

# Stimulation of river periphyton growth by ammoniacal-N vs.nitrate-N-



June 2018

Horizons Report 2018/EXT/1616



Prepared for:

Logan Brown Freshwater & Partnerships Manager June 2018 Report No. 2018/EXT/1616 ISBN 978-1-98-853768-9

#### Prepared by:

Cathy Kilroy, Amber Sinton, Janine Wech, Paul Lambert, Lisa Carlin, Otago University (student)

For any information regarding this report please contact: Cathy Kilroy Freshwater Ecologist Freshwater Ecology Telephone +64-3-343 7883 <u>cathy.kilroy@niwa.co.nz</u> National Institute of Water & Atmospheric Research Ltd PO Box 8602 Riccarton Christchurch 8011

NIWA Client Report No: 2018163CH NIWA Project: ENC18501

CONTACT	24 hr Freephone 0508 800 800	help@horizons.govt.nz	www.horizons.govt.nz	
SERVICE CENTRES	Kairanga Cnr Rongotea and Kairanga-Bunnythorpe Roads 	Taumarunui34 Maata StreetPalmerston North11-15 Victoria AvenueWhanganui181 Guyton Street	Levin 120–122 Hōkio Beach Road Taihape Torere Road Ohotu Woodville 116 Vogel Street	
POSTAL ADDRESS	Horizons Regional Council, Private Bag 110	25, Manawatū Mail Centre, Palmerston Nor	rth 4442 F 06 9522 929	



# Stimulation of river periphyton growth by ammoniacal-N vs. nitrate-N

An experimental investigation

Prepared for Envirolink (Horizons Regional Council)

June 2018

www.niwa.co.nz

#### Prepared by:

Cathy Kilroy Amber Sinton Janine Wech Paul Lambert Lisa Carlin, Otago University (student)

#### For any information regarding this report please contact:

Cathy Kilroy Freshwater Ecologist Freshwater Ecology +64-3-343 7883 cathy.kilroy@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd PO Box 8602 Riccarton Christchurch 8011

Phone +64 3 348 8987

NIWA CLIENT REPORT No:	2018163CH
Report date:	June 2018
NIWA Project:	ENC18501

Quality Assurance Statement							
Re	Reviewed by:	Juliet Milne					
Halcores	Formatting checked by:	Fenella Falconer					
C. Nonadwilliam.	Approved for release by:	Clive Howard-Williams					

© All rights reserved. This publication may not be reproduced or copied in any form without the permission of the copyright owner(s). Such permission is only to be given in accordance with the terms of the client's contract with NIWA. This copyright extends to all forms of copying and any storage of material in any kind of information retrieval system.

Whilst NIWA has used all reasonable endeavours to ensure that the information contained in this document is accurate, NIWA does not give any express or implied warranty as to the completeness of the information contained herein, or that it will be suitable for any purpose(s) other than those specifically contemplated during the Project or agreed by NIWA and the Client.

# Contents

Εχεςι	Executive summary5						
1	Introduction7						
2	Methods9						
	2.1	Site and experimental channels	9				
	2.2	Experimental design and sampling schedule	10				
	2.3	Sample processing	11				
	2.4	Invertebrate habitat	12				
	2.5	Data analysis	13				
3	Resu	lts	16				
	3.1	General observations	16				
	3.2	Nutrient treatments	16				
	3.3	Continuous water quality data	17				
	3.4	Periphyton biomass (chlorophyll a, AFDM)	17				
	3.5	Particulate N and P	20				
	3.6	Accrual rates	20				
	3.7	Periphyton community composition	21				
	3.8	Periphyton abundance relative to biomass	25				
	3.9	Invertebrate communities	25				
	3.10	Invertebrate abundance relative to periphyton biomass	27				
4	Discu	ssion	29				
	4.1	Effects of NH <sub>4</sub> -N vs. NO <sub>3</sub> -N on chlorophyll a and PN	29				
	4.2	Effects of NH <sub>4</sub> -N vs. NO <sub>3</sub> -N on periphyton community composition	30				
	4.3	The overall effect of added nutrients	30				
	4.4	Nutrient enrichment and invertebrate production	32				
5	Impli	cations for river health downstream of WWTP discharges	33				
6	Sumr	nary and conclusions	35				
7	Ackn	owledgements	37				
8	Refer	ences	38				
Арре	ndix A perip	Summary of differences between treatments in measured nutrient, hyton and invertebrate variables	, 44				

Appendix B	Periphyton taxa identified in each treatment on Days 10, 20 and 27	46
Appendix C	List of invertebrate taxa	49
Appendix D	Photographs of periphyton in different treatments	51

#### Tables

Table 2-1:	Invertebrate functional feeding group categories and definitions.	15
Table 3-1:	Measured concentrations DRP, NO <sub>3</sub> -N and NH <sub>4</sub> -N in the five experimental treatments.	17
Table 3-2:	Summary results of pairwise comparisons between treatments in the repeat	ed:
Table 3-3:	Mean percentage (across the three sampling dates) in each treatment of periphyton taxa making up about 85% of the community.	23
Table 3-4:	Pairwise comparisons following analysis of similarities (ANOSIM) on the periphyton community using all taxa, diatoms only, and diatoms functional groups.	24
Table 5-1:	NO <sub>3</sub> -N, NH <sub>4</sub> -N and DRP concentrations in discharges upstream and downstream of WWTPs in the Manawatu-Whanganui region.	34

# Figures

Figure 2-1:	The flow-through experimental channel system located beside the Kowai stock-water race, Springfield, Canterbury.	9
Figure 2-2:	Invertebrate habitat at the downstream end of one of the channels on Day 2 of the experiment.	4 13
Figure 3-1:	Continuous ambient light (top plot) and water temperature (lower plot) recorded during the experiment.	16
Figure 3-2:	Chlorophyll a and AFDM concentrations ( $\pm$ 1 standard error) plotted against time since the start of the experiment.	18
Figure 3-3:	Chlorophyll a plotted against AFDM, separated into treatments.	19
Figure 3-4:	Chlorophyll a and AFDM concentrations plotted against particulate N.	21
Figure 3-5:	Bar graphs showing abundance (left) and percentage (right) of periphyton ce in each treatment by cell size.	ells 22
Figure 3-6:	Bar graphs showing average composition of periphyton in each treatment by major algal groups.	/ 22
Figure 3-7:	Non-metric multi-dimensional scaling plot showing differences in periphytor communities (relative abundance) over time and between treatments.	י 24
Figure 3-8:	Chlorophyll a concentrations in each replicate channel plotted against total counts of algal cells on Day 10 and Day 20.	25
Figure 3-9:	Non-metric multi-dimensional scaling plot showing invertebrate communitie (abundances) coded by treatment.	es 26
Figure 3-10:	Bar graphs showing mean invertebrate community composition in each treatment by feeding group.	27
Figure 3-11:	Relationships between invertebrate densities and peak chlorophyll a and particulate N.	28

# **Executive summary**

This report forms the third part of an investigation initiated by Horizons Regional Council, to address the question: Do increasing concentrations or proportions of ammoniacal nitrogen (NH<sub>4</sub>-N) in river waters below the levels known to be toxic to aquatic life lead to faster periphyton growth and greater biomass than equivalent increases in nitrate-nitrogen (NO<sub>3</sub>-N)?

The question is related to discharges of nutrients into rivers in the Manawatu-Whanganui region from wastewater treatment plants (WWTPs), and the effects of those discharges on river health. In particular, periphyton chlorophyll *a* downstream of discharges can exceed recommended guidelines. Mitigation of the effects of WWTP discharges has focussed on reducing inputs of dissolved reactive phosphorus (DRP) into rivers, but this has not always resulted in reductions in chlorophyll *a* biomass.

Discharges may contain high concentrations of N as well as DRP, especially as  $NH_4$ -N.  $NH_4$ -N has long been known to be more readily assimilated by algae than  $NO_3$ -N, and could potentially stimulate periphyton growth in rivers to a greater extent than  $NO_3$ -N.

An MBIE Envirolink-funded literature review in 2016 cited multiple reports on differential effects by the two sources of N. However, most related to marine environments or concerned uptake rather than biomass; very few studies focussed on responses by stream periphyton to changes in NH<sub>4</sub>-N concentrations or proportions. Consequently, Horizons Regional Council coordinated funding from other interested Regional Councils to enable NIWA to carry out an experiment to address the above question. The experiment was carried out in March 2017. This report (also funded from the Envirolink fund) describes the experimental methods and results, and discusses the implications of the results.

The experiment was carried out in flow-through streamside channels located near Springfield, Canterbury. Background concentrations in the water supply from the Kowai River were relatively low (NO<sub>3</sub>-N, 106 mg m<sup>-3</sup>; NH<sub>4</sub>-N, 2.4 mg m<sup>-3</sup>; DRP, 1.1 mg m<sup>-3</sup>). Treatments were: control (no enrichment), enrichment with DRP only; and enrichment with dissolved inorganic nitrogen (DIN, the sum of NO<sub>3</sub>-N and NH<sub>4</sub>-N) to about 500 mg m<sup>-3</sup> with NH<sub>4</sub>-N making up <1%, 30% and 77% of DIN. Each of the five treatments was replicated in three channels.

Samples were collected at 2- to 5-day intervals between Day 3 and Day 27 of the experiment, and analysed (on selected days) for chlorophyll *a*, ash-free dry mass, particulate N and P (PN and PP), and periphyton community composition. Light and water temperature were monitored throughout the experiment. Habitat for invertebrates was provided in each channel to enable an evaluation of whether high NH<sub>4</sub>-N additions would affect invertebrate communities (which could indirectly affect periphyton through changing rates of grazing).

The experimental results showed that:

- enrichment of the water supply to periphyton growth surfaces in experimental channels with DRP (from < 1 to > 20 mg m<sup>-3</sup>) and DIN (from 110 to ~500 mg m<sup>-3</sup>) had different effects on periphyton biomass depending on the proportion of DIN that comprised NH<sub>4</sub>-N rather than NO<sub>3</sub>-N;
- under 77% NH<sub>4</sub>-N, periphyton chlorophyll *a* developed faster and, at its peak, was about 50% higher than under <1% NH<sub>4</sub>-N (i.e., 99% NO<sub>3</sub>-N). The periphyton growing with 77% NH<sub>4</sub>-N had more chlorophyll *a* and PN per unit weight of AFDM and per algal cell than periphyton with <1% NH<sub>4</sub>-N;

- periphyton grown with 30% NH<sub>4</sub>-N was generally intermediate between the <1% and 77% NH<sub>4</sub>N treatments (e.g., in chlorophyll *a* and PN) and few comparisons showed significant differences;
- AFDM was higher in all the N-enriched treatments than in the control and P treatments, but did not differ between them; the discrepancy between biomass as chlorophyll *a* and biomass as AFDM was likely caused by shifts in periphyton community composition as the proportion of NH<sub>4</sub>-N changed;
- periphyton community composition differed among treatments, although in all cases communities were dominated by diatoms;
- periphyton communities growing under both 30% and 77% NH<sub>4</sub>-N had higher proportions of small-sized algae than the other treatments, and under 77% NH<sub>4</sub>-N these algae had more chlorophyll *a* and PN per cell than in the other treatments;
- differences in accrual rates of chlorophyll *a* and ratios of PN : PP between all Nenriched treatments and the control and P treatments suggested that, at background concentrations, P and N both limited periphyton growth, even though low statistical power meant that corresponding differences in biomass as chlorophyll *a* could not be detected; and
- the highest NH<sub>4</sub>-N concentration applied in the treatments did not appear to negatively affect invertebrate production (through toxic effects), and there was no evidence that increased chlorophyll *a* in periphyton grown with 77% NH<sub>4</sub>-N was a consequence of lower invertebrate grazing pressure.

The results were reviewed in comparison to data on NO<sub>3</sub>-N, NH<sub>4</sub>-N and DRP concentrations upstream and downstream of WWTP discharges in the Manawatu-Whanganui region. While, based on the available data, we could not definitively attribute increased chlorophyll *a* at the downstream sites to the effect of NH<sub>4</sub>-N rather than DRP, it was a possibility for some WWTP discharges. In all cases, it would be informative to carry out studies to determine whether either DIN or DRP limits periphyton growth at the upstream sites. NH<sub>4</sub>-N at the downstream sites ranged between 1% and 40% of DIN, and it was noted that higher water temperatures than those measured during the experiment could accentuate the stimulatory effect of NH<sub>4</sub>-N on periphyton growth.

The overall conclusion from the experiment was that the answer to the question that prompted the study is generally "yes", recognising that: (a) the main biomass variable affected was chlorophyll a and not ash-free dry mass; and (b) the discrepancy between the two biomass measures is likely because changing the source of N (NO<sub>3</sub>-N or NH<sub>4</sub>-N) also led to changes in periphyton community composition. Finally, we note that these observations apply to the particular conditions of the experiment; outcomes may vary under different conditions, especially under different water temperatures.

# 1 Introduction

Concern about deterioration of river ecosystem health through increasing inputs of soluble nitrogen (N) is generally linked to nitrate and nitrite N (hereafter NO<sub>3</sub>-N), because most of the N that enters rivers by diffuse pathways (runoff, groundwater seepage) is already in an oxidised form (Aber et al. 2002, Dymond et al. 2013, Davis 2014). In contrast, discharges from point-sources such as wastewater treatment plants (WWTPs) can discharge high loads of N into receiving waters in its reduced form, mainly as ammoniacal-N (hereafter NH<sub>4</sub>-N) and organically-bound N (Figuero-Nieves 2014, 2016).

In the Manawatu-Whanganui region of New Zealand, at least eight WWTPs discharge into rivers, causing elevated concentrations of both DRP and NH<sub>4</sub>-N at downstream monitoring sites compared to upstream (Kilroy 2016). In most cases, mean periphyton chlorophyll a was also significantly higher downstream of the discharges than upstream. Periphyton becomes a concern when biomass exceeds One Plan river water quality targets or, more recently, the 'national bottom line' for ecosystem health in the National Policy Statement for Freshwater Management (NPF-FM, NZ Government 2017), set for the maintenance and protection of instream ecological health. In the past, most focus has been placed on managing the phosphorus in discharges (Roygard et al. 2012, Parfitt et al. 2013). Consequently, substantial investments have been made by the operators of WWTPs in the Manawatu-Whanganui region to remove soluble phosphorus during the treatment process. However, these investments have not resulted in the expected reductions in instream periphyton biomass. In the Manawatu River, recycling of particulate phosphorus was suggested as the reason for sustained high periphyton growth below a WWTP outfall after improvements to the treatment system (Hamill 2013). At the same time, apparent N-limitation of periphyton upstream of the discharge, but not downstream (Hamill 2013), suggesting that inputs of N (which were predominantly in the form of NH<sub>4</sub>-N) might also be responsible for high periphyton biomass.

All DIN taken up by the algal cells in periphyton must be subject to intra-cellular reduction to NH<sub>4</sub>-N before it can be assimilated. Therefore NH<sub>4</sub>-N is theoretically the most energy-efficient source of N for algae. Preferential uptake of reduced (e.g., N as the NH<sub>4</sub><sup>+</sup>) versus oxidised (e.g., N as NO<sub>3</sub><sup>-</sup>) forms of N in aquatic primary producers has been studied for decades (see review by Syrett 1981), but the implications for freshwater ecosystems of changes in the composition of N supplies to primary producers are complex (e.g., Glibert et al. 2016). The potential stimulatory effects of NH<sub>4</sub>-N on algal uptake of N (and therefore growth) are offset by suppression of algal production as concentrations rise (Glibert et al. 2016), and reductions in productivity have been documented in estuaries receiving wastewater discharges (Parker et al. 2012, Collos and Harrison 2014). At very high concentrations (e.g., > 2000 mg m<sup>-3</sup> or >2 g m<sup>-3</sup>) NH<sub>4</sub>-N may have direct toxic effects on both primary producers (Glibert et al. 2016) and other organisms (Camargo and Alonso 2006). Effects on algal community composition have also been observed. In particular, elevated NH<sub>4</sub>-N has been associated with shifts to dominance of phytoplankton by non-N-fixing cyanobacteria (Chaffin and Bridgeman 2014), or with alterations of diatom community composition in river periphyton (Kutka and Richards 1997).

