Eutrophication effects on stream macrophytes

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macrophyte **noun**

mac·ro·phyte, ˈma-krə-ˌfīt

: a member of the macroscopic plant life especially of a body of water.

From German *Makrophyt,* derived from Greek *makro-* (*makrós* - large) + *-phyt* (*phytón* - plant).

eutrophication **noun**

eu·tro·phi·ca·tion, yü-ˌtrō-fə-ˈkā-shən

: the process by which a body of water becomes enriched in dissolved nutrients.

From Greek *eutrophos*, "well-nourished".

Source: *Merriam-Webster's online dictionary*

Abstract

Macrophytes are large, autotrophic organisms residing in aquatic environments. In streams they play important roles as substrate, shelter, and food for higher trophic levels, alter water movements and perform biochemical transformations. Unfortunately, macrophyte diversity worldwide is under threat from multiple anthropogenic factors. Eutrophication, the process of nutrient enrichment following land use intensification, is a major driver of negative stream ecosystem change, often in combination with other anthropogenic pressures such as deforestation and introduction of non-native species. The current paradigm of macrophyte response to eutrophication notes the importance of the hydrological setting and substrate type, but always encompasses modification to competitive interactions between macrophyte growth forms as nutrient concentrations become less limiting and competition for light increases. However, details of the responses of macrophytes to increased nutrient inputs under multiple stressor environments are complex and still warrant investigation if effective guidance on macrophyte management is to be developed.

In this thesis, I have addressed questions related to nutrient concentrations at which macrophytes start to accrue excessive biomass, how this relates to community shifts, what these community shifts look like and how they may be modulated by other variables. Specifically, I have used a mix of field observations and mesocosm experiments to:

i) investigate differences in community composition of macrophyte communities in streams along a eutrophication gradient.

ii) experimentally explore how external nutrient concentrations affect internal nutrient status, and consequently the growth, morphology, and photosynthesis of stream macrophytes across a range of irradiances.

iii) investigate if these responses lead to differences in competitive outcomes between species in mixed cultures.

To investigate relationships between eutrophication and macrophytes in streams in Aotearoa New Zealand, I surveyed 30 lowland streams on the country's North Island, focusing on the Waikato region. I targeted sites with as few confounding factors as possible by using existing stream classification data to select reaches in each stream that had low slope, were unshaded, and a discharge of around 1 $m^3 s^{-1}$. From those meeting these criteria I used a pre-existing, land-use based nutrient yield model to selected streams expected to provide a range of nutrient concentration. At each site I sampled nutrients (N and P) in water and sediment and analyzed the dominant macrophyte species for internal N and P content. I quantified community structure on five transects across each stream.

To explore links between environment and macrophytes I combined stream descriptors from national datasets in combination with the macrophyte community data and a list of trait scores of the encountered species in a RLQ multivariate analysis. The results showed a gradient in macrophyte community structure, a striking feature of which was the change in percentage of non-native species in the streams: eutrophic streams have almost no native species present. Additionally, many nutrient enriched streams were heavily clogged by macrophyte biomass, with species showing traits related to high performance and effective dispersal. A subsidiary gradient in the RLQ analysis, driven by nitrate and sediment phosphorous, saw a high presence of emergent species in eutrophic streams. This can be because the emergent species have a high nitrogen use efficiency compared to submerged species and can deal with a high turbidity by growing out of the water. A second subsidiary gradient, driven by water column phosphorous, correlated with eutrophic streams having a high degree of submerged non-native species; *Ceratophyllum demersum*, *Egeria densa*, *Lagarosiphon major*, and *Elodea canadensis*, all species that disperse primarily through fragmentation and can utilize bicarbonate as a carbon source. Phosphorous and carbon availability probably is a major driver for the presence of these species in these streams.

To test inferences made from observational field investigations, growth, morphological, and photosynthetic responses to different levels of nutrient concentration were investigated in a new experimental mesocosm setup. The system comprised four flumes fed by local groundwater stripped of ions by a reverse osmosis system, then dosed with major ions, macro- and micronutrient elements in controlled amounts to ensure finely controlled growth conditions.

The first experiment addressed hypotheses around the impact of nitrate concentration on growth of two pondweed species common to Aotearoa New Zealand, *Potamogeton crispus* and *P. ochreatus,* and possible interactions between nutrient and irradiance. Four nutrient concentrations (control ~0 µg NO_3^-N L^{-1}, low ~20 µg NO_3^-N L^{-1}, medium ~250 µg NO_3^-N L^{-1}, and, high ~5000 µg NO_3^-N L^{-1}) were combined with four light levels (67.7, 44.5, 30.9, and 7.8 mol photons m^{-2} d^{1}) in a full factorial design. I measured internal nitrogen (N) and carbon (C) content, growth rates, and morphology and used pulse amplitude modulated fluorometry to make photosynthesis-irradiance curves. The four external nutrient concentrations gave rise to two distinct groupings of plants based on their final internal nutrient content: a high-N group (2-4 % N) from high and medium nitrate treatments and a low-N group (~1 % N) from the low and control nitrate treatments. High-N plants of both species had faster growth rates and grew wider and taller than the low-N plants, but *P. crispus* grew widest and branched more, especially at high light levels. Low light availability negated any significant difference between N groups and, notably, low-N individuals of *P. ochreatus* performed better in this treatment than those of *P. crispus*. In general, the native *P. ochreatus* performed better than the non-native *P. crispus* under low nutrient stress, and the latter was best at high light and high nutrients. The results suggest that *P. crispus* has a great proliferation potential in eutrophic areas, and that lowering

resource levels can help manage not only macrophyte proliferation, but also invasive species. A potential competitive advantage can be inferred for *P crispus* in eutrophic, well-lit waters.

The second mesocosm experiment investigated competitive interactions between the non-native and native pondweed. Four nutrient treatments were again used (0, 40, 120, and 400 μg NO_3^-N L^{-1}), combined with two light treatments (18 and 3 mol photons m^{-2} d^{-1}) in a full factorial design. In each treatment I compared growth of each species when plated in monoculture and mixed culture pots. For each replicate, growth rate and morphological traits (main stem length, lateral spread, branching degree, and root length) were measured. Neither species performed differently in mixed- or mono-specific cultures, indicating no inter-specific interactions under the experimental conditions. Rather, in mixed culture plots the outcome, in terms of growth differentials between taxa, reflected the responses of each species to the treatment. Increasing irradiance tended to result in *P. crispus* outperforming *P. ochreatus,* while increasing nutrient levels shifted allowed *P. ochreatus* to outperform *P. ochreatus,* particularly at low irradiance. Based on the results of this study, there is no direct short term competitive suppression of either species by the other, but differences in trait expression at different resource levels/combinations might still lead to competitive exclusion in a more natural setting.

In this thesis I have shown that macrophytes respond to increases in external nutrient level by taking up more nutrients, increasing growth and expressing morphological plasticity. This is species dependent, however, and it is these differences in the ability to respond that appear to shape the macrophyte communities in streams. Streams with high light and nutrient supplies are susceptible to high accrual of biomass by a subset of responsive species that may ultimately end up being the only species present in these streams. I found that the eutrophication threshold for nitrogen is quite low, below 250 μg NO_3-N L^{-1}. Macrophyte growth was evident at nitrate concentrations only seen in quite pristine streams and several nutrient species seem to determine community compositions in streams. Thus, management actions for the protection of stream ecosystems would benefit from managing resource levels in general rather than focusing on a specific stressor.

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Contribution and publications

Publications from this work:

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Conferences:

- Skovsholt L., Riis T., Matheson F., Hawes I. - Difference in growth responses of invasive and native aquatic plants from nitrogen and light variability. [Poster] RIVERS + NZFSS 2020, Invercargill, New Zealand
- Skovsholt L., Riis T., Matheson F., Hawes I. – Eutrophication effects on stream macrophytes. [Oral presentation] University of Waikato postgraduate conference 2021, Online, New Zealand
- Skovsholt L., Riis T., Matheson F., Hawes I. - Growth and trait responses of Potamogeton crispus and P. ochreatus to variations in nitrogen and shade. [Poster] INTECOL 2021, Online, New Zealand
- Skovsholt L., Riis T., Matheson F., Hawes I. - Effekt af næring og lystilgængelighed på vækstrespons hos en hjemmehørende og en invasiv vandaksart i New Zealand. [Poster] Ferskvandssymposiet 2023, Vingsted, Denmark
- Skovsholt L., Riis T., Matheson F., Hawes I. - Land use shapes aquatic macrophyte communities in unshaded slow flowing lowland streams by stressor specific responses. [Poster] Danish OIKOS Meeting 2023, University of Southern Denmark, Odense, Denmark
- Skovsholt L., Riis T., Matheson F., Hawes I. - Riverine plant community changes on a gradient of land use in lowland New Zealand streams. [Oral presentation] IAGP symposium 2023, Antwerpen, Belgium

Journal article drafts:

- Skovsholt L., Riis T., Matheson F., Hawes I. - Trait-specific groups of aquatic macrophytes respond differently to eutrophication of unshaded lowland streams.. Submitted to *Science of the Total Environment* May 2024

- Skovsholt L., Riis T., Matheson F., Hawes I. – Short term competition between a native and non-native macrophyte is not affected by nutrient levels. Projected submission in *Aquatic Botany* mid 2024

Other research collaborations:

- Skovsholt, L. J., Pastor, A., Docherty, C. L., Milner, A. M., & Riis, T. (2020). Changes in hydrology affects stream nutrient uptake and primary production in a high-Arctic stream. *Biogeochemistry*, *151*(2-3), 187-201.c

- Pastor, A., Skovsholt, L. J., Christoffersen, K. S., Wu, N., & Riis, T. (2021). Geomorphology and vegetation drive hydrochemistry changes in two Northeast Greenland streams. *Hydrological Processes*, *35*(10), e14369.

- Pastor, A., Wu, N., Skovsholt, L. J., & Riis, T. (2020). Biofilm growth in two streams draining mountainous permafrost catchments in NE Greenland. *Journal of Geophysical Research: Biogeosciences*, *125*(3), e2019JG005557.

- Pastor, A., Poblador, S., Skovsholt, L. J., & Riis, T. (2020). Microbial carbon and nitrogen processes in high-Arctic riparian soils. *Permafrost and Periglacial Processes*, *31*(1), 223-236.

- Pastor, A., Freixa, A., Skovsholt, L. J., Wu, N., Romaní, A. M., & Riis, T. (2019). Microbial organic matter utilisation in high-arctic streams: key enzymatic controls. *Microbial ecology*, *78*, 539-554.

