CHARACTERISATION AND POTENTIAL OPTIMISATION OF SEEPAGE WETLANDS FOR NITRATE MITIGATION IN NEW ZEALAND HILL COUNTRY

A thesis presented in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

in

Soil Science

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

SCHOOL OF AGRICULTURE AND ENVIRONMENT

Palmerston North, New Zealand

Suha Sanwar

2023

To empty stomachs, fighting souls, struggling people.

And to Ilma, Sanwar, Ferdowsi, Taha.

Please consider the factors listed below in your assessment of the work.

This statement has been prepared by the candidate's supervisor in consultation with the student and has been endorsed by the relevant Head of Academic Unit.

Student Name:	Suha Sanwar	ID Number:	[redacted]
Supervisor Name:	Dr. Lucy Burkitt	Date:	31-Mar-20
Thesis title:	CHARACTERISATION AND POTENTIAL OPTIMISATION OF SEEPAGE WETLANDS FOR NITRATE MITIGATION IN NEW ZEALAND HILL COUNTRY		

Considerations to be taken into account. Note: This statement will remain in the final copy of the thesis which will be available from the Massey University Library following the examination process. [*Enter key considerations here for the examiners. This can include but is not limited to change of scope, scale, topic, focus; limitations in relation to data collection, access to necessary literature or archival materials, laboratories, field sites; disruptions as a result of lockdown and various alert levels, medical or health considerations etc*]

This PhD thesis has been significantly impacted by the Covid-19 pandemic. These impacts have been both in terms of compromised data collection and also depleted research time due to not being able to physically progress experiments for weeks to months at a time. Specifically, there have been periods of time where data are missing or site sampling was not conducted at the same time of year, as the student was unable to travel to field sites to collect data. Government restrictions on movement and access to university facilities also impacted on the setup and sampling of the sediment column study.

As a result of concerns for future movement restrictions and depleted research time, the final experiment was converted to a laboratory scale study, whereas the original plan was to conduct this experimental work in situ, which would have strengthened the practical application of this thesis.

It is also important to be aware that the student was unable to attend any in person conferences and therefore was denied the opportunity to interact with academic peers and get feedback on her research.

Approved by DRC 10/Feb/2021
DRC 21/02/03
Updated September 2021

Confidential for Examiners Only: [*Please enter any other considerations which are confidential for examiners only and should not be placed in the final thesis version submitted to Library following the examination process]*

The student was granted a 3-month suspension (1 Jan-31 Mar 2022) and has also needed to extend her PhD enrolment for 1 year, but this was based on health and family challenges, rather than Covid-19 specifically.

Signed, confirming this is a fair reflection of the impact of Covid-19 on this research.

Student	SUHA SANWAR	Digitally signed by SUHA SANWAR Date: 2023.04.04 15:56:12 +12'00'
Supervisor	Lucy Burkitt	Digitally signed by Lucy Burkitt DN: cn=Lucy Burkitt, c=NZ, o=Massey University, ou=Farmed Landscapes Research Centre, email=L.Burkitt@massey.ac.nz Date: 2023.04.04 16:09:01 +12'00'
Head of Academic Unit (or nominee)	Diane Pearson	Digitally signed by Diane Pearson Date: 2023.04.05 10:06:24 +12'00'

Approved by DRC 10/Feb/2021
DRC 21/02/03
Updated September 2021

ABSTRACT

Diffuse nitrate (NO_3^-) loss to pastoral waterways in hill country headwater catchments is a water quality concern in many countries with pasture-dependent economies, including New Zealand (NZ). Sheep and beef farming is the dominant land use in NZ hill country which are often located in headwater catchments. As these primary industries strive toward production growth to meet global demand for meat exports, this agricultural intensification will introduce more NO_3^- to its waterways. This contrasts with the recently enacted National Policy Statement for Freshwater Management 2020 (NPS-FM) which recognises the significance and calls for the protection of small wetlands in recognition of their ecosystem services including nutrient regulation, water quality improvement as well as associated social well-being. Nitrate mitigation in low-order streams in pastoral headwater catchments are important due to their proportionally large catchment coverage and major contribution to the national NO_3^- load to NZ rivers. Seepage wetlands in hill country landscapes can be a N-sink and, therefore, is a potentially cost-effective and natural NO_3^--mitigation tool for improved water quality from the pastoral headwater catchments.

Seepage wetlands are features that occur along low-order streams in the low gradient of hill country landscapes. Their organic matter-rich sediment, saturated conditions and locations at the convergence of surface and subsurface NO_3^- rich flow pathways make seepage wetlands a unique landscape feature in terms of NO_3^- reduction via denitrification processes. However, denitrification is spatially and temporally variable as the process is influenced by the wetland sediment and hydrological properties. Several studies have demonstrated that seepage wetlands can be a potential NO_3^- sink and have quantified high sediment denitrification capacities in individual wetlands. However, variations in sediment and denitrification properties across a range of wetlands and a comprehensive study of seepage wetland hydrological characteristics that influence NO_3^- attenuation have not been undertaken, particularly in pastoral hill country landscapes in NZ.

This thesis has examined the spatial variabilities of seepage wetland denitrification and the denitrification-influencing sediment properties across four hill country seepage

wetlands within the Horizons Regional Council administrative boundary in NZ. The spatial gradients of sediment properties were examined vertically (at 15 cm depth intervals) and horizontally (within- and between- wetlands) in seepage wetland sites. Sediment physicochemical (water content (WC), pH, Eh) and chemical properties (dissolved organic carbon (DOC), NO_3^-, NH_4^+, %total carbon or %TC, %total nitrogen or %TN, C:N, dissolved Fe^{2+} and dissolved Mn^{2+}) and sediment denitrification enzyme activity (DEA), that represents sediment denitrification capacity, were quantified. The DEA values were highest at the surface depths across all wetland sites. Based on the wide range (560-5371 µg N_2O-N kg^{-1} DS h^{-1}) and distinctive surface DEA values, the seepage wetland study sites were categorised into high-performing H-DEA (>3000 µg N_2O-N kg^{-1} DS h^{-1}) and comparatively low-performing L-DEA (<1000 µg N_2O-N kg^{-1} DS h^{-1}) sites. The H-DEA sites measured 7 to 10 times higher surface DEA values compared to the L-DEA sites. Spatial variability of denitrification in seepage wetlands was mainly driven by sediment WC, NO_3^-, %TC, %TN, C:N, dissolved Fe^{2+} and dissolved Mn^{2+} ($p \leq 0.05$). The H-DEA site measured high WC (78%) which was above the threshold for denitrification and high sediment NO_3^- (15.9-18.5 mg NO_3-N/kg DS), in contrast to the L-DEA sites (WC 39.8-37.4%, 2.5-3.97 mg NO_3-N/kg DS). The heterogeneity of WC explained the heterogeneous distribution of DEA within the individual L-DEA sites. The sediment properties accounted for only 58-73% of the overall spatial variability in DEA, suggesting that additional wetland characteristics such as wetland hydrology, could have an important influence on denitrification in seepage wetlands.

The seepage wetland hydrology and associated NO_3^- removal were characterised in detail at one of the L-DEA sites located on Tuapaka farm. During the hydrological characterisation, streamflow discharge and water quality were monitored at inflow and outflow for a 2-year period (June 2019-May 2021). Shallow groundwater quality was monitored at the 0.5, 1 and 1.5 m depths at the inflow, midflow and outflow positions in the wetland for a 1.5-year period (November 2019-May 2021). The seepage wetlands site demonstrated a stream inflow-dominated hydrology (83-87%) with small seepage contributions (8-14%) to the seepage wetland hydrology. Precipitation was found to be the major hydrological and associated NO_3^- removal (means attenuation) driver in the seepage wetland site. The seepage wetland was found an overall NO_3^- sink that on an average removed 23% of the annual NO_3^- inflow. Compared to the stream inflow (<0.03 mg NO_3-N/L), higher shallow groundwater NO_3^- concentrations (<0.11 mg NO_3-N/L) suggests that seepage is potentially an important NO_3^- source in these wetlands. High flow conditions, high winter precipitation and direct grazing during low flow periods are potentially major NO_3^- loss hot moments. In contrast, initial rapid infiltration at the onset of high precipitation events in early winter and spring and dissipated flow conditions highlighted opportunities for NO_3^- attenuation in the wetland and were identified as major NO_3^- removal hot moments. An overall dissipated flow condition driven by seasonally equivalent precipitation (22% of annual precipitation in winter) facilitated considerably higher annual NO_3^- removal of 40.8% (2.78 kg NO_3-N) in the wetland in year 2, in contrast to very low NO_3^- removal (0.3%, ~0.02 kg NO_3-N) under an erratic annual precipitation distribution (38% of annual precipitation in winter) in year 1. These findings suggest there is scope to improve NO_3^- removal by optimising flow conditions to slow flow in seepage wetlands to minimise NO_3^- loss during NO_3^- loss hot moments.

In a follow-up laboratory-scale seepage wetland intact sediment column experiment, the effectiveness of diffuse flow, via subsurface outflow, was investigated for the optimisation of the wetland NO_3^- removal. During the experiment, the flow intervention altered the NO_3^- reduction-constraints observed in the preceding hydrological study and facilitated anaerobic conditions conducive to denitrification to capitalise on the sediment denitrification capacity, which was quantified during the preceding seepage wetland sediment characterisation study. The flow intervention involved vertical downwelling of NO_3^- rich (5 mg NO_3-N/L) pastoral surface runoff and subsequent horizontal discharge through a subsurface sediment column depth of 15 cm depth, collected from the Tuapaka seepage wetland site. The effectiveness of the subsurface drainage intervention for NO_3^- removal was assessed by monitoring the subsurface outflow water quality. The study showed that flow intervention achieved 50-96% NO_3^- removal from NO_3^- rich surface runoff. Based on the observations from the column study, two separate optimal operational HRTs of 2 and 13 hr are recommended to achieve large NO_3^- removal (50% from NO_3^- input of 5 mg NO_3-N/L) in a short period of time and large reduction in NO_3^- concentration at the outflow (<0.15 mg NO_3-N/L), respectively. The reasonably short period of HRT for such high NO_3^- removal efficiency

(50-96%) supports the potential for the application of subsurface outflow intervention as a practical *in-situ* NO_3^- mitigation strategy, which warrants further research. This study also acknowledges the associated technical limitations of translating the laboratory-based findings to the field scale and recommends future studies to overcome these research limitations including high sediment compressions during intact sediment column samplings from the field, for example.

The thesis not only demonstrates a flow intervention strategy to improve NO_3^- mitigation via flow regulation in seepage wetlands, but also guides future management by identifying the potential seepage wetland hot spots in the landscape (chapter 3) and the NO_3^- removal hot moments in the wetlands (chapter 4) and also by recommending necessary HRTs for flow intervention (chapter 5).

In summary, this thesis has generated a robust dataset that improves our understanding of seepage wetland characteristics and their influences on NO_3^- removal at spatial and temporal scales. From an application perspective, this research provides new knowledge as to 'where', 'when' and 'how' seepage wetlands can be targeted to enhance their role in NO_3^- mitigations in hill country landscapes. This information has the potential to accelerate the integration of seepage wetlands into the toolbox of NO_3^- management strategies that could be used at a farm scale to improve water quality leaving NZ pastoral headwater catchments.

ACKNOWLEDGEMENT

In the name of Allah, the most gracious, the most merciful Who has made this incredible journey happen.

I convey my sincere gratitude to my chief supervisor Dr. Lucy Burkitt for technical guidance, patience, encouragement, ready-to-hear and prompt-to-reply mindset throughout this research. I am intrigued by the seemingly simple scientific input and encouragement from my co-supervisor Dr. Ranvir Singh which have facilitated overcoming several important technical hurdles during this thesis. The critical science insights received from my other respected co-supervisors Dr. Mike Bretherton and Dr. Gabor Kereszturi are surely going to influence my future science enquiries.

I acknowledge everyone I am aware of, and beyond, who have contributed to this PhD journey directly and indirectly with support in all forms. I thank the School of Agriculture and Environment for logistic support. I am grateful to David Feek and Bob Toes for constructing equipment used in this research and for the immense assistance in the field. I am indebted for their mammoth hard work that included several occasions when sediment cores got stuck in the ground or parts of the sampling equipment broke down while standing knee-deep in mud and water in the middle of nowhere. My sincere acknowledgements to Dr. Peter Bishop for sharing technical expertise and tips and tricks in the laboratory. Thanks to Ian Furkert and Ross Wallace in the soils lab. I also acknowledge the Riddet Institute and Landcare Research for letting us use their laboratory facilities from time to time. I acknowledge Professor Mark Bebbington, Senior tutor Matt Irwin and Dr. Anja Moebis for their scientific inputs and cooperation.

Thanks to the landowners for logistic supports and access to the wetland sites.

Thanks for the inspiration provided by my colleagues and peers as we shared space and time and experiences. Federico Duranovich, Fernando Avendano, Stefania Romero Ramirez, Neha Jha, May Hedges, Gautam Shrestha, Ratna Hidayati, Akinson Tumbure, Khadija Malik, Grace Chibuike, Amira Mahmoud, Eli Shokri, Micaela Moll – thank you. I acknowledge the administrative support from Sharon Wright, Fiona Bardell, and many others for smoothly running the process without me even being aware of it.

I appreciate all the scholarships I have received before and during this PhD journey including the scholarships from the School of Agriculture and Environment at Massey University and the Helen E Akers doctoral scholarship, Peer Scholars Award, Covid-19 Bursary and additional funding support from the Farmed Landscapes Research Centre without which this research would be next to impossible for me to continue.

Ilma, my daughter, has been a great support. I can only pray for the very best for my parents and brother for their extraordinary support.

To every farmer who grows food for us, every worker whose shoulder this 'civilisation' stands upon, every stomach that meaninglessly goes empty as we watch on or choose not to while we make or are 'forced' to mind our ways, I take the opportunity to pay my tribute here, though that little helps. To the free-spirited birds out there who have consistently refused to accept the unacceptable - I send my love. Power to you.

This research has seen a global pandemic, inflation, and peoples' lives taking 180-degree turns in every part of this planet while I was trying to fathom the meaning of what we do as I kept doing it. Even that thought process has been a privilege, I assume.

Finally, back to where it all started, wetlands! Driven by a pure passion for wetlands, I landed in this part of the world. I sincerely hope this research contributes to safeguarding these little seepage wetlands hidden between the hill bends, so that they can continue to perform gigantic cycles of nutrient transformations that never cease to amaze me.

All praises to the Almighty.

RELEVANT PUBLICATIONS AND PRESENTATIONS

Publications to date

Conference/workshop proceedings

- Sanwar, S., Burkitt, L., Singh, R., Bretherton, M. and Kereszturi, G. (2022). Hill country wetland hydrological characterisation for attenuation. In: Ada*ptive Strategies for Future Farming, Occasional Report No. 34, 11p*. (Eds. C.L. Christensen, D.J. Horne and R. Singh). Palmerston North, New Zealand: Fertilizer and Lime Research Centre, Massey University.

Presentations

- Sanwar, S., Burkitt, L., Singh, R., Bretherton, M. and Kereszturi, G. (2023). “Characterisation of hydrology and denitrification potential in hill country seepage wetlands.” Presented at the 35th Annual FLRC Workshop 2023, 8-10 February 2023, Palmerston North, New Zealand.

- Sanwar, S., Burkitt, L., Singh, R., Bretherton, M. and Kereszturi, G. (2022). “Hill country wetland hydrological characterisation for attenuation.” Presented at the 34th Annual FLRC Workshop 2023, 9-11 February 2022, Palmerston North, New Zealand.

- Sanwar, S., Burkitt, L., Singh, R., Bretherton, M. and Kereszturi, G. (2021). “Sediment properties & denitrification capacity in seepage wetlands in pastoral hill country.” Presented at the 11th INTECOL International Wetlands Conference, 10-15 October 2021, Christchurch, New Zealand.

- Sanwar, S., Burkitt, L., Singh, R., Bretherton, M. and Kereszturi, G. (2021). “Hill country seepage wetland sediment characteristics and their denitrification capacity (New Zealand).” Presented at the Soils: Investigating in our futures-2021 Joint Conference Soil Science Australia and The New Zealand Society of Soil Science, 27 June-2 July 2021, Cairns, Australia.

- Sanwar, S., Burkitt, L., Singh, R., Bretherton, M. and Kereszturi, G. (2019). “Mitigating nitrate and phosphorus loss by optimizing the function of pastoral hill country seepage wetlands.” Poster presented at the SAE Symposium 2021, October 2019, Massey University, Palmerston North, New Zealand.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

C	Carbon
d	Day
DEA	Denitrification Enzyme Activity
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DNRA	Dissimilatory nitrate reduction to ammonium
DS	Dry sediment
Fe^{2+}	Available or dissolved ferrous (II) ion
h	Hour
ha	Hectare
HRT	Hydraulic residence time
GIS	Geographic Information System
GHG	Greenhouse gas
HRC	Horizons Regional Council
L	Litre
LUC	Land Use Capability
rpm	Rotation per minute
Mn^{2+}	Available or dissolved manganese (II) ion
N	Nitrogen
N_2O	Nitrous oxide
N_2	Dinitrogen gas
NIWA	National Institute of Water and Atmospheric Research
NO_3^-	Nitrate
NZ	New Zealand
NPS-FM	National Policy Statement for Freshwater Management
P	Phosphorus
OM	Organic Matter
rpm	Rotation per minute
TC	Total Carbon
TN	Total Nitrogen
WC	Water content
WFPS	Water Filled Pore Space

Chapter 1: Introduction

Nitrate (NO_3^-) loss from agriculture is a global water quality concern. Nitrate is a well-recognised water quality issue in New Zealand (NZ) as in many other countries with pastoral production as major land use (Bijay & Craswell, 2021; Chaubey et al., 2007; MfE & Stats NZ, 2021). In NZ, hill country pastoral beef and sheep farming is a major contributor to the national economy, but the headwater catchments that support these industries are also a source of NO_3^- to waterbodies (Lambert et al., 1985; McDowell et al., 2017).

Hill country is an undulating landform with slopes>15° and altitudes of <1,000 m above sea level. Hill country is a unique landscape feature of NZ that comprises 40% (10 million ha) of the country's land area (Blaschke et al., 1992). The elevated slopes, low soil fertility and high erodibility of hill country make it less well suited to cultivation. This has led to pastoral production for sheep and beef, which involves extensive grazing management and low nitrogen (N) input, to become the dominant land use in the hill country in NZ (8,765,000 ha and 63% of the NZ agricultural area) (Beef & Lamb New Zealand, 2022; MfE & Stats NZ, 2021). Pastoral products including sheep (49%) and beef (27%), comprise nearly half the NZ's foreign export income (48.3%), earning nearly 86 billion NZD in the 2022 fiscal year (Beef & Lamb New Zealand, 2022). Between 2011-2021, although sheep numbers and the total area under sheep farming have decreased, the intensity and livestock productivity of the industry have grown (37% and 118% increases in lamb weight and lamb production, respectively) (Beef & Lamb New Zealand, 2022; MfE & Stats NZ, 2021). Research shows without N-fertiliser use, the low-fertility hill country soil is able to support only 25% of the current pastoral crop production (Beef & Lamb New Zealand, 2022). As NZ works towards a domestic target of doubling the year 2012's primary industry export value by 2025 (Grimmond et al., 2014; Meat Industry Association, 2021; New Zealand Government, 2012), agricultural intensification will no doubt lead to additional farm N-input and increased risk of NO_3^- loss to waterways (Ledgard, 2001; Parfitt et al., 2012).

Due to the proportionally large N-contribution of low-order streams to rivers downstream, NO_3^- mitigation in low-order streams that drain hill country in headwater catchments has great potential for catchment water quality improvement (Alexander et al., 2007; Alexander et al., 2000; Dodds & Oakes, 2008). In NZ, low-order (1-3) streams make up 87% of total catchment areas nationally (McDowell et al., 2017). The disperse drainage networks allow low-order streams to intercept NO_3^- from large pastoral catchment areas (Hill, 2000; Lowrance et al., 1997). The large stream surface area to stream volume ratio of the low-order streams also favour greater NO_3^- uptake and retention (Peterson et al., 2001), in contrast to the channelised and deep cutting, high-order streams with smaller catchment coverage and smaller stream surface area to stream volume ratio (due to their deeper depths), in general.

Expected agricultural intensification in hill country pastoral headwater catchments is likely to increase N contribution to waterbodies. Nitrate in water can promote algal growth, groundwater contamination and hypoxia in global oceans (Boesch et al., 2001; Böhlke et al., 2004; Park et al., 2010). These environmental pressures put NZ, as a leading global meat exporter, in a challenging position as the primary industry targets global business growth without compromising water quality (MfE & MPI, 2018). In order to improve water quality, NZ has introduced a National Policy Statement for Freshwater Management 2020 (NPS-FM) (MfE, 2022b). The amended NPS-FM 2022 mandates the recognition of *Te Mana o Te Wai* (vitality of clean, healthy water) in any catchment and freshwater farm planning, which places the health of water as a core guiding principle (MfE, 2022b). The NPS-FM calls for the protection, management and enhancement of all freshwater bodies, including natural inland small wetlands (as small as <0.05 ha) (MfE, 2022b). Furthermore, the NPS-FM recognises the contribution of small wetlands to water quality and freshwater ecology and binds the regional government bodies (the regional councils) to set threshold limits of NO_3^- concentration from catchments and develop scientific knowledge on and the delineation of small wetlands that could include seepage wetlands in pastoral hill country landscapes (MfE, 2022b).

Seepage wetlands are natural, small, valley-bottom wetland features that frequently occur in the hill country landscape (Johnson & Gerbeaux, 2004; Rutherford et al., 2018). Like wetlands in general, seepage wetlands are analogous to sponges and are capable of retaining large water volumes within the porous wetland matrix that comprises soft, loose and unconsolidated sediment substrates (Sorrell & Gerbeaux, 2004). Their organic matter (OM)-rich sediments, saturated and reducing conditions make these small wetlands biogeochemical hotspots for redox reactions such as denitrification-based NO_3^- reduction processes (Burgin et al., 2010; Hill et al., 2000; McClain et al., 2003).

Seepage wetlands are sustained by hillslope hydrology and are fed by groundwater and low-order streams (Davie, 2004; Johnson & Gerbeaux, 2004). Being located at the low gradients in the pastoral hill country landscapes, seepage wetlands are at the convergence of surface and subsurface NO_3^- flow pathways from the surrounding uphill pastures (Anderson et al., 2015). These sediment and hydrological features enhance the NO_3^- removal capacities of seepage wetlands via denitrification, compared to the surrounding landscape features (Chibuike et al., 2019; Collins et al., 2005). This means seepage wetlands offer a natural and cost-effective edge-of-field opportunity to mitigate diffuse N loss to pastoral hill country waterways (Dodd et al., 2016; Goeller et al., 2020; McKergow et al., 2016).

Denitrification is a NO_3^- reduction process facilitated by microbes under oxygen-limited conditions. Nitrate is reduced to nitrous oxide (N_2O) and atmospheric inert dinitrogen gas (N_2). Due to the scope for environmentally safe and relatively permanent NO_3^- removal in the form of N_2 from soils, denitrification in wetlands offers the potential for NO_3^- mitigation in agricultural catchments (Barton et al., 1999). Contrasting denitrification, the other NO_3^- removal processes e.g. via plant uptake, or dissimilatory NO_3^- reduction to ammonium (DNRA) are temporary in the sense that NO_3^- can return to the soil upon plant and microbial decay. This advantage makes denitrification a preferred NO_3^- treatment strategy applied in natural and artificial wetlands (Carstensen et al., 2019). However, wetland denitrification is highly site-specific and temporally variable in natural environments regulated by wetland characteristics. Therefore, in order to achieve effective NO_3^- mitigation outcomes, the use of wetlands for treatment purposes depends on a sound knowledge of the key drivers that influence the wetland denitrification process.

Wetland sediment properties influence NO_3^- reduction process and are 'proximal regulators' (factors in the immediate environment that have direct effect) in denitrification. Wetland denitrification is influenced by sediment physicochemical (redox condition, pH, water content) and chemical (electron donors e.g. dissolved organic carbon, dissolved iron and dissolved manganese, and NO_3^- input) properties (Attard et al., 2011; Bai et al., 2017; Deng et al., 2020; Knowles, 1982; Ribas et al., 2017; Saggar et al., 2013; Sirivedhin & Gray, 2006; Wall et al., 2005; Wang et al., 2021). In general, a saturated, reducing and N and labile carbon-rich sediment environment enhances denitrification. Due to their strong relationship to denitrification, sediment characterisation is often used to explain denitrification variations in wetlands (Christensen et al., 1990; Hayakawa et al., 2012; Wallenstein et al., 2006; Wu et al., 2021). Sediment characterisation is also useful to understand the influence of 'distal regulators' (soil type, climate and land-use practice) on denitrification at spatial and temporal scales larger than the proximal regulators (Groffman et al., 1987; Martínez-Espinosa et al., 2021; Saggar et al., 2013). This is because, at the macro-scale, sediment physicochemical and chemical properties are derivatives of landscape properties such as baserock, topography, geographic position and land use (Groffman et al., 1987; Vidon & Hill, 2004b; Vidon & Smith, 2007). At a micro-scale, sediment properties as proximal regulators define the microbial niche, including denitrifier population composition and abundance (Liu et al., 2018; Martínez-Espinosa et al., 2021; Veraart et al., 2017; Xiong et al., 2017). The sediment analytical techniques are also comparatively convenient, compared to those associated with microbial analysis or investigations into landscape variables. Overall, the ability of sediment properties to represent denitrification-influencing parameters at multiple scales make wetland sediment characterisation a powerful and recommended assessment tool for the spatial gradient assessment of sediment denitrification capacity (Anderson et al., 2015; Han et al., 2017; Martínez-Espinosa et al., 2021; Xiong et al., 2015; Zhong et al., 2016).

Wetland hydrology has a regulatory role in denitrification-based NO_3^- removal processes in wetlands. Hydrological properties that influence NO_3^- removal in wetlands are hydrological connectivity, hydraulic conductivity, residence time and NO_3^- loading (Giles et al., 2012; Hill, 2019). Hydrology in headwater catchments is particularly dynamic because it is dominated by local hydrology, primarily precipitation in the immediate area, and has little connection to regional hydrology or groundwater systems (Denver et al., 2014; Winter, 1983, 1999). Highly dynamic quickflow (dominated by surface runoff which quickly flows to streams) is characterised by steep hydrographs and short residence times, which are typical of hill catchment hydrology (Davie, 2004). Dynamic hydrology in the wetland means fluctuations in NO_3^- load, residence time and perturbations in the NO_3^- reducing environment, resulting in overall fluctuating wetland NO_3^- removal efficiencies (Burt & Pinay, 2005; Peters et al., 2011). A wetlands' own hydrogeological features also influence NO_3^- attenuation. For example, seepage wetland studies have measured a wide range in NO_3^- removal from 24±5% from surface flow (Rutherford & Nguyen, 2004) to >90% removal in shallow groundwater (Burns & Nguyen, 2002), which implies that seepage wetland NO_3^- removal efficiency can vary with NO_3^- transport pathways. Cooper (1990) has observed high NO_3^- attenuation in the drainage that passes through organic soil, in contrast to the drainage through mineral soil in a wetland. Bypass of the wetland NO_3^- attenuation function altogether is also possible when there is a lack of hydrological connectivity, or NO_3^- in subsurface drainage bypasses the overlying OM-rich wetland sediment and directly discharges to streams (Burt et al., 1999; Puckett, 2004).

Overall, NO_3^- reduction processes in wetlands are a function of their sediment and hydrological properties that drive site-specific and temporally variable denitrification (Giles et al., 2012; Ranalli & Macalady, 2010; Rivett et al., 2008). A clear understanding of these sediment and hydrological properties and their fluxes is vital for the successful application and maximisation of wetland NO_3^- mitigation function.

1.1 Rationale of the thesis

In NZ, although it has been decades since the NO_3^- mitigation potential of seepage wetlands was first identified in pastoral hill country landscapes (Cooper & Cooke, 1984; Smith, 1989), sediment and hydrological properties remain poorly characterised in these wetlands. Separate seepage wetland studies have estimated the longitudinal and temporal gradients in NO_3^- removal in surface flow and shallow groundwater (Burns & Nguyen, 2002; Rutherford & Nguyen, 2004), quantified sediment denitrification capacities (Burns & Nguyen, 2002; Chibuike et al., 2019) and identified several associated sediment and hydrological factors (Chibuike et al., 2019; Nguyen et al., 1999a). Those studies have generated some evidence that seepage wetlands can be NO_3^- sink and have established the context that these small wetlands could be useful for NO_3^- mitigation in hill country landscapes. However, these previous studies are small in number and mostly single-site based. These studies mostly covered short temporal scales (<6 months) and have rarely attempted to characterise wetland properties (i.e., sediment and hydrology), NO_3^- attenuation and its temporal fluxes). The hydrological investigations rarely used an integrated approach, except the studies by Cooke and Dons (1988) and Cooke and Cooper (1988). Due to these research limitations, a comprehensive understanding of the seepage wetland features from the perspective of NO_3^- attenuation is currently lacking.

Also, most of the previous seepage wetlands studies in NZ assumed wetlands as primarily groundwater-fed and were mostly conducted in a dairy pastoral landscape (Hughes et al., 2013; McKergow et al., 2012; Zaman et al., 2008). Dairy pastures are generally located in low and flat lands with dense drainage networks and receive higher N-input, which contrasts with those in hill country pastoral headwater catchments. Thus, the transfer of the currently available knowledge of seepage wetlands to the wider context of hill country landscapes is difficult, and less likely to be accurate. Comprehensive knowledge of the seepage wetland features influencing NO_3^- attenuation in the context of hill country pastoral land use, is absent in the current literature. This knowledge gap precludes the use of seepage wetlands as a NO_3^- mitigation tool in catchment-based NO_3^- management.

Addressing this research gap is urgent because the NPS-FM requires a focus on 'on-farm' strategies to reduce nutrient loss from hill country farms. Considering the expansive and difficult terrain associated with hill country farms, harnessing the potential of NO_3^- removal via denitrification in natural and existing wetlands has great potential for NO_3^- mitigation in the landscape (Cooke & Cooper, 1988). However, harnessing this benefit requires sound knowledge of the seepage wetland system to begin with. Rigorous investigation into the function and capacity of seepage wetlands to improve water quality is necessary for both science and policy contexts, as uncertainty surrounding the actual environmental benefits of introduced technologies is one of the top three barriers to new technology adoption, as observed among sheep and beef farmers (Monaghan et al., 2007). A full-scale seepage wetland characterisation across the landscape will, 1) allow the generation of seepage wetland NO_3^- removal estimates, and 2) guide farm practices as to ‘where‘ in the landscape, ‘when‘ and ‘how’ to effectively use these natural wetlands for NO_3^- mitigation.

In this context, this thesis will characterise pastoral hill country seepage wetlands from the perspective of NO_3^- mitigation. To achieve this goal, this thesis will investigate the sediment properties that influence denitrification-based NO_3^- reduction in seepage wetlands and assess the spatial gradients with an intention to guide ‘where’ in the landscape the seepage wetlands potentially are more effective for NO_3^- mitigation. Secondly, this thesis will characterise the wetland hydrology and NO_3^- removal in a seepage wetland over various temporal scales, from daily to annual. This approach is expected to help identify temporal opportunities for enhanced NO_3^- attenuation in the wetlands and guide as to ‘when’ management interventions in seepage wetlands may be more effective. Finally, the thesis will examine the potential of a flow intervention using a laboratory-based intact sediment column study in order to improve our understanding of ‘how’ to potentially optimise NO_3^- removal in seepage wetlands.

1.2 Research hypothesis

1. Denitrification capacity varies with the spatial gradients of sediment properties in pastoral hill country seepage wetlands.

2. Nitrate removal is temporally variable in response to seepage wetland hydrology.

3. Nitrate removal from inflow surface runoff can be optimised via subsurface outflow intervention in seepage wetlands.

1.3 Research objectives

This thesis characterises the seepage wetland (sediment and surface and subsurface hydrologic properties) and potentially optimises NO_3^- removal in seepage wetlands in the pastoral hill country landscape. In particular, the objectives of the thesis are to:

1. Characterise the spatial gradients in denitrification and the denitrification-influencing sediment physicochemical and chemical properties in multiple seepage wetlands.

2. Characterise the seepage wetland hydrology and NO_3^- removal in a seepage wetland.

3. Optimise NO_3^- removal from pastoral surface runoff via a subsurface outflow intervention in a laboratory-based simulated seepage wetland, using an intact sediment column experiment.

1.4 Thesis outline

This thesis consists of six chapters which are briefly described as follows:

Chapter 1 introduces the context and rationale of the research. It states the research hypotheses and research objectives, before presenting an outline of the thesis.

Chapter 2 reviews the literature to provide a broad theoretical framework within which the thesis is conducted. The chapter begins by introducing hill country and hill country seepage wetland features. The chapter presents a conceptual framework of the hill country hydrology-NO_3^- transport continuum which is necessary to set the scene for subsequent experimental chapters. This chapter reviews wetland sediment and hydrological influences on NO_3^- removal with a particular focus on NZ pastoral hill country seepage wetland literature in order to identify the major research gaps. To facilitate the application of the thesis, available flow interventions for NO_3^- treatment in wetlands are also reviewed.

Chapters 3-5 address the specific objectives of this thesis. These experimental chapters have been constructed in the format of independent articles and therefore, may contain some repetitions, for example, in the introduction sections in individual chapters. **Chapter 3** explores the relationships between the spatial gradients of wetland sediment properties and sediment denitrification capacities in pastoral hill country seepage wetlands. **Chapter 4** examines the temporal dynamics in NO_3^- removal in response to the wetland surface and subsurface hydrology and identifies temporal opportunities for improved NO_3^- attenuation in seepage wetlands. **Chapter 5** examines the effectiveness of a subsurface outflow intervention to optimise NO_3^- removal from NO_3^- rich inflow, representing pastoral surface runoff, in a laboratory-based wetland sediment column study.

Finally, **chapter 6** discusses key findings from chapters 3-5 and explores the contribution of this research in the context of how seepage wetlands can be used for NO_3^- mitigation and improving water quality from NZ hill country beef and sheep farms. To this end, a geospatial analysis was conducted to scale up the research findings to the landscape scale and explore the broader application of this research.

Chapter 2: Literature review

2.1 Introduction

This literature review provides a theoretical framework, within which this thesis will be broadly conducted. The review focuses on the influence of seepage wetland properties and function on nitrate (NO_3^-) mitigation in pastoral hill country landscapes and therefore other water quality parameters will not be considered. The broad theoretical framework provides an umbrella under which a more detailed literature review associated with each experimental chapter is conducted.

This review starts by introducing the hill country landscape setting in which the thesis is conducted. It examines definitions, physiography, hill country farming practice and its NO_3^- water quality implications. Based on the New Zealand (NZ) wetland classification, major seepage wetland features are reviewed including seepage wetland sediment and vegetation and seepage wetland formation processes. Sediment physicochemical and chemical properties and their influences on denitrification in wetlands are explored, due to their influences on NO_3^- removal. Denitrification-based NO_3^- reduction process is also examined in detail particularly due to its scope for complete NO_3^- removal from wetlands via inert dinitrogen gas (N_2). Other NO_3^- reduction processes are only examined where relevant to the thesis. Considering the regulatory role of hydrology in NO_3^- transport and transformation, the hillslope hydrology and its implication for NO_3^- transport to seepage wetlands are then explored.

As the literature review develops, current pastoral hill country seepage wetland literature is examined from the perspective of denitrification-based NO_3^- removal in which the previous studies have been broadly categorised into 1) sediment studies, and 2) hydrological studies conducted in NZ.

This literature review also explores different flow regulation options used for NO_3^- treatment in natural and artificial wetlands in order to explore their potential for enhancing NO_3^- removal in seepage wetlands.

Finally, existing knowledge gaps limit the application of seepage wetlands for NO_3^- mitigation in pastoral hill country landscapes. Based on the identified gaps, the thesis scope is set within which the subsequent experiments are conducted.

2.2 Hill country

2.2.1 Definition

Hill country is defined as all Class 5, 6, 7 and 8 land forms from the NZ Land Resource Inventory (NZLRI) with grade D slopes and above (slopes>15°) and located below an altitude of 1,000 m above sea level (MfE, 2008). Hill country is a unique and prominent landscape feature of NZ that covers 40% (10 million ha) of the country's land area, most (63%) of which is in the North Island (6.3 million ha) while 37% is in the South Island (3.7 million ha) (Basher et al., 2008; Blaschke et al., 1992).

Hill country in NZ is a part of the chain of mountains that runs roughly south-west to north-east along the length of the country, which is in continuous tectonic uplifting and associated volcanic activities. Hill country soils are relatively young and soil order in hill country mostly consists of steepland variants of Cambisols and Regosols (Hodgson et al., 2019).

2.2.2 Physiographic features

In general, key physiographic features of hill country include 1) difficult terrain with undulating to steep slopes, 2) low fertility soil formed on thin and young parent material, 3) diverse microclimates driven by large variations in slope, aspect, soil types and vegetation, 4) rapid downslope movement of contaminants (e.g. sediment, N, P), and 5) a high degree of variability in microtopography (due to local lithologic, climatic variability, grazing management practices) with a high sensitivity to erosions (Basher et al., 2008; Jones et al., 2008; Kemp & Lopez, 2016).

2.2.3 Major land use features

The key physiographic features, described in Section 2.2.2, limit agricultural activities in hill country to pastoral-based industries supporting sheep and beef production, with approximately 52% of the North Island hill country under sheep and beef production

(Beef & Lamb New Zealand, 2022). However, this pastoral land use comes with severe environmental risks of erosion and moderate nutrient loss (N and P) that contribute to poor water quality (Dodd et al., 2016; Lambert et al., 1985; Mackay, 2008).

2.2.4 Nutrient loss risk

Pastoral activities, including grazing management, introduce anthropogenic NO_3^- into the N-cycle and influence N-loss from hill country landscapes. For N-supply, NZ hill country pasture primarily depends on biological fixation by legumes (largely white clover) (Parfitt et al., 2006). However, soil N-deficits are also addressed by N-fertiliser application (Gillingham et al., 2007). If N-fertiliser is used, it is commonly applied at a rate of ~35 kg/ha/yr and a large fraction of this N remains unutilised as N loss to water or the atmosphere are ~11 kg/ha/yr (Dodd et al., 2016). Animal grazing and resting/camping behaviours influence N distribution in the landscape. Animals prefer camping on flatter slopes which results in nutrient accumulation from animal excreta on these slopes (Kemp & Lopez, 2016) and this nutrient can be transported to wetlands located at the toeslopes of hills, during runoff events. Direct grazing in low-lying wetland areas introduces high NO_3^- concentrations, in urine patches, which readily leaches to subsurface flow (Rogers et al., 2023).

2.2.5 Water quality implications of hill country farming

The natural physiographic (Section 2.2.2) and hydrologic (discussed in detail in Section 2.4) features and processes and land modifications accelerate NO_3^- loss from hill country farms, which is particularly high during winter and spring when evaporation loss is low (Bargh, 1978; Basher et al., 2008; Dodd et al., 2016; McColl, 1979). Modelling in low-order streams (1-3) that commonly flow through hill country in headwater catchments supporting pastoral agriculture, has estimated that these streams contribute 77% of the national NO_3^- loads to water bodies (McDowell et al., 2017). These modelled results are supported by Dodd et al. (2016) who reported that pastoral waterways are the major carriers of NO_3^- from headwater catchments and contribute to poor water quality downstream.

The water quality implication of hill country farming becomes severe in regions with large hill country land area coverage e.g., in the Horizons Regional Council (HRC) jurisdiction which is located in the lower North Island of NZ. Approximately 60% of the Horizons Regional Council's 22 million ha land area is classified as hill country based on Land Use Capability (LUC) classes 6e, 7e and 8e which the HRC classifies as hill country (M Todd, pers. comm.). Although NO_3^- concentrations have improved in half of the water quality monitoring sites in the region over the last 10 years, a large number of sites do not meet the Horizons Regional Council targets (Horizons Regional Council, 2021). Concern around NO_3^- concentrations is also evident nationally, with 54% of the water quality monitoring sites declining in quality between 2001-2020 (Stats NZ, 2022). These trends highlight the need for NO_3^- mitigation options in these hill country landscapes to ensure regional and national freshwater quality targets are achieved.

2.3 Hill country seepage wetlands

2.3.1 Definition

Seepage wetlands are potential NO_3^- sinks in pastoral hill country landscapes (Cooke & Cooper, 1988). Seepage wetlands are naturally occurring, small (often <0.1 ha), natural and valley-bottom wetland features that occur between gullies in NZ hill country (Figure 2-1) (Rutherford & Nguyen, 2004). Hill country seepage wetlands can be both stand-alone or form part of a wetland complex. Seepage wetlands are defined as:

"wetland area on a slope which carries a moderate to steady flow of subsurface water, often also surface water, including water that has percolated to the land surface, the volume being less than that which would be considered as a stream or spring"
(Johnson & Gerbeaux, 2004).

Seepage wetlands tend to have a large surface area to volume ratio, which encourages surface water to flow slowly and interact with the wetland sediments. Considering this important function and based on their landform setting and the water regime (source, level, fluctuation, flow, chemistry, temperature, etc.), seepage wetlands are classified as one of the nine wetland classes in the semi-hierarchical wetland classification proposed for NZ by Johnson and Gerbeaux (2004), modified from Clarkson et al. (2003)

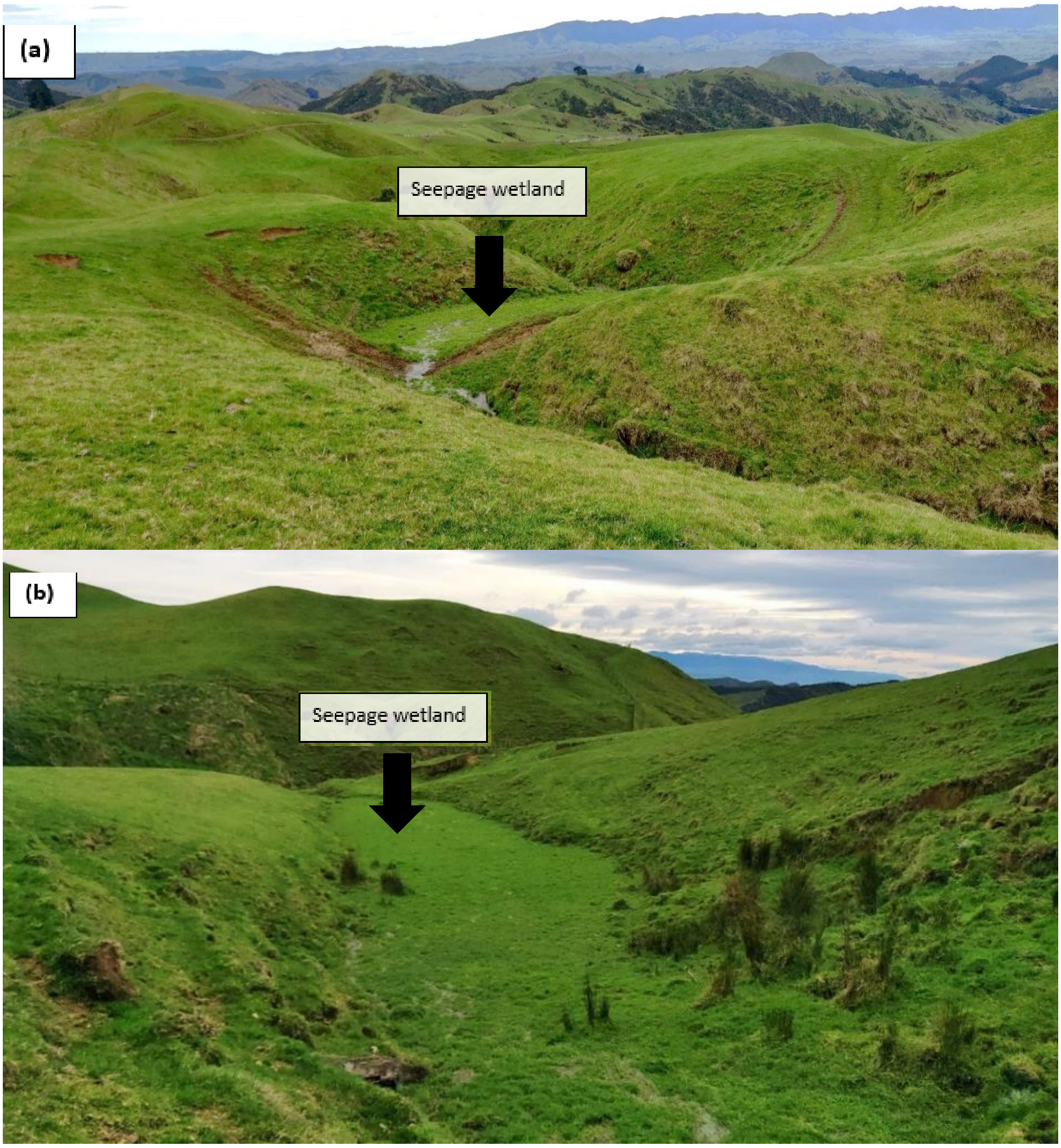


Figure 2-1. (a) An example of a seepage wetland in a pastoral hill country landscape, (b) a close-up view of a seepage wetland.

and Ward et al. (1999). Understanding seepage wetlands' role in nutrient management will increasingly become important as the recent National Policy Statement for Freshwater Management (NPS-FM) calls for the identification, delineation, protection, and management of small wetlands across NZ, in recognition of the importance of small wetlands in water quality improvements.

Terminologies used to describe these wetlands by NZ scientists have been arbitrary. These wetlands were initially referred to as "riparian swales" or "riparian wetlands" in some of the most influential research on this topic (Burns & Nguyen, 2002; Cooper, 1990; Cooper et al., 1995; Matheson et al., 2002; Nguyen & Downes, 1997; Nguyen et al., 1999a; Rutherford & Nguyen, 2004; Rutherford et al., 2009). Their reasons for a cautious use of terminologies was perhaps influenced by the suspicion that shallow groundwater contribution via seepage may have been less dominant, as a proportion of the total hydrological input into the wetland in pastoral hill country wetland studies. In contrast, dairy pastoral wetland studies have more confidently used the term “seepage wetlands” which is likely attributed to the groundwater dominance in the wetland hydrology of those studies (Hughes & Quinn, 2014; McKergow et al., 2016; McKergow et al., 2012). Over the course of time, the term “seepage wetlands” started to be used more universally (Parfitt et al., 2009; Zaman et al., 2008). irrespective of hill country or dairy (Parfitt et al., 2009; Rutherford et al., 2018; Uuemaa et al., 2018). This development is useful because using a single terminology to refer to these valley-bottom wetlands, 1) avoids unnecessary confusion created by using different terminologies for similar wetland systems, 2) facilitates the exchange of knowledge across different landscapes, and 3) allows the seepage wetland science to proliferate. In this context, we choose to use the term “seepage wetlands” throughout the thesis.

2.3.2 Seepage wetland formation process

Seepage wetlands form from the seepage of subsurface flow, which is prevented from further subsurface downward movement due to either the presence of an impermeable geological bedrock, change in slope, or the water table intersecting the bedrock forcing the subsurface flow to “seep” out through hillside seeps and toeslope. The seepage creates a saturated condition in the open valley-bottom floor, often aided by additional input from diffuse stream flow pathways. The saturated condition initiates seepage wetland formation as it slows down organic matter (OM) decomposition and promotes OM accretion, vegetation development and evolution, and eventually, accelerates the seepage wetland formation process. Landslips and slumps, due to forest clearing, can also facilitate wetland formation processes by blocking waterways perpendicularly (Knight, 2013; Uuemaa et al., 2018).

Mineral components of seepage wetland sediment substrates are mainly loess from upland erosion and organic matter accretion on impervious geological material (bedrock, clay, dense till, iron pan). This makes seepage wetland sediments generally shallow in depth (~1 m), which in turn supports a shallow groundwater system (Rutherford & Nguyen, 2004). Sediments may partly possess the upland soil properties they source from, modified over time by the unique hydrologic conditions in the seepage wetlands.

A conceptual diagram in Figure 2-2 shows a typical seepage wetland within a pastoral hill country landscape with major hydrogeological components and nutrient transport processes. Flow channels are typically only active and visible following rainfall events.

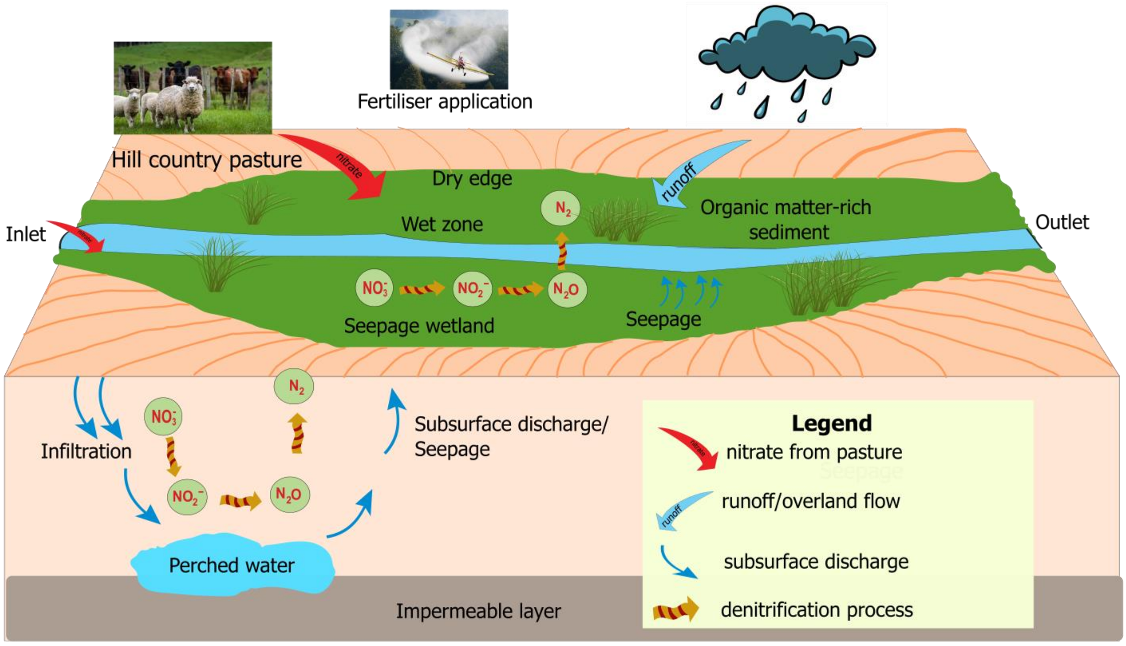


Figure 2-2. The conceptual diagram shows the major seepage wetland features including hydrologic and NO_3^- flow pathways in a pastoral hill country landscape.

2.3.3 Sediment

Seepage wetland sediment substrates range from raw to well-developed mineral soil and peats, with nutrient status and pH ranging from low to high and water table fluctuations between above-ground surface flow to slightly below-ground flow (Johnson & Gerbeaux, 2004). Seepage wetland sediment substrates are generally OM-rich, loose and unconsolidated at the surface depths. Sediment substrates consist of a thick root mat of wetland plants and organic mucks at the surface depths and organic flocks and decaying plant material have been found up to ~20 cm depths (McKergow et al., 2016). Sediment becomes consolidated with increasing depths consisting of mixed organic matter, clay and silt and becomes low in permeability. This results in increased bulk density, decreased porosity and decreased saturated hydraulic conductivities, with overall low water contents in contrast to that at the surface depths (McKergow et al., 2016; Rutherford & Nguyen, 2004). Gley soil which is bluish grey, silty clay, firm and low in permeability often occurs at ~50 cm depth and is indicative of very reduced conditions (Burns & Nguyen, 2002; Rutherford & Nguyen, 2004; Zaman et al., 2008).

2.3.4 Vegetation

Vegetation assists wetland formation processes and plays an important nutrient regulatory function by facilitating physical, chemical and biological nutrient cycling processes (Valkama et al., 2019). As the surface flow enters the wetland, its friction with the wetland vegetation dissipates the flow velocity and facilitates the physical settling of sediment and nutrients. Plants take up these nutrients and recycle them onto the surface of seepage wetlands. In addition, plant root-mediated oxygenated conditions in the rhizosphere have been found to favour denitrification-based NO_3^- reduction (Matheson et al., 2002). This is in contrast to the highly reduced sediment environment in the absence of vegetation, which induces dissimilatory NO_3^- reduction to NH_4^+ instead (Matheson et al., 2002). Vegetation in the seepage wetland is generally of low-lying stature (Johnson and Gerbeaux 2004) and commonly includes sedges, rushes, reeds, tall herbs and scrub (Johnson and Gerbeaux, 2004; McKergow et al., 2016). *Phormium, Carex, Coprosma, Gahnia, Typha, Cordyline, Dacrycarpus, Laurelia, Syzygium* are also key vegetation indicators for seepage wetlands (Johnson and Gerbeaux, 2004). Seepage wetland studies have also reported soft brome (*Bromus hordaceus L.)* with some floating glaucous sweet grasses (*Glyceria declinata Breb.),* soft rush (*Juncus effuses* L.), and wiwi (*Juncus edgariae L*.) (Zaman et al., 2008).

2.3.5 Hydrology and nutrient flow pathways

Seepage wetlands occurring at the valley bottom in the hill country landscape are driven by hillslope hydrology. Surface flow discharge tends to occur via low-order streams (1-3), which can be perennial to ephemeral in nature, and via event-based overland flow (Johnson & Gerbeaux, 2004). Literature shows low-order streams form the larger segments of the total stream lengths and drain larger proportions of the total catchment area (Andersen, 2004). The diffuse drainage network of low-order streams allows these streams to intercept NO_3^- from large hill country farm areas. Low-order streams are the largest NO_3^- load contributors to NZ rivers according to a modelled study (McDowell et al., 2017). As seepage wetlands occur at a low gradient in the hill country landscape (Rutherford & Nguyen, 2004), these wetlands are at the convergence zone of important surface and subsurface flow pathways and associated NO_3^- delivery from the upland pastoral catchment. As NO_3^--rich pastoral surface and subsurface flow reach seepage wetlands, the flow slows and allows NO_3^- laden water to spread across and interact with the large organic matter-rich wetland surface area to facilitate NO_3^--reduction via denitrification. This highlights the seepage wetlands' potential for NO_3^- load reduction from pastoral headwater streams before the pastoral drainage joins the drainage network downstream.

A detailed description of seepage wetland hydrological drivers is provided in the following section.

2.4 A theoretical framework for NO_3^- transport and reducing condition fluxes in hill country seepage wetlands

Dynamic flow condition is a key feature in hillslope hydrology. Major surface and subsurface flow pathways in hill hydrology consist of intermittent to perennial low-order streams, overland flow and seepage of shallow groundwater. The presence of impermeable layers at shallow depths in the wetlands in this landscape means, 1) a lack of connectivity to the regional hydrology and, therefore, an absence of deep groundwater, and 2) surface and shallow subsurface flow pathways connected to a local hydrology that is dynamic in response to precipitation. The dynamic flow condition means high variability in nutrient transport to the biogeochemical environment in seepage wetlands. These features mean that seepage wetland NO_3^- dynamics are influenced by hillslope hydrology-NO_3^- coupling, which is a conceptual framework that links the water flowpaths and the associated NO_3^- transport pathways from the upland areas to the wetlands (Burt & Pinay, 2005). Although Bowden et al. (2001) studied hillslope hydrology and McGlynn et al. (2002) have provided a conceptual framework for flow pathways in a hillslope-headwater wetland-stream in the Glendu catchment on the South Island of NZ, frameworks to assess hill country hydrology and NO_3^- removal functions of seepage wetlands are limited (Cooke & Cooper, 1988; Cooke & Dons, 1988). To aid future seepage wetland NO_3^- removal assessments, a simplified conceptual framework of hillslope hydrology-NO_3^- coupling is proposed here with a focus on NO_3^- delivery to and reducing conditions within seepage wetlands (Figure 2-3).

Since precipitation is the primary water source in hillslope hydrology, storm runoff potentially plays a strong regulatory role in surface and subsurface flow, and the associated NO_3^- transport to hill country seepage wetlands (Burt & Pinay, 2005; McGlynn & McDonnell, 2003a). Precipitation also indirectly influences the between-event subsurface discharges in these catchments with precipitation frequency, precipitation-less periods allowing NO_3^- concentration build up in the wetland soils (Burt & Pinay, 2005). Considering the substantial influence of storm runoff in hillslope hydrology, this theoretical framework explores storm runoff mechanisms from the context of NO_3^- transport to and the potential for NO_3^- reducing conditions within seepage wetlands.

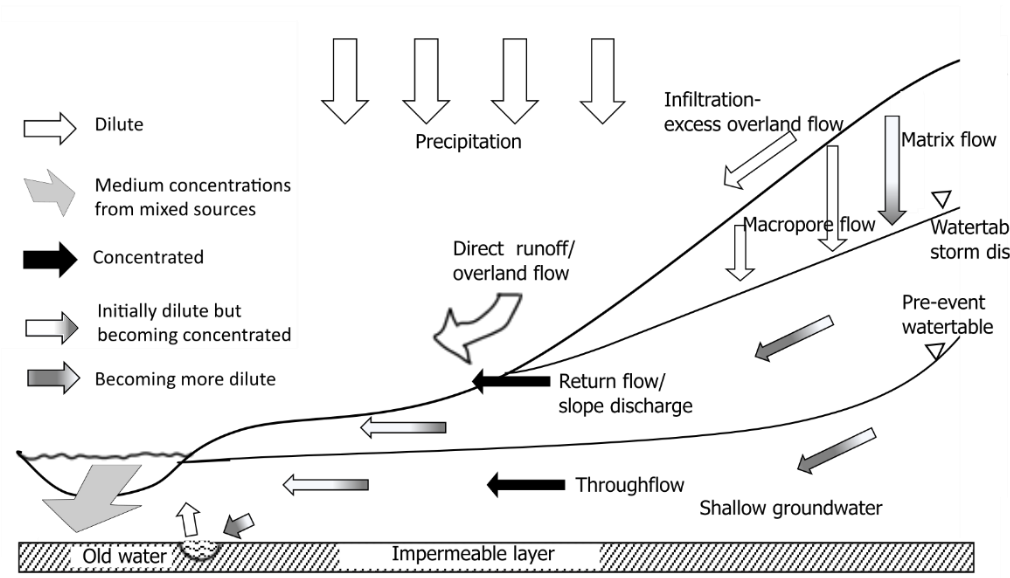


Figure 2-3. Schematic diagram of NO_3^- transport in hillslope based on Burt and Pinay (2005) and McGlynn et al. (2002)

2.4.1 Storm runoff processes

Winter and spring are major precipitation seasons in the temperate climates of NZ. Winter and spring precipitation introduces large hydrological flows to catchments and accelerates nutrient loads that account for most of the annual hill farm NO_3^- loss (Cooke & Dons, 1988; Elliott & Carlson, 2004; McColl, 1979; Quinn & Stroud, 2002). Although annual precipitation is generally equally distributed in many parts of NZ (Hodgson et al., 2019), a study investigating the storm runoff generation in a pastoral hill catchment in NZ reported that most of the storm runoff (65%) occurred in winter (June-August) (Cooke & Dons, 1988). Storm runoff processes include infiltration excess overland flow, saturation excess runoff and subsurface runoff.

2.4.1.1 Infiltration excess overland flow

The infiltration excess runoff, also known as Hortonian overland flow (Horton, 1933), occurs when precipitation intensity exceeds soil infiltration rate and results in overland flow. This type of storm runoff can generate surface flows from limited areas in the catchment that have large flood peaks and low residence time (Burt & Pinay, 2005). Due to its rapidity, infiltration excess runoff gets little opportunity to interact with the soil surface during its transport and often results in low solute concentrations (Burt & Pinay, 2005). Thus infiltration excess runoff is considered 'new' water to the system as it retains the chemical signature of precipitation to an extent (Figure 2-3).

However, there is an established understanding that the non-Hortonian runoff mechanisms of saturation excess and subsurface runoff are more prevalent in the hillslope hydrological context in NZ (Bowden et al., 2001; Cooke & Dons, 1988; Davie, 2004; Dunne & Black, 1970a; McColl & Gibson, 2012). Therefore, this conceptual framework of hillslope hydrology-NO_3^- coupling mainly explores the two non-Hortonian runoff mechanisms.

2.4.1.2 Saturation excess runoff

As winter begins, antecedent dry conditions from the preceding dry season allow the initial winter precipitation to infiltrate the unsaturated soil profile, fill up pore spaces, saturate the soil profile and eventually result in saturation excess overland flow when precipitation continues. Saturation excess runoff is aided by frequent storm events and low evaporative loss over winter and occurs when the soil profile becomes saturated with the infiltrated precipitation (Dunne & Black, 1970b). This type of runoff occurs in areas of permeable soil with moderate hydraulic conductivity, water table fluctuations, low slope angles and in areas of reduced moisture storage (Burt & Pinay, 2005). In terms of chemical properties, saturation excess runoff is a mixture of 'old' soil water and 'new' precipitation water unable to infiltrate (Burt & Pinay, 2005).

2.4.1.3 Subsurface runoff

In the upland subsurface environment, the infiltrated precipitation becomes 'subsurface runoff' as it moves through organic and permeable, unsaturated soil as throughflow and/or via macropore flow. Subsurface runoff can dominate storm hydrographs in areas where deep permeable soil overlies comparatively impermeable layers or bedrock (Davie, 2004; McGlynn et al., 2002).

Subsurface runoff plays a critical role in solute transport and transformation due to its residence time in the sediment matrix that can influence the NO_3^- concentration discharged to wetlands (Burt & Pinay, 2005) (Figure 2-3). During subsurface water movement, the throughflow interacts with the surrounding soil matrix and water can become relatively 'old' and enriched with solute. In contrast, macropore flow is rapid, has less interaction with the soil matrix and results in relatively 'new' water discharge (Figure 2-3).

Traveling further downward, as subsurface runoff reaches the mineral soil, the runoff can develop perched water over the impermeable layer or bedrock. With increasing throughflow, the water table rises and the perched water recharges the permeable soil horizon above as capillary fringe (McDonnell, 1990), where 'old', pre-event water carrying NO_3^- (built up in soil during preceding dry seasons) is ready to be discharged (Bowden et al., 2001). At this stage, the water table rise can 1) initiate slope discharge at locations of gradual or abrupt changes in hillslope or hydraulic conductivity, and/or 2) push out and displace the 'old', NO_3^--rich pre-event water from the permeable soil profile (Cooke & Cooper, 1988).

In contrast, low-NO_3^- groundwater discharge can 'dilute' the stream NO_3^- concentration and incorrectly be interpreted as NO_3^- removal in wetlands (Warwick & Hill, 1988). Such dilution may occur when newly infiltrated NO_3^--rich water in upslope subsurface displaces low-NO_3^- 'old' water in the deep subsurface and pushes it out into the wetland and ends up diluting the stream NO_3^- concentration (Davie, 2004; Hill, 1996; Petry et al., 2002).

Thus the subsurface runoff that reaches wetlands and streams is considered a translatory flow which has chemical properties different from the original precipitation composition (Davie, 2004; McGlynn et al., 2002). Subsurface runoff can contribute large flow volumes as studies have found at least half of the peak discharge stream flow was contributed by subsurface discharge (Buttle, 1994; Sklash et al., 1986). This means subsurface runoff can have a substantial influence on seepage wetland and stream water NO_3^- chemistry (Hill, 1996).

Although the different runoff mechanisms are discussed here separately, the processes are not necessarily mutually exclusive and their dominances may shift at different stages of a storm hydrograph (Bonell et al., 1990; Jürg & Mosley, 1998). For example, during a runoff study in Tussock grassland in the South Island of NZ, saturation excess runoff was generated during a large storm when quickflow volume was >10 mm and runoff was a mixture of 'old' and 'new' water from two separate sources. In contrast, at quickflow of <10 mm, subsurface runoff contributed 'old' water from shallow groundwater during the recession limb in a hydrograph of a precipitation event (Bonell et al., 1990). The variabilities in runoff mechanisms depend on catchment characteristics e.g. soil characteristics, geology, topography, precipitation intensity and frequency, local hydrology and land use-driven disturbance in soil structure (Bowden et al., 2001; Cooke & Dons, 1988).

2.4.2 Upland-wetland hydrological connectivity

2.4.2.1 High NO_3^- delivery vs. low residence time with influence on denitrification

As the water table rises into the unsaturated zone with increased subsurface runoff, an upland to wetland hydrological connectivity is established (McGlynn & McDonnell, 2003b), and flow becomes lateral. From the perspective of NO_3^- transport to and transformation within seepage wetlands, upland-wetland hydrological connectivity has important implications for denitrification in seepage wetlands. Firstly, precipitation and slope discharge contribute to the high-water table and keep seepage wetlands saturated. The presence of impermeable layers at shallow depths in seepage wetlands further maintains the saturated conditions and promotes an anaerobic environment in the wetland. Secondly, the upland-wetland hydrological connectivity provides a continuum for NO_3^- transport from the upland to wetland soils. During the rising limb in storm hydrographs, dissolved organic carbon (DOC) and NO_3^- (observed during winter-spring floods and also immediately following summer storm events) delivered from the hill country catchment (Cooke & Dons, 1988; McGlynn & McDonnell, 2003b) has potential to promote denitrification in the wetland (Willems et al., 1997), as infiltration of this NO_3^- rich runoff can accelerate subsurface denitrification in NZ pastoral soil (Luo et al., 2000). However, short residence time during storm events can limit NO_3^- reduction and can result in a 'bypass' of the wetland's NO_3^- attenuation function (Burt et al., 1999; Cooper & Cooke, 1984).

2.4.2.2 Potentials of long residence times

As wetland residence time increases, NO_3^- removal in the wetland becomes more effective (Cooke & Cooper, 1988). Delayed NO_3^- delivery via subsurface runoff to wetlands has been observed even after several days following precipitation events in pastoral catchments in NZ and Australia (Parfitt et al., 2009; Smethurst et al., 2014).

Between storm events, water in the unsaturated soil profile 'ages' and can become NO_3^--enriched during its drainage through the soil matrix. This can translate to NO_3^- rich shallow groundwater discharge to wetlands under baseflow conditions. In summer, slope discharge and water table height decrease, but slow slope discharge continues as baseflow (Burt & Pinay, 2005; Davie, 2004, 2008). Such baseflow can maintain saturated conditions in wetlands and feed the wetland for prolonged periods (up to 4 weeks), as observed in a bog (precipitation-fed wetland), located at hillslope at the Glendu catchment in South Island in NZ (Bowden et al., 2001). Under baseflow conditions, during summer-early autumn, NO_3^- can also accumulate on the soil surface and within the root zone from NO_3^- fertiliser inputs, dung and urine deposition and nitrification stimulated by high temperatures (Cooke & Cooper, 1988). These can drive periodic high streamflow NO_3^- concentrations and provide a ready NO_3^- supply for denitrification in wetlands. However, as slope discharge becomes less dominant and evaporation increases in summer, disrupted hydrological connectivity and the less-than-saturated conditions can disrupt the reducing condition and the NO_3^- supply to denitrification microsites and interrupt wetland denitrification (Burt et al., 1999). This can leave the NO_3^- accumulated within the surface sediment layer of the wetland, ready-to-be-flushed out in the next storm event.

This review highlights the discrepancy between the occurrences of high NO_3^- loads and the ideal hydrological conditions to facilitate a reducing condition in the wetland for effective NO_3^- removal. In winter, wetlands can receive large NO_3^- loads under high flow conditions, and result in short residence times that can overwhelm the denitrification capacity of the wetland soils. Whereas in summer, NO_3^- supply can get disrupted due to a lack of hydrological connectivity, despite the temperature and long residence times being supportive of NO_3^- reduction in the wetland (Burt et al., 1999; Cooper & Cooke, 1984).

2.5 Denitrification vs. dissimilatory NO_3^- reduction to ammonium (DNRA)

2.5.1 Denitrification

Denitrification is the microbe-mediated NO_3^- reduction to gaseous forms of nitrous oxide (N_2O) and dinitrogen (N_2) (Tiedje, 1983). In denitrification, NO_3^- is used as the electron acceptor for energy generation in oxygen-limited environments e.g., in saturated conditions in wetlands, and is reduced in a stepwise process as in Figure 2-4.

(+5)		(+3)		(+2)		(+1)		(0)
NO_3^-	→	NO_2^-	→	NO	→	N_2O	→	N_2
nitrate		nitrite		nitric oxide		nitrous oxide		dinitrogen

Figure 2-4. Denitrification reaction. Values inside parentheses show the oxidation states of the corresponding N-forms in the reaction.

Denitrification can be autotrophic (uses reduced forms of iron and manganese as electron donors) and heterotrophic (uses carbon for cell growth and as an electron donor) (Korom, 1992). The heterotrophic denitrification process dominates denitrification-based NO_3^- reduction in a natural environment (Knowles, 1982). Denitrifiers, microorganisms involved in the denitrification process, are ubiquitous in nature. Therefore, denitrification can occur under a wide range of environmental conditions (Korom, 1992). This feature makes denitrification a major NO_3^- reduction pathway in ecosystems ranging from terrestrial to aquatic, including wetland ecosystems.

Wetlands as a transitioning ecosystem between terrestrial and aquatic ecosystems have a particular advantage for the denitrification process (Hammer & Bastian, 2020; Martínez-Espinosa et al., 2021). Compared to terrestrial ecosystems, wetlands soils are saturated and oxygen-limited whereas, compared to aquatic ecosystems, wetlands are rich in electron donor supply (labile C) for a high denitrifier population. The biogeochemical environments in wetlands are supportive of high NO_3^- reduction and result in high denitrification activities that make wetlands potential denitrification hotspots (Barton et al., 1999; Groffman et al., 2009).

Although denitrification is generally a major NO_3^- reduction pathway in wetlands (Willems et al., 1997), their dominance as a NO_3^- reduction pathway can vary (Cooke & Cooper, 1988). For example, plant uptake can dominate NO_3^- reduction process during the active plant growing season (Cooper & Cooke, 1984; Zaman et al., 2008). However, in contrast to denitrification, the mitigation effects of most other NO_3^- reduction pathways (plant and microbial uptake and dissimilatory NO_3^- reduction to ammonium (DNRA)), in wetlands are only temporary. This is because the NO_3^-, which is assimilated by biological uptake and/or DNRA, can return to wetland soils upon plant and microbial decay. In contrast, the denitrification process offers a comparatively permanent mitigation scope as it reduces NO_3^- to an environmentally sound and inert N_2 gas, when the process is complete.

Because denitrification is a microbial process, the biogeochemical environment has a strong influence on denitrification in wetlands. Sediment physicochemical (water content, pH, redox potential) and chemical (electron donor and acceptor supply) properties define the wetland biogeochemical environment and thus have a regulatory role in denitrification in wetlands. Sediment properties are also the derivatives of geological and climatic properties and associated hydrologic processes that can vary widely across regions and landscapes and drive spatially variable sediment denitrification capacities. This means sediment properties have the added advantage of reflecting the factors affecting denitrification at multiple scales. It also means depending on sediment properties, denitrification outcomes can be highly variable as denitrification can vary even within close proximities of a few centimetres (Giles et al., 2012).

Sediment denitrification capacities are quantified by measuring denitrification enzyme assay (DEA). The DEA method provides an estimate of denitrifying enzyme activity in soil samples under short-term laboratory conditions. Different *in-situ* and laboratory-based DEA quantification techniques are available, however, one of the most commonly used DEA-measurement techniques is the acetylene inhibition technique (Tiedje, 1983; Yoshinari et al., 1977).

2.5.2 Dissimilatory NO_3^- reduction to ammonium (DNRA)

The dissimilatory NO_3^- reduction to ammonium (DNRA) is a dissimilatory NO_3^- reduction process in which NO_3^- is reduced to NH_4^+ in highly reducing and organic matter-rich sediment conditions. The DNRA occurs when denitrification becomes NO_3^- limited at low NO_3^- concentrations (~0.01 mg NO_3-N/L) (Handler et al., 2022; Tiedje, 1988; Wang et al., 2019a). In low-NO_3^- environments, further denitrification of NO_3^- and NO_2^- (an intermediate product in denitrification) becomes less energy efficient and NO_3^- reduction to NH_4^+ in DNRA becomes relatively more useful in microbial respiration (Pandey et al., 2020) (Figure 2-5).

Important factors affecting DNRA in the soil environment are sediment oxidation, high organic C/N ratio, organic C quality, NO_2^-/NO_3^- ratio and dissolved Fe^{2+} concentration (Fazzolari et al., 1998; Handler et al., 2022; Pandey et al., 2020; Rütting et al., 2011; Rütting et al., 2010). Nitrate concentration has also been associated with DNRA (Pandey et al., 2020) as Yin et al. (1998) suggest that DNRA occurs at a threshold DOC/NO_3-N ratio of >12.

Figure 2-5. Diagram showing the partitioning of denitrification and dissimilatory NO_3^- reduction to ammonium processes. Taken from Liu et al. (2021).

In comparison to denitrification, our awareness of the DNRA process in seepage wetlands is limited. Often this is because DNRA has long been considered only a minor NO_3^- reduction pathway mainly due to the limitations associated with DNRA assessment techniques. With technical tools like molecular technology becoming available, DNRA appears as an important NO_3^- reduction mechanism in seepage wetlands under certain sediment environmental conditions, e.g. unvegetated conditions (Matheson et al., 2002).