This report was commissioned by Horizons Regional Council, through MBIE Envirolink Medium Advice Grant HZLC142, and forms Step 3 in a process, outlined below, to address the question: **Do** increasing concentrations or proportions of NH<sub>4</sub>-N in river waters below the levels known to be toxic to aquatic life lead to faster periphyton growth and greater biomass than equivalent increases in NO<sub>3</sub>-N?

**Step 1:** Following discussion between NIWA and Horizons Regional Council (Logan Brown), an Envirolink Medium Advice Grant (HZLC124) was obtained to carry out a review of the literature on the effects of NH<sub>4</sub>-N versus NO<sub>3</sub>-N on periphyton in river, to address the above question.

The outcome of the review (Kilroy 2016) was that most of the published information found related to phytoplankton in marine environments. Multiple studies reported differential effects by the two sources of N, including effects on algal productivity, nutrient uptake rates and community composition. In contrast, few studies were located that focussed on responses by stream periphyton to changes in the concentrations or proportions of NH<sub>4</sub>-N. Consequently, no consistent patterns were identified. Nutrient-diffusing substrate assays generally indicated no difference in biomass between N augmentation as NH<sub>4</sub>-N and NO<sub>3</sub>-N. Species-specific effects on growth rates were detected in one study.

A further objective of the project was to design an experiment aimed at directly addressing the question. Given the lack of information available from the literature, this became particularly important. The report (Kilroy 2016) set out details of the proposed experiment.

**Step 2:** Horizons Regional Council coordinated funding from various local councils to allow NIWA to carry out the experiment described in Kilroy (2016). Supporting councils were: Horizons Regional Council, Northland Regional Council, Greater Wellington Regional Council, Waikato Regional Council, Tararua District Council, Ruapehu District Council and Palmerston North City Council. The experimental work and subsequent sample analyses were completed between January and August 2017. Preliminary findings were communicated to Horizons Regional Council, and the data were organised ready for a full data analysis.

**Step 3:** The current second MBIE Envirolink Medium Advice Grant was sought by Horizons Regional Council to complete analysis of the data and report fully on the results of the experiment. This report is the outcome of Step 3. The report includes:

- a full description of the experimental methods and results (Section 2 and Section 3); and
- discussion and interpretation of the results (Section 4) and an evaluation of their implications (Section 5).

# 2 Methods

# 2.1 Site and experimental channels

The experiment was conducted in 15 flow-through streamside channels (Figure 2-1). Water was sourced from the Kowai River, a tributary of the Waimakariri River, Canterbury, via a stock-water race. The intake from the Kowai River is ~1500 m upstream of the experimental channels. The Kowai River drains a predominantly beech-forest, tussock and alpine catchment, with minor farmland, within the catchment 15-20 km upstream of the experimental channel site. Background nutrient concentrations in the stock-water race at the time of the experiment (in mg m<sup>-3</sup>)<sup>1</sup> were:

- nitrate-nitrogen (NO<sub>3</sub>-N), 106;
- ammoniacal-nitrogen (NH<sub>4</sub>-N), 2.4;
- dissolved reactive phosphorus (DRP), 1.1.



**Figure 2-1:** The flow-through experimental channel system located beside the Kowai stock-water race, **Springfield, Canterbury.** The system has 24 channels, but only 15 were used in this experiment to ensure that sufficient water was available for each channel to maintain near-bed flow velocities within the range typical in rivers and streams.

The channels were constructed of 4.5 mm thick polycarbonate, with dimensions 2 m length, 0.19 m internal width, and 0.15 m high. Submersible pumps transferred water from the raceway to header

<sup>&</sup>lt;sup>1</sup> Throughout this report, concentrations of dissolved nutrients (N and P) are shown in milligrams per cubic metre, which is equivalent to micrograms per litre. These values are 1000 x milligrams per litre (mg/L) which is the most commonly regional council reported unit for nutrient concentrations in rivers. In addition, all units are presented using the International System of Units (SI units). For example, mg/m<sup>3</sup> is shown as mg m<sup>-3</sup>.

tanks. Water flow to the channels was gravity-fed from the header tanks and entered the channels via a head box and a series of baffles, which even out the flow. Flow was held at a constant rate of about 35 L min<sup>-1</sup> (i.e., 0.583 L s<sup>-1</sup>) in each channel, using a constant diameter of the delivery valve opening. This delivery rate translated to a water velocity and depth over the growing surfaces of, respectively, 0.15 m s<sup>-1</sup> and 17 mm.

During the experiment, polycarbonate covers were secured over the channels to screen out UV radiation, which can have a strong negative effect on algal growth in the very shallow water in the channels, potentially masking the effects of other variables being tested (Figure 2-1), The screens used were opaque to both UVA and UVB wavelengths and also reduced photosynthetically active radiation (PAR) by about 10% (Bothwell et al. 1993). In natural river waters, UVA and UVB wavelengths are largely attenuated in the top few centimetres of water, with variability depending on water chemistry, especially dissolved organic carbon (Frost et al. 2005). Frost et al. (2005) predicted that "appreciable quantities of UVB [the most damaging form of UV radiation (Bothwell et al. 1994)] will be found only in the shallowest areas of unshaded streams (i.e., shallow riffles or barely submerged rocks) with low DOC [dissolved organic carbon] concentrations." Since rivers below WWTP discharges relevant to this study are all larger, deeper waterways, it was important to ensure that the experimental treatments represented the rivers of interest as closely as possible.

All water was collected into a return flume, which directed water back into the stock-water race. Chemical amendments were delivered from reservoirs (20-L containers) via a peristaltic pump to the top of 35-mm internal diameter water delivery pipes leading into each channel. Turbulent water flow through a pipe length of at least 1.5 m ensured complete mixing before the treatment water entered the channels.

Algal growth surfaces were 50 x 50 mm patches of plastic with a felted surface, attached to concrete pavers (12 patches per paver) using water-resistant adhesive. Three pavers were prepared for each channel. All pavers had been pre-treated by immersion in the stock-water race for at least 2 weeks, to avoid leaching of any soluble residue from the concrete that might interfere with the nutrient treatments.

# 2.2 Experimental design and sampling schedule

### 2.2.1 Treatments

Five experimental treatments were applied to each of three replicate channels. We aimed to test the effect on periphyton growth of enrichment by dissolved inorganic nitrogen (DIN) to about 500 mg m<sup>-3</sup>, with the DIN addition comprising NO<sub>3</sub>-N, NH<sub>4</sub>-N or a mixture of NO<sub>3</sub>-N and NH<sub>4</sub>-N. The target DIN concentration of 500 mg m<sup>-3</sup> was selected for the experiment because this concentration was within the range of DIN measured at sites downstream of WWTP discharges in the Manawatu-Whanganui region (Kilroy 2016). DRP was added at a concentration high enough to avoid any growth limitation by P. Concentrations of DRP sufficient to saturate periphyton biomass development have been estimated to be 22 - 28 mg m<sup>-3</sup> (Bothwell 1989, Hill and Fanta 2005). Treatment targets were as follows (assuming background concentrations of NO<sub>3</sub>-N, 100 mg m<sup>-3</sup> and NH<sub>4</sub>-N, 2.5 mg m<sup>-3</sup>, based on previous measurements of nutrient concentrations in the stock-water race.

Control:no nutrients additions, background concentrations only;DRP:added DRP of 22 mg m-3;

P+NH <sub>4</sub> -N:	added DRP of 22 mg m <sup>-3</sup> , and added NH <sub>4</sub> -N of 400 mg m <sup>-3</sup> (i.e., NH <sub>4</sub> -N ~80% of DIN);
P+NO <sub>3</sub> +NH <sub>4</sub> <sup>2</sup>	added DRP of 22 mg m $^{-3},$ added NO $_3\text{-}N$ of 250 mg m $^{-3},$ added NH $_4\text{-}N$ of 140 mg m $^{-3}$ (i.e., NH $_4\text{-}N$ ~30% of DIN);
P+NO₃-N	added DRP of 22 mg m <sup>-3</sup> , and added NO <sub>3</sub> -N of 400 mg m <sup>-3</sup> (i.e., NH <sub>4</sub> -N <1% of DIN, background level).

DRP was added as sodium di-hydrogen orthophosphate (NaH<sub>2</sub>PO<sub>4</sub>), NO<sub>3</sub>N as sodium nitrate (NaNO<sub>3</sub>) and NH<sub>4</sub>-N ammonium chloride (NH<sub>4</sub>Cl)

#### 2.2.2 Sampling procedure

The experiment commenced on 1 March 2017 and continued until 27 March 2017. Nutrient enrichment was applied throughout the experimental period. Starting on Day 3 periphyton samples were collected at intervals of 2 to 5 days until the end of the experiment. On each sample collection, we removed one or two growth patches from each of the three pavers in each channel (pre-selected using a random number generator). All patches from the same channel (3 – 4 patches) were pooled into the same sample container. Samples were immediately placed on ice, and stored frozen (-20°C) until processing.

On three occasions (Days 6, 15, and 22) we collected a water sample from the outlet from each channel. Samples were filtered on site through 0.45  $\mu$ m cellulose filters, stored on ice and frozen immediately on return to the laboratory until analysis for nutrient concentrations.

#### 2.2.3 Water temperature and ambient light

During the experiments, water temperature was recorded at 15-min intervals using water temperature probes attached to a Starlogger (Unidata, Perth, Australia), and installed in three replicate channels. Integrated incident PAR in an unshaded location at the site was logged at one-hourly intervals using a LiCor (Lincoln, NE, USA) integrating light meter (LI-1000) coupled with a quantum sensor (LI-190SB).

#### 2.2.4 Continuous monitoring of pH and conductivity

As a further check on any potential ecological effects of adding NH<sub>4</sub>Cl and NaNO<sub>3</sub> we deployed datasondes (Greenspan Pty) to record near-continuous (5-min intervals) pH, temperature and conductivity in four channels for 48-hour periods, towards the end of the experiment, when biomass appeared to be at its peak. The sondes were placed in 20-litre buckets at the end of two control channel, and two channels with added NH<sub>4</sub>-N and NO<sub>3</sub>-N on Day 20. Water from the channels flowed into the buckets before overflowing into the return flume.

#### 2.3 Sample processing

#### 2.3.1 Water samples

All water samples were analysed for NO<sub>3</sub>-N, NH<sub>4</sub>-N and DRP using a Lachat QuikChem FIA+ 8000 series analyser (Lachat Instruments, Milwaukee, WI), with analytical detection limits of 0.5, 1.8 and 0.5 mg m<sup>-3</sup>, respectively.

<sup>&</sup>lt;sup>2</sup> The notation for this treatment should be **P+NH3-N+NO3-N**, for consistency. For brevity we chose to use **P+NH4+NO3** as the label for the treatment in which we added both NH4-N and NO3-N.

#### 2.3.2 Periphyton biomass and community composition

In the laboratory, after thawing of samples, periphyton was gently brushed off the patches with minimal rinse water, homogenised for 10 seconds using a hand-held electric blender, and made up to a known volume with distilled water. Aliquots of the resulting slurry were filtered through glass-fibre filters (Whatman GF/C, two per sample) for analysis for chlorophyll *a* and ash-free dry mass (AFDM). Chlorophyll *a* was extracted from one of the filters using hot ethanol extraction as described by Biggs and Kilroy (2000). For AFDM, the second filter (pre-ashed and pre-weighed) was dried at 105°C for 24 h and re-weighed, then ashed at 400°C for 4 h and weighed a third time.

For samples collected on Day 10 and Day 20, a further aliquot of the slurry was filtered through acidwashed, pre-ashed GF/F filters, and subsequently analysed for total (particulate) N and P. Particulate N and P (PN and PP)<sup>3</sup> were determined on a flow injection analyser (FIA) following persulphate digestion using auto cadmium reduction, and molybdenum blue, respectively.

For samples collected on Day 10, Day 20 and Day 27, a final aliquot of the slurry was taken to determine periphyton species composition. Counts were made on measured aliquots examined in an Utermohl chamber under a Leica DMIL inverted microscope (Leica Microsystems GmbH, Wetzlar, Germany). At least 500 cells of live or recently alive diatoms, single-celled chlorophytes or cyanobacteria, and small-celled filamentous algae (as determined by the presence of intact chloroplasts) were counted in random fields at 400 x magnification. In a second scan, an estimate of the total length in the sample of large filamentous algae was made in 10 random fields of view at 100 x magnification. Lengths were converted to cells using an estimate of the average cell length of each taxon counted. Cell counts were standardised to percentage abundances in each sample.

### 2.4 Invertebrate habitat

High *in-situ* concentrations of NH<sub>4</sub>-N can be toxic to invertebrates through release of free ammonia, with toxicity varying predictably depending on total concentration of ammoniacal N, pH and water temperature (Camargo and Alonso 2006). The toxic effects of NH<sub>4</sub>-N are managed in New Zealand rivers through inclusion of NH<sub>4</sub>-N as an attribute in the NPS-FM (NZ Government 2017). For example, annual median and maximum NH<sub>4</sub>-N concentrations between 240 and 1300 and 400 and 1400 mg m<sup>-3</sup>, respectively (based on a pH of 8 and water temperature of 20°C) are considered to provide 80% protection of aquatic life from the effects of ammonium toxicity (US EPA 2013, NZ Government 2017). The range of median NH<sub>4</sub>-N concentrations at the 80% protection level includes the target concentration in the P+NH<sub>4</sub>-N treatment in our experiment (400 mg m<sup>-3</sup>, assuming pH 8 and water temperature 20°C). We did not exclude invertebrates from entering the channels in the stream water during the experiment. Therefore, we considered that at the highest enrichment rate by NH<sub>4</sub>-N there was potential for adverse effects on invertebrates, which could influence grazing rates and periphyton biomass.

To check for the possibility that different invertebrate densities establishing in the different nutrient treatments could affect periphyton biomass though different grazing rates, we reserved a 600-mm length towards the downstream end of each channel for invertebrate habitat. Each channel section was lined with fine mesh and then filled with 5 L of river gravels (diameter 20 to 100 mm) to provide invertebrate habitat (Figure 2-2). Larger rocks placed at the downstream end prevented gravel washing out of the channel. At the end of the experiment (Day 27), all the gravel in each channel section was collected into the mesh, enabling removal of all the gravel without losing any

<sup>&</sup>lt;sup>3</sup> PN and PP provide an estimate of the total N and P incorporated into the algae in the sample. The amounts are not exact because periphyton includes other small organisms (e.g., small invertebrates) and also P, in particular, may be adsorbed onto inorganic particles.

invertebrates. Invertebrates inhabiting the gravels were rinsed out into containers and preserved immediately in isopropyl alcohol. Full counts were performed on the samples, to the lowest practicable taxonomic level.



Figure 2-2:Invertebrate habitat at the downstream end of one of the channels on Day 24 of theexperiment.Flow is from right to left. At the end of the experiment (Day 27), all the gravel was gathered into<br/>the mesh and invertebrates extracted from the entire sample in each channel.

### 2.5 Data analysis

#### 2.5.1 Biomass

Chlorophyll *a* and AFDM data in each treatment were first plotted over time. Repeated measures ANOVA (with treatment as a factor) (RM-ANOVA) was used to identify statistically different periphyton biomass (as chlorophyll *a* and AFDM) among the treatments. The biomass data were log-transformed to ensure the data met requirements for homogeneity of variance and normal distribution.