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N – Nitrogen

P – Phosphorous

PCA – Principal Component Analysis

NZ – New Zealand

FRE3 – Frequency of flows 3 times median flow

FRE7 – Frequency of flows 7 times median flow

CSR – Competitive, stress-tolerant, ruderal

RNA – Ribonucleic acid

EIV – Ellenberg Indicator Value

DIN – Dissolved inorganic nitrogen

I – Irradiance

TRP – Total reactive phosphorous

DW – Dry weight

PAM fluorometry – Pulse amplitude modulated fluorometry

PI – Photosynthesis-irradiance

PSII – Photo system II

Y – Quantum yield

F – Fluorescence

Fm’ – Fluorescence under saturating light

ETR – Electron transport rate

AF – Absorption factor

PAR – Photosynthetically active radiation

P_{max} – Maximum photosynthetic rate

I_c – Saturation irradiance

DRP – Dissolved reactive phosphorus

UV-VIS – Ultraviolet-visible

RGR – Relative growth rate

A – Absorbance

ANOVA – Analysis of variance

SD – Standard deviation

E_k – Saturation irradiance

Φ – Quantum yield

FIA – Flow injection analyser

CR – Competitive ratio

RD – Relative dominance

PC – Potamogeton crispus

PO – Potamogeton ochreatus

MSL – Main stem length

BD – Branching degree

LS – Lateral spread

RL – Root length

GIS – Geographic information system

TSS – Total suspended sediment

DIFN – Diffuse non-interceptance

TN – Total nitrogen

TP – Total phosphorus

SS – Suspended solids

FENZ – Freshwater ecosystems of New Zealand

H’ – Shannon’s diversity

J’ – Pielou’s evenness

NTU – Neophilic turbidity units

CA – Correspondence analysis

VIFs – Variance inflation factors

PNUE – Photosynthetic nitrogen use efficiency

Chapter I

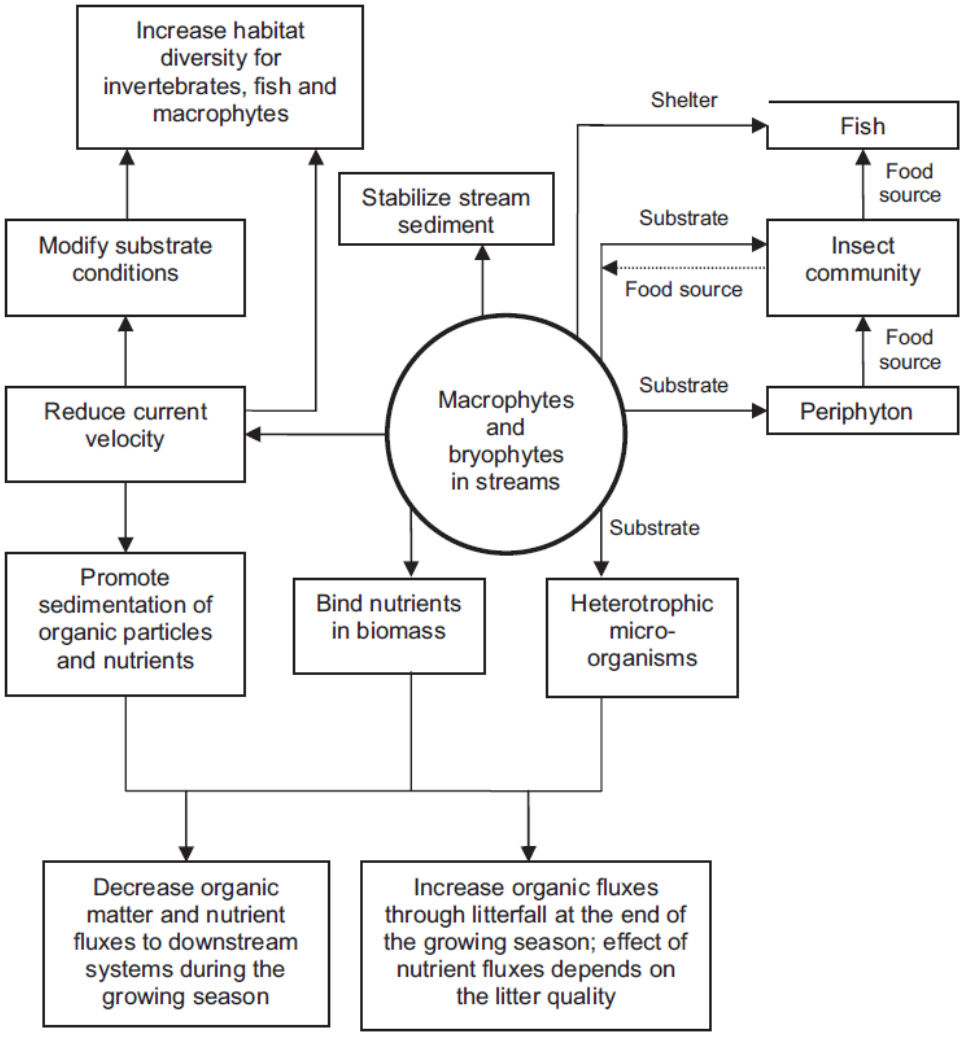
General introduction.

1.1 A general introduction to macrophytes in streams and rivers and the effect of eutrophication.

1.1.1 Importance of macrophytes in streams and rivers.

The term "Macrophyte" stems from the Greek words *makrós* and *phytón* and describes an organism that is large enough to be seen with the naked eye and can photosynthesize. It does not constitute a distinct taxonomic group; it is instead a functional classification, usually applied to aquatic plants, and encompassing bryophytes, pteridophytes, macroalgae and angiosperms that complete all or most of their lifecycle in a degree of submersion. Early attempts at a definition encompass a range of aquatic

Figure 1-1: *Ecological influences of macrophytes and bryophytes in streams (for details see Bowden et al. 2017).*



affinity from fully submerged to growth in soils where the water table is 50 cm below ground (Sculthorpe, 1967), however this thesis will proceed with a looser definition; that aquatic macrophytes are large autotrophs that can tolerate submergence and thus are found in waterbodies or water-saturated soils. Where present, aquatic macrophytes are important in the stream ecosystem (Fig 1.1). Their structure alone provides a multitude of ecological values, including substrate for epiphytic biofilms (Köhler et al., 2010), shelter from current for invertebrates (Carpenter & Lodge, 1986; Sand-Jensen et al., 1989) and habitat for fish (Dibble et al., 1996; Quirino et al., 2019). Macrophytes are also of great importance for stream functioning; in soft-bottom streams they are the main driver of stream metabolism and play an important role in biochemical processes (Levi et al., 2015; Sand-Jensen et al., 1989).

The ecosystem services provided by macrophytes are numerous. They can help to purify water, through attenuation of suspended sediments (Jones et al., 2012; Sand-Jensen, 1998) and nutrients (Bernot et al., 2006; Hall et al., 2005), a great benefit for downstream lakes or estuaries. Their presence in the stream can also have less beneficial effects particularly when growth is profuse. Thick macrophyte growths increases hydraulic resistance, slows and retains water (Riis et al., 2008; Sand-Jensen, 1998), which can contribute to flooding of the surrounding land in times of high precipitation in the catchment. They can also decrease oxygen levels near the sediment (Caraco & Cole, 2002).

1.1.2 Growth forms of Macrophytes.

Macrophytes are typically divided into different growth forms starting with whether they are rooted to the sediment or not. Rooted species are further divided into emergent, submerged and floating leaved types (Sculthorpe, 1967). The growth form classifications are not absolute, a species can belong to one or the other in various degrees, and heterophylly (the ability to produce different types of leaves) is relatively common (Sculthorpe, 1967).

Free floating macrophytes are usually found in sites of low water velocity such as pools or backwaters. They range from floating submerged to floating on the surface, and this can change with different life cycle stages. Although not attached to the sediment, some species still have an extensive root system, while others do not. Some species even have terrestrial morphotypes, if they happen to strand on marginal wet soils (Sculthorpe, 1967).

Emergent macrophytes can be found close to or on the banks of streams and in pools and backwaters. Their roots are often downward protruding and deep penetrating, making fine sediments their preferred substrate type, while the leaves are erect and at least partly in air. Common NZ examples include *Typha orientalis*, (raupō or bulrush) and *Eleocharis sphacelata* (kutakuta or tall spike sedge). The stiff, brittle structure of most emergents, combined with an extensive root network means that too much mechanical pressure, for example from vigorous water movement, will cause them to break

(Haslam, 1978). Additionally, they are restricted to water depths of a few metres (although exceptions exist) as they rely primarily on aerial biomass for gas exchange and reproduction. They are typically restricted to growing at the stream margins where water depth and velocity is low. Many species exhibit heterophylly, whereby the aerial leaves have a different morphology to the submerged ones. Some emergent species are sprawlers and spread across the ground or water surface via rhizomes or stolons (Sculthorpe, 1967). These include a range of species with various affinity for submergence e.g. *Nasturtium officinale* (watercress) which can sprawl across the water surface and occur in reasonably deep water, or *Glyceria maxima* (sweet reed grass), that is usually creeping into the stream from the banks or found floating in the upper parts of still water. Indeed, sprawling species can cover the water surface of small streams and trap sediment beneath, ultimately contributing to the transformation of aquatic environments into terrestrial, especially in backwater areas (Jones et al., 2012). Often sprouting adventitious roots above the sediment surface, sprawlers can acquire solutes from the water as well as the sediment.

Submerged macrophytes are usually also attached to the sediment, with their photosynthetic tissue below the water surface, but may produce reproductive organs above (Sculthorpe, 1967). Most species are rooted to the substrate and can in some cases produce intricate root networks that intertwine through coarse, stable substrate giving them good anchoring strength. Combined with a flexible stem, that lowers drag, this makes them well adapted to fast flowing waters (Haslam, 1978). Submerged species can be found at great depths in lakes, only limited by availability of light and pressure. The grouping includes species with well-developed roots that can acquire solutes from both water and sediment (Madsen & Cedergreen, 2002; Piepho, 2017; Schwarz et al., 2000; Sculthorpe, 1967). Some submerged species are without roots though, instead anchoring themselves to the substrate by other means, e.g. *Ceratophyllum dermersum* which anchors (weakly) by its lower leaves (Schutten et al., 2005). Characean algae are also a common form of submerged macrophyte in New Zealand and these develop rhizoid structures for anchoring to the sediment.

Floating leaved macrophytes attach themselves to the sediment and have stems that grow to the water surface where they produce floating leaves, spreading out across the water surface (e.g. the common non-native *Nymphaea alba*). The floating leaved growth form, albeit hydrologically constricted, has certain competitive advantages. The leaf is connected to both air and water, facilitating effective gas exchange while maintaining the ability of water column solute uptake. Additionally, these plants are often aerenchymatic, facilitating their roots to grow in eutrophic anoxic sediments, utilizing this as a source of carbon and nutrients (Sculthorpe, 1967). Finally, their leaves can cover a large proportion of the water surface, effectively shading-out submerged species (Mccreary, 1991). The petiole is the restricting element as to where true floating-leaved species can grow; high fragility confines them to slow moving or still water and gas/solute transport limits length and thus depth. In streams floating leaved species are thus often found in backwaters or growing from banks of large rivers with low

water velocity (Haslam, 1978). However, heterophylly does allow some taxa to have both submerged and floating leaves, for example, *Potamogeton cheesemanii*; in faster flowing water this species can be present as entirely submerged morphotypes, acting like true submerged species (see below), while in backwaters floating leaves can predominate.

1.1.3 Natural factors influencing macrophyte communities.

1.1.3.1 Light

Being autotrophic, macrophytes are dependent on light to sustain growth (Bal et al., 2011; Julian et al., 2011; Köhler et al., 2010; Zhang et al., 2010). The amount of incoming irradiation ultimately determines the photosynthetic rate that a plant can achieve and thus the maximum rate at which carbon can be incorporated into structural tissue (Carr et al., 1997). Each macrophyte species, however, has an optimal light intensity for growth and these can be very different (Ellawala Kankanamge et al., 2019). Furthermore, species with some growth habits can create a canopy that diminishes the light reaching species below and as such are highly competitive in some environments (Van Gerven et al., 2015).

Variations in irradiance in streams and rivers come from water column turbidity and shading, either from other macrophytes or riparian vegetation. Thus, community composition in shaded and unshaded streams can be expected to differ, as the species with high light preferences, typically with concurrent high growth rates, will have a competitive advantage where light is readily available. Adaptations to low light can be a valid strategy as species can survive below the canopy of faster growing species or be natural inhabitants of shaded streams (Baattrup-Pedersen et al., 2015; Mouton et al., 2019). While light may be important as a determinant of community composition, is needs to be thought of as being in interplay with other factors, as macrophytes will simultaneously compete for other resources (e.g., macro and micro-nutrients, space) and be affected by processes that remove biomass (e.g., floods, grazing, disease)(Riis & Biggs, 2001).