2.5.3 The contrast between denitrification and DNRA and its implication in wetland NO_3^- attenuation

While denitrification and DNRA are both dissimilatory NO_3^- reduction processes and have similar environmental requirements, their impact in terms of NO_3^--mitigation is contrasting. Denitrification and DNRA processes both require NO_3^-, organic C and reducing conditions. However, in denitrification, NO_3^- is reduced to environmentally sound N_2 when the process is complete. In contrast, NO_3^- is reduced to NH_4^+ in DNRA. Nitrate reduced in the DNRA process returns to sediment upon plant decay and via nitrification during water table fluctuations, and can become a future NO_3^- source for denitrification. This means that while the denitrification process offers scope for NO_3^- mitigation, DNRA can be a source of NO_3^- instead. Therefore, understanding the partitioning between these two processes is crucial to fully understand the NO_3^- mitigation capacity. However, their overlapping N-sources and outputs often make the distinction between the two NO_3^- reduction processes difficult (Giles et al., 2012).

An investigation into major NO_3^- reduction pathways in a seepage wetland soil microcosm study has suggested the soil oxidation conditions as a partitioning regulator between the DNRA and the denitrification processes (Matheson et al., 2002). Although knowledge of the DNRA process in seepage wetlands is rudimentary, it is to be aware of DNRA as a potential and alternative NO_3^- reduction pathway particularly in temperate climates (e.g. NZ) as suggested by Rütting et al. (2011). Under temperate climatic conditions, the seepage wetland study conducted in NZ by Matheson et al. (2002), found DNRA accounted for a large proportion of NO_3^- removal (49%) in an unvegetated condition. In contrast, denitrification accounted for 61-63% of NO_3^- removal under a vegetated condition, where plant root-facilitated aerated conditions repressed DNRA and allowed a greater proportion of denitrification-based NO_3^- removal.

2.6 Influence of wetland sediment properties on denitrification

2.6.1 Sediment water content, oxygen limitation and redox condition

Saturated condition is a major feature that makes wetlands particular hotspots for biogeochemical reactions that require reducing conditions e.g. NO_3^- reduction processes (Giles et al., 2012). Saturated conditions, characterised by high sediment water content and consequent low redox potentials (Eh), 1) restrict oxygen diffusion in sediments, 2) facilitate and maintain a NO_3^- reducing anoxic environment, 3) establish a continuum between the denitrification inputs (NO_3^- and labile organic carbon), and anaerobic microsites (Giles et al., 2012; Luo et al., 1999a), and 4) regulate redox chemical species availability (Burgin & Groffman, 2012; Burgin et al., 2010). Fluxes in saturated conditions, enhanced by hydrologic fluxes, regulate the perturbations in these effects, with consequences for denitrification.

The feedback among the dissolved oxygen (DO) concentration in water (includes surface and shallow groundwater), sediment water content, and their effects on the dominant redox reactions in wetland sediment environment, make it difficult to separate their individual effects. Thus, the quantifications of sediment water content (WC) and redox potentials and DO concentrations in surface and subsurface water in hydrologic studies, are reasonably measuring a similar phenomenon, i.e., the oxygen limitation in wetland environment, albeit using separate but interlinked parameters to explain the wetland NO_3^- reducing conditions.

Threshold limits exist for sediment WC and redox potential, based on their respective positive and negative associations with denitrification. The aerobic condition in air-filled pore spaces in sediments, at water contents below field capacity, can interrupt NO_3^- reduction. Soil WC at field capacity has been suggested as critical for denitrification in poorly drained silty clay pastoral soil in NZ (Ruz-Jerez et al., 1994). A 60% water-filled pore-space (WFPS) is recommended as a critical limit for denitrification (Bremner & Shaw, 1958). Denitrification reaction tends to become more complete as the WFPS increases. Weier et al. (1993) have shown a greater proportion of denitrification end-product is N_2O at 70% WFPS, in contrast to N_2 at WFPS >90%. With increasing water content, oxygen becomes restricted and redox potential (Eh) declines. The DO<2 mg/L or Eh<300 mV is considered ideal for NO_3^- reduction process (Bates & Spalding, 1998; Van Cleemput et al., 2007). With further decline in Eh, more redox chemical species become available as electron donors, e.g. Fe (III) hydroxides become available as Fe^{2+} to support subsurface denitrification where labile carbon is exhausted or absent, e.g. in deep groundwater (Di Capua et al., 2019; Knowles, 1982).

2.6.2 pH

The effect of pH on denitrification is indirect via its influence on the denitrifier gene composition and abundance, and the electron donor and acceptor supply for denitrification (Wallenstein et al., 2006). In general, soil pH negatively correlates with the N_2O/N_2 product ratio of denitrification (Šimek and Cooper 2002). This means at acidic pH, a greater fraction of the denitrification end-product is N_2O which is a potent GHG gas. Bowen et al. (2020) have shown high pH favours *nirS* (nitrite reductase gene) abundance, in contrast to high abundance of *nirK* (nitrite reductase gene) at acidic pH. The *nirK* lacks the N_2O reductase enzyme necessary for N_2O reduction to N_2. This means denitrification in acidic soils, can terminate before completion with N_2O as the end-product (Bergaust et al., 2010; Wallenstein et al., 2006) as there is positive association between low pH and N_2O emissions (Hefting et al., 2003).

Despite the negative influence of low pH on denitrification, the process can still occur as denitrification has been recorded at pH values as low as 4.8 (Bremner & Shaw, 1958), but can completely stop at pH of 3.5 (Parkin et al., 1985). As pH increases, between pH 5.1 and 9.4, the N_2O/N_2 ratio increasingly becomes proportionally equivalent as denitrification end-products (Scholefield et al., 1997). Studies show while N_2O can be the only end-product at pH <6 (Ha et al., 2015), N_2 becomes the principal end-product at pH>6 (Wang et al., 2021). The highest denitrification rate by the most common denitrifier occurs at neutral to alkaline soil pH of 7-9, suggesting a potential optimum pH range (Alyamani et al., 2020; Knowles, 1990). However, a universal optimum pH for denitrification is yet to be agreed on (Šimek & Cooper, 2002; Wang et al., 2021).

Sediment pH can also influence the availabilities of denitrification substrates (e.g., electron donors and acceptors) and alter their corresponding influence on denitrification. As organic matter decomposition slows down due to low microbial activity at low pH, organic C supply can become low. Conversely, low pH can facilitate dissolved Fe^{2+} availability via the dissolution of Fe(III) oxides and hydroxides, which is an alternate electron donor and can fuel autotrophic denitrification (Di Capua et al., 2019).

The influence of sediment pH is also evident in the influence of NO_3^- enrichment on denitrification. For example, partial denitrification in acidic submerged soil occurred when NO_3^- was increased from 0 to 5 mg NO_3-N/L with N_2O as the dominant end-product in study in Vietnam (Ha et al., 2015). In the same study, denitrification was more complete in a submerged agricultural soil at neutral pH at NO_3^- enrichment from 0 to up to 10 mg NO_3-N/L (Ha et al., 2015).

Since soil in NZ is acidic in general, the discussion above suggests a risk of N_2O emission from NZ pastoral soils. Sediment pH ranges 4.7-6.1 in pastoral seepage wetlands in hill country and dairy landscapes (Chibuike et al., 2019; Zaman et al., 2008). A NZ hill country seepage wetland study reported a pH of 6.1 at the 0-30 cm depth, which increased to pH 6.6 at the 60-100 cm depth. In contrast, pH value decreased with depth from 5.4 at 0-10 cm to 4.7 at 40-70 cm in a wetland studied in a NZ dairy catchment (Zaman et al., 2008).

2.6.3 NO_3^-

Because NO_3^- is the major electron acceptor in the denitrification process, NO_3^- enrichment generally accelerates the NO_3^- reduction process (Hanson et al., 1994a; Sartoris et al., 2000; Willems et al., 1997; Xue et al., 1999). For example, for NO_3^- enrichments of 5 and 10 mg NO_3-N/L in wetland soil, the NO_3^- removed (i.e. decrease in concentration over the incubation period) was 3.7 and 7.5 mg NO_3-N/kg respectively (Ha et al., 2015) and measured a strong positive linear regression (R^2=0.9) between NO_3^- enrichment and denitrification (Ha et al., 2015).

However, there is a limit of NO_3^- enrichment beyond which denitrification can become a N_2O-source due to the inhibitory effect of excessive NO_3^- on denitrification (Blackmer & Bremner, 1978). A study has shown denitrification becomes independent of NO_3^- concentrations >1mg/L, as the kinetics of denitrification measured zero in a freshwater sand and gravel aquifer in the USA (Smith & Duff, 1988). In another study, as NO_3^- increased from 4 to 44 mg NO_3^-/L, a submerged wetland soil in Vietnam became a N_2O-source (Ha et al., 2015). When NO_3^- is in excess, N_2O reduction to N_2 can become less energy-efficient and denitrification can terminate with a greater proportion of end-products as N_2O gas, instead of gaseous N_2 (Firestone et al., 1979; Weier et al., 1993). Nitrate concentrations have also shown positive correlations with N_2O emissions and negative correlations with N_2 generations in grazed grassland in Ireland (Jahangir et al., 2012).

In agricultural soils, denitrification is seldom NO_3^--limited generally due to the high soil NO_3^- contents from farm-input (such as urine, dung and fertiliser) (Barton et al., 1999). This can drive high denitrification rates in agricultural soils, compared to natural soils e.g. in forest ecosystems (Barton et al., 1999). Yet, Zaman et al. (2008) speculated denitrification might have been NO_3^--limited during their study in a seepage wetland in dairy pasture in NZ. Although NO_3^- concentration in groundwater and subsurface denitrification capacity can be low numerically (38 to 64 µg N kg^{-1} h^{-1}), groundwater denitrification can still account for substantial NO_3^- removal in wetlands (Jahangir et al., 2012; Smith & Duff, 1988). Investigation into the influence of NO_3^- concentration on denitrifier composition or abundance found no relationship (Veraart et al., 2017), which suggests the positive effect of NO_3^- on denitrification is primarily chemical. Yet there are suggestions that C-supply is more important in denitrification, compared to NO_3^- supply (Weier et al., 1993).

Seepage wetland sediment studies in NZ have generally demonstrated very low NO_3^- contents. Mean seepage wetland sediment NO_3^- contents of 0.11 and 0.12 mg NO_3-N/kg dry soil (DS) are reported in hill country and dairy pastoral landscapes, respectively (Chibuike et al., 2019; Zaman et al., 2008). Nitrate concentrations have also been found to be low (~0.01 mg NO_3-N/L) in surface and shallow groundwater in seepage wetlands, as observed in a dairy pastoral catchment (Zaman et al., 2008).

2.6.4 Labile carbon

Microbes use labile organic carbon (OC) in assimilatory (cellular growth) and dissimilatory (energy generation e.g., via denitrification) processes. Organic matter decomposition consumes oxygen that can facilitate oxygen-limiting conditions, necessary for NO_3^- reduction processes. The positive influence of OC in NO_3^- reduction processes make OC a key denitrification-influencing sediment property and OC is often recommended as an indicator of spatial variations of denitrification (Chibuike et al., 2019; Hill & Cardaci, 2004; Jahangir et al., 2012; Pfenning & McMahon, 1997; Schipper et al., 1993; Steele et al., 1984). Sediments in wetland surface depths are rich in OC from root exudates and detrital deposits generally measure high denitrification activities (Bernard-Jannin et al., 2017). As depth increases in the wetland, denitrification activity exponentially decreases due to low labile C in the subsurface (Burford & Bremner, 1975; Drury et al., 1991; Surey et al., 2020; Zarnetske et al., 2011b).

Dissolved organic carbon (DOC) quantity can also influence denitrification end-products as N_2O emission increases with soil C, as observed in paddy soil in China (Chen et al., 2018). Conversely, Saggar et al. (2013) have reviewed that the ratio of N_2O:N_2 as a denitrification end-product varies with soil N in temperate grassland soils.

In seepage wetlands, DOC and DEA are positively correlated with correlation coefficients ranging from 0.81-0.89, as observed in the USA and NZ (Anderson et al., 2014; Chibuike et al., 2019; Hill & Cardaci, 2004). In a pastoral hill country seepage wetland in NZ, DOC concentrations were three times higher in a seepage wetland (498 mg/kg DS at 0-30 cm), compared to the surrounding dry soil (109 mg/kg DS at 0-30 cm), which contributed to 7-69 times higher DEA in the wetland (Chibuike et al., 2019).

Dissolved OC quality, source and stoichiometry of C in sediment also influence the spatial gradient in NO_3^- reduction in wetland sediments (Bastviken et al., 2005; Giles et al., 2012; Grebliunas & Perry, 2016). For example, despite similar DOC concentrations of ~370 mg DOC/kg DS between different depths, low DOC quality (indicated by high DOC molecular weight) resulted in 94% lower denitrification capacity in wetland subsurface depths (20 µg N_2O-N kg^{-1} DS h^{-1} at 60-100 cm depth), in contrast to surface DEA (369 µg N_2O-N kg^{-1} DS h^{-1} at 0-30 cm depth), which had more labile DOC as indicated by the low DOC molecular weight (Chibuike et al., 2019). Another study comparing the effect of C availability from different sources (surface water vs groundwater carbon sources) reported higher N_2O production, which is an indication of greater efficiency of fulvic acid (labile DOC) derived from surface water, in contrast to less available groundwater-derived carbon (Pfenning & McMahon, 1997). Yet, buried OC at >1 m depths have been found to fuel shallow groundwater denitrification in pastoral landscapes in NZ (Stenger et al., 2018).

2.6.5 Topography

Topography influences fine particle soil deposition in the landscape, development of saturated condition, the associated sediment physical and chemical properties and denitrifier distribution with overall influence on denitrification in wetlands. Accumulations of DOC and NO_3^-, which originate from upland grazing activities (e.g., animal excreta from stock camping areas on hillslopes), can create a zone of high denitrification activity at toeslopes (Andersen, 2004; Kemp & Lopez, 2016; Saggar et al., 1990). Significantly higher DOC accumulation (498 mg/kg DS) in a hill country seepage wetland located at the toeslope has measured 7 to 69 times higher denitrification activities in contrast to the surrounding upland soils (109 mg DOC/kg DS) in NZ (Chibuike et al., 2019). Another study in a grazed pasture in NZ measured higher soil NO_3^- content, *nirK* abundance and denitrification enzyme activities at low slopes (0-12°) compared to steeper slopes (Zhong et al., 2016).

2.6.6 Texture

Soil texture influences the drainage characteristics and associated reducing conditions in soil with overall implications for denitrification. Soil texture determines pore space, soil aeration, hydraulic conductivity and water content that influence oxygen limitation, nutrient transport and residence time and associated microbial distribution in soils (Eppinger & Walraevens, 2014; Fredrickson et al., 1989). Threshold WFPS, the water content limit above which further increases in water content can enhance denitrification, is greater for soils with coarse texture (74-83% WFPS in sandy and sandy loam soils) compared to fine-textured soils (62-83% in loam and 50-74% WFPS in clay soils) (Barton et al., 1999). Soils high in clay content (>30% clay) exhibited high DEA values (>8.3 μg N g^{-1} DS day^{-1}), compared to low DEA values (<2.5 μg N g^{-1} DS day^{-1}) in sand-dominated soil (>80% sand) in Flemish agricultural soils (D'haene et al., 2003). In a USA study, fine-textured, poorly drained soils measured higher denitrification (40 kg N ha^{-1} yr^{-1}) that continued over a longer period of time, compared to moderately well-drained soils (<5 kg N ha^{-1} yr^{-1}) at 0-15 cm depths (Hanson et al., 1994a). However, fine-textured soils also have the potential to interfere with C diffusion and can constrain microbial distribution, and in that case, may limit denitrification in clay soils (Eppinger & Walraevens, 2014; Myrold & Tiedje, 1985).

2.7 Nitrate removal studies in New Zealand pastoral seepage wetlands

The first studies examining the potential of seepage wetlands to mitigate diffuse NO_3^- loss to water in pastoral hill country were reported in a study near Hamilton in late 1980s to early 1990s (Cooke & Cooper, 1988; Cooper, 1990; Cooper & Cooke, 1984; Smith et al., 1993). These studies made important scientific contributions by identifying the role small seepage wetlands play in NO_3^- transformation in agricultural headwater catchments and the research has been globally recognised (Hill, 1991, 1996). Between 1980-2005, there were several NZ seepage wetland studies on NO_3^- removal associated with surface and subsurface transport (Burns & Nguyen, 2002; Cooke & Dons, 1988; Nguyen et al., 1999a; Rutherford & Nguyen, 2004) and N-reduction processes (plant uptake, denitrification, DNRA) (Matheson et al., 2002), with these studies primarily based in pastoral hill country landscapes. However, before a comprehensive understanding of the biogeochemical and hydrologic function of hill country seepage wetlands in terms of NO_3^- reduction was developed, the research focus shifted away from the pastoral hill country towards the dairy pastoral catchments. It should be noted that mitigating NO_3^- near their source and within the boundaries of hill country farms has great environmental advantage as opposed to this NO_3^- enriched water joining denser drainage networks downstream. It is likely that higher downstream water volumes and NO_3^- concentrations will require more expensive mitigation strategies to treat this water. Nonetheless, the dairy-focused studies examined here were located in the mid to low floodplains where studies have quantified the NO_3^- delivery to seepage wetlands via different hydrological routes (surface and subsurface flow) and N-attenuation in the wetlands under various flow (baseflow, overland flow) and grazing conditions (Hughes et al., 2013; McKergow et al., 2012; Wilcock et al., 2012; Zaman et al., 2008). As a consequence, there is a dearth of knowledge on the biogeochemical and hydrologic function of hill country seepage wetlands.

The following section reviews the seepage wetland literature relevant to NO_3^- reduction with a focus on pastoral hill country and broadly categorises studies into 1) wetland sediment denitrification studies, and 2) hydrological studies that assessed NO_3^- removal in seepage wetlands.

2.7.1 Sediment properties in sheep and beef grazed pastoral hill country seepage wetlands

Seepage wetland studies located on sheep and beef pastoral farms have quantified sediment DEA and measured several sediment physicochemical (pH, Eh, WC, bulk density, porosity) and chemical parameters (DOC, TC, TN, C:N, NO_3^-, NH_4^+, dissolved Fe^{2+} and Mn^{2+}) that potentially influence wetland denitrification in general. However, the number of these studies is very small (Table 2-1) and are single-site based that have focussed on: 1) a hill country farm (Tuapaka) in the Manawatū region of the lower North Island, and 2) several wetland sites in both hill country and flat landscapes at the Whatawhata Hill Country Research Station located near Hamilton in the Waikato region (central North Island, NZ).

2.7.1.1 Sediment DEA

A review of the vertical distribution of seepage wetland sediment DEA shows high denitrification activity in surface depths in sheep and beef-grazed hill country. A wide range of surface DEA values 389-6480 µg N_2O-N kg^{-1} DS h^{-1} between 0-30 cm depths have been reported, with the value varying with sites, seasons and depths (Table 2-1). The previous NZ seepage wetland sediment DEA estimates are summarised in Table 2-1 which were mostly quantified in the laboratory using acetylene inhibition technique (Tiedje et al., 1989; Yoshinari et al., 1977). A mean surface DEA of 369 µg N_2O-N kg^{-1} DS h^{-1} was measured at the 0-30 cm depth at sheep and beef grazed Tuapaka located in Palmerston North in the lower North Island, referred to as the Tuapaka study from hereon (Chibuike et al., 2019). A much higher aggregated surface DEA of 4000 µg N_2O-N kg^{-1} DS h^{-1} (mean DEA of the 0-5, 5-10 and 10-25 cm depths calculated for the purpose of this comparison) was calculated at the sheep and beef grazed Whatawhata site (Nguyen & Downes, 1997). The large difference between the surface DEA values reported by Chibuike et al. (2019) and Nguyen and Downes (1997) could be due to the differences in site-properties, and also the depths sampled (Table 2-1).

Seasonal comparisons of DEA in a NZ study suggest denitrification activity in seepage wetlands can be high in late winter-early spring (6000 µg N_2O-N kg^{-1} h^{-1}) (Nguyen & Downes, 1997) in contrast to a DEA of 2500 µg N_2O-N kg^{-1} h^{-1} in summer at the surface 5-10 cm depth at the Whatawhata site (Nguyen & Downes, 1997). The seasonal DEA flux was also particularly higher in the subsurface depths as the DEA varied between 2000 (summer) and 6000 (winter) µg N_2O-N kg^{-1} h^{-1} at the 5-10 cm depth, compared to a much less seasonally contrasting 1000 (summer) and 2000 (winter) µg N_2O-N kg^{-1} h^{-1} at the surface 0-5 cm depths (Nguyen & Downes, 1997). In a separate study, streambed sediment in a similar wetland measured higher DEA of 2970 and 2050 mg N_2O-N m^{-2} h^{-1} during the winter months of June and August, respectively, compared to 1640 mg N_2O-N m^{-2} h^{-1} measured in the late spring month of November via *in-situ* DEA measurement in a pastoral seepage wetland (Cooper & Cooke, 1984).

Table 2-1. Denitrification enzyme activity (DEA) measured using *in-situ* and laboratory techniques at seepage wetlands at sheep-beef grazed hill country pastures in New Zealand.

Location	Site description	DEA	Reference
Laboratory-based DEA measurement - Acetylene inhibition technique (μg N_2O-N kg^{-1} h^{-1})			
Whatawhata (Hamilton, central North Island)	Seepage wetland sediment	<u>Summer (February)</u> 0-5 cm: 2500 ± 1000 5-10 cm: 2000 ± 1000 10-20 cm: 1000 ± 500 <u>Late winter-early spring (August-November)</u> 0-5 cm: 1000 ± 1500 5-10 cm: 6000 ± 4000 10-20 cm: 5000 ± 2500	Nguyen and Downes (1997)
Whatawhata (Hamilton, central North Island)	Seepage wetland sediment	<u>Early autumn (March-April)</u> 5700 ± 1800	Nguyen et al. (1999b)
Tuapaka (Palmerston North, lower North Island)	Seepage wetland sediment	<u>Spring (November)</u> 0-30 cm: 389 30-60 cm: 170 60-100 cm: <10	Chibuike et al. (2019)
Whatawhata (Hamilton, central North Island)	Seepage wetland sediment	<u>Autumn (April)</u> 4100 ± 1500	Rutherford and Nguyen (2004)
Scotman Valley (Hamilton)	Seepage zone sediment	0-5 cm: 6480	Cooke and Cooper (1988)
***In-situ* DEA measurement – Push-pull technique (mg N_2O m^{-2} h^{-1})**			
Whatawhata (Hamilton, central North Island)	Streambed sediment	Measured from increasing distance (m) from seepage source and denitrification rate <u>Winter (June)</u> 10 m: 29.7 ± 7.2, 25 m: 18.3 ± 3.1, 50 m: 5.1 ± 2.6 <u>Winter (August)</u> 10 m: 20.5 ± 3.5,25 m: 16.0 ± 2.0, 50 m: 9.1 ± 1.8 <u>Spring (November)</u> 10 m: 16.4 ± 3.2,25 m: 10.0 ± 2.0, 50 m: 5.1 ± 1.1	(Cooper & Cooke, 1984)

2.7.1.2 Sediment physicochemical and chemical properties

This section reviews the seepage wetland sediment physicochemical and chemical properties in sheep-beef grazed hill country pastures in NZ studies which are summarised in Table 2-2. The NZ studies have found wetland sediments neutral in pH (6.1-6.6) and in a highly reduced condition (Eh: <20 mV) that demonstrated characteristics of a NO_3^- reducing sediment environment. These studies also reported increasing bulk density and WC, and decreasing porosity and saturated hydraulic conductivity with increasing depths. Specifically, saturated hydraulic conductivity decreased sharply with depth, with a 50% decrease from 0-10 cm compared to the 0-20 cm depth and a 98% decrease within the top 30 cm depth measured (Rutherford & Nguyen, 2004). The high saturated hydraulic conductivity in the surface at the 0-10 cm depth observed by Rutherford and Nguyen (2004), is expected in these wetlands due to the loose and unconsolidated sediments at the wetland surface (Table 2-2). In comparison, the low hydraulic conductivities at subsurface depths have the potential to become a physical barrier to the lateral movements of shallow groundwater and drive larger shallow groundwater flow through upper subsurface depths of higher hydraulic conductivities. This finding indicates a potential depth for shallow flow intervention, should a need for flow regulation arise in order to improve NO_3^- attenuation.

In terms of mineral composition, a previous investigation suggested seepage wetland sediment is organic, based on the high sediment C:N ratio of 10:1 (Nguyen & Downes, 1997). The ratio was found to be consistent throughout the surface 25 cm depth in the Nguyen & Downes (1997) studied wetland. Carbon and N compounds that include DOC, total C and total N, are high in the surface depths due to organic matter deposition and they decrease with depth (Chibuike et al., 2019; Nguyen & Downes, 1997). In contrast, dissolved Fe^{2+} concentrations were found to increase with depth, which has the potential to fuel denitrification at subsurface depths low in organic C (Stenger et al., 2008).

Table 2-2. Sediment chemical and physicochemical properties in sheep-beef grazed pastoral hill country seepage wetlands in New Zealand.

Seepage wetland location	Depth (cm)	Chemical properties								Physicochemical properties					Reference
		NO_3-N	NH_4-N	DOC	Dissolved Mn^{2+}	Dissolved Fe^{2+}	TC	TN	C:N	pH	WC	Bulk density	Porosity	Vertical Sat. hyd. Conduct.	
		---------------------------(mg/kg DS) -----------------------------					-------%-------				(% DS)	(g/cm³)	(%)	(cm/day)	
Tuapaka Site (Palmerston North, lower North Island)	0-30 30-60 60-100	0.1	~0.4	498 490 370	1 0.6 0.75	30 35 40	-	-	-	6.1 6.3 6.6	40 22 20	-	-	-	Chibuike et al. (2019)
Whatawhata site (Hamilton, central North Island)	0-5 5-10 10-20	0.94 0.89 0.93	507±99 247±3.6 99±6.8	-	-	-	9.1±0.2 6.9±0.1 3.7±0.2	0.9 0.64 0.35	10.1 10.8 10.7		-	1.15 1.22 1.37	-	-	Nguyen and Downes (1997)
Whatawhata site (Hamilton, central North Island)	-	0.5-2	74±8.9	%OM 42±1.9	-	-	-	-	-	-	-	-	-	-	Nguyen et al. (1999b)
Whatawhata site (Hamilton, central North Island)	0-10 10-20 20-30		-	-	-	-	-	-		-	-	0.14±0.01 0.15±0.03 0.22±0.04 0.29±0.05	86±2 79±5 79±4 74±8	89±38 33±46 3.8±0.1 0.2±0.1	(Rutherford & Nguyen, 2004)

WC = water content, DOC = dissolved organic carbon, TC = total carbon, TN = total nitrogen, DS = Dry Sediment

The sediment NO_3^- concentration is very low (~<1 mg NO_3-N/kg DS) in NZ seepage wetlands (Chibuike et al., 2019; Nguyen & Downes, 1997). This indicates that denitrification has either occurred and/or a possible NO_3^--limitation for denitrification in these wetlands (Addy et al., 2002; Zaman et al., 2008). Compared to NO_3^- in sediments, ammonium (NH_4^+) forms the larger mineralizable N-fraction (NH_4^++ NO_3^-). Sediment NH_4^+ ranges from 4-507 mg NH_4-N/kg DS (Chibuike et al., 2019; Nguyen & Downes, 1997). The Whatawhata study also measured high sediment NH_4^+ concentrations of 507 mg NH_4-N/kg DS at the 0-5 cm depth and these concentrations decreased to 99 mg NH_4-N/kg DS at the 10-25 cm depth (Nguyen & Downes, 1997). In contrast, the Tuapaka study reported a consistently low NH_4^+ concentration of ~4 mg NH_4-N/kg DS throughout 1-m sediment depth. Ammonium in these wetlands can become an important NO_3^- source via nitrification when the wetland becomes aerobic, and thus, NH_4^+ can positively influence sediment DEA (Chibuike et al., 2019).

In order to understand which sediment property is responsible for driving surface DEA, when sediment properties were compared across the two major NZ seepage wetland studies, NH_4^+ concentration was the only investigated parameter that varied distinctively between the two studies (Chibuike et al., 2019; Nguyen & Downes, 1997). The Whatawhata site which measured 2-5 times higher mean DEA in the 0-25 cm depth, also measured several magnitudes higher NH_4^+ concentrations, compared to the Tuapaka study presented in Table 2-2 (Chibuike et al., 2019; Nguyen & Downes, 1997). That study also measured slightly higher TC and TN, compared to the Tuapaka study. While the Whatawhata study did not assess the influence of sediment properties on denitrification, Chibuike et al. (2019) observed a positive influence of NH_4^+ on DEA. However, the differences in methodological approaches such as the depth intervals and the sediment properties examined, between the only two published hill country pastoral seepage wetland studies make it difficult to infer the major factors influencing DEA variability between previously studied seepage wetlands.

The only seepage wetland study that attempted to quantify the sediment-influence on seepage wetland DEA, has reported positive correlations between DEA and the sediment NH_4^+, DOC, dissolved Mn^{2+} and WC (Chibuike et al., 2019). Additionally, that study reported a lack of correlation of DEA with sediment NO_3^- or sediment Fe^{2+}

concentrations in their seepage wetland study, despite the literature, in general, suggesting their positive associations with DEA in wetlands (Chibuike et al., 2019; Korom, 1992; Sirivedhin & Gray, 2006). Although Chibuike et al. (2019) quantified sediment properties and DEA at increasing depth intervals, assessing the associations between sediment properties and depth was outside their research scope. However, the knowledge of the spatial variation of DEA in seepage wetlands is necessary if future wetland interventions aim to optimise NO_3^- removal.

2.7.1.3 Knowledge gaps in previous NZ pastoral seepage wetland sediment studies

This sub-section identifies several key knowledge gaps preventing a comprehensive understanding of seepage wetland sediment denitrification in pastoral hill country in NZ. The previous studies:

1. Are very small in number and are mostly single-site based. Therefore, the previous seepage wetland sediment DEA estimates do not account for between-wetland DEA variabilities. As a result, they were unable to advance our understanding of how the spatial variabilities of DEA at landscape scale, for example, are influenced by different geology or farming practices.
2. Were varied in their investigated depth intervals and sediment column lengths as they examined either a coarse depth interval of every 30 cm in 1m column (Chibuike et al., 2019) or restricted depth resolution within the top 0.3 m (Nguyen & Downes, 1997).
3. Assessed small and often different sets of sediment variables.
4. Are limited in their investigation into the relationships between a) the spatial variabilities of sediment properties and b) the relationships between sediment properties and DEA.

2.7.2 Hydrological studies on NO_3^- removal by seepage wetlands

Due to the limited literature specific to hill country, this section explores the literature on seepage wetland NO_3^- removal from both the hill country and lower slope dairy pastoral catchments. Previous NZ seepage wetland NO_3^- removal studies have focused more on the hydrological aspects, compared to the wetland's sediment influence. Yet, the number of hydrological studies remains very small. Relevant research in hill country landscapes is even scarcer. This section reviews the literature on pastoral seepage wetland hydrology from the perspective of NO_3^- removal/attenuation.

Previous studies in seepage wetlands have 1) estimated NO_3^- removal based on the changes in NO_3^- concentrations during NO_3^- transport in surface and subsurface hydrological pathways through the wetlands (Burns & Nguyen, 2002; Rutherford & Nguyen, 2004), 2) quantified fluxes in the NO_3^- contributing pathways in wetlands under different hydrological conditions (Parfitt et al., 2009), and 3) investigated temporal variation in NO_3^- between events e.g. pre- and vs. post-event based fluxes that have included precipitation and livestock grazing (Hughes et al., 2013; McKergow et al., 2012). Most of the previous studies have reportedly occurred in groundwater-fed seepage wetlands which have meant greater scope for sediment-NO_3^- interactions and the potential for greater NO_3^- removal efficiencies, in contrast to streamflow-dominated wetlands (Hill, 1996).

2.7.2.1 NO_3^- removal efficiency

Seepage wetland subsurface flow has shown higher NO_3^- removal efficiency compared to the removal in surface flow. For example, subsurface NO_3^- transport resulted in >90% NO_3^- removal, on a mass-basis, during a tracer experiment that monitored shallow groundwater at the 15 and 30 cm depths during a 24-day study in a groundwater-fed pastoral hill seepage wetland at Whatawhata near Hamilton (Burns & Nguyen, 2002). During that study, most of the NO_3^- (93%) was removed in the first 4 days and within 30 cm of its total travel length of 100 cm (Burns & Nguyen, 2002). In a separate study in the same wetland, surface flow NO_3^- removal was much less (51%) based on NO_3-N inflow and outflow in the wetland during a 6-month study (Nguyen et al., 1999a). A lesser NO_3^- removal of 24±9% (mass-basis) occurred in a 'surface flow' monitored study in the surface 5 cm during a month-long tracer experiment in a 1.5-m long experimental enclosure across a seepage wetland located in the same area as the examples above (Rutherford & Nguyen, 2004). These comparisons imply a greater NO_3^- removal efficiency in flows via subsurface pathways in seepage wetlands compared to the surface flow pathways. Additionally, vertical mixing has been found to be critical to achieve effective NO_3^- reduction in these wetlands (Rutherford & Nguyen, 2004).

2.7.2.2 Flow pathways

Literature suggests flowpath NO_3^- contributions are temporally variable in small wetlands within headwater catchments. Yet, these variabilities have rarely been assessed in hill country seepage wetlands in an integrated manner except by Cooke and Dons (1988) and Cooke and Cooper (1988). Previous studies have identified seepage wetlands to be mainly groundwater-fed, but most of these studies occurred in dairy pastures located in low floodplain catchments (Hughes et al., 2013; McKergow et al., 2012), which have dense drainage networks of high-order streams and receive high farm NO_3^- inputs. In contrast, hill country hydrology located in headwater catchments, 1) comprises of low-order streams with disperse drainage networks, 2) connects to a local hydrology, 3) lacks deep groundwater and 4) depends on limited hydrological sources, mainly precipitation.

Seepage wetland hydrological studies in pastoral hill country have reported both groundwater-fed (Burns & Nguyen, 2002; Nguyen et al., 1999a) and surface-flow-dominated contributions (Rutherford & Nguyen, 2004). The dominance of flow pathways was based on their relative hydrological contributions to the total flow in the wetlands (Burns & Nguyen, 2002; Rutherford & Nguyen, 2004). Considering the high NO_3^- removal efficiencies of subsurface pathways, previously discussed in this review, the identification and characterisation of dominant flow pathways in wetlands have the potential to indicate whether a seepage wetland is likely to be a NO_3^- source (partial wetland NO_3^- attenuation) or sink (complete NO_3^- attenuation in the wetland) (Vidon & Smith, 2007). However, such assessments require integrated investigations of both surface and subsurface hydrological pathways which has been rare in previous NZ studies.

2.7.2.3 Seasonal variation in flow

Despite a general understanding that precipitation is a key hydrological driver in hillslope hydrology, the influence of precipitation on seepage wetland hydrology remains poorly studied (Cooke & Dons, 1988; Nguyen et al., 1999a). In hillslope hydrology, precipitation-driven stormflow of short duration accounts for disproportionately large proportions of annual NO_3^- loads (Bargh, 1978). In an analysis of headwater influence on downstream water quality, Dodds and Oakes (2008) have shown that a quarter of annual water volume can discharge within a month or two between May-July, with this period representing the high-flow winter period. Flow discharge and NO_3^- are generally positively associated in NZ water bodies (Dodds & Oakes, 2008). Winter precipitation drives high flow conditions and low hydraulic residence time (HRT), resulting in large NO_3^- loads leaving pastoral hill country catchments (Bargh, 1978; Drake et al., 2018; Quinn & Stroud, 2002; Uuemaa et al., 2018).

Low HRT and oxygenated flow conditions resulted in a negative NO_3^- removal (-29±5%) from surface runoff that occurred in a seepage wetland during a 3-year study in a dairy pastoral catchment in NZ (Wilcock et al., 2012). This negative value contradicts the general observation that these wetlands act as NO_3^- sinks. However, a modelling study shows seepage wetlands under similar landscape settings are capable of NO_3^- removal >75% during subsurface drainage in a dairy pastoral catchment (Uuemaa et al., 2018). Disturbances from direct grazing in the wetland under low flow conditions can provide additional challenges to the function of seepage wetlands and this highlights the importance of farm stock management for efficient farm NO_3^- mitigation via these wetlands (McKergow et al., 2012).

In summary, previous seepage wetland hydrological studies are useful as they provide several important preliminary insights into the hydrological influence on NO_3^- removals which are:

1. Nitrate removal in seepage wetlands varies between hydrological pathways (Burns & Nguyen, 2002; Nguyen et al., 1999a; Rutherford & Nguyen, 2004),
2. Subsurface pathways are comparatively more effective in terms of NO_3^- removal (Burns & Nguyen, 2002),

3. Precipitation can be an important hydrological driver that influences the surface and subsurface flow and their relative NO_3^- contributions in wetlands (Nguyen et al., 1999a), and
4. Hydrological properties e.g., flow volume contribution from different hydrological routes, subsurface hydraulic conductivity and the associated seepage flow rates are highly variable in these wetlands, where vertical mixing is necessary for effective NO_3^- removals (Rutherford & Nguyen, 2004).

2.7.2.4 Knowledge gaps in previous NZ pastoral seepage wetland hydrological studies

The current NZ seepage wetland hydrological studies provide a solid foundation to build our understanding of the hydrological features that may influence NO_3^- removal in these wetlands. However, these previous studies fall short of providing a comprehensive understanding of hydrological influences on NO_3^- attenuation processes as:

1. Most of these studies have investigated either the surface or the subsurface flow pathways, therefore, they lack an integrated hydrological understanding.
2. The majority of these studies were temporally limited in their study periods, of generally less than six months, which as a research timeframe is inadequate to understand NO_3^- removal fluxes over larger temporal scales, e.g., at annual scales, necessary for policy considerations.

These limitations highlight the need for further research to characterise seepage wetland hydrology and NO_3^- removal in order to identify periods of high NO_3^- removal (removal hot moments with disproportionately high NO_3^- attenuation in the wetlands over a short period of time) and NO_3^- loss hot moments (with disproportionately high NO_3^- bypassing wetland attenuation over a short period of time) (McClain et al., 2003). The identified research gaps are critical to be addressed to improve NO_3^- attenuation opportunities in pastoral hill country landscapes. Understanding the wetland hydrological conditions that enhance denitrification, could then be manipulated to enhance NO_3^- removal.

2.8 Flow regulation to improve NO_3^- removal in wetlands

While hydrologic conditions such as short wetland HRT and oxic flow conditions can limit NO_3^- removal in wetlands, these limiting conditions can be altered to improve NO_3^- removal by wetland systems. Flow regulation in natural and artificial wetlands offers opportunities to improve NO_3^- treatment in non-point source pollution such as pastoral drainage. A common approach in such interventions is to increase the HRT of the hydrological flow through the wetland in order to enhance the interaction between the NO_3^--rich pastoral runoff and the wetland sediment matrix, and also to facilitate redox conditions that promote denitrification-based NO_3^- removal.

2.8.1 Flow regulation intervention types

There is a range of different flow management intervention types for NO_3^- treatment in pastoral drainage (Table 2-3). These flow interventions can involve 1) surface flow management (free-water surface flow drainage), 2) subsurface flow management including vertical and horizontal subsurface flow regulation interventions (e.g. in controlled drainage, denitrifying bioreactors), and 3) integrated flow regulation that adopts features from both the surface and subsurface flow regulation types e.g. saturated buffer zones and integrated buffer zones (Carstensen et al., 2020) (Table 2-3).

In general, greater NO_3^- reductions have been observed in interventions that involved subsurface flow (Section 2.7.2). This is further supported by a recent global review which reported greater NO_3^- removal efficiencies in subsurface transport in which an average NO_3^- removal efficiency of >70% was reported for the control drainage types, in contrast to 41±21% via free-surface flow drainage (Bonaiti & Borin, 2010; Carstensen et al., 2020) (Table 2-3).

The differences in the *influent feeding pattern* during the subsurface flow interventions i.e., horizontal vs. vertical inflow can further influence NO_3^- removal (Rutherford & Nguyen, 2004; Tanner et al., 2005). The overall performance of these different inflow patterns in NO_3^- removal however is variable according to the current literature. For example, a horizontal subsurface intervention has measured higher NO_3^- removal rates of 2.16-2.32 g N m^{-3} d^{-1} and TN load reductions of 53-54%, compared to removal rates

Table 2-3. Different water flow regulation types and intervention strategies used in treatment wetlands summarised mainly from Carstensen et al. (2020).

Water flow regulation type and intervention strategies	Description	Principle	NO_3^- removal efficiency (%)	NO_3^- removal rate (g N m^{-2} yr^{-1})
		Surface drainage		
Free-surface flow Constructed Wetland (FWS)[a]	Surface flow through a series of deep ponds, channels and shallow vegetation, before discharging to a stream. Suitable for low permeable soils and hillslopes.	To increase the hydraulic residence time and promote sedimentation and denitrification.	41±21	60±69
		Subsurface drainage		
Controlled Drainage (CD)[b]	Water level is raised and drainage flow is restricted using a drainage flow control structure.	To alter the hydrological cycle.	50±20	1±1
Denitrifying Bioreactor[c]	Drainage water travels through a deep basin filled with carbon-rich material, vertically or horizontally. Alternative terminologies: biofilters, denitrifying beds, denitrifying bioreactor (DBR), subsurface flow constructed wetland.	To facilitate anaerobic conditions and supply C-source to promote denitrification.	40±27	594±481
Saturated Buffer Zone (SBZ)[d]	Recently developed intervention. Drainage water flows through riparian soil during subsurface lateral passage through perforated pipe. The infiltrating water saturates the riparian soil.	To facilitate anaerobic conditions and promote denitrification.	37±25	23±18
		Combined surface and subsurface drainage		
Integrated Buffer Zone (IBZ)[e]	Hydraulic residence time is increased via drainage water's passage through a retention pond before infiltrating through shallow vegetation.	Improved performance compared to traditional riparian buffers.	26±4	140±50

[a] Kovacic et al. (2000), [b] Kröger et al. (2011) , [c] Schipper et al. (2010), [d] Jaynes and Isenhart (2014), [e] Carstensen et al. (2021).

of 1.19-2.31 g N m^{-3} d^{-1} and TN load reductions of 38-60% via vertical subsurface flow intervention, in a subsurface flow constructed wetland woodchip bioreactor (Hoffmann et al., 2019). Conversely, a study comparing the different influent feeding patterns showed better performance in the vertical inflow with 95-100% NO_3^- removal, compared to ~90% under the integrated (horizontal + vertical) and 70-90% under horizontal only inflow in a denitrification constructed wetland study used to treat municipal tailwater in China (Wang et al., 2020). In that study, the high performance observed under the vertical inflow condition was attributed to its associated higher denitrifier abundance and diversity, compared to the other inflow patterns (Wang et al., 2020).

The effectiveness of wetland flow regulation in enhancing NO_3^- removal is assessed by quantifying several variables that include NO_3^- removal efficiencies, NO_3^- removal rate and HRT (APHA, 2005; Mendes, 2021; Reed et al., 1995; Woltemade & Woodward, 2008). The NO_3^- removal rate measures the rate at which NO_3^- is being removed per unit surface area or volume of the wetland media (sediment or woodchip in a bioreactor, for example) during flow and is measured as the difference between the NO_3^- fluxes in (inlet. flux) and out (outlet flux) of the system. However, NO_3^- removal efficiency is a more commonly reported parameter in wetland performance monitoring in nutrient removal and is defined as the percentage change in NO_3^- concentration during the subsurface flow, between the inlet and outlet. These definitions are useful to consider prior to flow regulation type selection because NO_3^- removal efficiency and NO_3^- removal rate is variable with flow regulation type (Table 2-3).

2.8.2 Hydraulic residence time for NO_3^- removal in flow regulation

Hydraulic residence time (HRT) is an important parameter used to quantify the effectiveness of flow manipulations (Hoffmann et al., 2019; Woltemade & Woodward, 2008; Xu et al., 2016). The HRT is the average duration that water carrying soluble compounds such as NO_3^- reside in a wetland and is measured as the mean travelling period a water parcel takes to travel between the point of entry and the point of exit in a treatment system (Woltemade & Woodward, 2008). Long HRT 1) enhances the contact between NO_3^- and the wetland sediment matrix, carrying microbes and electron donor supply, and 2) facilitates redox condition. Thus, increasing HRT improves microbial

NO_3^- reduction and facilitates NO_3^- concentration reductions along the flowpath (Jiang & Chui, 2022; Kröger et al., 2012; Woltemade & Woodward, 2008; Xu et al., 2016).

The HRT for effective NO_3^- removal varies in temporal scales and can range from hours to days, between different wetlands and under different drainage conditions (Table 2-4). For example, a surface flow-constructed wetland, that received subsurface seepage flow with high in NO_3^- concentrations from upland dairy pasture, has measured 11-49% NO_3^- removal efficiencies in 1.5-51 d in a modelling study, where the efficiency was affected by flow conditions (Tanner et al., 2005). The modelling study indicated that 11-36% NO_3^- removal occurred in 1.9-4.3 d under high flow conditions in winter, whereas NO_3^- removal increased to 44% in 1.5 d during summer and 46% in 51 d during autumn (Tanner et al., 2005). In contrast, a comparatively high N removal efficiency of 30-70% was achieved within a shorter HRT of 1.5 d in a restored spring-fed depressional wetland (USA), with similar geohydrologic features as seepage wetlands (Woltemade & Woodward, 2008).

While generally, a few hours of HRT is often adequate in bioreactors where optimum HRT is <10-12 h (Hoover et al., 2016; Zhao et al., 2013), lab-scale studies and constructed wetlands treating non-point source pollutants frequently report HRTs that range in the scale of days (Healy et al., 2012; Qian et al., 2018; Robins et al., 2000) (Table 2-4). Often these reports show large NO_3^- removal within the initial few hours into the drainage operation (Healy et al., 2012). For example, a 60-day-long study demonstrated that substantial NO_3^- removal can be achieved within 3.5 hr in a vertical flow regulation intervention (Wang et al., 2020). In another study, the majority of NO_3^- removal occurred within 2 h (Kröger et al., 2012). The wide range of the required HRT to achieve a similar range of NO_3^- removals under different drainage conditions, as Table 2-4 shows, emphasises the need to determine the associated optimum HRT for operational purposes before a flow regulation intervention is implemented.

Table 2-4. Estimates of NO_3^- removal in different flow regulation studies.

Flow regulation type	Pollutant type (location)	HRT (d= day, h=hr)	$\%NO_3^-$ removal	Change in NO_3-N concentration between inlet and outlet (mg N/L)	Reference
Natural and artificial wetlands					
Restored spring-fed depressional wetland	Limestone spring (USA)	1.5 d 0.5-3.2 d	30-70	from 8 to 1	Woltemade and Woodward (2008)
Surface flow constructed wetland	Daily pastoral Subsurface drainage (New Zealand)	1.9-4.3 d	11-36		Tanner et al. (2005)
Control drainage: Weir to raise water level	Agricultural drainage (USA)	-	79±7.5	From 23±5.7 g to 0.9±0.3 g	Kröger et al. (2012)
Horizontal subsurface flow constructed wetland	Synthetic wastewater (China)	2 h 12 h	20 80%	-	Gao et al. (2017)
Constructed wetland: emergent system	Urban water (Iran)	1 d 3 d 5 d	14 17.62 17.75	-	Nazarpoor et al. (2021)
Experiment examining influent feeding pattern - vertical vs. horizontal vs. integrated (horizontal + vertical)	Municipal tailwater (China)	60 d	95-100 (Vertical) ~90 (Horizontal + vertical) 70-85 (Horizontal)	From 42-50 to 0-1 From 40-45 to 0-3 From 32-40 to 3-5	Wang et al. (2020)
Vertical subsurface constructed wetland	Sewage effluent (Egypt)	0.5 d	Found not effective	-	Abdelhakeem et al. (2016)
Woodchip bioreactor, a subsurface flow constructed wetland examining horizontal and vertical upward and downward drainage	Tile drainage water (Denmark)	3 h	Quantified parameter is TN reduction Mean 20-30 Horizontal flow: 53-54 Vertical flow: 38-60	-	Hoffmann et al. (2019)

Flow regulation type	Pollutant type (location)	HRT (d= day, h=hr)	%NO_3^- removal	Change in NO_3-N concentration between inlet and outlet (mg N/L)	Reference
Woodchip denitrification bioreactor	Subsurface agricultural drainage (USA) with input 10, 30 and 50 NO_3-N/L	1.7 h 21.2 h	8 55	-	Hoover et al. (2016)
A compound natural treatment system (primary subsurface vertical flow wetland + submerged macrophyte oxidation pond + secondary subsurface vertical flow wetland)	Wastewater plant secondary effluent (China)	21.38 d	TN reduction of 87	-	Zhao et al. (2013)
			Laboratory-scale experiment		
Denitrification bioreactor	Treatment plant wastewater with 19.5 to 32.5 mg NO_3-N/L (Ireland)	4 d 22 d	>99%	-	Healy et al. (2012)
Constructed wetland	Biosolid application site using NO_3^- contaminated groundwater with low NO_3^- concentration (USA)	>3 d	>90	-	Misiti et al. (2011)
Bioreactor	Tile drainage with 10 mg NO_3-N/L (USA)	2.1 d 9.8 d	30 100	-	Greenan et al. (2009)
Experimental study	Treatment of river water polluted by wastewater treatment effluent (China)	1.5 d	70	-	Zhao et al. (2011)
Constructed wetland	Synthetic groundwater with 30 mg NO_3-N/L (USA)	1 d 3 d 7 d	82 98 88	from 30	Robins et al. (2000)
Continuous vertical upflow	Groundwater from lake receiving non-point NO_3^- (China)	120 h	-	from 100 to 1 mg/L	Qian et al. (2018)

2.9 Thesis scope

Wetland NO_3^- reduction processes such as denitrification, is a function of wetland sediment and hydrological properties that are spatially and temporarily variable. While previous seepage wetland studies have laid important scientific foundations and have recognised that these wetlands can be NO_3^- sinks, several existing knowledge gaps (listed in sections 2.7.1.3 and 2.7.2.3) due to the previous research limitations fall short in providing a comprehensive understanding of seepage wetland characteristics and spatial and temporal variations. For instance, the previous studies, mostly single-site based, were restricted to a single geographical location in NZ and were limited in research timeframes, whereas this knowledge is integral for the integration and utilisation of seepage wetlands for NO_3^- mitigation in the pastoral hill country landscapes. Such a knowledge gap currently prevents the enhanced and widespread application of a naturally occurring NO_3^- mitigation tool, i.e., seepage wetlands.

As such, this thesis will characterise the spatial gradient in seepage wetlands sediment denitrification capacities by quantifying denitrification enzyme activity (DEA) of a range of NZ seepage wetlands using the previously used acetylene inhibition technique to allow comparisons with previous studies. This thesis will identify the sediment properties that regulate spatial variation of DEA in seepage wetlands. This study will also characterise the surface and subsurface hydrology of a representative wetland over longer temporal scale than previously studied. Finally, the thesis will explore drainage manipulation opportunities to enhance NO_3^- removal in seepage wetlands, and potentially improve water quality leaving hill country farms.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Suha Sanwar
Name and title of main supervisor:	Dr. Lucy Burkitt
In which chapter is the manuscript/published work?	chapter 3
What percentage of the manuscript/published work was contributed by the student?	85%

Describe the contribution that the student has made to the manuscript/published work:

Suha assisted in designing this study and undertook all the field sampling and laboratory analysis. She statistically analysed the data, compiled all graphs and tables and drafted the manuscript with the support from supervisors.

Please select one of the following three options:

◯ **The manuscript/published work is published or in press**

Please provide the full reference of the research output:

◯ **The manuscript is currently under review for publication**

Please provide the name of the journal:

◉ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	SUHA SANWAR Digitally signed by SUHA SANWAR Date: 2023.04.03 13:21:44 +12'00'	Main supervisor's signature:	Lucy Burkitt Digitally signed by Lucy Burkitt DN: cn=Lucy Burkitt, c=NZ, o=Massey University, ou=Farmed Landscapes Research Centre, email=L.Burkitt@massey.ac.nz Date: 2023.04.03 12:27:51 +12'00'

This form should be placed at the beginning of each relevant thesis chapter.

Chapter 3: Spatial gradients in denitrification and sediment characteristics in pastoral hill country seepage wetlands

3.1 Introduction

Pastoral nitrate (NO_3^-) loss is a major water quality issue in many countries including New Zealand (NZ) where pastoral production is a major land use (Bijay & Craswell, 2021; Ferrier et al., 2001; MfE & Stats NZ, 2021). Hill country used predominantly for beef and sheep pastoral farming, is a unique landscape feature in NZ that is interspersed with naturally occurring seepage wetlands located between gullies at the valley bottoms (Johnson & Gerbeaux, 2004). Seepage wetlands are characterised by their saturated and organic matter-rich sediments, and are located at the convergence of the surface and subsurface NO_3^- flow pathways from the surrounding pastures, creating a denitrification hotspot in the pastoral hill landscape (Nguyen & Downes, 1997; Rutherford & Nguyen, 2004; Vidon & Hill, 2004a). However, wetland denitrification is site-specific (Dhondt et al., 2004) and is influenced by the heterogeneity of sediment properties (Moon et al., 2020; Ribas et al., 2017; Wall et al., 2005; Wu et al., 2021). Despite the critical need to understand the spatial variation in denitrification, our current knowledge is extremely limited in the pastoral hill country seepage wetlands in NZ.

Denitrification is a microbe-mediated NO_3^- reduction process in which NO_3^- is used by denitrifiers as electron acceptors for respiration under oxygen-limited conditions (Tiedje, 1983). During this process, NO_3^- is reduced to dinitrogen gas (N_2) in the presence of electron donors (dissolved organic carbon (DOC), dissolved iron (Fe^{2+}) and dissolved manganese (Mn^{2+})) (Hedin et al., 1998; Tiedje, 1983). Denitrification is a major and also a preferred pathway for NO_3^- removal (Saunders & Kalff, 2001; Vidon & Hill, 2004a) in pastoral wetlands, because in contrast to the other NO_3^- removal processes (e.g. plant and microbial uptake, dissimilatory NO_3^- reduction to ammonium (NH_4^+) called DNRA)), where NO_3^- retention is only temporary, denitrification can return NO_3^- to the inert, atmospheric gaseous N_2 form. However, denitrification is a stepwise process in which, depending on the wetland sediment environment, NO_3^- reduction can terminate at an intermediate stage and can result in nitrous oxide (N_2O) as the end-product which is a potent greenhouse gas (Hefting et al., 2003; Tiedje, 1983).

Sediment properties influence denitrification capacity in wetlands by defining the biogeochemical environment (Attard et al., 2011; Saggar et al., 2013; Wall et al., 2005; Wang et al., 2021). Major sediment physicochemical and chemical properties that influence denitrification in the wetland are redox potential (Eh), water content (WC), pH, DOC (major electron donor), alternative electron donors (dissolved Fe^{2+} and Mn^{2+}), electron acceptor (NO_3^-), C and N compounds and parameters that include NH_4^+, total nitrogen (TN), total carbon (TC) and C:N (Deng et al., 2020; Knowles, 1982; Sirivedhin & Gray, 2006; Wang et al., 2021). For example, a low sediment redox potential (Eh<300 mV) is necessary to trigger the denitrification process (Reddy & DeLaune, 2008; Van Cleemput et al., 2007). High WC in sediments e.g., in a saturated condition, facilitates the necessary oxygen limitation and establishes the continuum between the denitrification substrates (electron donor and acceptors) and denitrification hotspot microsites. In contrast, low WC can interrupt these functions and limit denitrification (Li et al., 2022). A positive influence of WC on denitrification enzyme activity (DEA), a parameter used to measure the sediment denitrification capacity, has been demonstrated in a previously limited anaerobic NZ pastoral soil, where the DEA accelerated after the soil was saturated (Luo et al., 1999a). A 60% water-filled pore space (WFPS) is considered a threshold for denitrification and as the WFPS increases, denitrification becomes more complete towards N_2-generation, as the major denitrification product shifts from N_2O at 70-80% WFPS to N_2 at around 90% WFPS (Weier et al., 1993). The DEA also generally accelerates with increased supplies of the denitrification substrate i.e., NO_3^- and electron donor supplies (Ribas et al., 2017). For example, the generally high DEA at the surface of wetlands is attributed to the NO_3^- received in pastoral drainage in addition to their generally high WC and organic carbon (OC) concentrations (Ha et al., 2015). In contrast, low denitrifier activities due to low NO_3^- and OC supplies, drive often exponentially lower denitrification activities in subsurface depths (Jahangir et al., 2012; Ribas et al., 2017). However, alternative electron donors such as dissolved Fe^{2+} and Mn^{2+} can fuel denitrification when DOC is limited such as in the subsurface depths. Relict C can also drive high DEA at subsurface depths (Barkle et al., 2007; Green et al., 2008; Song et al., 2016; Stenger et al., 2018). A pastoral hill country seepage wetland study has shown positive influences of dissolved Mn^{2+} and DOC on DEA (Chibuike et al., 2019). However, the influence of sediment properties on denitrification is often non-linear due to interferences from other sediment properties. For example, when NO_3^- is excessive and under acidic conditions, denitrification can terminate at N_2O generation because further denitrification to N_2 becomes less energy efficient (Hefting et al., 2003; Verhoeven et al., 2006; Weier et al., 1993). Conversely, when NO_3^- is low, highly reduced and labile C-rich sediments can drive NO_3^- reduction towards DNRA processes in which the denitrification intermediate products of NO_3^- or N_2O are reduced to NH_4^+. This process is more energy efficient, compared to the denitrification process (Bohrerova et al., 2004; De Laune et al., 1981). These examples demonstrate the strong influences of the sediment physicochemical and chemical properties on the denitrification outcome and its spatial gradients. Numerous studies have also recorded the associations between the vertical and horizontal spatial gradients of denitrification and sediment properties, in different wetlands, and based on which, have recommended sediment properties as important proxies for DEA (Giles et al., 2012; Luo et al., 1998; Wu et al., 2021).

Sediment characterisation is a powerful tool for improving our understanding of the spatial gradients of sediment denitrification capacity in wetlands. This is because sediment properties associate to multiple factors that affect denitrification at different scales, from macro (e.g., lithology, land use) to micro (denitrifier abundance) scales (Anderson et al., 2015; Han et al., 2017; Martínez-Espinosa et al., 2021; Xiong et al., 2015; Zhong et al., 2016). On a macro-spatial scale, wetland sediments derive their physicochemical and chemical properties from the long-term geological (baserock, topography, geographic position in the catchment) and hydrogeological (nutrient load and pulses, hydraulic conductivity, residence time) processes and land use activities (fertiliser application, grazing management) that drive the site-specificity of denitrification capacity (Liu et al., 2018; Veraart et al., 2017; Xiong et al., 2017). At the micro-scale, the sediment environment shapes the denitrifier composition and abundance by influencing substrate availability e.g., electron donor and pH. Many studies have found sediment properties, compared to denitrifier genes, as more reliable

DEA-predictors (Liu et al., 2018; Veraart et al., 2017; Xiong et al., 2017). Yet, while the role of sediment properties as DEA indicators is generally well established, sediment properties in pastoral hill seepage wetlands in NZ and their relationship to DEA remain poorly quantified.

There is general acceptance that seepage wetlands act as denitrification hotspots (McKergow et al., 2016; Rutherford et al., 2018), as several studies have attributed NO_3^- removal at hill country seepage wetlands mainly to sediment DEA (Burns & Nguyen, 2002; Rutherford & Nguyen, 2004). However, detailed studies of sediment properties that influence denitrification and their spatial gradients in pastoral hill country seepage wetlands in NZ largely remain absent. Among the few studies that have examined the sediment drivers in denitrification in pastoral hill seepage wetlands, Chibuike (2019) attributed the high denitrification capacity observed in the seepage wetland to its high sediment WC, DOC, NH_4^+ and dissolved Mn^{2+} concentrations and reported a lack of correlation between DEA and sediment NO_3^- and dissolved Fe^{2+}, from 1 m long wetland sediment columns. A separate NZ study measured DEA and sediment TC, TN, C:N, NH_4^+ and NO_3^- in the microbially active surface 0-25 cm depth of a wetland (Nguyen & Downes, 1997), whereas subsurface redox assessments have measured high denitrification even at subsurface depths >1 m, being fuelled by relict C or alternate electron donors, in NZ hill pasture (Barkle et al., 2007). These sporadic studies of denitrification in hill country seepage wetlands are limited due to 1) the small number of studies, 2) single-site based studies, and 3) differences in the depth intervals studied. Research examining the spatial variability of sediment characteristics and DEA in pastoral hill country wetlands is much less common. This current knowledge gap limits the potential for DEA prediction modelling between wetlands, an approach which would be helpful to improve NO_3^- management and enhance attenuation capacity across hill country landscapes.

This study characterises the spatial gradients in sediment properties and their denitrification capacities in four hill country seepage wetlands formed on two contrasting baserocks, in the lower North Island in NZ. The objectives of this study are to 1) quantify the sediment physicochemical properties and assess their spatial gradients vertically (within sediment column), and horizontally (within- and between-wetlands), 2) quantify the sediment denitrification capacities, and 3) determine the relationships between the sediment properties and the denitrification capacity.

3.2 Methodology

3.2.1 Study area

Four hill country seepage wetlands were selected as study sites which are located on two different baserocks – greywacke (Tuapaka and Ballantrae sites) and silt/mudstone (Rathmoy and Wairiri sites) in the lower North Island in NZ (Figures 3-1, 3-2, Table 3-1).

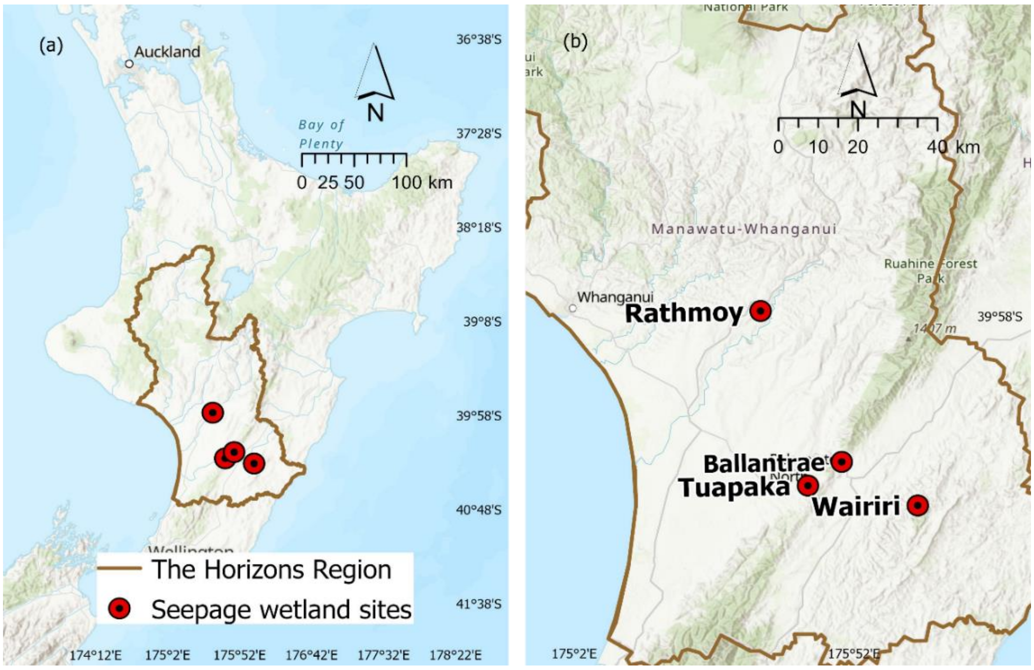


Figure 3-1. (a) The Horizons Regional Council jurisdiction, (b) seepage wetland study sites located across the Horizons Regional Council, Lower North Island, New Zealand.

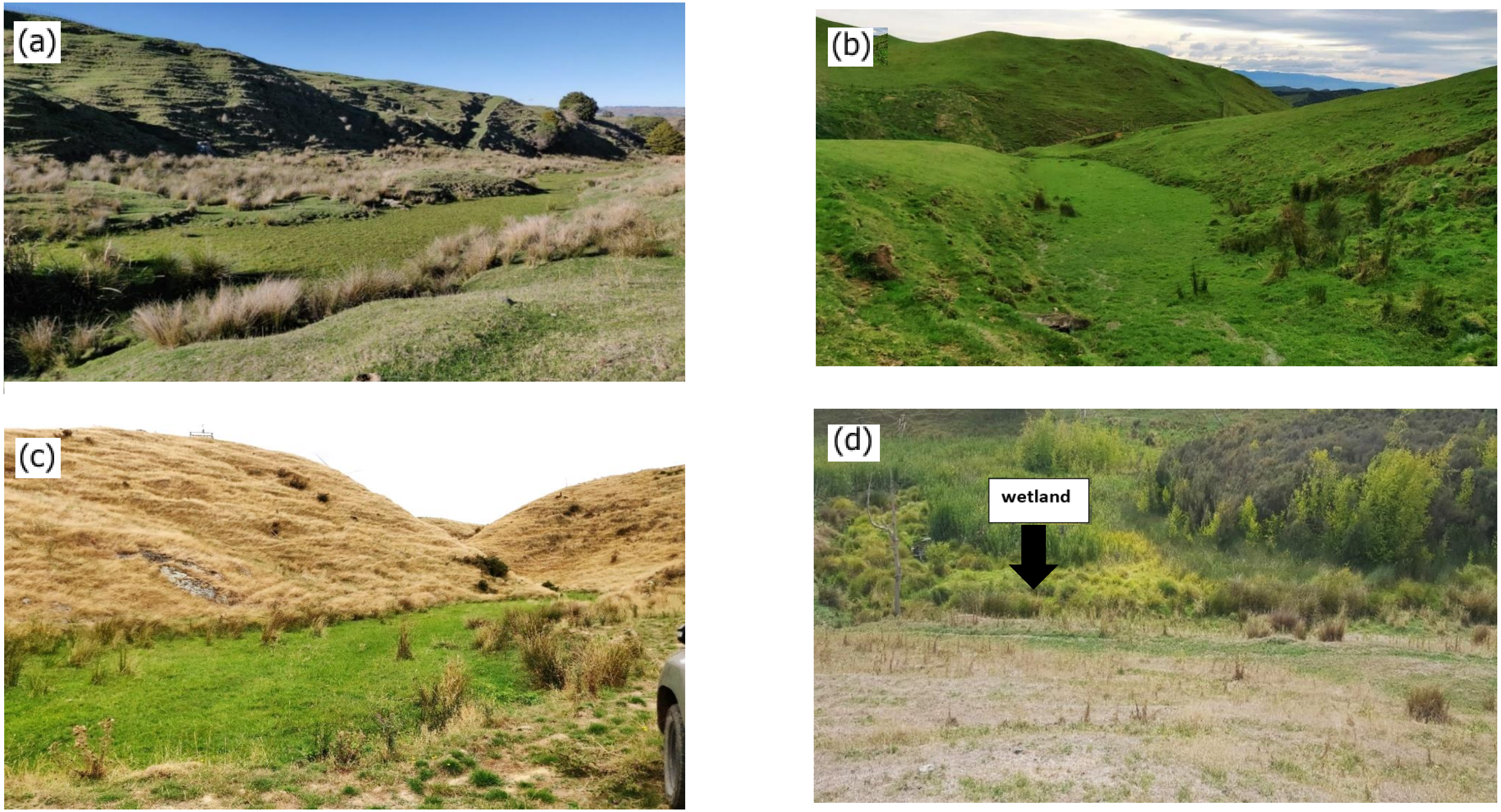


Figure 3-2. The (a) Rathmoy, (b) Wairiri, (c) Tuapaka, and (d) Ballantrae seepage wetland sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand).

Table 3-1. Seepage wetland study site descriptions with wetland area in parentheses.

Baserock[a], Soil series[b]	Soil drainage class[b]	Mean long-term annual precipitation (mm)	Location	Elevation above sea level[h] (m)
		Rathmoy site (0.92 ha)		
Siltstone, Huntersville series	Well-drained	937[d]	39°56'51.14" S, 175°36’18.84" E	357
		Wairiri site (0.1 ha)		
Greywacke, Korokoro series	Well-drained	1310[e]	40°24’10 S, 176°03’37” E	450
		Tuapaka site (0.08 ha)		
Greywacke, Makara steepland series[c]	Imperfect	1100[f]	40°21’11.8” S, 175°44’14.1” E	263
		Ballantrae site (0.09 ha)		
Mudstone, Maharahara series	Imperfect	1270[g]	40°17'58.19" S, 175°50’17.67" E	192

Sources: [a]Landcare Research (2010b), [b]Landcare Research (2010a), [c]Pollok and McLaughlin (1986), [d]NIWA (2022), [e]Farmer records, [f]Fransen et al. (2022), [g]Hoogendoorn et al. (2016), [h]Google map (2021)

3.2.2 Sampling technique

Intact, half-cylindrical wetland sediment columns (33 cm long and 7.5 cm diameter) (photo shown in Appendix 3-1) were sampled up to a depth of 1 m, where possible, based on the reported shallow wetland depth in previous seepage wetland studies (McKergow et al., 2016; Rutherford et al., 2018; Rutherford & Nguyen, 2004). Triplicate cores were sampled from three positions (inflow, midflow and outflow as in Figure 3-3) across the surface water flowline, at four seepage wetland sites (Figures. 3-2). Water depth above the wetland sediment surface was >5 cm during the sampling, suggesting saturated sediment condition. At each sampling position, cores were sampled, within 0.5 m of each other. The sediment column was cored using a hand-driven D-corer and sampled in several sections to minimise sediment compression (Appendix 3-1). As an exception, the unconsolidated sediments at the Ballantrae site required samples to be taken using 1 m aluminium tubes (7.2 cm diameter). The sampled sediment columns were sectioned into the depth intervals of 0-15, 15-30, 30-45, 45-60, 60-75 and 75-100 cm, placed into ziplock plastic bags, transported in chilled condition to the laboratory and stored at 4°C until chemical analysis. The sites were sampled between June and November in 2020 which included a gap due to Covid travel restrictions.

Child's tests (Childs, 1981) were conducted along the length of freshly sampled sediment cores to assess redox conditions. Blue-grey coloured gley soil was observed at the 40-60 cm depths of cores from all sites, except Ballantrae. Similar findings have been reported at the same depth ranges in pastoral hill country seepage wetlands (Rutherford & Nguyen, 2004). Fragments of wood debris, likely as a result of the conversion from native vegetative cover to pastoral cover during the early 1900s (Knight, 2013), were visible at depths of >75 cm, particularly at the Tuapaka site.

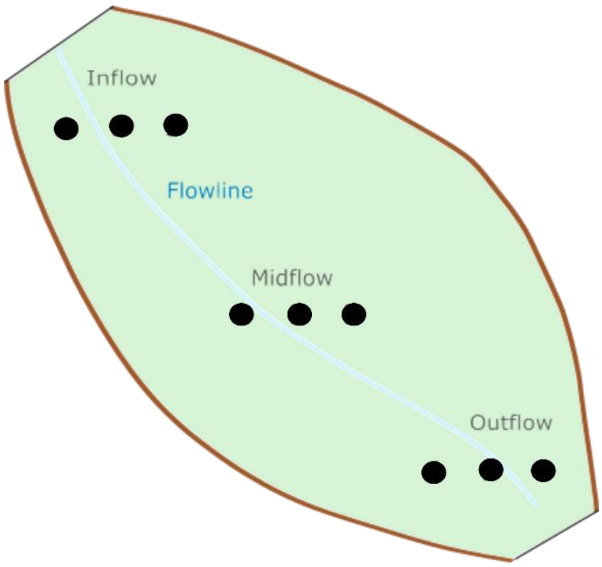


Figure 3-3. Sediment column coring positions (triplicate cores marked with circles) at the inflow, midflow and outflow of the seepage wetland study sites.

3.2.3 Laboratory analysis

3.2.3.1 Sediment subsampling

Within 3-5 days of collection from the field, the column depth samples were N_2-flushed to minimise oxygen contamination and were homogenised before subsampling. These field moist samples were then subsampled for physiochemical, electron donors, N species, DEA and water content (WC), with more detailed methods provided below. The DOC analysis and the DEA incubations were conducted within 3-10 days of sample collection.

3.2.3.2 Physico-chemical and chemical property analysis

For the physicochemical (pH, Eh, EC) and electron donor (DOC, dissolved Fe^{2+} and Mn^{2+}) analysis, stepwise sediment extraction was conducted in which 15 g of sediment was extracted with 37.5 mL of deionized water (at 1:2.5 wt/v) and shaken on a rotatory shaker for 1 h in 50 mL extraction tubes at room temperature. The extracts were centrifuged at 5000 rpm for 20 min at 20°C. A 25 mL sample of this extract was analysed for pH, Eh and EC. The rest of the extract was centrifuged again at 5000 rpm for 2 h, to separate particulate organic matter (in preference to 0.45 µm filtration) (Chibuike, 2019). The subsequent supernatant extract was preserved for 1) DOC (stored at 4°C) and 2) dissolved Fe^{2+} and Mn^{2+} (preserved in 2% HNO_3, at 20° C) analysis.