The data series of chlorophyll *a* were also used to estimate periphyton exponential growth rates in each channel. For these calculations we assumed that losses of periphyton from invertebrate grazing and detachment caused by flow fluctuations were low, but recognise that some losses were likely occurring. Therefore, we refer hereafter to accrual rates rather than growth rates (except as noted). Accrual rates were calculated for each treatment using data averaged across the three replicate channels.

Net accrual rate (per day, or d<sup>-1</sup>) was calculated as:

 $B_T = B_0 \exp(kT)$ 

where: B0 and BT are chlorophyll a (mg m<sup>-2</sup>) at, respectively the start and end of the period considered (T days),

k is the net accrual rate during the exponential growth phase.

Re-arranging the equation:

#### net accrual rate k = [Log<sub>e</sub> (B/a)]/T

The period considered was Day 3 to the day of maximum biomass, which occurred in all treatments on Day 20. Accrual rates were compared between treatments using one-way ANOVA, followed by pairwise tests, with significance determined from a Tukey's HSD test.

#### 2.5.2 Particulate N and P

PN and PP determined from the periphyton samples on Days 10 and 20 were standardised to unit area and compared among treatments using a two-way ANOVA with Day and Treatment as factors. The analysis was repeated for PN and PP standardised to chlorophyll *a* or AFDM. PN and PP were also compared with biomass (chlorophyll *a* and AFDM determined on the same day) using linear regression. All data were transformed to ensure the data met requirements for homogeneity of variance and normal distribution. Inconsistent variance within treatments precluded the use of parametric statistics (i.e., ANOVA) on data from individual days; in these cases, values were compared among treatments using non-parametric Kolmogorov-Smirnoff (KS) tests.

#### 2.5.3 Periphyton community composition

Quantitative counts of algal cells (as cells cm<sup>-2</sup>) were compared across time and treatments using two-way ANOVA. The taxa were classified into four arbitrary groups by cell size, and size classes were also compared across treatments. The four groups were: very small (vsmall) (< 25  $\mu$ m diameter or length), small (26 – 50  $\mu$ m diameter or length), medium (51 – 80  $\mu$ m diameter or length) and large (> 80  $\mu$ m diameter or length).

We used analysis of similarities (ANOSIM) to determine whether the raw periphyton community composition (taxa counts converted to percentages) differed between treatments. A matrix of Bray-Curtis similarities<sup>4</sup> was generated between all samples. ANOSIM uses a re-sampling procedure on the ranked similarity values to determine whether two sets of samples differ significantly (i.e., when the probability of a difference due to chance is less than 5%, or P < 0.05). To reduce "noise" in the data, rare taxa (e.g., total abundance across all samples of <1%) were not included. Data were square-root transformed prior to calculation of similarities, to down-weight the influence of very common species, as recommended by Clarke et al. (2014).

Non-metric multi-dimensional scaling (NMDS) was used to visualise the similarities in a twodimensional plot. In NMDS, the Bray-Curtis similarity between each pair of sites is ranked and the sites are then plotted with respect to the ranks so that sites similar to each other plot closer together than sites that are more dissimilar. ANOSIM and NMDS were performed using PRIMER-E software (Clarke et al. 2014).

In addition to the size classes (see above), we reduced the detailed taxonomic dataset to major taxonomic groups, based on biovolume (diatoms, green filamentous algae, red algae, other green algae, cyanobacteria), and diatom functional groups (high profile, low profile, motile, as defined by Passy 2007). Relative abundances (percentages) of each group were compared across treatments using non-parametric KS tests.

<sup>&</sup>lt;sup>4</sup> Bray-Curtis similarity is calculated from the proportion of species common to two samples, weighted by their abundance. The index ranges from 0 (no species in common) to 1 (all species in both samples with the same abundances).

#### 2.5.4 Invertebrate communities

ANOSIM and NMDS were used to determine whether invertebrate community composition differed between treatments. Square-root-transformed densities were used in the analysis. Three replicates per treatment limited generation of P values using resampling. The 15 samples were therefore compared across three groups: samples with no added N (i.e., the control and DRP treatments, six samples); samples with added NH<sub>4</sub>-N (i.e., the P+NH<sub>4</sub>-N and P+NO<sub>3</sub>+NH<sub>4</sub> treatments, six samples); and samples with added NO<sub>3</sub>-N only (the P+NO<sub>3</sub>-N treatment, three samples).

In addition, all invertebrates in the dataset were categorised according to functional feeding group (Table 2-1). Densities and proportions of the functional feeding groups were compared between treatments using non-parametric KS tests. Relationships between invertebrate densities and peak periphyton biomass, PN and PP in each channel were also compared using regression. Although peak biomass occurred in the channels earlier than the collection date for invertebrates, we judged that the comparison was valid because sloughing of biomass commenced in the artificial substrates in some channels from about day 22 onwards. Such sloughing was not observed in sections of natural cobble substrate used for the invertebrate component of the experiment

Feeding group	Definition	Examples
Browser	predominantly consume algae	Larvae of <i>Deleatidium</i> (mayfly), <i>Pycnocentrodes</i> (caddisfly)
Collector-filterer	filter material from streamflow using constructed nets	Larvae of Aoteaspyche (caddisfly)
Collector- gatherer	feed on organic deposits on stream bed	Larvae of <i>Chironomus</i> spp. and Orthocladiinae (two-winged flies)
Filter-feeder	filter material from flow using mouthparts or other anatomy	Larvae of Austrosimulium (sandfly); Cladocera and other crustaceans
Predator	feed on other invertebrate species	Larvae of Stenoperla (stonefly)

Table 2-1:	Invertebrate functional feeding group categories and definitions.	Note that there are other
categories (e	.g., shredders), but no representatives were found in the samples.	

Finally, the invertebrate data were used to calculate values for four invertebrate community indices sensitive to water quality and catchment land use: Macroinvertebrate Community Index (MCI), the MCI quantitative variant (QMCI), EPT (Ephemeroptera, Plecoptera and Trichoptera) Index and Percent EPT. MCI and QMCI scores are derived from tolerance values assigned to different taxa (Stark 1998, Stark and Maxted 2007). Taxa tolerant of poor water quality caused by organic pollution (low oxygen levels, siltation) have low values (e.g., worms, some midge larvae); taxa sensitive to these conditions have high values (e.g., mayfly and stonefly larvae). All four indices are widely used in New Zealand to assess stream health (e.g., Collier et al. 1998). Non-parametric KS-tests were used to compare the indices among treatments.

# 3 Results

For ease of reference, a quick summary of all results (main differences between treatments) is presented in Appendix A.

# 3.1 General observations

During the 27-day experiment ambient light and water temperature fluctuated considerably reflecting changeable weather conditions. The maximum hourly PAR recorded was on 2 March (6.59 mol  $m^{-2} h^{-1}$ ), and the minimum peak hourly PAR was 1.01 mol  $m^{-2} h^{-1}$  on the last day of the experiment (Figure 3-1).

The water temperature logger malfunctioned from 24 March. Therefore, the following statistics refer to data between 1 and 23 March only. Mean water temperature over the entire period was 13.8°C. The maximum temperature reached in the channels was 20.6°C (on 20 March), with peaks > 20°C on 1, 2 and 5 March (Figure 3-1).



**Figure 3-1:** Continuous ambient light (top plot) and water temperature (lower plot) recorded during the experiment. Ambient light is recorded as photosynthetically available radiation (PAR). Water temperature is the mean of data from sensors placed in three random channels. The sensors were placed at the head of the channel close to the water inlet. The mean difference in readings between the sensors was <0.1°C.

# 3.2 Nutrient treatments

The additions of NO<sub>3</sub>-N and NH<sub>4</sub>-N achieved close to the target DIN of 500 mg m<sup>-3</sup> in all three Nenriched treatments, with percentages of NH<sub>4</sub>-N of 0.5% (background levels), 30% and 77%, on average (Table 3-1). PO<sub>4</sub>-P additions achieved channel DRP concentrations of between 21 and 24 mg m<sup>-3</sup> (target was 22 mg m<sup>-3</sup>) in all the treatments, on average. DIN did not differ significantly between the three N-enriched treatments (ANOVA pairwise comparisons, P > 0.62) and DRP did not differ between the four treatments with P-enrichment (ANOVA pairwise comparisons, P > 0.35). Table 3-1:Measured concentrations DRP, NO<sub>3</sub>-N and NH<sub>4</sub>-N in the five experimental treatments.Meansand standard deviations (sd) are shown from water samples collected from all channels on Day 3, Day 6 andDay 22 (i.e., n = 9). The last pair of columns shows the percentage of N as NH<sub>4</sub>-N in each treatment

	Nutrient concentrations (mg m <sup>-3</sup> )						Percei	Percentages		
Treatment	D	RP	NO	3- <b>N</b>	NH	4- <b>N</b>	D	IN	NH	4- <b>N</b>
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Control	0.9	0.1	112	4.9	2.7	0.9	115	5.2	2.4	0.7
DRP	21	11.7	112	4.5	2.1	0.5	114	4.7	1.8	0.4
P+NO3-N	24	6.2	498	112	2.2	0.7	500	112	0.5	0.2
P+NO3+NH4	24	6.6	379	57	160	30	539	86	30	1.3
P+NH4-N	22	6.7	113	5.3	389	90	502	88	77	4.3

# 3.3 Continuous water quality data

The 5-min pH data collected over 6 days indicated that the pH in the channels with added NH<sub>4</sub>Cl was approximately 0.2 to 0.3 pH units lower than in the control channels. Over the period tested, pH was consistently > 7 (range of 7.50 to 7.85 in control channels and 7.25 to 7.63 in added NH<sub>4</sub>-N channels). The range represented diurnal fluctuations.

Mean water temperature over the Sonde deployment period (Day 22 to Day 27, when the channel temperature logger malfunctioned) was lower than for the whole experimental period (12.5°C and 13.8°C, respectively), as was maximum temperature (16.1°C and 20.7°C, respectively).

# 3.4 Periphyton biomass (chlorophyll *a*, AFDM)

Chlorophyll *a* in the P+NH<sub>4</sub>-N treatment channels appeared to accumulate faster than in other treatments (Figure 3-2). Chlorophyll *a* concentrations differed significantly between treatments (RM-ANOVA,  $F_{(4,8)} = 6.052$ , P = 0.015). In a post-hoc pairwise comparison, chlorophyll *a* in the P+NH<sub>4</sub>-N channels was significantly higher than that in the control channels or the P+NO<sub>3</sub>-N channels (P < 0.01) (Table 3-2). Between Day 22 and Day 27, some sloughing of biomass was observed on the paver substrates, which led to lower or more variable chlorophyll *a* in all treatments by Day 27.

AFDM also differed between treatments (RM-ANOVA,  $F_{(4,8)} = 9.763$ , P = 0.004), but pairwise differences were weaker and had a different pattern from chlorophyll *a* (Figure 3-2, Table 3-2). AFDM in the control treatments was lower than that in all three N-enriched treatments, which did not differ in AFDM. Channels enriched with P only (DRP treatment) had lower AFDM than those with added NO<sub>3</sub>-N (i.e., the P+NO<sub>3</sub>-N and P+NO<sub>3</sub>+NH<sub>4</sub> treatments) but did not differ from channels with added NH<sub>4</sub>-N (i.e., the P+NH<sub>4</sub>-N treatment).

Mean chlorophyll *a* concentrations on Day 20 (the day of peak biomass) in the control, DRP, P+NH<sub>4</sub>-N, P+NO<sub>3</sub>-N, and P+NO<sub>3</sub>+NH<sub>4</sub> treatments was 227, 229, 433, 283 and 331 mg m<sup>-2</sup>, respectively. Thus, peak chlorophyll *a* in the P+NH<sub>4</sub>-N treatment was, on average, >50% higher than in the P+NO<sub>3</sub>-N treatment. Mean AFDM concentrations on Day 20 were 79, 83, 92, 104 and 104 g m<sup>-2</sup>, respectively (Figure 3-2).



**Figure 3-2:** Chlorophyll *a* and AFDM concentrations (± 1 standard error) plotted against time since the start of the experiment. For statistics refer to Table 3-2.

Table 3-2:	Summa	ry results of pairwise comparisons between treatments in	the repeated measures
analysis of va	riance.	Bolded values indicate statistically significant differences.	"Mean difference" shows the
relative magn	itude of	the differences between treatments in transformed units.	

		Chloroph	yll a	AFDM	
Treatment 1	Treatment 2	Mean difference	Р	Mean difference	Р
Control	Р	-0.032	0.662	-5.445	0.367
Control	P+NH <sub>4</sub> -N	-0.252	0.004	-12.235	0.015
Control	P+NO <sub>3</sub> -N	-0.036	0.390	-17.473	0.037
Control	P+NO <sub>3</sub> +NH <sub>4</sub>	-0.122	0.155	-20.505	0.054
Р	P+NH <sub>4</sub> -N	-0.220	0.106	-6.790	0.211
Р	P+NO <sub>3</sub> -N	-0.004	0.970	-12.029	0.055
Р	P+NO <sub>3</sub> +NH <sub>4</sub>	-0.089	0.230	-15.061	0.036
P+NH <sub>4</sub> -N	P+NO <sub>3</sub> -N	0.216	0.006	-5.238	0.114
P+NH4-N	P+NO <sub>3</sub> +NH <sub>4</sub>	0.130	0.158	-8.270	0.231
P+NO <sub>3</sub> -N	P+NO <sub>3</sub> +NH <sub>4</sub>	-0.086	0.333	-3.032	0.617

Peak measured AFDM occurred later than peak chlorophyll *a*, on Day 22, with an overall increase of 38 g m<sup>-2</sup> in AFDM between the two dates (paired t-test, P < 0.0001). In contrast, there was a mean decline in chlorophyll *a* between Day 20 and Day 22 of 35 mg m<sup>-2</sup> (paired t-test, P = 0.012) (Figure 3-2).

Photographs of the substrates on the two dates enabled us to attribute the discrepancy between chlorophyll *a* and AFDM accrual between Days 20 and 22 to loss of chlorophyll *a* in actively growing algae at the top of the mat during sampling of adjacent patches. By this stage of the experiment, the algae on the growth patches had coalesced into a single mat, and it was difficult to remove a patch without disturbing the algae on neighbouring patches. In contrast organic matter evidently continued to accumulate as new algal growth commenced. Consequently, we consider that the apparent decline in chlorophyll *a* after Day 20 was at least partly an artefact of the experimental sampling. The concentration of chlorophyll *a* on Day 20 is therefore considered to represent peak chlorophyll *a* because at this stage chlorophyll *a* accrual was still consistent across the treatments and was not affected by sloughing.

Chlorophyll *a* was strongly correlated with AFDM ( $R^2 = 0.86$ , across all samples, Day 3 to Day 27), but the two biomass measures were not correlated on individual days ( $R^2 < 0.08$  in all cases). The slope of the overall relationship between AFDM and chlorophyll *a* for the P+NH<sub>4</sub>-N treatment differed from that in all other treatments (Analysis of covariance, P < 0.05) but the slopes in the other four treatments did not differ (Figure 3-3).





# 3.5 Particulate N and P

PN differed between Treatment and Day (two-way ANOVA treatment effect,  $F_{(4,20)} = 6.96$ , P = 0.001; Day effect,  $F_{(1,20)} = 322.52$ , P < 0.001), with a non-significant interaction. Pairwise comparisons showed no difference between the DRP treatment and other treatments, and no difference between the three treatments receiving added N (in any form). However, PN in the three treatments with added N was significantly higher than in the control treatments. Looking at PN on Day 20 only, a KS test indicated that PN was significantly lower in the control and DRP treatments than in the treatments with added N, and PN was higher in the P+NH<sub>4</sub>-N treatment than the P+NO<sub>3</sub>-N treatment. Mean PN concentrations on Day 20 in the control, DRP, P+NH<sub>4</sub>-N, P+NO<sub>3</sub>-N, and P+NH<sub>4</sub>+NO<sub>3</sub> treatments were 2140, 2450, 3240, 2800 and 2940 mg m<sup>-2</sup>, respectively.