1.1.3.2 Inorganic carbon

Acquisition of inorganic carbon is a prerequisite for any photosynthesizing organism but poses a unique challenge for aquatic macrophytes, as opposed to terrestrial plants. The physical properties of water prohibit carbon dioxide from diffusing as fast as in air; indeed, the diffusion rate is 10,000 times slower in water. Moreover, at pHs normal for natural freshwaters, close to pH 7-8, chemical equilibrium processes mean that dissolved inorganic carbon is primarily in the form of bicarbonate (HCO_3^-). Floating leaved and emergent macrophytes have adapted by growing out of the water to access CO_2 in the air and Isoetids (a group of small stature plants that complete their entire life cycle submerged – *Isoetes alpinus* is the common NZ species) can utilize carbon dioxide (CO_2) from the sediment via their aerenchyma (Sculthorpe, 1967). Other submerged macrophytes exploit another solution, that is utilisation of bicarbonate for photosynthesis rather than relying on dissolved CO_2

(Sand-Jensen, 1983). Not all submerged macrophytes are equally capable of performing this feat, which requires the enzyme carbonic anhydrase. Elodeids (stem plants that have flowers above the waterline) rely on water column CO_2 or HCO_3^-. In lakes the still standing water can amplify shortage of CO_2 as little replacement takes place, however streams, being constantly in motion, usually do not suffer from this problem except maybe in the large, deep, slow-flowing rivers with very little turbulence (Allan et al., 2021). As such, the competition between HCO_3^- and CO_2 users might not be as important in rivers as it is in lakes (Sand-Jensen, 1983) (but see Maberly et al., 2015; and Sand-Jensen et al., 2022).

1.1.3.3 Temperature

Temperature regulates all biological functioning and thereby also affects macrophytes in streams (Lacoul & Freedman, 2006). The direct effect of temperature is relatively straightforward, namely the speed at which enzymes function, and thus their metabolic rate. However, the effects of temperature go beyond this as there are several more indirect effects. Temperature has been found to affect seed establishment success, thus affecting community composition (Riis et al., 2012). Furthermore, it has also been found to affect the competition between species, thereby affecting community composition (Riis et al., 2012). Temperature not only affects the macrophytes but also the organisms with whom they share the ecosystem. Consequently, there are likely to be numerous effects of temperature, not easily quantifiable, as this is expected to influence these other organisms that inhabit the stream, and these are in turn expected to influence the macrophytes. For example, nitrification and denitrification are two fundamental processes regulating the nitrogen pool in streams (Bernhardt et al., 2002; Cooper & Cooke, 1984) and, being bacterial processes, are highly susceptible to temperature changes (Schaeffer et al., 2013). By changing these, temperature can thus affect nitrogen availability.

1.1.3.4 Hydrology

The ability of macrophytes to persist in streams depends to a high degree on the flow conditions (Biggs, 1996). Water velocity controls boundary layer thickness and so regulates uptake of solutes, but it also imposes a constant physical stress on the plants which, dependent on the flow velocity, can prevent macrophytes being present at all (Poff et al., 1997). Another important hydrological factor to consider is the frequency with which the discharge increases to levels that are outside the norm (Haslam, 1978; Riis & Biggs, 2003), i.e., frequency of flood flows. Flood events are also termed flushing flows, as they can flush out most of the macrophyte biomass, essentially resetting the community biomass to low values. The variability of flushing flows, sometimes expressed as frequency of flow 3 times the annual median (FRE3) or 7 times the annual median (FRE7), is important in determining which species of macrophytes can persist , as species have very different tolerance towards this, both in terms of resistance and resilience (Riis & Biggs, 2001, 2003).

1.1.3.5 Grazing

As a disturbing factor, grazing can also be important in regulating macrophyte communities. Grazing can not only create biomass losses but also reduced flowering (O'hare et al., 2007) and reduced shoot length (Søndergaard et al., 1996). Grazers can also, by focusing their grazing on the lower parts of a macrophyte bed, cause collapse of an entire bed (Bakker et al., 2016). The effect of a grazer depends in part on the type of grazer. For example, Bakker et al. (2016) explains that among large herbivore grazers, fully aquatic species (manatees, dugongs, turtles) and semi-aquatic (swans) have the largest impact on both abundance and composition of submerged plant communities, as opposed to resident semi-aquatic and resident terrestrial species. Additionally, the effect of large herbivores was not limited to grazing, as they also can function as important dispersers and act as ecological engineers that can influence the local hydrology (Bakker et al., 2016). Studies from lakes have investigated the relative importance of grazers on macrophytes and have found waterfowl to be the most important relative to invertebrates or fish (Søndergaard et al., 1996). In transferring results from lakes to streams, it is important to note however, that water velocity has an effect as a determiner of the extent of grazing in that high velocities can deter grazers such as large waterfowl (Wood et al., 2018). Grazers in New Zealand include the aquatic caterpillars of the native moth *Hygraula nitens* (Redekop et al., 2018), and also in some areas the non-native grass carp *Ctenopharyngodon idella* (Wells et al., 2003) and rudd *Scardinius erythrophthalmus* (Lake et al., 2002).

1.1.3.6 Competition

In any natural biological system, there will be some resources that are scarcer than others, and these will be the source of competition between species, and indeed individuals of the same species (Lacoul & Freedman, 2006). These resources range from the more traditional kind, like nutrients or light, to the less obvious, like space or sexual partners. Competition is not limited to being amongst the same types of organisms, i.e. in streams, macrophytes compete not only with other macrophytes, but also with macroalgae and epiphytic microalgae (Hilton et al., 2006; Köhler et al., 2010) or, given sufficiently slow water velocity, phytoplankton in the water column (Hilton et al., 2006). The outcome of competition depends not only on a species' resource acquisition ability but also the disturbance regime of the system and the species ability to cope with this.

In the 1970's, Grime (2006) introduced the CSR (competitive, stress-tolerant, ruderal) framework of how plants have adapted to variations in resource availability and disturbance regime, and although it was developed for terrestrial vegetation, it can be applied to freshwater as well (Garbey et al., 2004). Plants that are tolerant of nutrient stress (low availability), are referred to as S-species. Predominantly perennial, they are slow growing and do not accumulate much biomass. These species are very good at utilizing the available nutrients and can maintain nutrients in their tissue for a longer time than most other species. Additionally, S-species are usually the types found in adverse environments, as they harbour various adaptations to cope with conditions that would be toxic for other plants, such as high

salinity areas. S-species are, however, not able to cope with a high degree of disturbance. Isoetids are examples of S-species (Riis & Biggs, 2001). Plants that are responsive to high levels of nutrient supply but are intolerant of disturbance are termed C-species. C-species are not found in areas of high disturbance either, but these are very competitive in areas with abundant resources. C-species grow slowly but attain a lot of biomass and usually grow to a size where they can out-shade smaller species, hence their competitive advantage. Trees are good examples of C-species on land, while aquatic species include *Aponogeton distachyos* and *Nymphea alba* (Riis & Biggs, 2001). Species that prevail in high resource areas with a high disturbance regime are classified as R-species. R-species have effective dispersal measures and as such, they can quickly colonize new areas, for example after a disturbing event like a forest fire. High performing, with a fast growth rate, they can rapidly accumulate biomass and set seed before the disturbances in the environment set in again. Not surprisingly, R-species are often annual species. Aquatic examples include *Potamogeton crispus* and *P. pectinatus* (Riis & Biggs, 2001). It is worthy to note that while effective as a broad ecological framework, the Grime classifications are not absolute and indeed, species can be classified as being in between one or more categories, e.g. *Chara globularis* (C & S) or *Myriophyllum triphyllum* (C & R)(Riis & Biggs, 2001). Furthermore, some species have a degree of plasticity that makes them able to shift between categories depending on their current environment, making them highly effective competitors (Garbey et al., 2004).

1.1.4 Anthropogenic factors affecting macrophyte communities.

1.1.4.1 Damming and channelisation.

The factors governing macrophyte communities in streams are subject to alteration by human activity. The water flow can be altered in various ways. Dams manage water flow, resulting in changes to the natural variability in stream and river discharge. This may result in variable flow and higher or lower water levels than under natural conditions. Low water levels can make it easier for the riparian vegetation to colonise (Johnson, 1994), while extreme unnatural water level fluctuations can result in only plants adapted to these extreme conditions surviving in the upper littoral zone (Hawes et al., 2003). Additionally, as the amount of water that passes through a hydroelectric dam depends on the Bneed for electricity, times of high-water availability and low demand can lead to extreme water releases, creating flushing flows downstream of the dam. If the interval between floods is not

sufficiently long for regrowth, macrophytes cannot persist in the stream. Furthermore, the timing and frequency of these floods will likely be different from what the natural community has been used to during its evolutionary history (e.g. spring floods from snowmelt, flushing flows associated with winter storms), thus, even if macrophytes do persist in the stream the community will shift towards a more disturbance resilient one, with species that have a high regrowth rate (Poff et al., 1997; Van Looy et al., 2019). Dams can have a hydro-chemical effect as well, where the biogeochemistry in reservoirs created behind dams affects the hydrochemistry of the effluent water (Ligon et al., 1995). Additionally, as many dam-reservoirs function like lakes, they can be a source of phytoplankton that alter the light environment, and disruption of dislodged macrophytes passing through dam structures can enhance the production of dispersal organs (i.e., clonal fragments) from macrophytes.

Channelisation of streams, that is, the removal of the meanders to facilitate drainage, also affects hydrology (Poff et al., 1997). With the meanders gone, so disappears the pattern of alternating riffle-pool sequences, and the differences in flow velocities created by the water going through a bend. As such, the effect of channelisation is the creation of a more homogenous habitat, which consequently leads to a lower diversity of macrophytes (Sand-Jensen et al., 2000). Furthermore, the lack of low velocity pool areas can lead to decreased sediment and water retention imposing greater flooding risk to downstream areas (Kundzewicz, 1999). These are both dependent on the amount of macrophytes present however, as they can lower water velocities and increase residence time.

1.1.4.2 Invasive species

Invasive species are another complication in macrophyte community dynamics. The continuing globalisation of our society means that the number of vectors that can carry species to foreign places are becoming more numerous (Vitousek et al., 1997). Unintentional dispersal by plane or ship is not uncommon, but the trade of exotic species as aquarium plants is also an important vector (Champion et al., 2013). The introduction of non-native species to an ecosystem does not automatically mean that it will be invasive in the sense that it disrupts existing ecosystems. A species can find a previously unexplored niche in the new ecosystem and not cause problems (Mooney & Cleland, 2001; Vilà & Weiner, 2004). Some introduced species, however, have a negative effect on the indigenous flora by reducing the biomass of natives in an area or even excluding them altogether, and thus become invasive (e.g. Mooney & Cleland, 2001). In some cases, the invasive species can introduce a different structure and function to the environment compared to the native species that it is outcompeting, and in this way, it can change the local microclimate, with implications for fish and invertebrates (Ren & Zhang, 2009; Schultz & Dibble, 2011). Additionally, higher trophic levels can be affected as invasives may have a different palatability and thus change grazing patterns of herbivores (Bakker & Nolet, 2014). Many hypotheses exist to explain why some exotics become so successful as invaders (see review by Fleming & Dibble, 2014). Some propose a lower pressure from pathogens, predators, or parasites, as in their new environment, no evolution has yet taken place to target the invader

(Fleming & Dibble, 2014). Others propose that invaders that come to dominate are species that are naturally good competitors and that they have excellent resource acquisition which makes them prone to outperform extant flora (Fleming & Dibble, 2014; Vilà & Weiner, 2004). Generally, invasive species seem to thrive in anthropogenically disturbed areas (Quinn et al., 2011).

1.1.4.3 Deforestation

Changes outside of the stream can also have profound effects on the in-stream vegetation. Deforestation can mean that a stream originally surrounded by forest suddenly has none and is subjected to a novel light regime. As irradiance is a key driver for macrophyte communities, deforestation can lead to changes in community structure, from a periphyton dominated system to a macrophyte dominated one, provided flow and substrate permits macrophyte presence. However, another effect of land use change is that the transition from a pristine to anthropologically affected area usually entails an increase in fine sediment transport. Removal of riparian vegetation to be able to farm adjacent to the stream or livestock having access to the stream are both examples of activities that increase erosion (Bear et al., 2012).

1.1.4.4 Eutrophication

Lastly, increasing population density and intensification of agriculture practices have caused an increased amount of nutrients to enter freshwater ecosystems, leading them to suffer the effects of eutrophication (Sand-Jensen et al., 2008; Snelder et al., 2017). Eutrophication is the transition of a system from a baseline state to a more productive (eutrophic) one, due to a change in factors influencing the system (Dodds, 2007). The cause of the transition, and the magnitude of change depends in part on the baseline trophic state of the system. A naturally eutrophic system might have different factors limiting further production than an oligotrophic one, as will an autotrophic compared to a heterotrophic one (Dodds, 2007) In the scope of this review and indeed, this thesis, eutrophication is meant in the sense of increases in the macronutrients N and P, which is the most widely accepted current interpretation of eutrophication (Hilton et al., 2006).