The pH and the Eh of the sediment extract were measured using a pH and Eh meter (Meter Lab®). The EC was determined on an EC meter (Hanna Instruments). The DOC concentration was determined using the dry combustion method using a total organic carbon (TOC) analyser (Shimadzu TOC-L). The dissolved Fe^{2+} and Mn^{2+} concentrations were determined on a 4200 Microwave Plasma-Atomic Emission Spectrometer – MPAES (Agilent Technologies).

Sediment NO_3-N and NH_4-N were extracted by adding 3 g to 30 mL of KCl (1:10 wt/v) and shaking on a rotatory shaker for 1 h at 20°C in 30 mL oak ridge bottles. Extracts were then filtered (Whatman filter paper No. 41) and preserved at <4°C until analysis. Nitrate and NH_4^+ concentrations were determined using the continuous flow analysis on Technicon® AutoAnalyser II.

Sub-samples for %TC and %TN analysis was air-dried. The %TC and %TN of air-dried samples (homogenised and sieved through 0.05 mm sieve) were analysed on an Elementar®/vario MICRO cube.

The gravimetric moisture content of the sediment was measured by oven-drying sediment (105°C for 16 h) of known weight. The measured moisture content was also used to express the sediment nutrient concentrations on an oven-dry sediment (DS) mass basis.

The sediment water content (WC) in this study represents the percentage of water content in the fresh sediments calculated as:

((Fresh sediment sample weight (g) – Oven-dry sediment weight (g)) / Fresh sediment sample weight (g)) x 100 (Eq. 3-1)

3.2.3.3 Denitrification enzyme activity (DEA)

Sediment denitrification capacity was determined by quantifying the DEA via the acetylene-inhibited anaerobic incubation technique (Tiedje et al., 1989; Yoshinari et al., 1977) with modifications of using the vacuum pouch method as detailed by Rivas et al. (2014). The DEA incubation was conducted on 20 gm of fresh sediment inside heat-sealed vacuum pouches. The sealed vacuum pouch was flushed with 50 mL of N_2, followed by consecutive additions of 20 mL of acetylene, 20 mL of DEA solution (50 µg NO_3^- g^{-1} DS + 10 ppm chloramphenicol) and 180 mL of N_2, into the pouch via a luer-lock valve. The addition of NO_3^- means that the DEA referred to in this thesis represents the 'potential' DEA. The addition of acetylene arrests N_2 production in denitrification and generates N_2O as an end-product. It allows sampling of this gas product as an alternative to N_2, avoiding contamination due to the ubiquitous presence of N_2 in the atmosphere. The enzyme inhibitor chloramphenicol, prevents *de novo* synthesis of enzymes during incubation (Dendooven et al., 1994). The pouches were incubated in the dark at 20°C, while rotating on a mechanical shaker (160 rpm) for 6 hours. The nitrous oxide (N_2O) gas was sampled from the pouches at 2-hr intervals, sampling at time 0 (initial gas sampling before incubation), 2, 4, and 6 hrs. Each 25 mL sample of N_2O gas was compressed into 12 mL vac-vials for N_2O-analysis on a Shimadzu Gas Chromatograph (GC) 17 A (Japan) equipped with a 63Ni electron capture detector and operating at a column and detector temperature of 55 and 330°C, respectively.

3.2.3.4 Quality control

Quality control protocols were maintained during the laboratory analyses, including the use of reference samples, sample duplicates, and blank samples. Triplicate analysis for a randomly selected depth interval in each sediment column was conducted for analytical precision. Chemical analyses were spiked with reference standards at regular intervals to check for instrument consistency. Triplicate blank analyses were conducted for each parameter investigated. Each batch of analyses incorporated separate calibration and instrumental consistency monitoring.

3.2.4 Data analysis

Mean values were compared between depths at each site, by using ANOVA with Tukey analysis, to assess the vertical gradient of the measured variables within sediment columns. ANOVA with Tukey analysis was also conducted to compare the mean values of the measured variables of all depths between the wetland positions to assess the horizontal gradient in sediment properties within the wetlands. The level of confidence interval for all statistical analyses reported in this study was set at 95% i.e., $p \leq 0.05$. Data was normalized where necessary for analysis of one-way variance (ANOVA) with Tukey's comparison procedure. Correlations between the measured variables were analysed by Spearman's rank correlation technique.

Principal component analysis (PCA) was conducted, by using the PCA package in R, in order to identify major sediment properties that define the sediment environment in which denitrification occurs, for each investigated depth interval in the study sites. The sediment variables that align closer along the principal components (i.e., PC1 and PC2) in the PCA biplot contribute the most to the associated sediment environment and, thus, to the corresponding DEA. Sediment variables with eigenvalues>1 significantly contribute to the dimensions in the PCA biplots and were identified for the first four components in the PCA analyses in the study sites.

The statistical analyses in this study were performed with the software Minitab (version 19.1.1) and R (RStudio 1.2.5033).

3.3 Results

3.3.1 Vertical gradients in seepage wetland sediment properties and DEA

3.3.1.1 Redox and associated sediment

The vertical changes in sediment properties are presented as means across the three sampling positions. However, it is acknowledged (Section 3.4.3) that selected horizontal variations in sediment properties were significantly ($p<0.05$) higher at the outflow and midflow sampling positions at Tuapaka and Ballantrae, respectively.

The pastoral hill country seepage wetland sediments across the four study sites were moderately reduced (Eh: -15 to 95 mV) and moderately acidic (pH: 4.5-6.7) (Table 3-2), with moderate to high WC (43-73%). Mean WC values were higher at the Rathmoy (77.0%) and the Wairiri (67.4%) sites, compared to the Tuapaka (46.7%) and the Ballantrae (33.87%) sites. The 0-15 cm depth, across the study sites, was less reduced (Eh 29.6 mV, $p\leq0.05$) and more acidic (mean pH 5.8, $p\leq0.05$) in general, compared to the deeper >15 cm depths.

The Eh and WC measurements were positively correlated with each other, and both decreased down the sediment column, while pH increased (Table 3-2, Figure 3-4), across the study sites. A strong negative correlation between Eh and pH was present ($r=-0.9$, $p<0.001$) in the current study (Table 3-3). Significantly lower Eh (19.5 mV) and significantly higher pH (6.0) were measured at the 30-45 cm depth ($p\leq0.05$), compared to the other depths across the study sites.

Table 3-2. Descriptive statistics of the sediment physicochemical and chemical properties in sediment column depth intervals at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). Letters next to the mean (±SD) values indicate the statistical differences between different depths within a study site significant at p≤0.05.

Depth (cm)	pH		Eh (mV)		EC (µS/cm)		C:N	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
				Rathmoy				
0-15	5.1-6.2	5.8±0.4[a]	5.8-61.9	29.6±21.1[a]	48.8-209.5	93.0±48.4[a]	12.7-16.5	15.2±1.2[a]
15-30	5.1-6.1	5.7±0.3[a]	14.8-64.3	30.9±14.6[a]	25.5-212.8	68.9±56.1[ab]	11.8-15.7	14.0±1.1[a]
30-45	5.7-6.4	6.0±0.2[a]	-5.7-33.4	18.9±10.5[a]	21.4-111.1	38.9±30.5[b]	11.5-15.9	13.5±1.2[a]
45-60	5.5-6.3	5.8±0.2[a]	3.7-91.5	30.4±20.2[a]	15.4-100.7	39.9±31.4[b]	11.4-15.7	12.8±1.2[a]
60-75	5.7-6.4	5.9±0.2[a]	-3.3-31	20.3±11.3[a]	7.87-88.7	27.3±23.8[b]	10.4-14.2	12.8±1.1[a]
75-100	5.8-6.3	6.0±0.2[a]	0.8-33.4	18.1±10.5[a]	16.3-28.1	20.4±4.2[b]	13.8-14.8	14.3±0.4[a]
				Wairiri				
0-15	5.6-6.0	5.8±0.1[b]	17.9-95	35.8±18.7[a]	17.3-62.1	34.5±14.0[a]	12.1-13.6	12.6±0.5[b]
15-30	5.5-6.0	5.8±0.1[b]	17.7-47.4	27.9±8.7[ab]	5.6-60	20.2±14.6[b]	12.2-13.3	12.7±0.4[b]
30-45	5.8-6.0	5.9±0.1[ab]	18.5-27.8	24.6±3.1[ab]	8.4-19.1	13.3±2.9[bc]	11.5-14.4	12.9±0.7[b]
45-60	5.7-6.0	5.8±0.1[b]	20.7-35.2	27.8±4.4[ab]	7.3-18.6	12.2±3.9[bc]	11.7-14.8	13.0±0.9[b]
60-75	5.5-6.0	5.7±0.2[b]	5.67-46.1	27.0±10.8[ab]	1.2-10.1	6.9±3.2[c]	12.8-15.9	14.1±1.1[a]
75-100	5.8-6.4	6.1±0.3[a]	-7.1-29.8	9.9±15.4[b]	2.3-8.2	4.4±2.6[bc]	13.1-14.3	13.5±0.6[ab]
				Tuapaka				
0-15	5.3-6.4	5.9±0.4[b]	2.2-61	29.3±20[a]	21.2-66.4	37.5±16.8[a]	10.7-13.0	11.9±0.7[b]
15-30	5.9-6.6	6.3±0.3[a]	-9.1-32.3	11.6±13.8[b]	18.8-74.2	38.1±17.5[a]	11.2-16.2	12.3±1.6[ab]
30-45	6.0-6.7	6.3±0.2[a]	-12.4-23.6	10.2±10.2[b]	12-42.2	24.5±9.2[a]	11.2-12.9	12.0±0.6[ab]
45-60	5.5-6.5	6.1±0.3[ab]	-0.2-48.7	18.3±13.6[ab]	5.18-45.5	14.2±10.1[a]	11.3-15.7	13.2±1.2[a]
60-75	5.8-6.0	6.1±0.2[ab]	5.6-32.6	20.9±10.5[ab]	6.4-30.5	16.7±8.8[a]	12.1-13.7	13.0±0.7[ab]
75-100	5.7-6.6	5.9±0.4[ab]	-4.55-39.6	26.8±18.7[ab]	17.1-41.9	23.5±10.4[a]	12.0-13.4	12.7±0.5[ab]
				Ballantrae				
0-15	5.5-6.4	5.9±0.3[b]	-5.8-44.9	22.3±16.0[a]	17.7-142.8	56.8±40.7[a]	8.1-13.3	10.6±1.5[a]
15-30	5.5-6.4	5.8±0.3[b]	1.8-41.8	24.5±14.4[a]	11.7-40.2	24.4±7.6[b]	7.8-11.0	9.8±1.0[a]
30-45	5.5-6.6	5.9±0.3[ab]	-15-41.1	22.6±14.9[a]	4.71-49.9	22.0±11.2[b]	8.1-10.5	9.6±0.8[ab]
45-60	5.6-6.3	5.9±0.2[ab]	1.5-40.8	24.6±12.5[a]	22.0-42.8	29.6±7.3[ab]	8.9-11.0	10.0±0.7[a]
60-75	6.2-6.5	6.4±0.1[a]	-3.2-7.8	2.2±4.9[a]	39.2-61.1	52.8±9.4[ab]	7.7-8.2	7.9±0.2[b]

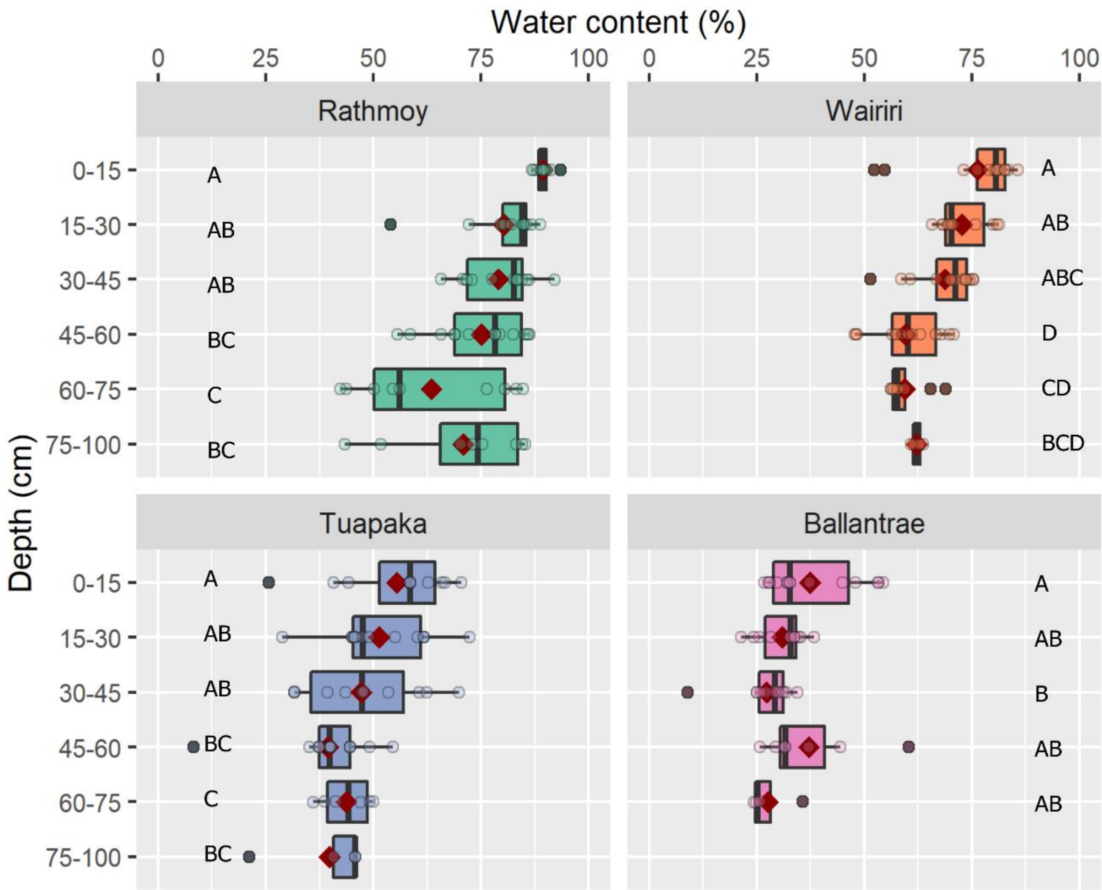


Figure 3-4. Boxplots showing sediment water content (%), expressed as percentage of fresh sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at p≤0.05.

Table 3-3. Spearman's rank correlation analysis of normalised sediment physicochemical and chemical properties at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council (Lower North Island in New Zealand). Bold values indicate significance at p≤0.05 (black), p≤0.01 (blue), p<0.001 (red).

	Sediment depth	Eh	pH	WC	EC	NO_3^-	NH_4^+	DOC	%TC	%TN	C:N	Fe^{2+}	Mn^{2+}
Eh	-0.13												
pH	**0.15**	**-0.93**											
WC	**-0.13**	**0.16**	**-0.18**										
EC	**-0.51**	-0.08	0.05	**0.15**									
NO_3^-	-0.12	**0.24**	**-0.30**	-0.12	0.02								
NH_4^+	0.02	0.00	0.01	0.02	**0.19**	-0.02							
DOC	**0.19**	0.02	0.06	**0.19**	**-0.26**	**0.30**	-0.11						
%TC	**-0.14**	**0.17**	**-0.17**	**0.91**	0.07	**0.74**	**-0.16**	**0.53**					
%TN	**-0.18**	**0.17**	**-0.17**	**0.90**	0.10	**0.75**	**-0.16**	**0.51**	**0.99**				
C:N	**0.18**	0.07	-0.04	**0.65**	**-0.17**	**0.42**	**-0.17**	**0.50**	**0.74**	**0.65**			
Fe^{2+}	0.01	0.10	-0.14	**0.25**	0.12	**0.39**	0.02	-0.01	**0.19**	**0.20**	0.01		
Mn^{2+}	0.09	0.11	**-0.17**	**0.32**	-0.07	**0.57**	-0.06	0.06	**0.27**	**0.29**	0.05	**0.79**	
DEA	**-0.61**	0.11	**-0.15**	**0.59**	**0.30**	**0.56**	-0.01	**0.20**	**0.57**	**0.59**	**0.23**	**0.15**	**0.18**

Eh = redox potential, WC = water content, EC = electrical conductivity, DOC = dissolved organic carbon, %TC = percentage total carbon, %TN= percentage total nitrogen, DEA = denitrification enzyme activity

3.3.1.2 DEA

The highest DEA values across the study sites were measured at the surface 0-15 cm depths, but varied widely between the sites. The mean surface DEA, on dry sediment (DS) mass basis, measured at Rathmoy (5371 µg N_2O-N kg^{-1} DS h^{-1}) and Wairiri (3868 µg N_2O-N kg^{-1} DS h^{-1}) were 7 to 10 times higher compared to the mean surface DEA values measured at Tuapaka (560 µg N_2O-N kg^{-1} DS h^{-1}) and Ballantrae (620 µg N_2O-N kg^{-1} DS h^{-1}) (Figure 3-5).

Based on the surface DEA, the study sites were categorised into 1) the high-performing H-DEA sites (mean surface DEA>3000 µg N_2O-N kg^{-1} DS h^{-1}) that included the Rathmoy and the Wairiri sites, and 2) the low-performing L-DEA sites (mean surface DEA <1000 µg N_2O-N kg^{-1} DS h^{-1}) which were the Tuapaka and the Ballantrae sites.

Across the study sites, DEA was highest in the surface 0-15cm depth (2496 µg N_2O-N kg^{-1} DS h^{-1}) and decreased sharply with increasing depth ($r=-0.5$, $p\leq0.05$) (Figure 3-5), to one-third of the surface depth DEA (819 µg N_2O-N kg^{-1} DS h^{-1}) at the 15-30 cm depth. Despite the sharp declines with increasing depth, the H-DEA sites continued to measure higher overall DEA values in the subsurface (>15 cm depth), compared to the L-DEA sites.

Between the H-DEA sites, the Wairiri site measured higher subsurface DEA (2099 µg N_2O-N kg^{-1} DS h^{-1} at the 15-30 cm depth) compared to that at the Rathmoy site (948 µg N_2O-N kg^{-1} DS h^{-1}). Thus, in terms of cumulative DEA at the surface 30 cm depths, DEA was higher at the Wairiri site, compared to the Rathmoy and remaining study sites.

Based on the Spearson's correlation ranking of the sediment properties (Table 3-3), DEA was positively correlated with WC, NO_3^-, DOC, TC, TN, C:N, dissolved Fe^{2+} and Mn^{2+} ($p\leq0.05$) concentrations and was negatively correlated with pH in the study sites.

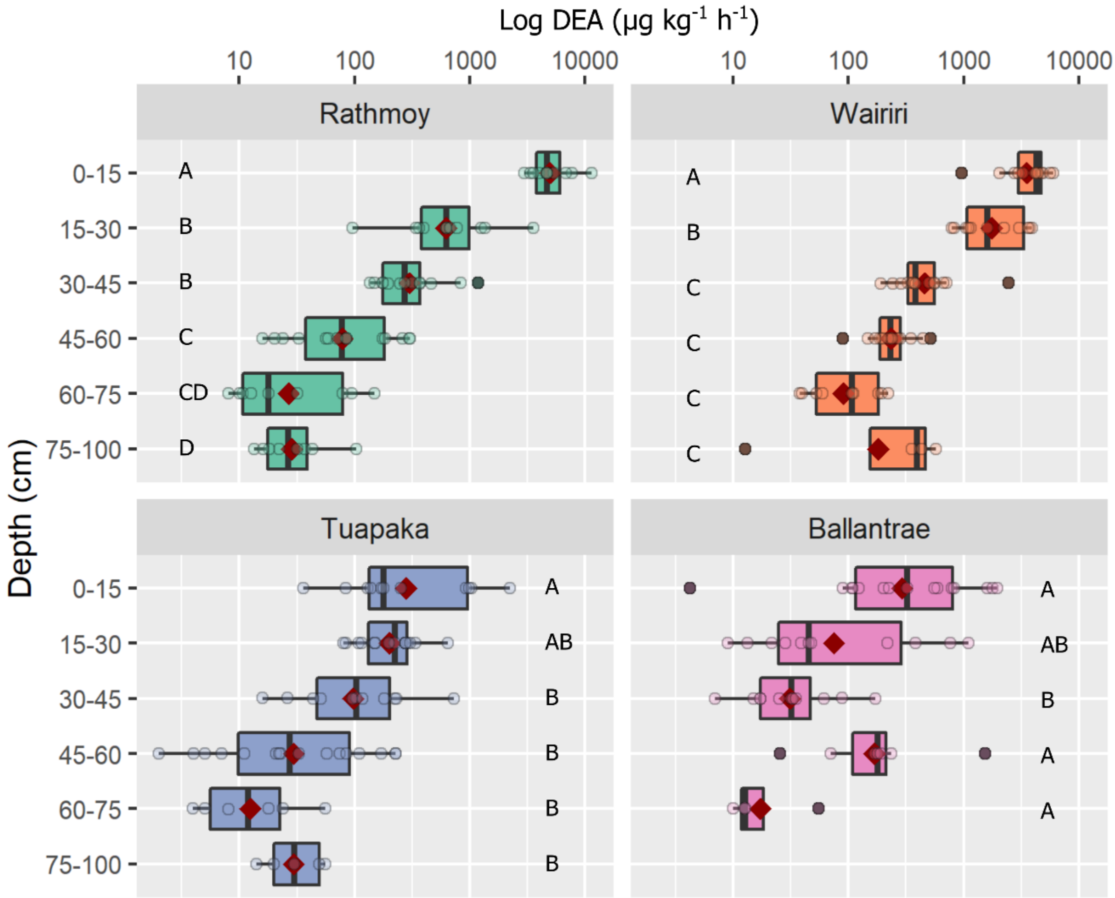


Figure 3-5. Boxplots showing sediment DEA (µg N_2O-N kg^{-1} DS h^{-1}), presented in log scale, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at p≤0.05.

3.3.1.3 Sediment chemical properties

3.3.1.3.1 NO_3^- and NH_4^+

The mean sediment NO_3^- concentrations were 6-7 times higher at the Rathmoy (15.9 mg NO_3-N/kg DS) and the Wairiri (18.5 mg NO_3-N/kg DS) sites compared to those at the Tuapaka (2.5 mg NO_3-N/kg DS) and the Ballantrae sites (3.97 mg NO_3-N/kg DS). Across the study sites, in general, the NO_3^- and the NH_4^+ concentrations contrasted each other where the highest mean NO_3^- concentration of 14.22 mg NO_3-N/kg DS (Figure 3-6) was measured at the surface 0-15 cm whereas there was a tendency of high NH_4^+ concentrations at the bottom ends of the sediment columns (Figure 3-7). Both NO_3^- and NH_4^+ tended to measure lower concentrations at the mid-column 30-45 and the 45-60 cm depths in the study sites ($p \leq 0.05$).

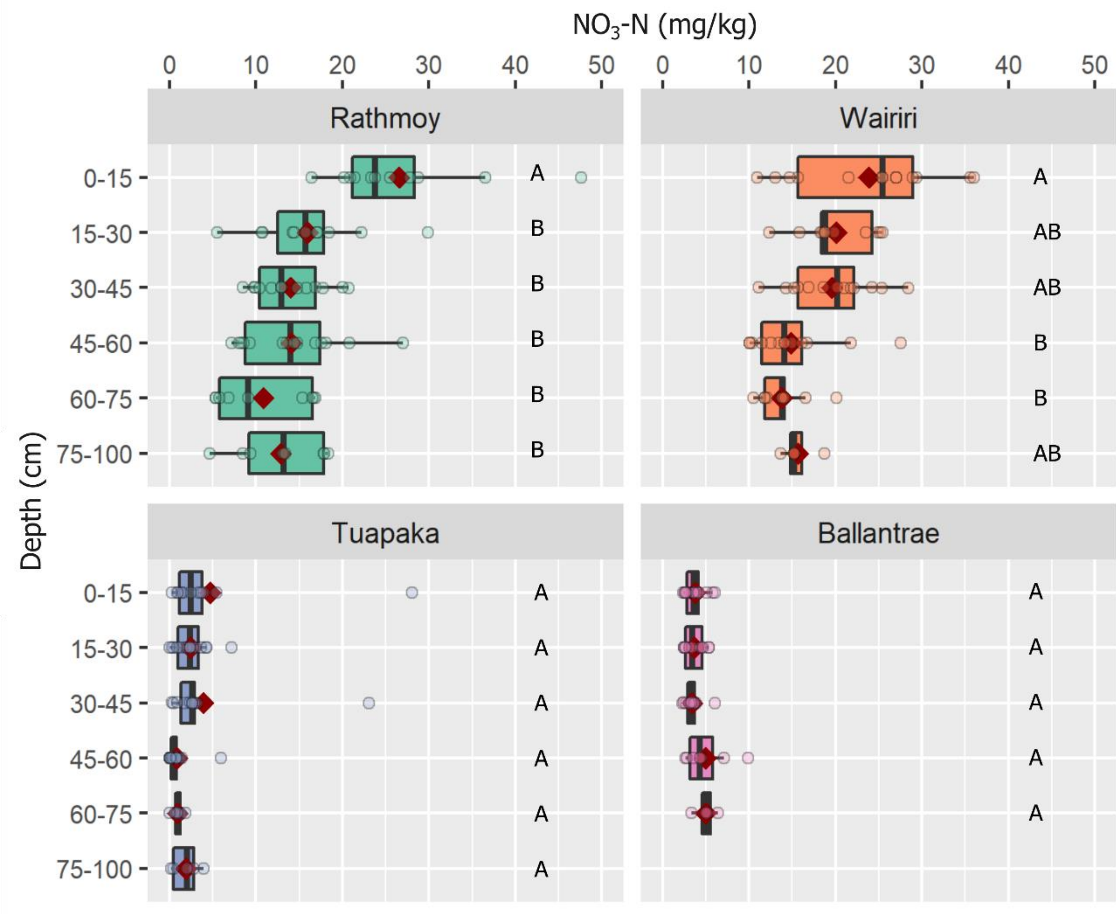


Figure 3-6. Boxplots showing sediment NO_3^- concentrations (mg NO_3-N/kg DS), on dry sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at $p \leq 0.05$.

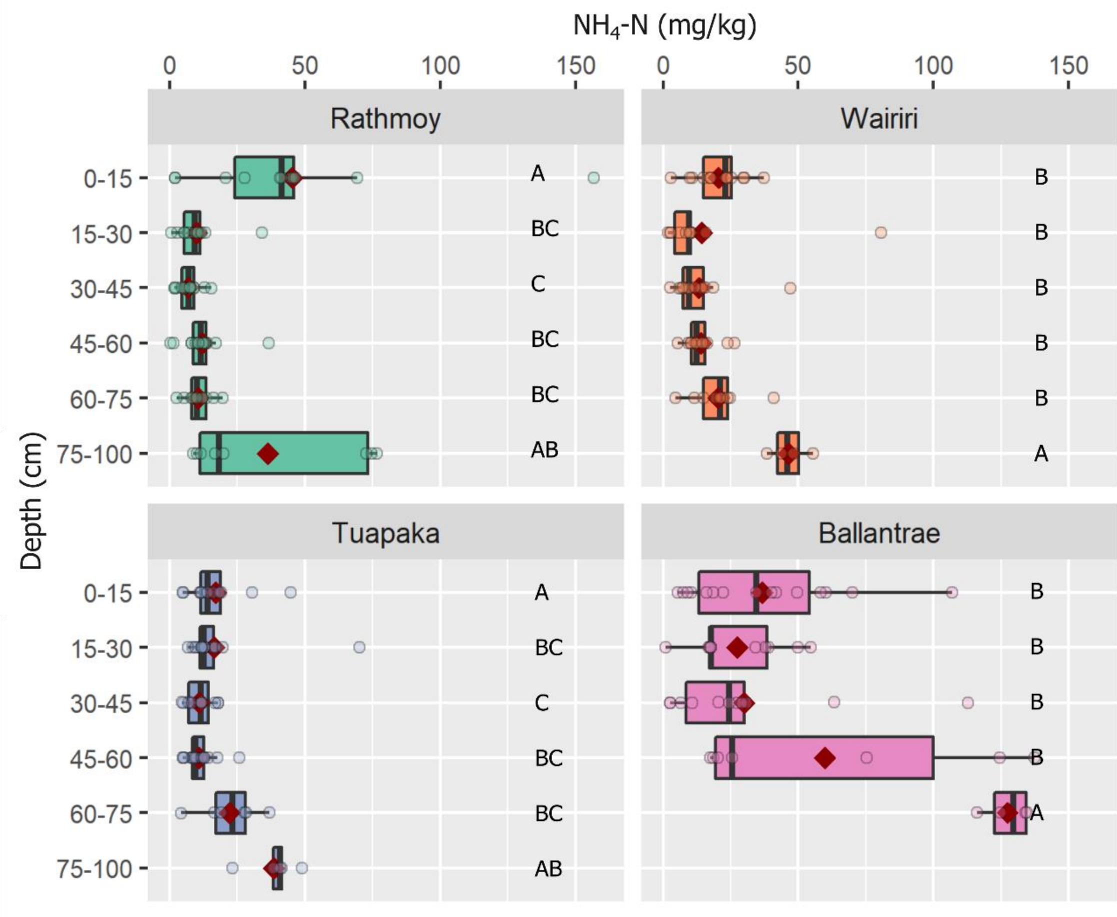


Figure 3-7. Boxplots showing sediment NH_4^+ concentrations (mg NH_4-N/kg DS), on dry sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at $p \leq 0.05$.

3.3.1.3.2 DOC

Overall, the mean DOC concentrations at the Tuapaka (580 mg/kg DS), Rathmoy (597 mg/kg) and Wairiri (711 mg/kg DS) sites were 5-6 times higher than those measured at the Ballantrae site (121 mg/kg DS). DOC accumulations, based on the high DOC values, were observed at the 60-75 and 75-100 cm depths at the Tuapaka (954 mg/kg DS; r=0.4, p≤0.05) and the Ballantrae sites (261 mg/kg DS; r=0.6, p≤0.05). In contrast, a significantly higher DOC concentration was measured at the 15-30 cm depth at the Wairiri site compared to the other depths at that site, while the Rathmoy site had a consistent DOC concentration throughout the sediment column (p>0.05) (Figure 3-8).

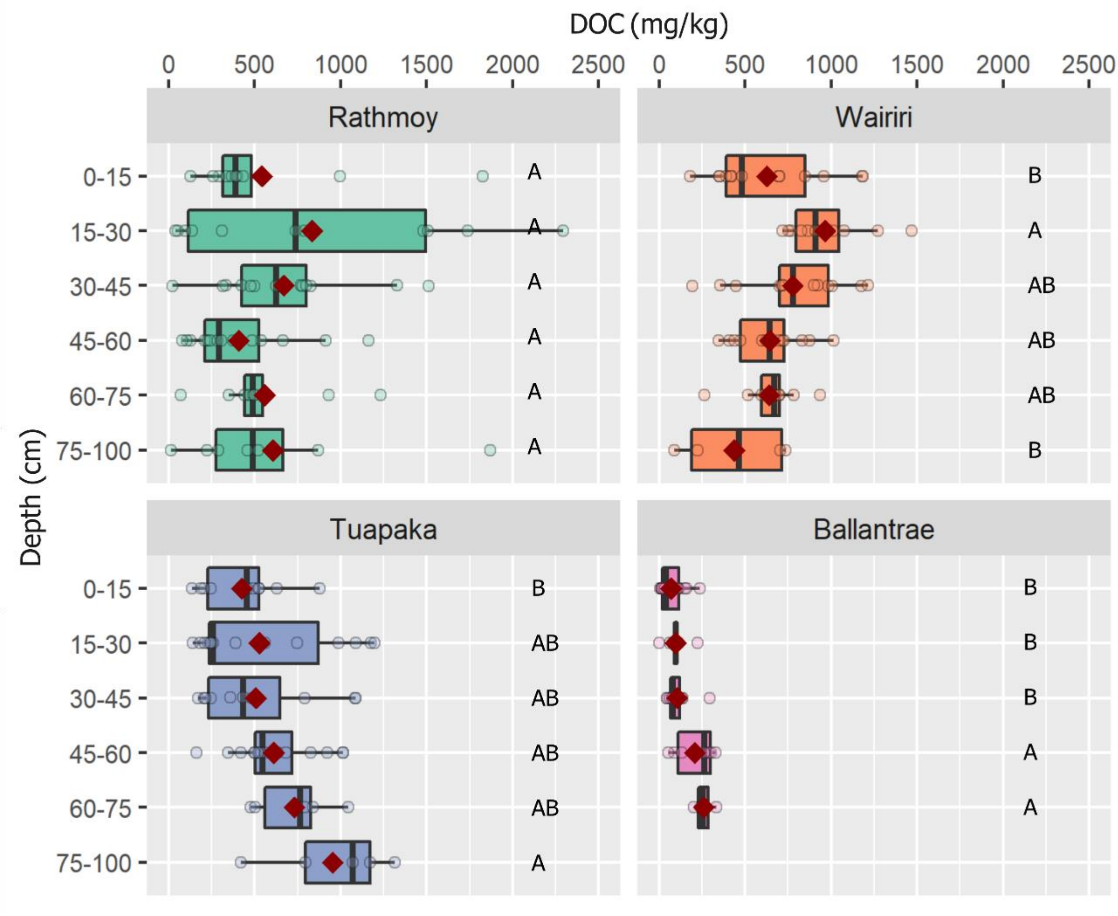


Figure 3-8. Boxplots showing sediment DOC concentrations (mg/kg DS), on dry sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at p≤0.05.

3.3.1.3.3 Dissolved Fe^{2+} and dissolved Mn^{2+}

The mean dissolved Fe^{2+} and Mn^{2+} concentrations (alternative electron donors in denitrification) were several magnitudes lower at Tuapaka (10.1 mg Fe^{2+}/kg DS and 1.7 mg Mn^{2+}/kg DS), compared to the other sites (36.4-44.6 mg Fe^{2+}/kg DS and 39.4-59.6 mg Mn^{2+}/kg DS) (Figures 3-9, 3-10). Across the study sites, the dissolved Fe^{2+} and Mn^{2+} highly positively correlated with each other ($r=0.7$, $p<0.001$). However, while Mn^{2+} concentration was positively correlated with depth across the study sites ($r=0.2$, $p\leq0.05$), the Fe^{2+} concentration was not. When all sites were compared, a higher mean

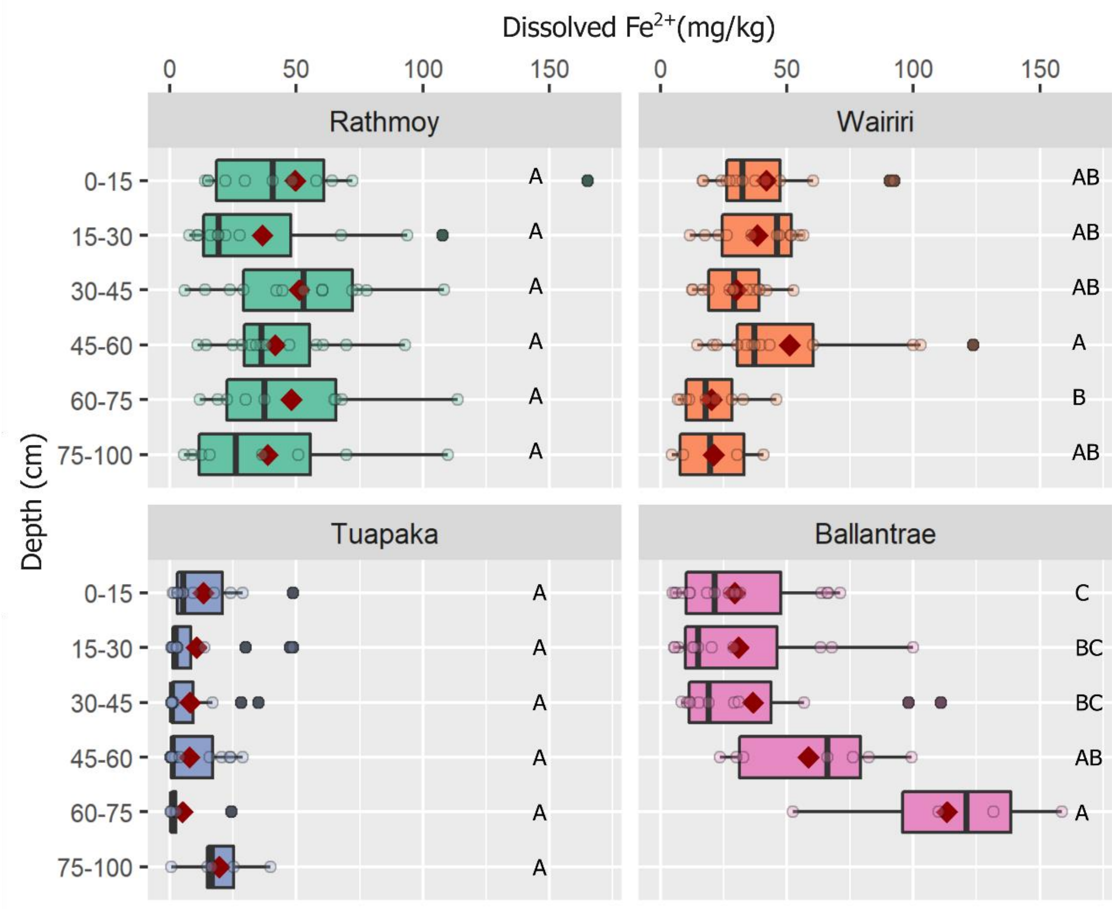


Figure 3-9. Boxplots showing sediment dissolved Fe^{2+} concentrations (mg/kg DS), on dry sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at p≤0.05.

Mn^{2+} concentration (51.1 mg/kg DS) was measured at the 45-60 cm depths. The Mn^{2+} concentration correlated positively with DEA at Tuapaka ($r=0.7$, $p<0.001$), but correlated negatively at Rathmoy ($r=-0.2$, $p\leq0.05$).

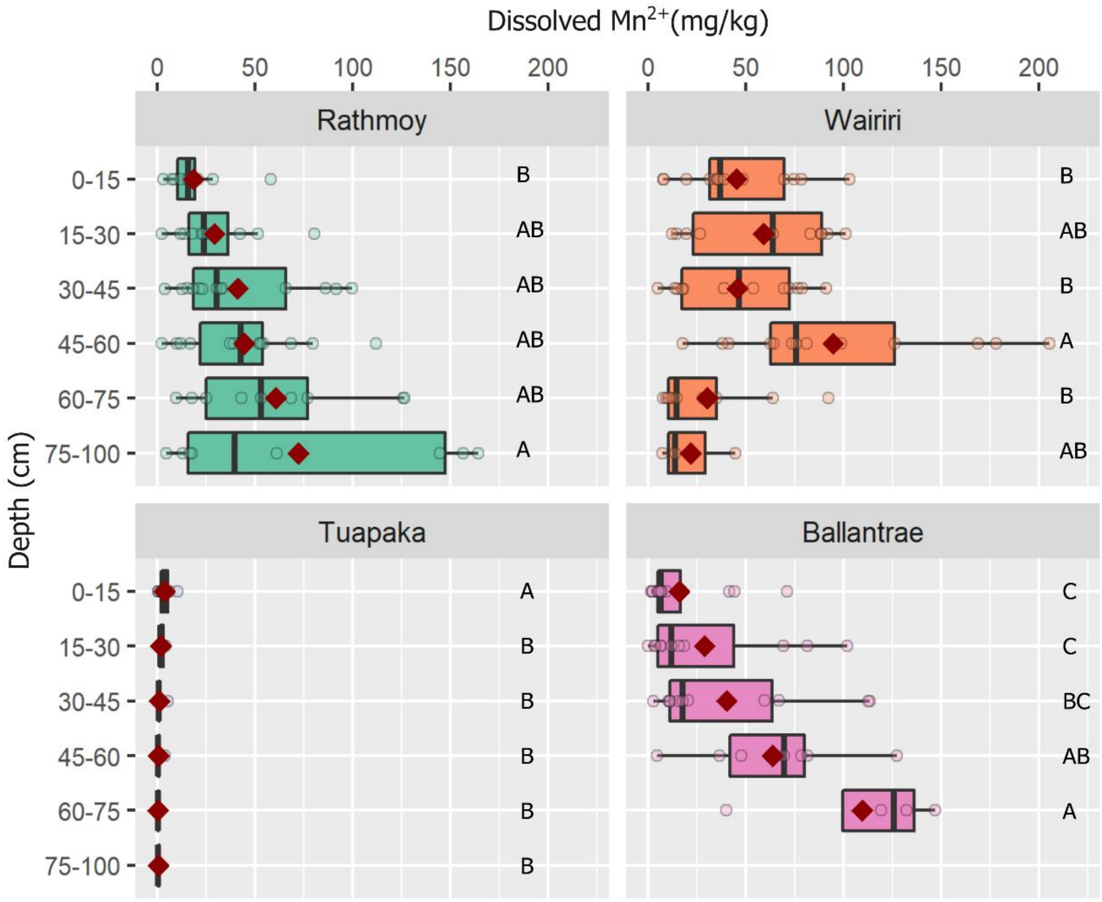


Figure 3-10. Boxplots showing sediment dissolved Mn^{2+} concentrations (mg/kg DS), on dry sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at $p\leq0.05$.

3.3.1.3.4 Total carbon and total nitrogen

The total carbon percentage (%TC) and total nitrogen percentage (%TN) were 2-5 times higher at Rathmoy (%TC=12.8, %TN=1.0) and at Wairiri (%TC=10.6, %TN=0.82), compared to the L-DEA sites (Figures 3-11, 3-12). The study sites measured the highest %TC and %TN values at the surface 0-15 cm depths (p≤0.05).

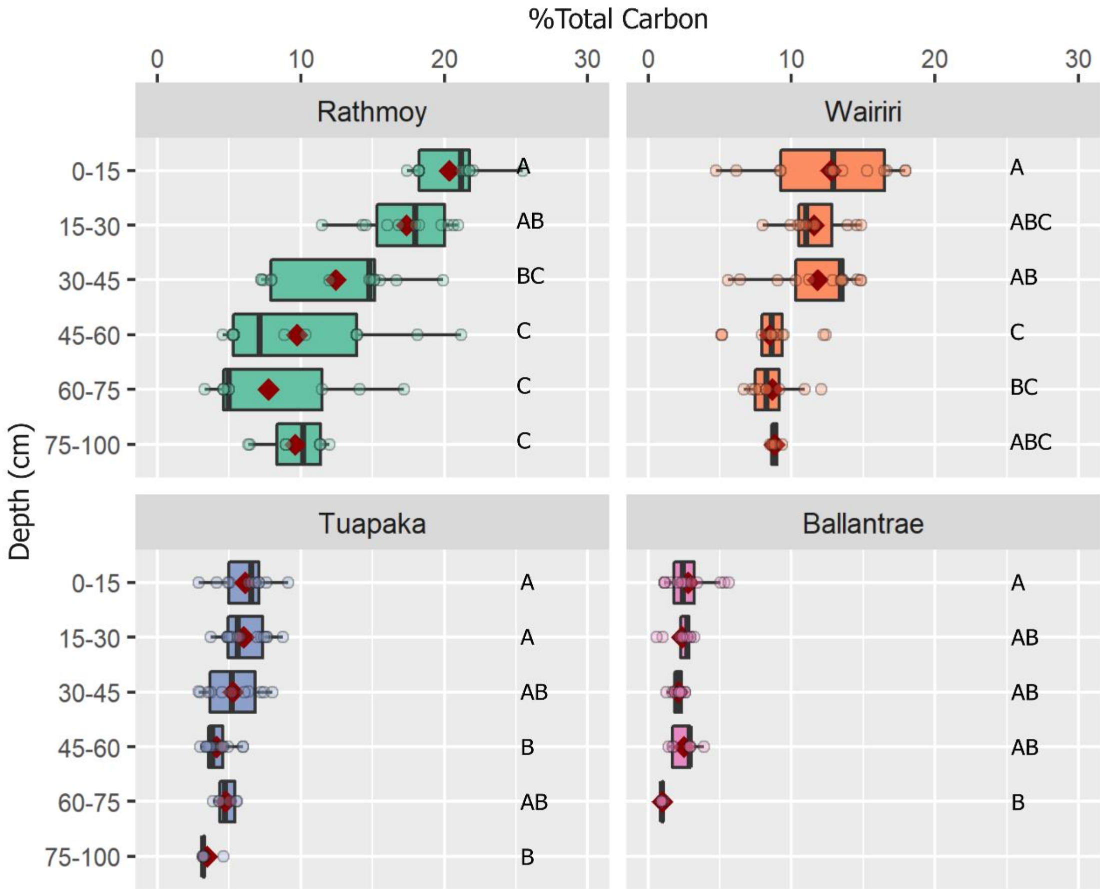


Figure 3-11. Boxplots showing sediment total carbon percentage i.e., %TC, on air-dried sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at p≤0.05.

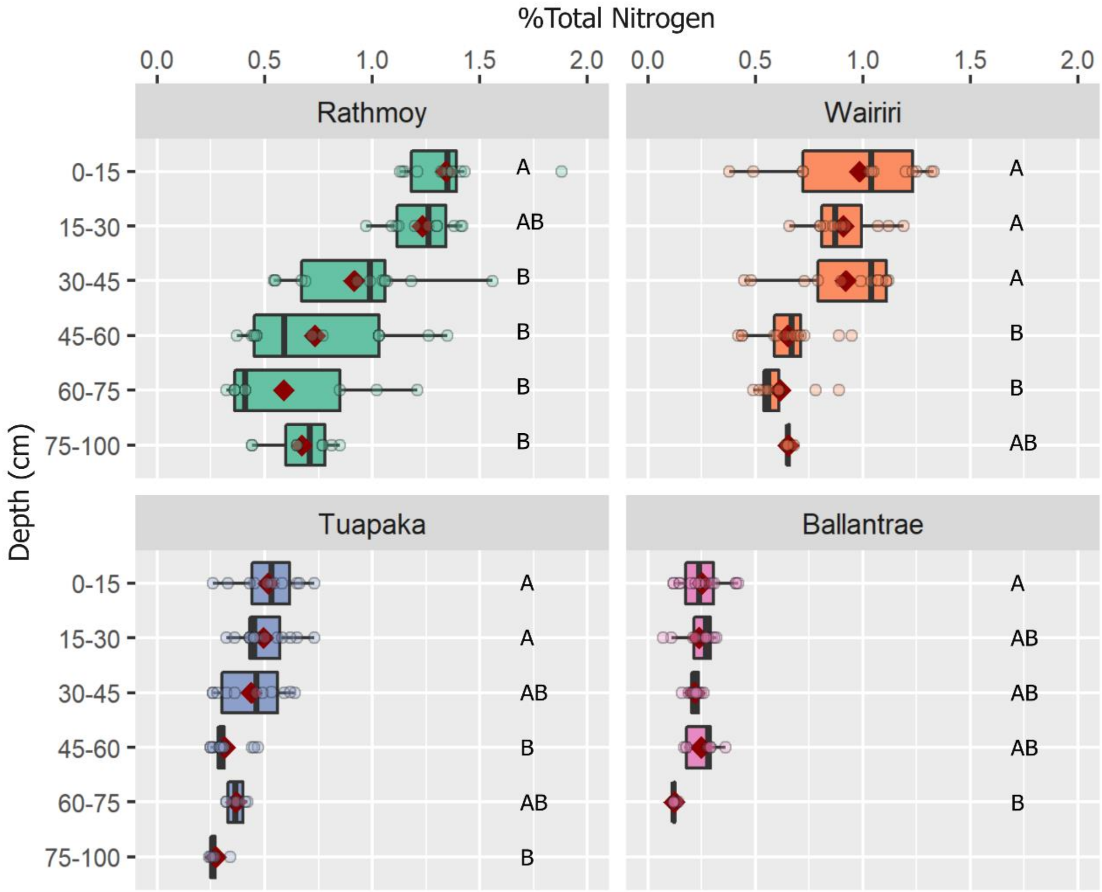


Figure 3-12. Boxplots showing sediment total nitrogen percentage i.e., %TN, on air-dried sediment mass-basis, with respect to depth in the pastoral hill country seepage wetland sediment columns at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Different letters indicate the mean differences in sediment properties between depths are significant at p≤0.05.

3.3.2 Horizontal gradients in seepage wetland sediment properties and DEA

The comparison of the mean DEA values in sediment columns at the inflow, midflow, and outflow wetland positions (Figure 3-3) found horizontal gradients in DEA were present at the L-DEA sites (Tuapaka and Ballantrae) ($p \leq 0.05$) (Table 3-4). Significantly higher DEA values were measured at the outflow position at the Tuapaka site (334 µg N_2O-N kg^{-1} DS h^{-1}) and at the midflow position at the Ballantrae site (589 µg N_2O-N kg^{-1} DS h^{-1}), compared to the other positions within the individual wetland ($p \leq 0.05$)

(Table 3-4). Within the wetland, one-way ANOVA analysis measured significantly higher WC (53.9%, r=0.6, p<0.001), NH_4^+ (26.1 mg/kg DS, r=0.4, p≤0.05), %TC (5.9, r=0.7, p<0.001) and dissolved Fe^{2+} (14.3 mg/kg DS, p≤0.05) at the outflow position at Tuapaka, which were also the sediment properties that positively correlated with DEA at Tuapaka. At Ballantrae, DEA positively correlated with TC (3.1%; r=0.7, p<0.001) and C:N (13.4; r=0.6, p<0.001) which measured higher values at the midflow position (Table 3-4), which was consistent with higher DEA values. In contrast, DEA tended to be homogenously distributed across the H-DEA sites (p>0.05) (Table 3-4).

Table 3-4. Within-wetland horizontal gradients in sediment properties and denitrification enzyme activity at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). High/Low = based on the mean values at wetland positions compared within a wetland site, with mean differences significant at p≤0.05.

Wetland site	Inflow	Midflow	Outflow
Rathmoy	High pH, WC, NO_3^-, DOC, %TC, C:N, dissolved Fe^{2+}	Low %TC, dissolved Fe^{2+}	High Eh, EC Low WC, pH, DOC, C:N
Wairiri	High pH Low Eh, dissolved Fe^{2+}	High C:N Low Eh	High Eh, TN, dissolved Fe^{2+} and Mn^{2+}
Tuapaka	Low DOC, NH_4^+, C:N, dissolved Fe^{2+}	High DOC, C:N; Low NH_4^+	High WC, NH_4^+, DOC, %TC, %TN, dissolved Fe^{2+}, DEA
Ballantrae	High DOC, %TC, dissolved Mn^{2+}, DEA	High EC, WC, %TC, %TN, C:N, DEA	Low EC, %TC, C:N

3.3.3 Relationships between sediment properties and DEA

The PCA biplots in Figure 3-13, showed the sediment properties that accounted for the majority of the variances (58-73%) in the seepage wetland sediment environment were WC, %TC, %TN, NO_3^-, C:N, dissolved Fe^{2+} and Mn^{2+} (with eigenvalues>1) (Figure 3-13). They are also the sediment properties that positively correlated to DEA (Table 3-3).

Water content, NO_3^-, %TC, %TN and C:N) aligned very closely to the DIM1 on the PCA biplots at all depths across the study sites, which suggests their strong influences on the sediment environment. With increasing depth, these sediment properties accounted for greater variances in the sediment environment, which increased from 61-62% at the 0-45 cm depths to 73% at the 60-75 cm depths.

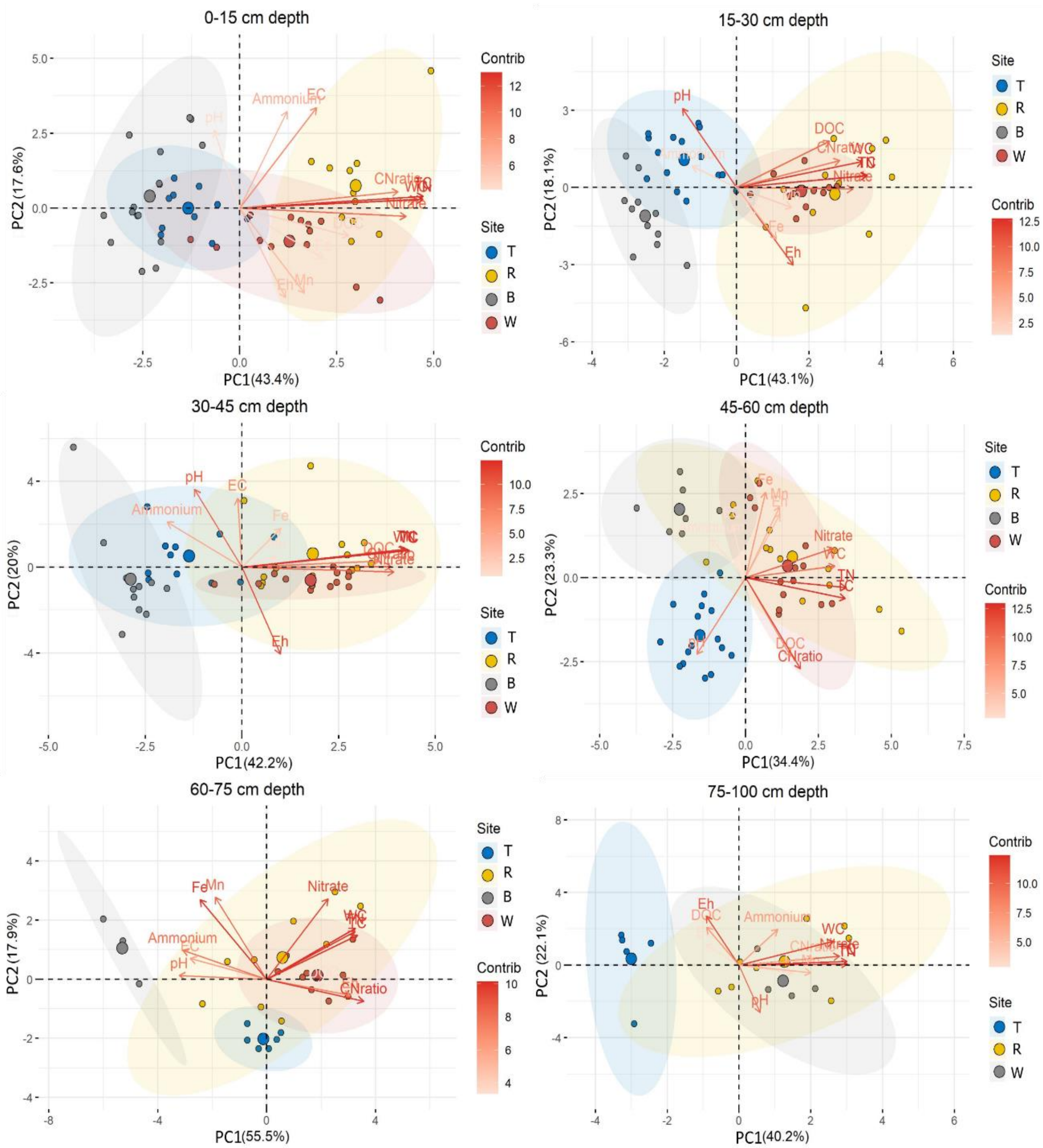


Figure 3-13. PCA biplots showing variances of sediment characteristics in the sediment column depth intervals at four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand). WC = water content, EC = electrical conductivity, TC = %Total Carbon, TN = %Total Nitrogen, Fe = dissolved Fe^{2+} and Mn = dissolved Mn^{2+}. T = Tuapaka, R = Rathmoy, B = Ballantrae, W = Wairiri.

3.4 Discussion

3.4.1 Sediment redox condition and associated sediment characteristics

Wetland sediments analysed from the four study sites had moderate to high WC (43-73%), moderately acidic to near neutral pH values (4.5-6.7) and moderately reduced redox conditions (-15 to 95 mV) (Section 3.3.1.1, Table 3-2). These results suggest NO_3^- reducing sediment environments according to Van Cleemput et al. (2007). The mean redox potentials from each site measured in the current study were <29 mV, which is well within the threshold Eh of <300 mV for NO_3^- reduction (Reddy & DeLaune, 2008). The previously reported ranges were narrower for sediment pH (6.1-6.6), Eh (20-38 mV) and WC (20-40%) in a single-site based seepage wetland study (referred from hereon as the Tuapaka study) (Chibuike et al., 2019) which was conducted near the Tuapaka site examined in the current study. While across the current study sites, the redox condition was similar, the wide variability in their DEA values (2-11,477 µg N_2O-N kg^{-1} DS h^{-1}) suggests other factors also influence DEA in these wetlands.

Sediment WC played an important regulatory role in denitrification across the four seepage wetlands. This finding is supported by the following observations 1) the strong positive correlation between WC and DEA ($r=0.59$, $p<0.001$) (Table 3-3), 2) WC was a major explanatory variable of DEA at all investigated depths as observed in the PCA-biplots (Figure 3-13), and 3) WC positively correlated with several other sediment properties, including %TC, %TN, C:N, dissolved Fe^{2+} and Mn^{2+} ($p<0.001$), that positively associated with DEA (Table 3-3). Numerous studies have shown the positive influence of WC on denitrification (Flite III et al., 2001; Han et al., 2017; Li et al., 2022) and the DEA-influencing sediment substrates (e.g. electron donors and acceptors) in wetlands (Hu et al., 2020). Denitrification is enhanced under saturated conditions as the high WC limits oxygen and transports denitrification substrates to denitrification microsites. This transport can be interrupted under low WC which affects denitrification (Li et al., 2022). Thus, changes in sediment WC influence DEA directly and indirectly, through its influence on other sediment properties that directly influence denitrification (Yu & Ehrenfeld, 2009).

3.4.2 Vertical gradients in seepage wetland sediment properties and DEA

The high DEA measured at the surface depths of the four seepage wetland sites and their sharp declines with depths (Figure 3-5) is likely influenced by the vertical gradients of sediment properties that are key controlling factors of denitrification in wetland sediments (Wu et al., 2021).

At the surface 0-15 and 15-30 cm depths, the high DEA values (Figure 3-5) are likely driven by the occurrences of high sediment WC, NO_3^-, DOC, %TC and %TN measured at those depths (Figures 3-4, 3-6, 3-8, 3-11, 3-12). Organic C and NO_3^- deposit on wetland surface from decomposing plant material, pastoral drainage, fertiliser, animal dung and urine inputs. Saturated condition in the wetland slows down organic matter decomposition (Sahrawat, 2004) and results in organic matter accumulation on the wetland surface, which explains the high organic C and N compounds (DOC, %TC and %TN) across the four study sites. High organic C and NO_3^- at surface depths, stimulate denitrifier activities (Hao & Huang, 2022) that may explain the high DEA measured at the surface 0-15 and 15-30 cm depths across the study sites. In contrast, low denitrification activity at subsurface depths is generally driven by low labile C and NO_3^- concentrations (Burt et al., 1999; Luo et al., 1998; Peterson et al., 2013).

When compared with previous seepage wetland studies, the aggregated mean DEA from the 0-15 and 15-30 cm depths at the Tuapaka site (a L-DEA site) of 372 µg N_2O-N kg^{-1} DS h^{-1} in the current study (Figure 3-5), compared well with the previous DEA value of 369 µg N_2O-N kg^{-1} DS h^{-1} measured at the same depth in the Tuapaka study (Chibuike et al., 2019). In contrast, the high DEA values measured at the H-DEA sites (3868-5371 µg N_2O-N kg^{-1} DS h^{-1} at the 0-15 cm depths at Rathmoy and Wairiri) (Figure 3-5) are more comparable to the mean DEA value of 4100±300 µg N_2O-N kg^{-1} DS h^{-1} measured at the same depths in a pastoral hill country seepage wetland near Hamilton in NZ (Burns & Nguyen, 2002; Rutherford & Nguyen, 2004).

Comparatively, low DEA values were measured at the 30-45 cm (45-585 µg N_2O-N kg^{-1} DS h^{-1}) and 45-60 cm (67-119 µg N_2O-N kg^{-1} DS h^{-1}) depths, despite the presence of denitrification-favourable sediment pH and Eh values (Table 3-2). The low DEA could be due to low microbial activity driven by low labile C and low hydraulic conductivity (indicated by the gley soils observed at three of the four study sites; Section 3.2.2) at the high bulk density subsurface depths (Araragi et al., 1979; Li et al., 2022). A previous hill country seepage wetland study has shown increased bulk density at subsurface depths and associated sharp decreases in saturated hydraulic conductivity from 89 cm day^{-1} at the 0-10 cm depth to 0.2 cm day^{-1} at the 20-30 cm depth (Rutherford & Nguyen, 2004). Also, gley soil, generally indicative of reduced conditions which were also measured in low Eh sediments in the current study, can facilitate electron release (Mansfeldt, 2004) and may explain the high Mn^{2+} concentrations measured at the 30-45 and 45-60 cm depths examined in the current study sites. Although the measured sediment DEA was low at the 30-45 and 45-60 cm depths, the low Eh, high pH and abundant electron donor supply (i.e., dissolved Mn^{2+}) demonstrated a potential supportive environment at these depths (Table 3-2 and Figure 3-10) for shallow groundwater NO_3^- removal. The literature also corroborates that subsurface environments low in DEA values can still account for substantial shallow groundwater NO_3^- removal via denitrification in wetlands (Jahangir et al., 2012; Jarvis & Hatch, 1994).

At the deeper 60-75 and 75-100 cm depths, sediment properties across the four wetland sites suggest potential for DNRA-based NO_3^- reduction. This suggestion is based on the following observations: 1) the high DOC (correlation to depth, $r=0.19$, $p\leq0.05$) and NH_4^+ concentrations (Figures 3-8, 3-7), 2) the DOC:NO_3-N ratio of 31 to 39×10^3 which are above the threshold (>12) for DNRA (Yin et al., 1998), and 3) the high dissolved Fe^{2+} concentration measured at the 60-100 cm depth (Figure 3-9). Studies have shown the DOC:NO_3^- ratio and the dissolved Fe^{2+} as strong predictors of DNRA (Handler et al., 2022; Pandey et al., 2020; Rütting et al., 2011). Additionally, the positive correlations between DOC and NH_4^+, particularly at the L-DEA sites (Tuapaka: $r=0.4$, $p<0.001$ and Ballantrae: $r=0.7$, $p<0.001$) further suggest the positive influence of DOC on NH_4^+ concentration in those sites. The high DOC concentration measured at the 60-75 and 75-100 cm depths (Figure 3-8) could be explained by the decomposing woody debris observed during the sampling events at the Tuapaka and Ballantrae sites (Section 3.2.2). In the presence of high labile C in a highly reduced environment with low NO_3^- concentrations, denitrification can become NO_3^--limited and NO_3^- reduction to NH_4^+ via DNRA becomes more energy efficient (Pandey et al., 2020). Incomplete organic matter decomposition under the reduced environment is another explanation for the measured high NH_4^+ concentrations (Enwezor, 1976). However, the possibility of DNRA in seepage wetlands has previously been speculated due to an overall DOC:NO_3-N ratio of >600 in the Tuapaka study (Chibuike et al., 2019). That study also measured a positive correlation between NH_4^+ and DEA that suggested a positive influence of NH_4^+ on DEA in these wetlands as a potential NO_3^--source via nitrification (Chibuike et al., 2019). While the current study reports lack of correlation between NH_4^+ and DEA ($p>0.05$, Table 3-3), the high NH_4^+ at the subsurface depths in the current study sites suggests a potential NO_3^- source if these wetlands are drained and become aerobic.

Compared to the L-DEA sites, high DEA values over deeper sediment column depths measured at the H-DEA sites suggest some seepage wetlands have greater NO_3^- mitigation capacities, via surface and subsurface denitrification. The H-DEA sites measured not only high surface DEA, but the aggregated mean DEA was also higher (2201 µg N_2O-N kg^{-1} DS h^{-1}) compared to the NO_3^--poor L-DEA sites (311 µg N_2O-N kg^{-1} DS h^{-1}) throughout the top 0-45 cm depths (Figure 3-5). Also, when the subsurface sediments were investigated at the 15-30 cm depth, the high DEA at the H-DEA sites (Rathmoy: 913 µg N_2O-N kg^{-1} DS h^{-1}, Wairiri: 2099 µg N_2O-N kg^{-1} DS h^{-1} at the 15-30 cm depths) were 2-10 times higher than even the highest DEA measured at the surface 0-15 cm at the L-DEA sites (Tuapaka: 560 µg N_2O-N kg^{-1} DS h^{-1}, Ballantrae: 243 µg N_2O-N kg^{-1} DS h^{-1}). These comparisons suggest higher NO_3^- removal potentials at the H-DEA sites, compared to the L-DEA sites.

The high DEA at the 15-30 cm depths of the H-DEA sites could be driven by the high sediment DOC and NO_3^- concentrations at those depths (834 mg DOC/kg DS and 16 mg NO_3-N/kg DS at Rathmoy; 965 mg DOC/kg DS and 20 mg NO_3-N/kg DS at Wairiri), and their possible positive influence on denitrifier population, in the deeper sediment depths, compared to the low DOC and NO_3^--poor L-DEA sites. Additionally, the high DOC concentrations at the 60-100 cm depth measured at the L-DEA sites (Figure 3-8) also have the potential to support subsurface groundwater NO_3^- removal, when NO_3^- becomes available, as previously observed in hillslope shallow groundwater in NZ (Stenger et al., 2018).

Concerning electron acceptors, the importance of NO_3^- for DEA appears to be relative in seepage wetlands. Significant correlations between sediment NO_3^- and DEA present at the H-DEA sites ($r = 0.54-0.65$, $p<0.001$; Rathmoy: 15.89 mg NO_3-N/kg DS, Wairiri 18.54 mg NO_3-N/kg DS) but absent at the L-DEA sites ($p>0.05$; Tuapaka: 2.49 mg NO_3-N/kg DS, Ballantrae: 3.97 mg NO_3-N/kg DS), suggests NO_3^- enrichment can enhance DEA more in the NO_3^--rich (>15 mg NO_3-N/kg DS) seepage wetlands. The multiple-site approach used in the current study enabled the identification of the relative importance of NO_3^- in seepage wetland DEA. Similar to the observations at the H-DEA sites, a number of studies have measured strong positive influences of NO_3^- on denitrification in wetlands (Han et al., 2017; Hanson et al., 1994b; Hu et al., 2020; Martin et al., 2001).

Contrary to the H-DEA sites, the lack of a correlation between *in-situ* sediment NO_3^- and DEA in the NO_3^--poor L-DEA sites ($p>0.05$), and also in the Tuapaka study (Chibuike et al., 2019), could be due to an inadequate conditioning period for the adaptation of microbes of NO_3^- poor soils to the NO_3^- enrichment as part of the DEA incubations. It should be noted that seepage wetland NO_3^- concentrations are generally low (<2 mg NO_3-N/kg DS) (Chibuike et al., 2019; Nguyen & Downes, 1997; Nguyen et al., 1999b). In NO_3^- poor and low DOC soils, Peterson et al. (2013) suggested a period of 24-36 h as a 'lag time' which is necessary for microbes to adjust before denitrification accelerates at 36-96 h. Compared to those time periods, a shorter incubation duration of 6 h in the current study, may have measured sediment DEA before it had fully accelerated particularly for the L-DEA sites and the corresponding DEA may have been underestimated. However, maintaining a constant sediment incubation period across the study sites was necessary to understand the spatial variability of potential DEA across these seepage wetlands.

Comparisons of the seepage wetland sediment properties between the current and the previous NZ studies are constrained by 1) the limited number of published research and 2) the differences in the studied depth intervals. Yet, the comparisons where possible, highlight that values measured in the current study agreed well with published values for several sediment properties that include sediment DOC and dissolved Fe^{2+}, %TC, %TN, C:N (Chibuike et al., 2019; Nguyen & Downes, 1997) in seepage wetlands. The sediment column mean DOC of 527 mg DOC/kg DS and dissolved Fe^{2+} of 32.91 mg

Fe^{2+}/kg DS across the four study sites compared well with the previous 452 mg DOC/kg DS and 30 mg Fe^{2+}/kg DS, respectively, measured in 1 m long sediment columns in the Tuapaka study (Chibuike et al., 2019). In contrast, several magnitude higher mean dissolved Mn^{2+} concentration of 35.5 mg/kg DS was measured across the study sites, compared to the previous value of ~1 mg Mn^{2+}/kg DS in the Tuapaka study (Chibuike et al., 2019). The measured mean %TC (8.26%) and %TN (0.65%) in the current study at the 0-15 cm depth, compared well with a seepage wetland near Hamilton which measured 3.7-9.7 %TC and 0.35-0.9 %TN (Nguyen & Downes, 1997).

The current study builds on the previously known positive influences of DOC and the dissolved Mn^{2+} on DEA as electron donors and adds that dissolved Fe^{2+}, %TC, %TN and NO_3^- are important sediment properties that correlate with DEA (Table 3-3) in pastoral hill seepage wetlands. As a product of Fe^{3+}-reduction (Fe-S reduction) and also as an electron donor in autotrophic denitrification, dissolved Fe^{2+} is indicative of and has the potential to fuel denitrification, where organic C is low e.g. in subsurface depths (Xu et al., 2021). This could be the case for the high Fe^{2+} concentrations measured at the subsurface at the Ballantrae and the Wairiri sites and could be indicative of denitrification favourable subsurface environments. Additionally, a strong positive correlation between Mn^{2+} and Fe^{2+} ($r=0.79$, $p>0.001$) suggests their co-occurrence across the study sites.

Comparing their corresponding correlations with DEA, %TC ($r=0.57$, $p<0.001$) and %TN ($r=0.59$, $p<0.001$) are likely to be more reliable predictors of DEA in the study sites, compared to DOC ($r=0.20$, $p\leq0.05$) that previously also had measured a similar correlation with DEA in the Tuapaka study. Strong linear regressions of %TC and %TN with DEA, have previously been reported for North Island pastoral soils (Steele et al., 1984). Additionally, the strong correlation between %TC and DOC measured in the current study ($r=0.53$, $p<0.001$), suggests %TC is a potential DOC-source in the study sites. Both a Hamilton site and the current study sites showed decreasing lower %TC and %TN with increasing wetland depths (Nguyen & Downes, 1997). Their positive associations with DEA suggest the decreasing %TC and %TN had influenced the low subsurface DEA in the current study, likely by influencing the denitrifier distribution (Chakrawal et al., 2022).

3.4.3 Horizontal gradients in seepage wetland sediment properties and DEA

The contrasting DEA values between the H-DEA and the L-DEA sites in the current study demonstrate the gradient of seepage wetland DEA at the landscape scale. Literature shows that DEA spatial variability can be largely driven by the spatial variabilities in sediment properties (Dhondt et al., 2004; Wall et al., 2005; Xiong et al., 2015).

3.4.3.1 Within-wetland horizontal gradients

Within the wetland, the non-uniform distribution of DEA-influencing sediment properties can drive horizontal gradients in DEA in seepage wetlands. In the current study, the wetland positions with significantly higher WC ($p \leq 0.05$) measured significantly higher DEA at the L-DEA sites ($p \leq 0.05$), as observed at the outflow position at Tuapaka (DEA 334 µg N_2O-N kg^{-1} DS h^{-1}, 53.9% WC) and the midflow at Ballantrae (DEA 589 µg N_2O-N kg^{-1} DS h^{-1}, 38.6% WC) (Table 3-4). In contrast, the H-DEA sites in general, measured uniform distributions for WC and DEA ($p > 0.05$) within the sites (Table 3-4). Non-uniform distribution of sediment saturation conditions e.g. under non-flooded conditions has previously shown non-uniform DEA distribution within wetlands (Christensen et al., 1990). As water is a major carrier of substrates, a less-than-saturated condition can mean interrupted supplies of NO_3^- and DOC to denitrification microsites. The NO_3^- poor sites, e.g. the L-DEA sites, are also likely to have low denitrifier abundance and different denitrifier composition compared to the NO_3^- rich H-DEA sites (Wang et al., 2019b). Comparatively less WC (Figure 3-4) and the non-uniform WC distributions (Table 3-4) within the L-DEA sites may have added to the effect of low sediment NO_3^- on DEA, by disrupting the NO_3^- distribution and the associated denitrifier composition and abundance, that resulted to an overall low DEA at the L-DEA sites, despite the NO_3^- enrichment during the DEA incubation in the laboratory.