Using ANOVA, PP did not differ between treatments or over time (P > 0.05). However, on Day 20 the data suggested very low PP in the control treatment samples compared to all other samples. The difference was confirmed by a two-sample KS test. Mean PP concentrations on Day 20 in the control, DRP, P+NH<sub>4</sub>-N, P+NO<sub>3</sub>-N, and P+NH<sub>4</sub>+NO<sub>3</sub> treatments were 78, 509, 325, 367 and 425 mg m<sup>-2</sup>, respectively.

PN and PP standardised to chlorophyll *a* and PP standardised to AFDM (as percentages, by weight) did not differ between treatments but differed over time, with significantly lower values on Day 20 than on Day 10 in all cases (detailed results not shown). The percentage of N in AFDM differed between treatments (two-way ANOVA treatment effect,  $F_{(4,20)} = 3.598$ , P = 0.023), and over time (P < 0.001). Pairwise comparisons showed that the treatment differences were driven by higher %N in AFDM in the P+NH<sub>4</sub>-N treatment than in the control treatment and the P+NO<sub>3</sub>-N treatment (P = 0.022, P = 0.046, respectively). Mean %N in AFDM was 3.2, 3.3, and 4.1 in the control, P+NO<sub>3</sub>-N and P+NH<sub>4</sub>-N treatments, respectively).

Using Day 10 and Day 20 data combined, PN was strongly and positively related to both chlorophyll *a* and AFDM, explaining 91% of the variance in both biomass measures ( $R^2 = 0.91$ . P < 0.001) (Figure 3-4). Using data from Day 20 only, PN was still significantly related to chlorophyll *a* and AFDM ( $R^2 = 0.55$ , P = 0.001,  $R^2 = 0.38$ , P <0.01, respectively) (Figure 3-4). Corresponding relationships from Day 10 were that PN was related to AFDM ( $R^2 = 0.53$ , P = 0.001) but not to chlorophyll *a*. AFDM and chlorophyll *a* were not correlated on either Day 10 or Day 20 ( $R^2 = 0$ , P > 0.50), despite the strong correlation between them across all dates (Figure 3-3). PP was not related to chlorophyll *a* or AFDM, either within the two sampling dates or across dates.

The ratio PN : PP was variable on Day 10 and did not differ between treatments. On Day 20, PN : PP was higher in the control treatment than in all other treatments (one-way ANOVA,  $F_{(4,10)} = 5.350$ , P = 0.014, post-hoc pairwise comparisons P < 0.05). Mean PN : PP in the control, DRP, P+NH<sub>4</sub>-N, P+NO<sub>3</sub>-N, and P+NH<sub>4</sub>+NO<sub>3</sub> treatments were 29.1, 6.4, 10.7, 9.7 and 7.9, respectively.

# 3.6 Accrual rates

Starting biomass (B<sub>0</sub>) was taken as the mean chlorophyll *a* across all treatments on Day 3, because at that stage there was no difference between treatments (ANOVA, F(4, 10) = 1.266, P = 0.346; chlorophyll  $a = 2.79 \pm 1.17$  (standard deviation) mg m<sup>-3</sup>. Mean exponential accrual rates in the control, DRP, P+NH<sub>4</sub>-N, P+NO<sub>3</sub>-N, and P+NH<sub>4</sub>+NO<sub>3</sub> treatments were, respectively, 0.258, 0.257, 0.297, 0.271 and 0.280. Accrual in the P+NH<sub>4</sub>-N treatment was higher than that in the control, DRP, and P+NO<sub>3</sub>-N treatments (one-way ANOVA pairwise comparisons, P < 0.05), but was not different from that in the P+NH<sub>4</sub>+NO<sub>3</sub> treatment (P = 0.248).



**Figure 3-4:** Chlorophyll *a* and AFDM concentrations plotted against particulate N. The top two plots show the relationships across all data from Day 10 and Day 20. The bottom two plots show the relationships for Day 10 and Day 20 separately.

### 3.7 Periphyton community composition

A list of all taxa identified, with their respective size class and functional group (for diatoms), is presented in Appendix B. Total cell abundance (calculated as cells cm<sup>-2</sup>) increased between Day 10 and Day 20, and then declined by Day 27, consistent with the biomass observations (Figure 3-5). Abundance (across all three days of counts) was higher in the P+NH<sub>4</sub>-N and P+NO<sub>3</sub>+NH<sub>4</sub> treatments than in the control treatment and was higher in the P+NH<sub>4</sub>-N treatment than the DRP treatment (post-hoc tests after a two-way ANOVA, with Day and Treatment as factors, P < 0.05). Taxa in four size classes also differed between treatments. For example, abundances of "very small" taxa were higher in the P+NH<sub>4</sub>-N and P+NO<sub>3</sub>+NH<sub>4</sub> treatments than in the control, P and P+NO<sub>3</sub>-N treatments (ANOVA, P < 0.05) (Figure 3-5).

Periphyton communities were dominated by diatoms on all three sampling dates (Figure 3-6). On the first sampling date (Day 10), most of the non-diatom periphyton comprised green filamentous algae (up to 35% in the control treatment). Proportions of green filamentous algae had declined by Day 20 and further declined by Day 27. Moderate proportions of cyanobacteria (up to > 10%) were present by Day 20, but this increased only in the P+NO<sub>3</sub>-N treatment by Day 27 (Figure 3-6).



**Figure 3-5:** Bar graphs showing abundance (left) and percentage (right) of periphyton cells in each treatment by cell size. Note different *y*-axis scales on the left-hand plots.



**Figure 3-6:** Bar graphs showing average composition of periphyton in each treatment by major algal groups. Data shown are counts weighted by biovolume. Note that red algae were present in some samples, but mostly in amounts too low to be visible on the plot.

The small diatom *Encyonema minutum* numerically dominated most samples, with an average relative abundance of 43% (range 17 – 62%). Other common taxa included the diatoms *Nitzschia acicularis* (mean abundance 8.5%), *Nitzschia palea* (4.3%) and *Cymbella kappii* (3.7%), and the cyanobacterium *Merismospedia* sp. (4.9%) (Table 3-3).

Differences between sampling dates seen in an NMDS plot (Figure 3-7) were driven by major shifts in abundance of some taxa over time. For example, *N. acicularis* abundance increased between Day 10 and Day 20, but by Day 27 was largely absent from all samples. *E. minutum* increased in abundance between Day 10 and Day 20, and remained common on Day 27. *N. palea* was most abundant on Day 10 but declined to low proportions (<2% in most samples) by Day 27. Green algae were most abundant on Day 10 and were represented mainly by *Microspora* sp. and *Oedogonium* sp. By Day 20, *Microspora* was at low abundance in all treatments, and *Oedogonium* was declining.

Differences between treatments indicated by, for example, separation of samples in the control treatments from other treatments in Figure 3-7, were driven by different relative abundances rather than major species turnover, although some taxa were persistently lowest in the control treatment (e.g., *N. acicularis* and the large filamentous diatom *Melosira varians*) (Table 3-3).

A two-way ANOSIM with Day and Treatment as factors showed significant differences between sampling day (Global R = 0.921, P = 0.001) and treatment (Global R = 0.323, P = 0.001). Communities in the control treatments differed significantly from those in all other treatments, and the P-NH<sub>4</sub>-N communities differed from those in the DRP and P+NO<sub>3</sub>-N treatments (pairwise comparisons, Table 3-4).

				Treatment		
Taxon	Туре	Control	DRP	P+NH <sub>4</sub> -N	P+NO <sub>3</sub> -N	P+NO <sub>3</sub> +NH <sub>4</sub>
Encyonema minutum	D	48.0	39.7	51.4	33.1	41.8
Nitzschia acicularis	D		10.1	7.5	10.0	13.7
Nitzschia palea	D	3.2	5.9	3.6	5.1	4.0
Cymbella kappii	D	5.9	2.7	3.3	3.7	2.7
Gomphonema parvulum	D	2.9	3.9	2.4	3.9	3.1
Diatoma vulgaris	D	4.6	2.4	1.6	2.6	
Melosira varians	D		2.1		3.2	1.9
Synedra ulna	D	2.7	2.6	4.2	2.4	3.2
Fragilaria capucina	D	2.9	2.2	2.4	3.0	2.4
Navicula cryptocephala	D		2.0	1.2	1.8	1.3
Navicula cf. margalithii	D	1.1	2.2	1.1	2.2	1.5
Gomphonema cf. minutum	D		2.1		2.0	1.6
Cymbella tumida	D	2.4	2.5	1.2	2.1	
Cocconeis placentula	D	1.2				
Achnanthidium minutissimum	D	2.4				
Tabellaria flocculosa	D	1.5				
Microspora sp.	GF	3.3				
Merismopedia sp.	С	2.2	3.4	4.3	8.0	6.7

 Table 3-3:
 Mean percentage (across the three sampling dates) in each treatment of periphyton taxa

 making up about 85% of the community.
 Under "Type", D = diatom, GF = green filament, C = cyanobacteria.



Figure 3-7: Non-metric multi-dimensional scaling plot showing differences in periphyton communities (relative abundance) over time and between treatments. The data shown are for all taxa. Using only diatoms produced a similar pattern. The stress value of 0.15 indicates that the data are reasonably well represented in two dimensions. Ellipses highlight groups that are separated and therefore potentially different.

The diatom data alone reflected the pattern in the whole periphyton community (Day, Global R = 0.919, P = 0.001; Treatment, Global R = 0.335, P = 0.001), as did the data based on functional groups (Day, Global R = 0.50, P = 0.001; Treatment, Global R = 0.343, P = 0.001). Pairwise comparisons also showed between-treatment differences similar to those for community composition based on all of the data (Table 3-4).

using all tax and indicate and 1 (mean	using all taxa, diatoms only, and diatoms functional groups. The R statistic is the main output from ANOSIM and indicates the strength of the difference. R can range from <0 (meaning effectively no groupings in the data) and 1 (meaning complete separation of the two groups). Shaded cells show significant differences (P < 0.05).												
		All perip	hyton	Diatom	s only	Diatom fun	ct. groups						
		R statistic	Р	R statistic	Ρ	R statistic	Р						
Control	DRP	0.654	0.002	0.654	0.002	0.704	0.005						
Control	P+NH <sub>4</sub> -N	0.802	0.002	0.802	0.002	0.765	0.001						
Control	P+NO₃-N	0.654	0.002	0.654	0.003	0.568	0.003						
Control	P+NO <sub>3</sub> +NH <sub>4</sub>	0.778	0.002	0.778	0.001	0.840	0.003						
DRP	P+NH4-N	0.358	0.012	0.358	0.008	0.247	0.074						
DRP	P+NO₃-N	-0.222	0.960	-0.222	0.956	-0.259	0.948						
DRP	P+NO <sub>3</sub> +NH <sub>4</sub>	0.012	0.472	0.012	0.471	-0.198	0.967						
P+NH <sub>4</sub> -N	P+NO <sub>3</sub> -N	0.346	0.026	0.346	0.014	0.333	0.019						
P+NH <sub>4</sub> -N	P+NO₃+NH₄	0.049	0.391	0.049	0.353	0.198	0.135						
P+NO₃-N	P+NO₃+NH₄	0.062	0.367	0.062	0.353	0.123	0.183						

Table 3-4: Pairwise comparisons following analysis of similarities (ANOSIM) on the periphyton community

#### 3.8 Periphyton abundance relative to biomass

Between Day 10 and Day 20, total cell abundance explained 83% and 84% of the variance in chlorophyll a and PN, respectively. Algal cells in the P-NH<sub>4</sub>-N treatment contained more chlorophyll a and PN per cell, on average, than cells in other treatments (ANCOVA, P < 0.05) (Figure 3-8).



Figure 3-8: Chlorophyll *a* concentrations in each replicate channel plotted against total counts of algal cells on Day 10 and Day 20. Data separated by treatment, with best fit least-squares regression lines. Combining all of the data,  $R^2 = 0.83$ . The equivalent plot for PN had a similar pattern (not shown).

### 3.9 Invertebrate communities

Sixty-one invertebrate taxa were identified from the 15 samples. Twenty taxa were rare (less than five individuals counted across all samples). The most common taxa were the Diptera (two-winged fly) species *Naonella forsythi* and *Cricotopus aucklandensis*, both of which belong to the collector-gatherer feeding group. The next most abundant taxon was the filter feeding caddis fly *Aoteapsyche* spp. These three taxa accounted for 51% of all the invertebrates counted. The ten most abundant taxa accounted for 89% of the total count. The number of taxa in each sample ranged from 29 to 36 (mean 32.7) and there was no statistically significant difference in taxa richness between treatments (KS-test, P > 0.05). Refer to Appendix C for the full list of taxa and a summary of counts.

Invertebrate community composition in the samples with added NH<sub>4</sub>-N separated from samples with no added N in a two-dimensional NMDS plot, and these groups of samples differed significantly

(ANOSIM, R = 0.333, P = 0.009). The samples receiving only NO<sub>3</sub>-N overlapped with the group receiving NH<sub>4</sub>-N and did not differ from them (ANOSIM, P > 0.05) (Figure 3-9).

Bar graphs of total invertebrate densities separated into feeding groups suggested higher total densities of invertebrates in the treatments enriched with N than in the control and DRP treatments (Figure 3-10). Two-sample KS tests confirmed that total invertebrate densities in the P+NH<sub>4</sub>-N treatments were higher than those in the control and DRP treatments. Variability among the three replicates meant that no other treatment pairs differed significantly. Collector-gatherers (which made up most of the invertebrate community) were significantly higher in both the P+NH<sub>4</sub>-N and P+NO<sub>3</sub>+NH<sub>4</sub> treatments than in the control and DRP treatments (KS tests, P = 0.000). Densities of collector-filterers were higher in the P+NH<sub>4</sub>-N and P+NO<sub>3</sub>+NH<sub>4</sub> treatments than in the DRP treatment (KS tests, P = 0.000). Densities of browsers did not differ among treatments.\

Three of the four invertebrate community indices calculated from each sample (MCI, QMCI, EPT index) did not differ between treatments. % EPT was lower in the P treatment than in the control or  $P+NO_3-N$  treatments, and was also higher in the  $P+NO_3-N$  than the  $P+NH_4-N$  treatment (KS-tests, P = 0), although neither of the latter two differed from the control.



**Figure 3-9:** Non-metric multi-dimensional scaling plot showing invertebrate communities (abundances) coded by treatment. The numbers next to each symbol are channel numbers. Control treatments were in channels 1, 8 and 18; P+NH<sub>4</sub>-N treatments were in channels 7, 14 and 20. Invertebrate communities in the channels receiving N (circled green and red symbols) are generally separated from communities in the control and DRP treatments (circled blue symbols), although the separation is not strong (e.g., channels 18 and 19 are close together, indicating similarity).



**Figure 3-10:** Bar graphs showing mean invertebrate community composition in each treatment by feeding group. Refer to Table 2-1 for explanations of the feeding groups.

#### 3.10 Invertebrate abundance relative to periphyton biomass

Total invertebrate density was positively related to peak chlorophyll *a* (as measured on Day 20) ( $R^2 = 0.32$ , P = 0.016) and also with PN on Day 20 ( $R^2 = 0.45$ , P = 0.004). These relationships were slightly stronger for densities of collector-gatherers only (chlorophyll *a*  $R^2 = 0.39$ , P = 0.009; PN  $R^2 = 0.52$ , P = 0.002) (Figure 3-11). Relationships between invertebrate densities (Day 27) and AFDM or TP (on Day 20) were not significant.

The relationships between total invertebrate and browser densities with chlorophyll *a* were weakened by one outlying value (indicated with red arrows on Figure 3-11). High invertebrate densities were recorded in this sample, but chlorophyll *a* was relatively low. The sample was from a  $P+NO_3-N$  treatment channel, located in the centre of the channel array (Figure 2-1). There was no obvious explanation for the outlier and it was retained in the regression. However, we note that chlorophyll *a* in the  $P+NO_3-N$  treatment was more variable than in other treatments towards the end of the experiment (as indicated by the large error bars in Figure 3-2).