1.1.5 Influence of nutrients on macrophytes.

1.1.5.1 Effects on abundance and biomass.

Macrophyte biomass accrual is limited when nitrogen and phosphorus, mostly found in organic forms or as ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) (Allan et al., 2021), are in short supply (Barko et al., 1991). The influence of elevated nutrient levels in streams on the growth of aquatic macrophytes is mostly related to replacing the limitation of nutrients with another limiting factor, usually light (O'hare et al., 2018). Evidence of relationships between nutrients and biomass of macrophytes in streams have been found on many occasions (see Appendix I: Table A1); for example, Mebane et al. (2014) found that increases in biomass were related to sediment and water column nitrogen and sediment loosely sorbed phosphorus and O'hare et al. (2010) found that increased

soluble reactive phosphorus in stream water yielded increases in biomass (for *Ranunculus penicillatus*), and that the relationship was strengthened when coupled with an increased HCO_3- availability. However, a relationship between nutrients and biomass is not always clear. Some studies that have investigated abiotic influences on macrophyte biomass have also found no apparent relationship between the two, but rather an effect of incoming irradiation or flow velocity (Canfield & Hoyer, 1988; Giorgi et al., 2005). Eutrophication has different effects in different environments (Artigas et al., 2013; O'hare et al., 2018) and species responses to nutrient increases are not uniform (James et al., 2006). However, in streams the combined effects of alleviation of growth limitation and presence of productive species in suitable physical environments can lead to excessive biomass production, termed "nuisance growth" due to its adverse effect on the ecosystem and its services (Matheson et al., 2012). Nuisance growth is especially found in nutrient rich lowland streams (Collins et al., 2018; Haslam, 1978).

1.1.5.2 Effect on other factors including plant traits.

Increases in nutrients can have other effects on macrophyte morphology than just growth. Physiologically, a plant will invest the largest amount of resources towards the organs responsible for taking up the resource that is most limiting for growth (Lambers et al., 2008). When nutrients are no longer limiting, the resources that are limiting (e.g. carbon, light) must be obtained from the water column or the air, thus the macrophyte can focus its production of biomass on the above-ground parts, leading to a lower root/shoot biomass ratio (Madsen & Cedergreen, 2002). Exceptions to this do occur, macrophytes with well-developed aerenchyma (e.g. *Lobelia dortmanna*) can facilitate transfer of CO_2 from sediments to photosynthetic tissue (Boston et al., 1987; Raven et al., 1988) and so need a large root biomass for this purpose. Additionally, roots maintain other functions like anchoring, so the variability of biomass allocation to this function would likely be also dependent on water velocity (Riis & Biggs, 2001). Internal nutrient concentrations in tissues have been found to increase with increases in available nutrients (Bracken et al., 2015; Li et al., 2018). This is likely an adaptation of plants to create a nutrient reserve in case the environmental nutrient availability declines, which may be the norm in natural streams with pulsed nutrient supply. Nutrient reserves are an advantage under fluctuating nutrient supply, but somewhat superfluous in eutrophicated streams that are relatively stable in their nutrient concentrations (e.g. Madsen & Cedergreen, 2002). Increased tissue nutrient content can be a contributing factor in increasing palatability to grazers (Bakker & Nolet, 2014).

1.1.5.3 Nutrients in the water column and the sediment.

Most river macrophytes can take up nutrients using both their above and below ground tissues (Barko et al., 1991; Barko & James, 1998). The main exception to this is emergent species that do not exhibit heterophylly and do not have the capacity for nutrient uptake through their leaves (e.g. *Glyceria* spp). Whether uptake takes place predominantly in the aboveground or belowground tissue has sparked some debate, especially as this could have potential implications for management. Madsen and

Cedergreen (2002) found that for four common submerged macrophytes of Danish lowland streams; *Elodea canadensis*, *Callitriche cophocarpa*, *Ranunculus aquatilis* and *Potamogeton crispus*, experimental removal of the roots did not alter growth rate compared with individuals that had their roots intact, proving that at least these species can satisfy their nutrient demand from the water column alone, when the water is nutrient rich. However, several studies have reported a growth response from macrophytes in enriched sediments (Carr & Chambers, 1998; Chambers et al., 1989; Ságová-Marečková et al., 2009). Additionally, large scale surveys have found general correlations between macrophyte abundance and sediment nutrients (Mebane et al., 2014). Thus, for many macrophytes species, it seems that the source of nutrient is not as important as whether nutrients are the limiting factor for growth.

The importance of water column relative to sediment nutrients is likely to be affected by the different growth forms of macrophytes and the root morphology of the individual species. Submerged species without well-developed roots are restricted to meeting their nutrient demands by foliar uptake from the water column alone (Madsen & Cedergreen, 2002), while erect emergent species depend largely on root uptake of sediment nutrients (Sculthorpe, 1967). Submerged species with well-developed roots can exploit both sources and will therefore likely be concentrating uptake where nutrients are more readily available (Bowden et al., 2017). Some emergent species, however, have adventitious roots (e.g. sprawling types like *Nasturtium officinale*), that enable them to utilize nutrients from the water column as well as from sediment (Steffens & Rasmussen, 2015).

Additionally, different species have different root characteristics. Some species have long, vertically penetrating roots (e.g. *Sparganium erectum*) and are usually found in fine grained sediment, while others have shallower roots that intertwine around a usually coarser substrate, creating an intricate network with a good anchoring strength (e.g. the rheophilic *Ranunculus* species) (Haslam, 1978). The difference in substrate grain size has a consequence for nutrient availability as fine grained substrate typically is more nutrient rich than coarse grained (Stutter et al., 2007), and thus root uptake is more likely to be of consequence. Furthermore, it is important to consider not only the nutrient content in the sediment but the bioavailability of these nutrients. The availability of phosphorus in the sediments depends not only on how much orthophosphate is present but also how much aluminium (Al) and iron (Fe) is present in the sediment as these bind with the phosphate making it less available for biological uptake. For example, Mebane et al. (2014) found that normalizing P to Fe or Al in sediments was a better indicator for macrophyte biomass than was P alone. Nutrient turnover in sediments can also affect the amount of nutrients available for macrophytes and indeed the species of nutrients available. Again, substrate type is of importance as finer grained sediments tend to contain more organic matter, which can fuel microbial turnover, increasing bioavailable nutrients (Trimmer et al., 2009). Fine sediment deters interstitial water replacement and large amounts of organic matter fuels heterotrophic respiration thus leading to anoxic conditions, affecting biogeochemical processes that determine

speciation of nutrients (Jones et al., 2012). Anoxia, for example, constrains what nitrogen transformations can take place, favouring ammonification and denitrification over nitrification (Bernhardt et al., 2002). However, macrophytes with extensive root networks can aerate the rhizosphere, facilitating a powerful nitrification-denitrification coupling (Risgaard-Petersen & Jensen, 1997). Furthermore, Trimmer et al. (2009) reports that the submerged macrophyte *Ranunculus penicillatus* var. *calacereous* favoured uptake of NH_4^+ produced in the sediment over NO_3^- from the water column, showing how important these transformations can be.

Given the importance of substrate type on nutrient dynamics in streams, the local hydrological conditions will also be of importance in determining where macrophytes will focus their nutrient uptake. Smaller particles require a lower water velocity to move and is thus more readily translocated with the current. Thus, sites of low water velocity are more prone to deposition of fine sediment, while those of high water velocity tend to be dominated by coarser sediment, with the finer sediment removed by currents. Macrophytes themselves alter the water velocity, and in this way alter the sedimentation rate (Sand-Jensen, 1998). The magnitude of water velocity reduction is different between species (Sand-Jensen, 1998), but if significant, a macrophyte bed can by this mechanism create a nutrient rich, fine substrate, potentially depleted in oxygen (Jones et al., 2012). There is a temporal perspective to this of course, because substantially increased sedimentation can only occur after the macrophyte stand has developed a certain amount of biomass (Jones et al., 2012).

Water velocity also has another effect that could influence where nutrient uptake occurs. A higher water velocity will decrease the diffusive boundary layer surrounding the leaves, making nutrient uptake more effective (Bishop et al., 1997), potentially making this the preferred pathway (Bowden et al., 2017). However, uptake will be lowered within the macrophyte stands as there is lowered water velocity. While hydrology has several important implications for the question of sediment or water column uptake, the most important effect is the influence on the distribution of the different species of macrophytes, in particular with regards to the different growth forms (Sand-Jensen et al., 1989).

The picture that emerges from this brief review of the role of sediments and water column nutrients in supporting stream macrophyte growth is one of complex interactions between multiple variables. At present, there is no simple paradigm for predicting macrophyte response to “eutrophication” analogous to the type of nutrient-biomass relationships developed for phytoplankton in lakes (e.g. Vollenweider, 1968).

1.1.5.4 Which is the most important nutrient in streams, N or P?

With a global meta-analysis of growth experiments, Elser et al. (2007) found that primary producers across ecosystem types generally show a greater response of co-addition of N and P than either of them independently. In freshwater ecosystems, the pattern recurs for the benthic compartment of streams, with N and P individually prompting equal responses and the combination of the two roughly

thrice that (Elser et al., 2007). However, while the study does elegantly illuminate these trends, its transfer to macrophyte responses is not without caveats. For example, the studies included for the stream benthic compartment are from periphyton, and thus do not to account for the complications arising from considering the availability of nutrients in both the water column and the sediment (as discussed above). The conceptual framework of Hilton et al. (2006) revised by O'hare et al. (2018), builds largely on P as the influencing factor. However, various studies relating macrophyte biomass to nutrients have found both N and P to have an effect (see Appendix I Table A.1.1), but responses vary, not only regarding type and form of nutrient, but also among species. The somewhat vague but nonetheless prudent conclusion remains that the in situ limiting nutrient will vary depending on nutrient load to the stream (underlying catchment geology, land use, etc.), in-stream transformations, the species of macrophytes present and indeed other factors limiting growth.

Expectations are that the future for anthropogenically impacted waters will lean toward a prevalence of limitation by P however, as the use of N fertilizer is increasing relative to P, which is rather stagnant (Peñuelas et al., 2013). Sediment erosion will still be a source of particulate bound P, but the dissolved inorganic N:P ratio in rivers globally has indeed increased (Vilmin et al., 2018). This altered nutrient stoichiometry imposed on freshwater ecosystems has several potential consequences for the macrophyte communities (Peñuelas et al., 2013). N:P ratios of foliage of plants have been shown to be negatively correlated with net photosynthetic rate (Reich et al., 2009), production of biomass (Gusewell, 2004) and plant growth (Elser et al., 2010). Indeed, high growth rates have been correlated to low N:P ratios, as this relates to an internal ratio of RNA to protein (Loladze & Elser, 2011). As such, fast growing species will have a lower N:P ratio (Rivas-Ubach et al., 2012), and lower environmental N:P ratios have been correlated with lower species richness, although in terrestrial environments (Seastedt & Vaccaro, 2001). Additionally, stoichiometric changes can affect other trophic levels (Giling et al., 2012) along with food web structure and nutrient recycling (Hall, 2009).

1.1.6 Influence of nutrients on community composition.

The effect of nutrients on the community composition of macrophytes species assemblages in streams is two-fold. Increases in nutrients can give a competitive advantage to species favouring fast growth and effective resource acquisition, and thus to an exclusion of other species (Hilton et al., 2006; O'hare et al., 2018). Additionally, as these competitive traits are very often found in invasive exotic species, increased nutrient levels can often help to facilitate invasion success (Fleming & Dibble, 2014). However, whether these effects manifest depends on the local hydrological, photic and thermal setting, thus complicating the untangling of nutrient effects and effects of other factors (Steffen et al., 2014).