3.4.3.2 Between-wetland gradients

Between-wetlands, the sites in the same DEA-category (Section 3.3.1.2) shared similar ranges of values for several major DEA-influencing sediment properties (WC, NO_3^-, %TC, %TN, C:N, dissolved Mn^{2+}) in the current study where the H-DEA sites, in general, measured the higher values for the DEA-influencing sediment properties (WC, NO_3^-,

%TC, %TN, dissolved Mn^{2+}), compared to the values at the L-DEA-sites. For example, WC which is essential to maintain nutrient transport and oxygen limitation, was higher and well above the threshold limit for denitrification (WC>60%) (Weier et al., 1993) at the H-DEA sites (mean WC 67-77%) compared to the below threshold WC measured at the L-DEA sites (mean WC 33-47%) (Figure 3-4). Also, the concentrations of the electron donors and the electron acceptor (20 mg NO_3-N/kg DS at the H-DEA vs 3.6 mg NO_3-N/kg DS at the L-DEA) were higher at the H-DEA sites, compared to the L-DEA sites (Figure 3-6). Nitrate positively associates with sediment DEA in general (Bowen et al., 2020; Wall et al., 2005). Furthermore, while the mean electron donor concentrations were 731 mg DOC/kg DS, 41.3 mg Fe^{2+}/kg DS and 40.5 mg Mn^{2+}/kg DS at the H-DEA sites, their corresponding mean concentrations at the L-DEA sites were 193.5 mg DOC/kg DS, 21.5 mg Fe^{2+}/kg DS and 14.8 mg Mn^{2+}/kg DS at the 0-45 cm depths, respectively. These occurrences of the DEA-influencing sediment properties in higher quantities at the H-DEA site have likely facilitated their distinctively high DEA values. The key influence of sediment properties on the spatial gradient of DEA in seepage wetlands is further confirmed by the large variances (58-73%) the sediment properties accounted for in the seepage wetland environment in which denitrification occurs, as the PCA-biplots show (Figure 3-13).

Compared to the wetland sediment properties, the baserock of the wetland appears to be a poor indicator of seepage wetland DEA, as both a L-DEA (Tuapaka) and a H-DEA (Wairiri) were located on greywacke bedrock (Table 3-1). This suggests bedrock properties have contributed little to the site-specific wetland influences on DEA in the current study.

The current study confirms the site-specific sediment influence on DEA. For example, 1) sediment NO_3^- and dissolved Fe^{2+} strongly influenced DEA at the H-DEA sites, whereas such correlation was absent at the L-DEA sites, 2) the dissolved Mn^{2+} concentrations demonstrated opposite correlations with DEA at the Tuapaka ($r=0.5$, $p \leq 0.05$; a L-DEA site on greywacke bedrock) and the Rathmoy ($r=-0.3$, $p \leq 0.05$; a H-DEA site on mudstone bedrock) sites while the Ballantrae (L-DEA site on mudstone bedrock) or the Wairiri (H-DEA site on greywacke bedrock) sites exhibited no such correlations ($p>0.05$).

While the PCA-biplot suggests sediment properties are important, a considerable fraction of the wetland influence (27-42%) on DEA remains unexplained by sediments alone. Across different wetlands, sediment properties have accounted for 43-66% of the variances ranging from a NO_3^--reduction stream (France) to an urban riparian wetland (China) (Wei et al., 2020; Wu et al., 2021), while in another study, only 40% the variance in sediment denitrification rate was explained by the sediment characteristics or the water physicochemical properties alone (Liu et al., 2018). Hydrogeological properties (flow pathways and connectivity, pulsing) and fluxes also affect denitrification in wetlands (Arnon et al., 2007; Cooper et al., 1995; Giles et al., 2012; Hill et al., 2000; Ye et al., 2017). Thus, future studies on seepage wetland hydrological properties, unexplored in the current chapter, may further improve our understanding of the wetland influence on denitrification which is necessary for improved use of these wetlands for NO_3^- mitigation. Future study on N_2O emission potential from seepage wetlands is also necessary, which was outside the current research scope. This is particularly important there are risks of N_2O emissions from the NO_3^--rich H-DEA sites examined in the current study, as previous studies have shown denitrification can terminate at the intermediate stage, resulting in N_2O emissions (Groffman & Hanson, 1997; Verhoeven et al., 2006; Weier et al., 1993).

3.4.4 Comparisons of seepage wetland DEA

Based on the aggregated mean DEA measured at the surface 0-45 cm depths, the current study sites rank as follows: Rathmoy (2219 µg N_2O-N kg^{-1} DS h^{-1}) > Wairiri (2184 µg N_2O-N kg^{-1} DS h^{-1}) > Tuapaka (319 µg N_2O-N kg^{-1} DS h^{-1}) > Ballantrae (302 µg N_2O-N kg^{-1} DS h^{-1}). To compare, the previous surface DEA values were 369 µg N_2O-N kg^{-1} DS h^{-1} at the 0-30 cm depth in the Tuapaka study (Chibuike et al., 2019), 4100 µg N_2O-N kg^{-1} DS h^{-1} at the 0-15 cm depth and aggregated mean of 3550 µg N_2O-N kg^{-1} DS h^{-1} at the 0-10 cm depth at seepage wetlands near Hamilton (Nguyen & Downes, 1997; Rutherford & Nguyen, 2004) (Table 3-5). A slightly higher but comparable surface DEA range of 560-5371 µg N_2O-N kg^{-1} DS h^{-1} at the 0-15 cm depths in the current study sites 1) gives us confidence about the analytical quality of the measurements in this study, 2) shows these wetlands vary widely in their denitrification capacities, and 3) extends the previously known upper limit of the surface DEA in the pastoral hill seepage

Table 3-5. Comparisons of the seepage wetland DEA values with global estimates across a range of terrestrial ecosystems

Site	DEA ($\mu g\ N_2O\text{-}N\ kg^{-1}\ DS\ h^{-1}$)	Reference
Global mean DEA in terrestrial ecosystems (range with the upper limit from the humid subtropical climate and the lower limit from the dry climate)	146±3.79 (40.2±6.34 - 176±6.34)	Li et al. (2022)
20 pastoral soils in the North Island, NZ (0-5 cm depth)	180 - 183 x 10^3	Steele et al. (1984)
Seepage wetland at the Whatawhata research centre near Hamilton, central North Island (0-15 cm depth)	4100±300	Rutherford and Nguyen (2004)
Site same as above, aggregated mean (range) (0-10 cm depth)	Winter-early spring: 1500 (1000 - 2000) Summer: 3550 (1000 - 6000)	Nguyen and Downes (1997)
The H-DEA sites lower North Island, NZ (0-15 cm depth)	Rathmoy: 5371 Wairiri: 3868	The current study
The L-DEA sites lower North Island, NZ (0-15 cm depth)	Tuapaka: 560 Ballantrae: 720	The current study
Tuapaka seepage wetland, North Island, NZ (0-30 cm depth)	369	Chibuike et al. (2019)
Hill country dry soil at Tuapaka, North Island, NZ (0-30 cm depth)	51	Chibuike et al. (2019)
Dairy-grazed rain-fed seepage wetland near Hamilton, NZ	4900 - 7900	Tanner et al. (2005)
Dairy pastoral seepage wetland, North Island, NZ (0-10 cm depth)	8500±1010	Zaman et al. (2008)

wetlands which was 4100 µg N_2O-N kg^{-1} DS h^{-1} at the 0-15 cm depth at a site near Hamilton (Rutherford & Nguyen, 2004). Furthermore, the sediment DEA measurements under similar incubation condition for multiple sites in the current study provides a more reliable surface DEA range than previously available for the pastoral hill country seepage wetlands in NZ. A comparison with DEA values across various ecosystems in Table 3-5, also showed that DEA in pastoral hill seepage wetlands is higher than the current average global estimates from terrestrial ecosystems (Li et al., 2022).

However, the sediment DEA may have been overestimated in the current study due to the measurement technique involved. A previous study using a similar measurement technique as the one used in the current study measured a laboratory-based potential DEA which was 5-fold higher (Luo et al., 1999a; Luo et al., 2000) than the *in-situ* DEA measurements in pastoral soil measured in the current study. The previous study attributed the high laboratory DEA values to the physical disruption of sediments, NO_3^--enrichment and forced anaerobic condition associated with the acetylene inhibition technique (Luo et al., 1999a; Luo et al., 2000; van den Heuvel et al., 2009).

The measured sediment DEA can be used to estimate the areal NO_3^- removal, in order to model the potential for NO_3^- attenuation in seepage wetlands. Nitrate removal was estimated in the current study using the sediment DEA values measured at the 0-15 cm depths. The associated measurement technique and the bulk density data are presented in Appendices 3-2 and 3-3. The areal NO_3^- removals in the current study areas were estimated to be 0.9±0.9 g N/m^2/day (Rathmoy), 5.0±3.6 g N/m^2/day (Wairiri), 0.6±0.8 g N/m^2/day (Tuapaka) and 1.3±1.3 g N/m^2/day (Ballantrae). Despite measuring the lowest DEA values among the 0-15 depths of all four sites, the comparatively high NO_3^- removal measured at the Ballantrae site can be attributed to the particularly high bulk density at that site. Previous areal NO_3^- removal rates have been estimated to be 0.9±0.3 g N/m^2/day (equivalent to 3,285 kg NO_3-N/ha/yr) for a hill country seepage wetland based on surface flow and seepage velocity within the surface 10 cm of the wetland, and sediment DEA (Rutherford & Nguyen, 2004). The comparison shows, on an area-basis, some wetlands e.g. Wairiri (1.8x10^5 ± 1.3x10^5 g N/m^2/day) show greater potential for NO_3^- attenuation via the denitrification process than has previously been measured in the hill country landscape. However, the generally high estimated areal NO_3^- removal rates could be elevated due to the high NO_3^- input applied during the DEA incubation and caution and extrapolation to a seepage wetland under natural conditions is not advised.

3.5 Conclusion

The seepage wetland DEA is spatially variable, both vertically (within wetland sediment columns) and horizontally (within and between wetlands), which closely follows the spatial gradients in sediment properties. The surface depths measured the highest DEA values across all four wetland sites. The surface DEA values measured between the sites were distinctive, and on that basis, they were categorised as H-DEA (>3000 µg N_2O-N kg^{-1} DS h^{-1}) and L-DEA (<1000 µg N_2O-N kg^{-1} DS h^{-1}) sites. Sediment properties have a strong influence on denitrification in these wetlands, as sediment properties were able to explain the majority of the variances (58-73%) in the spatial gradients in the wetland sediment environment in which denitrification occurs. Major sediment properties that influenced the spatial gradients of DEA in seepage wetlands were WC, NO_3^-, %TC, %TN, DOC, dissolved Fe^{2+} and Mn^{2+} and they measured positive correlations with DEA in the current study. The occurrences of these properties in higher quantities at the H-DEA sites, compared to the L-DEA sites, explain the distinctively higher DEA at the H-DEA sites. The WC was found to be a strong driver of the horizontal gradient in DEA within wetlands, as the L-DEA sites with less than saturated conditions measured significantly higher DEA at specific wetland positions and these were associated with higher WCs. In contrast, DEA was uniformly distributed within the H-DEA sites, where WC was high and more uniformly distributed. Baserock was found to be a poor DEA indicator in the current study. Instead, sediment properties are recommended as more reliable DEA-predictors. Still, a large fraction of the variances in the sediment environment (~30%) remained unexplained, suggesting future studies are necessary to understand other possible wetland influences e.g., wetland hydrological properties that affect DEA.

In contrast to the previous single-site based seepage wetland studies, the current multi-site study generated a robust dataset that will be critical to improving our understanding of the spatial gradients of denitrification in these wetlands across the landscape. This multi-site study builds on the previously known positive influences of WC, DOC and dissolved Mn^{2+} on DEA in seepage wetlands (Chibuike et al., 2019), by adding sediment N and C compounds (NO_3^-, %TC, %TN, C:N) and dissolved Fe^{2+}. Sediment NH_4^+ may not be as important for seepage wetland DEA as suggested earlier by Chibuike et al. (2019). The findings from the current study provide important insights into the drivers that regulate denitrification in pastoral hill country seepage wetlands. This new knowledge is critical if enhancement of denitrification in these seepage wetlands is desired, in order to improve catchment water quality.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Suha Sanwar
Name and title of main supervisor:	Dr. Lucy Burkitt
In which chapter is the manuscript/published work?	chapter 4
What percentage of the manuscript/published work was contributed by the student?	85%

Describe the contribution that the student has made to the manuscript/published work:

Suha assisted in designing this study and undertook all the field sampling and laboratory analysis. She statistically analysed the data, compiled all graphs and tables and drafted the manuscript with the support from supervisors.

Please select one of the following three options:

(●) The manuscript/published work is published or in press

Please provide the full reference of the research output:

Sanwar, S., Burkitt, L., Singh, R., Bretherton, M. and Kereszturi, G. (2022). Hill country wetland hydrological characterisation for attenuation. In: Adaptive Strategies for Future Farming, Occasional Report No. 34, 11p. (Eds. C.L. Christensen, D.J. Horne and R. Singh). Palmerston North, New Zealand: Fertilizer and Lime Researc

( ) The manuscript is currently under review for publication

Please provide the name of the journal:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Student's signature:	SUHA SANWAR Digitally signed by SUHA SANWAR Date: 2023.04.03 13:23:11 +12'00'	Main supervisor's signature:	Lucy Burkitt Digitally signed by Lucy Burkitt DN: cn=Lucy Burkitt, c=NZ, o=Massey University, ou=Farmed Landscapes Research Centre, email=L.Burkitt@massey.ac.nz Date: 2023.04.03 12:28:30 +12'00'

This form should be placed at the beginning of each relevant thesis chapter.

Chapter 4: Hydrological and nitrate removal characterisations of a pastoral hill country seepage wetland

---

At the end of the sediment characterisation in chapter 3, a large fraction of the wetland influence on denitrification remained unaccounted for. To understand what other factors were influencing wetland denitrification, temporal changes in hydrological and NO_3^- removal characteristics were assessed in chapter 4.

4.1 Introduction

Nitrate (NO_3^-) loss from pastoral headwater catchments is a water quality concern in New Zealand (NZ) (Bijay & Craswell, 2021; Dodd et al., 2016; Hooda et al., 1997; MfE & Stats NZ, 2021). Beef and sheep farming is the dominant land use in NZ hill country and these farms are primarily located in headwater catchments. Low-order (1-3) streams in headwaters cover an estimated 87% of a river's total catchment area in NZ and contribute 77% of the NO_3^- load to NZ water bodies (McDowell et al., 2017). These estimates suggest that NO_3^- load reductions in low-order streams have the potential to benefit catchment water quality (Parfitt et al., 2006). In that case, effective utilisation of edge-of-field and catchment mitigation practices such as seepage wetlands may help to reduce diffuse N loss from pastoral hill country farming systems.

Seepage wetlands are small wetlands that occur along low-order streams in low-gradients in the hill country and have the potential to be NO_3^- mitigation tools in pastoral hill country landscapes (Hill, 1996; McKergow et al., 2016; Rutherford et al., 2018; Rutherford & Nguyen, 2004). Seepage wetlands form from colluvium and alluvium sourced from surrounding upland pastoral soils and their underlying rocks. This deposition occurs on more consolidated rock which is exposed by the downcutting of streambeds in areas where the slope is gentler. Due to the presence of an impermeable layer at shallow depths, seepage wetland soil is shallow in thickness (~1 m) (Rutherford

& Nguyen, 2004). Hillslope hydrology dominates seepage wetland hydrology (Burt & Pinay, 2005). Subsurface input (slope discharge, shallow groundwater discharges) and/or overland flow (stream, seasonally dependent Hortonian flow) keep the adjacent soil profiles and the seepage wetland saturated and promote a reducing environment. Seepage wetlands, with saturated and organic matter (OM)-rich sediment are located at the convergent zones of important surface and subsurface NO_3^- input from upland pastures, provide a unique NO_3^- reducing opportunity (Burt & Pinay, 2005; Chibuike et al., 2019). However, due to the dynamic nature of hillslope hydrology and its influence on NO_3^- delivery, residence time and fluxes in the NO_3^- reducing environment in the wetlands (Jencso et al., 2009; Ocampo et al., 2006), NO_3^- removal can be highly variable over temporal scales in seepage wetlands.

Previous studies have reported variable NO_3^- removal estimates in seepage wetlands under different hydrological conditions. Burns and Nguyen (2002) measured 90% NO_3^- removal (from an input of 193.8 mg NO_3-N/L) in the shallow groundwater within first 4 days of a 24-day tracer experiment in a groundwater-fed hill pastoral seepage wetland at Whatawhata near Hamilton in the central North Island of NZ. In contrast, in the same study area, 51% of NO_3^- was removed from surface runoff, during a 6-month surface flow monitoring study between August-March (Nguyen et al., 1999a). While that study claimed to have monitored most of the annual wetland flow, their NO_3^- removal estimate may not represent the seepage wetland NO_3^- removal of winter, when the majority of the NO_3^- runoff occurs in pastoral hill country (Bargh, 1978; Nguyen et al., 1999a; Quinn & Stroud, 2002). In a separate study, the same wetland measured 24±9% NO_3^- (from 4.6 g to 3.5 g NO_3-N) removal from surface runoff, including a vertical mixing at the top 0-5 cm depth, that travelled a 1.5 m distance in an enclosed experimental tray in a month-long *in-situ* tracer study (Rutherford & Nguyen, 2004). In contrast, a negative NO_3^- removal (-29±5%) was measured in a seepage wetland located on a dairy farm in the Waikato region in NZ and was attributed to a prevalent aerobic condition in the wetland over a 3-year study (Wilcock et al., 2012). A NO_3^- removal simulation found that a groundwater-fed seepage wetland has the potentials for >75% NO_3^- removal in dairy catchments (Uuemaa et al., 2018). Such a wide range of seepage wetland NO_3^- removal rates under different hydrological conditions, and associated variations in hydrological routes and water residence times, emphasise the importance of understanding seepage wetland hydrological properties (e.g., major N delivery routes and fluxes). This knowledge is necessary in order to optimise the NO_3^- mitigation function of seepage wetlands in pastoral hill country landscapes.

A sound understanding of critical flow pathways, in the context of local catchment hydrology, is also key because of their influence on NO_3^- delivery routes and fluxes. For instance, at a seepage wetland at Ballantrae in the North Island (NZ), seepage zones delivered higher NO_3^- concentration (7.3 mg NO_3-N/L) during a quickflow event in winter in a year with higher than average precipitation, as compared to the concentration in an average precipitation year winter (4.8 mg NO_3-N/L) (Parfitt et al., 2009). Studies have shown there can be a lag time before such subsurface runoff appears in the wetland following precipitation events (Cooke & Dons, 1988; Davie, 2004). For example, a subsurface NO_3^- delivery to stream water initiated two days after the cessation of a storm event in a pastoral headwater catchment in Australia (Smethurst et al., 2014). As upland-wetland hydrological connectivity is established following precipitation events, associated subsurface fluxes in NO_3^--delivery can influence denitrification-based NO_3^- removal (Burt & Pinay, 2005; Luo et al., 1999a; Ocampo et al., 2006). In contrast, low-NO_3^- groundwater discharge can 'dilute' the stream NO_3^- concentration and incorrectly be interpreted as NO_3^- removal in wetlands (Warwick & Hill, 1988). Such dilution may occur when newly infiltrated NO_3^--rich water in upslope subsurface displaces low-NO_3^- 'old' water in the deep subsurface and pushes it out into the wetland and dilutes stream NO_3^- concentrations (Davie, 2004; Hill, 1996; Petry et al., 2002).

Due to the dynamic nature of hillslope hydrology and its regulatory role in the fluxes of NO_3^- transport to and its residence time in seepage wetlands, there can be hot moments when potential NO_3^- removal either is disproportionately enhanced termed 'NO_3^- removal hot moment' or is disrupted during 'NO_3^- loss hot moment'. Based on the 'hot moments concept' provided by McClain et al. (2003), NO_3^- removal hot moments are the short periods of time (<20% of the total period monitored) with disproportionately high NO_3^- removal due to a prevalence of a NO_3^--reducing condition in the wetland. In contrast, 'NO_3^- loss hot moment' are the short period of time (<20% of the total period monitored) when disproportionately high NO_3^- flux occur in seepage wetlands and are typically driven by high flow events e.g., precipitation or snowmelt (Vidon et al., 2010). Assessment of seepage wetland hydrology and NO_3^- removal over annual scales may help identify such hot moments. Identifications of these hot moments can help 1) understand when a seepage wetland is in the highly supportive condition for NO_3^- removal or at high risk of NO_3^- loss, and 2) address that information in farm NO_3^- management.

Current understanding of the dynamic nature of the seepage wetland hydrology and its influence on NO_3^- removal is extremely limited for pastoral hill country landscapes in NZ. Previous studies have sporadically assessed either surface or subsurface hydrology in seepage wetlands (Burns & Nguyen, 2002; Rutherford & Nguyen, 2004), which have ranged in timeframes from less than a month to 6 months (Burns & Nguyen, 2002; Nguyen et al., 1999a; Rutherford & Nguyen, 2004), mostly outside of the winter season when most NO_3^- loads from the hill country occur (Bargh, 1978). Also, these studies are extremely small in number to cover a comprehensive understanding of the feedback between surface-subsurface hydrology and their influence on the NO_3^- removal and their temporal variations in seepage wetlands. While a sound knowledge of seepage wetlands' hydrological and NO_3^- removal characteristics is fundamental for the application of these seepage wetlands as NO_3^- mitigation tools in pastoral hill country landscapes, the current literature is extremely limited in this scope.

In this study, seepage wetland hydrology and NO_3^- removal were characterised based on hydrological and water quality monitoring for a 2-year period at a seepage wetland in a hill country farm in the lower North Island in NZ. The objectives were to 1) characterise the surface and subsurface hydrology, 2) quantify the wetland NO_3^- removal, and 3) identify the hydrological 'hot moments' related to NO_3^- removal in a pastoral hill country seepage wetland.

4.2 Methodology

4.2.1 Study area

The study area is a seepage wetland located at Massey University's sheep and beef hill country research farm (Tuapaka) near Palmerston North, in the lower North Island, NZ (40°21'11.8"S, 175°44'14.1"E) (Figure 4-1a). The study area has a humid temperate climate with predominantly dry summers and a long-term average temperature of 12.5°C. It is located about 320 m above sea level. The long-term annual average precipitation of 1100 mm (Fransen et al., 2022).

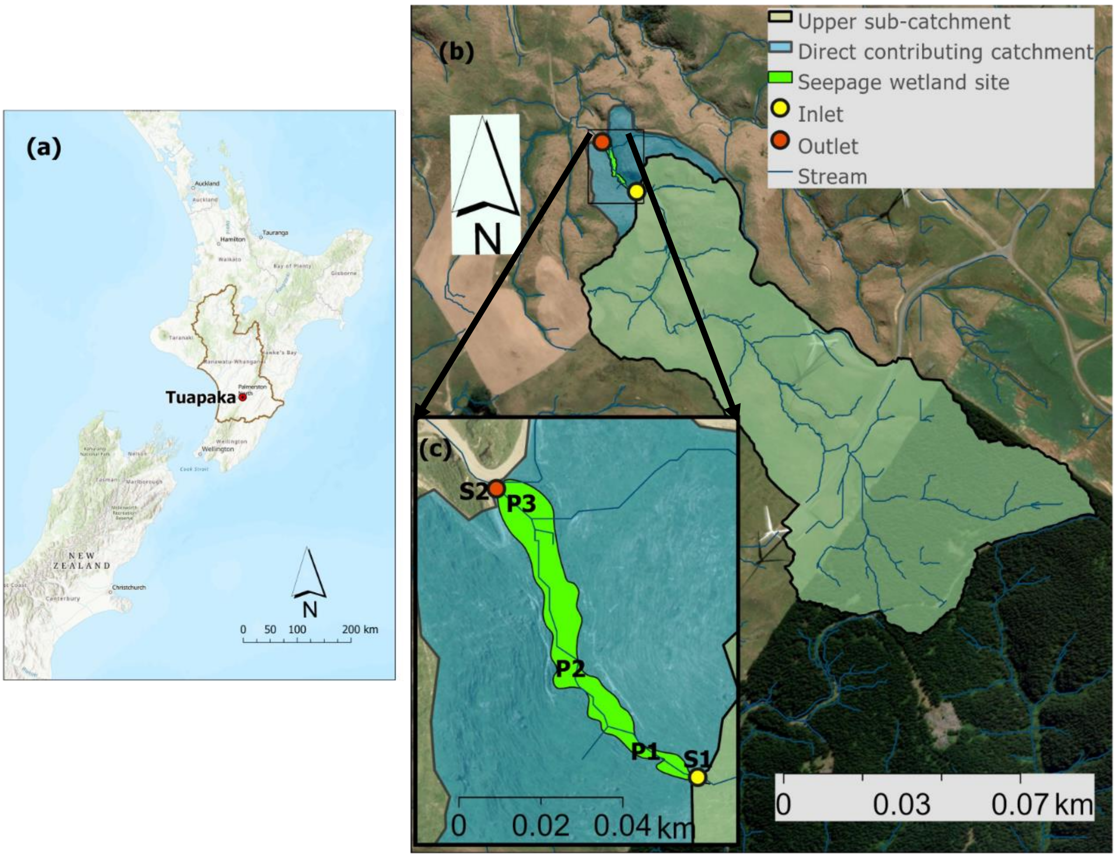


Figure 4-1. (a) Location of seepage wetland site at a hill country farm at Tuapaka (Palmerston North, lower North Island, New Zealand). (b) The 25.2 ha catchment area (= upper sub-catchment of 23.5 ha + direct contributing catchment of 1.6 ha) of the seepage wetland study area. (c) The seepage wetland (0.08 ha) where the surface flow discharge and water quality were monitored at the inlet (S1) and outlet (S2) between June 2019-May 2021. Shallow groundwater quality was monitored in piezometers at the 0.5, 1 and 1.5 m depths at the inflow (P1), midflow (P2) and outflow (P3) positions between November 2019-May 2021.

The seepage wetland site is a funnel-shaped, narrow, valley-bottom wetland zone located on a second-order perennial stream (Figure 4-1b). The 0.08 ha wetland receives drainage at the wetland outlet point from a catchment area of 25.2 ha, which comprises an upper catchment (23.5 ha) and a direct contributing catchment (1.6 ha) (Figure 4-1b). The direct contributing catchment area is the sub-catchment that discharges directly into the wetland between the wetland inlet and outlet points, which are shown in Figure 4-1c. Three ephemeral flow pathways carrying hill runoff, including one very close to the outlet, discharge from the direct contributing catchment into the wetland (Figure 4-1c). Pastoral (sheep and beef) production is the major land use in the catchment, with 33.5% of the upper sub-catchment area in plantation forestry (*Pinus radiata*) outside the farm boundary (Figure 4-1b). The perennial stream enters the wetland through a clearly defined inlet pipe (S1 in Figure 4-1c) and exits through an outlet pipe (S2 in Figure 4-1c). The stream flow is shallow and reasonably dispersed across the wetland during low-flow conditions. The stream retreats ~1 m from the wetland edges over summer when flow is low, but the dispersed flow maintains an average waterhead of about 5 cm over the wetland surface across the stream flow area. During high-flow events, a wide channel forms across the wetland. A farm track passes over the outlet pipe that can add nutrients and suspended sediment to the outflow during overland flow events with little opportunity for interaction with the wetland. Similar overland flows can also be expected from the ephemeral stream joining the outflow near the end of the wetland and can be a source of high NO_3^- concentrations in the outflow as it washes off nutrients that accumulate on hillslopes used by livestock for grazing (McDowell, 2023).

4.2.1.1 Wetland soil

The wetland soil is a mix of colluvium of primarily Makara steepland soil (Typic Orthic Brown soil) and alluvium consisting of Ramiha soil (Acidic Allophanic Brown Soil) and Korokoro soil (Typic Firm Brown Soil) from the surrounding upland hills (Pollok & McLaughlin, 1986). Makara steepland soil formed directly from greywacke baserock, a highly compacted sedimentary sandstone (Pollok & McLaughlin, 1986). The Makara soil has a thin (10 cm) topsoil of very dark greyish brown silt loam with a fine nutty structure, friable in consistency and gritty due to its formation from small greywacke fragments.

The subsoil is thick (25 cm), pale yellow with weak yellowish-brown mottling, and has a clay loam texture with many interspersed angular fragments. It is underlaid by a slightly weathered greywacke (Cowie, 1978).

4.2.2 Surface water monitoring

Surface flow (stream) discharge and quality were monitored at the inlet (inflow) and the outlet (outflow) at the seepage wetland site between June 2019-May 2021. Throughout this study, year 1 refers to the monitoring period between 1 June 2019-31 May 2020 and year 2 refers to 1 June 2020-31 May 2021.

The inflow and outflow discharge rates were recorded at 15-min intervals using an ultrasonic doppler technique with automated Unidata Starloggers 6526G-512K (model G) (Appendix 4-1). Water quality was monitored at the same monitoring points from grab samples collected fortnightly and also during high flow events, using manual height samplers which collected samples as rising water heights reached 10, 20, 30, and 40 cm above the base of the discharge logger (Appendix 4-1). Water quality sampling was disrupted during the study by field work restrictions 1) between 20 March 2020-17 June 2020 due to the Covid-19 pandemic, and 2) between 1 August 2019-30 September 2019 and 1 August 2020-30 September 2020 due to lambing seasons which prevented access to the farm due to associated animal welfare issues.

Surface water samples were kept cool during the transport to the laboratory, filtered (<0.45 µm micropore filter) within hours of the collection, and then stored frozen until chemical analysis within a period of two months.

4.2.2.1 Precipitation and evapotranspiration data collection

Daily precipitation data was collected from an on-site weather station located within 100 m of the site located and at the same elevation. However, the last five months of the 2-year study period, between January 2021-May 2021, the precipitation data were collected from a weather station 2 km down the hill on flat topography due to the malfunctioning of the on-site weather station. The alternative weather station was the closest weather monitoring station available. Substitution of precipitation data from the alternative weather station was considered acceptable as the weather station malfunction occurred during a period of comparatively less precipitation and a paired comparison between precipitation collected between the two sites for the preceding period found similar precipitation. Daily precipitation data recorded in the study area were categorised as: low (<10 mm/day), moderate (10-25 mm/day), moderately high (>25-50 mm/day) and high (>50 mm/day).

Daily evapotranspiration (ET) data was assumed equivalent to the daily potential evapotranspiration (PET), collected from the NZ's National Climate Database CliFlo (station id 3243) located within an 11 km radius of the study area (NIWA, 2023).

4.2.3 Shallow groundwater monitoring

4.2.3.1 Piezometer installation

Piezometers were installed to sample shallow groundwater at three different depths (0.5, 1, and 1.5 m) at three positions (inflow, midflow and outflow) on the wetland flowline (Figure 4-1c). Piezometers were made of PVC pipe with an internal diameter of 2.5 cm and with the bottom 50 cm of the pipes slotted (0.5 cm i.e., 0.5 m) and wrapped with a nylon screen (0.25 µm) to allow shallow groundwater flow into the piezometers with minimal clogging. The piezometers were installed by manually screwing them into the soft and unconsolidated wetland sediments so that their 0.5 m screened bottom ends were positioned to collect water from the 0-0.5 m (referred as the 0.5 m depth in this study), 0.5-1 m (1 m) and 1-1.5 m (1.5 m) depths below the wetland surface. Gaps between the piezometer and wetland sediments were sealed off using bentonite to prevent surface water entry. Piezometers were kept capped throughout the monitoring period to minimise gas exchange. The removable caps had permanently attached sampling tubes fitted, each reaching the intended shallow groundwater sampling depths. The exposed ends of the tubes were always sealed, including the period between their purging and sampling, to minimise atmospheric oxygen contamination of the shallow groundwater recharging for sampling.

4.2.3.2 Shallow groundwater sampling and in-situ measurement

Shallow groundwater physicochemical (redox potential (Eh), pH, electrical conductivity (EC), dissolved oxygen (DO)), and chemical (NO_3^-, SO_4^{2-}, dissolved Fe^{2+} and Mn^{2+}, dissolved organic carbon (DOC)) properties were monitored fortnightly using piezometers for 1.5 years (November 2019-May 2021). Shallow groundwater sampling was conducted using the national protocol for groundwater sampling in NZ (Daughney et al., 2006), and modified using the low-flow purging technique recommended by Wilde et al. (1998) to overcome the long recharge time in the piezometers.

Piezometers were purge-sampled using a peristaltic pump. Shallow groundwater three times the piezometer volume was purged to remove any standing water and ensure fresh shallow groundwater sampling from the intended wetland depths. The sampling tubes were kept sealed off between the purging and the sampling in order to maintain suction in the sampling tube during shallow groundwater recharge. Low recharge was a frequent challenge during the study period in piezometers at the 1 m depths, and at the 1.5 m depths on several occasions, at the midflow and outflow positions in the wetland. In case the shallow groundwater recharge was very slow, the intended purged volume was reduced to a single piezometer volume.

In cases where blinding of the piezometer screens occurred, the piezometers were disassembled so that the screens could be cleaned, reassembled, sealed to prevent air and water contamination, and re-purged before repeating the sampling. If the problem persisted, sampling from that piezometer was skipped for that day.

A smarTROLL Multiparameter (MP) instrument was used for *in-situ* measurement of the physicochemical properties (DO, pH, temperature, EC) of shallow groundwater in the piezometers. The collected shallow groundwater samples were filtered (through <0.45 µm micropore filter) and subsampled separately for anion, DOC and dissolved Fe^{2+} and Mn^{2+} analyses. Samples for dissolved Fe^{2+} and Mn^{2+} analyses were preserved with 2% HNO_3 to prevent oxidation of the species. Samples for DOC measurement was collected in opaque brown glass bottles to reduce contact with light. The collected samples were transferred to the laboratory in chilled condition and kept frozen in the laboratory until further analysis. An exception was the samples for DOC analysis which were stored at 4°C and analysed within 3 days of the collection.

4.2.4 Laboratory analysis

The surface and shallow groundwater samples were analysed for anion concentrations (NO_3^- and SO_4^{2-}) using Dionex Aquion Ion Chromatography (IC) with detection limits of 0.01 mg/L. Dissolved Fe^{2+} and dissolved Mn^{2+} were analysed using Agilent 4200 Microwave Plasma Atomic Emission Spectroscopy (MPAES). DOC analyses were performed on a TOC analyser (Shimadzu TOC-L), based on the combustion technique by Bremner and Tabatabai (1971).

4.2.5 Data analysis

4.2.5.1 The conceptual framework for data analysis

Data analysis in the current study was conceptually based on the water balance components in a seepage wetland illustrated in Figure 4-2. The terminologies used in the diagram and their assumptions are explained elaborately in Appendix 4-2.

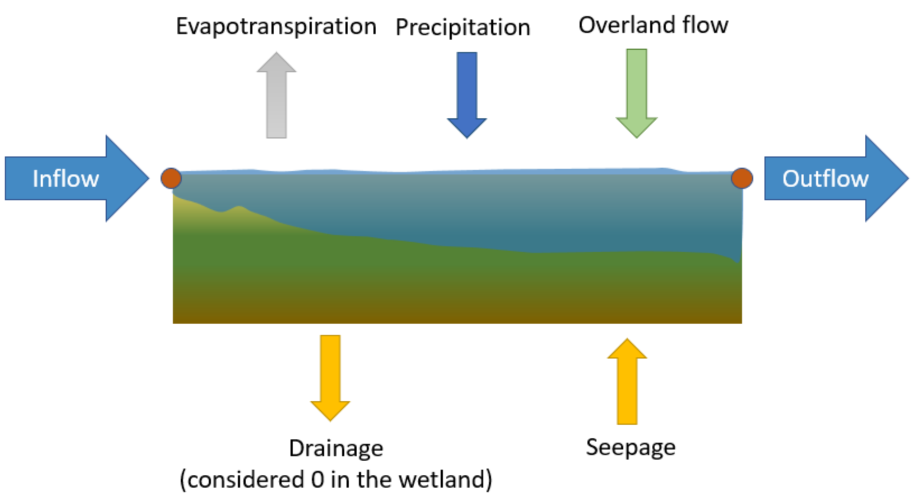


Figure 4-2. Conceptual diagram shows the water balance components in a seepage wetland. In this diagram, inflow represents the stream flow entering and outflow represents the stream flow leaving the wetland. Precipitation represents the water added via precipitation. Evapotranspiration is the water lost by the wetland vegetation and is assumed equivalent to the potential evapotranspiration in the study area. Overland flow is the surface runoff and seepage (originating in the total catchment area that includes the directly contributing catchment area) is the subsurface discharge inputs from the directly contributing catchment area between the inflow and outflow points.

The inflow and outflow data were analysed in order to estimate 1) the flow in the wetland under baseflow vs. quickflow-dominated conditions, and 2) the baseflow input necessary for the runoff estimations.

4.2.5.2 Baseflow separation

Baseflow separations were conducted on inflow and outflow discharge rates using the BFI+ (version 3.0) from the HydroOffice tool (Gregor, 2010). The baseflow separation was conducted separately for each monitored year. Local minimum filter was used during the baseflow separation in which the daily baseflow values are estimated by linear interpolations between local minimums. A local minimum is the lowest discharge in one-half the interval minus 1-day (0.5(2N*-1) days) before and after the day being considered. Finally, local minimums are connected by straight lines to adjacent local minimums (Gregor, 2010).

The analysis generated annual BFI values and daily timeseries of the BFI. The annual BFI values obtained were averaged across the 2-year study period to estimate the average BFI for the study area.

Daily BFI values were categorised into summer (December-February), spring (March-May), winter (June-August) and autumn (September-November) in order to compare seasonal baseflow contributions in the wetland.

4.2.5.3 Quickflow estimation

In order to assess the NO_3^- dynamics in the seepage wetland under different flow conditions, the daily outflow values were categorised as: (a) quickflow-dominated, if daily BFI<0.65 and (b) baseflow-dominated, if daily BFI>0.65, the cut-off value selected based on the average BFI at the outflow for the 2-year study period (Buss & Achten, 2022). Similar criteria were applied to categorise surface water quality into the quickflow-dominated and the baseflow-dominated samples. In this way, out of the total 48 surface water quality sampling events during the 2-year study period, 71% of the samples belonged to the baseflow-dominated conditions, while the baseflow-dominated conditions occurred in 66% of the monitoring period. Thus, quickflow samples are slightly underrepresented in this study.

In order to compare the outflow NO_3^- concentrations between quickflow-dominated vs. baseflow-dominated conditions, the quickflow and the baseflow water quality samples from the outlet were subsampled. During the subsampling, the water samples were ordered from the highest to the lowest BFI values. To increase their representativeness, samples with BFI>0.9 were selected as the baseflow-dominated samples and samples with BFI<0.3 were assigned as the quickflow-dominated samples in the corresponding data analysis.

4.2.5.4 Wetland water balance

4.2.5.4.1 Daily water balance

To assess the hydrological fluxes in the wetland at short temporal scales, a daily water balance was calculated for the wetland study area. The daily water balance is quantified as the water depth that the wetland retained from the inflow, i.e., inflow volume retained by unit wetland area (in mm/day), calculated using the Eq. 4-1:

Daily water balance (mm/day) = daily inflow depth (mm/day) - daily outflow depth (mm/day) (Eq. 4-1)

where,

Daily inflow or outflow depth (mm) = (daily inflow or outflow (m^3) / wetland area (m^2)) * 1000 (Eq. 4-2)

Daily inflow depth > daily outflow depth was considered a positive water balance and indicated a net wetland water gain (Figure 4-3a). Daily inflow depth < daily outflow depth was considered a negative balance and indicated a net wetland water loss from the wetland (Figure 4-3b).

To assess the seasonal flow variations in the wetland, the daily water balance across the 2-year study period was categorised into summer (December-February), spring (March-May), winter (June-August) and autumn (September-November).

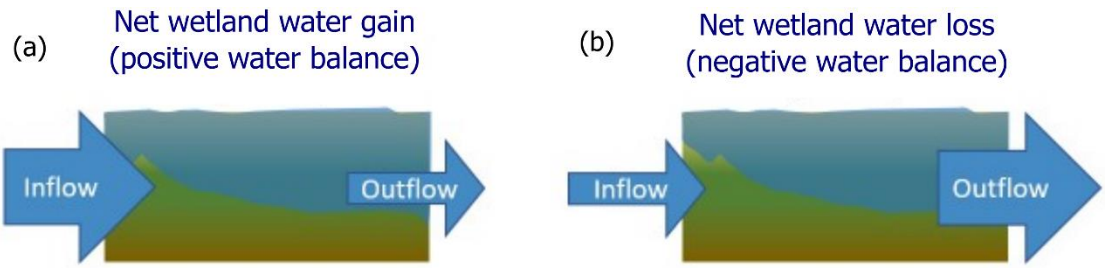


Figure 4-3. Conceptual diagram to interpret daily water balance at the current seepage wetland study site. The diagram shows, (a) net wetland water gain occurs during positive water balance when daily inflow depth > daily outflow depth, and (b) net wetland water loss occurs during a negative water balance occurs when daily inflow depth < daily outflow depth in the wetland.

4.2.5.4.2 Annual water balance and seepage estimation

The annual water balance (Eq. 4-3) was constructed to help identify the major flow pathways and estimate their relative contributions to the wetland hydrology (Figure 4-2), using Eq. 4-3:

Annual water balance (m^3/yr) = (Precipitation + Inflow + Lateral flow) (m^3/yr) - (Outflow + Evapotranspiration + Drainage) (m^3/yr) (Eq. 4-3)

From Eq. 4-3, the lateral flow (*LF*) was estimated as in Eq. 4-4. The lateral flow in the current study is the overland flow (*OF*) and seepage (*S*) combined that discharge from the direct contributing catchment into the wetland (Figure 4-2). The associated terminologies and the underlying assumptions of the water balance are explained elaborately in Appendix 4-2.

Lateral flow = (Outflow + Evapotranspiration + Drainage) – (Precipitation + Inflow) (Eq. 4-4)

where, Lateral flow, $LF = OF + S$ (Eq. 4-5)

Annual lateral flow value (Eq. 4-5) was further disintegrated to estimate the seepage using an indirect estimation technique. In this approach, the first step involved the estimation of the overland flow (*OF*) from the direct contributing catchment. The assumption was that the upper sub-catchment and the direct contributing catchment shared similar hydrogeological properties (Figures 4-1b, c). Therefore, the overland flow rate in the direct contributing catchment was assumed equivalent to the overland flow in the upper sub-catchment. Based on this assumption, the overland flow from the direct contributing catchment was calculated using the following stepwise procedure:

1. The annual baseflow in the upper sub-catchment (m^3) = wetland inflow BFI * annual wetland inflow (m^3) (Eq. 4-6)
2. The annual quickflow in the upper sub-catchment (m^3) = annual inflow (m^3) - annual baseflow in the upper sub-catchment (m^3). (Eq. 4-7)
3. Overland flow rate in the upper sub-catchment (m) = annual quickflow in the upper sub-catchment (m^3) / upper sub-catchment area (m^2) (Eq. 4-8)
4. The overland flow (*OF*) from the direct contributing catchment to the wetland was estimated as:

Overland flow from the direct contributing catchment (*OF*) (m^3) = Overland flow rate in the upper sub-catchment (m) * direct contributing catchment area (m^2) (Eq. 4-9)

Using the Eq. 4-4 and Eq. 4-9, the seepage (*S*) was estimated as in Eq. 4-10. The seepage in this study is the shallow groundwater originating from the total catchment area but discharging directly into the wetland via the direct contributing catchment area.

$S = LF - OF$ (Eq. 4-10)

4.2.5.5 Wetland NO_3^- removal estimations

4.2.5.5.1 Annual NO_3^- load estimation

In order to identify the flow condition driving major NO_3^- loads in and out of the wetland in the study area, annual NO_3^- loads were estimated for the inflow and the outflow using the flow-stratified technique (Elwan et al., 2018). In this technique, the measured flow rates were ranked from the highest to the lowest and were assigned to 5 flow bins, as this method has been found to be a more accurate way of assessing nutrient loads for New Zealand waterways (Elwan et al., 2018). The average NO_3^- load for a flow bin was the product of the average flow rate and the mean NO_3^- concentration measured in the flow bin. The annual NO_3^- load was the sum of the average NO_3^- loads from the 5 flow bins for a monitored year. The annual NO_3^- loads from the lowest 80% flow were calculated as the sum of the average loads from the flow bins 2-5 for a monitored year and were considered the NO_3^- load in the wetland under low flow conditions. The annual NO_3^- loads from the top 20% flow were considered to represent the NO_3^- load in the wetland under high flow conditions.

4.2.5.5.2 Annual NO_3^- balance

To estimate the NO_3^- removal by the seepage wetland, a simplified approach was adopted. The N-input from precipitation was considered negligible (Parfitt et al., 2012). The annual NO_3^- balance for the wetland was calculated separately for the monitored years as:

NO_3^- balance (kg NO_3-N/yr) = outflow NO_3^- load (kg NO_3-N/yr) - inflow NO_3^- load (kg NO_3-N/yr) - overland flow NO_3^- load (kg NO_3-N/yr) - seepage NO_3^- load (kg NO_3-N/yr) (Eq. 4-11)

In Eq. 4-11, the inflow and the outflow NO_3^- loads are the annual NO_3^- loads at the inflow and the outflow, respectively (Section 4.2.5.5.1). The overland flow NO_3^- load is the product of the annual overland flow from the direct contributing catchment in a monitored year (Eq. 4-9) and the mean inflow NO_3^- concentration under quickflow-dominated conditions, because overland flow generates during quickflow-dominated conditions (Section 4.2.5.3) across the 2-year study period. The seepage NO_3^- load is the product of the annual seepage volume indirectly estimated from the direct contributing catchment in a monitored year (Eq. 4-10) and the mean NO_3^- concentration in shallow groundwater at the 1.5 m depths across the wetland inflow, midflow and outflow positions across the shallow groundwater monitoring period. The seepage NO_3^- load estimation in the current study was based on the mean shallow groundwater NO_3^- concentration at the 1.5 m depths because 1) the seepage in this study is the subsurface input (originating in the total catchment area) from the direct contributing catchment area into the wetland (Section 4.2.5.1, Figures 4-1b,c, 4-2, Appendix 4-2), and 2) the deepest subsurface input i.e. the shallow groundwater monitored depth in the current study was the 1.5 m depth in the wetland and was considered closer to the seepage source, compared to the remaining shallower monitored 0.5 and 1.0 m depths.

4.2.5.5.3 Daily NO_3^- dynamics

To assess the dynamics of NO_3^- removal from inflow in the wetland, a daily NO_3^- dynamics was estimated as the difference between the daily inflow and daily outflow NO_3^- loads on surface water quality sampled days (Eq. 4-12). For simplicity, all other surface or subsurface pathways of NO_3^- input and loss were considered absent.

Daily NO_3^- dynamics (g NO_3-N/day) = daily inflow NO_3^- load (g NO_3-N/day) – daily outflow NO_3^- load (g NO_3-N/day) (Eq. 4-12)

The wetland was considered a NO_3^- sink during a positive daily NO_3^- dynamics if daily inflow NO_3^- load > daily outflow NO_3^- load. The wetland was considered a NO_3^- source during a negative daily NO_3^- dynamics occur if daily inflow NO_3^- load < daily outflow NO_3^- load.

Daily inflow and daily outflow NO_3^- loads were calculated using (Eq. 4-13), for example, at inflow:

Daily inflow NO_3^- load (g NO_3-N/day) = mean inflow discharge rate of the surface water quality sampled day (L/s) * corresponding inflow NO_3^- concentration (mg NO_3-N/L) (Eq. 4-13)

4.2.5.6 Subsurface redox assessment

Because NO_3^- reduction via denitrification is a redox reaction, assessment of the redox environment can indicate the potential for subsurface NO_3^- reduction in wetlands. The subsurface redox categorisation technique, developed by McMahon and Chapelle (2008), is a commonly used approach in which subsurface environments are assigned to the redox categories of oxic, mixed, or anoxic, based on the quantified available electron acceptors (O_2, NO_3^-, Fe^{3+}, Mn^{4+}, SO_4^{2-} and CO_2). Many studies in NZ have used this technique to determine how supportive the subsurface environment is for subsurface NO_3^- reduction across different landscapes in NZ (Clague, 2013; Clague et al., 2019; Close et al., 2016; Collins et al., 2017; Rivas, 2018; Sarris et al., 2019; Wilson et al., 2018), mostly in deep groundwater (>2 m depth). In order to characterise the wetland subsurface NO_3^- reducing environment in the seepage wetland site, a subsurface redox assessment was conducted in the current study. Major redox categories for the collected shallow groundwater samples were determined, and the indicators of the NO_3^- reducing subsurface environments were obtained using the method of McMahon and Chapelle (2008) with modifications (increased threshold concentration for DO concentration) for NZ conditions based on Wilson et al. (2016) (Table 4-1). During the assessment, the *in-situ* measured shallow groundwater DO concentration was used for the O_2 concentration.

Table 4-1. Threshold concentrations of shallow groundwater properties used for the subsurface redox assessment in the current study, modified from McMahon and Chapelle (2008) and Wilson et al. (2016).

Redox category	Redox process	DO	NO_3-N	Dissolved Mn^{2+}	Dissolved Fe^{2+}	SO_4^{2-}
		mg/L				
Oxic	O_2 reduction	> 2	-	< 0.05	< 0.1	
Suboxic		< 2	< 0.5	< 0.05	< 0.1	
Anoxic	NO_3^- reduction	< 2	≥ 0.5	< 0.05	< 0.1	
	Mn^{4+} reduction	< 2	<0.5	≥ 0.05	< 0.1	
	Fe^{3+}/SO_4^{2-} reduction	< 2	<0.5	-	≥ 0.1	≥0.5
	Methanogenesis	< 2	< 0.5	-	≥ 0.1	<0.5
Mixed	When more than one redox process criteria is present	-	-	-	-	-

4.2.6 Statistical analysis

In order to characterise the wetland hydrological properties in the study area, descriptive statistical analysis (range, mean, median and standard deviation) was conducted on 1) surface flow discharge rates, and 2) shallow groundwater properties. By using the Analysis of Variance by Tukey technique, NO_3^- concentrations were compared in 1) outflows under the quickflow- vs. the baseflow-dominated conditions, 2) shallow groundwater between the monitored depths, and 3) shallow groundwater between summer (December-May) and winter (June-November), within a 95% confidence interval ($p \leq 0.05$) using Minitab (version 19.1.1). The relationships between shallow groundwater properties, including their categorical variable i.e., depth, were assessed by conducting a Kendal-Tau rank non-parametric correlation (significant at p≤0.05) on the entire database of shallow groundwater by using R (RStudio 1.2.5033). Plots were generated using MS Excel and R.

4.3 Results

4.3.1 Seepage wetland hydrology: flow characteristics and water balance

The seepage wetland received 83% of the total annual flow from stream inflow (Figure 4-4a, Table 4-2). This suggests a stream inflow-dominated hydrology. In the wetland, outflow discharge rates were generally higher (median 1.1 L/s, mean 2.5 L/s, range 0.4-67.6 L/s) than inflow discharge rates (median: 0.4 L/s, mean: 2.1 L/s, range 0.4-93.4 L/s), indicating an additional flow input (13.1 x 10^3 m^3/yr which is 14% of the outflow averaged across the 2-year study) from the direct contributing catchment. Most of this additional flow input was from seepage (11 x 10^3 m^3/yr) that accounted for about 84% of total input (13.1 x 10^3 m^3/yr) from the direct contributing catchment area (Figure 4-4a, Eq. 4-10). The wetland showed a flow-dampening effect, observed in the gentler slope of the outflow's flow exceedance curve, in contrast to the steeper curve observed at the inflow (Figure 4-4b). Low-flow conditions tended to prevail in the wetland area where the outflow discharge rate was <0.7 L/s during 70% of the monitored period (Figure 4-4b).

The seepage wetland was baseflow-dominated as suggested by the mean outflow BFI of 0.66 across the 2-year study, meaning an estimated 66% of the outflow was baseflow, while the remaining 34% of the outflow was quickflow. The inflow BFI was 0.53 during the study. The mean baseflow discharge rates in the outflow were observed to be higher in the winter and spring (1.6 and 0.9 L/s, respectively) compared to summer and autumn (0.4 and 0.5 L/s, respectively). A more detailed seasonal comparison of baseflow during the study period is illustrated in Figure 4-6.

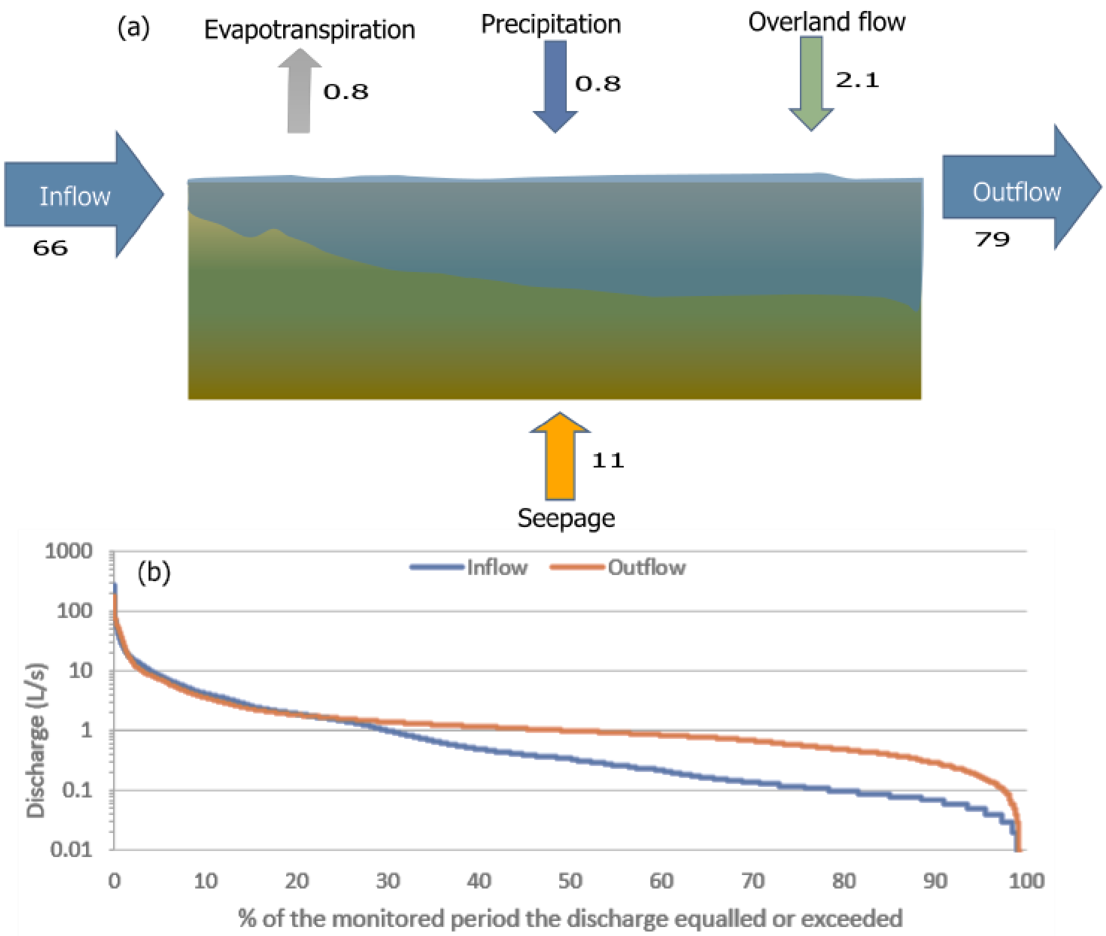


Figure 4-4. (a) Average annual water balance (10^3 m^3/yr) and (b) inflow and outflow exceedance curves based on their corresponding flow discharge rates (L/s) at 15-min intervals in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) in year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021).

During the study years, the annual precipitation was similar between the study years (year 1: 906 mm, year 2: 915 mm), with precipitation being 17-18% less than the long-term average annual precipitation of 1100 mm for the study area (Fransen et al., 2022). The contribution of precipitation as input to the wetland's annual water balance was found to be negligible (0.8%) and was equivalent to the annual evapotranspiration loss (Figure 4-4a). However, as a driver of short-duration but major hydrological events (shown as large fluxes in Figure 4-4b), precipitation played a significant role in the wetland hydrology over the 2-year study.

Table 4-2. Annual water balances in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) in year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021). The deep drainage from the wetland was assumed negligible and not accounted for in the water balance.

Water balance components	**Year 1**	**Year 2**
	(10^3 m^3)	
Precipitation, *P*	0.8	0.8
Inflow, *I*	68.9	62.3
Outflow, *O*	85.3	72.4
Evapotranspiration, *ET*	0.8	0.8
Input = *P* + *I* + *LF* (lateral flow)	69.6	63.0
Output = *O* + *ET*	86.1	73.2
Lateral flow considered equivalent to the residual of the water balance, LF = (*O* + *ET*) – (*P* + *I*)	16.5	10.2
Overland flow, *OF*	2.2	2.1
Seepage, *S* (= *LF* – *OF*)	14.3	8.1
	%	
Lateral flow contribution to the outflow	19	14
Seepage contribution to the lateral flow	87	80
Seepage contribution to the outflow	17	11

4.3.2 Hydrological responses to precipitation

The wetland hydrological fluxes on a daily to a seasonal basis, showed similar patterns to the corresponding precipitation fluxes in the study area (Figure 4-5).

4.3.2.1 Daily water balance

At the onset of the moderately high to high precipitation events (as defined in Section 4.2.2.1), that were frequent in winter and spring, both the inflow and the outflow rates rose sharply (Figure 4-5a). Initially, the outflow rate is lower than the inflow rate, which caused a net wetland water gain before a prolonged net wetland water loss (Figures 4-3a, b; 4-5a, b). For example, at the onset of a high precipitation event of 72 mm on 13/06/2019, an initial rapid net gain of 2674 mm/day of water depth occurred as the wetland absorbed water from the inflow (Figure 4-5b). However, as

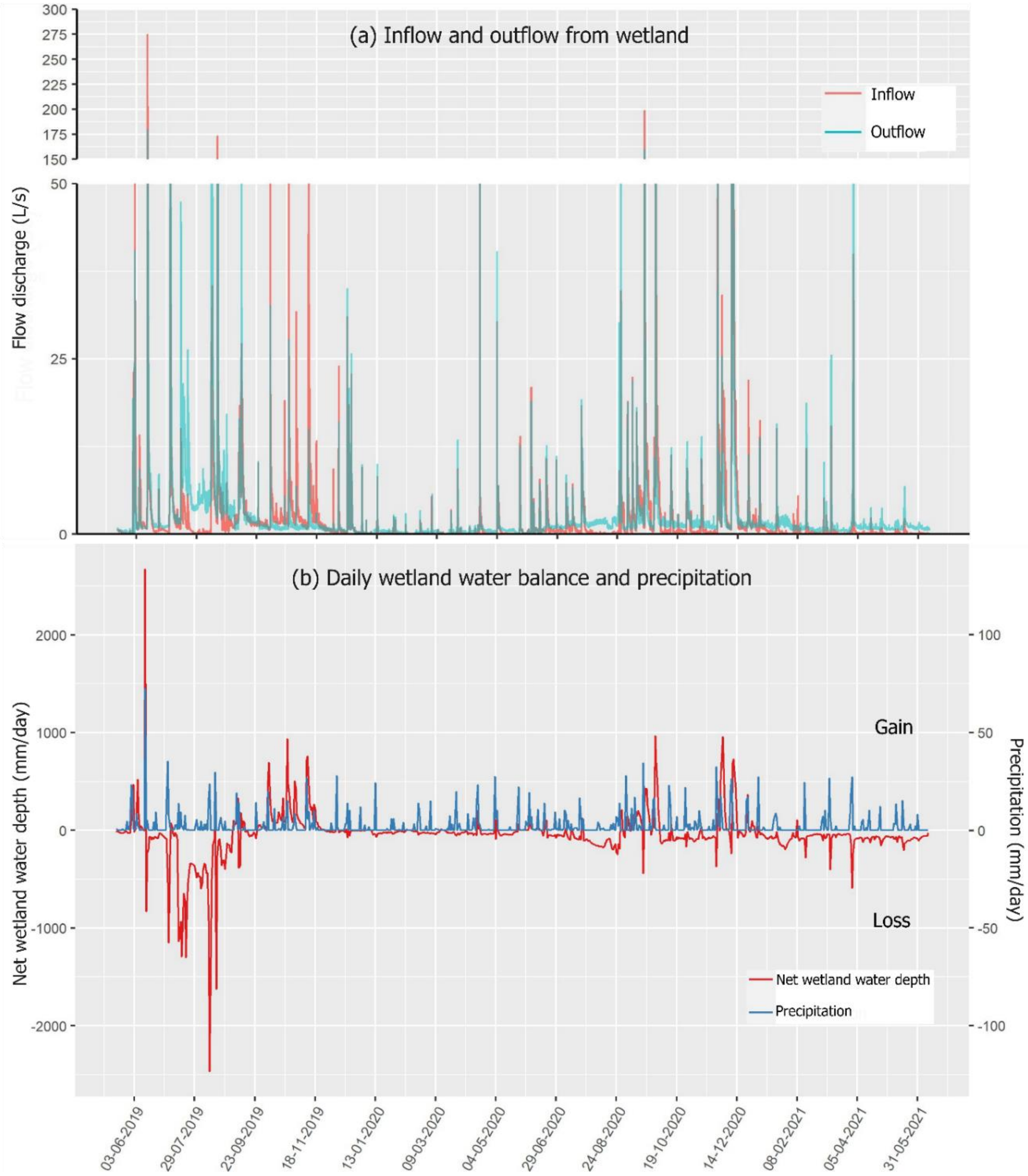


Figure 4-5. (a) Surface flow i.e., stream inflow and outflow rates (L/s), and (b) the daily water balance (= inflow - outflow, in mm/day) and the daily precipitation (mm/day) in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) between year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021). Gain = positive wetland water balance or net wetland water gain, Loss = negative wetland water balance or net wetland water loss at daily scales.

throughflow (a shallow subsurface component of the stormflow) to the wetland increased, the outflow rate began to exceed the inflow rate. Consequently, a prolonged net wetland water loss occurred (14/06/2019-16/08/2019, Figures 4-3b, 4-5b). These losses were larger in volume compared to the initial rapid net gain, with additional hydrological contributions from a series of low to moderately high rainfall events during this period. Several events of such initial rapid net gains occurred in the wetland driven by moderately high to high daily rainfall between June and December, i.e., winter and spring, during both study years.

4.3.2.2 Seasonal distribution of flow in seepage wetland

A seasonal investigation conducted by summing up the daily water balances for each season, showed there was a seasonally cyclic pattern in the net wetland water gains and losses in the studied wetland. Across the 2-year study period, spring measured a net wetland water gain (8 x 10^3 m^3), whereas summer (-1 x 10^3 m^3), autumn (-4 x 10^3 m^3) and winter (-14 x 10^3 m^3) measured net wetland water losses from the wetland. The magnitudes of net wetland water losses were smaller during summer and autumn as compared to the net wetland water loss in winter (-14 x 10^3 m^3) (Figure 4-5b).

The seasonal outflow distribution from the wetland closely reflected the seasonal distribution of the precipitation measured during both monitored years (Figure 4-6). For instance, year 1 measured concentrated winter precipitation (345 mm precipitation between 1 June – 30 August) that approximated ~38% of the total annual precipitation (Figure 4-6). Larger fractions of the annual flow (both outflow and its baseflow component) also left the wetland during winter in year 1 (Figure 4-6). This resulted in a disproportionately large net wetland water loss (85% of the annual net wetland water loss) from the wetland during winter in year 1 (Figure 4-5b). Smaller fractions of total annual precipitation received in the non-winter seasons of year 1 resulted in lower fractions of annual outflow during those seasons, compared to their winter values (Figure 4-6).

The concentrated winter precipitation in year 1 also likely had driven the higher lateral flow (16.5 x 10^3 m^3) (Table 4-2), which conforms from overland flow and seepage

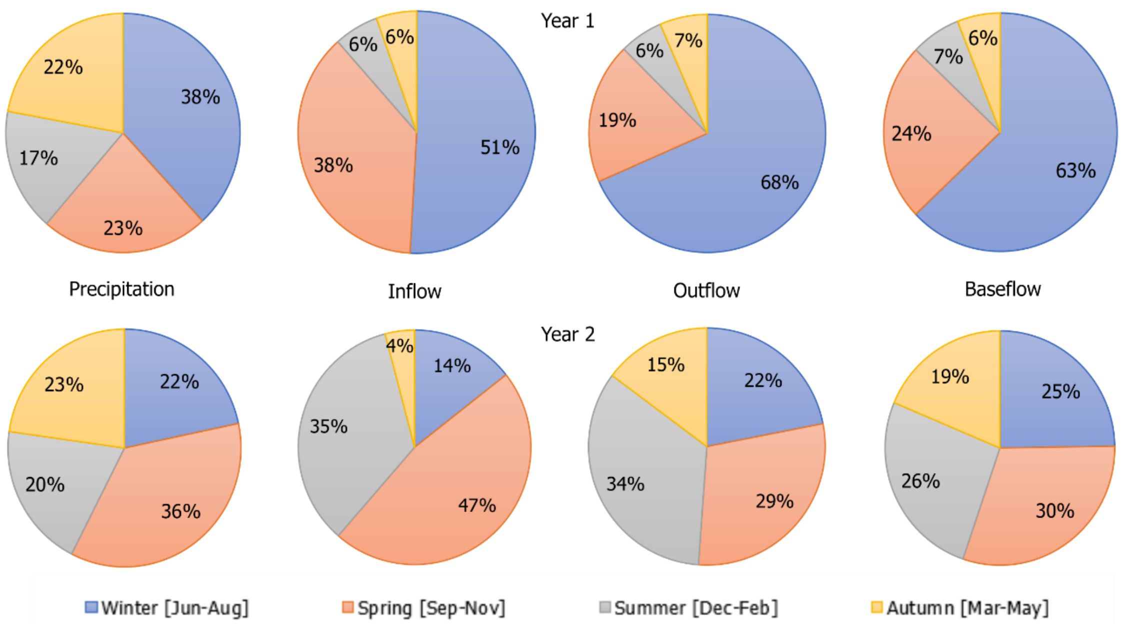


Figure 4-6. Seasonal distribution (%) of annual precipitation, inflow, outflow and baseflow (in outflow) observed in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) during the year 1 (June 2019-May 2020) and the year 2 (June 2020-May 2021).

(Section 4.2.5.1), into the wetland in year 1, as compared to the lateral flow (10.2 x 10^3 m^3) in the year 2, which received less winter precipitation (Figure 4-6). In contrast to the concentrated winter precipitation (345 mm, 38% of the annual precipitation) in year 1, there was less winter precipitation in year 2 (199 mm, 22% of the annual precipitation (Figure 4-6). It generated less outflow (22%) and less baseflow (25%) during winter in year 2 (Figure 4-6). It resulted in only 26% of the annual net wetland water loss in winter in year 2, compared to 85% in year 1 (indicated in Figure 4-5b). When compared with the precipitation, the proportional seasonal distributions in outflow suggest 1) the strong influence of precipitation on the outflow, and 2) an overall dissipated hydrological flow through the wetland in year 2 (Figure 4-6).

The seasonal inflow distribution was also different between the monitored years in the wetland. In year 1, the wetland received most of the annual inflow during winter (51%, 35 x 10^3 m^3), followed by spring (38%, 26 x 10^3 m^3), summer (6%, 4.2 x 10^3 m^3) and autumn (6%, 3.8 x 10^3 m^3). In contrast, in year 2, most of the total annual inflow occurred during spring (47%, 29 x 10^3 m^3) and summer (35%, 21 x 10^3 m^3), while winter received only 14% of the annual inflow (8.9×10^3 m^3) and autumn received a small fraction (4%, 2.5×10^3 m^3) (Figure 4-6).

4.3.3 Effect of seepage wetland hydrology on NO_3^- load and removal

4.3.3.1 Flowrate contribution to NO_3^- load in and out of the wetland

In general, high flow conditions contributed to most of the inflow and outflow NO_3^- loads (Table 4-3). However, in contrast to year 1, a substantial proportion of the outflow NO_3^- load also occurred in low flow conditions in year 2 as the sum of the outflow NO_3^- loads in the lower flow bins 2-5 (1.8 kg NO_3-N/yr at an average flow rate of 0.6-1.5 L/s) was approximately equivalent to the high NO_3^- load measured in the highest flow bin in year 2 (flow bin 1, 2.2 kg NO_3-N/yr at an average flow rate of 7.2 L/s) (Table 4-3). The wetland also lost more NO_3^- load in outflow in low flow conditions than it received in inflow, in year 2, as the outflow NO_3^- load in the low flow bins of 2-5 (1.8 kg NO_3-N/yr at an average flow rate of 0.6-1.5 L/s) that year was greater than the inflow NO_3^- load (0.5 kg NO_3-N/yr at an average flow rate of 0.1-0.8 L/s) (Table 4-3).

Compared to year 1, the wetland received 56% higher inflow NO_3^- load in year 2, but 26% less outflow NO_3^- from the wetland through the outlet (Table 4-3). This non-linear

Table 4-3. Annual NO_3^- loads measured in the inflow and outflow in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) in year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021), estimated by the flow-stratification technique, with the flowrates ranked from the highest to the lowest and assigned to 5 flow bins. [a] flow bin 1. [b] flow bins 2-5.

Study year	Flow monitoring point	NO_3^- load (kg NO_3-N/yr)							
		Average load in flow bins					Annual load	In highest 20% flow[a]	In lowest 80% flow[b]
		1	2	3	4	5			
Year 1	Inflow	2.92	0.60	0.06	0.04	0.02	3.6	2.9	0.7
	Outflow	5.00	0.24	0.07	0.11	0.03	5.4	5.0	0.5
Year 2	Inflow	5.2	0.1	0.05	0.28	0.08	5.7	5.2	0.5
	Outflow	2.2	0.1	0.5	1.0	0.2	4.0	2.2	1.8

disparity, in the inflow and outflow NO_3^- loads of the wetland could be affected by variations in the hydrological fluxes observed during the study years (Figures 4.5, 4.6).

4.3.3.2 Annual NO_3^- balance of the wetland

The average annual NO_3^- balance indicated that the wetland served as an overall NO_3^- sink. However, there was a wide annual variation in NO_3^- removal across the study years 1 and 2 (Table 4-4). The wetland removed an average of 23% of the surface and subsurface NO_3^- input combined during the 2-year study period (Figure 4-7, Table 4-4). However, in year 1, the wetland only removed 0.3% of the NO_3^- input load, compared to 40.8% in year 2 (Table 4-4). The annual NO_3^- removal capacity in the seepage wetland varied between 0.21 kg NO_3-N/ha/yr (year 1) and 33 kg NO_3-N/ha/yr (year 2).

The lateral flow contributed an estimated 25% of the total NO_3^- input to the wetland averaged across the 2-year study (33.4% in year 1 and 16.4% in year 2) to the wetland (Table 4-4). While the lateral flow NO_3^- contribution appears disproportionately large compared to the total catchment NO_3^- contribution, it should be noted that the lateral flow accounts for both the overland flow and seepage discharging from the direct contributing catchment into the wetland, where seepage can originate from the total catchment area. Our estimate shows that the majority of the lateral NO_3^- input reached the wetland as the seepage NO_3^- input, which accounted for 20.5% of the total

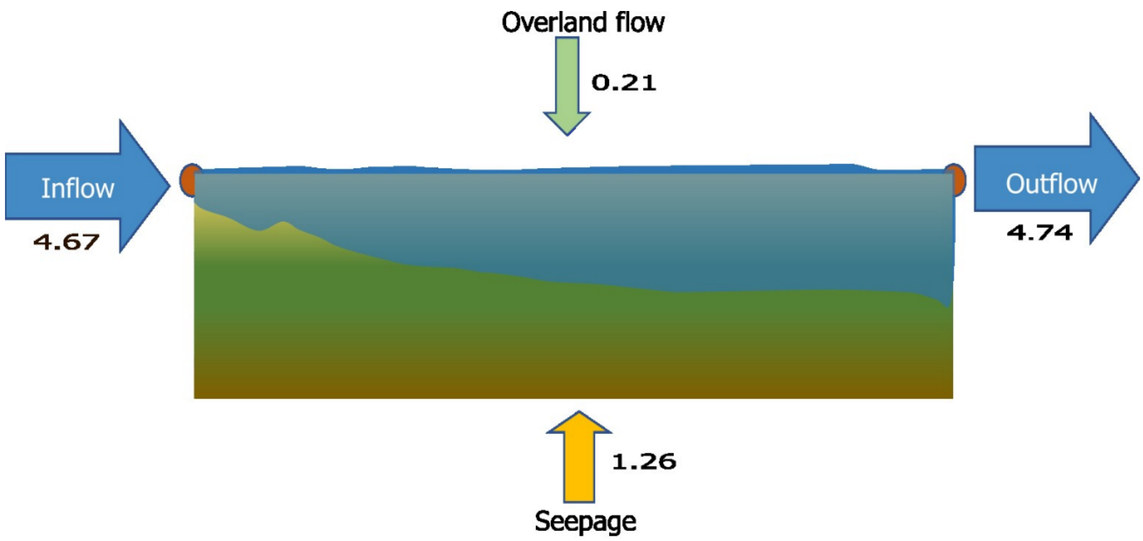


Figure 4-7. Average annual wetland NO_3^- balance (kg NO_3-N/yr) in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) averaged across the 2-year study period of year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021).

NO_3^- input into the wetland (Figure 4-7). The estimated seepage NO_3^- input was particularly high in year 1 (1.6 kg NO_3-N/yr), compared to year 2 (0.9 kg NO_3-N/yr) (Table 4-4).

Table 4-4. The annual NO_3^- balances in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) in year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021).