**Figure 3-11:** Relationships between invertebrate densities and peak chlorophyll *a* and particulate N. Invertebrate data are counts made from samples collected on Day 27. Periphyton data were collected on Day 20. The top two plots show total densities; the lower two plots show densities of browsers only (see Table 2-1). The red arrows show an outlying value. Refer to text.

# 4 Discussion

### 4.1 Effects of NH<sub>4</sub>-N vs. NO<sub>3</sub>-N on chlorophyll *a* and PN

Preferential uptake of the reduced form of N (e.g.,  $NH_4^+$ ) in algae over oxidised forms of N has been recognised since the 1930s (see reviews by Syrett 1981; Glibert et al., 2016), and is interpreted as both a consequence of the lower energetic cost to cells of obtaining N in its reduced form and inhibition of NO<sub>3</sub><sup>-</sup> uptake in the presence of NH<sub>4</sub><sup>+</sup> (see below).<sup>5</sup> Once in the cell, NO<sub>3</sub><sup>-</sup> must be reduced to  $NH_4^+$  prior to assimilation, which requires the enzyme nitrate reductase and uses energy. Nitrate reductase activity is linked to photosynthesis in many algae, and so cannot occur in the dark (Clark et al. 2002). Early reports of preferential uptake of reduced N by algae have been confirmed by many observations that in a mixed medium, and N-limited conditions, laboratory cultures of algae take up  $NH_4^+$  first, and  $NO_3^-$  is taken up only when  $NH_4^+$  is depleted. Furthermore, uptake rates of  $NH_4^+$  can far exceed cell requirements following exposure to nitrogen-depleted conditions (McCarthy and Goldman 1979, Tapia et al. 1996, Rees 2007). Consequently, the primary finding in this experiment should not be unexpected; namely that DIN at a concentration of about 500 mg m<sup>-3</sup> comprising 77% NH<sub>4</sub>-N (i.e., the P+NH<sub>4</sub>-N treatment) appeared to stimulate chlorophyll *a* accumulation over time to a greater extent than DIN at the same concentration but comprising >99% NO<sub>3</sub>-N (i.e., the P+NO<sub>3</sub>-N treatment). Accrual rates were faster and peak chlorophyll a was more than 50% higher with 77%  $NH_4$ -N than with <1%  $NH_4$ -N.

Despite the well-documented differential uptake rates of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> by algal cells, as far as we are aware, a direct stimulatory effect of NH<sub>4</sub>-N, compared to NO<sub>3</sub>-N, has not previously been demonstrated for periphyton chlorophyll *a* in fresh waters, although the effect has been demonstrated experimentally for phytoplankton in lakes (Donald et al. 2011, 2013). In streams, differential responses by periphyton to NH<sub>4</sub>-N and NO<sub>3</sub>-N enrichment have been investigated in several nutrient diffusing substrate (NDS) experiments. In these studies, either no biomass responses were detected (Kutka and Richards 1997, Hollein et al. 2010), or apparent depression of biomass by high NH<sub>4</sub>-N was observed (Ribot et al. 2015). In the present experiment, periphyton receiving N as 77% NH<sub>4</sub>-N also contained more N per unit of AFDM and also more chlorophyll *a* and PN per algal cell than periphyton receiving <1% NH<sub>4</sub>-N. These observations suggest higher rates of N uptake when the N source was primarily NH<sub>4</sub>-N rather than NO<sub>3</sub>-N, although the experiment was designed to compare biomass responses rather than uptake.

The preferential uptake of  $NH_4^+$  over  $NO_3^-$  is largely driven by suppression of the assimilation of  $NO_3^-$  through inhibition of nitrate reductase production (Syrett 1981 and references therein) in the presence of  $NH_4^+$ . The concentrations of  $NH_4$ -N above which  $NO_3^-$  usage by cells is suppressed have often been assumed to be around 15-30 mg m<sup>-3</sup>  $NH_4$ -N (Glibert et al. 2016). Dugdale et al. (2007) observed a threshold of ~60 mg m<sup>-3</sup> above which diatom-dominated phytoplankton blooms did not develop. However, thresholds for inhibition of  $NO^{3^-}$  uptake can be much higher (e.g., >400 mg m<sup>-3</sup>, Maestrini et al. 1986; >200 mg m<sup>-3</sup>, Dortch 1990). Studies in marine environments showed that  $NO_3^-$  uptake inhibition is  $NO_3$ -N concentration dependent (e.g., Harrison et al. 1996), summarised by Glibert et al. (2016) as:

"Cells growing on highly elevated  $NO_3$ -N concentrations, as in the case of a nutrient-rich environment may require considerably more  $NH_4$  to repress cellular  $NO_3$  activity than is

<sup>&</sup>lt;sup>5</sup> In this discussion, the abbreviations NO<sub>3</sub>-N and NH<sub>4</sub>-N are used when referring to concentrations of the N in the two different forms. When referring to uptake and assimilation, the notation for the nitrate and ammonium ions (NO3- and NH4+) is used because the ions are transported into the cell and subsequently assimilated.

the case for a cell with a low cellular  $NO_3$  content, as in oligotrophic environments" (Glibert et al. 2016, p. 169).

Related to the present experimental results, the above observations from previous studies are consistent with highest rates of inhibition of NO<sub>3</sub><sup>-</sup> uptake in the treatment with 77% NH<sub>4</sub>-N (i.e., lower NO<sub>3</sub>-N relative to NH<sub>4</sub>-N). Presumably both the high concentration and ratio of NH<sub>4</sub>-N to NO<sub>3</sub>-N led to more rapid uptake of NH<sub>4</sub>-N (with associated faster growth and PN accumulation) than in the treatment with 30% NH<sub>4</sub>-N. A relatively cool water temperature may have further reduced the inhibitory effect of NH<sub>4</sub>-N at 30% of N on NO<sub>3</sub><sup>-</sup> uptake because NO<sub>3</sub><sup>-</sup> uptake inhibition is temperature dependent, with lower rates of inhibition at lower temperatures (Lomas and Glibert 1999). Water temperature over the course of the experiment was (13.8°C) and in the middle of the range of  $4 - 20^{\circ}$ C tested by Lomas and Glibert (1999). The physiological explanation for reduced inhibition is that nitrate reductase activity has an inverse relationship with temperature over the range from 25°C to 12°C (Berges et al. 2002, Glibert et al. 2016). Increased rates of enzyme activity in the lower temperature range counteract the inhibitory effect of NH<sub>4</sub><sup>-</sup>, the uptake of which increases as temperature increases (Lomas and Glibert 1999b).

# 4.2 Effects of NH<sub>4</sub>-N vs. NO<sub>3</sub>-N on periphyton community composition

Research over the past two decades has clarified that different algal groups and species have different responses to NH<sub>4</sub>-N and NO<sub>3</sub>-N as sources of N. For example, Lomas and Glibert (1999) identified stronger NH<sub>4</sub><sup>+</sup>suppression of NO<sub>3</sub><sup>-</sup> uptake in dinoflagellates than in diatoms but in both groups inhibition rates were lowest at cool temperatures. In enclosure experiments in a lake, NH<sub>4</sub>-N additions to phosphorus-rich water resulted in higher biomass (compared to a control) than NO<sub>3</sub>-N additions, and there were also many species-specific responses, with *Planktothrix* and *Phormidium* (non-N-fixing cyanobacteria), dominating assemblages enriched with NH<sub>4</sub>-N, and *Cyclotella* (a diatom) dominating following NO<sub>3</sub>-N additions (Donald et al. 2011, 2013). Glibert et al. (2016) summarised literature demonstrating that diatoms tend to be "NO<sub>3</sub>-opportunists", whereas very small-sized picoplankton (including cyanobacteria) tend to respond to N-enrichment by NH<sub>4</sub>-N.

In addition to different responses by major algal groups, species-specific responses to  $NH_4-N$  vs.  $NO_3-N$  have been reported in earlier studies for diatom-dominated communities, such as those reported in the present experiment. For example, growth rates of the freshwater taxa *Cyclotella menghiniana* and *Nitzchia* sp. were more strongly negatively affected by  $NH_4-N$  additions than the stalked diatom *Gomphonema parvulum* (Zhang et al. 2013). A preference by *G. parvulum* for  $NH_4^+$  and by *Cyclotella* sp. to  $NO_3^+$  was also reported by Kutka et al. (1997) in nutrient diffusing experiments. In the present study, diatoms dominated in all nutrient treatments, and, consistent with Zhang et al. (2013), different proportions of diatoms led to a significant difference in periphyton community composition between channels receiving  $NH_4-N$  as 77% and <1% of N supply. Variability among replicates meant that no significant difference in individual species could be identified, but community difference was driven largely by markedly higher densities of small-sized algal taxa (< 25 µm in diameter) in the communities supplied with 77%  $NH_4-N$  than with <1%  $NH_4-N$  and <1%  $NH_4-N$ , suggesting some congruity of response to  $NH_4-N$  across the gradient of 30% to 77%  $NH_4-N$ .

# 4.3 The overall effect of added nutrients

While mean chlorophyll *a* concentration was significantly higher in periphyton receiving DIN with 77%  $NH_4$ -N than in the periphyton receiving DIN with <1%  $NH_4$ -N and in the control treatment, it was

surprising that no differences were detected among the other pairs of treatments. Nutrient concentrations in the control treatment of 112 mg m<sup>-3</sup> for DIN and 0.9 mg m<sup>-3</sup> for DRP would generally be expected to limit periphyton growth so that adding N and P would stimulate chlorophyll *a* (e.g., Keck and Lepori 2012, but see Tank and Dodds 2003 for a discussion). The issue may be one of low statistical power to detect significant differences as a result of an experimental design with only three replicates, given that the mean values of peak chlorophyll *a* followed the expected pattern of lowest in the control and DRP treatments (see Appendix A). Nevertheless, use of only three replicate channels is generally an acceptable design in field experiments (Underwood 2009). Furthermore, the fact that a difference was detected between the P+NH<sub>4</sub>-N and P+NO<sub>3</sub>-N treatments, despite only three replicates, suggests that the difference was meaningful, at least under the particular conditions of the experiment.

In contrast to chlorophyll a, periphyton biomass measured as AFDM followed a more predictable pattern. AFDM in the control treatment was evidently limited by both DRP and DIN supply, as indicated by increased AFDM concentration when both DRP and DIN were added, but no difference from the control with P-enrichment only. A clear pattern in the experiment was that the equivalent amount of AFDM in the treatment receiving 77% NH<sub>4</sub>-N contained significantly more chlorophyll a and PN than all the other treatments. AFDM is usually strongly correlated with chlorophyll a (Rodman and Scott 2017), as it was in this study, across all treatments and time. However, discrepancies can occur as a result differing periphyton community composition (e.g., Kilroy et al. 2015), and different rates of senescence of algae in the mat (Saunders et al. 2016). In this case, a combination of these two processes could have operated. First, diatoms produce less copious mucilage (which adds to AFDM) under nutrient-replete conditions (Alcoverro et al. 2000). Under these conditions small, chloroplast-rich diatoms, with a high surface area to volume ratio (i.e., high capacity for N uptake relative to their size) were apparently favoured (e.g., Sunda and Hardison 2007). Second, rapid uptake of  $NH_4^+$  could have maintained a thicker layer of live cells than in the treatments receiving predominantly  $NO_3^-$ , leading to higher chlorophyll *a* and higher TN concentration relative to AFDM. Photographs taken during the experiment show clear differences between treatments in mat density and cover. Refer to Appendix D for examples.

In the experiment, the levels of nutrient addition were intended to create saturated conditions for periphyton growth. Calculated accrual rates (for chlorophyll a) between Day 3 and Day 20 indicated relatively rapid growth in all treatments. For example, the range of accrual rates in this experiment  $(0.26 - 0.30 d^{-1})$  exceeded the maximum accrual rates reported under DIN and DRP saturation for AFDM (0.24 d<sup>-1</sup>) by Hill and Fanta (2008). Bothwell (1988) developed a relationship for maximum cellular growth rates for diatoms, under replete nutrient conditions, as a function of temperature<sup>6</sup>. Comparing the calculated maximum with the net accrual rate when N was supplied as 77% NH<sub>4</sub>-N showed that the rate was almost 75% of the theoretical maximum. In the control and DRP treatments, accrual rates were about 65% of the theoretical maximum, and were 68% and 70% in the treatments receiving<1% and 30% of DIN as NH<sub>4</sub>-N, respectively. The differences between treatments were therefore small, but over a period of 17 days resulted in large differences in chlorophyll a concentration. Because the accrual rates were net of any losses due to invertebrate grazing or cell sloughing, the rates observed in the DIN-enriched treatments very likely indicate nutrient-saturated growth rates, but with the channels receiving N as 77% NH<sub>4</sub>-N supporting a higher growth rate. Furthermore, cellular PN : PP ratios on Day 20, were consistent with P-limitation in the control treatment, N-limitation in the P treatment, and neither N- nor P-limitation (or co-limitation)

Stimulation of river periphyton growth by ammoniacal-N vs. nitrate-N

<sup>&</sup>lt;sup>6</sup> The relationship is: maximum growth rate (cell divisions per day) = 0.189 + (0.0278\*mean temperature) (Bothwell 1988).

in all the treatments with added N, using the criteria: <7:1 (N-limited), between 7:1 and 15:1 (colimited), and >15:1 (P-limited) (McDowell et al. 2009). These ratios reflected N : P ratios in the overlying water (see Appendix A).

#### 4.4 Nutrient enrichment and invertebrate production

The purpose of assessing invertebrate communities in the experiment was to determine whether additions of  $NH_4$ -N at high concentrations would impact on invertebrate communities by either reducing densities or changing community composition to favour more tolerant taxa, through the toxic effects of disassociated  $NH_3$  (Camargo and Alosno 2006). In particular, reduced densities of invertebrates that consume algae could indirectly lead to higher periphyton biomass in treatments with high concentrations of  $NH_4$ -N.

Invertebrate grazing rates in the experimental channels are not known, but we observed few invertebrates on the periphyton growth surfaces during the course of the experiment. This suggested that, in this experiment, the periphyton responses observed were a direct result of the nutrient enrichment. However, effects on invertebrates in the channel areas with more suitable invertebrate habitat become relevant when interpreting the results with reference to what might occur in a river. Invertebrate grazing almost always exerts a negative effect on periphyton biomass (Liess and Hillebrand 2004), and that effect can be substantial. For example, in one experiment, excluding grazers resulted in increased periphyton biomass of >55% (Taylor et al. 2002). In a very productive stream, invertebrate grazing reduced periphyton up to 60-fold over an accrual period of 16 days (Sturt et al. 2011).

In the present experiment, grazing taxa (browsers) made up from 5 to 15% of the invertebrate community, and browser densities did not differ between treatments. There was some evidence for slightly lower quality invertebrate communities in the enriched treatment with 77% NH<sub>4</sub>-N compared to <1% NH<sub>4</sub>-N (from differences in %EPT). However, the main difference between treatments was that channels enriched with both P and N had total higher invertebrate densities than control channels or those enriched only with P. Furthermore, total invertebrate densities were positively correlated with peak chlorophyll a and PN, suggesting that the effect of enrichment with DIN and DRP was to increase invertebrate productivity in tandem with periphyton productivity, possibly though improved food quality at the higher DIN concentrations (Liess et al. 2012). Higher invertebrate densities occurred regardless of the source of N ( $NH_4$ -N or  $NO_3$ -N). We therefore conclude that the concentrations of NH<sub>4</sub>-N tested in the experiment did not impact on invertebrates in a way that could have indirectly led to increased periphyton biomass through suppression of grazing. Furthermore, toxic effects of NH<sub>4</sub>-N on invertebrates were unlikely in any case, because adding NH<sub>4</sub>-N slightly reduced the pH of the water, which would reduce toxicity. The toxic effect of NH<sub>4</sub>-N depends on the proportion of measured N that is free ammonia in its unionised form (i.e., NH<sub>3</sub> rather than NH<sub>4</sub><sup>+</sup>). The two forms separate in a predictable way depending on pH, water temperature and salinity. A quick rule of thumb is that the ratio of  $NH_3$  to  $NH_4^+$  increases 10-fold with every 1 pH unit increase and two-fold with every 10°C increase in water temperature between 0 and 30°C (Erikson 1985).