1.1.6.1 Effect on species richness of communities.

The intermediate disturbance hypothesis has been applied to a theoretical nutrient-macrophyte relation framework, thus predicting that species richness should peak at intermediate nutrient concentrations (Bornette & Puijalon, 2011; Lacoul & Freedman, 2006). However, studies of species richness usually find no relationship with nutrients, while a few find a linear response (Appendix I Table A.1.1). Barker et al. (2008) found an increase in nutrients to have a diminishing effect on species richness in mesocosm experiments, a result that is predicted by others to take effect at high nutrient concentrations as only dominant competitive species are thought to thrive in these conditions (Bornette & Puijalon, 2011; Lacoul & Freedman, 2006; O'hare et al., 2018). Indeed, this is mentioned as a contributing factor in species decline several places in Europe (Sand-Jensen et al., 2000; Steffen et al., 2013). The reason the decline in species richness is not often seen in the field can be due to several factors. Some authors propose that lotic waters are generally thought of as nutrient rich due to the constant replenishment of nutrients by water flow (Demars & Harper, 2005), however this is inconsistent with other observations (O'hare et al., 2018). Another explanation is the importance of other factors affecting the macrophyte community; various other factors has been presented as better predictors of species richness. Son et al. (2018) found suspended solids and phytoplankton (quantified as suspended chlorophyll *a*) to have a negative effect on the richness of submerged macrophytes in South-Korean rivers, while Giorgi et al. (2005) considered water velocity to be the most important variable in determining richness, although water column P was a contributing factor. Finally, eutrophication can change community composition (Quinn et al., 2011) in that it has a beneficial effect on non-native taxa (Fleming & Dibble, 2014) and thus not change richness *per se*, but still have a detrimental effect on native species richness.

As with species richness (the total number of species), community composition (which species, and the proportional distribution of these) does not always show a response to changing nutrient concentration when investigated in the field (Demars & Harper, 2005; Giblin, 2017; Mebane et al., 2014), and again this is likely due the influence of other factors. Factors that have been found to be more important than nutrients in driving community composition are for example; spatial, longitudinal and between-rivers connectivity (Demars & Harper, 2005), concentration of suspended solids (Giblin, 2017), water velocity (Giorgi et al., 2005) and shading (Canfield & Hoyer, 1988). Mebane et al. (2014) even found several factors other than nutrients to affect the community composition. However, several studies have indeed found eutrophication, either alone or in interplay with other factors, to play a role in structuring macrophyte communities. Quinn et al. (2011) found that anthropogenic disturbances, including eutrophication, promotes a higher fraction of exotic species, not unlike the results of Mouton et al. (2019). Although watercourse size had greatest effect on community composition, a tendency for species associated with eutrophication to dominate downstream from sewage plants was found by Demars and Harper (1998), as well as a weak

relationship between eutrophic species and soluble phosphate and nitrate. Carbiener et al. (1990) correlated different macrophyte communities with different nutrient conditions as NH_4^+ and PO_4^{3-}, similar to Feijoo and Lombardo (2007) and Kennedy et al. (2015). The resultant conclusion ends up being that eutrophication does indeed have an effect on the community of stream macrophytes, in that it fosters the dominance of competitive species (O'hare et al., 2018) (Appendix I Table A.1.1 – *effects on community composition*), however it is still more important for a community whether the flow environment is suitable for macrophyte growth (e.g. O'hare et al., 2011) or if there is enough incoming irradiation (e.g. Julian et al., 2011).

1.1.6.2 Traits of competitive species.

Plant traits of species living in a high nutrient environment are centred around either enhanced acquisition of light or the ability to tolerate declines in incoming irradiation (Baattrup-Pedersen et al., 2015), as the competition for light is so important when nutrients are no longer limiting. In a study of 772 European lowland streams, Baattrup-Pedersen et al. (2015) found that traits associated with nutrient enrichment (measured as agricultural influence) was inherently connected to light gathering or utilisation. Species in high nutrient environments grew from apical or multi-apical meristems or were tolerant of low light intensities (Baattrup-Pedersen et al., 2015). This is coherent with the predictions of O'hare et al. (2018), who suggests the remaining macrophytes in eutrophic streams will be highly competitive species, in possession of these traits (Riis & Biggs, 2001). As noted by Riis and Biggs (2001), most of the macrophytes in Aotearoa New Zealand streams falling under the "C" and "R" category (sensu Grime) are exotic species while native macrophytes dominate the "S" category. Similarly, (Mouton et al., 2019) found that disturbed and eutrophicated stream systems were dominated by exotic species that often possess traits related to effective colonisation and resource gathering. The invasion success of these species is in part due to their plasticity in traits relating to light acquisition (Ellawala Kankanamge et al., 2019).

Palatability can also be expected to change with increasing nutrients; from studies in ponds, there is experimental evidence that increases in nutrient inputs leads to increases in grazing pressure by waterfowl (Bakker & Nolet, 2014). By exposing fertilized and unfertilized ponds to consumers for 8 days, Bakker and Nolet (2014) observed a 50 % decrease in macrophyte biomass in fertilized ponds. Following the nutrient increase there was an increase in internal nutrient concentration and a dominance of *Elodea nuttallii*, whereas in the unfertilized ponds the dominant species was *Chara globularis*, considering the inherent difference in palatability of the two species, the authors suggest the increases in grazing pressure to be the result of higher palatability due to increased nutrient levels (Bakker & Nolet, 2014). Whether this result is transferable to streams is uncertain, but as mentioned before, water velocities can limit the impact of waterfowl grazers (Wood et al., 2018) which could insinuate that elevated grazing pressures induced by eutrophication is restricted to lentic systems or slow flowing rivers.

1.1.6.3 Interplay with epiphytes. Is the competition between the plants and the algae more important than between the plants themselves?

Due to the importance of light, cover of epiphytic algae can adversely affect macrophyte growth (Ham et al., 1981; Spink et al., 1993), however this shading mostly affects the submerged growth types, as emergent and floating leaved types can escape by growing out of the water. This imposed limitation is potentially exacerbated by increases in water column nutrients due to increased algae growth rates (Elser et al., 2007). Thus, at non-limiting nutrient levels, epiphytes always have the potential to out-shade submerged macrophytes, given they can cover the macrophytes enough to reduce light to insufficient levels for growth (O'hare et al., 2018).

Whether algal growth shades macrophytes enough to curtail growth depends on the amount of incoming light (Bowes et al., 2012), macroinvertebrate grazing (Neckles et al., 1993) or hydrology (Biggs, 1996; O'hare et al., 2010). Furthermore, macrophyte species can vary in tolerance of light reduction (Kern-Hansen & Dawson, 1978) or ability to grow faster than the epiphytes can cover them (O'hare et al., 2010). Hydrologic conditions suitable for epiphytic development will increase with decreasing stream flow, though stream flow also determines which growth form of macrophyte will be dominant (e.g. O'hare et al., 2011) and at the conditions where epiphytes have best conditions macrophytes will predominantly be of floating leaved or emergent types, and thus not that susceptible to epiphytic smothering. Where epiphytes can persist on submerged vegetation however, they can have significant detrimental effects on macrophyte growth, although in combination with other shading influences (phytoplankton and riparian vegetation) (Köhler et al., 2010). Still, the rather specific conditions necessary for epiphytes to affect macrophyte growth probably results in a limited overall impact under further eutrophication, which mean that competition between macrophyte species will be the dominant form of competition, rather than between macrophytes and epiphytes. Additionally, under eutrophic conditions, macrophytes will be competing for light (Baattrup-Pedersen et al., 2015), thus the epiphytes will not change the resource around which the competition is revolving.

1.2 Thesis structure and objectives.

The state of knowledge concerning eutrophication effects on stream plants has improved much since just twenty years ago, where concepts from lake ecology (whose attention from the scientific community dwarfed that of streams at the time) started to be tested in lotic settings (Hilton et al., 2006). Much remains to be investigated, however, not least because the evident interplay between variables and plant traits, coupled with the temporal dynamics of lotic systems, makes attribution of cause and effect difficult. By use of a field survey and a novel experimental setup this study aims to fill several research gaps:

- How does eutrophication affect submerged macrophytes specifically in open lowland streams in New Zealand?
- Does eutrophication help facilitate invasive species and if so, how?

Intertwined in these questions lies the questions; what are the thresholds nutrient concentrations where eutrophication effects are seen (i.e. at what concentration does community composition shift in the field and at what concentration does eutrophication start to facilitate the competitive superiority of non-native species over natives) and is imposing limitations on nutrient inputs to streams a viable management strategy?

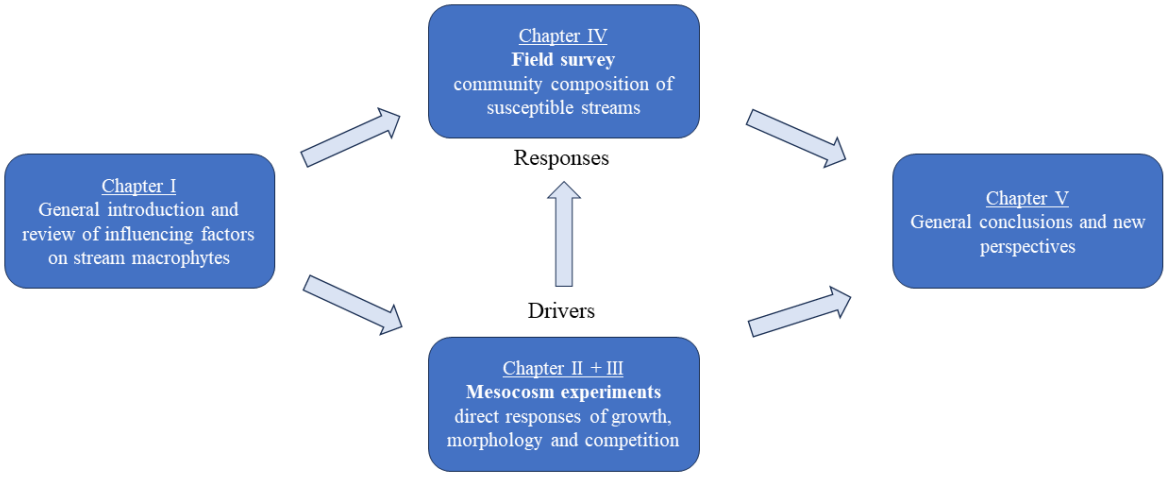
Logically, the thesis was structured around the idea that the primary driver of community composition change following eutrophication is the difference between species in their growth responses when exposed to high nutrient levels (Figure 1.2). This will lead to competition for space, that some species will lose and therefore be excluded. Following this idea, the thesis investigates eutrophication effects on stream macrophytes by specifically looking at:

i) Growth responses of macrophytes at different nutrient levels (Chapter II), which affects:

ii) Competitive interactions at different nutrient levels (Chapter III), which, lastly is the driver of:

iii) Community composition changes along a gradient of nutrient input to streams in vivo (Chapter IV).

Figure 1-2: Thesis logical structure and chapter relationships.



The chapters will address the research questions as follows.

1.2.1 Chapter II - Growth response to nitrate enrichment helps facilitate success of an alien *Potamogeton* in New Zealand streams.

One proposed factor facilitating invasion success of non-native species is the ability to grow well in high resource environments (Fleming & Dibble, 2014). In this chapter, a full factorial experiment is performed to elucidate the effects of different nitrate concentrations on the growth, morphology, and photosynthetic response in two sets of non-native and native macrophyte species of the same genus. The aim of this chapter is to find out whether the performance of the non-native species in eutrophic water is greater than that of the native one and thus if this difference can help explain the invasion success of the exotic. Additionally, knowledge will be gained relating the growth of aquatic macrophytes to the concentrations of water column nutrients, which will aid in determining nutrient concentration thresholds of eutrophication effects. The hypotheses to be tested is as follows:

(i) internal nutrient status of the plants will scale with water column nutrient concentration and
(ii) plant performance will reflect the nutrient status of the plant.

Furthermore, I hypothesized that
(iii) a low irradiance level would negate the effects of an increased nitrate level. In relation to (ii) I hypothesized that
(iv) the traits of the alien species would enable it to outperform the native species where both the availability of light and nutrient resources was high.

1.2.2 Chapter III – Direct competition between an alien and a native Aotearoa New Zealand macrophyte: Stronger effects of inherent trait expression than nutrient level.