NO_3^- balance components	**Year 1**	**Year 2**
	(kg NO_3-N//yr)	
Inflow NO_3^- load, *I* (measured)	3.64	5.69
Outflow NO_3^- load, *O* (measured)	5.45	4.03
Overland flow NO_3^- input, *OF* (estimated)	0.21	0.20
Seepage NO_3^- input, *S* (estimated)	1.61	0.92
Input = *I* + *OF* + *S*	5.47	6.81
Output = *O*	5.45	4.03
NO_3^- balance = Input – Output	0.02	2.78
%NO_3^- removal from the input	0.3	40.8
Mean NO_3^- removal across the 2-year study (kg NO_3-N/yr)	1.4	
Mean NO_3^- removal across the 2-year study (%)	22.8 (~23)	

4.3.3.3 Daily NO_3^- dynamics

The wetland's performance as a NO_3^- source or sink was influenced by the daily precipitation fluxes (Figure 4-5b). Comparison between the daily NO_3^- dynamics (Figure 4-8) and the daily net wetland water gains and losses (Figure 4-5b), which were closely associated with precipitation (Figure 4-5b), shows that the wetland can become a NO_3^- sink (positive daily NO_3^- dynamics) during initial rapid net water gains during moderately high to high precipitation events (Section 4.3.2.1). For example, at the onset of an exceptionally high precipitation event of 72 mm/day on 13/06/2019, an initial rapid net water gain accounted for a high and positive daily NO_3^- dynamic of 106 g NO_3-N/day in the wetland the same day in year 1 (Figures 4-5b, 4-8, Section 4.3.2.1).

On the contrary, the wetland became a net NO_3^- source and measured a high and negative daily NO_3^- dynamic (-777 g NO_3-N/day on 12/08/2019 in Figure 4-8) that resulted from prolonged net wetland water losses (between June-August in year 1 on

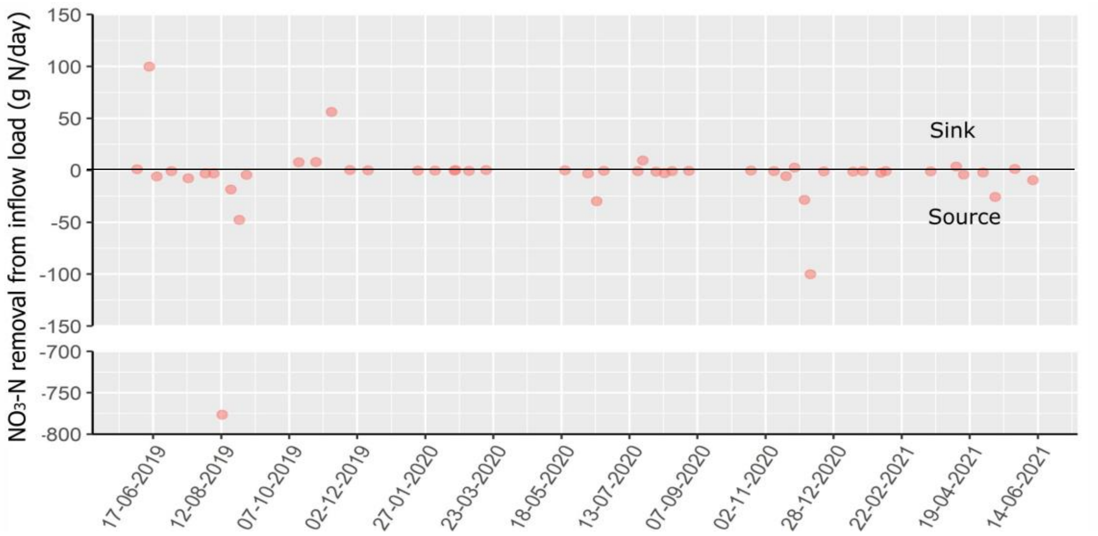


Figure 4-8. Daily NO_3^- removal dynamics (g NO_3-N/day) in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand). The daily NO_3^- dynamics is daily inflow NO_3^- load - daily outflow NO_3^- load which was calculated only for the surface water quality sampling days in year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021). Sink = positive daily NO_3^- dynamics when daily inflow NO_3^- load > daily outflow NO_3^- load. Source = negative daily NO_3^- dynamics when daily inflow NO_3^- load < daily outflow NO_3^- load.

Figure 4-5b, Section 4.3.2.1). The exceptionally high precipitation event (of 72 mm/day on 13/06/2019) and a following series of moderately high to high precipitation events between June-August in year 1 had likely developed high surface and subsurface runoff and generated high NO_3^- loads with low residence time that may have limited the NO_3^- removal in the wetland that year.

However, because annual precipitation is generally equivalently distributed in the region where the study area was located (Chappell, 2015), the event that measured high negative daily NO_3^- dynamics (-777 g NO_3-N/day on 12/08/2019) in winter in year 1 was not included in the seasonal analysis of the wetland's daily NO_3^- dynamics.

Seasonal investigation into the daily NO_3^- dynamics across the 2-year study (Figure 4-8) showed the wetland is mainly 1) a NO_3^- source during winter, but 2) a NO_3^- sink during spring, both linked to the corresponding seasonal net wetland water losses and gains, respectively (Figure 4-5b). These exhibit a strong influence of a precipitation-driven stream inflow-dominated hydrology on the NO_3^- removal dynamics in the seepage wetland site. Spring accounted for an estimated 31% of the measured total positive daily NO_3^- dynamics (seasonal mean 39.1 g NO_3-N/day across the 2-year study). In contrast, 54% of the negative daily NO_3^- dynamics in the wetland occurred during winter (seasonal mean -61.9 g NO_3-N/day across the 2-year study). In comparison, spring measured a very low negative daily NO_3^- dynamics of 4% (mean -6.1 g NO_3-N/day across the 2-year study) and suggested overall scope for substantial positive daily NO_3^- dynamics in the wetland during spring.

4.3.4 Shallow groundwater physicochemical and redox characteristics

4.3.4.1 Shallow groundwater physicochemical properties

The shallow groundwater which was monitored at the 0.5, 1 and 1.5 m depths in the seepage wetland was found to be predominantly oxic (mean DO of 5.5 mg/L) with a pH of 6.5-6.3. These ancillary data are presented in Appendix 4-3. Low DO (<2 mg/L) was occasionally measured at the 1.5 m depth, particularly in the inflow and outflow wetland positions. Shallow groundwater remained predominantly low in NO_3^- concentration (mean 0.07-0.11 mg NO_3-N/L) throughout the study area, but measured an abundant supply of available electron donors (mean 5.8-11.7 mg DOC/L, 14.1-29.3 mg Fe^{2+}/L and 2.7-4.1 mg Mn^{2+}/L) at the monitored three depths (Figure 4-9).

4.3.4.2 Depth variation in shallow groundwater properties

Several shallow groundwater physicochemical properties (pH, DOC and SO_4^{2-}) that can affect subsurface NO_3^- reduction, are negatively correlated with wetland depth ($p \leq 0.05$; Figure 4-9) and positively with DO concentrations (Appendix 4-4). The mean concentrations of DO (6.99 mg/L), SO_4^{2-} (1.59 mg/L) and DOC (11.65 mg/L) were significantly higher at the 0.5 m depth, compared to the values at the 1 and 1.5 m wetland depths (Figures 4-9a, e-f) ($p \leq 0.05$). Dissolved Fe^{2+} was present in significantly higher concentrations (29.5 mg/L; $p \leq 0.05$) at the 1 m depth, where dissolved Mn^{2+} concentration was also high (4.19 mg/L, $p > 0.05$) (Figures 4-9c, d). Dissolved Fe^{2+} negatively correlated with both DO and SO_4^{2-}, which were positively correlated with each other (Appendix 4-4). The NO_3^- concentration was consistent and low throughout the investigated wetland depths (~0.1 mg NO_3-N/L, $p > 0.05$, Figure 4-9b).

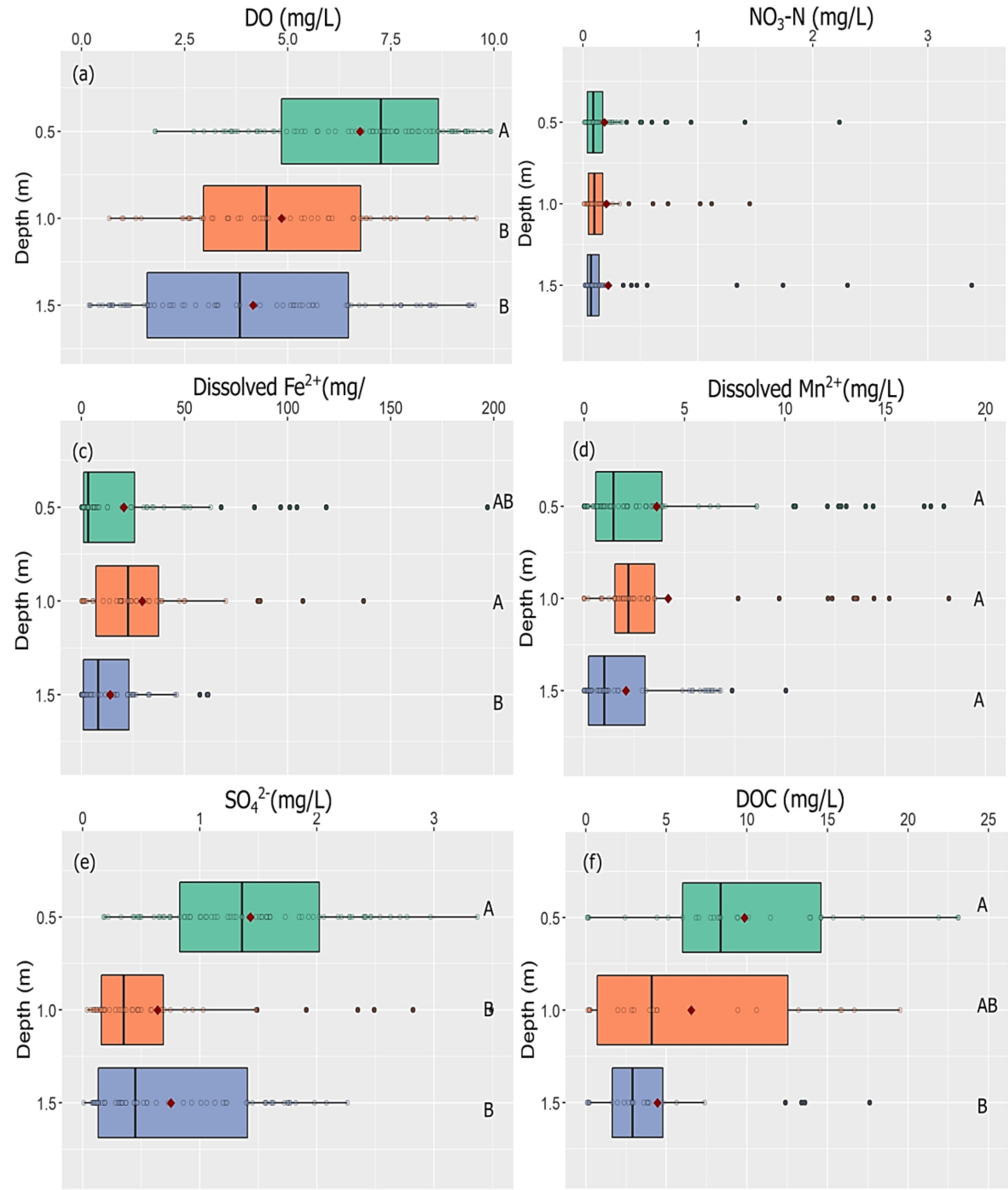


Figure 4-9. Boxplots show shallow groundwater (a) dissolved oxygen (DO), (b) NO_3-N, (c) dissolved Fe^{2+}, (d) dissolved Mn^{2+}, (e) SO_4^{2-} and (f) dissolved organic carbon (DOC) concentrations (mg/L) measured at the 0.5, 1 and 1.5 m depths in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) between November 2019-May 2021. The first and third quartiles represent 25% and 75% of the measured values, respectively. The median value is indicated by the vertical line inside the boxplot. The mean value is shown with the red diamond. Solid dots are outliers. Different letters indicate significant differences (p≤0.05) in mean concentrations compared between wetland depths. Two measurements of >9 mg NO_3-N/L from the 1 m depths at the inflow and the outflow positions that were measured in summer 2020-21 are not shown.

4.3.4.3 Subsurface redox categorisation for NO_3^- reduction

As per the subsurface redox assessment criteria (Table 4-1), O_2-based redox reaction was found to dominate in shallow groundwater at the seepage wetland site at all 3 depths. Deviations only occurred on five occurrences, between April and July, (i.e., late autumn-early winter), when the redox category was assessed as mixed oxic-anoxic at the 0.5 m depths among which three were O_2-Fe^{3+}/SO_4^{2-} and two were O_2-CH_4 driven redox processes. At the 1 m depth, on three occasions, the mixed oxic-anoxic redox characteristics were observed, with one O_2-Fe^{3+}/SO_4^{2-} and two O_2-CH_4-driven redox processes. At the 1.5 m depths, three suboxic and one anoxic (i.e. NO_3^--driven redox process) occurrences were observed (McMahon & Chapelle, 2008). All these deviations occurred between April and July during the study period. No clear effect of precipitation on these five occurrences could be drawn because half of the events occurred two days after a precipitation event, while the remaining events had no prior precipitation events at least five days prior to the corresponding sampling events.

4.3.5 Comparison of seepage wetland surface flow and shallow groundwater NO_3^- concentrations in seepage wetland under different hydrological conditions

During the study, the mean outflow NO_3^- concentration was significantly higher (0.10 mg NO_3-N/L) under quickflow-dominated condition than in the baseflow-dominated condition (0.03 mg NO_3-N/L, $p<0.05$) (Figure 4-10). The median NO_3^- concentration was 0.02 mg NO_3-N/L under both conditions.

When surface flow and shallow groundwater NO_3^- concentrations were compared, as in Figures 4-11a-d, the shallow groundwater NO_3^- values across the wetland depths (mean: 0.25, 0.20, 0.43 mg NO_3-N/L and median: 0.06, 0.10, 0.09 mg NO_3-N/L at the inflow, midflow and outflow wetland positions, respectively) were higher than the NO_3^- in surface flow i.e. inflow (mean: 0.08, median: 0.03 mg NO_3-N/L) and outflow (mean: 0.07, median: 0.03 mg NO_3-N/L). Also, the shallow groundwater NO_3^- concentrations averaged across the wetland positions, were 0.19 (median: 0.09 mg NO_3-N/L), 0.57 (median: 0.11 mg NO_3-N/L), 0.22 mg NO_3-N/L (median: 0.07 mg NO_3-N/L)

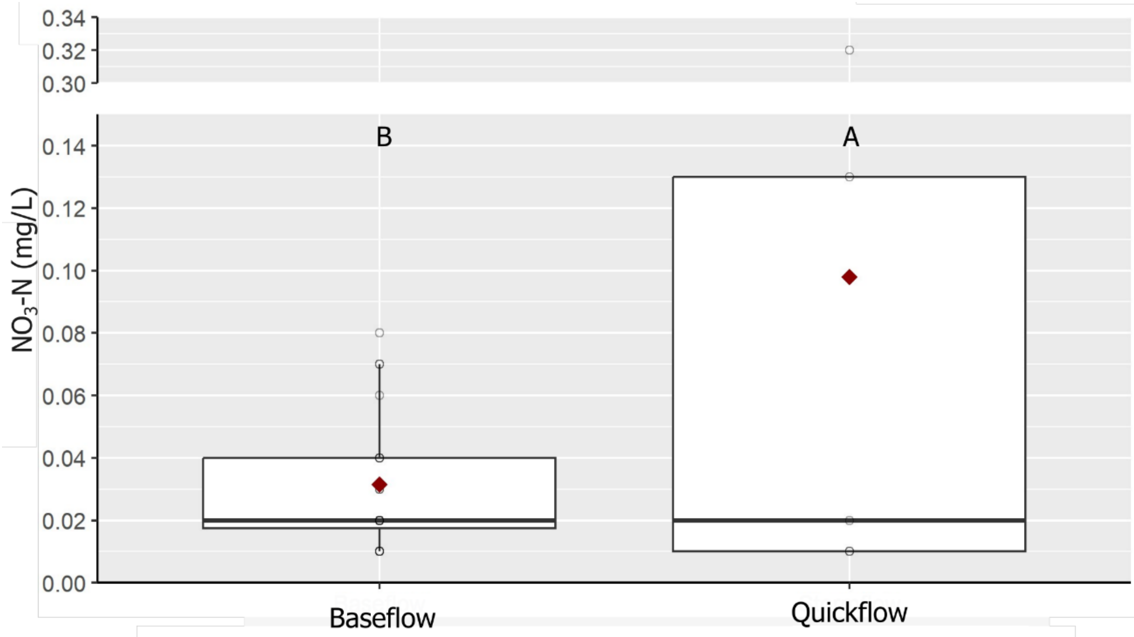


Figure 4-10. The outflow NO_3^- concentrations (mg NO_3-N/L) compared between the quickflow-dominated and baseflow-dominated conditions in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) between June 2019-May 2021. The first and third quartiles represent 25% and 75% of the measured values, respectively. The median value is indicated by the horizontal line inside the boxes. Mean values are depicted by a red diamond. Different letters indicate significant difference ($p \leq 0.05$) in the mean NO_3^- concentrations.

at the 0.5, 1 and 1.5 m depths, respectively, and were higher than the mean surface inflow (mean: 0.08, median: 0.03 mg NO_3-N/L) and outflow NO_3^- (mean: 0.07, median: 0.03 mg NO_3-N/L) concentrations. These comparisons suggested overall higher NO_3^- concentrations in shallow groundwater as compared to the surface inflow and outflow in the wetland.

Within the subsurface, the lack of seasonal differences in most of the monitored parameters ($p>0.05$), indicated reasonable stability in shallow groundwater properties throughout the year. The only exceptions were the DO and NO_3^- concentrations. The mean DO concentration was higher in winter (7.97 mg DO/L) than in summer (6.37 mg DO/L) at the 0.5 depth ($p \leq 0.05$) (Figure 4-12a). Conversely, the mean NO_3^- concentration was higher in summer (0.24 mg NO_3-N/L) than in winter (0.08 mg NO_3-N/L) at the 0.5 depth ($p \leq 0.05$) (Figure 4-12b). The seasonal comparison, however, kept two observations of NO_3^- concentrations >9 mg NO_3-N/L, in shallow groundwater at the 1 m depth in the wetland as outliers.

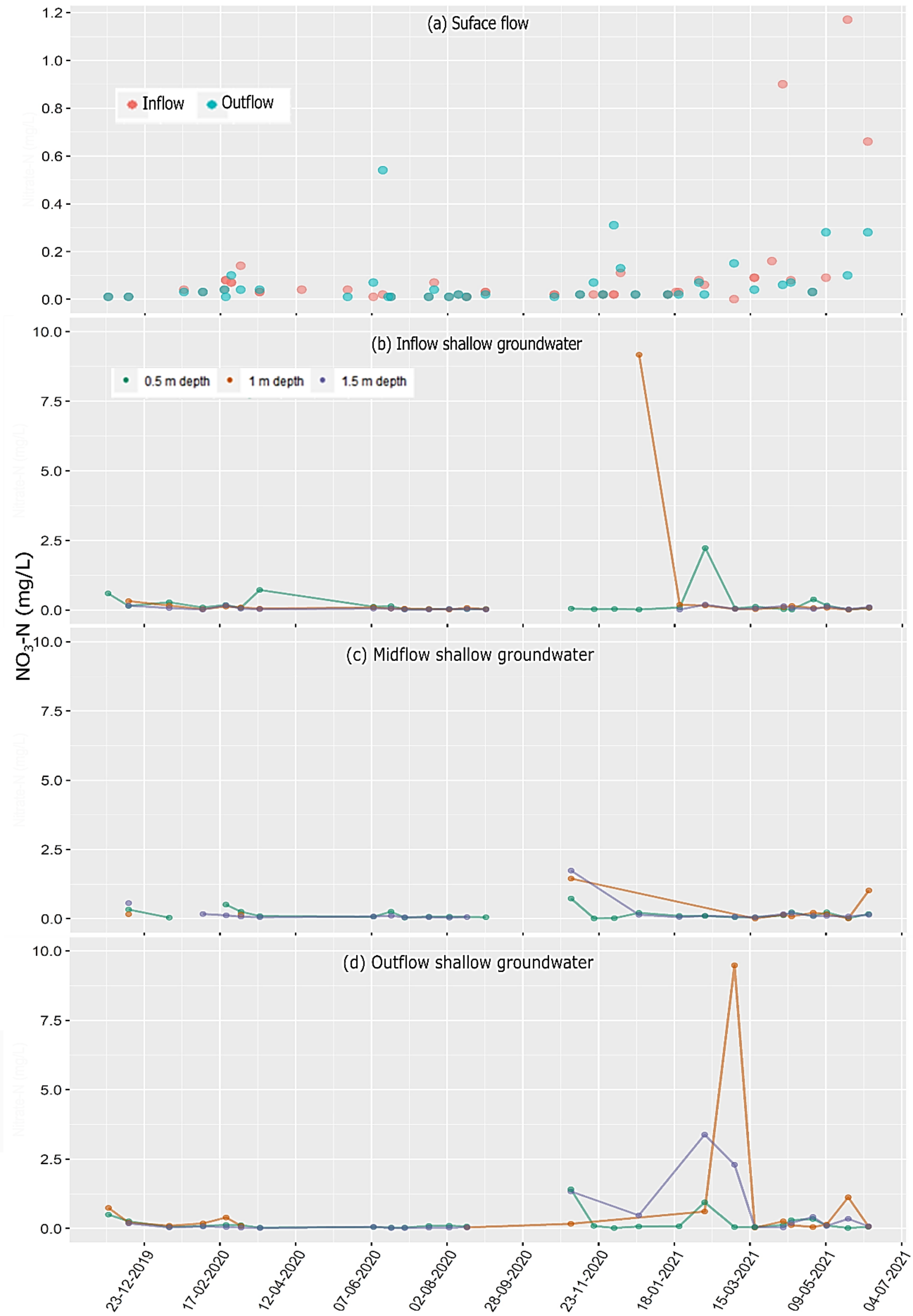


Figure 4-11. The NO_3^- concentrations (mg NO_3-N/L) compared between the surface flow and shallow groundwater at the inflow, midflow and outflow positions in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) between November 2019-May 2021.

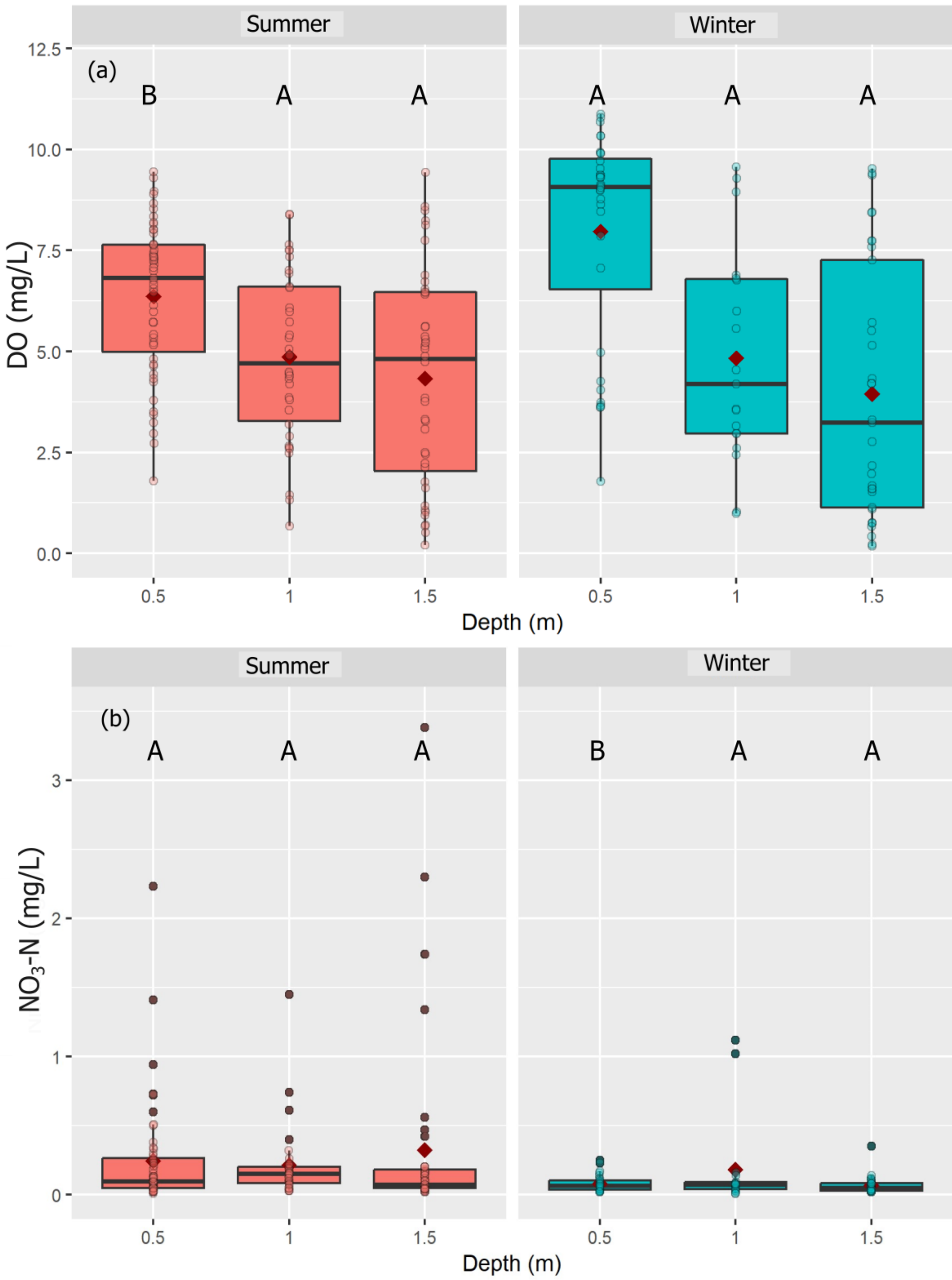


Figure 4-12. Shallow groundwater (a) DO (mg/L), and (b) NO_3^- concentrations (mg NO_3-N/L) compared between summer (November-April, in red) and winter (May-October, in blue) within a depth in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) between November 2019-May 2021. The first and third quartiles represent 25% and 75% of the measured values, respectively. Medians are represented by the horizontal line inside the box. Red dots represent the mean values. Solid dots are outliers. Different letters indicate significant difference ($p \leq 0.05$) between seasonal mean concentrations at a depth in shallow groundwater. Two measurements of > 9 mg NO_3-N/L from the 1 m depths at the inflow and the outflow positions that were measured in the summer 2020-21 are not shown.

During this study, shallow groundwater measured particularly high NO_3^- concentrations of 0.45-9.5 mg NO_3-N/L on several sampling occasions. Most of the elevated shallow groundwater NO_3^- concentrations occurred during summer (90%), under aerobic conditions (>2 mg DO/L) and at the 1 and 1.5 m depths (Appendix 4-5). The elevated shallow groundwater NO_3^- concentrations mostly in summer, indicate NO_3^- input mechanisms possibly include shallow groundwater NO_3^- input, grazing and nitrification in wetland soils (Appendix 4-5).

4.4 Discussion

4.4.1 Seepage wetland hydrology and its influence on NO_3^- dynamics

The hydrological characterisation of the seepage wetland in this study shows a precipitation-driven stream inflow-dominated hydrology. The wetland received most of its hydrological input (83%) from the stream that strongly responded to precipitation events in the study catchment (Table 4-2). According to the NZ wetland classification, devised by Johnson and Gerbeaux (2004), seepage wetlands receive hydrological inputs from surface flow (i.e. streams, springs) and subsurface seepage. Although previous seepage wetland studies have often reported these wetlands mainly as groundwater-fed (Burns & Nguyen, 2002; Hughes et al., 2013; Uuemaa et al., 2018), seepage contribution has been relatively minor in the current study. A previous study also showed a low seepage contribution of only 1-8% (at the 0-30 cm depths) to the total wetland water flow in a pastoral hill country seepage wetland, in the Waikato region in NZ (Rutherford & Nguyen, 2004). Similarly, in the current study, the seepage contribution to the total wetland outflow was low (8-14%), as estimated in the annual water balance of the wetland (Table 4-2). However, the inflow BFI of 0.53 (Section 4.3.1) suggests the stream that feeds the wetland receives large subsurface input from the catchment. Thus, the wetland is indirectly exposed to substantial subsurface hydrological and the associated NO_3^- loads from the catchment.

From a NO_3^- mitigation perspective, an important implication of the stream inflow-dominance in the seepage wetland hydrology, is that the wetland NO_3^- removal is likely to be low, compared to the groundwater-fed wetlands (Valkama et al., 2019; Walton et al., 2020). An additional potential limitation for NO_3^- reduction in the seepage wetland is the oxic subsurface observed (Section 4.3.4.3). Wetlands are effective nutrient traps due to their physical (vegetational friction) and biogeochemical features (OM-rich, saturated, reducing condition) that increase residence time, enhance sediment-NO_3^- interaction and facilitate a NO_3^- reducing environment (Valkama et al., 2019). Similar effects can be expected in the flow-dampening effect of the wetland (Section 4.3.1, Figure 4-3b), which suggests the wetland slows down the stream inflow and increases NO_3^- residence time in the wetland. Yet, the generally short residence time of stream flow can be a challenge for efficient NO_3^- removal (McPhillips et al., 2015). For example, Shabaga and Hill (2010) have shown that a fast-discharging spring with short residence time was a NO_3^- source in a valley bottom wetland study in Canada. In contrast, in the same study, a low NO_3^- concentration that discharged from a diffuse flow demonstrated the positive effect of long residence time for NO_3^- reduction in wetlands.

4.4.2 NO_3^- removal in seepage wetland

The wetland in the study area was found to be an overall NO_3^- sink. An estimated 23% NO_3^- removal occurred in the wetland averaged across the 2-year study (Section 4.3.3.2). This NO_3^- removal estimate is based on the seepage wetland's annual water and NO_3^- balances that combined surface and subsurface NO_3^- inputs into the wetland (Section 4.2.5.5.2). This is a novel approach in pastoral hill country seepage wetland NO_3^- removal studies. Previous seepage wetland NO_3^- removal estimates were based on either the surface or the subsurface hydrological pathways (Burns & Nguyen, 2002; Nguyen et al., 1999a; Rutherford & Nguyen, 2004). While the estimated 23% NO_3^- removal in the current study is comparable to that reported in other seepage wetlands under surface-flow prevalent conditions (24±9% NO_3-N removal within 1.5 m flow distance) (Rutherford & Nguyen, 2004), it is less than the NO_3^- removal of >75% measured in shallow groundwater-dominated pastoral natural seepage and constructed wetlands in NZ (Burns & Nguyen, 2002; Uuemaa et al., 2018).

Precipitation is a key driver in NO_3^- removal fluxes in seepage wetlands, as this study has shown influences of precipitation on NO_3^- removal on daily (Section 4.3.2.1) to annual scales. The wide annual variation in NO_3^- removals (0.3% in year 1 vs. 40.8% in year 2) (Table 4-4), and particularly the very low NO_3^- removal in year 1, highlights the effect of precipitation-driven hydrological fluxes on seepage wetland NO_3^- removal. Year 1 experienced higher winter precipitation than year 2 (Figure 4-5a). The stream-inflow dominated seepage wetland hydrology also showed strong responses to precipitation throughout the study, at a daily through to a seasonal and annual scale (Figures 4-5, 4-6). A previous seepage wetland study has also observed a reduction in NO_3^- removal during high precipitation events (Nguyen et al., 1999a). A pastoral hill country seepage wetland observed during a 6-month study near Hamilton, went from being a NO_3^- sink, as the wetland measured 51% NO_3^- removal from surface flow under no- to-low precipitation conditions, to being a NO_3^- source during high flow conditions (≥75 m^3/day) driven by high precipitation (Burns & Nguyen, 2002; Nguyen et al., 1999a). The current study reaffirms similar precipitation effects on NO_3^- removals in seepage wetlands and expands our understanding of the temporal hydrological dynamics previously observed in studies which ranged from a few weeks to a 6-month study, to the annual scale observed in the current study.

The large annual variation in NO_3^- removals observed in the current study is also a warning against the generalisation of seepage wetlands as NO_3^- sinks, as is frequently suggested in the literature (Hughes et al., 2013; Uuemaa et al., 2018; Zaman et al., 2009). Additionally, the large annual variation in NO_3^- removals also indicates the need to identify 1) major temporal NO_3^--loss and NO_3^--removal hot moments, and 2) the drivers of spatial and temporal variabilities in NO_3^- dynamics in seepage wetlands. A sound understanding of these hot moments, which have disproportionately high effects on wetland NO_3^- removals over short periods of time (McClain et al., 2003; Vidon et al., 2010), could help optimise NO_3^- removal in seepage wetlands and reduce farm NO_3^- losses to receiving waters.

4.4.3 NO_3^- loss hot moment in wetland

Results of this study suggest that major NO_3^- loss hot moments in the seepage wetland surface and subsurface flows are triggered by precipitation-driven high flow conditions. The study demonstrated, high and frequent precipitation events (Figure 4-5) drive

1) high flow conditions that account for a large proportion of annual NO_3^- loads in (including surface and subsurface NO_3^- input) and out of the seepage wetland (Section 4.3.3.1, Table 4-3), 2) large outflow from the seepage wetland (Figures 4-5, 4-6), and eventually, 3) large fluxes in NO_3^- removal in the wetland (Figure 4-8).

The concentrated winter precipitation in year 1 is a particularly supporting example of precipitation as the key driver of NO_3^- loss hot moments (Table 4-3). In hillslope hydrology, high precipitation can generate high subsurface flow, saturation excess overland flow, and stream flow of short duration and low wetland residence time that can result in large NO_3^- inflow loads from pastoral hill country catchments (Bargh, 1978; Cooke & Dons, 1988; Davie, 2004). Precipitation has a generally equivalent seasonal distribution in the North Island where the study area is located (Chappell, 2015). However, during the current study, precipitation in year 1 was concentrated in winter, with frequent events of moderately high to high precipitation events (Section 4.2.2.1) including an event of exceptionally high precipitation (72 mm/day on 13/06/2019) (Section 4.3.2.1 and 4.3.2.2, Figures 4-5b, 4-6). The 72 mm rain in 24 hrs has a return period of 5 years in the region where the study area is located in (Chappell, 2015). The concentrated winter precipitation in year 1 (38% of the annual precipitation, Figure 4-6) resulted in 68% of the annual outflow (Figure 4-6) and 85% of the annual net wetland water loss during winter (Figure 4-5b). That year, 91.7% of the annual outflow NO_3^- load (5 kg NO_3-N/yr in flow bin 1 outflow in year 1 shown in Table 4-3) occurred during the high flow conditions, with little opportunity for NO_3^- removal in the wetland. As a consequence, a very low NO_3^- removal occurred in the wetland in year 1 (Figure 4-8, Table 4-4). In contrast, year 2 received similar annual precipitation as year 1 (Section 4.3.1), but precipitation had an equivalent seasonal distribution (Figure 4-6). In year 2, the outflow NO_3^- load (2.2 kg NO_3-N/yr in flow bin 1) under high flow conditions accounted for only 55% of the annual outflow NO_3^- load (4.0 kg NO_3-N/yr) under high flow conditions (Table 4-3) and resulted in a relatively higher NO_3^- removal (40.8%) in the wetland compared to year 1 (0.3%). A previous study has also shown NO_3^- load increased with precipitation in seepage wetlands in a highly fertilised, hill country beef and sheep farm, as annual NO_3^- loss doubled from 23 kg NO_3-N/ha in an average-precipitation year to 44 kg NO_3-N/ha during a year with above-average precipitation

(Parfitt et al., 2009), similar to the concentrated winter precipitation in year 1 of the current study. Nguyen et al. (1999a) showed NO_3^- removal decreased in seepage wetlands in high flow conditions when flowrates exceeded 30 L/s following a high precipitation event of 45 mm.

Precipitation can also drive elevated seepage NO_3^- input into seepage wetlands potentially via subsurface runoff. Several indirect observations in this study suggest, the relatively higher direct NO_3^- input in the wetland in year 1 (Table 4-3) could be sourced from subsurface runoff via seepage (Table 4-4), potentially during the concentrated winter precipitation that year. Firstly, the wetland water balance shows most (84%) of the direct hydrological input into the wetland, between the inlet and outlet points, is contributed by seepage contribution from the direct contribution catchment (Table 4-2). Thus, seepage was the more likely contributing pathway of the additional NO_3^- input, as the outflow NO_3^- load measured a higher value than the inflow NO_3^- load in year 1 (Table 4-3). The wetland water balance shows (Table 4-2) that the estimated seepage was nearly double in year 1 (14.3 m^3) to that in year 2 (8.1 m^3) and contributed 1.6 kg NO_3-N in year 1 in contrast to 0.9 kg NO_3-N in year 2 (Table 4-4). Based on these observations, the current study supports that subsurface runoff can deliver NO_3^- to seepage wetlands (Burt & Pinay, 2005) and further emphasises precipitation as a major driver in NO_3^- loss hot moments in these wetlands. Previous studies have also shown increased subsurface NO_3^- input via seepage following high precipitation events in pastoral hill country seepage wetlands (Burt et al., 2022; Parfitt et al., 2009). A small first-order stream in the mid-Atlantic coastal plain has also shown greater average baseflow NO_3^- load in winter than in summer, and higher during a wet year in contrast to a dry year (Angier & McCarty, 2008). However, there can be a lag time in such subsurface runoff NO_3^- delivery as stream water recorded high NO_3^- concentration two days after the cessation of a storm event in a pastoral headwater wetland in Australia (Smethurst et al., 2014).

4.4.4 NO_3^- removal hot moment in wetland

The NO_3^- removal hot moments, i.e., opportunities for NO_3^- attenuation in the wetland, were identified in the study area during the periods of 1) net wetland water gain

(Section 4.3.2.1), and 2) NO_3^- pulses during low flow conditions (Table 4-3). The identified NO_3^- removal hot moments were observed at different temporal scales that ranged from a daily scale (during the initial rapid net gains in winter and spring), through a seasonal (spring) to an annual scale (at low flow conditions, highlighted in year 2).

At a daily scale, the initial rapid net water gain, as observed at the onset of high precipitation event in early winter (Section 4.3.2.1, Figure 4-5b), presents an opportunity for NO_3^- removal as indicated by the positive daily NO_3^- dynamics (106 g NO_3-N/day on 13/06/2019 in Figure 4-8). This high NO_3^- attenuation was likely supported by the antecedent unsaturated condition in soils ready to absorb water and retain the NO_3^- pulse (from nitrification, pastoral deposits from the preceding, comparatively dry months) delivered to the wetland (Bergstermann et al., 2011; Murphy et al., 2014; Peterson, 2016). Precipitation-driven infiltration of NO_3^- and labile carbon was observed to enhance subsurface denitrification in pastoral riparian soils in NZ (Luo et al., 1999b). Thus, initial periods of high flow conditions can have a positive effect on NO_3^- removal in seepage wetlands. For example, due to an increase in NO_3^- loading under high flow conditions, the denitrification rate measured a 400% increase in a constructed wetland in the USA (Poe et al., 2003). Despite winter being found to be largely a season of large negative daily NO_3^- dynamics (Section 4.3.3.3), several mixed redox reactions (indicating aerobic and anaerobic reactions occurring within short distance) in shallow groundwater during winter (Section 4.3.4.3) suggest supportive subsurface environments that can benefit positive daily NO_3^- dynamics, for example, during the initial rapid net water gains.

At a seasonal scale, several observations suggest scope for enhanced NO_3^- removal during spring. Firstly, throughout the study period, spring accounted for a high positive water balance in the wetland (4×10^3 m^3 averaged across the 2-year study period), in contrast to the non-spring seasons (Section 4.3.2.2). Secondly, spring had intermittent low to moderate precipitation (Figure 4-5b), which is likely to drive water table fluctuations and spasmodic NO_3^- inputs. Additionally, spring also accounted for several initial rapid water gains by the wetland during the study (Section 4.3.2.1). The generally warmer air temperatures, compared to the preceding winter, further accelerate denitrification. These wetland features in springs can support denitrification in the study area (Batson et al., 2012; Busnardo et al., 1992; Davie, 2004; Dodd et al., 2016; Mander et al., 2011; Tomasek et al., 2019) and may have driven an overall large positive daily NO_3^- dynamics of 39.1 g NO_3-N/day during spring, averaged across the 2-year study (Section 4.3.3.3, Figure 4-8). A previous seepage wetland study also reported a similar seasonal positive effect on NO_3^- removal at a site near Hamilton, where they measured NO_3^- removal of 10-30 g NO_3-N/day in early spring (Nguyen et al., 1999a). The cyclic nature of hillslope hydrology (Section 4.3.2.2), also observed by Campbell and Jackson (2004) in similar hydrologic conditions, further suggests that the positive NO_3^- dynamics of spring can be recurring in seepage wetlands. However, the estimated NO_3^- removal in spring could also be due to plant uptake which is likely to be elevated during the optimum growth conditions of spring (Cooper & Cooke, 1984; Zaman et al., 2008). In that case, the NO_3^- removal can be temporary until plants decay and organic N is mineralised, unless plants are grazed and excrement is transferred. The generally high evapotranspiration during the spring growing season, in contrast to winter, could also be incorrectly interpreted as a net wetland water gain in spring that would affect the daily NO_3^- dynamics estimates in the current study.

Thirdly, NO_3^- removal appears to improve when seepage wetland is under low and dissipated flow conditions, as the flowrate NO_3^- load contribution shows (Table 4.3). An example of this was observed in year 2 when high net NO_3^- removal (40.8%) occurred in the wetland (Section 4.3.3.2, Table 4-4). The seasonally equivalent distribution of precipitation in year 2 (Figure 4-6) which may have facilitated a diffuse flow through the wetland (Section 4.3.2), increased residence time and interaction between sediment-NO_3^-, which promotes NO_3^- removal (Jahangir et al., 2016; Woltemade & Woodward, 2008). As a result, in year 2, the wetland acted as a NO_3^- sink with a high positive NO_3^- balance (2.78 kg NO_3-N, 40.8% NO_3^- removal).

The NO_3^- removal of 40.8% quantified under a comparatively dissipated flow condition in year 2 can be considered representative of the wetland NO_3^- removal in the study area. This is because the study area is under low flow conditions most of the time, as the wetland's hydrological characterisation suggests (Section 4.3.1). In contrast, the average 23% NO_3^- removal estimated across the 2-year study (Table 4-4) has the risk of underestimating the NO_3^- balance for the study area due to the interference from the very low 0.3% NO_3^- removal (0.02 kg NO_3-N/yr) measured in year 1, which had concentrated winter precipitation including an exceptionally high precipitation event (72 mm/day) in June 2019 (Section 4.3.2.1, Figure 4.5b).

Additionally, the benefit of the dissipated flow condition for NO_3^- removal was also particularly evident in year 2 when the wetland received relatively higher inflow NO_3^- loads, as compared to year 1, but discharged lower NO_3^- loads (Table 4-3). The dissipated flow condition allowed 2.78 kg NO_3-N (40.8% of the annual input) of NO_3^- removal in year 2, in contrast to 0.02 kg NO_3-N (0.3% of the annual input) in year 1 (Figure 4-5, Table 4-4). This suggests the positive effect of dissipated flow conditions in improving wetland NO_3^- removal, as also has been observed in spring-fed valley bottom wetlands that measured 25-80% decrease in NO_3^- concentrations under diffuse surface flow conditions in spring and summer in Canada (Shabaga & Hill, 2010).

The NO_3^- removal hot moments, indicated by the positive NO_3^- balance during the net wetland water gains and the dissipated flow condition in the current study, have demonstrated the benefit of wetland residence time for NO_3^- removal in the study area (Briggs et al., 2014; Zarnetske et al., 2011a). It also suggests scope for the optimisation of NO_3^- removal in seepage wetlands by flow interventions that increase the residence time of stream inflow in the wetland (Jahangir et al., 2016; Shabaga & Hill, 2010).

4.4.5 Limitations for NO_3^- removal in the study area

Several naturally occurring physical and hydrological features and land-use practices in the study area could limit NO_3^- removal in seepage wetlands. Major hydrological limitations identified in the current study were 1) the stream inflow-dominance, and 2) the oxic subsurface environment in the wetland (Section 4.3.4.3).

While reducing condition benefits NO_3^- reduction in wetlands (Gillham & Cherry, 1978), the observed oxic shallow groundwater may have been a natural limitation in the current study area (Section 4.3.4.3). The oxic subsurface may have added to the possible negative effect of oxygenated surface flow condition and short residence time in the steam inflow-dominated wetland and may have contributed to the NO_3^- loss hot moments during the current study (Section 4.4.3) (Thayalakumaran et al., 2008).

Negative influence of oxic flow conditions on NO_3^- removal has previously been demonstrated in a seepage wetland (Wilcock et al., 2012). In that study, a prevalent aerobic flow condition promoted particulate NO_3^- formation that allowed a temporary NO_3^- removal via physical settling of the particles in the wetland in low flow conditions, but to resuspend and leave the wetland in high flow conditions. This mobilisation of resuspended NO_3^- accounted for an overall negative NO_3^- balance (-29±5% NO_3^- removal) in a pastoral dairy seepage wetland in NZ (Wilcock et al., 2012). The oxic subsurface in the current study area is another important limitation for overall NO_3^- removal as the wetland receives 13-29% of the total annual NO_3^- input via seepage (Table 4-4), and shallow groundwater NO_3^- concentration was often 4-5 times higher than the surface flow NO_3^- concentration (Section 4.3.5, Figure 4-11). A lack of reducing conditions at the subsurface may partly be contributing to the comparatively higher shallow groundwater NO_3^- concentration. The subsurface oxic environment, however, can be altered via flow management to increase residence time and promote reducing conditions in the seepage wetland. Under the suggested waterlogging-facilitated condition, substantially high DOC and dissolved Fe^{2+} concentrations (Figure 4-9) in the subsurface suggest that the seepage wetland will be able to sustain electron donor demand by the intended NO_3^- reduction process.

Land use practice, e.g., direct grazing in the wetland, could be another limitation for NO_3^- removal in the study area. Direct grazing on riparian waterways is common in low-flow conditions when the alternative upland pasture growth is limited due to water stress. Grazing causes physical disturbance via sediment compaction and adds NO_3^- via urine and dung deposition with an overall negative influence on the wetland NO_3^- removal (McDowell, 2023; McKergow et al., 2012; Yi et al., 2022). Although this study did not monitor direct grazing in the wetland, 1) the general grazing rotation (for an approximate 2-week-period every 45 days) practiced in the study area (J Brophy pers. comm), and 2) the frequently visible dung deposits in and adjacent to the wetland which were observed during field visits, suggest dung and particularly urine deposition are possible contributors to the NO_3^- pulse observed in this study in year 2 (Section 4.3.5, Figures 4-11, 4-12b). Additionally, the high NO_3^- concentration in the wetland in low flow conditions could also be sourced from 1) seepage in the baseflow-dominated condition (observed in the elevated NO_3^- in the shallow groundwater summer) (Appendix 4-5), and 2) nitrification (of organic nitrogenous compounds under aerobic conditions) in the dry wetland edges (Butturini et al., 2003).

Several other natural physical features could also limit NO_3^- removal in the study area that include the greywacke parent material, small wetland catchment ratio, and narrow wetland width. The high hydraulic conductive greywacke rock can drive oxic wetland subsurface conditions and low residence time, compared to finer particle parent material with lower hydraulic conductivity such as mudstone, resulting in higher residence time. The wetland width (average width <10 m) and the catchment area coverage (0.3% of the catchment area) in the study area are also less than the wetland width of 15-25 m and the catchment area coverage of 1-5% which are recommended for effective NO_3^- removal by a wetland (Mayer et al., 2007; Tanner et al., 2005).

4.4.6 Study limitations

The current study has several limitations that require to be noted. The water balance in the current study has in-built uncertainties due to the lack of direct measurements of several important water balance components, including seepage and overland flow, from the direct contributing catchment. The uncertainties may have been carried on to the wetland annual NO_3^- balance because its estimation was based on the water balance. Additionally, the NO_3^- balance had a coarse temporal resolution with possibilities of under- or overestimation in the current study. The small number of water quality samples due to the manual grab-sampling approach, were low in sampling frequency and are partly responsible for the coarse temporal resolution (as in Figure 4-8). Particularly, the daily NO_3^- dynamics estimation used a simplified approach (Figure 4-8). In addition, site access restrictions due to wet tracks, seasonal farming activities (e.g., lambing in spring), and the Covid-19 pandemic have also decreased the number of water quality samples that could be collected in the current study (Section 4.2.2). The seasonal hydrological and NO_3^- assessment may also have overlapping effects during seasonal transitions.

Future studies can focus on improving the accuracy of the seepage wetland NO_3^- removal estimates. Future research could consider adding to the current study techniques e.g., *in-situ* NO_3^- measurements in lateral flow (i.e., in seepage and overland flow), event-based and automated water samplings which cover all precipitation and grazing events. Furthermore, similar future studies at multiple sites will add robustness to the scientific understanding of the seepage wetland hydrological and NO_3^- removal characteristics.

4.4.7 Study implications

In summary, this study has characterised the hydrology and its influences on NO_3^- removal in a pastoral hill country seepage wetland in temperate climatic conditions of the lower North Island, NZ. The study has 1) quantified the surface and subsurface hydrologic and NO_3^- contributions in the wetland, 2) quantified the NO_3^- removal in the wetland and delineated the NO_3^- removal fluxes over a range of temporal scales - from a daily to an annual scale, and finally, 3) identified the major hot moments for the NO_3^- loss and the NO_3^- removal in the pastoral hill seepage wetland. The study found that the seepage wetland can be primarily stream inflow-fed and adds to the previous understanding of mainly groundwater-fed seepage wetlands. It also means climatic events, such as precipitation, are the key hydrological driver in the seepage wetland and seepage wetland NO_3^- removal. The seepage wetland receives substantial NO_3^- load in surface and subsurface runoff. While the seepage hydrological contribution can be small (8-14%), it can account for substantial annual NO_3^- input (13-29%) to the wetland, as shallow groundwater often measured 4-5 times higher NO_3^- concentration compared to the surface flow in the wetland.

Spring with low and dissipated flow conditions was found to be a season for overall positive NO_3^- removal. In contrast, NO_3^- removal is disrupted by high flow conditions and large outflow NO_3^- load from the wetland in winter.

The seepage wetland study area has an annualised NO_3^- removal of 1.40 kg NO_3-N/yr (areal removal rate 16.7 kg NO_3-N/ha/yr) based on the NO_3^- balance (based on both surface and shallow groundwater NO_3^- concentrations monitored) averaged across the 2-year study. A previous study that monitored only shallow groundwater reported an annualised NO_3^- removal of ~50 kg NO_3-N/yr in a pastoral hill country seepage wetland, located in the central North island of NZ (Burns and Nguyen 2002). Compared to the current study, the high NO_3^- removal in the previous study could be due to the particularly greater NO_3^- removal efficiency in subsurface flow pathways (Burns & Nguyen, 2002).

Compared to the mean areal removal rate of 16.7 kg NO_3-N/ha/yr measured in the current study, the areal NO_3^- removal rate measured in year 2 (33.3 kg NO_3-N/ha) is more likely to reflect the areal NO_3-N removal rate of the study area. This is because the wetland study area received seasonally equivalent precipitation in year 2 which is typical for the region the study area is located in (Chappell, 2015). To compare, another hill country seepage wetland study reported an areal NO_3^- removal rate of 3,285 kg NO_3-N/ha/yr (i.e. 0.9±0.3 g N/m^2/day) estimated based on surface flow and seepage velocity, within top 10 cm sediment depth, and combined with sediment DEA (Rutherford & Nguyen, 2004). Such a high areal NO_3^- removal rate is possibly due to the sediment DEA quantification technique, which applies an additional NO_3^- input in the laboratory- incubation. Such DEA values represent the 'potential' denitrification enzyme activity (DEA) of sediments, whereas the *in-situ* DEA values are likely to be lower. Compared to that study, a lower areal removal rate (16.7 kg NO_3-N/ha/yr)) was measured in the current study as it was based on the measured changes in NO_3^- concentrations in the hydrological pathways of surface and shallow groundwater flow, and is more likely to reflect NO_3^- removal in seepage wetlands under natural conditions.

However, a high fluctuation in the wetland NO_3^- removal measured in the current study warns of the risks of over-generalisation of the seepage wetlands as NO_3^- sinks. The strong influences of precipitation and its seasonal distribution, as drivers of NO_3^- removal flux, indicate the potential negative impact of erratic precipitation (e.g., climate change-driven increases in precipitation intensity and frequency) on seepage wetland NO_3^- removal.

This study also demonstrated NO_3^- loss risks during both high and low flow conditions in the seepage wetland, but due to different drivers. Despite the study demonstrating a positive effect of the low-flow condition on NO_3^- removal, a substantial NO_3^- load was bypassing the NO_3^- removal function in the wetland under low-flow conditions contributed by direct grazing. This NO_3^- loss in low flow conditions, however, highlights an opportunity for NO_3^- load management as dissipated flow conditions benefit NO_3^- removal in these wetlands. Targeted flow regulation might be useful in this case. Targeted flow regulation that facilitates dissipated flow through the wetland during NO_3^- pulses and allows slow or intermittent drainage through the wetland, during and after heavy precipitation in winter and after grazing in low flow conditions, could improve NO_3^- removal by 1) increasing residence time, 2) facilitating a reducing condition in the subsurface, and 3) enhancing wetland-NO_3^- interaction (McPhillips et al., 2015). Targeted NO_3^- pulse control could be used to avoid continuous drainage control operations and improve the seepage wetlands' NO_3^- removal with overall improved pastoral catchment water quality outcomes (Stålnacke et al., 2014). Future studies are required to identify the residence time for such targeted flow regulation to optimise the NO_3^- removal in pastoral hill seepage wetlands.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Suha Sanwar
Name and title of main supervisor:	Dr. Lucy Burkitt
In which chapter is the manuscript/published work?	Chapter 5
What percentage of the manuscript/published work was contributed by the student?	85%

Describe the contribution that the student has made to the manuscript/published work:

Suha assisted in designing this study and undertook all the field sampling and laboratory analysis. She statistically analysed the data, compiled all graphs and tables and drafted the manuscript with the support from supervisors.

Please select one of the following three options:

- ○ **The manuscript/published work is published or in press**
  Please provide the full reference of the research output:
- ○ **The manuscript is currently under review for publication**
  Please provide the name of the journal:
- ● **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	SUHA SANWAR Digitally signed by SUHA SANWAR Date: 2023.04.03 13:22:35 +12'00'	Main supervisor's signature:	Lucy Burkitt Digitally signed by Lucy Burkitt DN: cn=Lucy Burkitt, c=NZ, o=Massey University, ou=Farmed Landscapes Research Centre, email=L.Burkitt@massey.ac.nz Date: 2023.04.03 12:28:55 +12'00'

This form should be placed at the beginning of each relevant thesis chapter.

Chapter 5: Managed subsurface outflow to optimise nitrate removal: a laboratory-scale intact sediment column experiment

-----

The hydrological and NO_3^- removal characterisation conducted in chapter 4 identified a stream inflow-dominated hydrology in the seepage wetland study site, where NO_3^- attenuation is highly compromised under high flow conditions due to a bypassing of the wetland's NO_3^- attenuation function. Based on these observations, the effectiveness of a subsurface outflow intervention was examined in a laboratory-based intact sediment column study in order to increase the interaction between NO_3^- laden water and the wetland sediment in chapter 5.

5.1 Introduction

Seepage wetlands in pastoral hill country offer a scope to reduce nutrient losses including nitrate (NO_3^-) from farms to receiving waters. However, NO_3^- attenuation via denitrification processes in seepage wetlands depends on adequate wetland sediment-water interactions. Seepage wetlands are generally known as NO_3^- sinks (Cooke & Cooper, 1988; McKergow et al., 2016; Rutherford et al., 2018; Rutherford & Nguyen, 2004). However, their NO_3^- removal efficiency can be compromised under certain hydrologic conditions (Schaafsma et al., 1999; Tanner et al., 2005). Yet, the NO_3^- removal-limiting hydrologic conditions can be altered via water flow regulation, an approach which is in wide use for non-point source NO_3^- mitigations in natural and in artificial wetlands (Billen & Garnier, 1999; Gao et al., 2017; Hoffmann et al., 2019; Hoover et al., 2016; Kovacic et al., 2000; Kröger et al., 2012; Wang et al., 2020; Woltemade & Woodward, 2008). Yet, flow regulation has been rarely examined in natural seepage wetlands from the perspective of NO_3^- mitigation in the pastoral hill country landscape.

Hydrologic conditions such as fast-discharging and oxygenated flow conditions with short residence times can limit NO_3^- reduction in wetlands (Schaafsma et al., 1999). Water flows bypassing the wetlands' natural NO_3^- attenuation function have been reported in different pastoral catchments (Billen & Garnier, 1999; Burt et al., 1999; Puckett, 2004; Schaafsma et al., 1999). A fast-discharging spring measured little NO_3^- removal during a 100 m transport as overland flow in a wetland, in contrast to higher (50-95%) NO_3^- removals in a diffuse flow nearby, in an agricultural catchment in Canada (Shabaga & Hill, 2010). In a seepage wetland, prevalent aerobic flow conditions were found responsible for negative NO_3^- removal of -29±5% in a dairy pastoral catchment in NZ (Wilcock et al., 2012). Compared to surface flow, subsurface outflow is more efficient often measuring >90% NO_3^- removal efficiencies (Burns & Nguyen, 2002; Hill, 1996). These examples demonstrate the advantages of diffuse and subsurface flow conditions and highlight the potential for optimising NO_3^- removal in seepage wetlands via regulated subsurface outflows (Ramesh et al., 2020).

Flow regulation has been applied to agricultural drainage feeding into wetlands to improve NO_3^- treatment for decades (Chescheir et al., 1992; Kovacic et al., 2000; Schaafsma et al., 1999; Vymazal, 2022). Different types of flow-regulating interventions have been summarised in Table 2-3 (in chapter 2) that are used in various edge-of-field practices including wetlands for improving water quality. These include manipulation of surface and subsurface flow through denitrifying bioreactors, control drainage, saturated buffer zones and integrated buffer zones (Carstensen et al., 2020). These flow regulation interventions may vary in their NO_3^- removal rates and NO_3^- removal efficiencies which make them suitable for different drainage conditions and treatment objectives (Table 2-3) (Carstensen et al., 2020). A principal strategy common across various flow interventions (Table 2-3) involves enhancing interaction between sediment and drainage waters by increasing hydraulic retention time (HRT) and facilitating subsurface flow, which potentially increases NO_3^- removal via denitrification in the subsurface environment.

The NO_3^- removal rate and NO_3^- removal efficiency are commonly used parameters to measure the performance of wetlands in attenuation of NO_3^- flowing through. The NO_3^- removal rate is measured as the NO_3^- removed per unit wetland surface area per unit of time required for the drainage waters to travel through the wetland (Mendes, 2021). The NO_3^- removal efficiency is the percentage changes in NO_3^- inflow loads as the drainage travels through wetlands (Mendes, 2021). The NO_3^- removal rate and NO_3^- removal efficiency occur at the expense of each other due to their opposite relationships with the HRT (Kadlec, 2005; Lepine et al., 2016; Trepel & Palmeri, 2002). This makes it necessary to set treatment objectives and determine their associated optimum HRTs for effective NO_3^- removal by regulation of the flow through seepage wetlands. For example, if the treatment objective is to achieve a low NO_3^- concentration in the outlet discharge, the flow regulation should target an operational HRT that achieves a NO_3^- removal efficiency close to its maximum limit. Conversely, if the treatment objective is to achieve a high NO_3^- load reduction within a short period of time, the flow intervention should target an operational HRT when NO_3^- removal rate is high. Successful treatment outcomes from the flow regulation require an optimum HRT.

Flow interventions that involve subsurface flows are generally the more efficient ones in terms of NO_3^- removal (Carstensen et al., 2020; Xue et al., 1999). Compared to surface flow, slow flow through subsurface transport increases HRT and enhances drainage water-sediment interaction and facilitates NO_3^- reducing conditions which tend to have a positive influence on microbial NO_3^- reduction processes (Jiang & Chui, 2022; Kröger et al., 2012; Woltemade & Woodward, 2008; Xu et al., 2016). The benefit of subsurface flow is further supported by a recent global review that reports higher mean NO_3^- removal efficiency under control drainage involving subsurface flow, compared to the surface flow intervention types summarised in Table 2-3 (Carstensen et al., 2020). Seepage wetland studies have also shown a similar impact during subsurface transport which measured >90% NO_3^- removal (Burns & Nguyen, 2002). In contrast, NO_3^- removal efficiency was only 24±9% in surface flow in the same wetland in the pastoral hill country in NZ (Rutherford & Nguyen, 2004).

The effectiveness of flow interventions for NO_3^- removal is also influenced by the inflow feeding patterns, i.e., horizontal vs. vertical inflow (Abdelhakeem et al., 2016; Hoffmann et al., 2019; Qian et al., 2018; Wang et al., 2020). For example, a horizontal subsurface outflow intervention measured higher total nitrogen (TN) removal rates of 2.7-2.37 g $N/m^3/d$ and TN load reduction of 53-54%, compared to the lower TN removal rate of

1.2-2.3 g N/m^3/d and TN load reduction of 38-60% via vertical subsurface outflow intervention in a woodchip bioreactor (Hoffmann et al., 2019). Conversely, a study comparing different inflow feeding patterns found vertical inflow was more efficient as it measured 95-100% NO_3^- removal, and decreased from ~90% under integrated (horizontal + vertical) to 70-85% under horizontal inflow conditions, in a denitrification constructed wetland treating municipal tailwater in China (Wang et al., 2020). In that study, the high performance of the vertical inflow was attributed to its unique denitrifier abundance and diversity, compared to that in the other experimented inflow feeding patterns (Wang et al., 2020). An intact sediment column experiment simulating vertical downward percolation of surface runoff has measured 98% NO_3^- removal as NO_3^- concentration decreased from 60 to <1 mg NO_3-N/L during the flow intervention (Mwagona et al., 2019b). This emphasis on the importance of vertical mixing in surface sediments to become an effective NO_3^- sink resonates with the current seepage wetland study findings in this thesis and has previously been alluded to by Rutherford and Nguyen (2004).

While there is strong evidence that subsurface flow benefits NO_3^- removal in wetlands, it has rarely been tested to improve NO_3^- removal in pastoral hill country seepage wetlands. Due to the costs involved in testing flow interventions *in-situ*, laboratory sediment column studies using sediment cores taken from seepage wetlands, offer an opportunity to study these processes in a more controlled and feasible way. Although there are both advantages and limitations associated with field and laboratory-scale studies (Fenton et al., 2009; Kellman, 2004), sediment column flow experiments at the laboratory-scale allow 1) better control of the sediment substrate and flow regulation, 2) eliminate unwanted hydrological interferences and effects of climatic fluxes, and 3) allow robust monitoring which is often difficult over the long term in field experiments (Fenton et al., 2009; Mwagona et al., 2019b).

In this study, a set of laboratory-scale intact sediment column experiments was developed and the effectiveness of a subsurface outflow intervention was examined, in order to optimise NO_3^- removal in seepage wetland sediments that were sampled from a pastoral hill country farm in North Island in NZ. This study simulated a subsurface outflow of NO_3^--rich pastoral surface runoff water through the 15 cm depth of seepage wetland sediment columns. The research objectives were to 1) quantify the NO_3^- removal during the subsurface outflow intervention, 2) determine optimum HRT associated with optimum NO_3^- removal, 3) identify the sediment properties which influence NO_3^- removal, and 4) investigate the changes in the sediment properties following subsurface outflow intervention. The study results are expected to contribute to a sound understanding of flow regulation and its potential effects on NO_3^- dynamics and attenuation in seepage wetlands in pastoral hill county landscapes.

5.2 Methodology

5.2.1 Study area

The study area (40°21’11.8”S, 175°44’14.1”E) is a seepage wetland located at Tuapaka farm, which is a Massey University hill country research farm near Palmerston North in the lower North Island of NZ. The same wetland site also has been studied for seepage wetland sediment, hydrological and NO_3^- removal characterisations in chapters 3 and 4 of this thesis. General physical and soil characteristics of the wetland are available in chapters 3 and 4 (Sections 3.2, 4.2). The seepage wetland site is a valley bottom riparian zone fed mainly by a second-order perennial stream (Figure 4-1, Section 4.3.1) and receives minor seepage contributions (Table 4-2).

5.2.2 Sediment column coring technique

For the laboratory-scale sediment column experiment, nine intact sediment column cores were collected from the seepage wetland site. The cores were sampled in a 1m x 1m plot at the wetland outflow position (see Figure 3-2), selected for its comparatively deeper sediment depth of >1 m in the wetland.

Vegetation at the wetland surface was removed before coring to prevent plant NO_3^- uptake during the sediment column experiment. Sediment columns from the wetland were cored manually by screwing a cylindrical aluminium tube (length: ~150 cm, internal diameter of 7.2 cm), with both ends open, into the soft sediment until the core was obstructed by a dense clay layer. To prevent the soft sediment column from slipping out on removal of the aluminium tube (Al tube), suction was created inside the tube by sealing the top of the tube with a bund, before extraction.

As depth to the dense clay layer within the plot naturally varies, there were slight variations in the sediment column lengths collected. Some sediment compressions occurred during core collections. The sediment compression was measured *in-situ* using Eq. 5-1 and demonstrated in Figure 5-1:

Compression, C (cm) = A (cm) - B (cm) (Eq. 5-1)

Where A = the wetland sediment column depth in the field before coring (cm), B = sediment column length after coring (cm) as illustrated in Figure 5-1. Due to the high water contents and somewhat fluid nature of the sediments, particularly at the surface depths, the compression was high and ranged between 48-62%.

After the cores were extracted from the wetland, the ends of the tubes were sealed to keep the sediment columns submerged with the overlying stream water, as they were *in-situ*. The columns were kept standing upright during their transport to the laboratory to minimise sediment disturbance and were maintained in the same position when installed in the laboratory experiment setup (Figure 5-1).

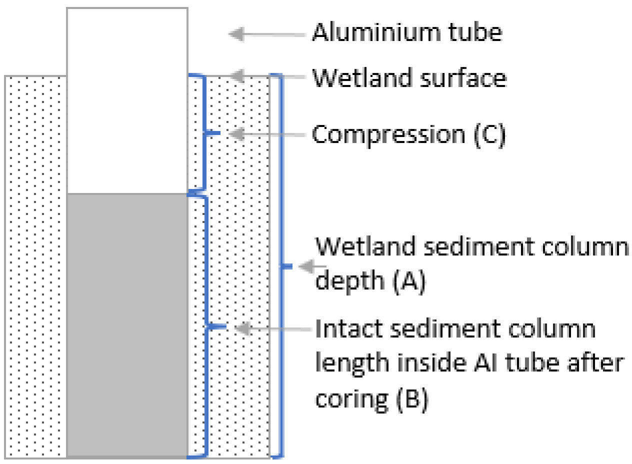


Figure 5-1. Diagram explaining sediment compression measurement technique, where compression C = A-B.

5.2.3 Laboratory experiment setup

The laboratory-scale subsurface outflow column experiment (Figure 5-2) was designed to simulate a subsurface outflow of pastoral surface runoff in wetlands and create a denitrification-conducive environment in order to promote NO_3^- removal in sediment columns. The simulated subsurface outflow involved a vertical downwelling of known pore-volumes (PV) (Eq. 5-2) of surface water and its subsequent horizontal discharge through a subsurface outflow depth at the 15 cm depth in the sediment column. All other flow interactions e.g. upwelling, and lateral subsurface flow were eliminated in the experiment setups. The 15 cm sediment column depth was selected as the intervened outflow depth because of the high denitrification enzyme activity (DEA) and sediment dissolved organic carbon (DOC) concentration, which is an electron donor in denitrification, measured at this depth and shown in Figures 3-4 and 3-7.

The PV for the intervened subsurface outflow depth was estimated using Eq. 5-2, in which the sediment column was considered as a cylindrical sphere:

$$PV = \pi r^2 \, d \, P_e \qquad \text{(Eq. 5-2)}$$

Where, r = radius of the sediment column (based on the internal diameter of the AI tube (cm), d = the intervened subsurface outflow depth (cm), P_e = effective porosity of 0.5, assuming half of the sediment sphere was water-filled pore space.

The column experiment setup consisted of six intact sediment columns as replicates. The remaining three columns were destructively sampled to measure pre-experiment sediment physicochemical and chemical properties. The columns received inflow through an inlet from a common supply tank, as shown in Figure 5-2. The inflow comprised stream water collected from the Tuapaka seepage wetland and enriched with 5 mg NO_3-N/L (from KNO_3), in order to represent pastoral surface runoff water quality. Because the sediment columns varied in their hydraulic conductivities, as observed before the experiment was initiated, inflow rates to each column varied. However, a constant 10 cm waterhead was maintained over the sediment top across all columns (Figure 5-2).

The columns were allowed to equilibrate for 2 weeks in the laboratory after the field sampling, to adapt to the laboratory temperature (20°C) and light conditions and to establish hydraulic conductivity and nutrient transport. During the equilibrium period, the columns received the inflow and drained through a narrow subsurface outlet (3 mm diameter) at the 15 cm sediment column depth where the narrow outlet acted as a physical barrier to the outflow and increased PV residence time in columns. The outflow samples discharged through the drainage outlet via a tube into an outflow sample collector (Figure 5-2), which was designed to seal off after a PV had accumulated and to spill over to a second container allowing consecutive measurements of the PV passages.

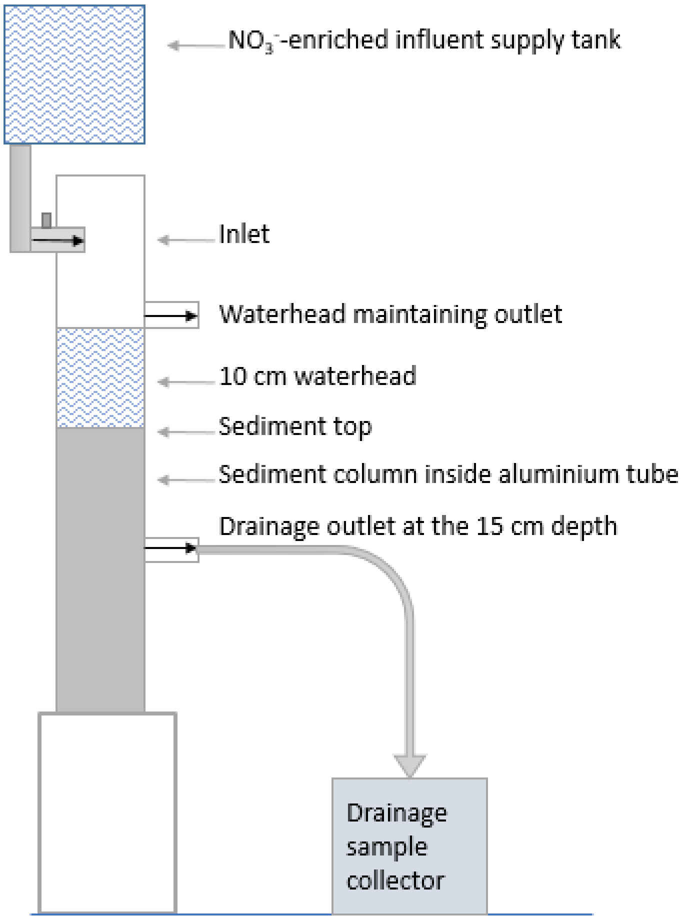


Figure 5-2. Diagram of the laboratory-scale subsurface outflow column experiment setup.

5.2.4 Subsurface outflow sediment column study

Following the equilibrium period, the subsurface outflow column experiment was initiated. During the experiment, sixteen consecutive PVs were collected as replicate outflow samples for analysis. The hydraulic residence time (HRT) in the current study is the time taken for each PV to accumulate in the outflow sample collector and was recorded manually.

5.2.5 Laboratory analysis

5.2.5.1 Pre- and post-experiment sediment physicochemical and chemical property analysis

A total of three pre- and six post-experiment sediment cores were analysed for sediment physicochemical that include pH, Eh, electrical conductivity (EC), water content, particle size analysis and chemical properties that included DOC, KCl-extractable NH_4-N and NO_3-N contents at the 0-15, 15-25 and >25 cm depth intervals in the sediment columns. Additionally, sediment porosity, particle size density, bulk density and hydraulic conductivity were measured, and organic matter content (OM) and organic carbon percentage (%OC) were quantified in the post-outflow study. The analytical techniques for the sediment physicochemical and chemical parameters have been described in Section 3.2.3 in chapter 3. Additionally in this study, OM was determined by loss on ignition technique in which soils were dried at 105°C for 24 h, then combusted at 400°C for 2 h (Nelson & Sommers, 1996). The percentage of organic carbon in sediments i.e., %org C was estimated as 58% of the OM content. Sediment porosity was determined by direct measurement using the gas pycnometer technique (Danielson & Sutherland, 1986). Due to the very fluid nature of the sediments, direct saturated hydraulic conductivity measurement was not possible. Alternatively, hydraulic conductivity was estimated based on the relationship between porosity and hydraulic conductivity known as Kozeny-Carman equation (Steiakakis et al., 2012). Sediment particle size was analysed by the slurry sampler on a Horiba Laser Scattering Particle Size Distribution Analyser (PSD) LA 950V.

