysis, fieldwork, writing. SG: conceptualisation, methodology, data analysis, fieldwork, writing. SF: conceptualisation, methodology, data analysis, fieldwork, writing.

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Conflicts of interest: The authors declare no conflicts of interest.

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