Anthropogenically disturbed stream ecosystems are more prone to be occupied by non-native species than pristine ones (Mouton et al., 2019; Quinn et al., 2011). Among the disturbances, eutrophication and removal of riparian vegetation are among the most widespread. In this chapter the competitive ability of a non-native species relative to a native species of the same genus is investigated under increasing light and nutrient availability. The experiment uses two light treatments, four nutrient treatments and X species composition treatments in a full factorial design. The aim of this chapter is to assess whether the non-native species perform better than the closely related native species when grown together, and if so, whether this performance gap increases with higher resource availability. Additionally, this chapter will elucidate the relative importance of nutrients and light on the outcome of macrophyte competition. Hypothesis is as follows:

"That the non-native *P. crispus* is the better competitor at high nutrient levels and high light and that the native *P. ochreatus* competes better at low nutrient and light levels."

1.2.3 Chapter IV - Human induced catchment changes cause nuisance macrophyte biomass and functional trait changes in susceptible New Zealand streams.

Evidence suggests that when nutrients are no longer limiting, competition for light becomes the dominant factor determining species composition in macrophyte-inhabited streams and dominant species will be those with better light acquisition strategies. With a field survey of streams in the Waikato region spanning a wide range of nutrient concentrations, the relationship between the stream environment and community structure of submerged macrophytes will be investigated. The focus will be on streams that are 'susceptible', that is, streams that are open, slow flowing, medium sized, and with fine substrate. The aim is to distinguish effects of different types of nutrients in different phases (water column vs sediment), and to see how this affects the internal nutrient status of the plants. In the field survey the concentrations of nutrients will be in focus, but several other factors will be measured (e.g., alkalinity, turbidity, water velocity etc.) to compare their importance with that of nutrients. On top of investigating the effects of type of nutrient, the aim is also to find threshold concentrations for eutrophication effects, i.e., the concentration at which community shifts starts to occur. The chapter has the following expectations:

(i) Streams receiving higher nutrient inputs have greater macrophyte biomass and a community composition dominated by non-native species and a lower overall macrophyte species diversity.
(ii) The nutrient content of less dynamic reservoirs in the streams (i.e., macrophyte tissues and sediments) would better correlate with nutrient inputs from land, and thus better reflect the nutrient supply available to macrophytes than one-off water samples collected from sites at the time of survey.

1.2.4 Chapter V – Knowledge gained, a revised synthesis on eutrophication effects on stream macrophytes.

In this chapter I synthesise an updated version of the eutrophication effect framework, based on the findings of this thesis. I present the logical structure of the thesis which provides the foundation for the arguments that leads to the conclusions of this project. I collate the results and conclusions and put these into perspective, specifically with regards to the threshold concentrations on eutrophication effects. Additionally, I recommend further avenues of research.

References

Allan, J. D., Castillo, M. M., & Capps, K. A. (2021). *Stream ecology: structure and function of running waters*: Springer Nature.

Artigas, J., García-Berthou, E., Bauer, D. E., Castro, M. I., Cochero, J., Colautti, D. C., . . . Sabater, S. (2013). Erratum: Global pressures, specific responses: effects of nutrient enrichment in streams from different biomes. *Environmental Research Letters, 8*(1). DOI:10.1088/1748-9326/8/1/019601

Bakker, E. S. & Nolet, B. A. (2014). Experimental evidence for enhanced top-down control of freshwater macrophytes with nutrient enrichment. *Oecologia, 176*(3), 825-836. DOI:10.1007/s00442-014-3047-y

Bakker, E. S., Pages, J. F., Arthur, R., & Alcoverro, T. (2016). Assessing the role of large herbivores in the structuring and functioning of freshwater and marine angiosperm ecosystems. *Ecography, 39*(2), 162-179. DOI:10.1111/ecog.01651

Bal, K. D., Bouma, T. J., Buis, K., Struyf, E., Jonas, S., Backx, H., & Meire, P. (2011). Trade-off between drag reduction and light interception of macrophytes: comparing five aquatic plants with contrasting morphology. *Functional Ecology, 25*(6), 1197-1205. DOI:10.1111/j.1365-2435.2011.01909.x

Barker, T., Hatton, K. O. C., Connor, L., & Moss, B. (2008). Effects of nitrate load on submerged plant biomass and species richness: results of a mesocosm experiment. *Fundamental and Applied Limnology, 173*(2), 89-100. DOI:10.1127/1863-9135/2008/0173-0089

Barko, J. W., Gunnison, D., & Carpenter, S. R. (1991). Sediment interactions with submersed macrophyte growth and community dynamics. *Aquatic Botany, 41*(1-3), 41-65.

Barko, J. W. & James, W. F. (1998). Effects of Submerged Aquatic Macrophytes on Nutrient Dynamics, Sedimentation, and Resuspension. In E. Jeppesen, M. Søndergaard, & K. S. Christoffersen (Eds.), *The Structuring Role of Submerged Macrophytes in Lakes* (Vol. 131): Springer Science & Business Media.

Bear, D. A., Russell, J. R., Tufekcioglu, M., Isenhart, T. M., Morrical, D. G., & Kovar, J. L. (2012). Stocking Rate and Riparian Vegetation Effects on Physical Characteristics of Riparian Zones of Midwestern Pastures. *Rangeland Ecology & Management, 65*(2), 119-128. DOI:10.2111/Rem-D-11-00133.1

Bernhardt, E. S., Hall, J., Robert O, & Likens, G. E. (2002). Whole-system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook Experimental Forest. *Ecosystems, 5*, 419-430.

Bernot, M. J., Tank, J. L., Royer, T. V., & David, M. B. (2006). Nutrient uptake in streams draining agricultural catchments of the midwestern United States. *Freshwater Biology, 51*(3), 499-509. DOI:10.1111/j.1365-2427.2006.01508.x

Biggs, B. J. F. (1996). Hydraulic habitat of plants in streams. *Regulated Rivers-Research & Management, 12*(2-3), 131-144. DOI:Doi 10.1002/(Sici)1099-1646(199603)12:2/3<131::Aid-Rrr385>3.3.Co;2-O

Bishop, P. L., Gibbs, J. T., & Cunningham, B. E. (1997). Relationship between concentration and hydrodynamic boundary layers over biofilms. *Environmental Technology, 18*(4), 375-385. DOI:Doi 10.1080/09593331808616551

Bornette, G. & Puijalon, S. (2011). Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences, 73*(1), 1-14. DOI:10.1007/s00027-010-0162-7

Boston, H. L., Adams, M. S., & Pienkowski, T. P. (1987). Utilisation of Sediment CO2 by Selected North American Isoetids. *Annals of Botany, 60*(5), 485-494. DOI:10.1093/oxfordjournals.aob.a087471

Bowden, W. B., Glime, J. M., & Riis, T. (2017). Macrophytes and bryophytes. In *Methods in Stream Ecology, Volume 1* (pp. 243-271): Elsevier.

Bowes, M. J., Ings, N. L., McCall, S. J., Warwick, A., Barrett, C., Wickham, H. D., . . . Singer, A. C. (2012). Nutrient and light limitation of periphyton in the River Thames: implications for

catchment management. *Sci Total Environ, 434*, 201-212. DOI:10.1016/j.scitotenv.2011.09.082
Bracken, M. E. S., Hillebrand, H., Borer, E. T., Seabloom, E. W., Cebrian, J., Cleland, E. E., . . . Smith, J. E. (2015). Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos, 124*(2), 113-121. DOI:10.1111/oik.01215
Baattrup-Pedersen, A., Gothe, E., Larsen, S. E., O'Hare, M., Birk, S., Riis, T., & Friberg, N. (2015). Plant trait characteristics vary with size and eutrophication in European lowland streams. *J Appl Ecol, 52*(6), 1617-1628. DOI:10.1111/1365-2664.12509
Canfield, D. E. & Hoyer, M. V. (1988). Infuence of Nutrient Enrichment and Light Availability on the Abundance of Aquatic Macrophytes in Florida Streams. *Canadian journal of fisheries and aquatic sciences, 45*, 1467-1472.
Carbiener, R., Tremolieres, M., Mercier, J. L., & Ortscheit, A. (1990). Aquatic Macrophyte Communities as Bioindicators of Eutrophication in Calcareous Oligosaprobe Stream Waters (Upper Rhine Plain, Alsace). *Vegetatio, 86*(1), 71-88. DOI:Doi 10.1007/Bf00045135
Carpenter, S. R. & Lodge, D. M. (1986). Effects of Submersed Macrophytes on Ecosystem Processes. *Aquatic Botany, 26*(3-4), 341-370. DOI:Doi 10.1016/0304-3770(86)90031-8
Carr, G. M. & Chambers, P. A. (1998). Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. *Freshwater Biology, 39*(3), 525-536. DOI:10.1046/j.1365-2427.1998.00300.x
Carr, G. M., Duthie, H. C., & Taylor, W. D. (1997). Models of aquatic plant productivity: a review of the factors that influence growth. *Aquatic Botany, 59*(3-4), 195-215.
Cavalli, G., Riis, T., & Baattrup-Pedersen, A. (2012). Bicarbonate use in three aquatic plants. *Aquatic Botany, 98*(1), 57-60. DOI:10.1016/j.aquabot.2011.12.007
Chambers, P. A., Prepas, E. E., Bothwell, M. L., & Hamilton, H. R. (1989). Roots Versus Shoots in Nutrient-Uptake by Aquatic Macrophytes in Flowing Waters. *Canadian journal of fisheries and aquatic sciences, 46*(3), 435-439. DOI:DOI 10.1139/f89-058
Champion, P., Rowe, D., Smith, B., Wells, R., Kilroy, C., & de Winton, M. (2013). *Freshwater pests of New Zealand*. Retrieved from NIWA, Wellington:
Collins, K. E., Febria, C. M., Warburton, H. J., Devlin, H. S., Hogsden, K. L., Goeller, B. C., . . . Harding, J. S. (2018). Evaluating practical macrophyte control tools on small agricultural waterways in Canterbury, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 53*(2), 182-200. DOI:10.1080/00288330.2018.1487454
Cooper, A. B. & Cooke, J. G. (1984). Nitrate loss and transformation in 2 vegetated headwater streams. *New Zealand Journal of Marine and Freshwater Research, 18*(4), 441-450.
Demars, B. O. L. & Harper, D. M. (1998). The aquatic macrophytes of an English lowland river system: assessing response to nutrient enrichment. *Hydrobiologia, 384*, 75-88. DOI:Doi 10.1023/A:1003203512565
Demars, B. O. L. & Harper, D. M. (2005). Distribution of aquatic vascular plants in lowland rivers: separating the effects of local environmental conditions, longitudinal connectivity and river basin isolation. *Freshwater Biology, 50*(3), 418-437. DOI:10.1111/j.1365-2427.2004.01329.x
Dibble, E. D., Killgore, K. J., & Harrel, S. L. (1996). Assessment of fish-plant interactions. *Multidimensional Approaches to Reservoir Fisheries Management, 16*, 357-372.
Dodds, W. K. (2007). Trophic state, eutrophication and nutrient criteria in streams. *Trends Ecol Evol, 22*(12), 669-676. DOI:10.1016/j.tree.2007.07.010
Ellawala Kankanamge, C., Matheson, F. E., & Riis, T. (2019). Shading constrains the growth of invasive submerged macrophytes in streams. *Aquatic Botany, 158*. DOI:10.1016/j.aquabot.2019.06.004
Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., . . . Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett, 10*(12), 1135-1142. DOI:10.1111/j.1461-0248.2007.01113.x
Elser, J. J., Fagan, W. F., Kerkhoff, A. J., Swenson, N. G., & Enquist, B. J. (2010). Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytol, 186*(3), 593-608. DOI:10.1111/j.1469-8137.2010.03214.x

Feijoo, C. S. & Lombardo, R. J. (2007). Baseline water quality and macrophyte assemblages in Pampean streams: a regional approach. *Water Res, 41*(7), 1399-1410. DOI:10.1016/j.watres.2006.08.026

Fleming, J. P. & Dibble, E. D. (2014). Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia, 746*(1), 23-37. DOI:10.1007/s10750-014-2026-y

Garbey, C., Thiebaut, G., & Muller, S. (2004). Morphological plasticity of a spreading aquatic macrophyte, Ranunculus peltatus, in response to environmental variables. *Plant Ecology, 173*(1), 125-137. DOI:10.1023/B:VEGE.0000026336.44576.ff

Giblin, S. M. (2017). Identifying and quantifying environmental thresholds for ecological shifts in a large semi-regulated river. *Journal of Freshwater Ecology, 32*(1), 433-453. DOI:10.1080/02705060.2017.1319431

Giling, D. P., Reich, P., & Thompson, R. M. (2012). Riparian vegetation removal alters consumer - resource stoichiometry in an Australian lowland stream. *Marine and Freshwater Research, 63*(1). DOI:10.1071/mf11092

Giorgi, A., Feijoó, C., & Tell, G. (2005). Primary producers in a Pampean stream: temporal variation and structuring role. *Biodiversity and Conservation, 14*(7), 1699-1718. DOI:10.1007/s10531-004-0694-z

Grime, J. P. (2006). *Plant strategies, vegetation processes, and ecosystem properties*: John Wiley & Sons.

Gusewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytol, 164*(2), 243-266. DOI:10.1111/j.1469-8137.2004.01192.x

Hall, S. R. (2009). Stoichiometrically Explicit Food Webs: Feedbacks between Resource Supply, Elemental Constraints, and Species Diversity. *Annual Review of Ecology, Evolution, and Systematics, 40*(1), 503-528. DOI:10.1146/annurev.ecolsys.39.110707.173518

Hall, S. R., Smith, V. H., Lytle, D. A., & Leibold, M. A. (2005). Constraints on primary producer N : P stoichiometry along N : P supply ratio gradients. *Ecology, 86*(7), 1894-1904. DOI:Doi 10.1890/04-1045

Ham, S. F., Wright, J. F., & Berrie, A. D. (1981). Growth and recession of aquatic macrophytes on an unshaded section of the River Lamhourn, England, from 1971 to 1976. *Freshwater Biology, 11*, 381-390.

Haslam, S. M. (1978). River plants; the macrophytic vegetation of watercourses.

Hawes, I., Riis, T., Sutherland, D., & Flanagan, M. (2003). Physical constraints to aquatic plant growth in New Zealand lakes. *Journal of Aquatic Plant Management, 41*, 44-52.

Hilton, J., O'Hare, M., Bowes, M. J., & Jones, J. I. (2006). How green is my river? A new paradigm of eutrophication in rivers. *Sci Total Environ, 365*(1-3), 66-83. DOI:10.1016/j.scitotenv.2006.02.055

James, C. S., Eaton, J. W., & Hardwick, K. (2006). Responses of three invasive aquatic macrophytes to nutrient enrichment do not explain their observed field displacements. *Aquatic Botany, 84*(4), 347-353. DOI:10.1016/j.aquabot.2006.01.002

Johnson, W. C. (1994). Woodland Expansion in the Platte River, Nebraska - Patterns and Causes. *Ecological Monographs, 64*(1), 45-84. DOI:Doi 10.2307/2937055

Jones, J. I., Collins, A. L., Naden, P. S., & Sear, D. A. (2012). The Relationship between Fine Sediment and Macrophytes in Rivers. *River Research and Applications, 28*(7), 1006-1018. DOI:10.1002/rra.1486

Julian, J. P., Seegert, S. Z., Powers, S. M., Stanley, E. H., & Doyle, M. W. (2011). Light as a first-order control on ecosystem structure in a temperate stream. *Ecohydrology, 4*(3), 422-432. DOI:10.1002/eco.144

Kennedy, M. P., Lang, P., Grimaldo, J. T., Martins, S. V., Bruce, A., Hastie, A., . . . Murphy, K. J. (2015). Environmental drivers of aquatic macrophyte communities in southern tropical African rivers: Zambia as a case study. *Aquatic Botany, 124*, 19-28. DOI:10.1016/j.aquabot.2015.03.002

Kern-Hansen, U. & Dawson, F. H. (1978). *The Standing Crop of Aquatic Plants in Lowland Streams in Denmark and the Interrelationships of Nutrients in Plant, Sediment, and Water*. Paper presented at the Proc. EWRS 5th Symp. on Aquatic Weeds.

Kundzewicz, Z. W. (1999). Flood protection—sustainability Issues. *Hydrological Sciences Journal, 44*(4), 559-571. DOI:10.1080/02626669909492252
Köhler, J., Hachoł, J., & Hilt, S. (2010). Regulation of submersed macrophyte biomass in a temperate lowland river: Interactions between shading by bank vegetation, epiphyton and water turbidity. *Aquatic Botany, 92*(2), 129-136. DOI:10.1016/j.aquabot.2009.10.018
Lacoul, P. & Freedman, B. (2006). Environmental influences on aquatic plants in freshwater ecosystems. *Environmental Reviews, 14*(2), 89-136. DOI:10.1139/A06-001
Lake, M. D., Hicks, B. J., Wells, R. D. S., & Dugdale, T. M. (2002). Consumption of submerged aquatic macrophytes by rudd (Scardinius erythrophthalmus L.) in New Zealand. *Hydrobiologia, 470*(1-3), 13-22. DOI:Doi 10.1023/A:1015689432289
Lambers, H., Chapin, F. S., & Pons, T. L. (2008). *Plant physiological ecology* (Vol. 2): Springer.
Levi, P. S., Riis, T., Alnøe, A. B., Peipoch, M., Maetzke, K., Bruus, C., & Baattrup-Pedersen, A. (2015). Macrophyte Complexity Controls Nutrient Uptake in Lowland Streams. *Ecosystems, 18*(5), 914-931. DOI:10.1007/s10021-015-9872-y
Li, W., Li, Y., Zhong, J., Fu, H., Tu, J., & Fan, H. (2018). Submerged Macrophytes Exhibit Different Phosphorus Stoichiometric Homeostasis. *Front Plant Sci, 9*, 1207. DOI:10.3389/fpls.2018.01207
Ligon, F. K., Dietrich, W. E., & Trush, W. J. (1995). Downstream Ecological Effects of Dams. *Bioscience, 45*(3), 183-192. DOI:Doi 10.2307/1312557
Loladze, I. & Elser, J. J. (2011). The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio. *Ecol Lett, 14*(3), 244-250. DOI:10.1111/j.1461-0248.2010.01577.x
Maberly, S. C., Berthelot, S. A., Stott, A. W., & Gontero, B. (2015). Adaptation by macrophytes to inorganic carbon down a river with naturally variable concentrations of CO2. *J Plant Physiol, 172*, 120-127. DOI:10.1016/j.jplph.2014.07.025
Madsen, T. V. & Cedergreen, N. (2002). Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology, 47*(2), 283-291. DOI:10.1046/j.1365-2427.2002.00802.x
Matheson, F. E., Quinn, J., & Hickey, C. (2012). *Review of the New Zealand instream plant and nutrient guidelines and development of an extended decision making framework: Phases 1 and 2 final report* (HAM2012-081). Retrieved from
McCreary, N. J. (1991). Competition as a Mechanism of Submersed Macrophyte Community Structure. *Aquatic Botany, 41*(1-3), 177-193. DOI:Doi 10.1016/0304-3770(91)90043-5
Mebane, C. A., Simon, N. S., & Maret, T. R. (2014). Linking nutrient enrichment and streamflow to macrophytes in agricultural streams. *Hydrobiologia, 722*(1), 143-158. DOI:10.1007/s10750-013-1693-4
Mooney, H. A. & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proc Natl Acad Sci U S A, 98*(10), 5446-5451. DOI:10.1073/pnas.091093398
Mouton, T. L., Matheson, F. E., Stephenson, F., Champion, P. D., Wadhwa, S., Hamer, M. P., . . . Riis, T. (2019). Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape. *Sci Total Environ, 659*, 1370-1381. DOI:10.1016/j.scitotenv.2018.12.277
Neckles, H. A., Wetzel, R. L., & Orth, R. J. (1993). Relative Effects of Nutrient Enrichment and Grazing on Epiphyte-Macrophyte (Zostera-Marina L) Dynamics. *Oecologia, 93*(2), 285-295. DOI:Doi 10.1007/Bf00317683
O'Hare, J. M., O'Hare, M. T., Gurnell, A. M., Dunbar, M. J., Scarlett, P. M., & Laizé, C. (2011). Physical constraints on the distribution of macrophytes linked with flow and sediment dynamics in British rivers. *River Research and Applications, 27*(6), 671-683. DOI:10.1002/rra.1379
O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lazar, A. N., . . . Bowes, M. J. (2018). Responses of Aquatic Plants to Eutrophication in Rivers: A Revised Conceptual Model. *Front Plant Sci, 9*, 451. DOI:10.3389/fpls.2018.00451
O'Hare, M. T., Clarke, R. T., Bowes, M. J., Cailes, C., Henville, P., Bissett, N., . . . Neal, M. (2010). Eutrophication impacts on a river macrophyte. *Aquatic Botany, 92*(3), 173-178. DOI:10.1016/j.aquabot.2009.11.001

O'Hare, M. T., Stillman, R. A., McDonnell, J. O., & Wood, L. R. (2007). Effects of mute swan grazing on a keystone macrophyte. *Freshwater Biology, 52*(12), 2463-2475. DOI:10.1111/j.1365-2427.2007.01841.x

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., . . . Janssens, I. A. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat Commun, 4*, 2934. DOI:10.1038/ncomms3934

Piepho, M. (2017). Assessing maximum depth distribution, vegetated area, and production of submerged macrophytes in shallow, turbid coastal lagoons of the southern Baltic Sea. *Hydrobiologia, 794*(1), 303-316. DOI:10.1007/s10750-017-3107-5

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., . . . Stromberg, J. C. (1997). The natural flow regime. *Bioscience, 47*(11), 769-784.

Quinn, L. D., Schooler, S. S., & Van Klinken, R. D. (2011). Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions, 17*(1), 132-143. DOI:10.1111/j.1472-4642.2010.00726.x

Quirino, B. A., Carniatto, N., Thomaz, S. M., Aleixo, M. H. F., & Fugi, R. (2019). The amphibian macrophyte Polygonum punctatum as a temporary habitat and feeding ground for fish. *Aquatic Ecology, 53*(3), 441-452. DOI:10.1007/s10452-019-09700-9

Raven, J. A., Handley, L. L., Macfarlane, J. J., Mcinroy, S., Mckenzie, L., Richards, J. H., & Samuelsson, G. (1988). The role of CO2 uptake by roots and CAM in acquisition of inorganic C by plants of the isoetid life-form: a review, with new data on Eriocaulon decangulare L. *New Phytologist, 108*(2), 125-148. DOI:https://doi.org/10.1111/j.1469-8137.1988.tb03690.x

Redekop, P., Gross, E. M., Nuttens, A., Hofstra, D. E., Clayton, J. S., & Hussner, A. (2018). Hygraula nitens, the only native aquatic caterpillar in New Zealand, prefers feeding on an alien submerged plant. *Hydrobiologia, 812*(1), 13-25. DOI:10.1007/s10750-016-2709-7

Reich, P. B., Oleksyn, J., & Wright, I. J. (2009). Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. *Oecologia, 160*(2), 207-212. DOI:10.1007/s00442-009-1291-3

Ren, M. X. & Zhang, Q. G. (2009). The relative generality of plant invasion mechanisms and predicting future invasive plants. *Weed Research, 49*(5), 449-460. DOI:10.1111/j.1365-3180.2009.00723.x

Riis, T. & Biggs, B. J. F. (2001). Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply - a synthesis and conceptual model. *New Zealand Journal of Marine and Freshwater Research, 35*(2), 255-267. DOI:10.1080/00288330.2001.9516996

Riis, T. & Biggs, B. J. F. (2003). Hydrologic and hydraulic control of macrophyte establishment and performance in streams. *Limnology and Oceanography, 48*(4), 1488-1497. DOI:10.4319/lo.2003.48.4.1488

Riis, T., Olesen, B., Clayton, J. S., Lambertini, C., Brix, H., & Sorrell, B. K. (2012). Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. *Aquatic Botany, 102*, 56-64. DOI:10.1016/j.aquabot.2012.05.002