5.2.5.2 Outflow sample analysis

During the experiment, 16 PV outflow sample was collected from each of the six columns in the subsurface outflow column experiment setup. The NO_3^- and DOC concentrations in the inflow and outflow were measured using an OPUS UV spectral NO_3^- sensor (TriOS, Germany) with a path length of 10 mm, precisions of ±0.005 mg NO_3-N/L and ±0.1 mg DOC/L and detection limits of 0.03 mg NO_3-N/L and 1 mg DOC/L. For NH_4^+ analysis, the samples were filtered (0.45 µm) and stored at <4°C until measured via continuous flow analysis (Technicon® AutoAnalyser II) with the detection limit of 0.25 mg N/L.

Quality control protocols were maintained during the laboratory analyses, including the use of reference samples, sample duplicates and blank samples. Triplicate analysis of sediment properties for a randomly selected depth interval in each sediment column was conducted to verify analytical precision. Randomly selected water samples also underwent triplicate analysis. Chemical analyses were spiked with reference standards at regular intervals to check for instrumental consistency. Triplicate blank analyses were conducted for each parameter investigated. Each chemical analysis undertaken in batches incorporated separate calibration and instrumental consistency monitoring.

5.2.6 Data analysis

5.2.6.1 NO_3^- removal efficiency and rate

The NO_3^- removal efficiency is defined as the percentage reduction of NO_3^- concentration during the subsurface flow between the inlet and the outlet (Figure 5-2), calculated using Eq. 5-3 as:

$$NO_3^- \text{ removal efficiency (\%)} = ((C_i - C_o) / C_i) * 100 \qquad \text{(Eq. 5-3)}$$

where C_i = inflow NO_3^- concentration (mg NO_3-N/L) at the PV passage start; C_o = outflow NO_3^- concentration (mg NO_3-N/L).

The NO_3^- removal rate is defined as the rate at which NO_3^- is removed from PV per unit sediment surface area during the subsurface outflow and is calculated using Eq. 5-4 as:

$$NO_3^- \text{ removal rate } (\mu g\ NO_3\text{-N cm}^{-2}\ \text{hr}^{-1}) = ((C_i - C_o) \times D_r)/\pi r^2 \qquad \text{(Eq. 5-4)}$$

where C_i = inflow NO_3^- concentration (mg NO_3-N/L); C_o = the outflow NO_3^- concentration (mg NO_3-N/L), r = radius of the sediment column (cm), D_r = PV drainage flowrate which was calculated as the PV/HRT (mL/hr).

The measured NO_3^- removal efficiency was interpreted using the conceptual diagram in Figure 5-3. Similar estimation techniques (Eq. 5-3 to 5-4) and concepts (Figure 5-3) were also adopted to estimate the removal rates and the removal efficiencies of NH_4^+ and DOC in this study.

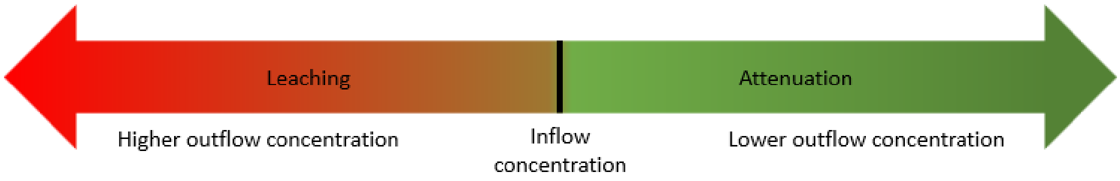


Figure 5-3. A conceptual diagram to describe the NO_3^-, NH_4^+ and DOC removals in the subsurface outflow column experiment.

5.2.6.2 Statistical analysis

The influence of subsurface outflow intervention on seepage wetland sediment properties was assessed by comparing the pre- and post-experiment sediment column properties using paired t-tests. The relationship between the NO_3^- removal efficiencies and their corresponding HRT, measured during the column experiment, was fitted to a non-linear regression using Gauss-Newton iteration. The derivative function of the non-linear regression equation was used to estimate the HRTs for targeted or modelled NO_3^- removal efficiencies (of 50, 65, 75, 80, 90 and 95%) and for the modelled rate of $\%NO_3^-$ removed starting at 0 hr of HRT for validation purposes. The modelled rate of $\%NO_3^-$ removed is the rate at which $\%NO_3^-$ is removed per hour during the experiment.

ANOVA Tukey analysis was conducted to compare the outlet NH_4^+ concentrations between different HRTs>20hr. To identify sediment properties that influenced the combined NO_3^-, NH_4^+ and DOC removals during the flow intervention, a redundancy analysis (RDA) was conducted on the full dataset where the post-experiment sediment properties were the explanatory variable and the response variables were the NO_3^-, NH_4^+ and DOC-related parameters measured during the experiment. Data were log-transformed prior to the RDA analysis. All statistical analyses were considered significant at $p \leq 0.05$. The statistical analyses for this study were conducted using Minitab (version 19.1.1) and R program (RStudio 1.2.5033). Plots in this study were generated using the R program.

5.3 Results and Discussion

5.3.1 Influence of subsurface outflow intervention on seepage wetland sediment column properties

The pre-experiment sediment columns were assessed as acidic and in moderately reduced condition. The mean pH values were 4.5, 4.9 and 4.9 and the mean Eh values were 64, 47 and 49 mV at the 0-15, 15-25 and >25 cm depths in the sediment columns, respectively (Table 5-1). The low sediment pH values were in a range slightly detrimental to denitrifier activity (Bremner & Shaw, 1958; Parkin et al., 1985). The low Eh values, however, suggested an N-reducing environment in the columns (Van Cleemput et al., 2007).

The subsurface outflow intervention at 15 cm sediment column depth resulted in a more denitrification-conducive sediment environment, as the post-experiment sediments showed less acidic and more reduced conditions compared to the pre-experiment sediment properties ($p<0.05$) (Table 5-1). A denitrification bed treating point source NO_3^- also observed increases in pH from pH 6 to 7 following flow intervention (Warneke et al., 2011). A prolonged waterlogged condition had driven a steep decline in redox potential during a column experiment on the hydrologic control of redox and N-dynamics in a peatland soil (Rubol et al., 2012). Additionally, post-experiment sediment properties in the current study measured significantly lower sediment NO_3^- (mean decrease of 165 mg NO_3-N/kg DS; from 197 mg NO_3-N/kg DS to 31 mg NO_3-N/kg DS, $p\leq0.01$) and DOC concentrations (mean decrease of 54.6 mg/kg DS; from 181 mg/kg DS to 127 mg/kg DS, $p\leq0.05$) (Table 5-1). Although there was a slight increase in the mean sediment NH_4^+ concentration (mean increase 6.3 mg NH_4-N/kg DS; from 39 mg NH_4-N/kg DS to 45.2 mg NH_4-N/kg DS), this increase was not significant ($p>0.05$) (Table 5-1).

The decrease in sediment NO_3^- concentration following the subsurface outflow intervention indicates NO_3^- reduction via denitrification within the columns (Addy et al., 2016). It further suggests the prolonged waterlogged conditions created during the subsurface outflow intervention can potentially drive NO_3^--limitation in sediments, suggested by a very low outflow NO_3^- concentration of <0.15 mg NO_3-N/L and the denitrification capacity of the sediment (quantified in chapter 3) may have remained underutilised. In that case, the subsurface outflow intervention will continue to support NO_3^- reduction in the presence of additional NO_3^- input (Zaman et al., 2008). However, NO_3^- replenishment via the inflow had likely been impeded by the low hydraulic conductivity (3.20 cm/d) (Table 5-1) and/or low outflow rates at the intervened 0-15 cm outflow depth in the columns. A previous study measured a higher saturated hydraulic conductivity of 89 ± 38 cm/d in cores of shallower depth (0-10 cm), likely due to less sediment compression compared to that measured in the current study (Section 5.2.2). The high bulk density and low hydraulic conductivity that were observed in the current study (Table 5-1), could potentially interrupt NO_3^- diffusion, carbon supply and associated denitrifier distribution resulting in low NO_3^- removal via denitrification in subsurface depths (Fenton et al., 2009). These limitations need to be kept in mind when interpreting the results of the current subsurface outflow study.

Table 5-1. Pre-experiment and post-experiment sediment properties in the sediment columns sampled from a pastoral hill country seepage wetland at the Tuapaka farm (Palmerston North, New Zealand) and used in the subsurface outflow column experiment. Data presented show mean values (± standard deviation) at the three investigated depth intervals in the sediment columns. DS = Dry Sediment.

Depth (cm)	Bulk density (g/cm^3)	Particle size density	Porosity	Hydraulic conductivity (cm/d)	% Sand	% Silt	% Clay	pH	Eh (mV)	Electrical conductivity (µS/cm)	Water content (%)	NO_3-N	NH_4-N	DOC	OM	Org C
												(mg/kg DS)			(%)	
								Pre-experiment (n=3)								
0-15	0.9±0.0	-	-	-	14	85	0	4.5±0.1	64.1±8.8	362±142	65.8±3.5	225±65	37.6±5	154±95	-	-
15-20	0.1±0.2	-	-	-	14	85	0	4.9±0.1	47±11.2	110±25	57.8±9.3	10±16	40.2±10.2	453±264	-	-
>25	1.2±0.4	-	-	-	17	82	0	4.9±0.2	49±4.3	144±75	55.1±2.6	2.4±2	40.0±5.2	386±153	-	-
								Post-experiment (n=6)								
0-15	1.2±0.38	2.3±0.2	45±14	3.2	13	86	0	5.3±0.7	30±39.8	162±109	64.1±6.3	32±25	45.2±53.6	126±109	18.0±3.3	10.5±1.9
15-20	1.3±0.3	2.3±0.3	40±17	3.2	16	83	0	5.3±0.5	29±28	38±38	55.0±8.0	10±14	55.7±29.6	130±36	16.1±3.6	9.3±2.1
>25	2.2±1.0	2.2±0.2	16±24	2.6	14	85	0	5.5±0.4	16±21	66±81	50.3±6.1	1.7±0.6	47.9±12.5	133±37	13.7±2.9	8±1.7

5.3.2 NO_3^- removal efficiency and NO_3^- removal rate

The subsurface outflow intervention measured a high mean NO_3^- removal efficiency of 91.3 (±17)% (Figure 5-4a), as the mean NO_3^- concentration decreased from 5 mg NO_3-N/L in the inflow to <0.15 mg NO_3-N/L in the outflow collected at 15 cm outflow depth. The associated HRT values ranged 0.91-457 hr (Figure 5-4) in the current study experiment. The measured mean NO_3^- removal rate was 1.7(±4.6) µg NO_3-N cm^{-2} hr^{-1} across the sediment columns (Figure 5-4b). The NO_3^- removal efficiency showed that 96% of the NO_3^- was removed in the first 13 hr (Figures 5-4a, b). This estimate agrees well with several other flow intervention studies that have measured similar high

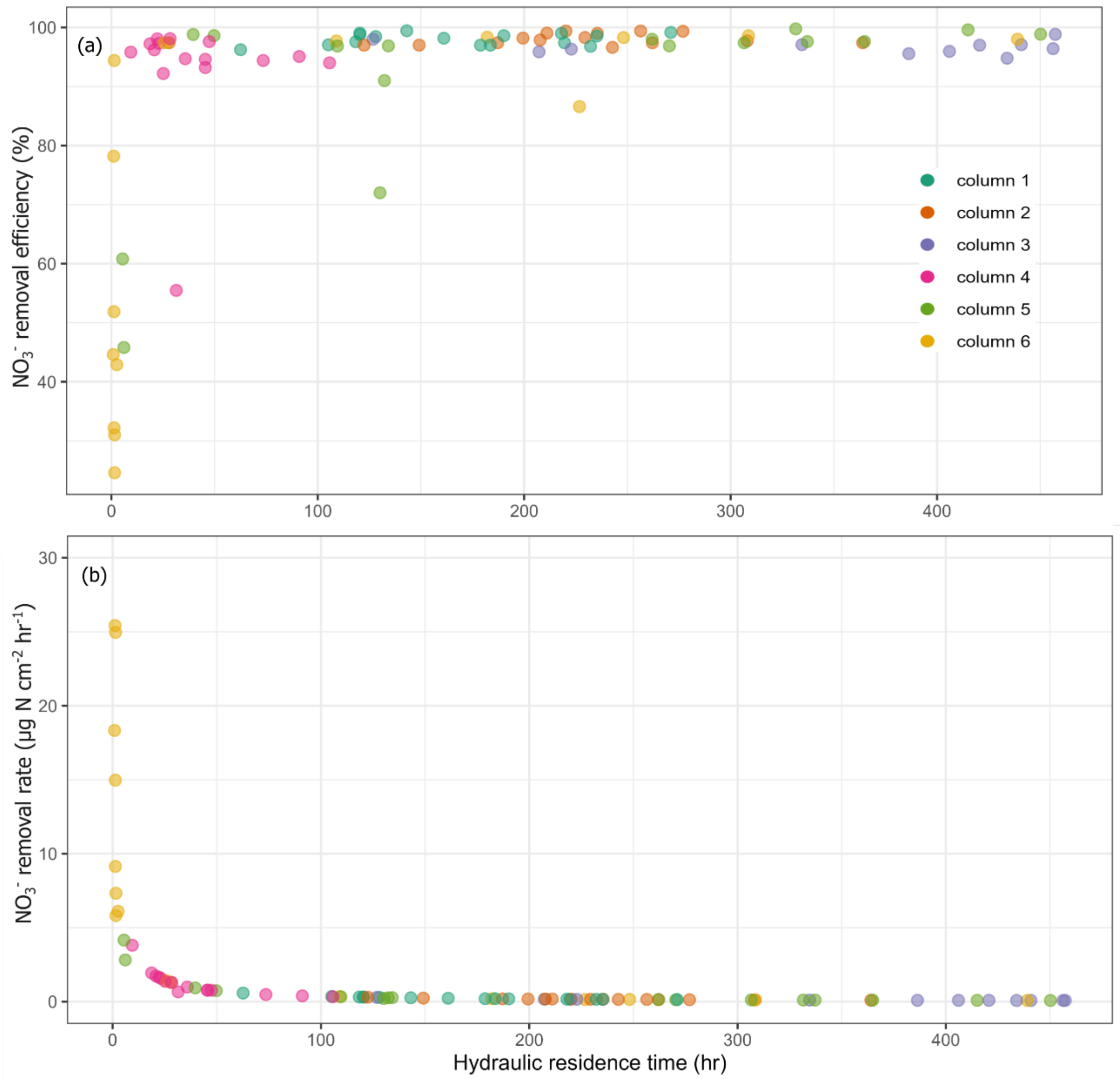


Figure 5-4. The measured (a) NO_3^- removal efficiency (%) and (b) NO_3^- removal rate (µg NO_3-N cm^{-2} hr^{-1}) at different hydraulic residence time (hr) during subsurface outflow at the 15 cm depth in the subsurface outflow column experiment.

NO_3^- removal efficiency within a comparable HRT under different flow interventions (Gao et al., 2017; Hoffmann et al., 2019; Wang et al., 2020).

5.3.3 Optimum hydraulic residence time for modelled NO_3^- removal

The measured NO_3^- removal efficiency and measured HRT, from the subsurface outflow sediment column study, were fitted to a non-linear regression (Eq. 5-5) using Gauss-Newton iteration, with 95% confidence interval (Figure 5-5):

$$NO_3^- \text{ removal efficiency (\%)} = 96.9264 - (89.4702 * \text{Exp}(-0.324418 * \text{HRT})) \quad \text{(Eq. 5-5)}$$

The modelled rate of $\%NO_3^-$ removed (%/hr) was based on the derivative function of Eq. 5-5 as in Eq. 5-6:

$$\text{Modelled rate of } \%NO_3^- \text{ removed (/hr)} = -89.4702 * (-0.324418 * \text{Exp}(-0.324418 * \text{HRT}) \quad \text{(Eq. 5-6)}$$

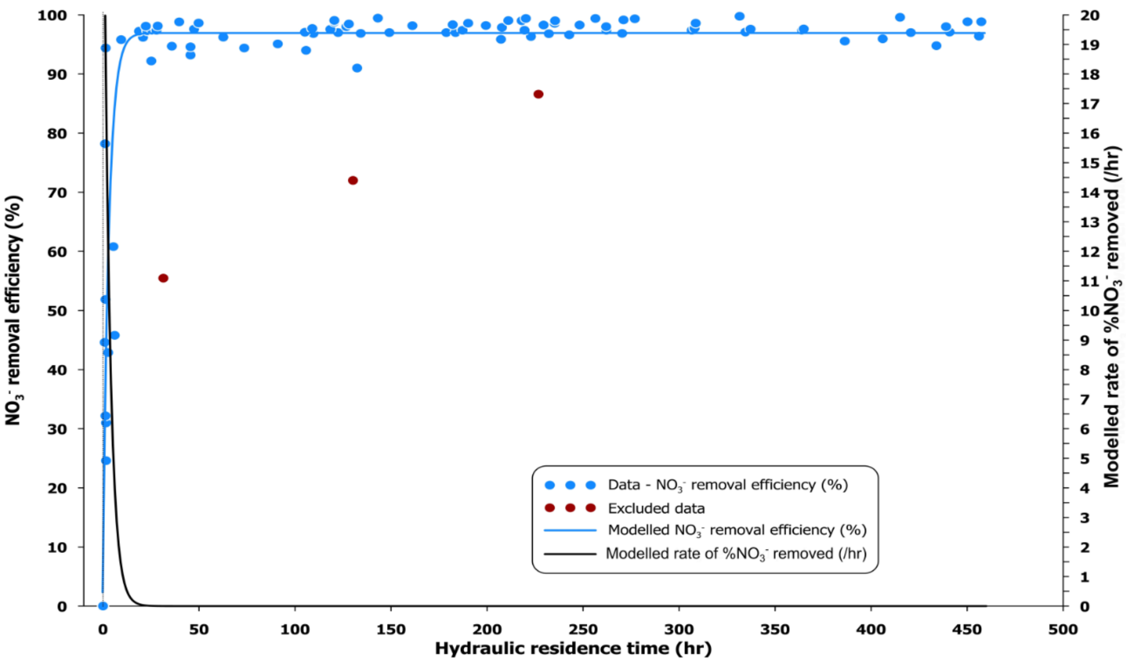


Figure 5-5. The estimated hydraulic residence time (hr) for the modelled NO_3^- removal efficiency (%) and modelled rate of $\%NO_3^-$ removed (/hr) which were derived from the Gauss-Newton non-linear regression fitted between the measured NO_3^- removal efficiency (%) and measured HRT during subsurface outflow at the 15 cm depth in the subsurface outflow column experiment.

Figure 5-5 illustrates the measured and the modelled NO_3^- removal efficiencies and also the modelled rate of %NO_3^- removed which are presented against the required HRT (hr). The modelled estimates were validated by initiating the modelling at 0 hr of HRT. Table 5-2 summarises several modelled NO_3^- removal efficiencies that can be targeted during the subsurface outflow intervention and lists the associated HRT and the modelled rate of %NO_3^- removed, i.e., the rate at which a percentage of NO_3^- will be removed per hour. The highest modelled rate of %NO_3^- removed of 20 /hr, was estimated to occur in the first 1 hr of HRT (Figure 5-5, Table 5-2), suggesting that the first hour of subsurface outflow intervention is important for NO_3^- removal.

As per the model predictions in Table 5-2, a large fraction of the NO_3^- removal occurs within the first few hours of subsurface outflow initiation, as a modelled 50% of NO_3^- removal efficiency occurred in the first 2 hr of HRT during the subsurface outflow intervention (Figure 5-5, Table 5-2). This finding aligns well with studies that had shown major NO_3^- removal can occur in the initial few hours of the flow interventions. A vertical flow intervention achieved 96% NO_3^- removal within 3.5 hr of HRT during municipal tailwater treatment in a denitrification constructed wetland in China (Wang et al., 2020).

Table 5-2. Modelled NO_3^- removal efficiency (%) and modelled rate of %NO_3^- removed (/hr) and the required estimated hydraulic residence times (hr), estimated based on the associated non-linear regression equations from the subsurface outflow column experiment.

Modelled NO_3^- removal efficiency (%)	Modelled rate of %NO_3^- removed (/hr)	Required estimated HRT (hr)
35	20	1
50	15	2
65	10	3.2
75	>5 to <10	4.4
80	5	5.2
90	<5	8.1
95	<5	13.1

A control drainage via weir installation measured 79% NO_3^- removal (equivalent to 23±4.8 g NO_3-N) within 2 hr of HRT in an agricultural wetland in USA (Kröger et al., 2012). This confirms that a large NO_3^- load reduction does not necessarily require extended flow intervention periods. Based on the gradients in NO_3^- removal along different HRTs (Figure 5-5), the current study recommends an HRT of 2 hr as the optimum operational HRT, because this is the shortest period during the intervention when the largest (50%) NO_3^- removal can occur. This recommended operational HRT can be particularly useful when a large NO_3^- removal within short periods of time becomes critical. Such a demand can rise when wetlands receive large NO_3^- loads but experience short residence times, e.g. in high-flow wetland conditions following precipitation events (Tanner et al., 2005).

However, further research is required before the recommended operational HRT values can be applied to field conditions because the specific NO_3^- loading rates with respect to the wetland drainage conditions need to be factored in. Nonetheless, this study shows that subsurface outflow intervention has the potential for large NO_3^- reductions over a short period of time in seepage wetlands.

While 50% of the modelled NO_3^- removal efficiency, equivalent to NO_3^- load reduction, occurred in 2 hr of HRT (Table 5-2), columns would still be discharging substantially high outlet NO_3^- concentrations of ~2.5 mg NO_3-N/L. This has the potential to become a water quality issue when the discharge enters streams and rivers (Goeller et al., 2019). In order to increase NO_3^- load reduction further, extending the HRT beyond 2 hr shows that 75% NO_3^- removal efficiency can be achieved in 4.4 hr and 90% at 8.1 hr of HRTs (Table 5-2). An additional 5 hr of HRT reported only a 5% gain in the NO_3^- removal efficiency, with a modelled 95% NO_3^- removal efficiency possible within 13 hr of HRT (Figure 5-5, Table 5-2). The modelled rate of %NO_3^- removed becomes critically low at 13 hours HRT (Table 5-2). This HRT of 13 hr is recommended if maximum NO_3^- removal and minimal NO_3^- outlet concentration is desired as this has the potential to achieve outlet NO_3^- concentrations of <0.25 mg NO_3-N/L.

Further extending the HRT to >13 hr becomes inefficient. This is because 1) there is little increase in NO_3^- removal efficiencies as NO_3^- removal rates reach low values (Table 5-2), 2) columns possibly become NO_3^--limited for denitrification, which is suggested by the low outlet NO_3^- concentration of <0.25 mg NO_3-N/L (Table 5-2), indicative of efficient denitrification as suggested by Addy et al. (2016), and 3) the NH_4^+ loss risk increases as elevated outlet NH_4^+ concentrations increase at long HRT observed in the current study (discussed in detail in the following Section 5.3.4).

While operating the subsurface outflow intervention for the recommended duration of operational HRTs may not remove all NO_3^-, it will substantially minimise NO_3^- loss within short period of time of 2 hr, for example. By strategically applying short-duration HRTs to a hill country seepage wetland, farm NO_3^- management could benefit under high-flow hydrological conditions. Conversely, a longer operational HRT of 13 hr could be effective in hydrologic conditions when seepage wetlands receive high input NO_3^- concentrations from nitrification, seepage input, N fertiliser application, and urine deposition from grazing stock during dry seasons or under baseflow-dominated conditions (McKergow et al., 2012).

5.3.4 NH_4^+ and DOC loss

The column experiments show NH_4^+ and DOC losses can occur during the subsurface outflow intervention (Figures 5-6, 5-7). Compared to the negligible mean inflow NH_4^+ concentration of 0.03±0.25 mg NH_4-N/L, elevated outlet NH_4^+ concentrations of 1.1-4.0 mg NH_4-N/L occurred in 45% of the outflow samples, between HRTs of 22-456 hr (Figure 5-6a, b). This study suggests NH_4^+ concentrations in the outflow increases at high HRT (Figure 5-6b), a finding also supported by Zhang et al. (2020), as elevated NH_4^+ concentrations >1 mg NH_4-N/L largely occurred at HRT>100 hr (Figure 5-6b).

Possible NH_4^+ formation mechanisms during the subsurface outflow intervention in the current study are OM mineralisation and dissimilatory NO_3^- reduction to NH_4^+ (DNRA) (Covatti et al., 2022; Pandey et al., 2020). The outflow sampling strategy (Section 5.2.3) in the current study did not permit NH_4^+ analysis on fresh outflow. The elevated outlet NH_4^+ concentrations in the outflow and in the post-experiment sediments (Section 5.3.1, Table 5-1) indicated NH_4^+ formations during the experiment. A column

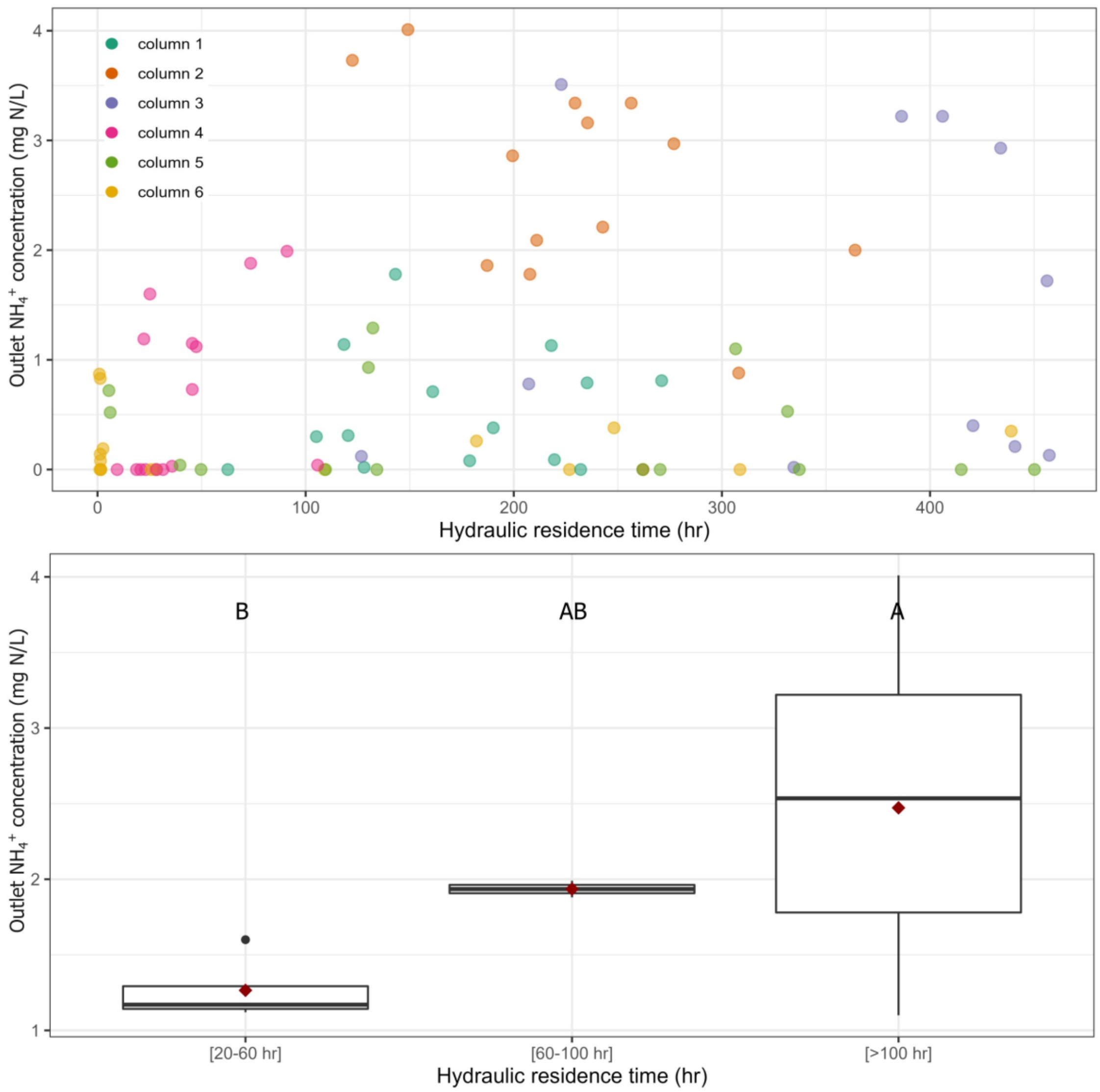


Figure 5-6. (a) Outlet NH_4^+ concentrations (mg NH_4-N/L) in relation to hydraulic residence time (HRT) (hr), and (b) Boxplot of outlet NH_4^+ concentrations at different HRT ranges of >20 hr measured at the 15 cm outflow depth in the subsurface outflow column experiment. First and third quartiles represent 25% and 75% of the measured values, respectively. Median value is indicated by the vertical straight line inside the boxes. Mean value is indicated by the red diamond. Solid dots are outliers. Letters next to the boxplots indicate the mean differences in outlet NH_4^+ concentrations between the HRT values >20 hr significant at $p \leq 0.05$.

experiment on riverbed sediments attributed the NH_4^+ formed during the study to OM mineralisation under reduced conditions driven by low infiltration rate during the riverbed infiltration (Covatti et al., 2022). In their study, the effluent NH_4^+ concentration was up to 1 mg NH_4-N/L against the inflow NH_4^+ concentration of 0.01 mg NH_4-N/L (Covatti et al., 2022). Likewise, in the current study, OM mineralisation may have been responsible for the elevated outlet NH_4^+ concentrations >0.25 mg NH_4-N/L in the sediment columns that measured very low hydraulic conductivity across the columns

(Section 5.3.1). Additionally, DNRA is the likely driver of the elevated NH_4^+ of >1 mg NH_4-N/L because the corresponding samples had longer HRTs (22-456 hr) (Figure 5-6b), by which time the outlet NO_3^- concentration had decreased to <0.45 mg NO_3-N/L (indirectly shown in Figure 5-4a) that indicated high NO_3^- reduction. The NO_3^- limited (Section 5.3.1), DOC-rich and reduced conditions in the post-experiment sediment are conducive to DNRA (Pandey et al., 2020; Zhang et al., 2021). This has implications in terms of sediment NO_3^- attenuation as the NO_3^- removed from the system by NH_4^+ formation via DNRA is only temporary, meaning the NH_4^+ formed can return to the environment as NO_3^- via nitrification when the system becomes aerobic. Also, NH_4^+ loss in pastoral discharge can trigger harmful algal growth in waterbodies. In contrast, complete denitrification reduces NO_3^- to atmospheric dinitrogen in the form of N_2, and is the expected termination point in the NO_3^- reduction process for NO_3^- mitigation.

Compared to the inflow DOC concentration of ~11 mg/L, outlet DOC concentrations ranged from 0.07-96.9 mg/L which suggested DOC loss throughout the subsurface outflow intervention (Figure 5-7). Many bioreactor studies have suggested the treatment performance of the subsurface outflow intervention declines over the longer-term due to the continuous loss of DOC in outflow, which causes a C-limitation (Cameron & Schipper, 2010; Christianson et al., 2021; Healy et al., 2012). Considering that seepage wetlands are natural wetland features receiving organic matter from various

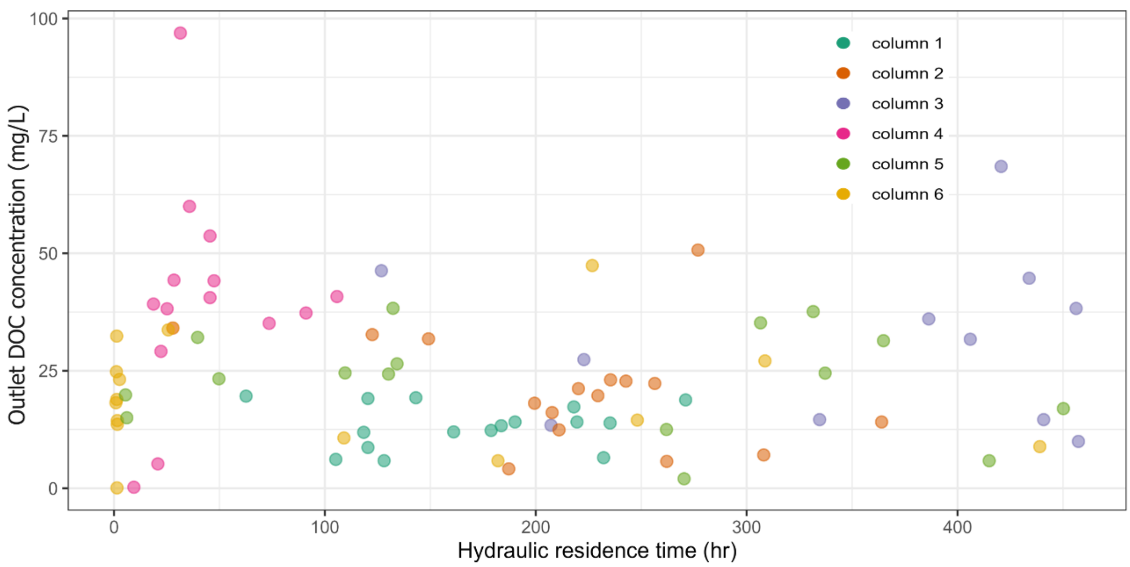


Figure 5-7. Outlet DOC concentration (mg/L) in relation to hydraulic residence time (hr) measured at the 15 cm outflow depth in the subsurface outflow column experiment.

sources (including grazing deposits, plant decay) in pastoral landscapes, C-limitation is considered less of a concern in the current subsurface outflow intervention.

This study shows an NH_4^+ loss risk is low at HRT <13 hr during subsurface outflow intervention. In this regard, applying subsurface outflow interventions only when needed and for short durations of HRTs of 2-13 hr, will help minimise the potential for DNRA-based NH_4^+ formations, the risk of which is speculated to occur at HRTs >20 hr (Figure 5-6). Based on these findings, this research suggests NO_3^- attenuations by subsurface outflow intervention also should carefully assess the possibility of pollution swapping by NH_4^+ formation before the application of such interventions.

5.3.5 Influence of seepage wetland sediment properties on NO_3^- removal

Flowrate, hydraulic conductivity, and bulk density were found to be the major sediment properties that influenced the overall removal of NO_3^-, NH_4^+ and DOC during the subsurface outflow intervention, as the redundancy analysis (RDA) shows in Figure 5-8. These sediment properties explained 42% of the variance in the nutrient removals, as the first two axes in the RDA accounted for 42.1% of the variances in the response variables (R^2-adjusted 0.40) (Figure 5-8). This analysis shows sediment flowrate,

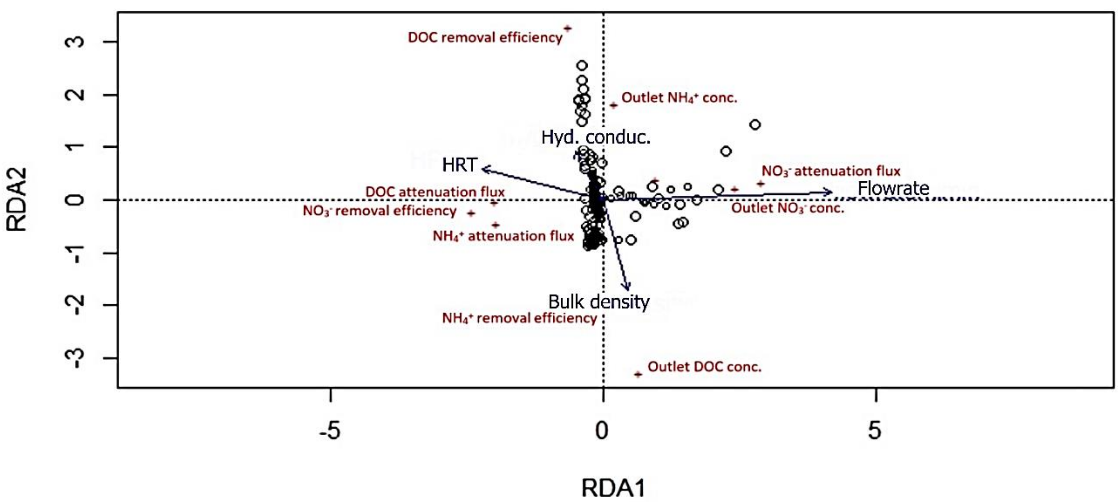


Figure 5-8. The redundancy analysis (RDA) plot shows wetland sediment properties that influence removals of NO_3^-, NH_4^+ and DOC during the subsurface outflow measured at the 15 cm outflow depth in the subsurface outflow column experiment.

hydraulic conductivity and bulk density have important influences on the NO_3^- removals during the subsurface outflow intervention. These findings agree well with the literature, showing that HRT, hydraulic conductivity and associated sediment drainage properties have important influences on wetland NO_3^- removal (Christianson et al., 2021; Chun et al., 2009; Fenton et al., 2009). This is due to their role in defining the overall sediment N-reducing environment. For example, high bulk density and low hydraulic conductivity drive slow drainage that increases residence time, improves drainage-sediment interaction and facilitates an oxygen-limited environment favourable for microbial NO_3^- reduction (Fenton et al., 2009; Hoffmann et al., 2019).

5.3.6 Study implications

This subsurface outflow column study has demonstrated that its vertical downwelling and subsequent horizontal subsurface discharge has potential as a NO_3^- mitigation strategy in sediments sampled from a seepage wetland. These findings highlight the opportunities for applying such strategies in field seepage wetlands. Previous evidence supporting the potential of subsurface outflow intervention, particularly the effectiveness of the vertical flow, in seepage wetlands had been variable. The NO_3^- removal efficiency values under subsurface flow involving vertical flow have ranged in the literature from negative values at a constructed wetland receiving sewage effluent (Egypt) (Abdelhakeem et al., 2016) to 98% removal (from 100 mg NO_3-N/L to 1 mg NO_3-N/L) achieved after 120 hr during a continuous vertical upflow of groundwater in a lake receiving nonpoint source pollutants in China (Qian et al., 2018). In comparison, a horizontal subsurface outflow intervention measured NO_3^- removal of 80% in 12 hr, with electrolysis support integrated into the subsurface flow constructed wetland (Gao et al., 2017). The vertical downwelling as part of the subsurface outflow intervention examined in the current study measured 95% NO_3^- removal in 13 hr of HRT. This aligns well with a recent comparison of different inflow patterns that found the vertical downwelling more effective, as it measured 95-100% NO_3^- removal (from 42-50 mg NO_3-N/L to <1 mg NO_3-N/L), in contrast to 70-85% NO_3^- removal (from 32-40 mg NO_3-N/L to <1 mg NO_3-N/L) during horizontal flow intervention of municipal tailwater (Wang et al., 2020).

Furthermore, the recommendation of two separate operational HRTs in this research could be applied to achieve different NO_3^- treatment objectives. It provides some flexibility to the subsurface outflow intervention to suit varied hydrologic conditions and increases the robustness of its potential application in the field.

When compared with artificial treatment installations e.g., bioreactors, seepage wetlands have greater advantages in that they are natural features, and could require minimal intervention to achieve improved NO_3^- removal at lower HRTs as this study has indicated. When compared with bioreactors, the flow intervention of the current study shows higher efficiency with 50% NO_3^- removal from 5 NO_3-N/L of NO_3^- within 2 hr of HRT. In contrast, one bioreactor removed 88% NO_3^- from a NO_3^- concentration of 8 mg NO_3-N/L to 1 mg NO_3-N/L in 24 hr of HRT (Krauter, 2001). Another woodchip bioreactor measured 7% NO_3^- removal in 1.7 hr of HRT from an inflow NO_3^- concentration of 30 mg NO_3-N/L (Hoover et al., 2016). A review on woodchip bioreactor shows HRT <6 hr is insufficient and recommends 6-20 or >20hr of HRT for efficient NO_3^- removal (Addy et al., 2016). Furthermore, the application of subsurface outflow intervention to existing natural seepage wetlands in difficult hill country terrain is feasible due to its minimal cost and fewer logistical challenges. Bioreactors are usually constructed in comparatively low-slope terrains and receive drainage from large flat areas in the landscape and involve installation and maintenance costs (Christianson et al., 2021). In contrast, relatively low-cost and low-maintenance installation of structures to 'control' subsurface outflows in the existing natural seepage wetlands, offer an opportunity to optimise their NO_3^- attenuation capacity in pastoral hill country landscapes.

The current thesis proposes a conceptual design of a physical structural barrier (Figure 5-9) to 'control' of subsurface outflows in seepage wetlands. The structure consists of a 1.5 m long liftable barrier which is attached to a frame permanently installed at the outlet in the wetland. When in operation, the barrier could be lowered down to 1 m depth into the wetland sediment. The remaining 0.5 m of the shaft would protrude above the surface of the wetland and block the free-flowing surface stream outflow. Overall, the aim is to slow flow in the wetland and increase the residence time for an intended period of time. In this way, the retained flow could be forced to infiltrate (vertical downwelling) and subsequently discharge through the subsurface outlets which are perforated on the barrier, at a depth of 15 cm below the wetland surface. This intervention has the potential to enhance wetland sediment and drainage interaction and promote NO_3^- reduction in seepage wetlands. When flow regulation is not required, the barrier could be fully lifted above the wetland surface, and the stream can flow unimpeded.

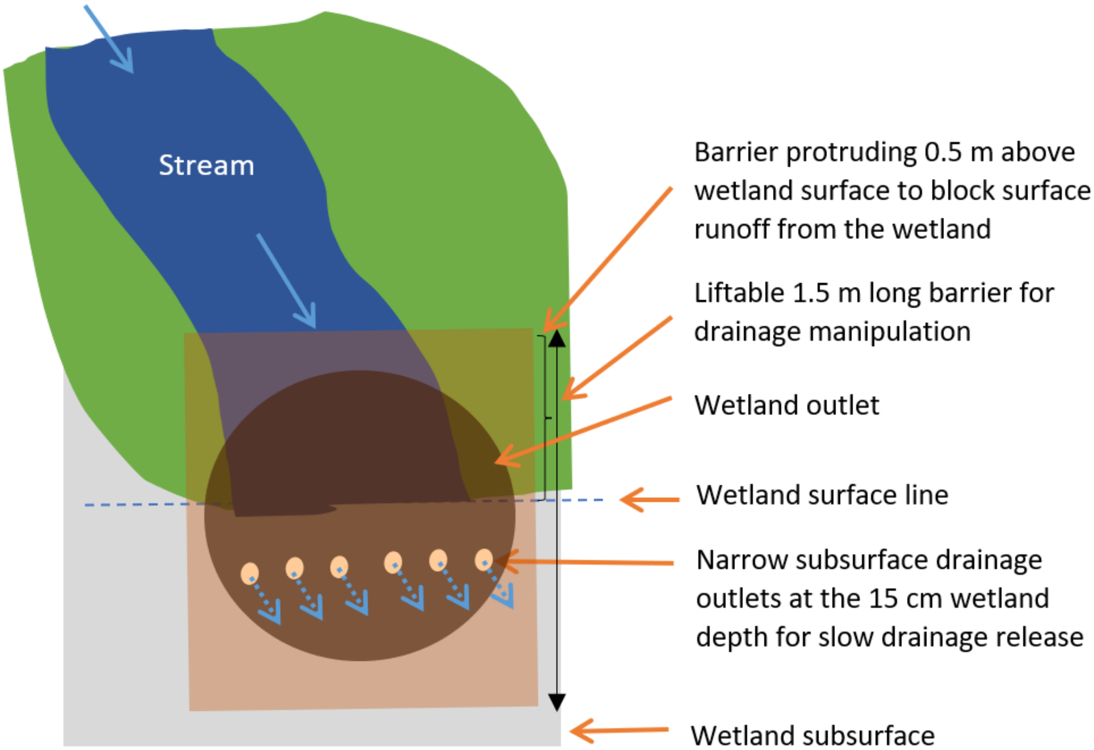


Figure 5-9. Conceptual diagram of the proposed "controlled" subsurface outflow intervention structure to optimise NO_3^- removal in seepage wetlands.

5.3.7 Study limitations and future research

There were several limitations in the column experiment. Firstly, the high sediment compression that occurred during sediment coring in the field had increased the sediment bulk density in the columns with possible influences on the hydraulic conductivity and interferences with HRT and associated NO_3^- removal, as they are interrelated according to the redundancy analysis (Figure 5-8). The low hydraulic conductivity in sediment columns is less likely to occur in field conditions with generally loose and unconsolidated sediments in the surface depths (Kellman, 2004). Secondly, groundwater interactions were eliminated in the experiment (Section 5.2.2). However, in natural wetlands, major N-removal processes switch between dilution, denitrification and the other N-cycling processes e.g. via plant uptake, coupled nitrification-denitrification in response to hydrological regimes and seasonal fluxes with different consequences in terms of NO_3^- mitigations (Buss et al., 2005). Thirdly, the sentiment columns used in the experiment varied in their hydraulic conductivities part of which could be due to the compressions caused during the sediment sampling. These challenges are unique to artificial column studies, and we would expect NO_3^- removal rates to be different during subsurface flow under *in-situ* wetland conditions (Kellman, 2004).

Overcoming some of these challenges is critical to strengthening the confidence about the viability of the proposed flow intervention. Future research is needed to confirm that denitrification process is the major N-reduction process during such flow interventions. Therefore, future laboratory-scale and *in-situ* studies are required to 1) compartmentalise the major N-reduction processes, and 2) quantify N_2O and N_2 fluxes in seepage wetlands under the 'controlled' subsurface outflow intervention. These future studies could consider the use of tracers and time-lapsed automated outflow sampling to develop an understanding of the 1) simultaneous NH_4^+ generations, and 2) the gaseous N_2O and N_2 productions (Healy et al., 2015; Mwagona et al., 2019a), which could not be answered in the current study due to the limitation of the sampling strategy involved. In the current study, the sampling of PV required the accumulation of samples over several days, meaning samples were unsuitable for continuous NH_4^+ monitoring, or denitrification gas analysis.

5.4 Conclusion

This research has examined the effectiveness of subsurface outflow intervention to optimise NO_3^- removal in seepage wetlands. In a laboratory-scale subsurface outflow sediment column study, flow intervention via subsurface outflow was simulated in a seepage wetland where the surface runoff was restricted to vertical downwelling and subsequent horizontal outflow discharge through sediment column at 15 cm sediment column depth.

The simulated subsurface outflow was found to be an effective flow intervention strategy in which seepage wetland sediment achieved 50-95% NO_3^- removal efficiency within 2-13 hr of HRT. Based on this result, an operational HRT of 13 hr appears to achieve maximum NO_3^- removal via the regulated subsurface outflow intervention in seepage wetland sediments. If that HRT becomes logistically infeasible due to hydrologic conditions in the wetland, such as high flow events with the potential of overspilling, a lower operational HRT of 2 hr can be targeted. This lower operational HRT has the potential to allow substantial NO_3^- load reductions of up to 50%.

Although the current study suggests the possibility of partially converting NO_3^- to NH_4 via DNRA process at higher hydraulic residence time (Section 5.3.4), such losses can be potentially managed by optimising management of flow regulation within the operational HRT recommended in this study.

The laboratory-scale sediment column experiment in this research demonstrates potential application of a 'controlled' subsurface outflow intervention in a naturally occurring seepage wetland to improve NO_3^- management in pastoral headwater catchments in NZ. This is a useful finding that requires further *in-situ* studies as although the installation of low-structural installations requiring 'controlled' subsurface outflow intervention are likely to face practical challenges associated with high flow events and the presence of farm animals, they are likely to be cost-effective in a hill country environment and therefore worth exploring further.

Chapter 6: Synthesis and conclusions

A sound understanding of wetland influence on its NO_3^- removal function is the key to formulating new catchment management practices that can mitigate NO_3^- and its adverse effect on water quality. Seepage wetlands are potential NO_3^- sinks that occur in the pastoral hill country landscapes in New Zealand (Cooke & Cooper, 1988; Rutherford et al., 2018). Seepage wetlands are very similar to valley bottom wetlands that occur in headwater catchments and have been widely studied in the USA and Canada (Drake et al., 2018; Gold et al., 2001; Hill, 1996; Vidon et al., 2010). Sediment and hydrology play integral roles in wetland NO_3^- reduction processes e.g., via denitrification (Hefting et al., 2004; Hill & Cardaci, 2004; Martínez-Espinosa et al., 2021; Wall et al., 2005). While global literature is exhaustive on the influence of wetland sediments on NO_3^- removal fluxes, for example in valley bottom wetlands, relevant knowledge is critically limited for pastoral hill country seepage wetlands in NZ (Chibuike et al., 2019; Nguyen et al., 1999a; Rutherford & Nguyen, 2004).

Although some previous studies have made important scientific contributions by identifying the potential of seepage wetlands as NO_3^- mitigation tools in the pastoral landscape in NZ (Cooke & Cooper, 1988; Nguyen et al., 1999a; Rutherford & Nguyen, 2004), the seepage wetland sediment and hydrological characteristics that influence NO_3^- attenuations in these wetlands had several important research gaps prior to this thesis. Previous studies provided a fragmented understanding based on primarily single-site studies that lacked insights into the spatio-temporal variations in denitrification and associated NO_3^- removal (Burns & Nguyen, 2002; Nguyen et al., 1999a; Rutherford & Nguyen, 2004). Due to such research limitations, previous seepage wetland studies haven't provided a comprehensive understanding of the spatial variability of denitrification and its sediment and hydrological drivers or whether there is an opportunity to optimise NO_3^- removal in seepage wetlands. These research gaps are currently limiting the application and policy endorsement of seepage wetlands for NO_3^- mitigation in the pastoral hill country landscapes, despite their known potential for NO_3^- attenuation (Cooke & Cooper, 1988).

This thesis has addressed several pre-existing research gaps, by characterising the sediment, hydrological and NO_3^- removal properties and by exploring the potential for NO_3^- removal optimisation in seepage wetland sediment columns in pastoral hill country landscapes. This thesis had the following specific objectives:

1. To characterise the spatial gradients of denitrification enzyme activity (DEA) and the DEA-influencing sediment properties in seepage wetlands.
2. To characterise seepage wetland hydrology and the associated NO_3^- removal in a seepage wetland.
3. To investigate the effectiveness of subsurface outflow intervention for optimisation of NO_3^- removal from NO_3^- rich pastoral surface runoff in seepage wetlands using a laboratory-based sediment column study.

The expected outcome of the thesis was to generate improved understanding of seepage wetland characteristics from the perspective of NO_3^- mitigation, with a focus on denitrification. A geospatial analysis was undertaken as part of this final chapter to assess the extent of hill country landscape properties within the Horizons Regional Council, which has the potential to support denitrification. This is a preliminary step towards assessing the contribution of seepage wetlands at a catchment scale, to mitigate NO_3^- loss. The longer-term objective of the thesis is to improve water quality from pastoral headwater catchments by including seepage wetlands in the NO_3^- management tools available to hill country farmers and policymakers.

6.1 Spatial gradients of seepage wetland denitrification enzyme activity (DEA) and DEA-influencing sediment properties

In this thesis, the spatial gradients of DEA and the DEA-influencing sediment properties in seepage wetlands have been assessed in 36 sediment columns, by sampling three sediment columns across the flowlines at four seepage wetland sites. These sites were located on sheep and beef grazed hill country farms located across the Horizons Regional Council, in the lower North Island of NZ (Chapter 3).

6.1.1 Sediment physicochemical and chemical properties

The seepage wetland sediment characterisation confirmed a NO_3^- reducing sediment environment which was slightly acidic to neutral (pH 5.1-6.7) and had moderately high reducing conditions (Eh<29 mV). The high water contents (WC) measured (43-73%) were likely to influence denitrification directly as well as indirectly by creating oxygen limitation, which can initiate the NO_3^- reduction process and maintain nutrient supply to the wetland denitrification sites within sediments (Burgin et al., 2010; Giles et al., 2012; Wang et al., 2021). Based on the sediment carbon to nitrogen (C:N) ratio (mostly above >12:1), the seepage wetland sediments were found to be organic in nature (Nguyen & Downes, 1997). The sediment chemical properties ranged in values from 87-2291 mg DOC/kg DS (Dissolved Organic Carbon; Dry Sediment), 0.01-47.6 mg NO_3-N/kg DS, 0.01-16.5 %TC (total carbon), 0.02-8.15 %TN (total nitrogen), 0.19-165 mg dissolved Fe^{2+}/kg DS, 0.00-205 mg dissolved Mn^{2+}/kg DS at the study sites, with higher values at the surface depths for DOC, NO_3^-, %TC and %TN which mean there is adequate presence of potential electron donors (DOC, %TC) for denitrification in the seepage wetland sediment environment.

6.1.2 Spatial DEA gradients in seepage wetlands

In agreement with the current literature (Christensen et al., 1990; Giles et al., 2012; Hayakawa et al., 2012), the DEA in the current study varied vertically and horizontally in the four seepage wetlands studied, with DEA highest at the surface 0-15 cm depths (mean value range of 560-5371 µg N_2O-N kg^{-1} DS h^{-1}) and decreasing DEA with increasing wetland depth. The wide between-wetland DEA variability suggests some seepage wetlands are potential 'denitrification hotspots' in the pastoral hill country landscape.

Based on this variation, the seepage wetland study sites were categorised into high-performing H-DEA sites (criteria: DEA>3000 µg N_2O-N kg^{-1} DS h^{-1}, 3868-5371 µg N_2O-N kg^{-1} DS h^{-1} at the 0-15 cm depths) and low-performing L-DEA sites (criteria: DEA<1000 µg N_2O-N kg^{-1} DS h^{-1}, 560-620 µg N_2O-N kg^{-1} DS h^{-1} at the 0-15 cm depths). This approach is the first step in spatially characterising seepage wetland NO_3^- mitigation capacity in order to apply this knowledge to a broader landscape scale.

6.1.3 Relationships between sediment properties and DEA

Wetland sediment plays a regulatory role that drives site-specific denitrification (Christensen et al., 1990; Giles et al., 2012; Hayakawa et al., 2012). Therefore, the identification of sediment properties that drive spatial variabilities in seepage wetland DEA can be used to identify potential denitrification hotspots at the landscape scale (Groffman et al., 2009). Guided by this concept, this thesis assessed the relationships between the seepage wetland sediment properties and DEA. The assessment showed the dominant DEA-influencing sediment properties in seepage wetlands are sediment WC, NO_3^-, %TC, %TN, C:N, dissolved iron Fe^{2+}, dissolved manganese (Mn^{2+}) and DOC concentrations ($p \leq 0.05$). The findings from this research 1) agree well with the global literature in terms of key sediment properties that influence denitrification in wetlands (Burgin et al., 2010; Li et al., 2022; Sirivedhin & Gray, 2006), and 2) expand on our previous knowledge by adding NO_3^-, %TC, %TN, C:N and dissolved Fe^{2+} as key DEA-influencing seepage wetland sediment properties (Chibuike et al., 2019).

The spatial variabilities of DEA across the four wetlands were well explained by the DEA-influencing sediment properties. The H-DEA sites which measured 7 to 10 times higher surface DEA compared to the L-DEA sites, also measured higher mean sediment WC (67-77%), DOC (597-711 mg DOC/kg DS) and NO_3^- (15.9-18.5 mg NO_3-N/kg DS) concentrations at surface depths, compared to 46.7-33.9% WC, 149-580 mg DOC/kg DS, 2.5-4 mg NO_3-N/kg DS at the L-DEA sites, respectively.

Sediment WC is a particularly important sediment property that influences DEA in seepage wetlands ($p \leq 0.05$). Sediment WC influences DEA both directly and indirectly, via the WC influences on other sediment chemical properties that included %TC, %TN and NO_3^- ($p \leq 0.05$). Furthermore, the within-wetland DEA variabilities in seepage wetlands were also explained by variations in sediment WC. For example, WC values varied within-wetlands at the L-DEA sites (34-47% WC, $p \leq 0.05$), whereas the H-DEA sites measured higher WC (67-77%) and showed no within-wetland variations ($p > 0.05$). As a possible consequence, at the L-DEA sites, the DEA was higher at the wetland position which had a higher sediment WC. In contrast, the DEA was homogeneous at the H-DEA sites (i.e., no within-wetland variability, $p > 0.05$).

The study findings suggest that seepage wetlands on more intensively managed hill country farms have the potential to become denitrification hotspots if DEA-influencing sediment properties such as WC, NO_3^-, %TC, %TN, C:N, dissolved Fe^{2+}, dissolved Mn^{2+} and DOC concentrations are conducive. Both H-DEA sites were located on more intensively managed hill country farms, meaning higher stocking rate and N-input may explain the higher sediment NO_3^- concentrations of 15.9-18.5 mg NO_3-N/kg DS compared to the lower NO_3^- concentrations measured at the L-DEA sites (2.5-4 mg NO_3-N/kg DS). In this case, 'denitrification hotspot' seepage wetlands can potentially offer more intensive farms an efficient NO_3^- mitigation strategy to improve NO_3^- water quality outcomes in pastoral headwater catchments. Such application, however, requires prior assessment of greenhouse gas emission potential because denitrification in NO_3^--rich soils can become a N_2O source, resulting in pollution swapping to a potent greenhouse gas (Weier et al., 1993).

The strong influence of sediment properties on DEA measured in this research gives us confidence that wetland sediment properties can be used as a proxy for the spatial variation in DEA both within and between seepage wetlands. This can inform 1) catchment-based NO_3^- management intervention by allowing the identification of potential 'denitrification hotspot seepage wetlands' by comparing the known sediment property values within the catchment, 2) possibly upscaling the measured DEA to other seepage wetlands with similar properties, and 3) future modelling of seepage wetland DEA (Groffman et al., 2009). The current study which examined four seepage wetlands builds on the previously limited single-site research and addresses the research gap in spatial assessments of wetland denitrification hotspots to overcome challenges associated with national and global denitrification models (Groffman et al., 2009; Martínez-Espinosa et al., 2021; Seitzinger et al., 2006).

Finally, these thesis findings highlight the need to explore additional wetland influences on denitrification-based NO_3^- removal, because sediment properties were only able to explain 58-73% of the spatial gradients in seepage wetland DEA. This suggests that other wetland processes could have important influences on NO_3^- removal in these wetlands.

6.2 Hydrological processes and temporal fluxes in NO_3^- removal

Seepage wetland hydrology and associated NO_3^- removal were characterised at one of the L-DEA sites, located at Tuapaka. At this site, the seepage wetland hydrological and NO_3^- removal characteristics were based on surface flow monitored for 2-year and shallow groundwater monitored at the 0.5, 1 and 1.5 m depths for 1.5-year period. Seepage wetland NO_3^- removal at an annual scale was estimated based on the wetland water balance and NO_3^- balance based on surface and subsurface observations. Such an integrated hydrological and NO_3^- removal assessment approach is novel for NZ seepage wetlands.

6.2.1 Seepage wetland hydrological characteristics

The hydrological characterisation identified a stream inflow-dominated seepage wetland hydrology, where most of the hydrological flow (83%) to the wetland was via a perennial, second-order stream. The estimated seepage contribution to the wetland was comparatively low and accounted for 8-14% of the total hydrological flow during the 2-year study period. An oxic subsurface redox environment was prevalent. This may have contributed to the higher shallow groundwater NO_3^- concentrations (median: 0.06, 0.1, 0.09 mg NO_3-N/L at inflow, midflow and outflow) in the study area, compared to the surface flow NO_3^- concentrations (median: 0.03 and 0.03 mg NO_3-N/L at stream inflow and outflow, respectively), that can make seepage potentially an important NO_3^- source. During this study, the seepage NO_3^- contribution to the wetland ranged from 1.6 kg NO_3-N/yr in year 1 to 0.9 kg NO_3-N/yr in year 2, which were 29.5 and 13.5% of the annual NO_3^- inputs, respectively. However, the median NO_3^- concentration in the current study site was <0.11 mg NO_3-N/L, which is comparatively higher than the NO_3^- concentration of <0.012 mg NO_3-N/L reported at a daily pastoral seepage wetland (Zaman et al., 2008), the only other study that reports seepage wetland shallow groundwater NO_3^- concentrations.

6.2.2 Temporal opportunities and risks in wetland NO_3^- removal

The seepage wetland examined in the current study was primarily a NO_3^- sink. On average, the seepage wetland NO_3^- removal was 23% of the annual NO_3^- input. However, annual NO_3^- removal in the seepage wetland varied widely. The seepage wetland NO_3^- balance showed the wetland attenuated 0.02 kg NO_3-N/yr in year 1 and 2.78 kg NO_3-N/yr in year 2, accounting for 0.3% and 40.8% of the annual NO_3^- inputs, respectively.

The observed wide annual variation in wetland NO_3^- removal was largely driven by precipitation and its seasonal distribution during the 2-year study. For example, year 1 measured concentrated winter precipitation as it received 38% of the annual precipitation during winter, which resulted in 68% of the annual outflow. Hill country pastoral NO_3^- loss is generally higher during winter and spring in the temperate climate of NZ. The large outflow results in low residence time creating elevated NO_3^- loss as a hot moment that resulted in a very low annual NO_3^- removal of 0.02 kg NO_3-N, equivalent to 0.3% of the annual NO_3^- input, by the wetland in year 1. During the research, major NO_3^- loss hot moments in the seepage wetland were identified during 1) large and frequent precipitation events in winter, 2) high flow conditions, and also 3) low flow conditions, possibly due to direct grazing in the wetland by farm stock during precipitation-free periods.

In contrast, a proportionally equivalent seasonal precipitation distribution in year 2 facilitated a dissipated flow condition in the wetland throughout the year. Additionally, a greater proportion of annual inflow also reached the wetland during spring, in year 2, when the temperature is favourable for denitrification. As a consequence, the annual NO_3^- removal greatly improved to 40.8% of the annual NO_3^- input, equivalent to 2.78 kg NO_3-N in year 2. During this research, major NO_3^- removal (attenuation) hot moments occurred in the seepage wetland during 1) initial rapid gain at the onset of precipitation events between early winter and spring at a daily scale, 2) spring at a seasonal scale, and 3) during diffuse flow conditions at an annual scale. The positive effect of diffuse flow on NO_3^- removal, observed during the study, indicates scope for optimisation of NO_3^- removal via flow management in this seepage wetland.

The hot moments, identified in the current thesis, can be targeted to practically manipulate the flow conditions under which seepage wetlands are most likely to attenuate NO_3^-. Flow interventions can consider using a physical barrier to restrict streamflow from the wetland and allow only subsurface outflow. The conceptual design of such a subsurface outflow intervention has been proposed in chapter 5. Such subsurface outflow intervention would increase flow residence time, improve the interaction between sediment and NO_3^--carrying surface runoff and facilitate a reducing subsurface environment, enhance wetland NO_3^- removal and improve downstream water quality.

6.3 Subsurface outflow intervention as a tool to improve NO_3^- removal in seepage wetlands

Based on the observations in Chapter 4, a subsurface outflow column study was developed to alter the NO_3^- reduction-constraints and facilitate anaerobic conditions conducive to denitrification, in order to test if NO_3^- removal could be enhanced in seepage wetlands. Based on the benefit of subsurface flow manipulation on NO_3^- attenuations found in the literature review, the flow intervention targeted a subsurface outflow of pastoral surface runoff via a subsurface 0-15 cm sediment column depth, selected due to the comparatively high sediment DEA (560 µg N_2O-N kg^{-1} DS h^{-1}) and DOC concentrations (580 mg/kg DS) at that depth at Tuapaka as measured in chapter 3.

A laboratory-scale subsurface outflow intervention was initiated and the effectiveness of the intervention on NO_3^- removal from NO_3^--rich inflow was measured. The experiment involved restricting the flow of surface water (simulating surface runoff in a seepage wetland) to achieve subsurface outflow through intact sediment cores, using a vertical downwelling and subsequent horizontal discharge at a depth of 15 cm. The effectiveness of the subsurface outflow intervention in removing NO_3^- was assessed by monitoring NO_3^- in the outflow water.

This thesis highlights the potential of low-cost flow interventions to lower the outflow NO_3^- concentrations, as this thesis has demonstrated that subsurface outflow intervention can achieve 50-96% NO_3^- removal from the NO_3^- rich inflow (5 mg NO_3-N/L) within reasonably short hydraulic residence time (HRT) of 2-13 hr under experimental conditions. To compare, NO_3^- removal of 0.3-40.8% (mean 23%) was measured under natural flow conditions at the Tuapaka seepage wetland during a 2-year-long hydrological characterisation study (chapter 5), whereas the laboratory-scale column study suggests >90% of the influent NO_3^- could be removed via subsurface outflow intervention. A structural design for a possible *in-situ* subsurface flow intervention has been proposed in Figure 5-9. The proposed intervention is an example of a practical mitigation strategy that could be implemented on hill country farms to minimise NO_3^- load during NO_3^- loss hot moments. An added advantage of such a mitigation strategy is that the outflow intervention does not require long hydraulic residence times (HRT). Furthermore, the two operational HRTs of 2 and 13 hr identified in the column study could theoretically be applied in an *in-situ* seepage wetland environment to achieve different NO_3^- removal treatment objectives and add more flexibility to a proposed intervention.

This thesis acknowledges the limitation of transferring the research findings from the lab-based column experiment to the field conditions. The optimal operational HRTs identified in this thesis were determined in a laboratory-scale sediment column study. *In-situ* seepage wetland studies would be needed to define field-based HRTs. The outflow intervention and measurement techniques developed in the column study could be adapted to determine the optimum operational HRTs *in-situ*. Seepage wetlands are prevalent across many hill country farms; therefore, the proposed flow intervention could potentially be upscaled to a catchment scale. Such upscaling, however, requires a spatial understanding of the relevant landscape features associated with seepage wetlands within the pastoral hill country landscape.

6.4 Geospatial analysis of hill country landscape properties with the potential to support denitrification

Seepage wetland characteristics are derivatives of landscape properties (e.g., lithology, soil drainage condition) that underpin the NO_3^- attenuations at a catchment scale. In order to explore how the findings of this thesis could be applied to a broader scale, a geospatial analysis was undertaken within the Horizons Regional Council (HRC) administrative boundary, to map several landscape features e.g., baserock, soil drainage class and soil permeability class that favour denitrification. The selection of these landscape properties was based on the assumption of their representativeness of the sediment properties identified as important for denitrification (chapter 3). Firstly, sediment WC was found to be a key DEA-influencing sediment property (chapter 3) and can be approximated by soil drainage class in a GIS analysis. Secondly, wetland depth plays an important role in NO_3^- reductions (Hill, 1990, 1996). Plant rooting depth has also shown positive influences on DEA in contrast to unvegetated conditions in a pastoral hill country seepage wetland study (Matheson et al., 2002). Soil permeability in the NZ Land Use Classification was used to approximate plant rooting depth. Thirdly, numerous wetland studies show lithology has a strong influence on NO_3^- reduction environments in agricultural headwater catchments, as observed in the USA and Canada (Gold et al., 2001; Hill, 1996; Hill et al., 2004). Parent material influences the hydraulic conductivity and redox chemistry, with fine-grained baserocks having longer residence time which enhances NO_3^- reduction, in contrast to coarse-grained bedrocks that have higher hydraulic conductivity and oxic conditions (Böhlke et al., 2007). However, it is important to point out that the sediment characterisation of the four sites examined in the current thesis did not show a clear link between baserock and oxic condition, with two contrasting baserock types (greywacke and mudstone) underlaying the two H-DEA sites. Regardless, this geospatial analysis used baserock as a landscape property as lithology has been shown to influence denitrification (Hill et al., 2004).

First-order zonal statistical analysis using the baserock, soil drainage class and soil permeability class layers, show the four seepage wetlands studied in detail (in chapter 3), represent some of the most frequent underlying landscape properties within the Horizons Regional Council. To elaborate, the seepage wetland sites studied in the current thesis, are located in areas where the most frequent two baserocks (mudstone and greywacke), three soil drainage classes (well, moderately well and imperfect drainage) and two permeability classes (moderately and moderately to slowly permeable) exist (Table 6-1 and Appendices 6-5 to 6-7) and in these contexts, the study areas represent 1264 km^2 or 8.8% of the Horizons Regional Council (Table 6-1).

Table 6-1. Estimated land area coverages of the hill country landscape properties, which occur in the hill country seepage wetland study areas investigated in the current thesis, within the Horizons Regional Council jurisdiction based on a geospatial analysis. Hill country occupies 14,416 km^2 of the Horizons Regional Council jurisdiction (22,000 km^2), of which 6,956 km^2 is under a land use for sheep and beef pastoral farming. Data sourced from Landcare Research (2000), Landcare Research (2010a), Landcare Research (2010b) and Lynn et al. (2009).

Seepage wetland study site	Landscape properties (% hill country area, %pastoral hill area coverages)			Area represented with similar combinations of the landscape properties in the Horizons Regional Council	
	Baserock	Soil drainage class	Soil permeability class	Hill country (km^2)	% Sheep and beef pastoral hill country
Rathmoy (H-DEA site)	Sandstone or coarse siltstone (46%, 21%)	Well (60%, 26%)	Moderately (78%, 39%)	1,024	14.7
Wairiri (H-DEA site)	Greywacke (15%, 2%)	Moderately well (26%, 14%)	Moderately (78%, 39%)	30	0.43
Tuapaka (L-DEA site)	Greywacke (15%, 2%)	Imperfect (12%, 9%)	Moderate over slowly (8%, 4%)	22	0.31
Ballantrae (L-DEA site)	Mudstone or fine siltstone massive (25%, 19%)	Imperfect (12%, 9%)	Moderate over slowly (8%, 4%)	188	2.7

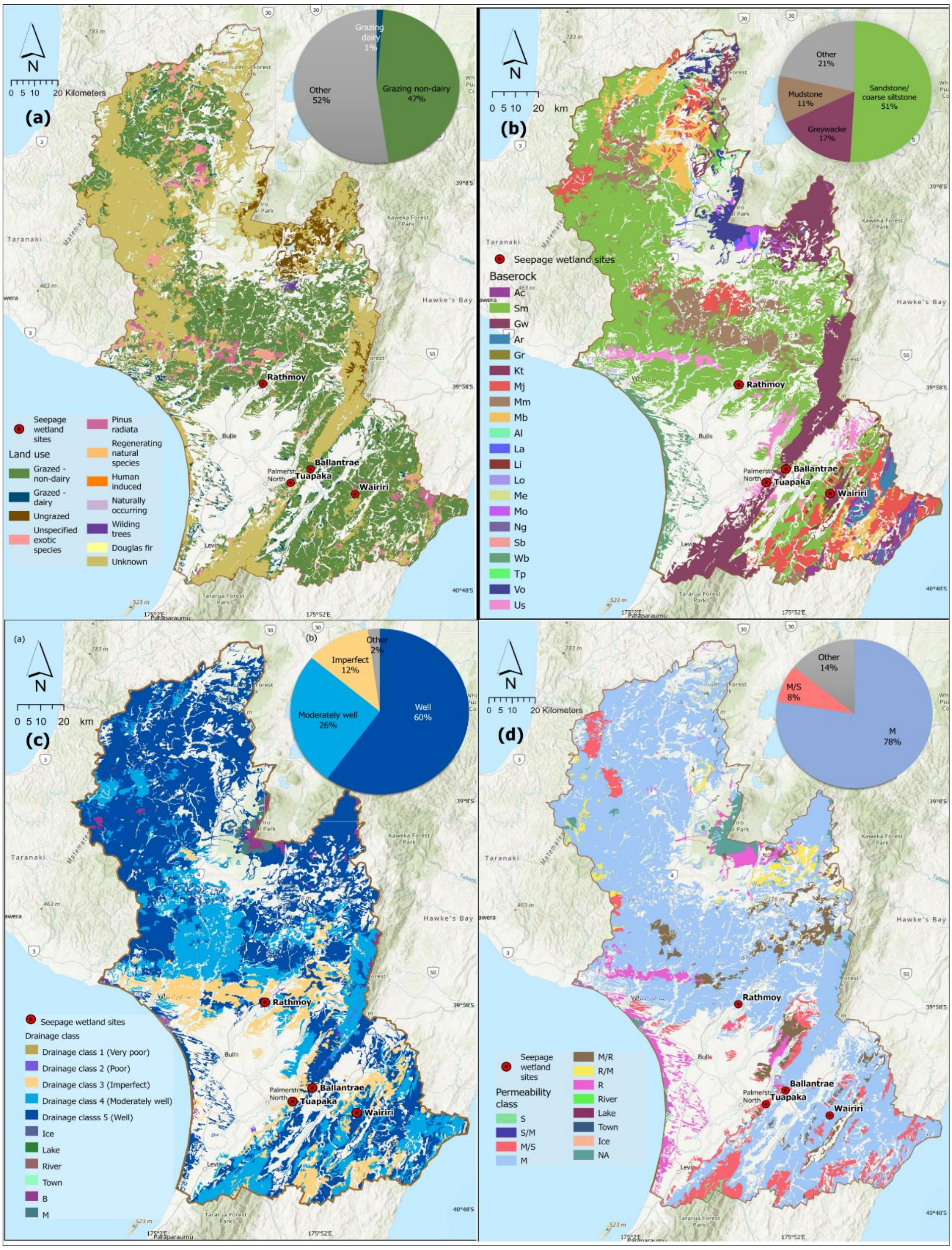


Figure 6-1. Hill country landscape properties in the Horizons Regional Council jurisdiction (a) land use, (b) baserock, (c) soil drainage classes and (d) soil permeability classes (1:50,000) obtained from geospatial analysis conducted in the current study. The location of the seepage wetland study sites investigated in this thesis are also shown on this map to indicate their representativeness of the landscape properties. The pie charts in the insets show the percentage hill country land area coverages by the landscape property. Data sources are available on in Appendix 6-1. Enlarged and detailed versions of these maps are available in Appendices 6-4 to 6-7.