# 5 Implications for river health downstream of WWTP discharges

In relating the results of the experiment back to the original question (*Do increasing concentrations or proportions of ammoniacal nitrogen* (*NH*<sub>4</sub>-*N*) *in river waters below the levels known to be toxic to aquatic life lead to faster periphyton growth and greater biomass than equivalent increases in nitrate-nitrogen* (*NO*<sub>3</sub>-*N*)?), it is important to remember that this experiment was just one trial carried out under a particular set of conditions. Therefore, as with all experiments, extrapolation to "real life" should be treated with caution (e.g., Carpenter 1996, Spivak et al. 2011, but see Benton et al. 2007).

Some realism in experimental channels was provided by an outdoor setting and also a time-scale for periphyton accrual (3 – 4 weeks) that approached typical accrual time in some rivers (Biggs 2000). We also took account of the potential effects of UV light in the shallow water of the experimental channels (by excluding UV light), recognising that UV effects are unlikely to be significant in the deeper waters of large rivers (Frost et al. 2005). However, the variable hydraulic conditions in large rivers cannot easily be replicated at a small scale. In addition, the values of chlorophyll *a* attained were higher than would be expected even in enriched rivers because of the sampling method, which targeted small patches of algae. Nevertheless, the general responses of periphyton chlorophyll *a* we observed can easily be reconciled with what is already known about the effect of NH<sub>4</sub>-N vs. NO<sub>3</sub>-N on algae, based on substantial research dating back over decades (see Section 4.1).

The experimental treatments and results can be reviewed relative to observations upstream and downstream of WWTPs in the Manawatu-Whanganui region using data from about 2009 to 2015. The target DIN concentration of 500 mg m<sup>-3</sup> was selected for the experiment because this concentration was within the range of DIN measured at sites downstream of WWTPs (Table 5-1).

Median chlorophyll *a* concentrations for seven of the eight WWTPs listed in Table 5-1 were significantly higher at the downstream site than at the upstream site, and at all seven sites at least two of median NH<sub>4</sub>-N, NO<sub>3</sub>-N and DRP concentrations were also significantly higher at the downstream site. NH<sub>4</sub>-N increased appreciably (i.e., to at least 50 mg m<sup>-3</sup>, with a final percentage of DIN of >30%) at downstream sites compared to upstream at Manawatu @ PNCC, Mangatera @ Dannevirke, Mangawhero @ Ohakune, and Oroua @ Feilding.

In view of the finding in the experiment that DIN with 30% was starting to show some effect in stimulating chlorophyll *a* compared to the same DIN supplied as NO<sub>3</sub>-N, then periphyton growth at three of these sites (Manawatu @ PNCC, Mangawhero @ Ohakune, and Oroua @ Feilding) may be affected by  $NH_4$ -N.

The exception to the above was Mangatera @ Dannevirke. Here, there was a very large increase between the upstream and downstream sites in median concentrations of DRP and NH<sub>4</sub>-N. Given that DRP at the upstream site was already very high (> 40 mg m<sup>-3</sup> on average) and above concentrations thought to saturate periphyton growth (~ 28 mg m<sup>-3</sup>, Bothwell 1989), it is possible that increased DIN was responsible for the increase in biomass rather than increased DRP. Further investigation would be needed to confirm this. In all cases it would be informative to carry out studies to determine whether either DIN or DRP limits periphyton growth at the upstream sites (assuming this information is not already available).

In all cases, warmer temperatures than the mean of  $13.8^{\circ}$ C recorded in our experiment would be expected to accentuate the stimulatory effect of NH<sub>4</sub>-N on periphyton (Lomas and Glibert 1999).

Table 5-1:NO<sub>3</sub>-N, NH<sub>4</sub>-N and DRP concentrations in discharges upstream and downstream of WWTPs inthe Manawatu-Whanganui region.Data are medians calculated from at least six years of monthly data. Thechlorophyll a data shown are  $92^{nd}$  percentiles of monthly data over the same period. Grey-shaded cells indicatesignificant differences between upstream (us) and downstream (ds) sites (paired t-tests, P < 0.05). Table</td>adapted from Kilroy (2016).

	Site	NH <sub>4</sub> -N	NO <sub>3</sub> -N	DIN	DRP	% NH <sub>4</sub> -N	Chlor. <i>a</i> (mg/m <sup>2</sup> )
Makotuku @ Raetihi	us	11	370	381	9	2	140
	ds	43	368	411	17	12	238
Manawatu @ PNCC	us	5	401	406	12	3	69
	ds	190	409	599	19	31	315
Mangatainoka	us	3	859	868	7	1	90
	ds	13	869	882	9	1	122
Mangatera @ Dannevirke	us	16	456	472	41	4	40
	ds	417	594	1011	176	34	96
Mangawhero @ Ohakune	us	8	137	145	14	6	50
	ds	50	152	202	21	31	75
Oroua @Feilding	us	50	267	317	15	24	33
	ds	500	779	1279	16	40	136
Porewa @ Hunterville	us	2	12	14	17	5	152
	ds	5	88	93	16	13	161
Waitangi @ Waiouru	us	7	262	269	33	3	91
	ds	78	371	449	60	16	185

A further aspect of the story is that periphyton in rivers downstream of WWTPs can be an effective means of removing excess N from the overlying water (Ogura et al. 2009, Ribot et al. 2012, 2013). However, removal capacity may be confounded by the fact that very high DIN (both NO<sub>3</sub>-N and NH<sub>4</sub>-N) concentrations can suppress periphyton growth (e.g., Ribot et al. 2015) and this has implications for the export of DIN downstream (Mulholland et al. 2008). The threshold(s) at which this effect begins are unclear.

# 6 Summary and conclusions

The main findings of the experiment were:

- enrichment of the water supply to periphyton growth surfaces in experimental channels with DRP (from < 1 to > 20 mg m<sup>-3</sup>) and DIN (from 110 to ~500 mg m<sup>-3</sup>) had different effects on periphyton biomass depending on the proportion of DIN that comprised NH<sub>4</sub>-N rather than NO<sub>3</sub>-N;
- under 77% NH<sub>4</sub>-N, periphyton chlorophyll *a* developed faster and, at its peak, was about 50% higher than under <1% NH<sub>4</sub>-N (i.e., 99% NO<sub>3</sub>-N). The periphyton growing with 77% NH<sub>4</sub>-N had more chlorophyll *a* and PN per unit weight of AFDM and per algal cell than periphyton with <1% NH<sub>4</sub>-N;
- periphyton grown with 30% NH<sub>4</sub>-N was generally intermediate between the <1% and 77% NH<sub>4</sub>N treatments (e.g., in chlorophyll *a* and PN) and few comparisons showed significant differences;
- AFDM was higher in all the DIN-enriched treatments than in the control and DRP treatments, but did not differ between them; the discrepancy between biomass as chlorophyll *a* and biomass as AFDM was likely caused by shifts in periphyton community composition as the proportion of NH<sub>4</sub>-N changed;
- periphyton community composition differed among treatments, although in all cases communities were dominated by diatoms;
- periphyton communities growing under both 30% and 77% NH<sub>4</sub>-N had higher proportions of small-sized algae than the other treatments, and under 77% NH<sub>4</sub>-N these algae had more chlorophyll *a* and PN per cell than in the other treatments;
- differences in accrual rates of chlorophyll *a* and ratios of PN : PP between all DINenriched treatments and the control and DRP treatments suggested that, at background concentrations, P and N both limited periphyton growth, even though low statistical power meant that corresponding differences in biomass as chlorophyll *a* could not be detected; and
- the highest NH<sub>4</sub>-N concentration applied in the treatments did not appear to negatively affect invertebrate production (through toxic effects), and there was no evidence that increased chlorophyll *a* in periphyton grown with 77% NH<sub>4</sub>-N was a consequence of lower invertebrate grazing pressure.

The results were reviewed relative to nutrient concentrations upstream and downstream of WWTP discharges in the Manawatu-Whanganui region. Based on the available data, we could not definitively attribute increased chlorophyll *a* at the downstream sites to the effect of NH<sub>4</sub>-N rather than DRP, but it was a possibility for some WWTP discharges. In all cases, it would be informative to carry out studies to determine whether either DIN or DRP limits periphyton growth at the upstream sites.

The overall conclusion from the experiment was "yes", increasing concentrations or proportions of ammoniacal nitrogen ( $NH_4$ -N) in river waters below the levels known to be toxic to aquatic life do lead to faster periphyton growth and greater biomass than equivalent increases in nitrate-nitrogen ( $NO_3$ -

*N*). This is a qualified "yes" because: (a) the main biomass variable affected was chlorophyll *a* and not ash-free dry mass; and (b) the discrepancy between the two biomass measures is likely because changing the source of N (NO<sub>3</sub>-N or NH<sub>4</sub>-N) also led to changes in periphyton community composition. Under high NH<sub>4</sub>-N we observed higher concentrations of small diatoms, with high chlorophyll *a* and particulate N content. Finally, we note that these observations apply to the particular conditions of the experiment; outcomes may vary under different conditions, especially under different water temperatures.

# 7 Acknowledgements

This report was funded by the MBIE Envirolink Fund, in a project sponsored by Horizons Regional Council (Envirolink Medium Advice Grant contract C01X1709). Funding to run the experiment was jointly provided by Horizons Regional Council, Northland Regional Council, Greater Wellington Regional Council, Waikato Regional Council, Tararua District Council, Ruapehu District Council, and Palmerston North City Council. Initial discussions with Logan Brown, Horizons Regional Council, were appreciated.

The successful running of the experiment was largely due to help from Neil Barr, Bruce Dudley and Brendan Smith (NIWA) in optimising the performance of the experimental channels. Brendan Smith implemented improvements to the apparatus, which were funded through NIWA's Sustainable Water Allocation research programme.

We thank the NIWA, Hamilton, Water Quality Laboratory for nutrient analyses, and Colin Grace (NIWA Instrument Systems) for supply and calibration of the Water Quality sondes. Thanks also to Dara Dunlop (University of Birmingham) for assistance with preparing the pavers for the experiment and general laboratory and field help.

Appropriate permissions were obtained for installing and running the channels, including for discharging surplus enrichment additions into the stockwater race (i.e., Selwyn District Council, Canterbury Regional Council). We are grateful to Rhys Cleland, Annavale Station, Springfield for permission to operate the channels on Annavale Station property.

Review comments on earlier versions of the report by Juliet Milne and Clive Howard-Williams were much appreciated.

#### 8 References

- Aber, J.D., Ollinger, S.V., Driscoll, C.T., Likens, G.E., Holmes, R.T., Freuder, R.J., Goodale, C.L. (2002) Inorganic nitrogen losses from a forested ecosystem in response to physical, chemical, biotic, and climatic perturbations. *Ecosystems*, 5(7): 648-658.
- Alcoverro, T., Conte, E., Mazzella, L. (2000) Production of mucilage by the Adriatic epipelic diatom *Cylindrotheca closterium* (Bacillariophyceae) under nutrient limitation. *Journal of Phycology*, 36(6): 1087-1095.
- Benton, T.G., Solan, M., Travis, J.M.J., Sait, S.M. (2007) Microcosm experiments can inform global ecological problems. *Trends in Ecology & Evolution*, 22(10): 516-521.
- Berges, J.A., Varela, D.E., Harrison, P.J. (2002) Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). *Marine Ecology Progress Series*, 225: 139-146.
- Biggs, B.J.F. (2000) Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society*, 19: 17-31.
- Biggs, B.J.F., Kilroy, C. (2000) *Stream Periphyton Monitoring Manual*. Published by NIWA for MfE.
- Bothwell, M., Sherbot, D., Roberge, A., Daley, R. (1993) Influence of natural ultraviolet radiation on lotic periphyton community growth, biomass accrual, and species composition: short-term versus long-term effects. *Journal of Phycology*, 29: 24-35.
- Bothwell, M.L., Sherbot, D.M.J., Pollock, C.M. (1994) Ecosystems response to solar ultraviolet-B radiation: Influence of trophic-level interactions. *Science*, 265(1 July 1994): 97 - 100.
- Bothwell, M.L. (1988) Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: The influence of temperature and light. *Canadian Journal of Fisheries and Aquatic Sciences* 45: 261 270.
- Bothwell, M.L. (1989) Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences*, 46: 1293-1301.
- Camargo, J.A., Alonso, A. (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, 32(6): 831-849.
- Carpenter, S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology*, 77(3): 677-680.
- Chaffin, J.D., Bridgeman, T.B. (2014) Organic and inorganic nitrogen utilization by nitrogenstressed cyanobacteria during bloom conditions. *Journal of Applied Phycology*, 26(1): 299-309.

- Clark, D.R., Flynn, K.J., Owens, N.J.P. (2002) The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. *New Phytologist*, 155(1): 101-108.
- Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M. (2014) Change in marine communities: an approach to statistical analysis and interpretation, 3rd edition. PRIMER-E: Plymouth.
- Collier, K.J., Wilcock, R.J., Meredith, A.S. (1998) Influence of substrate type and physicochemical conditions on macroinvertebrate faunas and biotic indices of some lowland Waikato, New Zealand, streams. *New Zealand Journal of Marine and Freshwater Research*, 32: 1-19.
- Collos, Y., Harrison, P.J. (2014) Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Marine Pollution Bulletin*, 80(1-2): 8-23.
- Donald, D.B., Bogard, M.J., Finlay, K., Bunting, L., Leavitt, P.R. (2013) Phytoplankton-Specific Response to Enrichment of Phosphorus-Rich Surface Waters with Ammonium, Nitrate, and Urea. *PLOS one*, 8(1): 14. 10.
- Donald, D.B., Bogard, M.J., Finlay, K., Leavitt, P.R. (2011) Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnology and Oceanography*, 56(6): 2161-2175.
- Dortch, Q. (1990) The interaction between ammonium and nitrate uptake in phytoplankton. *Marine Ecology Progress Series*, 61(1-2): 183-201.
- Dugdale, R.C., Wilkerson, F.P., Hogue, V.E., Marchi, A. (2007) The role of ammonium and nitrate in spring bloom development in San Francisco Bay. *Estuarine Coastal and Shelf Science*, 73(1-2): 17-29.
- Dymond, J.R., Ausseil, A.G.E., Parfitt, R.L., Herzig, A., McDowell, R.W. (2013) Nitrate and phosphorus leaching in New Zealand: a national perspective. *New Zealand Journal of Agricultural Research*, 56(1): 49-59.
- Erickson, R.J. (1985) An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms. *Water Research*, 19(8): 1047-1058.
- Figueroa-Nieves, D., McDowell, W.H., Potter, J.D., Martinez, G. (2016) Limited uptake of nutrient input from sewage effluent in a tropical landscape. *Freshwater Science*, 35(1): 12-24.
- Figueroa-Nieves, D., W. H. McDowell, J. D. Potter, G. Martinez, and J. R. Ortiz-Zayas. 2014. Effects of sewage effluents on water quality in tropical streams. *Journal of Environmental Quality*, 43:2053–2063.
- Frost, P.C., Larson, J.H., Kinsman, L.E., Lamberti, G.A., Bridgham, S.D. (2005) Attenuation of ultraviolet radiation in streams of northern Michigan. *Journal of the North American Benthological Society*, 24(2): 246-255.

- Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M. (2016) Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnology and Oceanography*, 61(1): 165-197.
- Hamill, K.D. (2013) Processes driving periphyton growth in the Manawatu River and implications for wastewater treatment. River Lake Ltd. Prepared for Palmerston North City Council.

http://www.pncc.govt.nz/media/2163129/pncc\_wwtp\_investigations\_2013\_revised\_v2signed.pdf.

- Harrison, W.G., Harris, L.R., Irwin, B.D. (1996) The kinetics of nitrogen utilization in the oceanic mixed layer: Nitrate and ammonium interactions at nanomolar concentrations. *Limnology and Oceanography*, 41(1): 16-32.
- Hill, W.R., Fanta, S.E. (2008) Phosphorus and light colimit periphyton growth at subsaturating irradiances. *Freshwater Biology*, 53(2): 215-225.
- Hoellein, T.J., Tank, J.L., Kelly, J.J., Rosi-Marshall, E.J. (2010) Seasonal variation in nutrient limitation of microbial biofilms colonizing organic and inorganic substrata in streams. *Hydrobiologia*, 649(1): 331-345.
- Keck, F., Lepori, F. (2012) Can we predict nutrient limitation in streams and rivers? *Freshwater Biology*, 57(7): 1410-1421.
- Kilroy, C. (2016) The effects of increases in ammoniacal-N on river periphyton: A literature review and proposed experiment. *NIWA Client Report* CHC2015\_044. Envirolink-funded report for Horizons Regional Council. 45 p.
- Kilroy, C., Wech, J. (2015) Spatial and temporal patterns in nutrient concentrations and periphyton in the Hurunui River. *NIWA Client Report* CHC2015\_086. For Ngai Tahu Forest Estates Ltd. 96 p.
- Kutka, F.J., Richards, C. (1997) Short-term nutrient influences on algal assemblages in three rivers of the Minnesota River basin. *Journal of Freshwater Ecology*, 12(3): 411-419.
- Liess, A., Hillebrand, H. (2004) Invited review: Direct and indirect effects in herbivore periphyton interactions. *Archiv Fur Hydrobiologie*, 159(4): 433-453.
- Liess, A., Le Gros, A., Wagenhoff, A., Townsend, C.R., Matthaei, C.D. (2012) Landuse intensity in stream catchments affects the benthic food web: consequences for nutrient supply, periphyton C:nutrient ratios, and invertebrate richness and abundance. *Freshwater Science*, 31(3): 813-824.
- Lomas, M.W., Glibert, P.M. (1999) Interactions between NH4+ and NO3- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. *Marine Biology*, 133(3): 541-551.
- Lomas, M.W., Glibert, P.M. (1999) Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. *Limnology and Oceanography*, 44(3): 556-572.

- Maestrini, S.Y., Robert, J.M., Leftley, J.W., Collos, Y. (1986) Ammonium thresholds for simultaneous uptake of ammonium and nitrate by oyster-pond algae. *Journal of Experimental Marine Biology and Ecology*, 102(1): 75-98.
- McCarthy, J.J., Goldman, J.C. (1979) Nitrogenous nutrition of marine-phytoplankton in nutrient-depleted waters. *Science*, 203(4381): 670-672.
- McDowell, R.W., Larned, S.T., Houlbrooke, D.J. (2009) Nitrogen and phosphorus in New Zealand streams and rivers: control and impact of eutrophication and the influence of land management. *New Zealand Journal of Marine and Freshwater Research*, 43(4): 985-995.
- Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O., Hamilton, S.K., Peterson, B.J., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Findlay, S.E.G., Gregory, S.V., Grimm, N.B., Johnson, S.L., McDowell, W.H., Meyer, J.L., Valett, H.M., Webster, J.R., Arango, C.P., Beaulieu, J.J., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Johnson, L.T., Niederlehner, B.R., O'Brien, J.M., Potter, J.D., Sheibley, R.W., Sobota, D.J., Thomas, S.M. (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature*, 452(7184): 202-205.
- New Zealand Government (2017) National Policy Statement for Freshwater Management 2014 (Amended 2017). <u>http://www.mfe.govt.nz/publications/fresh-water/national-policy-statement-freshwater-management-2014-amended-2017</u>.
- Ogura, A., Takeda, K., Nakatsubo, T. (2009) Periphyton contribution to nitrogen dynamics in the discharge from a wastewater treatment plant. *River Research and Applications*, 25(2): 229-235.
- Parfitt, R.L., Frelat, M., Dymond, J.R., Clark, M., Roygard, J. (2013) Sources of phosphorus in two subcatchments of the Manawatu River, and discussion of mitigation measures to reduce the phosphorus load. *New Zealand Journal of Agricultural Research*, 56(3): 187-202.
- Parker, A.E., Dugdale, R.C., Wilkerson, F.P. (2012) Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary. *Marine Pollution Bulletin*, 64(3): 574-586.
- Passy, S.I. (2007) Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany*, 86(2): 171-178.
- Rees, T.A.V. (2007) Metabolic and ecological constraints imposed by similar rates of ammonium and nitrate uptake per unit surface area at low substrate concentrations in marine phytoplankton and macroalgae. *Journal of Phycology*, 43(2): 197-207.
- Ribot, M., Marti, E., von Schiller, D., Sabater, F., Daims, H., Battin, T.J. (2012) Nitrogen processing and the role of epilithic biofilms downstream of a wastewater treatment plant. *Freshwater Science*, 31(4): 1057-1069.
- Ribot, M., von Schiller, D., Peipoch, M., Sabater, F., Grimm, N.B., Marti, E. (2013) Influence of nitrate and ammonium availability on uptake kinetics of stream biofilms. *Freshwater Science*, 32(4): 1155-1167.

- Ribot, M., von Schiller, D., Sabater, F., Marti, E. (2015) Biofilm growth and nitrogen uptake responses to increases in nitrate and ammonium availability. *Aquatic Sciences*, 77(4): 695-707.
- Rodman, A.R., Scott, J.T. (2017) Comparing two periphyton collection methods commonly used for stream bioassessment and the development of numeric nutrient standards. *Environmental Monitoring and Assessment*, 189(7): 14.
- Roygard, J.K.F., McArthur, K.J., Clark, M.E. (2012) Diffuse contributions dominate over point sources of soluble nutrients in two sub-catchments of the Manawatu River, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 46(2): 219-241.
- Saunders, L.L., Kilham, S.S., Verb, R. (2016) Diatom responses to microenvironment structure within metaphyton mats. *Inland Waters*, 6(2): 224-233.
- Spivak, A.C., Vanni, M.J., Mette, E.M. (2011) Moving on up: can results from simple aquatic mesocosm experiments be applied across broad spatial scales? *Freshwater Biology*, 56(2): 279-291.
- Stark, J.D. (1998) SQMCI: A biotic index for freshwater macroinvertebrate coded-abundance data. *New Zealand Journal of Marine and Freshwater Research*, 32(1): 55-66.
- Stark, J.D., Maxted, J. (2007) A user guide for the Macroinvertebrate Community Index. Prepared for the Ministry for the Environment. Cawthron Report No. 1166, 58 p.
- Sturt, M.M., Jansen, M.A.K., Harrison, S.S.C. (2011) Invertebrate grazing and riparian shade as controllers of nuisance algae in a eutrophic river. *Freshwater Biology*, 56(12): 2580-2593.
- Sunda, W.G., Hardison, D.R. (2007) Ammonium uptake and growth limitation in marine phytoplankton. *Limnology and Oceanography*, 52(6): 2496-2506.
- Syrett, P.J. (1981) Nitrogen metabolism of microalgae. *Canadian Bulletin of Fisheries and Aquatic Sciences* (210): 182-210.
- Tank, J.L., Dodds, W.K. (2003) Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology*, 48(6): 1031-1049.
- Tapia, M.I., deAlda, J., Llama, M.J., Serra, J.L. (1996) Changes in intracellular amino acids and organic acids induced by nitrogen starvation and nitrate or ammonium resupply in the cyanobacterium *Phormidium laminosum*. *Planta*, 198(4): 526-531.
- Taylor, B., McIntosh, A., Peckarsky, B. (2002) Reach-scale manipulations show invertebrate grazers depress algal resources in streams. *Limnology and Oceanography*, 47: 893-899.
- Underwood, A.J. (2009) Components of design in ecological field experiments. *Annales Zoologici Fennici*, 46(2): 93-111.
- US EPA (2013) Aquatic life ambient water quality criteria for ammonia freshwater 2013. EPA 822-R-13-001. United States Environmental Protection Agency, Office of Water, Washington D.C.

Zhang, J.Y.Y., Ni, W.M.M., Zhu, Y.M.M., Pan, Y.D.D. (2013) Effects of different nitrogen species on sensitivity and photosynthetic stress of three common freshwater diatoms. *Aquatic Ecology*, 47(1): 25-35.

# Appendix A Summary of differences between treatments in measured nutrient, periphyton and invertebrate variables

For ease of reference, mean values for the main variables measured during the experiment are listed in the table below for each treatment. The day of the measurement is shown where appropriate. Response variables in which we detected significant differences among treatments (either using ANOVA or non-parametric KS tests) are shaded in grey. Pink shading signifies a significant pairwise difference between the P+NO<sub>3</sub>-N treatments and the P+NH<sub>4</sub>-N or P+NO<sub>3</sub>+NH<sub>4</sub> treatments.

\*means that the statistical test was carried out using RM-ANOVA across the duration of the experiment.

Variable	Experimental treatment (see Section 2.2.1 for details)											
variable	Units	Day	Control	DRP	P+NH <sub>4</sub> -N	P+NO <sub>3</sub> -N	P+NO <sub>3</sub> +NH <sub>4</sub>					
Treatments (nutrients)												
DRP	mg m <sup>-3</sup>	3, 6, 22	0.9	21	24	24	22					
NO <sub>3</sub> -N	mg m <sup>-3</sup>	3, 6, 22	112	112	379	498	113					
NH <sub>4</sub> -N	mg m⁻³	3, 6, 22	2.7	2.1	160	2.2	389					
%NH <sub>4</sub> -N	mg m <sup>-3</sup>	3, 6, 22	0.7	0.4	77	0.2	30					
DIN : DRP	ratio	3, 6, 22	127	5.4	22.8	20.8	22.5					
RESPONSE VARIABLES												
Periphyton biomass an	d particulate	e N and P										
Chlorophyll a	mg m <sup>-2</sup>	20*	227	229	433	283	331					
AFDM	g m-2	20*	79	83	92	104	104					
Chlorophyll a accrual	d-1	3 – 20	0.258	0.257	0.297	0.271	0.280					
PN	mg m <sup>-2</sup>	10	807	1030	1136	1101	1189					
	mg m <sup>-2</sup>	20	2140	2450	3240	2800	2940					
PP	mg m <sup>-2</sup>	10	287	225	164	281	176					
	mg m <sup>-2</sup>	20	78	509	325	367	425					
%N in AFDM	%	10, 20	3.2	3.7	4.2	3.3	3.6					
PN : PP	ratio	20	29.1	6.4	10.7	9.7	7.9					
Perinhyton communiti	es.											
Community (multivaria	te analysis)	10, 20, 27										
Total cell density	cm <sup>-2</sup>	10*	2139	3595	4664	3131	3429					
, Total cell density	cm⁻²	20*	10463	12949	16046	12517	20307					
Total cell density	cm⁻²	27*	10768	7736	13646	10095	12397					
, Density, vsmall cells	cm⁻²	10*	1132	1970	2734	1610	1914					
Density, vsmall cells	cm <sup>-2</sup>	20*	6544	9099	11930	7578	15031					
Density, vsmall cells	cm⁻²	27*	6992	4604	9251	5461	7928					

Invertebrates

Verieble		Experimental treatment (see Section 2.2.1 for details)									
variable	Units	Day	Control	DRP	P+NH <sub>4</sub> -N	P+NO <sub>3</sub> -N	P+NO <sub>3</sub> +NH <sub>4</sub>				
Total density	No./ samp.	27	967.3	884.0	1471.0	1334.7	1333.3				
browser	No./ samp.	27	110	79	132	80	87				
collector-filterer	No./ samp.	27	179	102	205	264	190				
collector-gatherer	No./ samp.	27	600	658	1045	904	954				
filter-feeder	No./ samp.	27	8	2	3	8	7				
predator	No./ samp.	27	31	22	48	42	58				
browser	%	27	11.8	8.9	8.9	6.5	6.4				
collector-filterer	%	27	17.9	11.3	13.9	19.0	14.2				
collector-gatherer	%	27	61.7	75.0	71.1	68.0	71.9				
filter-feeder	%	27	0.9	0.2	0.2	0.6	0.5				
predator	%	27	3.2	2.4	3.3	3.1	4.2				
MCI	Index	27	87.0	87.7	82.1	88.1	86.3				
QMCI	Index	27	3.30	3.18	3.20	3.26	3.19				
EPT index	Count	27	12.3	12.0	11.7	13.3	12.0				
%EPT	%	27	28.2	18.9	23.3	27.4	22.1				

# Appendix B Periphyton taxa identified in each treatment on Days 10, 20 and 27

Mean percentage abundances are shown across the three replicates for each collection day. The total count is the total of all counts per square centimetre in all treatments and days. Data for Day 20 are grey-shaded to aid reading of the table.

Algal group: cy, cynaobacteria; dia, diatoms; gf, green filamentous algae; ngf, green non-filamentous; r, red algae.

Diatom guild: lp, low profile; hp, high profile; m, motile.

Size group: vs, very small (< 25 μm diameter); s, small (26 – 50 μm diameter); m, medium (51 – 80 μm diameter); l, large (> 80 μm diameter).