6.4.1 Limitation of the geospatial analysis

One of the challenges associated with this geospatial analysis is the coarse spatial resolution of the currently available landscape properties (1:50,000) that were used in the analysis (Section 6.4). Such coarse resolution is likely to be inadequate to reflect the micro-variability in soil properties that occur within short distances in the hill country landscapes (Kemp & Lopez, 2016), and may miss landscape properties associated with individual seepage wetlands that are inherently small in area (1 to 1000 m^2). This causes challenges with the geospatial assessment of ecosystem services of small wetlands e.g., estimating NO_3^- attenuations in seepage wetlands. Although generally formed from colluvium and alluvium of upland soils, seepage wetlands can be expected to retain some of the properties of their original parent materials, including their drainage properties and subsequent influences on DEA. Based on this assumption, the estimations summarised in Table 6-1 suggest the poorly drained wetland soil at the Tuapaka site would have high WC and the well-drained soil at Rathmoy site would have had low WC. Instead, the seepage wetland sediment characterisation in chapter 3 showed the opposite, as the high-DEA performing Rathmoy site measured higher WC than the Tuapaka site. We propose the site-specific wetland hydrogeological influences including seepage input and wetland topography may partly have overridden the influence of the landscape properties at the smaller spatial scales in the wetland study sites. Also, the sediment characterisation has shown that seepage wetland denitrification is strongly influenced by sediment DOC, NO_3^-, %TC, %TN, C:N, dissolved Fe^{2+} and dissolved Mn^{2+} concentrations in the wetlands. Several of these variables, e.g., DOC and NO_3^-, are associated with farm and grazing management practices. In chapter 3, in addition to WC, sediment DOC, NO_3^- concentrations were several magnitudes higher at the H-DEA sites that can be attributed to the more intensive farming practices on those farms. We propose, future seepage wetland geospatial analysis should additionally consider the wetland hydrology and farm management variables in order to use landscape properties effectively to understand spatial variations in DEA at catchment scales.

6.5 Thesis contributions to seepage wetland science and its boundaries

The detailed understanding of seepage wetland characteristics gained in this research is crucial to inform the science and policy aspects of farm NO_3^- management and mitigation. The knowledge generated in this thesis can potentially contribute to reducing hill country farming impacts on water quality, not only within the Horizons Regional Council region, where the research has been conducted, but also in other pastoral hill country catchments across NZ and globally. Through its contribution to our understanding of the seepage wetland properties and processes, this research also enriches the relevant global literature dedicated to denitrification-based NO_3^- attenuation in headwater agricultural catchments.

The major research findings, their implications and study limitations have already been discussed in individual chapters. This thesis synthesis takes a broader look into the thesis contribution to seepage wetland science. In addition, by compiling the thesis limitations and their possible implications, this section also sets the boundaries within which the thesis findings should be interpreted.

6.5.1 Improved spatial understanding of seepage wetland denitrification capacities

This multiple-site based seepage wetland study is the first of its kind in NZ to study variation in denitrification between wetland sites and also at multiple spatial scales from vertical (within sediment columns) to horizontal (within- and between-wetlands), across hill country pastoral farms. This approach greatly improves our spatial understanding of pastoral hill country seepage wetland properties. Prior to this thesis, previous studies into the relationships between sediment properties and denitrification and their spatial variabilities, were limited. The current thesis has addressed this research gap and has made an important contribution to improving our understanding of seepage wetland characteristics and how they influence NO_3^- mitigation in pastoral hill country landscapes. The sediment characterisation in the current study showed that not all seepage wetlands are equal in their denitrification capacities, and the study identified denitrification hotspot seepage wetlands within the profile of wetlands examined.

6.5.1.1 Research limitations and associated implications

When reviewing the research findings from this thesis, it is important to highlight that, firstly, this study provides only a single temporal snapshot of the seepage wetland sediment characteristics and their denitrification capacities. Because sediments were sampled only once at each seepage wetland site during the study, the sediment characterisation results may not reflect the seasonal dynamics in denitrification in these wetlands, which is expected to occur in wetlands in general. Previous studies have reported seasonal differences in wetland denitrification capacities between summer and winter. The current sediment DEA estimates were measured in winter and late spring, under saturated conditions when elevated NO_3^- and labile C deliveries to denitrification hotspots can accelerate denitrification in NZ pastoral landscapes, potentially representing the upper limits. Therefore, these research findings may not reflect the seepage wetland sediment properties in summer when the denitrification capacity is likely to be comparatively low.

Secondly, some of the observed between-wetland variations in sediment properties may have been driven by the temporal gaps (of approximately a month) between the sediment samplings at different study sites throughout winter-late spring. The study was designed to investigate sediments in as fresh conditions as possible in the laboratory. The associated resource limitations resulted in long temporal gaps between the fresh sediment analysis at a site and the subsequent sediment samplings at the remaining study areas.

6.5.2 Improved temporal understanding of wetland hydrology and associated NO_3^- removal

The identification of wetland NO_3^- loss hot moments and NO_3^- removal hot moments in this thesis, improves our understanding of the temporal variabilities of seepage wetland NO_3^- removals. Farm managers can potentially benefit from the research findings by timing their farm NO_3^- management actions with the identified hot moments to potentially reduce farm NO_3^- loss to pastoral waterways. The implications to improve freshwater health, subsequently help the stakeholders (regional councils, farmers, catchment managers) to meet the freshwater quality objectives outlined in the National Policy Statement for Freshwater Management.

6.5.2.1 Research limitations and associated implications

The NO_3^- measurements in surface and shallow groundwater in the seepage wetland study area largely relied on manual grab water sampling. In contrast, 1) real time NO_3^- monitoring at wetland inflow and outflow, 2) tracer-based subsurface flow velocities, and 3) measurement of all surface runoff sources into the wetland would greatly improve the NO_3^- estimations and the understanding of the associated temporal fluxes.

Also, this thesis conducted a hydrological study at only one site, which is inadequate to provide a generalised understanding of the seepage wetland hydrology across hill country landscapes. Similar studies in a larger number of sites would provide a better understanding of the relative contributions of different hydrological pathways into these wetlands and their potential for NO_3^- removal.

6.5.3 Potential for enhanced seepage wetland NO_3^- removal

This thesis demonstrated the potential effectiveness of a subsurface outflow management intervention to optimise NO_3^- removal in seepage wetlands, using a laboratory column study. Stakeholders could adopt the underlying principle of the demonstrated flow intervention technique in order to trial the enhancement of NO_3^- removal when the wetlands are at risk of NO_3^- loss, e.g., during NO_3^- loss hot moments. Such flow intervention could provide stakeholders with a readily available and natural tool to enhance NO_3^- mitigations in their farming landscapes. However, more research is needed before such interventions can be endorsed for *in-situ* applications, as their *in-situ* effectiveness needs to be tested and there are likely to be practical challenges associated with high flow events and disturbance from grazing cattle.

6.5.3.1 Research limitations and associated implications

The subsurface flow in the column experiment study was challenged by several technical difficulties, including heterogeneity between the replicate sediment columns that led to differences in their hydraulic conductivities and associated hydraulic residence times. Minimisation of sediment compressions during the sediment column samplings would reduce such challenges. Possible solutions could consider sampling of wider sediment columns or larger chunks of wetlands. In addition, flow intervention at shallower wetland depths (e.g. 5 cm depth), compared to the drainage depth of 15 cm examined in the column study, could be useful in reducing the intervention period, i.e. the experiment's duration in the laboratory.

Additionally, the following features could be adopted for the sediment outflow column experiment:

1. Additional treatments including different NO_3^- removal concentration as inputs and NO_3^- loading rates;
2. Continuous NO_3^- removal concentration measurement in the outflow drainage, instead of NO_3^- removal concentrations being measured as a function of pore volume, as used in the current thesis; and
3. Monitoring NO_3^- removal fluxes in the 1) overlying water columns, and 2) at the sediment-water interface would also add additional knowledge about the possible NO_3^- dynamics at the hyporheic zones in seepage wetlands under flow interventions.

6.5.4 Comparison of the seepage wetland NO_3^- removal estimates in this thesis

Based on the seepage wetland sediment DEA quantified in this thesis (chapter 3), seepage wetland study areas measured potential areal NO_3^- removal rates of 0.6±0.8 to 4.9±3.6 g N/m^2/day (equivalent to 2,433±2954 to 18,081±12,974 kg NO_3-N/ha/yr). A previous hill country seepage wetland study reported an areal NO_3^- removal rate of 0.9±0.3 g N/m^2/day, i.e. 3,285 kg NO_3-N/ha/yr based on surface flow and seepage velocity that was measured in a tracer-based experiment within the surface 10 cm sediment depth and was combined with sediment DEA (Rutherford & Nguyen, 2004). It should be noted that sediment DEA values in these studies can be expected to be much higher than the *in-situ* DEA values because sediment DEA in the laboratory are measured under enhanced denitrification-conducive environments (additional NO_3^- input and enforced saturated and anaerobic conditions).

Comparatively, a much lower areal NO_3^- removal rate of 16.7±23.3 kg NO_3-N/ha/yr was estimated in a seepage wetland study site at Tuapaka (with low sediment DEA) under natural conditions. This estimation was based on surface and shallow groundwater NO_3^- concentrations monitored in a 2-year long hydrological study in this thesis (chapter 4). At this rate, the Tuapaka site measured an annualised NO_3^- removal of 1.40 kg NO_3-N/yr, based on the NO_3^- balance. A previous study that monitored shallow groundwater, reported a higher annualised NO_3^- removal of ~50 kg NO_3-N/yr in a pastoral hill country seepage wetland, located in the central north Island of NZ (Burns & Nguyen, 2002). This could be due to the greater NO_3^- removal efficiency in subsurface flow pathways (Burns & Nguyen, 2002). In contrast, the NO_3^- removal of 1.40 kg NO_3-N/yr measured in this thesis is more realistic, as the estimation technique considered both hydrological pathways. Furthermore, the NO_3^- removal rate measured in year 2 (33.3 kg NO_3-N/ha) in the current study, is more likely to reflect the areal NO_3-N removal rate of the study area, because that year, the wetland received a seasonally equivalent precipitation which is typical for the region the study area is located in (Chappell, 2015).

This thesis also showed, that the naturally low areal NO_3^- removal rate of the wetland can be improved by altering the hydrological flow conditions in the seepage wetland (chapter 5) and with an experimental potential of achieving a mean NO_3^- removal rate of 1489.2 ± ($4x10^9$) kg NO_3-N/ha/yr with the flow intervention. However, this is unlikely to be achieved realistically.

Overall, the comparisons of the NO_3^- removal estimates across the thesis and with the previous estimates:

1. Strengthen our understanding of the variabilities in seepage wetland NO_3^- removal based on the different hydrological routes investigated and the estimation techniques involved.
2. Indicate a wide gap between the high wetland potential, yet low performance in terms of NO_3^- removal. This means seepage wetlands under natural conditions may underperform in terms of NO_3^- removal and the wetland NO_3^- removal capacity may remain under-utilised.

6.5.5 Summary

This thesis has contributed to an improved understanding of the seepage wetland sediment and hydrology, which is required to integrate seepage wetlands as a catchment-based NO_3^- management tool. In terms of seepage wetland science, this research has identified that sediment WC, NO_3^-, %TC, %TN, dissolved Fe^{2+} and Mn^{2+}, DOC regulates the spatial variability of DEA. Temporally, winter is generally a season of NO_3^- loss in seepage wetlands. In contrast, denitrification-based NO_3^- removal potentially improves in spring. Additionally, the high NO_3^- removal measured under dissipated flow conditions in this thesis, indicated the potential to improve NO_3^- removal via flow regulation in these wetlands. This research has progressed this concept by demonstrating that flow regulation can be used to improve NO_3^- reduction, even in seepage wetlands with low denitrification capacities. This contribution to our understanding of seepage wetland function and potential is critical at a time when the implementation of the Wetland policy under the National Policy Statement for Freshwater Management (NPS-FM) is being rolled out in NZ.

These research findings are likely to inform possible mitigation options that can be implemented by farmers, catchment groups, and regional councils. According to the NPS-FM, regional councils are required to set NO_3^- concentration limits to manage periphyton growth in water bodies (MfE & MPI, 2020). To achieve this, practical mitigation strategies are required both at the farm and catchment scale. In this context, the stakeholders will potentially benefit from the thesis findings through the use of nature-based solutions, e.g., natural seepage wetlands to reduce NO_3^- concentrations in farm discharge and reduce risks to freshwater.

In conclusion, this thesis has made a significant contribution to improving our understanding of the "where", "when" and "how" pastoral hill country seepage wetlands can be used to optimise NO_3^- mitigation, knowledge of which is critical for improving the NO_3^- water quality leaving pastoral hill country farms. This thesis provides an important link between previous and future seepage wetland studies and provides new knowledge that will facilitate the application of seepage wetlands for more effective NO_3^- mitigation in a pastoral hill country landscape. Finally, this research lays the foundations for using geospatial data to identify landscape features likely to support denitrification in seepage wetlands. The step will be critical for future modelling of seepage wetlands denitrification as a possible mitigation strategy at the catchment scale.

The thesis concludes by making important future study recommendations which are necessary to expand the current thesis findings.

6.6 Recommended future research

1. *In-situ* DEA measurements with no additional NO_3^- input are needed across multiple seepage wetlands to validate the current DEA estimates.

2. It is recommended that wetland sediment and hydrological characterisations are replicated across multiple sites in order to improve our *in-situ* understanding of spatial gradients and their influence on NO_3^- removal across pastoral hill country landscapes. This more comprehensive knowledge will be necessary for robust seepage wetland denitrification modelling in the future.

3. Future hydrology studies should consider automated and event-based water sampling or real-time NO_3^- sensors for surface flow and a more intensive piezometer network for subsurface flow, to improve the accuracy of the wetland NO_3^- balance. Such approaches will improve the NO_3^- estimates during hot moments e.g. high flow conditions following large precipitation events, different stages of storm flow and post-grazing wetland conditions.

4. Monitoring of subsurface flow and runoff studies in the future will 1) improve the wetland water and NO_3^- balances, and 2) provide greater confidence about the relative contributions of different hydrological pathways to NO_3^- mitigation.

5. In order to overcome the challenges faced during the subsurface outflow column study, several design modifications in the experimental setup are recommended that include 1) constant loading rates across all sediment columns, 2) drainage monitoring at more frequent time intervals using automated sensors for example, and 3) including N_2O gas sampling from the outflow. Future laboratory-based

drainage studies should also consider other treatments e.g., different NO_3^- loading rates in order to improve the drainage intervention for application in the field.

6. Wetland sediment coring techniques should be explored in future studies to minimise sediment compression.

7. Routine monitoring of physical/chemical properties e.g., DO, redox potential, dissolved Fe^{2+} and dissolved Mn^{2+} in the inflow water into sediment columns and in the outflow may provide additional insights into the redox dynamics during the subsurface outflow interventions.

8. Future studies should also explore N-reduction pathways other than denitrification e.g., DNRA and plant uptake in these wetlands. Compartmentalisation of the N-reduction pathways in future seepage wetlands studies would increase confidence regarding the extent of NO_3^- mitigation in these wetlands.

9. As higher resolution LIDAR data for the HRC become available, future studies should investigate whether the soil drainage properties in the landscape surrounding the seepage wetlands, as well as wetland hydrology, can be used as indicators for seepage wetland DEA, by using finer-resolution spatial data and a larger number of seepage wetland sites.

10. Future research could spatially integrate landscape properties to model the likely NO_3^- removal rate across the wider landscape, based on the seepage wetland properties and DEA measured in the current study.

11. Future investigations should consider additional GIS information such as on-farm NO_3^- applications and local hydrology because these properties may influence denitrification in pastoral hill country seepage wetlands.

12. We also suggest a series of multi-site studies similar to the current thesis are required to firmly establish relationships between sediment and hydrological characteristics, and DEA in seepage wetlands, in order to effectively model the potential for NO_3^- attenuation at a catchment scale.

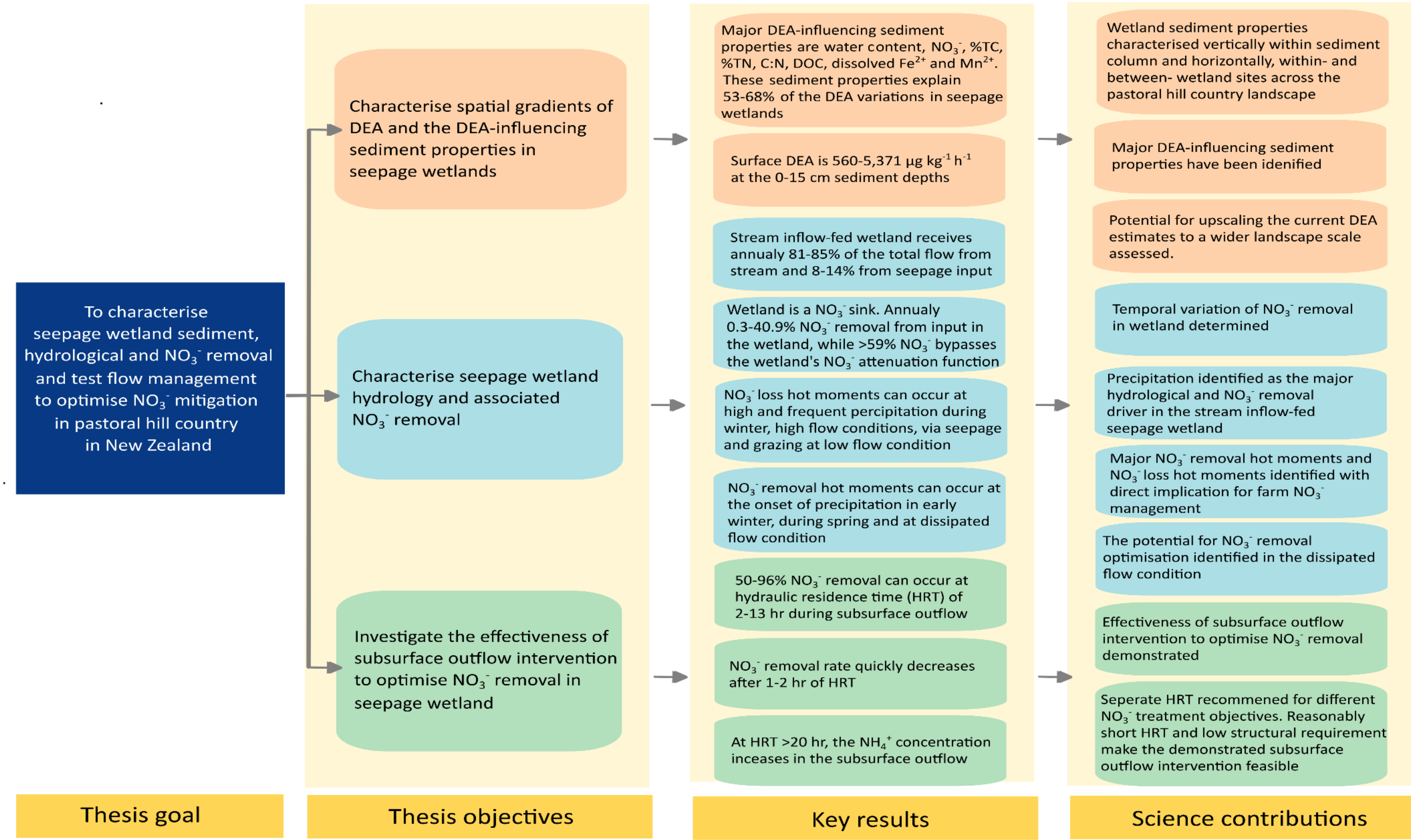


Figure 6-2. Thesis goal, objectives, key results and contributions.

References

Abdelhakeem, S. G., Aboulroos, S. A., & Kamel, M. M. (2016). Performance of a vertical subsurface flow constructed wetland under different operational conditions. *Journal of Advanced Research*, *7*(5), 803-814. https://doi.org/https://doi.org/10.1016/j.jare.2015.12.002

Addy, K., Gold, A. J., Christianson, L. E., David, M. B., Schipper, L. A., & Ratigan, N. A. (2016). Denitrifying bioreactors for nitrate removal: A meta-analysis. *Journal of Environmental Quality*, *45*(3), 873-881. https://doi.org/10.2134/jeq2015.07.0399

Addy, K., Kellogg, D., Gold, A. J., Groffman, P. M., Ferendo, G., & Sawyer, C. (2002). In situ push–pull method to determine ground water denitrification in riparian zones. *Journal of Environmental Quality*, *31*(3), 1017-1024.

Alexander, R. B., Boyer, E. W., Smith, R. A., Schwarz, G. E., & Moore, R. B. (2007). The role of headwater streams in downstream water quality. *JAWRA Journal of the American Water Resources Association*, *43*(1), 41-59.

Alexander, R. B., Smith, R. A., & Schwarz, G. E. (2000). Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature*, *403*(6771), 758-761.

Alyamani, E. J., Booq, R. Y., Bahkali, A. H., & Alharbi, S. A. (2020). Effect of denitrifying bacterial biomass and carbon sources on nitrate removal. *Journal of Pure and Applied Microbiology*, *14*(4), 2417-2424, Article 6597. https://doi.org/10.22207/jpam.14.4.19

Andersen, H. E. (2004). Hydrology and nitrogen balance of a seasonally inundated Danish floodplain wetland. *Hydrological Processes*, *18*(3), 415-434. https://doi.org/10.1002/hyp.1277

Anderson, T. R., Groffman, P. M., Kaushal, S. S., & Walter, M. T. (2014). Shallow groundwater denitrification in riparian zones of a headwater agricultural landscape. *Journal of Environmental Quality*, *43*(2), 732-744. https://doi.org/10.2134/jeq2013.07.0303

Anderson, T. R., Groffman, P. M., & Walter, M. T. (2015). Using a soil topographic index to distribute denitrification fluxes across a northeastern headwater catchment. *Journal of Hydrology*, *522*, 123-134. https://doi.org/https://doi.org/10.1016/j.jhydrol.2014.12.043

Angier, J. T., & McCarty, G. W. (2008). Variations in base-flow nitrate flux in a first-order stream and riparian zone. *JAWRA Journal of the American Water Resources Association*, *44*(2), 367-380. https://doi.org/10.1111/j.1752-1688.2007.00153.x

APHA. (2005). *Standard Methods for the Examination of Water and Wastewater* (21 ed.). American Public Health Association (APHA).

Araragi, M., Phetchawee, S., & Tantitanapat, P. (1979). Micro flora related to the nitrogen cycle in the tropical upland farm soils. *Soil Science and Plant Nutrition*, *25*(2), 235-244. https://doi.org/10.1080/00380768.1979.10433164

Arnon, S., Gray, K. A., & Packman, A. I. (2007). Biophysicochemical process coupling controls nitrogen use by benthic biofilms. *Limnology and Oceanography*, *52*(4), 1665-1671. https://doi.org/10.4319/lo.2007.52.4.1665

Attard, E., Recous, S., Chabbi, A., De Berranger, C., Guillaumaud, N., Labreuche, J., Philippot, L., Schmid, B., & Le Roux, X. (2011). Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. *Global Change Biology*, *17*(5), 1975-1989. https://doi.org/10.1111/j.1365-2486.2010.02340.x

Bai, J., Wang, X., Jia, J., Zhang, G., Wang, Y., & Zhang, S. (2017). Denitrification of soil nitrogen in coastal and inland salt marshes with different flooding frequencies. *Physics and Chemistry of the Earth, Parts A/B/C*, *97*, 31-36. https://doi.org/10.1016/j.pce.2017.01.015

Bargh, B. J. (1978). Output of water, suspended sediment, and phosphorus and nitrogen forms from a small agricultural catchment. *New Zealand Journal of Agricultural Research*, *21*(1), 29-38.

Barkle, G., Clough, T., & Stenger, R. (2007). Denitrification capacity in the vadose zone at three sites in the Lake Taupo catchment, New Zealand. *Soil Research*, *45*(2), 91. https://dx.doi.org/10.1071/SR06141

Barton, L., McLay, C. D. A., Schipper, L. A., & Smith, C. T. (1999). Annual denitrification rates in agricultural and forest soils: A review. *Soil Research*, *37*(6), 1073. https://doi.org/10.1071/sr99009

Basher, L., Botha, N., Dodd, M., Douglas, G., Lynn, I., Marden, M., McIvor, I., & Smith, W. (2008). *Hill Country Erosion: A Review of Knowledge on Erosion Processes, Mitigation Options, Social Learning and Their Long-Term Effectiveness in The Management of Hill Country Erosion*.

Bastviken, S. K., Eriksson, P. G., Premrov, A., & Tonderski, K. (2005). Potential denitrification in wetland sediments with different plant species detritus. *Ecological Engineering*, *25*(2), 183-190. https://doi.org/10.1016/j.ecoleng.2005.04.013

Bates, H. K., & Spalding, R. F. (1998). Aquifer denitrification as interpreted from in situ microcosm experiments. *Journal of Environmental Quality*, *27*(1), 174-182. https://doi.org/10.2134/jeq1998.00472425002700010025x

Batson, J. A., Mander, U., & Mitsch, W. J. (2012). Denitrification and a nitrogen budget of created riparian wetlands. *Journal of Environmental Quality*, *41*(6), 2024-2032. https://doi.org/10.2134/jeq2011.0449

Beef & Lamb New Zealand. (2022). *Compendium of New Zealand - Farm facts no. 46*. Beef+Lamb New Zealand.

Bergaust, L., Mao, Y., Bakken, L. R., & Frostegård, A. s. (2010). Denitrification response patterns during the transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrogen oxide reductase in Paracoccus denitrificans. *Applied and Environmental Microbiology*, *76*(19), 6387-6396.

Bergstermann, A., Cárdenas, L., Bol, R., Gilliam, L., Goulding, K., Meijide, A., Scholefield, D., Vallejo, A., & Well, R. (2011). Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N2O during denitrification. *Soil Biology & Biochemistry*, *43*(2), 240-250.

Bernard-Jannin, L., Sun, X., Teissier, S., Sauvage, S., & Sánchez-Pérez, J.-M. (2017). Spatio-temporal analysis of factors controlling nitrate dynamics and potential denitrification hot spots and hot moments in groundwater of an alluvial floodplain. *Ecological Engineering*, *103*(Part B), 372-384. https://doi.org/10.1016/j.ecoleng.2015.12.031

Bijay, S., & Craswell, E. (2021). Fertilizers and nitrate pollution of surface and ground water: an increasingly pervasive global problem. *SN Applied Sciences*, *3*(4), 518. https://doi.org/10.1007/s42452-021-04521-8

Billen, G., & Garnier, J. (1999). Nitrogen transfers through the Seine drainage network: A budget based on the application of the 'Riverstrahler' model. *Hydrobiologia*, *410*, 139-150.

Blackmer, A. M., & Bremner, J. M. (1978). Inhibitory effect of nitrate on reduction of N2O to N2 by soil microorganisms. *Soil Biology & Biochemistry*, *10*(3), 187-191.

Blaschke, P. M., Trustrum, N. A., & DeRose, R. C. (1992). Ecosystem processes and sustainable land use in New Zealand steeplands. *Agriculture, Ecosystems & Environment*, *41*(2), 153-178.

Boesch, D. F., Brinsfield, R. B., & Magnien, R. E. (2001). Chesapeake bay eutrophication: Scientific understanding, ecosystem restoration, and challenges for agriculture. *Journal of Environmental Quality*, *30*(2), 303-320. https://doi.org/10.2134/jeq2001.302303x

Böhlke, J. K., Harvey, J. W., & Voytek, M. A. (2004). Reach-scale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, midcontinent United States. *Limnology and Oceanography*, *49*(3), 821-838. https://doi.org/10.4319/lo.2004.49.3.0821

Böhlke, J. K., O'Connell, M. E., & Prestegaard, K. L. (2007). Ground water stratification and delivery of nitrate to an incised stream under varying flow conditions. *Journal of Environmental Quality*, *36*(3), 664-680.

Bohrerova, Z., Stralkova, R., Podesvova, J., Bohrer, G., & Pokorny, E. (2004). The relationship between redox potential and nitrification under different sequences of crop rotations. *Soil and Tillage Research*, *77*(1), 25-33.

Bonaiti, G., & Borin, M. (2010). Efficiency of controlled drainage and subirrigation in reducing nitrogen losses from agricultural fields. *Agricultural Water Management*, *98*(2), 343-352.

Bonell, M., Pearce, A. J., & Stewart, M. K. (1990). The identification of runoff-production mechanisms using environmental isotopes in a tussock grassland catchment, eastern Otago, New Zealand. *Hydrological Processes*, *4*(1), 15-34.

Bowden, W. B., Fahey, B. D., Ekanayake, J., & Murray, D. L. (2001). Hillslope and wetland hydrodynamics in a tussock grassland, South Island, New Zealand. *Hydrological Processes*, *15*(10), 1707-1730.

Bowen, H., Maul, J. E., Cavigelli, M. A., & Yarwood, S. (2020). Denitrifier abundance and community composition linked to denitrification activity in an agricultural and wetland soil. *Applied Soil Ecology*, *151*.

Bremner, J. M., & Shaw, K. (1958). Denitrification in soil. II. Factors affecting denitrification. *Journal of Agricultural Science*, *51*(1), 40-52. https://doi.org/10.1017/s0021859600032779

Bremner, J. M., & Tabatabai, M. (1971). Use of automated combustion techniques for total carbon, total nitrogen, and total sulfur analysis of soils. In *Instrumental Methods for Analysis of Soils and Plant Tissue* (pp. 1-15).

Briggs, M. A., Lautz, L. K., & Hare, D. K. (2014). Residence time control on hot moments of net nitrate production and uptake in the hyporheic zone. *Hydrological Processes*, *28*(11), 3741-3751. https://doi.org/10.1002/hyp.9921

Burford, J. R., & Bremner, J. M. (1975). Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. *Soil Biology & Biochemistry*, *7*(6), 389-394. https://doi.org/https://doi.org/10.1016/0038-0717(75)90055-3

Burgin, A. J., & Groffman, P. M. (2012). Soil O2 controls denitrification rates and N2O yield in a riparian wetland. *Journal of Geophysical Research*, *117*(G1). https://doi.org/10.1029/2011jg001799

Burgin, A. J., Groffman, P. M., & Lewis, D. N. (2010). Factors regulating denitrification in a riparian wetland. *Soil Science Society of America Journal*, *74*(5), 1826-1833. https://doi.org/10.2136/sssaj2009.0463

Burns, D., & Nguyen, M. (2002). Nitrate movement and removal along a shallow groundwater flow path in a riparian wetland within a sheep-grazed pastoral catchment: Results of a tracer study. *New Zealand Journal of Marine and Freshwater Research*, *36*(2), 371-385.

Burt, T., Matchett, L., Goulding, K., Webster, C., & Haycock, N. (1999). Denitrification in riparian buffer zones: the role of floodplain hydrology. *Hydrological Processes*, *13*(10), 1451-1463.

Burt, T., & Pinay, G. (2005). Linking hydrology and biogeochemistry in complex landscapes. *Progress in Physical Geography*, *29*(3), 297-316.

Burt, T., Pinay, G., Worrall, F., & Howden, N. (2022). Slopes: solute processes and landforms. *Geological Society, London, Memoirs*, *58*(1), 191-204. https://doi.org/doi:10.1144/M58-2021-5

Busnardo, M. J., Gersberg, R. M., Langis, R., Sinicrope, T. L., & Zedler, J. B. (1992). Nitrogen and phosphorus removal by wetland mesocosms subjected to different hydroperiods. *Ecological Engineering*, *1*(4), 287-307.

Buss, J., & Achten, C. (2022). Spatiotemporal variations of surface water quality in a medium-sized river catchment (Northwestern Germany) with agricultural and urban land use over a five-year period with extremely dry summers. *Science of the Total Environment*, *818*, Article 151730. https://doi.org/10.1016/j.scitotenv.2021.151730

Buss, S., Rivett, M., Morgan, P., & Bemment, C. (2005). *Attenuation of nitrate in the sub-surface environment*.

Buttle, J. (1994). Isotope hydrograph separations and rapid delivery of pre-event water from drainage basins. *Progress in Physical Geography*, *18*(1), 16-41.

Butturini, A., Bernal, S., Nin, E., Hellin, C., Rivero, L., Sabater, S., & Sabater, F. (2003). Influences of the stream groundwater hydrology on nitrate concentration in unsaturated riparian area bounded by an intermittent Mediterranean stream. *Water Resources Research*, *39*(4), SBH121-SBH1213. https://doi.org/10.1029/2001WR001260

Cameron, S. G., & Schipper, L. A. (2010). Nitrate removal and hydraulic performance of organic carbon for use in denitrification beds. *Ecological Engineering*, *36*(11), 1588-1595.

Campbell, D., & Jackson, R. (2004). Hydrology of wetlands. In *Freshwaters of New Zealand* (pp. 20.21-20.14). New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc.

Carstensen, M. V., Børgesen, C. D., Ovesen, N. B., Poulsen, J. R., Hvid, S. K., & Kronvang, B. (2019). Controlled drainage as a targeted mitigation measure for nitrogen and phosphorus. *Journal of Environmental Quality*, *48*(3), 677-685.

Carstensen, M. V., Hashemi, F., Hoffmann, C. C., Zak, D., Audet, J., & Kronvang, B. (2020). Efficiency of mitigation measures targeting nutrient losses from agricultural drainage systems: A review. *Ambio*, *49*(11), 1820-1837. https://doi.org/10.1007/s13280-020-01345-5

Carstensen, M. V., Zak, D., van't Veen, S. G. M., Wisniewska, K., Ovesen, N. B., Kronvang, B., & Audet, J. (2021). Nitrogen removal and greenhouse gas fluxes from integrated buffer zones treating agricultural drainage water. *Science of the Total Environment*, *774*, 145070. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.145070

Chakrawal, A., Calabrese, S., Herrmann, A. M., & Manzoni, S. (2022). Interacting bioenergetic and stoichiometric controls on microbial growth. *Frontiers in Microbiology*, *13*, Article 859063.

Chappell, P. R. (2015). *The climate and weather of Manawatu-Wanganui*. NIWA, Taihoro Nukurangi.

Chaubey, I., Sahoo, D., Haggard, B. E., Matlock, M. D., & Costello, T. A. (2007). Nutrient retention, nutrient limitation, and sediment-nutrient interactions in a pasture-dominated stream. *Transactions of the ASABE*, *50*(1), 35-44.

Chen, G., Kolb, L., Cavigelli, M. A., Weil, R. R., & Hooks, C. R. R. (2018). Can conservation tillage reduce N2O emissions on cropland transitioning to organic vegetable production? *Science of the Total Environment*, *618*, 927-940. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.08.296

Chescheir, G. M., Skaggs, R. W., & Gilliam, J. W. (1992). Evaluation of wetland buffer areas for treatment of pumped agricultural drainage water. *Transactions of the American Society of Agricultural Engineers*, *35*(1), 175-182.

Chibuike, G. (2019). *Investigating nitrate attenuation capacity and processes in pastoral hill country landscapes* [Doctoral, Massey University].

Chibuike, G., Burkitt, L., Camps-Arbestain, M., Singh, R., Bretherton, M., Bishop, P., & Shen, Q. (2019). Denitrification capacity of hill country wet and dry area soils as influenced by dissolved organic carbon concentration and chemistry. *Wetlands*, *40*(4), 681-691. https://doi.org/10.1007/s13157-019-01223-1

Childs, C. (1981). Field tests for ferrous iron and ferric-organic complexes (on exchange sites or in water-soluble forms) in soils. *Soil Research*, *19*(2), 175-180.

Christensen, S., Simkins, S., & Tiedje, J. M. (1990). Spatial variation in denitrification: Dependency of activity centers on the soil environment. *Soil Science Society of America Journal*, *54*(6), 1608-1613.

Christianson, L. E., Cooke, R. A., Hay, C. H., Helmers, M. J., Feyereisen, G. W., Ranaivoson, A. Z., McMaine, J. T., McDaniel, R., Rosen, T. R., Pluer, W. T., Schipper, L. A., Dougherty, H., Robinson, R. J., Layden, I. A., Irvine-Brown, S. M., Manca, F., Dhaese, K., Nelissen, V., & Von Ahnen, M. (2021). Effectiveness of denitrifying bioreactors on water pollutant reduction from agricultural areas. *Transactions of the ASABE*, *64*(2), 641-658. https://doi.org/10.13031/trans.14011

Chun, J., Cooke, R., Eheart, J., & Kang, M. (2009). Estimation of flow and transport parameters for woodchip-based bioreactors: I. laboratory-scale bioreactor. *Biosystems Engineering*, *104*(3), 384-395.

Clague, J. C. (2013). *Denitrification in the shallow groundwater system of two agricultural catchments in the Waikato, New Zealand* [Doctoral, Lincoln University].

Clague, J. C., Stenger, R., & Morgenstern, U. (2019). The influence of unsaturated zone drainage status on denitrification and the redox succession in shallow groundwater. *Science of the Total Environment*, *660*, 1232-1244. https://doi.org/10.1016/j.scitotenv.2018.12.383

Clarkson, B. R., Sorrell, B. K., Reeves, P. N., Champion, P. D., Partridge, T. R., & Clarkson, B. D. (2003). *Handbook for Monitoring Wetland Condition*. Ministry for the Environment.

Clayden, B., & Webb, T. H. (1994). *Criteria for Defining the Soilform, the Fourth Category of the New Zealand Soil Classification*. Landcare Research, New Zealand.

Close, M. E., Abraham, P., Humphries, B., Lilburne, L., Cuthill, T., & Wilson, S. (2016). Predicting groundwater redox status on a regional scale using linear discriminant analysis. *Journal of Contaminant Hydrology*, *191*, 19-32. https://doi.org/10.1016/j.jconhyd.2016.04.006

Collins, R., Elliott, S., & Adams, R. (2005). Overland flow delivery of faecal bacteria to a headwater pastoral stream. *Journal of Applied Microbiology*, *99*(1), 126-132.

Collins, S., Singh, R., Rivas, A., Palmer, A., Horne, D., Manderson, A., Roygard, J., & Matthews, A. (2017). Transport and potential attenuation of nitrogen in shallow groundwaters in the lower Rangitikei catchment, New Zealand. *Journal of Contaminant Hydrology*, *206*, 55-66. https://doi.org/10.1016/j.jconhyd.2017.10.002

Cooke, J., & Cooper, A. (1988). Sources and sinks of nutrients in a New Zealand hill pasture catchment III. Nitrogen. *Hydrological Processes*, *2*(2), 135-149.

Cooke, J. G., & Dons, T. (1988). Sources and sinks of nutrients in a New Zealand hill pasture catchment I. Stormflow generation. *Hydrological Processes*, *2*(2), 109-122.

Cooper, A. (1990). Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. *Hydrobiologia*, *202*(1-2), 13-26. https://doi.org/10.1007/bf00027089

Cooper, A., & Cooke, J. (1984). Nitrate loss and transformation in 2 vegetated headwater streams. *New Zealand Journal of Marine and Freshwater Research*, *18*(4), 441-450. https://doi.org/10.1080/00288330.1984.9516065

Cooper, A. B., Smith, C. M., & Smith, M. J. (1995). Effects of riparian set-aside on soil characteristics in an agricultural landscape: Implications for nutrient transport and retention. *Agriculture, Ecosystems & Environment*, *55*(1), 61-67. https://doi.org/10.1016/0167-8809(95)00605-R

Covatti, G., Grischek, T., & Burghardt, D. (2022). Tracing sources and transformations of ammonium during river bank filtration by means of column experiments. *Journal of Contaminant Hydrology*, *249*. https://doi.org/10.1016/j.jconhyd.2022.104050

Cowie, J. (1978). *Soils and agriculture of Kairanga County, North Island, New Zealand*. New Zealand Soil Bureau Lower Hutt, New Zealand.

D'haene, K., Moreels, E., De Neve, S., Chaves Daguilar, B., Boeckx, P., Hofman, G., & Van Cleemput, O. (2003). Soil properties influencing the denitrification potential of Flemish agricultural soils. *Biology and Fertility of Soils*, *38*(6), 358-366.

Danielson, R., & Sutherland, P. (1986). Porosity. In *Methods of Soil Analysis: Part 1 Physical and Mineralogical Methods* (Vol. 5, pp. 443-461).

Daughney, C., Jones, A., Baker, T., Hanson, C., Davidson, P., Zemansky, G., Reeves, R., & Thompson, M. (2006). *A National Protocol for State of The Environment Groundwater Sampling in New Zealand*. Ministry for the Environment.

Davie, T. (2004). Soil water, runoff, streamflow generation. In J. S. Harding, P. Mosley, C. Pearson, & B. Sorrell (Eds.), *Freshwater of New Zealand* (pp. 4.1-4.10). New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc.

Davie, T. (2008). *Fundamentals of Hydrology* (3 ed.). Routledge.

De Laune, R., Reddy, C., & Patrick Jr, W. (1981). *Effect of pH and redox potential on concentration of dissolved nutrients in an estuarine sediment* (0047-2425).

Dendooven, L., Splatt, P., & Anderson, J. M. (1994). The use of chloramphenicol in the study of the denitrification process - some side-effects. *Soil Biology & Biochemistry*, *26*(7), 925-927. https://doi.org/10.1016/0038-0717(94)90309-3

Deng, D., Pan, Y., Liu, G., Liu, W., & Ma, L. (2020). Seeking the hotspots of nitrogen removal: A comparison of sediment denitrification rate and denitrifier abundance among wetland types with different hydrological conditions. *Science of the Total Environment*, *737*, 140253. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.140253

Denver, J. M., Ator, S. W., Lang, M. W., Fisher, T. R., Gustafson, A. B., Fox, R., Clune, J. W., & McCarty, G. W. (2014). Nitrate fate and transport through current and former depressional wetlands in an agricultural landscape, Choptank Watershed, Maryland, United States. *Journal of Soil and Water Conservation*, *69*(1), 1-16. https://doi.org/10.2489/jswc.69.1.1

Dhondt, K., Boeckx, P., Hofman, G., & Van Cleemput, O. (2004). Temporal and spatial patterns of denitrification enzyme activity and nitrous oxide fluxes in three adjacent vegetated riparian buffer zones. *Biology and Fertility of Soils*, *40*(4), 243-251. https://doi.org/10.1007/s00374-004-0773-z

Di Capua, F., Pirozzi, F., Lens, P. N., & Esposito, G. (2019). Electron donors for autotrophic denitrification. *Chemical Engineering Journal*, *362*, 922-937.

Dodd, M. B., McDowell, R. W., & Quinn, J. M. (2016). A review of contaminant losses to water from pastoral hill lands and mitigation options. *NZGA: Research and Practice Series*, *16*, 137-147. https://doi.org/10.33584/rps.16.2016.3269

Dodds, W. K., & Oakes, R. M. (2008). Headwater influences on downstream water quality. *Environmental Management*, *41*(3), 367-377.

Drake, C. W., Jones, C. S., Schilling, K. E., Amado, A. A., & Weber, L. J. (2018). Estimating nitrate-nitrogen retention in a large constructed wetland using high-frequency, continuous monitoring and hydrologic modeling. *Ecological Engineering*, *117*, 69-83. https://doi.org/https://doi.org/10.1016/j.ecoleng.2018.03.014

Drury, C. F., McKenney, D. J., & Findlay, W. I. (1991). Relationships between denitrification, microbial biomass and indigenous soil properties. *Soil Biology & Biochemistry*, *23*(8), 751-755. https://doi.org/https://doi.org/10.1016/0038-0717(91)90145-A

Dunne, T., & Black, R. D. (1970a). An experimental investigation of runoff production in permeable soils. *Water Resources Research*, *6*(2), 478-490.

Dunne, T., & Black, R. D. (1970b). Partial area contributions to storm runoff in a small New England watershed. *Water Resources Research*, *6*(5), 1296-1311.

Elliott, A., & Carlson, W. (2004). Effects of sheep grazing episodes on sediment and nutrient loss in overland flow. *Soil Research*, *42*(2), 213-220.

Elwan, A., Singh, R., Patterson, M., Roygard, J., Horne, D., Clothier, B., & Jones, G. (2018). Influence of sampling frequency and load calculation methods on quantification of annual river nutrient and suspended solids loads. *Environmental Monitoring and Assessment*, *190*(2), 1-18.

Enwezor, W. (1976). The mineralization of nitrogen and phosphorus in organic materials of varying C: N and C: P ratios. *Plant and Soil*, *44*(1), 237-240.

Eppinger, R., & Walraevens, K. (2014). Spatial distribution of nitrate in Cenozoic sedimentary aquifers controlled by a variable reactivity system. In *Nitrates in Groundwater* (1st ed., pp. 10). CRC Press.

Fazzolari, É., Nicolardot, B., & Germon, J. C. (1998). Simultaneous effects of increasing levels of glucose and oxygen partial pressures on denitrification and dissimilatory nitrate reduction to ammonium in repacked soil cores. *European Journal of Soil Biology*, *34*(1), 47-52.

Fenton, O., Richards, K. G., Kirwan, L., Khalil, M. I., & Healy, M. G. (2009). Factors affecting nitrate distribution in shallow groundwater under a beef farm in South Eastern Ireland. *Journal of Environmental Management*, *90*(10), 3135-3146. https://dx.doi.org/10.1016/j.jenvman.2009.05.024

Ferrier, R., Helliwell, R., Cosby, B., Jenkins, A., & Wright, R. (2001). Recovery from acidification of lochs in Galloway, south-west Scotland, UK: 1979-1998. *Hydrology and Earth System Sciences*, *5*(3), 421-432.

Firestone, M., Smith, M., Firestone, R., & Tiedje, J. (1979). The influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. *Soil Science Society of America Journal*, *43*(6), 1140-1144.

Flite III, O. P., Shannon, R. D., Schnabel, R. R., & Parizek, R. R. (2001). Nitrate removal in a riparian wetland of the appalachian valley and ridge physiographic province. *Journal of Environmental Quality*, *30*(1), 254-261.

Fransen, P., Burkitt, L., Chibuike, G., Bretherton, M., Hickson, R., Morris, S., Hedley, C., & Roudier, P. (2022). Reducing nutrient and sediment losses in surface runoff by selecting cattle supplement feeding areas based on soil type in New Zealand hill country. *New Zealand Journal of Agricultural Research*, 1-18. https://doi.org/10.1080/00288233.2022.2086888

Fredrickson, J., Garland, T., Hicks, R., Thomas, J., Li, S., & McFadden, K. (1989). Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties. *Geomicrobiology Journal*, *7*(1-2), 53-66.

Gao, Y., Xie, Y. W., Zhang, Q., Wang, A. L., Yu, Y. X., & Yang, L. Y. (2017). Intensified nitrate and phosphorus removal in an electrolysis-integrated horizontal subsurface-flow constructed wetland. *Water Research*, *108*, 39-45. https://doi.org/10.1016/j.watres.2016.10.033

Giles, M., Morley, N., Baggs, E. M., & Daniell, T. J. (2012). Soil nitrate reducing processes - drivers, mechanisms for spatial variation, and significance for nitrous oxide production. *Frontiers in Microbiology*, *3*, 407. https://doi.org/10.3389/fmicb.2012.00407

Gillham, R. W., & Cherry, J. A. (1978). Field evidence of denitrification in shallow groundwater flow systems. *Water Quality Research Journal*, *13*(1), 53-72. https://doi.org/10.2166/wqrj.1978.006

Gillingham, A. G., Morton, J. D., & Gray, M. H. (2007). Pasture responses to phosphorus and nitrogen fertilisers on East Coast hill country: Total production from easy slopes. *New Zealand Journal of Agricultural Research*, *50*(3), 307-320. https://doi.org/10.1080/00288230709510299

Goeller, B. C., Febria, C. M., McKergow, L. A., Harding, J. S., Matheson, F. E., Tanner, C., & McIntosh, A. R. (2020). Combining tools from edge-of-field to in-stream to attenuate reactive nitrogen along small agricultural waterways. *Water*, *12*(2), 383.

Goeller, B. C., Febria, C. M., Warburton, H. J., Hogsden, K. L., Collins, K. E., Devlin, H. S., Harding, J. S., & McIntosh, A. R. (2019). Springs drive downstream nitrate export from artificially-drained agricultural headwater catchments. *Science of the Total Environment*, *671*, 119-128. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.03.308

Gold, A. J., Groffman, P. M., Addy, K., Kellogg, D. Q., Stolt, M., & Rosenblatt, A. E. (2001). Landscape attributes as controls on ground water nitrate removal capacity of riparian zones. *Journal of the American Water Resources Association*, *37*(6), 1457-1464.

Google map. (2021). *Elevation map of Rathmoy, Wairiri, Tuapaka and Ballentare seepage wetland sites*. https://earth.google.com/web/

Grebliunas, B. D., & Perry, W. L. (2016). The role of C:N:P stoichiometry in affecting denitrification in sediments from agricultural surface and tile-water wetlands. *SpringerPlus*, *5*(1), 1. https://doi.org/10.1186/s40064-016-1820-6

Green, C. T., Puckett, L. J., Böhlke, J. K., Bekins, B. A., Phillips, S. P., Kauffman, L. J., Denver, J. M., & Johnson, H. M. (2008). Limited occurrence of denitrification in four shallow aquifers in agricultural areas of the United States. *Journal of Environmental Quality*, *37*(3), 994-1009. https://doi.org/10.2134/jeq2006.0419

Greenan, C. M., Moorman, T. B., Parkin, T. B., Kaspar, T. C., & Jaynes, D. B. (2009). Denitrification in wood chip bioreactors at different water flows. *Journal of Environmental Quality*, *38*(4), 1664-1671.

Gregor, M. (2010). *User's Manual: BFI+ 3.0*. HydrOffice Software Package, Water Science. https://hydrooffice.org/Tool/BFI

Grimmond, D., Bell, B. D., & Yap, M. (2014). *Future capability needs for the primary industries in New Zealand*. Ministry for Primary Industries.

Groffman, P. M., Butterbach-Bahl, K., Fulweiler, R. W., Gold, A. J., Morse, J. L., Stander, E. K., Tague, C., Tonitto, C., & Vidon, P. (2009). Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry*, *93*(1-2), 49-77. https://doi.org/10.1007/s10533-008-9277-5

Groffman, P. M., & Hanson, G. C. (1997). Wetland denitrification: influence of site quality and relationships with wetland delineation protocols. *Soil Science Society of America Journal*, *61*(1), 323-329.

Groffman, P. M., Tiedje, J. M., Robertson, G. P., & Christensen, S. (1987). Denitrification at different temporal and geographical scales: Proximal and distal controls. In J. R. Wilson (Ed.), *Advances in Nitrogen Cycling in Agricultural Systems* (pp. 174–192). CAB International.

Ha, T. K. T., Maeda, M., Fujiwara, T., Nagare, H., & Akao, S. (2015). Effects of soil type and nitrate concentration on denitrification products (N2O and N2) under flooded conditions in laboratory microcosms. *Soil Science and Plant Nutrition*, *61*(6), 999-1004.

Hammer, D. A., & Bastian, R. K. (2020). Wetlands ecosystems: natural water purifiers? In *Constructed Wetlands for Wastewater Treatment* (1st ed., pp. 5-19). CRC Press.

Han, L., Huang, W., Yuan, X., Zhao, Y., Ma, Z., & Qin, J. (2017). Denitrification potential and influencing factors of the riparian zone soils in different watersheds, Taihu basin. *Water, Air, & Soil Pollution*, *228*(3), 108. https://doi.org/10.1007/s11270-017-3287-7

Handler, A. M., Suchy, A. K., & Grimm, N. B. (2022). Denitrification and DNRA in urban accidental wetlands in Phoenix, Arizona. *Journal of Geophysical Research: Biogeosciences*, *127*(2). https://doi.org/10.1029/2021jg006552

Hanson, G. C., Groffman, P. M., & Gold, A. J. (1994a). Denitrification in riparian wetlands receiving high and low groundwater nitrate inputs. *Journal of Environmental Quality*, *23*(5), 917-922. https://doi.org/10.2134/jeq1994.00472425002300050011x

Hanson, G. C., Groffman, P. M., & Gold, A. J. (1994b). Symptoms of nitrogen saturation in a riparian wetland. *Ecological Applications*, *4*(4), 750-756. https://doi.org/10.2307/1942005

Hao, L., & Huang, J. (2022). Effect of aquaculture reclamation on sediment nitrates reduction processes in mangrove wetland. *Journal of Marine Science and Engineering*, *10*(7), Article 857. https://doi.org/10.3390/jmse10070857

Hayakawa, A., Nakata, M., Jiang, R., Kuramochi, K., & Hatano, R. (2012). Spatial variation of denitrification potential of grassland, windbreak forest, and riparian forest soils in an agricultural catchment in eastern Hokkaido, Japan. *Ecological Engineering*, *47*, 92-100. https://doi.org/https://doi.org/10.1016/j.ecoleng.2012.06.034

Healy, M. G., Barrett, M., Lanigan, G. J., João Serrenho, A., Ibrahim, T. G., Thornton, S. F., Rolfe, S. A., Huang, W. E., & Fenton, O. (2015). Optimizing nitrate removal and evaluating pollution swapping trade-offs from laboratory denitrification bioreactors. *Ecological Engineering*, *74*, 290-301. https://doi.org/https://doi.org/10.1016/j.ecoleng.2014.10.005

Healy, M. G., Ibrahim, T. G., Lanigan, G. J., Serrenho, A. J., & Fenton, O. (2012). Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. *Ecological Engineering*, *40*, 198-209. https://doi.org/10.1016/j.ecoleng.2011.12.010

Hedin, L. O., Von Fischer, J. C., Ostrom, N. E., Kennedy, B. P., Brown, M. G., & Robertson, G. P. (1998). Thermodynamic constraints on nitrogentransformations and other biogeochemicalprocesses at soil–stream interfaces. *Ecology*, *79*(2), 684-703.

Hefting, M., Clément, J. C., Dowrick, D., Cosandey, A. C., Bernal, S., Cimpian, C., Tatur, A., Burt, T. P., & Pinay, G. (2004). Water table elevation controls on soil nitrogen cycling in riparian wetlands along a european climatic gradient. *Biogeochemistry*, *67*(1), 113-134.

Hefting, M. M., Bobbink, R., & de Caluwe, H. (2003). Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones. *Journal of Environmental Quality*, *32*(4), 1194-1203. https://acsess.onlinelibrary.wiley.com/doi/abs/10.2134/jeq2003.1194

Hewitt, A. E. (1993). *Methods and rationale of the New Zealand soil classification*. Manaaki Whenua Press. https://doi.org/doi:10.7931/DL1-LRSS-2

Hill, A. (1990). Ground water flow paths in relation to nitrogen chemistry in the near-stream zone. *Hydrobiologia*, *206*(1), 39-52.

Hill, A. (1991). A ground-water nitrogen budget for a headwater swamp in an area of permanent ground-water discharge. *Biogeochemistry*, *14*(3), 209-224. https://doi.org/10.1007/bf00000808

Hill, A. (1996). Nitrate removal in stream riparian zones. *Journal of Environmental Quality*, *25*(4), 743-755. https://doi.org/10.2134/jeq1996.00472425002500040014x

Hill, A. (2000). Stream chemistry and riparian zones. In J. Jones & P. J. Mulholland (Eds.), *Streams and Ground Waters* (pp. 83-110). Academic Press.

Hill, A. (2019). Groundwater nitrate removal in riparian buffer zones: a review of research progress in the past 20 years. *Biogeochemistry*, *143*(3), 347-369. https://doi.org/10.1007/s10533-019-00566-5

Hill, A., & Cardaci, M. (2004). Denitrification and organic carbon availability in riparian wetland soils and subsurface sediments. *Soil Science Society of America Journal*, *68*(1), 320-325. https://doi.org/10.2136/sssaj2004.0320

Hill, A., Devito, K. J., Campagnolo, S., & Sanmugadas, K. (2000). Subsurface denitrification in a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon. *Biogeochemistry*, *51*(2), 193-223.

Hill, A., Vidon, P. G., & Langat, J. (2004). Denitrification potential in relation to lithology in five headwater riparian zones. *Journal of Environmental Quality*, *33*(3), 911-919.

Hodgson, J., Cameron, K., Clark, D., Condron, L., Fraser, T., Hedley, M., Holmes, C., Kemp, P., Lucas, R., & Moot, D. (2019). New Zealand's pastoral industries: efficient use of grassland resources. In *Grasslands: Developments Opportunities Perspectives* (pp. 181-205). CRC Press.

Hoffmann, C. C., Larsen, S. E., & Kjaergaard, C. (2019). Nitrogen removal in woodchip-based biofilters of variable designs treating agricultural drainage discharges. *Journal of Environmental Quality*, *48*(6), 1881-1889. https://doi.org/10.2134/jeq2018.12.0442

Hooda, P., Moynagh, M., Svoboda, I., Thurlow, M., Stewart, M., Thomson, M., & Anderson, H. (1997). Streamwater nitrate concentrations in six agricultural catchments in Scotland. *Science of the Total Environment*, *201*(1), 63-78.

Hoogendoorn, C., Newton, P., Devantier, B., Rolle, B., Theobald, P., & Lloyd-West, C. (2016). Grazing intensity and micro-topographical effects on some nitrogen and carbon pools and fluxes in sheep-grazed hill country in New Zealand. *Agriculture, Ecosystems & Environment*, *217*, 22-32.

Hoover, N. L., Bhandari, A., Soupir, M. L., & Moorman, T. B. (2016). Woodchip denitrification bioreactors: Impact of temperature and hydraulic retention time on nitrate removal. *Journal of Environmental Quality*, *45*(3), 803-812. https://doi.org/10.2134/jeq2015.03.0161

Horizons Regional Council. (2021). *Regional state of environment summary 2020-21*. https://www.horizons.govt.nz/HRC/media/Media/Regional-Catchment-Summary-2020-21.pdf?ext=.pdf

Horton, R. E. (1933). The role of infiltration in the hydrologic cycle. *Eos, Transactions American Geophysical Union*, *14*(1), 446-460.

Hu, J., Inglett, K. S., Wright, A. L., Clark, M. W., & Reddy, K. R. (2020). Nitrous oxide dynamics during denitrification along a hydrological gradient of subtropical grasslands. *Soil Use and Management*, *36*(4), 682-692.

Hughes, A., McKergow, L., Tanner, C., & Sukias, J. (2013). Influence of livestock grazing on wetland attenuation of diffuse pollutants in agricultural catchments. *National Institute of Water and Atmospheric Research. Hamilton*.

Hughes, A. O., & Quinn, J. M. (2014). Before and after integrated catchment management in a headwater catchment: changes in water quality. *Environmental Management*, *54*(6), 1288-1305. https://doi.org/10.1007/s00267-014-0369-9

Jahangir, M. M. R., Khalil, M. I., Johnston, P., Cardenas, L. M., Hatch, D. J., Butler, M., Barrett, M., O'flaherty, V., & Richards, K. G. (2012). Denitrification potential in subsoils: A mechanism to reduce nitrate leaching to groundwater. *Agriculture, Ecosystems & Environment*, *147*, 13-23. https://doi.org/10.1016/j.agee.2011.04.015

Jahangir, M. M. R., Richards, K. G., Healy, M. G., Gill, L., Müller, C., Johnston, P., & Fenton, O. (2016). Carbon and nitrogen dynamics and greenhouse gas emissions in constructed wetlands treating wastewater: A review. *Hydrology and Earth System Sciences*, *20*(1), 109-123. https://doi.org/10.5194/hess-20-109-2016

Jarvis, S., & Hatch, D. (1994). Potential for denitrification at depth below long-term grass swards. *Soil Biology & Biochemistry*, *26*(12), 1629-1636.

Jaynes, D. B., & Isenhart, T. M. (2014). Reconnecting tile drainage to riparian buffer hydrology for enhanced nitrate removal. *Journal of Environmental Quality*, *43*(2), 631-638.

Jencso, K. G., McGlynn, B. L., Gooseff, M. N., Wondzell, S. M., Bencala, K. E., & Marshall, L. A. (2009). Hydrologic connectivity between landscapes and streams: Transferring reach-and plot-scale understanding to the catchment scale. *Water Resources Research*, *45*(4).

Jiang, L., & Chui, T. F. M. (2022). A review of the application of constructed wetlands (CWs) and their hydraulic, water quality and biological responses to changing hydrological conditions. *Ecological Engineering*, *174*, 106459.

Johnson, P., & Gerbeaux, P. (2004). *Wetland Types in New Zealand*. Department of Conservation.

Jones, H., Clough, P., Hock, B., & Phillips, C. (2008). *Economic costs of hill country erosion and benefits of mitigation in New Zealand: Review and recommendation of approach*. Ministry of Agriculture and Forest.

Jürg, M., & Mosley, M. P. (1998). Hydrological behaviour of pastoral hill country modified by extensive landsliding, northern Hawke's Bay, New Zealand. *Journal of Hydrology (New Zealand)*, *37*(2), 113-139.

Kadlec, R. H. (2005). Nitrogen farming for pollution control. *Journal of Environmental Science and Health*, *40*(6-7), 1307-1330.

Kellman, L. (2004). Nitrate removal in a first-order stream: Reconciling laboratory and field measurements. *Biogeochemistry*, *71*(1), 89-105.

Kemp, P., & Lopez, C. (2016). Hill country pastures in the southern North Island of New Zealand: an overview. *NZGA: Research and Practice Series*, *16*, 289-297.

Knight, C. (2013). Creating a pastoral world through fire: the case of the Manawatu, 1870-1910. *Journal of New Zealand Studies*(16), 100-120.

Knowles, R. (1982). Denitrification. *Microbiological Reviews*, *46*(1), 43-70.

Knowles, R. (1990). Acetylene inhibition technique: development, advantages, and potential problems. In N. P. Revsbech & J. Sorensen (Eds.), *Denitrification in Soil and Sediment* (pp. 151-166). Springer.

Korom, S. F. (1992). Natural denitrification in the saturated zone: a review. *Water Resources Research*, *28*(6), 1657-1668.

Kovacic, D. A., David, M. B., Gentry, L. E., Starks, K. M., & Cooke, R. A. (2000). *Effectiveness of constructed wetlands in reducing nitrogen and phosphorus export from agricultural tile drainage* (0047-2425).

Krauter, P. W. (2001). Using a wetland bioreactor to remediate ground water contaminated with nitrate (mg/L) and perchlorate (μg/L). *International Journal of Phytoremediation*, *3*(4), 415-433. https://doi.org/10.1080/15226510108500068

Kröger, R., Moore, M. T., Farris, J. L., & Gopalan, M. (2011). Evidence for the use of low-grade weirs in drainage ditches to improve nutrient reductions from agriculture. *Water, Air, & Soil Pollution*, *221*, 223-234.

Kröger, R., Pierce, S. C., Littlejohn, K. A., Moore, M. T., & Farris, J. L. (2012). Decreasing nitrate-N loads to coastal ecosystems with innovative drainage management strategies in agricultural landscapes: An experimental approach. *Agricultural Water Management*, *103*, 162-166. https://doi.org/https://doi.org/10.1016/j.agwat.2011.11.009

Lambert, M. G., Devantler, B. P., Nes, P., & Penny, P. E. (1985). Losses of nitrogen, phosphorus, and sediment in runoff from hill country under different fertiliser and grazing management regimes. *New Zealand Journal of Agricultural Research*, *28*(3), 371-379. https://doi.org/10.1080/00288233.1985.10430441

Landcare Research. (2000). *FSL Permeability Profile*. Landcare Research NZ Ltd. https://lris.scinfo.org.nz/layer/48105-fsl-permeability-profile/metadata/

Landcare Research. (2010a). *FSL Soil Drainage Class*. Landcare Research NZ Ltd. https://lris.scinfo.org.nz/layer/48104-fsl-soil-drainage-class/

Landcare Research. (2010b). *NZLRI Rock*. Retrieved 26/1/23 from https://lris.scinfo.org.nz/layer/48065-nzlri-rock/

Landcare Research. (2021). *NZLRI Land Use Capability 2021*. https://lris.scinfo.org.nz/layer/48076-nzlri-land-use-capability-2021/

Ledgard, S. F. (2001). Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil*, *228*, 43-59.

Lepine, C., Christianson, L., Sharrer, K., & Summerfelt, S. (2016). Optimizing hydraulic retention times in denitrifying woodchip bioreactors treating recirculating aquaculture system wastewater. *Journal of Environmental Quality*, *45*(3), 813-821. https://doi.org/10.2134/jeq2015.05.0242

Li, Z., Tang, Z., Song, Z., Chen, W., Tian, D., Tang, S., Wang, X., Wang, J., Liu, W., Wang, Y., Li, J., Jiang, L., Luo, Y., & Niu, S. (2022). Variations and controlling factors of soil denitrification rate. *Global Change Biology*, *28*(6), 2133-2145. https://doi.org/10.1111/gcb.16066

Liu, S., Dai, J., Wei, H., Li, S., Wang, P., Zhu, T., Zhou, J., & Qiu, D. (2021). Dissimilatory nitrate reduction to ammonium (DNRA) and denitrification pathways are leveraged by cyclic AMP receptor protein (CRP) paralogues based on electron donor/acceptor limitation in Shewanella loihica PV-4. *Applied and Environmental Microbiology*, *87*(2), e01964-01920.

Liu, W., Yao, L., Jiang, X., Guo, L., Cheng, X., & Liu, G. (2018). Sediment denitrification in Yangtze lakes is mainly influenced by environmental conditions but not biological communities. *Science of the Total Environment*, *616-617*, 978-987. https://doi.org/10.1016/j.scitotenv.2017.10.221

Lowrance, R., Altier, L. S., Newbold, J. D., Schnabel, R. R., Groffman, P. M., Denver, J. M., Correll, D. L., Gilliam, J. W., & Robinson, J. L. (1997). Water quality functions of riparian forest buffers in Chesapeake Bay watersheds. *Environmental Management*, *21*(5), 687-712.

Luo, J., Tillman, R., & Ball, P. (1999a). Factors regulating denitrification in a soil under pasture. *Soil Biology & Biochemistry*, *31*(6), 913-927.

Luo, J., Tillman, R., & Ball, P. (1999b). Grazing effects on denitrification in a soil under pasture during two contrasting seasons. *Soil Biology & Biochemistry*, *31*(6), 903-912.

Luo, J., Tillman, R. W., & Ball, P. R. (2000). Nitrogen loss through denitrification in a soil under pasture in New Zealand. *Soil Biology & Biochemistry*, *32*(4), 497-509. https://doi.org/10.1016/S0038-0717(99)00179-0

Luo, J., Tillman, R. W., White, R. E., & Ball, P. R. (1998). Variation in denitrification activity with soil depth under pasture. *Soil Biology & Biochemistry*, *30*(7), 897-903. https://doi.org/10.1016/s0038-0717(97)00206-x

Lynn, I., Manderson, A., Page, M., Harmsworth, G., Eyles, G., Douglas, G., Mackay, A., & Newsome, P. (2009). *Land use capability survey handbook: a New Zealand handbook for the classification of land* (3 ed.). AgResearch; Landcare Research New Zealand; Institute of Geological and Nuclear Sciences. https://doi.org/10.7931/DL1MG6

Mackay, A. (2008). Impacts of intensification of pastoral agriculture on soils: Current and emerging challenges and implications for future land uses. *New Zealand Veterinary Journal*, *56*(6), 281-288. https://doi.org/10.1080/00480169.2008.36848

Mander, U., Maddison, M., Soosaar, K., & Karabelnik, K. (2011). The impact of pulsing hydrology and fluctuating water table on greenhouse gas emissions from constructed wetlands. *Wetlands*, *31*(6), 1023-1032. https://doi.org/10.1007/s13157-011-0218-z

Mansfeldt, T. (2004). Redox potential of bulk soil and soil solution concentration of nitrate, manganese, iron, and sulfate in two Gleysols. *Journal of Plant Nutrition and Soil Science*, *167*(1), 7-16.

Martin, L. A., Mulholland, P. J., Webster, J. R., & Valett, H. M. (2001). Denitrification potential in sediments of headwater streams in the southern Appalachian Mountains, USA. *Journal of the North American Benthological Society*, *20*(4), 505-519.

Martínez-Espinosa, C., Sauvage, S., Al Bitar, A., Green, P. A., Vörösmarty, C. J., & Sánchez-Pérez, J. M. (2021). Denitrification in wetlands: A review towards a quantification at global scale. *Science of the Total Environment*, *754*, 142398. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.142398

Matheson, F. E., Nguyen, M., Cooper, A. B., Burt, T. P., & Bull, D. C. (2002). Fate of 15N-nitrate in unplanted, planted and harvested riparian wetland soil microcosms. *Ecological Engineering*, *19*(4), 249-264. https://doi.org/10.1016/S0925-8574(02)00093-9

Mayer, P. M., Reynolds Jr, S. K., McCutchen, M. D., & Canfield, T. J. (2007). Meta-analysis of nitrogen removal in riparian buffers. *Journal of Environmental Quality*, *36*(4), 1172-1180.

McClain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., Hart, S. C., Harvey, J. W., Johnston, C. A., & Mayorga, E. (2003). Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, *6*(4), 301-312.

McColl, R. H. S. (1979). Factors affecting downslope movement of nutrients in hill pasture. *Progress in Water Technology*, *11*(6), 15.

McColl, R. H. S., & Gibson, A. R. (2012). Downslope movement of nutrients in hill pasture, Taita, New Zealand. *New Zealand Journal of Agricultural Research*, *22*(1), 143-150. https://doi.org/10.1080/00288233.1979.10420854

McDonnell, J. J. (1990). A rationale for old water discharge through macropores in a steep, humid catchment. *Water Resources Research*, *26*(11), 2821-2832.

McDowell, R. (2023). The longevity of fencing out livestock as a method of decreasing contaminant concentrations in a headwater stream. *Journal of Environmental Quality*, *52*(1), 173-179. https://doi.org/10.1002/jeq2.20417

McDowell, R., Cox, N., & Snelder, T. (2017). Assessing the yield and load of contaminants with stream order: Would policy requiring livestock to be fenced out of high-order streams decrease catchment contaminant loads? *Journal of Environmental Quality*, *46*(5), 1038-1047. https://doi.org/10.2134/jeq2017.05.0212

McGlynn, B. L., McDonnel, J. J., & Brammer, D. D. (2002). A review of the evolving perceptual model of hillslope flowpaths at the Maimai catchments, New Zealand. *Journal of Hydrology*, *257*(1-4), 1-26.

McGlynn, B. L., & McDonnell, J. J. (2003a). Quantifying the relative contributions of riparian and hillslope zones to catchment runoff. *Water Resources Research*, *39*(11), 1310. https://doi.org/10.1029/2003wr002091

McGlynn, B. L., & McDonnell, J. J. (2003b). Role of discrete landscape units in controlling catchment dissolved organic carbon dynamics. *Water Resources Research*, *39*(4), 1090. https://doi.org/10.1029/2002wr001525

McKergow, L., Matheson, F., & Quinn, J. (2016). Riparian management: A restoration tool for New Zealand streams. *Ecological Management & Restoration*, *17*(3), 218-227. https://doi.org/10.1111/emr.12232

McKergow, L., Rutherford, J., & Timpany, G. (2012). Livestock-generated nitrogen exports from a pastoral wetland. *Journal of Environmental Quality*, *41*(5), 1681-1689. https://doi.org/10.2134/jeq2010.0435

McMahon, P., & Chapelle, F. (2008). Redox processes and water quality of selected principal aquifer systems. *Groundwater*, *46*(2), 259-271.

McPhillips, L. E., Groffman, P. M., Goodale, C. L., & Walter, M. T. (2015). Hydrologic and biogeochemical drivers of riparian denitrification in an agricultural watershed. *Water, Air, & Soil Pollution*, *226*(6). https://doi.org/10.1007/s11270-015-2434-2

Meat Industry Association. (2021, 30 Sep 2021). *Soaring demand for beef drives 26 per cent increase in New Zealand red meat exports in August*. Meat Industry Association (MIA). Retrieved 13/04/2022 from https://mia.co.nz/news-and-views/soaring-demand-for-beef-drives-26-per-cent-increase-in-new-zealand-red-meat-exports-in-august/

Mendes, L. R. D. (2021). Nitrogen removal from agricultural subsurface drainage by surface-flow wetlands: Variability. *Processes*, *9*(1), 156. https://doi.org/10.3390/pr9010156

MfE. (2008). *Soil erosion*. Ministry for the Environment.

MfE. (2022a). *LUCAS NZ Land Use Map 1990 2008 2012 2016 v011*. Ministry for the Environment. Retrieved 26/01/23 from https://data.mfe.govt.nz/layer/52375-lucas-nz-land-use-map-1990-2008-2012-2016-v011/

MfE. (2022b). *National Policy Statement for Freshwater Management 2020*. https://environment.govt.nz/publications/national-policy-statement-for-freshwater-management-2020-amended-december-2022/

MfE, & MPI. (2018). *Essential Freshwater: Healthy Water, Fairly Allocated.* Ministry for the Environment and Ministry for Primary Industries.

MfE, & MPI. (2020). Action for healthy waterways: Information on attributes for managing the ecosystem health and human contact values in the National Policy

Statement for Freshwater (2020). In: Ministry for the Environment and Ministry for Primary Industries.

MfE, & Stats NZ. (2021). *New Zealand's Environmental Reporting Series: Our land 2021*. https://environment.govt.nz/publications/our-land-2021/

Milne, J., Clayden, B., Singleton, P., & Wilson, A. (1995). *Soil Description Handbook* (Revised 1st ed.). Manaaki Whenua Press.

Misiti, T. M., Hajaya, M. G., & Pavlostathis, S. G. (2011). Nitrate reduction in a simulated free-water surface wetland system. *Water Research*, *45*(17), 5587-5598. https://doi.org/10.1016/j.watres.2011.08.019

Monaghan, R. M., Hedley, M. J., Di, H. J., McDowell, R. W., Cameron, K. C., & Ledgard, S. F. (2007). Nutrient management in New Zealand pastures—recent developments and future issues. *New Zealand Journal of Agricultural Research*, *50*(2), 181-201.

Moon, J. B., Wardrop, D. H., Fennessy, M. S., Ingram, H. M., Britson, A., & Okoro, M. H. (2020). Variation in surface and subsurface nitrogen cycling in headwater floodplain wetlands due to soil type and wetland condition. *Wetlands Ecology and Management*, *28*(5), 727-751.

Murphy, J. C., Hirsch, R. M., & Sprague, L. A. (2014). Antecedent flow conditions and nitrate concentrations in the Mississippi River basin. *Hydrology and Earth System Sciences*, *18*(3), 967-979.

Mwagona, P. C., Yao, Y., Yuanqi, S., & Yu, H. (2019a). Greenhouse gas emissions from intact riparian wetland soil columns continuously loaded with nitrate solution: a laboratory microcosm study. *Environmental Science and Pollution Research*, *26*(32), 33702-33714. https://doi.org/10.1007/s11356-019-06406-1

Mwagona, P. C., Yao, Y., Yuanqi, S., & Yu, H. (2019b). Laboratory study on nitrate removal and nitrous oxide emission in intact soil columns collected from nitrogenous loaded riparian wetland, Northeast China. *PLoS ONE*, *14*(3). https://doi.org/10.1371/journal.pone.0214456

Myrold, D. D., & Tiedje, J. M. (1985). Diffusional constraints on denitrification in soil. *Soil Science Society of America Journal*, *49*(3), 651-657.

Nazarpoor, R., Farasati, M., Fathaabadi, H., & Gholizadeh, M. (2021). Nitrate removal from water by using cyperus alternifolius plants in surface flow constructed wetlands. *Desalination and Water Treatment*, *224*, 197-205. https://doi.org/10.5004/dwt.2021.27162

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis: Part 3 Chemical Methods* (Vol. 5, pp. 961-1010).

New Zealand Government. (2012). *Building Innovation* (ISBN 978-0-478-40106-6). (The Business Growth Agenda Progress Reports, Issue.

https://img.scoop.co.nz/media/pdfs/1208/BGAprogressreportbuildinginnovationaugust2012.pdf

Nguyen, M., & Downes, M. (1997). Riparian wetlands as buffer zones against nutrient contamination in receiving waters: nitrogen, phosphorus and sulphur biogeochemistry in riparian soils. UNEP, IETC conference on Environmental Technologies for Wastewater Management, Murdoch University, Perth, Western Australia.

Nguyen, M., Downes, M., Mehlhorn, J., & Stroud, M. (1999a). Riparian wetland processing of nitrogen, phosphorus and suspended sediment inputs from a hill country sheep-grazed catchment in New Zealand. Proceedings of the Second Australian Stream Management Conference, Adeleide.

Nguyen, M., Rutherford, J., & Burns, D. (1999b). Denitrification and nitrate removal in two contrasting riparian wetlands. Modelling of land treatment systems, proceedings of the 20th New Zealand Land Treatment Collective Technical Session, New Plymouth, New Zealand, 14-15 October 1999.

NIWA. (2022). *The National Climate Database-database query*. National Institute of Water and Atmospheric Research. https://cliflo.niwa.co.nz/pls/niwp/wgenf.genform1

NIWA. (2023). *The National Climate Database-database query*. National Institute of Water and Atmospheric Research. https://cliflo.niwa.co.nz/pls/niwp/wgenf.genform1

Ocampo, C. J., Oldham, C. E., & Sivapalan, M. (2006). Nitrate attenuation in agricultural catchments: Shifting balances between transport and reaction. *Water Resources Research*, *42*(1). https://doi.org/10.1029/2004wr003773

Pandey, C. B., Kumar, U., Kaviraj, M., Minick, K. J., Mishra, A. K., & Singh, J. S. (2020). DNRA: A short-circuit in biological N-cycling to conserve nitrogen in terrestrial ecosystems. *Science of the Total Environment*, *738*, 139710. https://www.sciencedirect.com/science/article/pii/S0048969720332307

Parfitt, R. L., MacKay, A. D., Ross, D. J., & Budding, P. J. (2009). Effects of soil fertility on leaching losses of N, P and C in hill country. *New Zealand Journal of Agricultural Research*, *52*(1), 69-80. https://doi.org/10.1080/00288230909510490

Parfitt, R. L., Schipper, L. A., Baisden, W. T., & Elliott, A. H. (2006). Nitrogen inputs and outputs for New Zealand in 2001 at national and regional scales. *Biogeochemistry*, *80*(1), 71-88. https://doi.org/10.1007/s10533-006-0002-y

Parfitt, R. L., Stevenson, B. A., Dymond, J. R., Schipper, L. A., Baisden, W. T., & Ballantine, D. J. (2012). Nitrogen inputs and outputs for New Zealand from 1990 to 2010 at national and regional scales. *New Zealand Journal of Agricultural Research*, *55*(3), 241-262. https://doi.org/10.1080/00288233.2012.676991

Park, J.-H., Duan, L., Kim, B., Mitchell, M. J., & Shibata, H. (2010). Potential effects of climate change and variability on watershed biogeochemical processes and water quality in Northeast Asia. *Environment International*, *36*(2), 212-225.

Parkin, T. B., Sexstone, A. J., & Tiedje, J. M. (1985). Adaptation of denitrifying populations to low soil pH. *Applied and Environmental Microbiology*, *49*(5), 1053-1056.

Peters, N. E., Böhlke, J. K., Brooks, P. D., Burt, T. P., Gooseff, M. N., Hamilton, D. P., Mulholland, P. J., Roulet, N. T., & Turner, J. V. (2011). Hydrology and biogeochemistry linkages. In P. Wilderer (Ed.), *Treatise on Water Science* (pp. 271-304). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-444-53199-5.00038-5

Peterson, B. J., Wollheim, W. M., Mulholland, P. J., Webster, J. R., Meyer, J. L., Tank, J. L., Martí, E., Bowden, W. B., Valett, H. M., & Hershey, A. E. (2001). Control of nitrogen export from watersheds by headwater streams. *Science*, *292*(5514), 86-90.

Peterson, M. A. (2016). *The effect of the antecedent dry conditions on nitrogen removal for a modified bioretention system* [Doctoral, University of South Florida].

Peterson, M. E., Curtin, D., Thomas, S., Clough, T. J., & Meenken, E. D. (2013). Denitrification in vadose zone material amended with dissolved organic matter from topsoil and subsoil. *Soil Biology & Biochemistry*, *61*, 96-104. https://doi.org/10.1016/j.soilbio.2013.02.010

Petry, J., Soulsby, C., Malcolm, I. A., & Youngson, A. F. (2002). Hydrological controls on nutrient concentrations and fluxes in agricultural catchments. *Science of the Total Environment*, *294*(1), 95-110. https://doi.org/https://doi.org/10.1016/S0048-9697(02)00058-X

Pfenning, K. S., & McMahon, P. B. (1997). Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. *Journal of Hydrology*, *187*(3-4), 283-295. https://doi.org/10.1016/s0022-1694(96)03052-1

Poe, A. C., Piehler, M. F., Thompson, S. P., & Paerl, H. W. (2003). Denitrification in a constructed wetland receiving agricultural runoff. *Wetlands*, *23*(4), 817-826.

Pollok, J., & McLaughlin, B. (1986). A user-friendly guide to the soils of Tuapaka Farm, vol Publication No. 3. *Massey University Tuapaka Farm Series, Palmerston North*.

Puckett, L. (2004). Hydrogeologic controls on the transport and fate of nitrate in ground water beneath riparian buffer zones: results from thirteen studies across the United States. *Water Science and Technology*, *49*(3), 47-53.

Qian, J. Z., Wang, X. X., Ma, L., Wang, L. P., Liu, J. K., & Yang, Z. X. (2018). Simulation of denitrification in groundwater from Chaohu Lake Catchment, China. *Water Science and Engineering*, *11*(2), 114-119. https://doi.org/10.1016/j.wse.2018.07.004

Quinn, J., & Stroud, M. (2002). Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use. *New Zealand Journal of Marine and Freshwater Research*, *36*(2), 409-429. https://doi.org/10.1080/00288330.2002.9517097

Ramesh, R., Anderson, C. J., & Kalin, L. (2020). Characterizing nitrogen attenuation by headwater slope wetlands across different land uses. *Ecological Engineering*, *149*. https://doi.org/10.1016/j.ecoleng.2020.105833

Ranalli, A. J., & Macalady, D. L. (2010). The importance of the riparian zone and in-stream processes in nitrate attenuation in undisturbed and agricultural watersheds–A review of the scientific literature. *Journal of Hydrology*, *389*(3-4), 406-415.

Reddy, K. R., & DeLaune, R. D. (2008). *Biogeochemistry of wetlands: Science and applications*. CRC Press.

Reed, S. C., Crites, R. W., & Middlebrooks, E. J. (1995). *Natural Systems for Waste Management and Treatment*. McGraw-Hill, Inc.

Ribas, D., Calderer, M., Marti, V., Johnsen, A. R., Aamand, J., Nilsson, B., Jensen, J. K., Engesgaard, P., & Morici, C. (2017). Subsurface nitrate reduction under wetlands takes place in narrow superficial zones. *Environmental Technology*, *38*(21), 2725-2732. https://www.ncbi.nlm.nih.gov/pubmed/28004595

Rivas, A. A. (2018). *Characterisation of denitrification in the subsurface environment of the Manawatū catchment, New Zealand* [Doctoral, Massey University].

Rivett, M. O., Buss, S. R., Morgan, P., Smith, J. W., & Bemment, C. D. (2008). Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. *Water Research*, *42*(16), 4215-4232. https://doi.org/10.1016/j.watres.2008.07.020

Robins, J. P., Rock, J., Hayes, D. F., & Laquer, F. C. (2000). Nitrate removal for platte valley, nebraska synthetic groundwater using a constructed wetland model. *Environmental Technology (United Kingdom)*, *21*(6), 653-659. https://doi.org/10.1080/09593332108618076

Rogers, K. M., van der Raaij, R., Phillips, A., & Stewart, M. (2023). A national isotope survey to define the sources of nitrate contamination in New Zealand freshwaters. *Journal of Hydrology*, *617*, 129131. https://doi.org/https://doi.org/10.1016/j.jhydrol.2023.129131

Rubol, S., Silver, W. L., & Bellin, A. (2012). Hydrologic control on redox and nitrogen dynamics in a peatland soil. *Science of the Total Environment*, *432*, 37-46. https://doi.org/10.1016/j.scitotenv.2012.05.073

Rutherford, J., McKergow, L., Hughes, A., & Matheson, F. (2018). Natural seepage wetlands: can they reduce nitrogen losses? *DairyNZ Technical Series: Science in Action*, *39*, 10-13.

Rutherford, J., & Nguyen, M. (2004). Nitrate removal in riparian wetlands: interactions between surface flow and soils. *Journal of Environmental Quality*, *33*(3), 1133-1143.

Rutherford, J., Schroer, D., & Timpany, G. (2009). How much runoff do riparian wetlands affect? *New Zealand Journal of Marine and Freshwater Research*, *43*(5), 1079-1094.

Rütting, T., Boeckx, P., Müller, C., & Klemedtsson, L. (2011). Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences*, *8*(7), 1779-1791.

Rütting, T., Clough, T. J., Mueller, C., Lieffering, M., & Newton, P. C. (2010). Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture. *Global Change Biology*, *16*(9), 2530-2542.

Ruz-Jerez, B. E., White, R. E., & Ball, P. R. (1994). Long-term measurement of denitrification in 3 contrasting pastures grazed by sheep. *Soil Biology & Biochemistry*, *26*(1), 29-39. https://doi.org/10.1016/0038-0717(94)90192-9

Saggar, S., Jha, N., Deslippe, J., Bolan, N., Luo, J., Giltrap, D., Kim, D.-G., Zaman, M., & Tillman, R. (2013). Denitrification and N2O:N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of the Total Environment*, *465*, 173-195.

Saggar, S., Mackay, A. D., Hedley, M. J., Lambert, M. G., & Clark, D. A. (1990). A nutrient-transfer model to explain the fate of phosphorus and sulphur in a grazed hill-country pasture. *Agriculture, Ecosystems & Environment*, *30*(3-4), 295-315. https://doi.org/10.1016/0167-8809(90)90112-Q

Sahrawat, K. L. (2004). Organic matter accumulation in submerged soils. *Advances in Agronomy*, *81*, 169-201.

Sarris, T. S., Close, M. E., & Moore, C. (2019). Uncertainty assessment of nitrate reduction in heterogeneous aquifers under uncertain redox conditions. *Stochastic Environmental Research and Risk Assessment*, *33*(8-9), 1609-1627. https://doi.org/10.1007/s00477-019-01715-w

Sartoris, J. J., Thullen, J. S., Barber, L. B., & Salas, D. E. (2000). Investigation of nitrogen transformations in a southern California constructed wastewater treatment wetland. *Ecological Engineering*, *14*(1-2), 49-65.

Saunders, D., & Kalff, J. (2001). Nitrogen retention in wetlands, lakes and rivers. *Hydrobiologia*, *443*(1), 205-212.

Schaafsma, J. A., Baldwin, A. H., & Streb, C. A. (1999). An evaluation of a constructed wetland to treat wastewater from a dairy farm in Maryland, USA. *Ecological Engineering*, *14*(1-2), 199-206.

Schipper, L., Cooper, A., Harfoot, C., & Dyck, W. (1993). Regulators of denitrification in an organic riparian soil. *Soil Biology & Biochemistry*, *25*(7), 925-933.

Schipper, L. A., Robertson, W. D., Gold, A. J., Jaynes, D. B., & Cameron, S. C. (2010). Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. *Ecological Engineering*, *36*(11), 1532-1543.

Scholefield, D., Hawkins, J. M. B., & Jackson, S. M. (1997). Use of a flowing helium atmosphere incubation technique to measure the effects of denitrification controls applied to intact cores of a clay soil. *Soil Biology & Biochemistry*, *29*(9-10), 1337-1344. https://doi.org/10.1016/s0038-0717(97)00059-x

Seitzinger, S., Harrison, J. A., Böhlke, J. K., Bouwman, A. F., Lowrance, R., Peterson, B., Tobias, C., & Van Drecht, G. (2006). Denitrification across landscapes and waterscapes: A synthesis. *Ecological Applications*, *16*(6), 2064-2090.

Shabaga, J. A., & Hill, A. R. (2010). Groundwater-fed surface flow path hydrodynamics and nitrate removal in three riparian zones in southern Ontario, Canada. *Journal of Hydrology*, *388*(1-2), 52-64. https://doi.org/10.1016/j.jhydrol.2010.04.028

Šimek, M., & Cooper, J. E. (2002). The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, *53*(3), 345-354. https://dx.doi.org/10.1046/j.1365-2389.2002.00461.x

Sirivedhin, T., & Gray, K. A. (2006). Factors affecting denitrification rates in experimental wetlands: Field and laboratory studies. *Ecological Engineering*, *26*(2), 167-181. https://doi.org/10.1016/j.ecoleng.2005.09.001

Sklash, M., Stewart, M., & Pearce, A. (1986). Storm runoff generation in humid headwater catchments: 2. A case study of hillslope and low-order stream response. *Water Resources Research*, *22*(8), 1273-1282.

Smethurst, P. J., Petrone, K. C., Langergraber, G., Baillie, C. C., Worledge, D., & Nash, D. (2014). Nitrate dynamics in a rural headwater catchment: measurements and modelling. *Hydrological Processes*, *28*(4), 1820-1834. https://doi.org/10.1002/hyp.9709

Smith, C. M. (1989). Riparian pasture retirement effects on sediment, phosphorus, and nitrogen in channellised surface run-off from pastures. *New Zealand Journal of Marine and Freshwater Research*, *23*(1), 139-146.

Smith, M. J., Cooper, A. B., & Quinn, J. M. (1993). *Land-water interactions research at whatawhata: the start* (Vol. 4). NIWA-Ecosystems.

Smith, R. L., & Duff, J. H. (1988). Denitrification in a sand and gravel aquifer. *Applied and Environmental Microbiology*, *54*(5), 1071-1078.

Song, X., Wang, S., Wang, Y., Zhao, Z., & Yan, D. (2016). Addition of Fe2+ increase nitrate removal in vertical subsurface flow constructed wetlands [Article]. *Ecological Engineering*, *91*, 487-494. https://doi.org/10.1016/j.ecoleng.2016.03.013

Sorrell, B., & Gerbeaux, P. (2004). Wetland ecosystems. In J. S. Harding, P. Mosley, C. Pearson, & B. Sorrell (Eds.), *Freshwater of New Zealand* (pp. 28.21-28.15). New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc.

Stålnacke, P., Aakerøy, P. A., Blicher-Mathiesen, G., Iital, A., Jansons, V., Koskiaho, J., Kyllmar, K., Lagzdins, A., Pengerud, A., & Povilaitis, A. (2014). Temporal trends in nitrogen concentrations and losses from agricultural catchments in the Nordic and Baltic countries. *Agriculture, Ecosystems & Environment*, *198*, 94-103. https://doi.org/https://doi.org/10.1016/j.agee.2014.03.028

Stats NZ. (2021). *Livestock numbers*. Retrieved 29/01/2023 from https://www.stats.govt.nz/indicators/livestock-numbers

Stats NZ. (2022). *River water quality: nitrogen*. Retrieved 06/03/2023 from https://www.stats.govt.nz/indicators/river-water-quality-nitrogen#:~:text=Measured%20data%20for%2020%2Dyear,and%208%20percent%20were%20indeterminate

Steele, K. W., Bonish, P. M., & Sarathchandra, S. U. (1984). Denitrification potentials and microbiological characteristics of some northern North Island soils. *New Zealand Journal of Agricultural Research*, *27*(4), 525-530. https://doi.org/10.1080/00288233.1984.10418014

Steiakakis, E., Gamvroudis, C., & Alevizos, G. (2012). Kozeny-carman equation and hydraulic conductivity of compacted clayey soils. *Geomaterials*, *02*(02), 37-41. https://doi.org/10.4236/gm.2012.22006

Stenger, R., Barkle, G., Burgess, C., Wall, A., & Clague, J. (2008). Low nitrate contamination of shallow groundwater in spite of intensive dairying: the effect of reducing conditions in the vadose zone—aquifer continuum. *Journal of Hydrology (New Zealand)*, 1-24.

Stenger, R., Clague, J. C., Morgenstern, U., & Clough, T. J. (2018). Vertical stratification of redox conditions, denitrification and recharge in shallow groundwater on a volcanic hillslope containing relict organic matter. *Science of the Total Environment*, *639*, 1205-1219. https://doi.org/10.1016/j.scitotenv.2018.05.122

Surey, R., Lippold, E., Heilek, S., Sauheitl, L., Henjes, S., Horn, M. A., Mueller, C. W., Merbach, I., Kaiser, K., Böttcher, J., & Mikutta, R. (2020). Differences in labile soil organic matter explain potential denitrification and denitrifying communities in a long-term fertilization experiment. *Applied Soil Ecology*, *153*. https://doi.org/10.1016/j.apsoil.2020.103630

Tanner, C., Nguyen, M., & Sukias, J. (2005). Nutrient removal by a constructed wetland treating subsurface drainage from grazed dairy pasture. *Agriculture, Ecosystems & Environment*, *105*(1-2), 145-162. https://doi.org/10.1016/j.agee.2004.05.008

Thayalakumaran, T., Bristow, K. L., Charlesworth, P. B., & Fass, T. (2008). Geochemical conditions in groundwater systems: Implications for the attenuation of agricultural nitrate. *Agricultural Water Management*, *95*(2), 103-115. https://doi.org/https://doi.org/10.1016/j.agwat.2007.09.003

Tiedje, J. M. (1983). Denitrification. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties* (Vol. 9, pp. 1011-1026).

Tiedje, J. M. (1988). Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *Biology of anaerobic microorganisms*, 179-244.

Tiedje, J. M., Simkins, S., & Groffman, P. M. (1989). Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. *Plant and Soil*, *115*(2), 261-284. https://doi.org/10.1007/bf02202594

Tomasek, A. A., Hondzo, M., Kozarek, J. L., Staley, C., Wang, P., Lurndahl, N., & Sadowsky, M. J. (2019). Intermittent flooding of organic-rich soil promotes the formation of denitrification hot moments and hot spots. *Ecosphere*, *10*(1), Article e02549. https://doi.org/10.1002/ecs2.2549

Trepel, M., & Palmeri, L. (2002). Quantifying nitrogen retention in surface flow wetlands for environmental planning at the landscape-scale. *Ecological Engineering*, *19*(2), 127-140.

Uuemaa, E., Palliser, C., Hughes, A., & Tanner, C. (2018). Effectiveness of a natural headwater wetland for reducing agricultural nitrogen loads. *Water*, *10*(3). https://doi.org/10.3390/w10030287

Valkama, E., Usva, K., Saarinen, M., & Uusi-Kämppä, J. (2019). A meta-analysis on nitrogen retention by buffer zones. *Journal of Environmental Quality*, *48*(2), 270-279. https://doi.org/10.2134/jeq2018.03.0120

Van Cleemput, O., Boeckx, P., Lindgren, P.-E., & Tonderski, K. (2007). Denitrification in wetlands. In *Biology of the Nitrogen Cycle* (pp. 359-367). Elsevier.

van den Heuvel, R. N., Hefting, M. M., Tan, N. C., Jetten, M. S., & Verhoeven, J. T. (2009). N2O emission hotspots at different spatial scales and governing factors for small scale hotspots. *Science of the Total Environment*, *407*(7), 2325-2332. https://doi.org/10.1016/j.scitotenv.2008.11.010

Veraart, A. J., Dimitrov, M. R., Schrier-Uijl, A. P., Smidt, H., & de Klein, J. J. M. (2017). Abundance, activity and community structure of denitrifiers in drainage ditches in relation to sediment characteristics, vegetation and land-use. *Ecosystems*, *20*(5), 928-943.

Verhoeven, J. T., Arheimer, B., Yin, C., & Hefting, M. M. (2006). Regional and global concerns over wetlands and water quality. *Trends in Ecology and Evolution*, *21*(2), 96-103. https://www.ncbi.nlm.nih.gov/pubmed/16701481

Vidon, P., Allan, C., Burns, D., Duval, T. P., Gurwick, N., Inamdar, S., Lowrance, R., Okay, J., Scott, D., & Sebestyen, S. (2010). Hot spots and hot moments in riparian zones: potential for improved water quality management. *JAWRA Journal of the American Water Resources Association*, *46*(2), 278-298.

Vidon, P., & Hill, A. R. (2004a). Denitrification and patterns of electron donors and acceptors in eight riparian zones with contrasting hydrogeology. *Biogeochemistry*, *71*(2), 259-283.

Vidon, P., & Hill, A. R. (2004b). Landscape controls on nitrate removal in stream riparian zones. *Water Resources Research*, *40*(3).

Vidon, P., & Smith, A. P. (2007). Upland controls on the hydrological functioning of riparian zones in glacial till valleys of the Midwest. *JAWRA Journal of the American Water Resources Association*, *43*(6), 1524-1539. https://doi.org/10.1111/j.1752-1688.2007.00125.x

Vymazal, J. (2022). The historical development of constructed wetlands for wastewater treatment. *Land*, *11*(2), 174. https://doi.org/10.3390/land11020174

Wall, L. G., Tank, J. L., Royer, T. V., & Bernot, M. J. (2005). Spatial and temporal variability in sediment denitrification within an agriculturally influenced reservoir. *Biogeochemistry*, *76*(1), 85-111. https://doi.org/10.1007/s10533-005-2199-6

Wallenstein, M. D., Myrold, D. D., Firestone, M., & Voytek, M. (2006). Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecological Applications*, *16*(6), 2143-2152.

Walton, C. R., Zak, D., Audet, J., Petersen, R. J., Lange, J., Oehmke, C., Wichtmann, W., Kreyling, J., Grygoruk, M., Jablonska, E., Kotowski, W., Wisniewska, M. M., Ziegler, R., & Hoffmann, C. C. (2020). Wetland buffer zones for nitrogen and phosphorus retention: Impacts of soil type, hydrology and vegetation. *Science of the Total Environment*, *727*, 138709. https://doi.org/10.1016/j.scitotenv.2020.138709

Wang, C., Amon, B., Schulz, K., & Mehdi, B. (2021). Factors that influence nitrous oxide emissions from agricultural soils as well as their representation in simulation models: A review. *Agronomy*, *11*(4), 770. https://doi.org/10.3390/agronomy11040770

Wang, H., Li, Y., Zhang, S., Li, D., Liu, X., Wang, W., Liu, L., Wang, Y., & Kang, L. (2020). Effect of influent feeding pattern on municipal tailwater treatment during a sulfur-based denitrification constructed wetland. *Bioresource Technology*, *315*, Article 123807. https://doi.org/10.1016/j.biortech.2020.123807

Wang, X., Tamiev, D., Alagurajan, J., Dispirito, A. A., Phillips, G. J., & Hargrove, M. S. (2019a). The role of the NADH-dependent nitrite reductase, Nir, from Escherichia coli in fermentative ammonification. *Archives of Microbiology*, *201*(4), 519-530. https://doi.org/10.1007/s00203-018-1590-3

Wang, Y., Ji, H., Wang, R., & Guo, S. (2019b). Responses of nitrification and denitrification to nitrogen and phosphorus fertilization: does the intrinsic soil fertility matter? *Plant and Soil*, *440*(1-2), 443-456.

Ward, J. C., Lambie, J., & Clarkson, B. D. (1999). *Monitoring Changes in Wetland Extent: An Environmental Performance Indicator for Wetlands*. Lincoln Environmental.

Warneke, S., Schipper, L. A., Bruesewitz, D. A., McDonald, I., & Cameron, S. (2011). Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. *Ecological Engineering*, *37*(3), 511-522. https://doi.org/10.1016/j.ecoleng.2010.12.006

Warwick, J., & Hill, A. R. (1988). Nitrate depletion in the riparian zone of a small woodland stream. *Hydrobiologia*, *157*(3), 231-240.

Wei, H., Gao, D., Liu, Y., & Lin, X. (2020). Sediment nitrate reduction processes in response to environmental gradients along an urban river-estuary-sea continuum. *Science of the Total Environment*, *718*, 137185. https://www.sciencedirect.com/science/article/pii/S0048969720306951

Weier, K. L., Doran, J. W., Power, J. F., & Walters, D. T. (1993). Denitrification and the dinitrogen nitrous-oxide ratio as affected by soil-water, available carbon, and nitrate. *Soil Science Society of America Journal*, *57*(1), 66-72. https://doi.org/10.2136/sssaj1993.03615995005700010013x

Wilcock, R. J., Müller, K., Van Assema, G. B., Bellingham, M. A., & Ovenden, R. (2012). Attenuation of nitrogen, phosphorus and E. coli inputs from pasture runoff to surface waters by a farm wetland: The importance of wetland shape and residence time. *Water, Air, & Soil Pollution*, *223*(2), 499-509. https://doi.org/10.1007/s11270-011-0876-8

Wilde, F., Radke, D., Gibs, J., & Iwatsubo, R. (1998). *Handbooks for Water-Resources Investigations: National Field Manual for the Collection of Water-Quality Data. Field Measurements* (Vol. 9). US Department of the Interior and US Geological Survey.

Willems, H. P., Rotelli, M. D., Berry, D. F., Smith, E. P., Reneau Jr, R. B., & Mostaghimi, S. (1997). Nitrate removal in riparian wetland soils: effects of flow rate, temperature, nitrate concentration and soil depth. *Water Research*, *31*(4), 841-849.

Wilson, S., Close, M., & Abraham, P. (2016). *Predicting Groundwater Redox Status in the Southland Region* (0169-7722).

Wilson, S., Close, M. E., & Abraham, P. (2018). Applying linear discriminant analysis to predict groundwater redox conditions conducive to denitrification. *Journal of Hydrology*, *556*, 611-624. https://doi.org/https://doi.org/10.1016/j.jhydrol.2017.11.045

Winter, T. C. (1983). The interaction of lakes with variably saturated porous media. *Water Resources Research*, *19*(5), 1203-1218.

Winter, T. C. (1999). Relation of streams, lakes, and wetlands to groundwater flow systems. *Hydrogeology Journal*, *7*, 28-45. https://doi.org/10.1007/s100400050178

Woltemade, C. J., & Woodward, J. (2008). Nitrate removal in a restored spring-fed wetland, Pennsylvania, USA. *Journal of the American Water Resources Association*, *44*(1), 222-234. https://doi.org/10.1111/j.1752-1688.2007.00149.x

Wu, X., Probst, A., Barret, M., Payre-Suc, V., Camboulive, T., & Granouillac, F. (2021). Spatial variation of denitrification and key controlling factors in streams and ponds sediments from a critical zone (southwestern France). *Applied Geochemistry*, *131*, 105009. https://doi.org/https://doi.org/10.1016/j.apgeochem.2021.105009

Xiong, Z., Guo, L., Zhang, Q., Liu, G., & Liu, W. (2017). Edaphic conditions regulate denitrification directly and indirectly by altering denitrifier abundance in wetlands along the Han River, China. *Environmntal Science & Technology*, *51*(10), 5483-5491. https://doi.org/10.1021/acs.est.6b06521

Xiong, Z., Li, S., Yao, L., Liu, G., Zhang, Q., & Liu, W. (2015). Topography and land use effects on spatial variability of soil denitrification and related soil properties in riparian wetlands. *Ecological Engineering*, *83*, 437-443. https://doi.org/10.1016/j.ecoleng.2015.04.094

Xu, B. K., Shi, L. S., Zhong, H., & Wang, K. (2021). Investigation of Fe(II) and Mn(II) involved anoxic denitrification in agricultural soils with high manganese and iron contents. *Journal of Soils and Sediments*, *21*(1), 452-468. https://doi.org/10.1007/s11368-020-02776-z

Xu, J. H., He, S. B., Wu, S. Q., Huang, J. C., Zhou, W. L., & Chen, X. C. (2016). Effects of HRT and water temperature on nitrogen removal in autotrophic gravel filter. *Chemosphere*, *147*, 203-209. https://doi.org/10.1016/j.chemosphere.2015.12.136

Xue, Y., David, M. B., Gentry, L. E., Mulvaney, R. L., Kovacic, D. A., & Lindau, C. W. (1999). In situ measurements of denitrification in constructed wetlands. *Journal of Environmental Quality*, *28*(1), 263-269. https://doi.org/10.2134/jeq1999.00472425002800010032x

Ye, C., Cheng, X., Zhang, K., Du, M., & Zhang, Q. (2017). Hydrologic pulsing affects denitrification rates and denitrifier communities in a revegetated riparian ecotone. *Soil Biology & Biochemistry*, *115*, 137-147. https://doi.org/10.1016/j.soilbio.2017.08.018

Yi, J., Hu, W., Beare, M., Liu, J., Cichota, R., Teixeira, E., & Guo, L. (2022). Treading compaction during winter grazing can increase subsequent nitrate leaching by enhancing drainage. *Soil and Tillage Research*, *221*, 105424. https://doi.org/https://doi.org/10.1016/j.still.2022.105424

Yin, S., Shen, Q., Tang, Y., & Cheng, L. (1998). Reduction of nitrate to ammonium in selected paddy soils of China. *Pedosphere*, *8*(3), 221-228.

Yoshinari, T., Hynes, R., & Knowles, R. (1977). Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. *Soil Biology & Biochemistry*, *9*(3), 177-183.

Yu, S., & Ehrenfeld, J. G. (2009). The effects of changes in soil moisture on nitrogen cycling in acid wetland types of the New Jersey Pinelands (USA). *Soil Biology & Biochemistry*, *41*(12), 2394-2405.

Zaman, M., Nguyen, M., Gold, A. J., Groffman, P. M., Kellogg, D. Q., & Wilcock, R. J. (2008). Nitrous oxide generation, denitrification, and nitrate removal in a seepage wetland intercepting surface and subsurface flows from a grazed dairy catchment. *Soil Research*, *46*(7). https://doi.org/10.1071/sr07217

Zaman, M., Saggar, S., Blennerhassett, J. D., & Singh, J. (2009). Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. *Soil Biology & Biochemistry*, *41*(6), 1270-1280. https://doi.org/10.1016/j.soilbio.2009.03.011

Zarnetske, J. P., Haggerty, R., Wondzell, S. M., & Baker, M. A. (2011a). Dynamics of nitrate production and removal as a function of residence time in the hyporheic zone. *Journal of Geophysical Research*, *116*(G1). https://doi.org/10.1029/2010jg001356

Zarnetske, J. P., Haggerty, R., Wondzell, S. M., & Baker, M. A. (2011b). Labile dissolved organic carbon supply limits hyporheic denitrification. *Journal of Geophysical Research: Biogeosciences*, *116*(G4).

Zhang, M., Huang, J.-C., Sun, S., Rehman, M. M. U., He, S., & Zhou, W. (2021). Nitrogen removal through collaborative microbial pathways in tidal flow constructed wetlands. *Science of the Total Environment*, *758*, 143594. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.143594

Zhang, Q., Luo, S., Zhao, L., Zhang, P., Wang, S., Sun, C., & Zhang, L. (2020). Effect of Darcy flux on the release of dissolved organic matter and nitrogen from coal gangue in a coal mine underground reservoir: Column experiments. *Chemical Geology*, *545*, Article 119652. https://doi.org/10.1016/j.chemgeo.2020.119652

Zhao, H., Xu, X., Ke, F., Li, W., Feng, M., & Zhang, H. (2013). Nitrogen removal from wastewater plant secondary effluent in a compound natural treatment system. *Ecological Engineering*, *57*, 361-365. https://doi.org/10.1016/j.ecoleng.2013.04.026

Zhao, Z., Zhang, Y., Yan, F., Yuan, C., & Zhao, P. (2011). Influence of HRT and temperature on treatment of micro-polluted river water by using integrated horizontal-flow constructed wetland. 2011 2nd International Conference on Mechanic Automation and Control Engineering, MACE 2011 - Proceedings,

Zhong, L., Hoogendoorn, C. J., Bowatte, S., Li, F. Y., Wang, Y., & Luo, D. (2016). Slope class and grazing intensity effects on microorganisms and nitrogen transformation processes responsible for nitrous oxide emissions from hill pastures. *Agriculture, Ecosystems & Environment*, *217*, 70-78. https://doi.org/https://doi.org/10.1016/j.agee.2015.11.009

Appendices

Appendix 3-1. (a) The D-corer equipment used in sediment column samplings, (b) D-corer in use for sediment coring, (c) fresh sediment columns, (d) sediment columns sectioned into the intended depth intervals collected from four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand).

Appendix 3-2. Sediment mean bulk density at the 0-15 cm wetland depths at the four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand).

Seepage wetland site	Bulk density at 0-15 cm wetland depth ($g\ cm^3$)	
	Mean	SD
Rathmoy	0.18	0.15
Wairiri	0.72	0.19
Tuapaka	0.35	0.08
Ballantrae	0.59	0.28

Appendix 3-3. Areal NO_3^- removal ($g\ N/m^2/day$) estimation technique based on sediment DEA and bulk density measured at the 0-15 cm depths at the four seepage wetland study sites in pastoral hill country landscapes located in the Horizons Regional Council jurisdiction (Lower North Island in New Zealand).

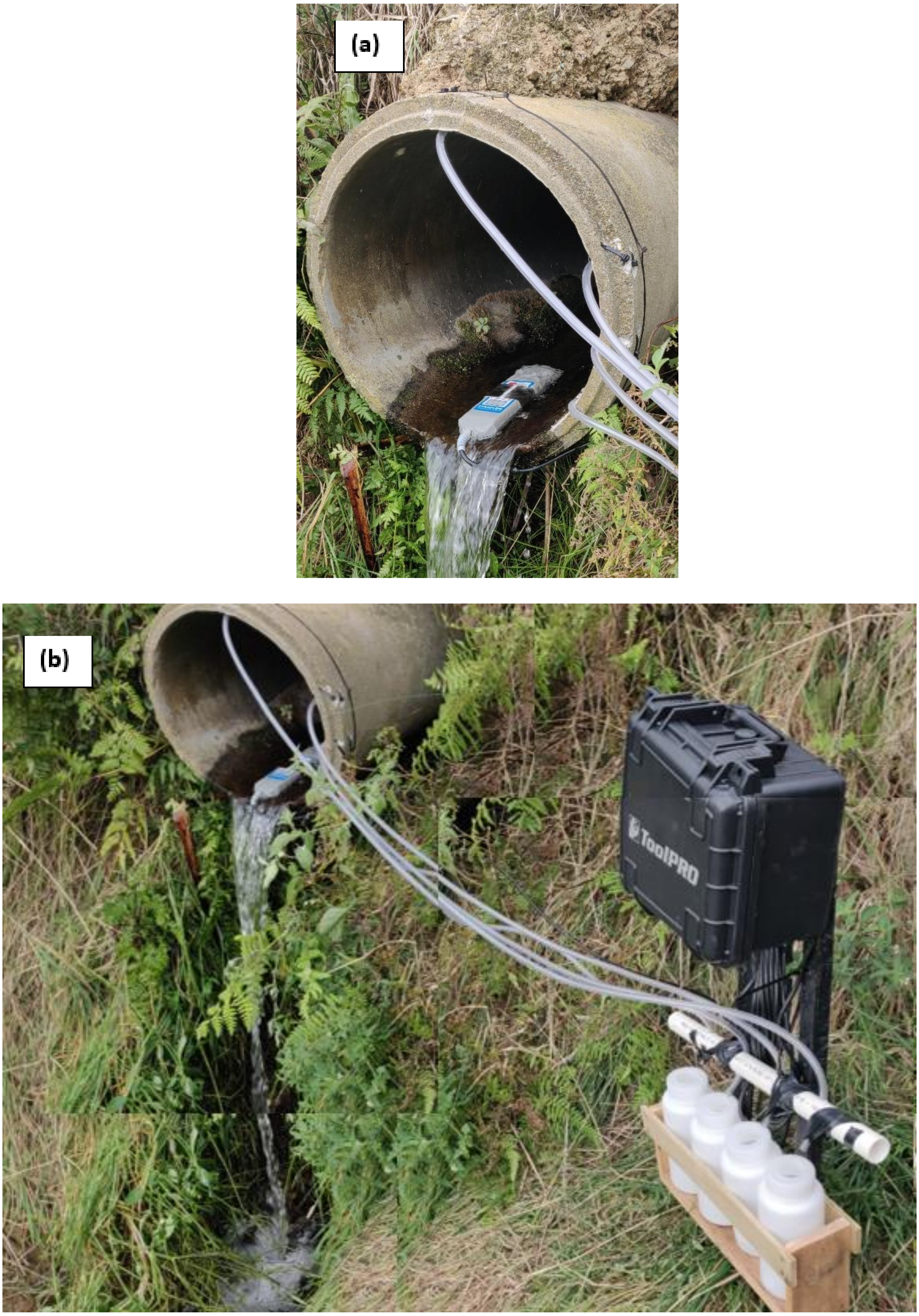
First, sediment column weight is determined using Eq. 3-2 for the 0-15 cm depth for 1 m^2 (=10000 cm^2) seepage wetland surface area.

Sediment column weight (kg) = Sediment bulk density (g/cm^3) x 15 cm x 10000 cm^2 /1000 (Eq. 3-2)

The areal NO_3^- removal was estimated using Eq. 3-3 that used the mean bulk density (Appendix 3-2) and sediment DEA measured at the seepage wetland study sites at the 0-15 cm depths.

Areal NO_3^- removal ($g\ N/m^2/yr$) = Sediment column weight (kg) x Sediment DEA ($\mu g\ N_2O$-N/kg/h) x 24 x 365 / 1000000 (Eq. 3-3)

Appendix 4-1. (a) Unidata Starlogger used for monitoring stream flow discharge (L/s) at 15-minute intervals, and (b) height samplers, at inflow and outflow in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) between year 1 (June 2019-May 2020) and year 2 (June 2020-May 2021). Photos were taken at the outlet. Similar setup was established at the inlet as well.



Appendix 4-2. Terminology used for water balance components.

The terminologies that have been used in the current study to describe different balance components for the wetland in the study area are detailed out here. A supporting conceptual diagram shown in Figure 4-2 is repeated here.

Precipitation (P) (Figure 4-2) is the water added via precipitation to the wetland, measured using an on-site weather station.

Evapotranspiration (ET) (Figure 4-2) is the water lost via evapotranspiration from vegetation growing on the surface of the wetland. Assumptions are: ET in the wetland is not constrained by water deficit because the wetland is under pastoral vegetation throughout the year in the study area. Therefore, the ET in the wetland is assumed equivalent to the potential evapotranspiration (PET) in the study area, which was obtained from NIWA's CLIFLO from station #3243.

The **inflow (I)** (Figure 4-2) is the stream flow entering the wetland measured every 15 minutes using an auto-logger at the inlet (Figure 4-2, Appendix 6-2). Inflow is the integrated response to all waterflow pathways operating within the upper sub-catchment (23.5 ha) located above the inlet (shown in Figure 4-1).

The **outflow (O)** (Figure 4-2) is the stream flow leaving the wetland measured every 15 minutes using an auto-logger at the outlet (Figure 4-1). Outflow is the integrated response to all waterflow pathways operating in the catchment area (25.2 ha) (shown in Figure 4-1).

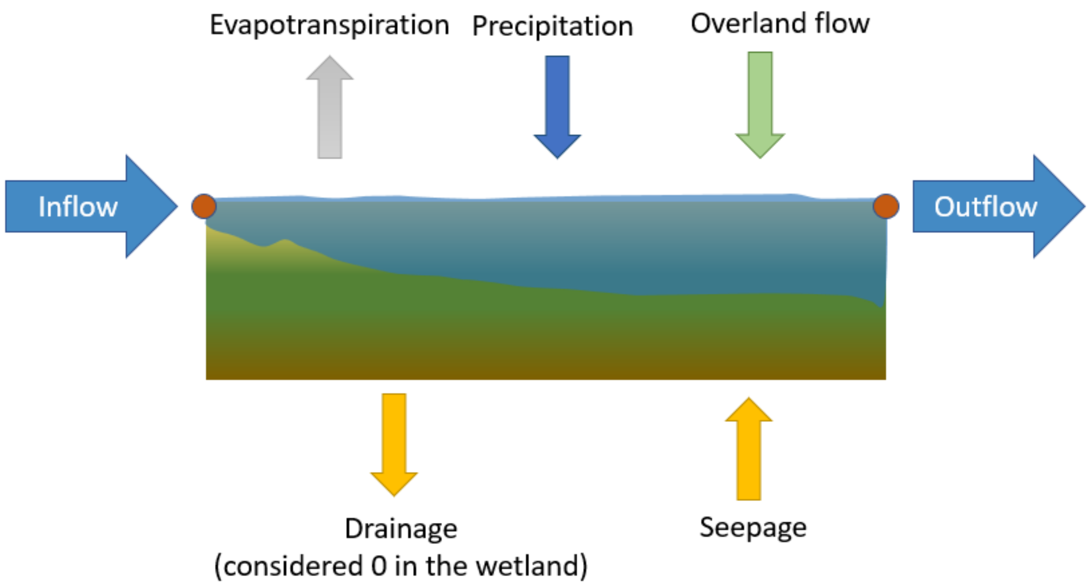


Figure 4-2. Conceptual diagram shows the water balance components in a seepage wetland.

Drainage (Figure 4-2) is any water in the wetland that is lost through the wetland base. It is assumed zero in this study. Given the very low infiltration rates of the sediment (chapter 5) covering the wetland greywacke base, it is likely the drainage value is quite low.

Lateral flow is the total discharge from the direct contributing catchment area into the wetland (shown in Figure 4-1). Conceptually, the lateral flow in this study is:

Lateral flow = (Overland flow) + (Seepage) (Eq. 4-5)

The lateral flow was estimated from the residual of the annual water balance of the wetland.

Overland flow (Figure 4-2) is any precipitation in the direct contributing catchment (Figure 4-1) that does not infiltrate and moves across the soil surface to the wetland. The net flow rate response of overland flow, at the outflow point of the wetland (Figure 4-1), is relatively rapid compared to the seepage (Figure 4-2). The assumption is that the upper
sub-catchment and the direct contributing catchment shared similar hydrogeological properties. Therefore, the overland flow rate in the direct contributing catchment (Figure 4-1) was assumed equivalent to the runoff rate in the upper sub-catchment.

The overland flow from the direct contributing catchment was calculated using the following stepwise procedure:

1. The annual baseflow in the upper sub-catchment (m^3) = wetland inflow BFI * annual wetland inflow (m^3) (Eq. 4-6)
2. The annual quickflow in the upper sub-catchment (m^3) = annual inflow (m^3) - annual baseflow in the upper sub-catchment (m^3). (Eq. 4-7)
3. Overland flow rate in the upper sub-catchment (m) = annual quickflow in the upper sub-catchment (m^3) / upper sub-catchment area (m^2) (Eq. 4-8)
4. The overland flow (*OF*) from the direct contributing catchment to the wetland was estimated as:

Overland flow from the direct contributing catchment (*OF*) (m^3) = Overland flow rate in the upper sub-catchment (m) * direct contributing catchment area (m^2) (Eq. 4-9)

Seepage is any shallow groundwater that can originate from the total catchment area but discharge from the direct contributing catchment into the wetland. The net flow rate of seepage response, at the outflow point of the wetland, is relatively slow compared to the overland flow. In this study seepage is:

Seepage = (Lateral flow) – (Overland flow) (Eq. 4-10)

Appendix 4-3. Descriptive statistics for shallow groundwater properties at the 0.5, 1 and 1.5 m depths in a pastoral hill country seepage wetland study site at Tuapaka farm (Palmerston North, New Zealand) between November 2019-May 2021. SD = standard deviation.

Parameter	Wetland depth (m)	Median	Range	Mean	SD
Temperature (°C)	0.5	17.16	6.49-21.77	17.92	6.07
	1	16.83	7.12-15.06	18.11	5.47
	1.5	16.47	7.8-13.5	17.27	4.96
Conductivity (µS/cm)	0.5	197.02	0.25-1001.7	266.93	198.48
	1	320.43	33.31-967.57	363.43	206.13
	1.5	283.94	0.54-620.77	291.56	151.02
pH	0.5	6.47	5.3-9.31	6.51	0.5
	1	6.25	5.51-7.26	6.24	0.28
	1.5	6.27	5.32-7.72	6.38	0.34
Eh (mV)	0.5	520.43	251.3-1220.7	459.9	399.46
	1	483.94	249.9-1188	468.8	346.59
	1.5	397.02	207.3-1206.4	448.3	329.1

Appendix 4-4. Kendall's Tau correlation coefficient between shallow groundwater properties in a pastoral hill country seepage wetland study site at Tuapaka farm (Palmerston North, New Zealand) between November 2019-May 2021. Bold indicates significant correlation at p≤0.05 level.

	Wetland depth	Temp.	DO	EC	pH	Eh	Fe^{2+}	Mn^{2+}	SO_4^{2-}	NO_3^-
Temp.	-0.03									
DO	**-0.34**	0.05								
EC	**0.11**	-0.05	**-0.16**							
pH	**-0.13**	0.03	**0.27**	0.03						
Eh	0.11	0.09	-0.02	-0.01	0.01					
Fe^{2+}	0.00	**-0.16**	**-0.23**	**0.34**	**-0.13**	-0.08				
Mn^{2+}	-0.10	-0.06	-0.03	**0.49**	-0.06	-0.00	**0.51**			
SO_4^{2-}	**-0.30**	0.03	**0.26**	**-0.30**	0.09	0.04	**-0.16**	-0.09		
NO_3^-	-0.05	**0.26**	0.04	0.04	-0.02	0.04	**0.12**	0.08	0.04	
DOC	**-0.30**	**0.21**	**0.24**	-0.02	0.04	0.02	-0.06	-0.00	**0.25**	0.08

Appendix 4-5. Chronologically presented elevated NO_3^- concentrations observed in a pastoral hill country seepage wetland at Tuapaka farm (Palmerston North, New Zealand) between November 2019-May 2021. NA = not available.

Observation date	Piezometer position in the wetland	Wetland depth (m)	NO_3-N (mg N/L)	DO (mg/L)
26/11/2019	Inflow	0.5	0.60	9.3
26/11/2019	Outflow	0.5	0.50	4.25
26/11/2019	Outflow	1	0.74	6.92
11/12/2019	Midflow	1.5	0.56	5.13
21/02/2020	Midflow	0.5	0.51	6.66
17/03/2020	Inflow	0.5	0.72	7.63
02/11/2020	Midflow	0.5	0.73	8.15
02/11/2020	Midflow	1	1.45	8.38
02/11/2020	Midflow	1.5	1.74	8.23
02/11/2020	Outflow	0.5	1.41	3.79
02/11/2020	Outflow	1.5	1.34	8.59
22/12/2020	Inflow	1	9.17	3.54
22/12/2020	Outflow	1.5	0.47	4.88
09/02/2021	Inflow	0.5	2.23	5.22
09/02/2021	Outflow	0.5	0.94	8.19
09/02/2021	Outflow	1	0.61	8.39
09/02/2021	Outflow	1.5	3.38	0.94
03/03/2021	Outflow	1	9.48	NA
03/03/2021	Outflow	1.5	2.30	NA
26/05/2021	Outflow	1	1.12	4.19

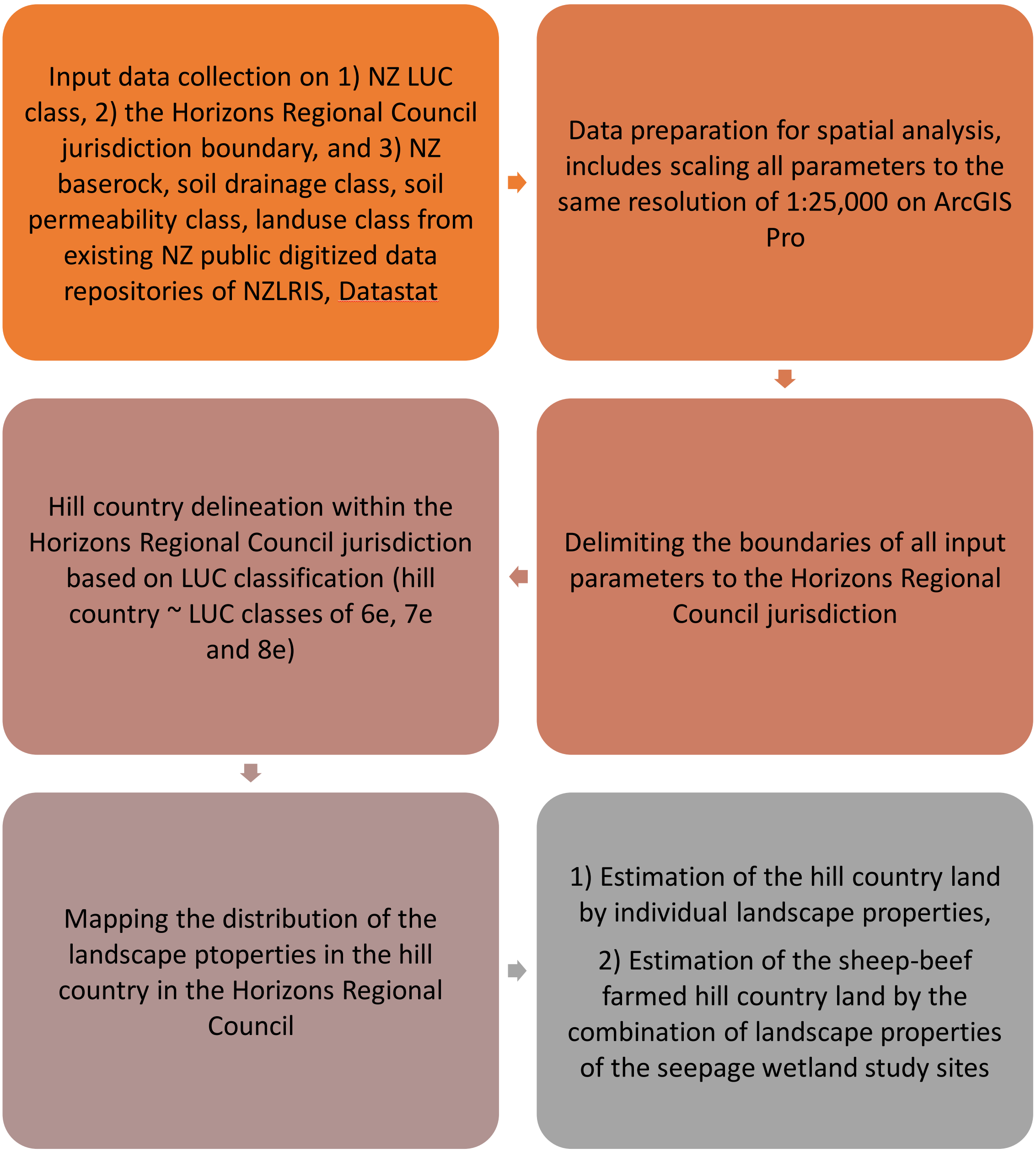
Appendix 6-1. List of input data, including their sources and brief description, which were used in the geospatial analysis in the current study.

Digitised input data	Brief data description according to the sources	Source
Horizons Regional Council jurisdiction	Regional council boundary for 2014 as defined by the Local Government Commission and/or regional councils themselves but maintained by Statistics New Zealand.	Stats NZ (2021)
Baserock	Baserock is a rock attribute, which denotes the principal underlying lithology (Lynn et al., 2009). Data originates from stereo aerial photograph interpretation, field verification and measurement as part of the 1:50000 scale New Zealand Land Resource Inventory and were later digitised for NZ Fundamental Soil Layer (FSL)[1].	Landcare Research (2010b)
Soil drainage class	Soil drainage classes are assessed using criteria of soil depth and duration of water tables inferred from soil colours and mottles (Lynn et al., 2009). The soil drainage classes are based on the NZ Soil Classification (Hewitt, 1993), outlined by Milne et al. (1995), and later digitised for NZ Fundamental Soil Layer (FSL)[1].	Landcare Research (2010a)
Soil permeability class	Soil permeability is the rate at which water travels through saturated soil. The soil permeability classes are related to potential rooting depth, depth to a slowly permeable horizon and internal soil drainage (Lynn et al., 2009). Permeability classes are from Clayden and Webb (1994) later digitised for NZ Fundamental Soil Layer (FSL)[1].	Landcare Research (2000)
Land use	Land use attributes with information on the pastoral land use differentiated into the non-dairy which mostly sheep and beef grazed vs. dairy. Data obtained from the LUCAS NZ Land Use data which is composed of New Zealand-wide land use classifications.	MfE (2022a)

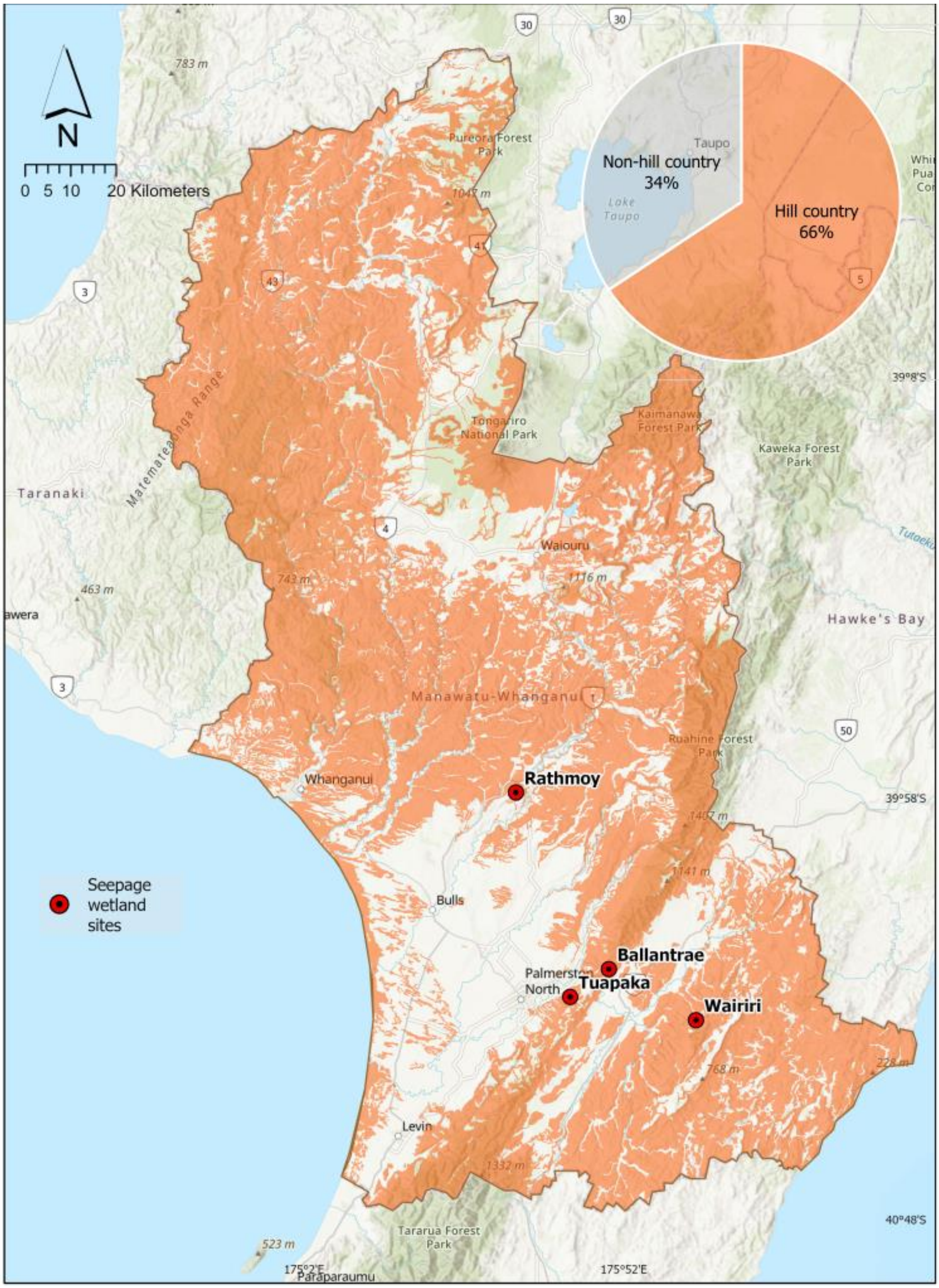
Digitised input data	Brief data description according to the sources	Source
Land use capability (LUC) class	A GIS database developed by Landcare Research developed that classifies land based on its ability to support agriculture and forestry production, based on soil, rock, slope, erosion and vegetation cover. The classification, referred to as Land Use Capability (LUC), gives an indication of the land use the land is able to support in the long term. The LUC classes 6e, 7e and 8e are used as criteria for hill country.	Landcare Research (2021)
Seepage wetland site GPS coordinates	-	Field visit

The [1]New Zealand Fundamental Soil Layer (FSL) is based on the relational joint features from two databases- 1) the New Zealand Land Resource Inventory (NZLRI), and 2) the National Soils Database (NSD). The New Zealand Land Resource Inventory Survey (NZLRIS) is a national polygon database of NZ physical land resources. NZLRIS has original data at approximately 1:50,000 scale. Data in NZLRIS is based on either the National Soils Database (NSD) or the professional estimates by pedologists. The NSD is a point database of soil physical, chemical and mineralogical characteristics of soil.

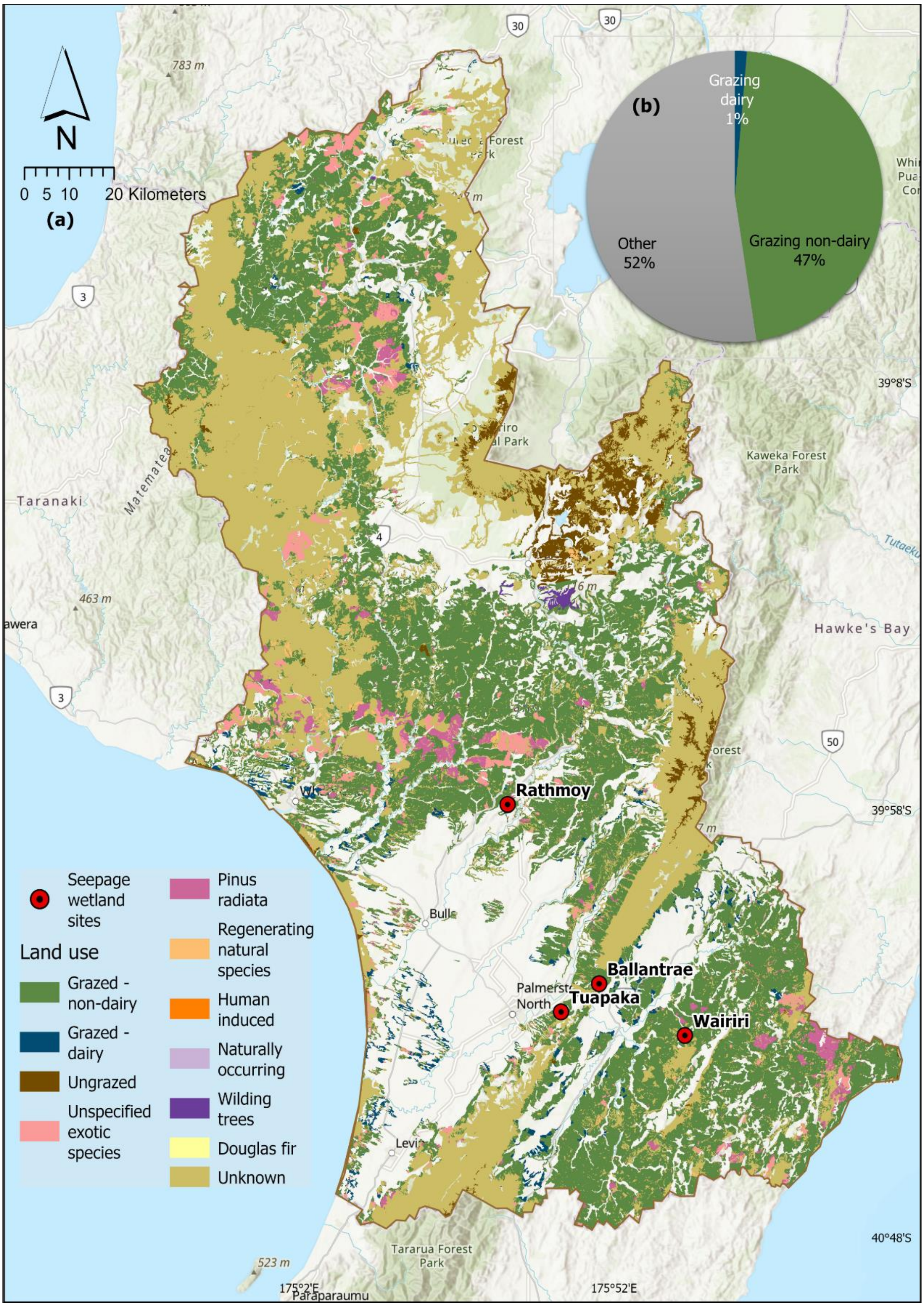
Appendix 6-2. Geospatial analysis workflow diagram for section 6.4.



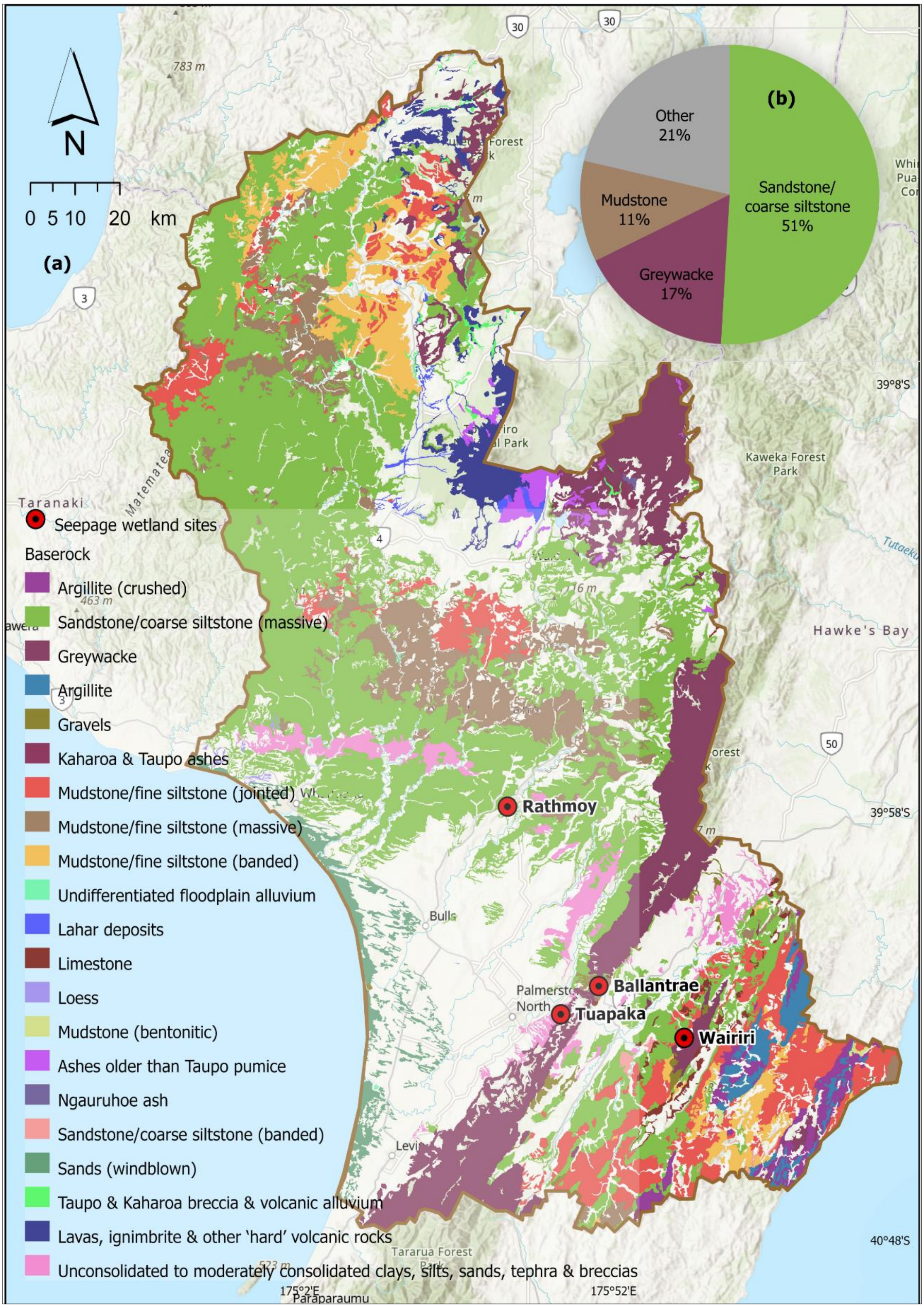
Appendix 6-3. (a) Hill country in the Horizons Regional Council jurisdiction (in Lower North Island, New Zealand) that comprises the LUC classes 6e, 7e, 8e (1:50,000) and obtained by a geospatial analysis conducted in the current study. (b) The pie chart shows the percentage hill country land area coverages by the corresponding landscape properties. Data was obtained from Landcare Research (2021).



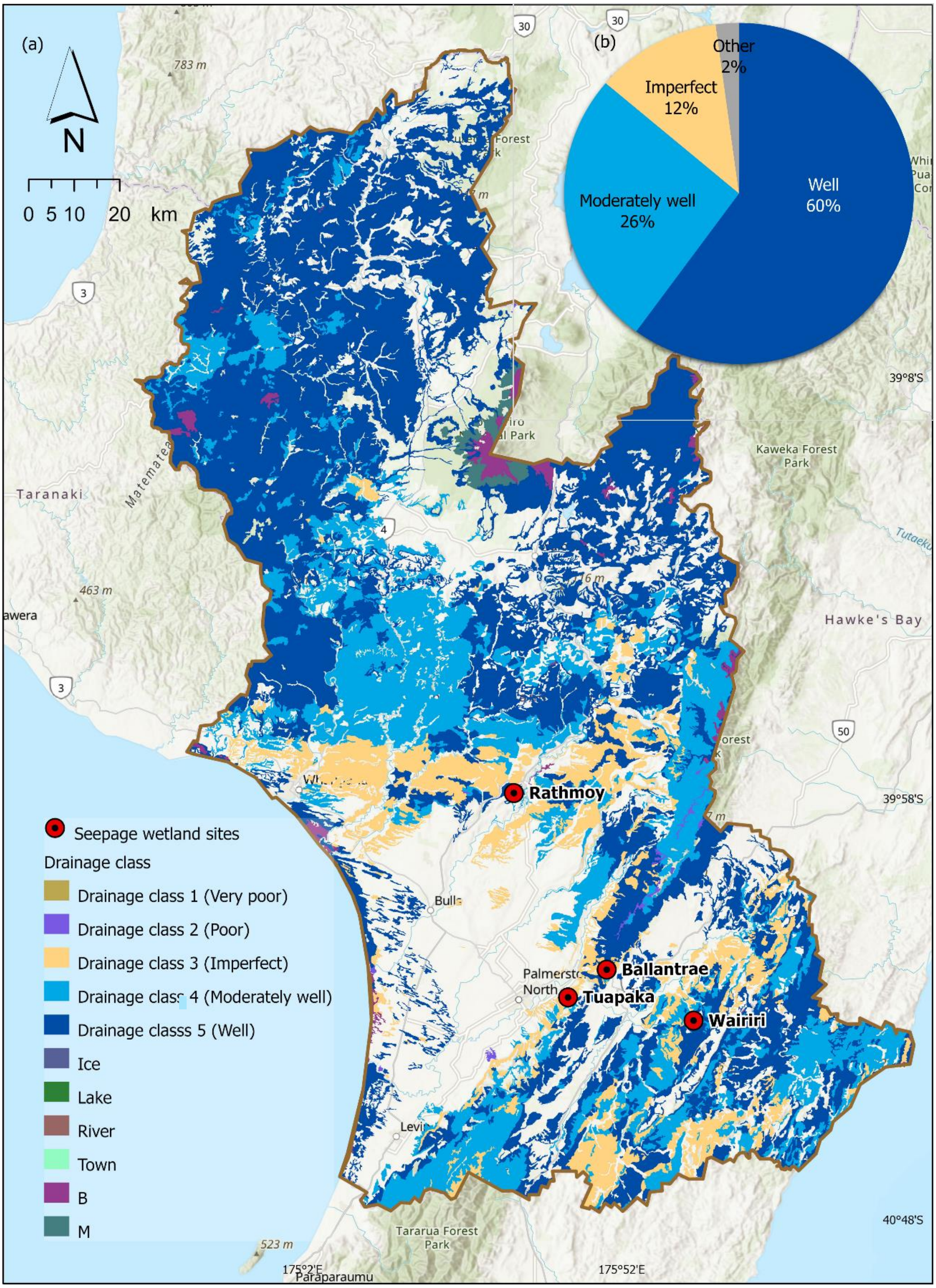
Appendix 6-4. (a) Grazed non-dairy land use area representing mainly sheep-beef grazed area in hill country in the Horizons Regional Council jurisdiction (1:50,000) obtained from geospatial analysis conducted in the current study. (b) The pie chart shows the hill country land area coverages by the land uses from the map. Data was obtained from MfE (2022a).



Appendix 6-5. (a) Baserock distribution in hill country in the Horizons Regional Council jurisdiction (1:50,000) obtained by a geospatial analysis conducted in the current study. (b) The pie chart shows the percentage hill country land area coverage by the baserocks. For simplicity, the mudstone pie on the pie chart combined the joined, banded and bentonitic mudstone types. Data obtained from Landcare Research (2010b).



Appendix 6-6. (a) Soil drainage classes in the hill country in the Horizons Regional Council jurisdiction (1:50,000) obtained from geospatial analysis conducted in the current study. (b) The pie chart shows the percentage hill country land area coverage by the soil drainage classes. Data was obtained from Landcare Research (2010a).



Appendix 6-7. (a) Soil permeability classes in the hill country in the Horizons Regional Council jurisdiction (1:50,000) obtained from geospatial analysis conducted in the current study. S = slow, M = moderate, and R = rapid permeabilities. S/M indicates slow over moderately permeable layer, for example. (b) The pie chart shows the percentage hill country land area coverage by the soil permeability classes on which 'M' covers moderately and moderate over slowly soil permeable classes. Data was obtained from Landcare Research (2000).