		Diatom	Sizo	Total			Day 10					Day 20					Day 27		
Group	Taxon	guild	gr.	count	Control	DRP	P+NH4- N	P+NO <sub>3</sub> - N	P+NO <sub>3</sub> + NH <sub>4</sub>	Control	DRP	P+NH4- N	P+NO <sub>3</sub> - N	P+NO <sub>3</sub> + NH <sub>4</sub>	Control	DRP	P+NH4- N	P+NO₃- N	P+NO₃+ NH₄
су	Coleodesmium		m	1749	0.6	0.2	0.7	0.7	0.6	2.3	0.7	0.2	0.4	0.1	0.2	0.1		0.1	
су	Merismopedia		S	25441	1.8	3.6	1.8	1.6	1.6	3.1	2.3	5.3	8.9	8.0	1.5	4.3	5.7	13.6	10.5
су	Phormidium		S	30											0.0	0.0		0.0	0.0
dia	Achnanthes exigua	lp	VS	218	0.3	0.1	0.3	0.3	0.5						0.0		0.1		0.1
dia	Achnanthidium minutissimum	lp	VS	2009	5.7	1.4	0.8	2.0	1.2	0.5	0.3	0.2	0.1	0.1	0.9	0.5	0.2	0.4	0.4
dia	Cocconeis placentula	lp	s	4069	1.3	1.5	1.1	1.9	2.1	1.3	0.8	0.6	0.9	0.4	1.0	1.2	1.1	1.2	1.1
dia	Cynbella aspera	hp	Ι	525	0.2	0.2		0.1		0.2	0.1	0.1	0.4	0.0	0.1	0.2	0.1	0.1	0.0
dia	Cymbella cuspidata	hp	m	187				0.2	0.1		0.1		0.1	0.1			0.1	0.1	
dia	Cymbella cf. helvetica	hp	m	238	0.1										0.2	0.3	0.0	0.1	0.1
dia	Cymbella kappii	hp	s	17640	1.5	1.5	1.5	1.7	1.6	5.9	2.2	2.6	4.2	2.2	10.4	4.3	5.8	5.4	4.4
dia	Cymbella tumida	hp	m	9301	1.1	0.5	0.7	0.6	0.3	2.3	3.2	1.4	2.8	1.8	3.8	3.7	1.4	2.8	2.1
dia	Diatoma mesodon	hp	s	335	0.2	0.1	0.0	0.1	0.2	0.3		0.0	0.2	0.1		0.0	0.1	0.0	
dia	Diatoma tenuis	hp	VS	710	0.7	0.1	0.5		0.3					0.4	0.3	0.3	0.0	0.1	0.1
dia	Diatoma vulgaris	hp	m	8423	5.8	3.5	2.3	3.7	2.6	5.2	2.2	1.2	1.9	0.5	3.0	1.5	1.3	2.1	1.3
dia	Didymosphenia geminata	hp	Ι	124		0.0	0.0		0.3				0.1	0.1					
dia	Diploneis elliptica	lp	s	1236	0.3	0.1	0.2	0.2	0.3	0.4	0.2	0.2	0.3	0.2	0.5	0.5	0.2	0.4	0.5
dia	Encyonema gracile	lp	s	203						0.2	0.1	0.0	0.1	0.0					0.1
dia	Encyonema minutum	lp	VS	200137	33.3	28.7	34.2	21.8	31.5	54.0	42.0	60.5	36.1	40.2	56.8	48.5	59.5	41.5	53.7
dia	Eunotia sp.	lp	s	233		0.2	0.0	0.2			0.0	0.0		0.1			0.1	0.1	0.2
dia	Fragilaria capucina	hp	S	8267	5.6	3.6	3.6	5.2	4.3	1.7	1.5	2.0	1.8	1.2	1.4	1.5	1.8	1.9	1.5

Stimulation of river periphyton growth by ammoniacal-N vs. nitrate-N

		Distom	Sizo	Total			Day 10					Day 20					Day 27		
Group	Taxon	guild	gr.	count	Control	DRP	P+NH4- N	P+NO₃- N	P+NO <sub>3</sub> + NH <sub>4</sub>	Control	DRP	P+NH4- N	P+NO₃- N	P+NO <sub>3</sub> + NH <sub>4</sub>	Control	DRP	P+NH4- N	P+NO₃- N	P+NO <sub>3</sub> + NH <sub>4</sub>
dia	Fragilariforms viridens	hp	S	95									0.2	0.0					
dia	Fragilaria vaucheriae	hp	vs	2829	1.8	0.8	1.0	1.8	0.7	0.8	0.4	0.4	0.4	0.3	0.9	1.1	1.1	0.8	0.4
dia	Frustulia crassinervia	hp	m	254		0.3		0.1		0.1		0.0	0.2	0.1					
dia	Frustulia vulgaris	hp	m	222	0.2	0.0		0.2	0.1	0.1		0.0	0.0		0.0	0.0	0.1	0.1	0.1
dia	Gomphonema acuminatum	hp	m	160	0.4	0.0	0.1	0.2		0.1			0.0		0.0	0.0			0.1
dia	Gomphonema angustum	hp	m	127	0.1			0.1		0.0	0.1				0.2				0.0
dia	Gomphonema clavatum	hp	m	441	0.2	0.1	0.0	0.1	0.3	0.3	0.1	0.0	0.2	0.1	0.1	0.2	0.1	0.0	0.0
dia	Gomphoneis minutum	hp	m	562		0.0	0.2	0.1	0.2	0.2	0.2	0.0	0.3	0.0	0.1	0.1	0.1	0.3	0.0
dia	Gomphonema minutum	lp	vs	6000	3.9	1.4	1.9	2.0	1.7	0.6	0.8	0.5	1.0	0.4	1.1	4.1	1.8	3.0	2.6
dia	Gomphonema parvulum	lp	s	11254	4.1	5.0	3.6	5.2	4.8	2.3	2.9	1.5	3.1	2.0	2.2	3.8	2.2	3.3	2.3
dia	Gomphonema truncatum	hp	m	381	0.3		0.1		0.1	0.4	0.2	0.1	0.0	0.2			0.0	0.0	
dia	Hantzschia sp.	m	S	78		0.0	0.1	0.1	0.2					0.0	0.0				0.0
dia	Melosira varians	hp	m	6954	0.3	1.7	1.4	2.7	2.7	0.1	1.7	0.6	2.4	1.1	0.2	2.9	1.5	4.5	2.0
dia	Navicula lanceolata	m	m	713	0.2	0.1	0.1	0.2	0.3	0.1	0.2	0.1	0.3	0.3	0.2	0.1	0.1	0.3	0.2
dia	Navicula capitatoradiata	m	s	2298	0.1	0.2	0.3	0.5	0.3	0.1	0.3	0.1	0.4	0.1	0.3	0.9	1.1	1.4	1.2
dia	Navicula cf. cincta	m	S	800			0.1	0.2							0.2	0.7	0.8	0.3	0.2
dia	Navicula cryptocephala	m	S	6086	1.1	1.7	1.1	1.8	1.2	1.0	2.3	1.2	2.0	1.3	0.4	1.9	1.3	1.6	1.2
dia	Navicula cf. margalithii	m	S	6444	1.2	2.2	0.9	2.7	1.6	1.4	2.2	1.4	1.6	1.3	0.8	2.0	1.0	2.2	1.5
dia	Navicula radiosa	m	m	273				0.1		0.0	0.1		0.2	0.0	0.2		0.1		0.1
dia	Navicula rhynchocephala	m	m	63	0.1			0.1						0.0		0.1	0.0		0.0
dia	Navicula sp. (small)	m	S	108				0.4	0.1	0.0	0.1			0.0					
dia	Nitzschia acicularis	m	VS	42748	2.0	12.6	13.1	14.3	12.3	0.2	17.4	8.7	15.4	27.7		0.3	0.9	0.3	1.0
dia	Nitzschia dissipata	m	vs	614	0.1	0.1	0.2	0.4	0.1		0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.3	0.2
dia	Nitzschia intermedia	m	s	630			0.3	1.0	1.6		0.2		0.6	0.0					
dia	Nitzschia linearis	m	s	3029	1.4	1.3	0.6	0.3	0.6	0.3	0.6	0.2	0.4	0.4	0.7	1.8	0.9	0.8	0.8
dia	nitzschia palea	m	vs	17314	4.0	8.4	5.6	6.4	5.7	5.1	7.2	3.4	6.4	4.2	0.6	2.0	1.8	2.4	2.0
dia	Nitzschia sp. (vsmall)	m	vs	4800	0.4	0.0									2.9	3.2	1.8	3.0	2.5
dia	Pinnularia viridis	m	m	113	0.1		0.1	0.1		0.0		0.0			0.1	0.1		0.0	
dia	Planothidium lanceolatum	lp	s	1924	1.4	1.5	1.0	1.2	1.1	0.3	0.6	0.3	0.3	0.3	0.4	0.1	0.6	0.4	0.1
dia	Reimeria sinuata	lp	VS	1680	0.3	0.2	0.3	0.9	0.2	0.6	0.5	0.4	0.3	0.3	0.6	0.3	0.2	0.5	0.5

		Distom	Size	Total			Day 10					Day 20					Day 27		
Group	Taxon	guild	gr.	count	Control	DRP	P+NH4- N	P+NO <sub>3</sub> - N	P+NO₃+ NH₄	Control	DRP	P+NH4- N	P+NO <sub>3</sub> - N	P+NO₃+ NH₄	Control	DRP	P+NH4- N	P+NO <sub>3</sub> - N	P+NO₃+ NH₄
dia	Rhoicosphenia abbreviata	lp	VS	449	0.3		0.1	0.1	0.2	0.2	0.2	0.1	0.0	0.1	0.1		0.1	0.0	0.1
dia	Rossithidium linearis	lp	VS	1699	0.3	0.4	0.3	1.6	1.4	0.7	0.3	0.4	0.2	0.3	0.6	0.2	0.1	0.5	0.3
dia	Sellaphora pupula	m	S	66			0.0		0.1			0.0		0.0	0.0				0.0
dia	Staurosirella leptostauron	hp	S	179	0.1	0.1		0.1	0.2	0.0	0.0			0.1	0.1	0.1		0.0	0.0
dia	Surirella angusta	m	S	1134		1.1	1.2	0.5	1.2		0.1	0.1	0.4	0.2		0.4	0.2	0.5	0.1
dia	Surirella tenera	m	Ι	337		0.1		0.3		0.0	0.0	0.3	0.0	0.1		0.1			0.1
dia	Synedra acus	hp	m	2622	1.2	2.1	1.7	0.4	1.2	0.5	0.4	0.5	0.3	0.3	1.2	0.6	0.6	0.7	0.4
dia	Synedra rumpens	hp	VS	127									0.2				0.1		
dia	Synedra ulna	hp	m	8298	4.6	5.3	9.2	4.9	6.5	0.8	0.6	0.1	0.5	0.3	2.6	1.8	3.3	1.6	2.6
dia	Synedra cf. ulna v. ramesi	hp	S	4667			0.1		0.2	2.6	1.8	2.5	2.5	1.5	0.1	0.1	0.1	0.0	
dia	Tabellaria floculosa	hp	S	3929	1.5	0.9	0.1	1.1		1.6	1.1	1.9	0.5	0.2	1.5	2.6	0.1	0.5	0.5
gf	Cladophora		Ι	4											0.0			0.0	
gf	Microspora		m	5137	8.2	5.8	6.3	6.2	5.0	1.1	0.6	0.3	0.9	0.4	0.7	0.6	0.2	0.4	0.2
gf	Mougeotia		Ι	8												0.0	0.0	0.0	0.0
gf	Oedogonium		Ι	1288	1.0	0.8	0.7	0.8	0.7	0.4	0.5	0.2	0.4	0.2	0.4	0.1	0.1	0.2	0.1
gf	Spirogyra		Ι	413	0.3	0.2	0.1	0.1	0.1	0.3	0.2	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0
gf	Stigeoclonium		S	175	0.1		0.1		0.0	0.2	0.1			0.1					
gf	Ulothrix		m	2												0.0			
nfg	Ankistrodesmus		m	54		0.0		0.4	0.1										
nfg	Staurastrum		Ι	448			0.1	0.2							0.4	0.3	0.3	0.0	0.3
r	Audouinella		S	14	0.0			0.1								0.0			0.0

# Appendix C List of invertebrate taxa

All taxa counted are listed. Total counts in each treatment (i.e., the sum of three replicates) are shown. The MCI score for each taxon is shown in the shaded column

				Total cou	nts in each t	reatment	
Taxon	Feeding group (see Table 2-1)	MCI score	Control	DRP	P+NH <sub>4</sub> -N	P+NO <sub>3</sub> - N	P+NO <sub>3</sub> + NH4
EPHEMEROPTERA							
Coloburiscus humeralis	filter-feeder	9	5	4	1	22	11
Deleatidium spp.	browser	8	81	68	116	87	70
PLECOPTERA							
Stenoperla prasina	predator	10	0	1	0	0	0
Zelandobius spp	browser	5	1	1	3	1	0
Zelandoperla decorata	browser	10	0	0	0	1	0
Zelandoperla sp	browser	10	0	0	0	2	0
TRICHOPTERA							
Aoteapsyche catherinae	collect_filt	4	10	19	13	17	6
Aoteapsyche colonica	collect_filt	4	3	5	3	3	7
Aoteapsyche spp	collect_filt	4	525	281	598	771	558
Costachorema spp	predator	7	17	3	19	25	19
Hudsonema amabile	predator	6	2	4	21	4	0
Hydrobiosis spp.	predator	6	61	35	63	57	102
Hydrobiosis copis	predator	6	0	0	1	1	0
Hydrobiosis parumbripennis	predator	6	1	0	4	6	3
Olinga feredayi	browser	9	12	10	12	4	14
Oxyethira albiceps	browser	2	6	3	5	9	3
Psilochorema bidens	predator	8	8	9	22	21	33
Psilochorema leptoharpax	predator	8	0	1	0	0	0
<i>Pycnocentria</i> sp	browser	7	17	6	26	30	7
Pycnocentria evecta	browser	7	2	1	0	0	0
Pycnocentrodes sp	browser	5	79	57	120	54	60
DIPTERA			_				
Aphrophila neozelandica	browser	5	7	1	13	10	15
Austrosimulium sp	filter-feeder	3	0	0	0	1	1
Chironomus zelandicus	collector_gath	2	0	1	3	2	0
Corynoneura sp.	collector_gath	2	0	2	0	0	0
Cricotopus auckianaensis	collector_gath	3	482	515	746	549	/60
Cricotopus planus	collector_gath	3	16	70	38	60 270	32
Chicotopus zealanaicus	collector_gath	3	158	210	279	278	250
Chironomidae (P)	collector_gath	3	0	194	394	301	1/4
Emplaidae	collector_gath	2	0	4	0	0	1
Epinyunuae Erioptorini group	browsor	4	2	0	4	0	1
Eukiefferiella sp	collector gath	2	108	170	295	225	270
Lahodiamesa sp	collector gath	5	108	1	295	255	275
Maaridiamesa sp	collector gath	3	226	357	/59	500	531
Muscidae	collector gath	3	1	5	433 2	200	3
Ngonella forsythi	collector gath	2	611	/10	857	7/3	766
Orthocladiinae	collector gath	2	0	0	0	0	0
Orthocladiinae sn C	collector gath	2	6	11	16	7	25
Podonominae	collector gath	8	0	0	1	2	1
Polypedilum spp.	collector gath	3	0	0	0	0	1
Psychodidae	collector gath	1	4	1	19	4	3
Stictoclaudius spp.	collector gath		0	2	1	9	0
Tanypodinae	predator	5	2	2	5	6	11
Tanytarsus spp.	predator	3	1	0	0	0	0
Tanytarsus vespertinus	predator	3	1	7	9	6	5
Zelandotipula sp	unk	6	2	1	0	3	4
COLEOPTERA					-		

	Feedbac many		Total counts in each treatment								
Taxon	(see Table 2-1)	score	Control	DRP	P+NH <sub>4</sub> -N	P+NO <sub>3</sub> - N	P+NO <sub>3</sub> + NH <sub>4</sub>				
Elmidae (L)	collector_gath	6	17	10	19	16	26				
Elmidae (A)	collector_gath	6	0	1	2	5	1				
Scirtidae	collector_gath	8	3	1	0	0	0				
MOLLUSCA											
Physa sp.	browser	3	2	6	0	2	0				
Potamopyrgus antipodarum	browser	4	122	84	101	41	92				
ANNELIDA											
<i>Eiseniella</i> sp		1	0	0	1	0	1				
Lumbriculidae		1	5	0	6	2	8				
Naididae		1	60	38	50	44	64				
Enchytraeidae		1	52	25	56	52	32				
PLATYHELMINTHES	predator		0	0	0	1	0				
CRUSTACEA											
Cladocera	filter-feeder	5	1	1	0	0	0				
Copepoda	filter-feeder	5	0	0	1	0	0				
Harpacticoida	filter-feeder	5	1	0	0	0	0				
Ostracoda	filter-feeder	3	17	0	8	1	8				
ACARINA	predator	5	0	3	0	0	0				
COLLEMBOLA		6	0	2	1	7	5				
INDICES	Taxa Richness		32.0	31.7	31.7	35.0	32.3				
(means of three replicates)	Taxa Total		967.3	884.0	1471.0	1334.7	1333.3				
	MCI		87.0	87.7	82.1	88.1	86.3				
	QMCI		3.3	3.2	3.2	3.3	3.2				
	EPT index		12.3	12.0	11.7	13.3	12.0				
	%EPT		28.2	18.9	23.3	27.4	22.1				

# Appendix D Photographs of periphyton in different treatments

Photographs of growth surfaces in different nutrient treatments, taken on Day 17 of the experiment. Each photograph shows periphyton growth in a separate replicate channel (i.e., two of the three replicates are shown). Note the darker algae in the  $P+NH_4-N$  and  $P + NO_3+NH_4$  treatments.





11-15 Victoria Avenue Private Bag 11 025 Manawatu Mail Centre Palmerston North 4442 T 0508 800 800 F 06 952 2929 help@horizons.govt.nz www.horizons.govt.nz