Riis, T., Suren, A. M., Clausen, B., & Sand-Jensen, K. A. J. (2008). Vegetation and flow regime in lowland streams. *Freshwater Biology, 53*(8), 1531-1543. DOI:10.1111/j.1365-2427.2008.01987.x

Risgaard-Petersen, N. & Jensen, K. (1997). Nitrification and denitrification in the rhizosphere of the aquatic macrophyte Lobelia dortmanna L. *Limnology and Oceanography, 42*(3), 529-537. DOI:DOI 10.4319/lo.1997.42.3.0529

Rivas-Ubach, A., Sardans, J., Perez-Trujillo, M., Estiarte, M., & Penuelas, J. (2012). Strong relationship between elemental stoichiometry and metabolome in plants. *Proc Natl Acad Sci U S A, 109*(11), 4181-4186. DOI:10.1073/pnas.1116092109

Ságová-Marečková, M., Petrusek, A., & Květ, J. (2009). Biomass production and nutrient accumulation in Sparganium emersum Rehm. after sediment treatment with mineral and organic fertilisers in three mesocosm experiments. *Aquatic Ecology, 43*(4), 903-913. DOI:10.1007/s10452-008-9214-7

Sand-Jensen, K. (1983). Photosynthetic Carbon-Sources of Stream Macrophytes. *Journal of Experimental Botany, 34*(139), 198-210. DOI:DOI 10.1093/jxb/34.2.198
Sand-Jensen, K. (1998). Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology, 39*(4), 663-679. DOI:10.1046/j.1365-2427.1998.00316.x
Sand-Jensen, K., Jeppesen, E., Nielsen, K., Vanderbijl, L., Hjermind, L., Nielsen, L. W., & Iversen, T. M. (1989). Growth of Macrophytes and Ecosystem Consequences in a Lowland Danish Stream. *Freshwater Biology, 22*(1), 15-32. DOI:DOI 10.1111/j.1365-2427.1989.tb01080.x
Sand-Jensen, K., Pedersen, N. L., Thorsgaard, I., Moeslund, B., Borum, J., & Brodersen, K. P. (2008). 100 years of vegetation decline and recovery in Lake Fure, Denmark. *Journal of Ecology, 96*(2), 260-271. DOI:10.1111/j.1365-2745.2007.01339.x
Sand-Jensen, K., Riis, T., & Martinsen, K. T. (2022). Photosynthesis, growth, and distribution of plants in lowland streams—A synthesis and new data analyses of 40 years research. *Freshwater Biology, 67*(7), 1255-1271. DOI:https://doi.org/10.1111/fwb.13915
Sand-Jensen, K., Riis, T., Vestergaard, O., & Larsen, S. E. (2000). Macrophyte decline in Danish lakes and streams over the past 100 years. *Journal of Ecology, 88*(6), 1030-1040. DOI:10.1046/j.1365-2745.2000.00519.x
Schaeffer, S. M., Sharp, E., Schimel, J. P., & Welker, J. M. (2013). Soil–plant N processes in a High Arctic ecosystem, NW Greenland are altered by long‐term experimental warming and higher rainfall. *Global change biology, 19*(11), 3529-3539.
Schultz, R. & Dibble, E. (2011). Effects of invasive macrophytes on freshwater fish and macroinvertebrate communities: the role of invasive plant traits. *Hydrobiologia, 684*(1), 1-14. DOI:10.1007/s10750-011-0978-8
Schutten, J., Dainty, J., & Davy, A. J. (2005). Root anchorage and its significance for submerged plants in shallow lakes. *Journal of Ecology, 93*(3), 556-571. DOI:10.1111/j.1365-2745.2005.00980.x
Schwarz, A. M., Howard‐Williams, C., & Clayton, J. (2000). Analysis of relationships between maximum depth limits of aquatic plants and underwater light in 63 New Zealand lakes. *New Zealand Journal of Marine and Freshwater Research, 34*(1), 157-174. DOI:10.1080/00288330.2000.9516923
Sculthorpe, C. D. (1967). *The biology of aquatic vascular plants*.
Seastedt, T. R. & Vaccaro, L. (2001). Plant Species Richness, Productivity, and Nitrogen and Phosphorus Limitations across a Snowpack Gradient in Alpine Tundra, Colorado, U.S.A. *Arctic, Antarctic, and Alpine Research, 33*(1), 100-106. DOI:10.1080/15230430.2001.12003410
Snelder, T. H., Larned, S. T., & McDowell, R. W. (2017). Anthropogenic increases of catchment nitrogen and phosphorus loads in New Zealand. *New Zealand Journal of Marine and Freshwater Research, 52*(3), 336-361. DOI:10.1080/00288330.2017.1393758
Son, D., Cho, H., & Lee, E. J. (2018). Determining factors for the occurrence and richness of submerged macrophytes in major Korean rivers. *Aquatic Botany, 150*, 82-88. DOI:10.1016/j.aquabot.2018.07.003
Spink, A. J., Murphy, K. J., Smith, S. M., & Westlake, D. F. (1993). Effects of Eutrophication on Ranunculus and Potamogeton. *Journal of Aquatic Plant Management, 31*, 113-117.
Steffen, K., Becker, T., Herr, W., & Leuschner, C. (2013). Diversity loss in the macrophyte vegetation of northwest German streams and rivers between the 1950s and 2010. *Hydrobiologia, 713*(1), 1-17. DOI:10.1007/s10750-013-1472-2
Steffen, K., Leuschner, C., Müller, U., Wiegleb, G., & Becker, T. (2014). Relationships between macrophyte vegetation and physical and chemical conditions in northwest German running waters. *Aquatic Botany, 113*, 46-55. DOI:10.1016/j.aquabot.2013.10.006
Steffens, B. & Rasmussen, A. (2015). The Physiology of Adventitious Roots. *Plant Physiology, 170*(2), 603-617. DOI:10.1104/pp.15.01360
Stutter, M. I., Langan, S. J., & Demars, B. O. (2007). River sediments provide a link between catchment pressures and ecological status in a mixed land use Scottish River system. *Water Res, 41*(12), 2803-2815. DOI:10.1016/j.watres.2007.03.006

Søndergaard, M., Bruun, L., Lauridsen, T., Jeppesen, E., & Madsen, T. V. (1996). The impact of grazing waterfowl on submerged macrophytes: In situ experiments in a shallow eutrophic lake. *Aquatic Botany, 53*(1-2), 73-84. DOI:Doi 10.1016/0304-3770(95)01013-0

Trimmer, M., Sanders, I. A., & Heppell, C. M. (2009). Carbon and nitrogen cycling in a vegetated lowland chalk river impacted by sediment. *Hydrological Processes, 23*(15), 2225-2238. DOI:10.1002/hyp.7276

van Gerven, L. P., de Klein, J. J., Gerla, D. J., Kooi, B. W., Kuiper, J. J., & Mooij, W. M. (2015). Competition for light and nutrients in layered communities of aquatic plants. *Am Nat, 186*(1), 72-83. DOI:10.1086/681620

Van Looy, K., Tonkin, J. D., Floury, M., Leigh, C., Soininen, J., Larsen, S., . . . Wolter, C. (2019). The three Rs of river ecosystem resilience: Resources, recruitment, and refugia. *River Research and Applications, 35*(2), 107-120. DOI:10.1002/rra.3396

Vilà, M. & Weiner, J. (2004). Are Invasive Plant Species Better Competitors Than Native Plant Species? - Evidence from Pair-Wise Experiments. *Oikos, 105*, 229-238.

Vilmin, L., Mogollon, J. M., Beusen, A. H. W., & Bouwman, A. F. (2018). Forms and subannual variability of nitrogen and phosphorus loading to global river networks over the 20th century. *Global and Planetary Change, 163*, 67-85. DOI:10.1016/j.gloplacha.2018.02.007

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human Domination of Earth's Ecosystems. *Science, 277*(5325), 494-499. DOI:10.1126/science.277.5325.494

Vollenweider, R. (1968). Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. *Paris Rep, 192*, 14.

Wells, R. D. S., Bannon, H. J., & Hicks, B. J. (2003). Control of macrophytes by grass carp(Ctenopharyngodon idella)in a Waikato drain, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 37*(1), 85-93. DOI:10.1080/00288330.2003.9517148

Wood, K. A., Stillman, R. A., Clarke, R. T., Daunt, F., & O'Hare, M. T. (2018). Water velocity limits the temporal extent of herbivore effects on aquatic plants in a lowland river. *Hydrobiologia, 812*(1), 45-55. DOI:10.1007/s10750-016-2744-4

Zhang, M., Cao, T., Ni, L., Xie, P., & Li, Z. (2010). Carbon, nitrogen and antioxidant enzyme responses of Potamogeton crispus to both low light and high nutrient stresses. *Environmental and Experimental Botany, 68*(1), 44-50. DOI:10.1016/j.envexpbot.2009.09.003

Chapter II

Growth response to nitrate enrichment helps facilitate success of an alien *Potamogeton* in New Zealand streams.

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2.1 Abstract

Motivated by stream ecosystem degradation by eutrophication, we mimicked slow flowing lowland stream conditions with a novel experimental setup to further our understanding of aquatic plant responses to increases in nitrate and light. We conducted a mesocosm growth experiment of two species from the genus *Potamogeton*: *P. crispus* (alien) and *P. ochreatus* (native), grown at four nitrate and four light levels. We hypothesised that (i) internal nutrient status of the plants would scale with water column nutrient concentration, and that (ii) plant performance would reflect the nutrient status of the plant. Furthermore, we hypothesised that (iii) a low irradiance level would negate the effects of an increased nitrate level. In relation to (ii) we hypothesised that (iv) the traits of the alien species would enable it to outperform the native species where both the availability of light and nutrient resources was high. Internal tissue N content was broadly similar in the two higher (> 250 µg NO_3^- L^{-1}) and the two lower nutrient treatments (< 20 µg NO_3^- L^{-1}) in both species and plants were therefore collapsed into high and low N-groups. High-N individuals had higher growth rates than low-N ones regardless of species or light treatment and plants had reduced growth rates at the lowest light treatment, however this response was less evident for *P. crispus*. The highest growth rate was found at the high-N individuals of *P. crispus* at the highest light treatment, and correspondingly, in this treatment this species exhibited an increase in branching degree and lateral spread from the low-N plants. As *P. crispus* spreads by fragmentation, our results show it to be a highly effective competitor in anthropogenically impacted areas compared to its native counterpart. Our study exemplifies how light can influence eutrophication responses of plants and how both need to be accounted for in management decisions.

2.2 Introduction

Aquatic submerged macrophytes support many important services in stream ecosystems. They provide food and habitat for invertebrates and fish (Collier et al., 1999; Giorgi et al., 2005; Paice et al., 2017; Quirino et al., 2019), alter the physico-chemical environment (Sand-Jensen, 1998) and affect nutrient cycling (Riis et al., 2019). However, increased anthropogenic influences have impacted stream macrophyte communities and the services they support (Mouton et al., 2019; Sand-Jensen et al., 2000; Steffen et al., 2013). Of particular relevance to macrophytes has been the global increase in fertilizer use, which has led to widespread eutrophication across ecosystems (Vilmin et al., 2018) and excessive biomass production (Hilton et al., 2006; O'hare et al., 2018), also termed 'nuisance growth' (Haslam, 1978; Matheson et al., 2012).

Nuisance biomass accrual by macrophytes is dependent on the complex interplay of multiple environmental and biotic factors, and is most frequently occurring in lowland reaches, where water velocity is sufficiently low to reduce scouring (Riis & Biggs, 2003) and light is not limiting macrophyte growth (Mouton et al., 2019). When these conditions are met, in-stream biomass scales with nutrient levels (Baattrup-Pedersen et al., 2016; Carr et al., 2003; Garbey et al., 2004; Heaney et al., 2001; Mebane et al., 2014; O'hare et al., 2010; Riis et al., 2010; Sosiak, 2002). When these conditions are not met, no direct link between macrophyte biomass and stream nutrient concentrations is observed (Canfield & Hoyer, 1988; Kern-Hansen & Dawson, 1978; Kouwe, 1983; Lévesque et al., 2016), making simple relationships unlikely and underpins the importance of disentangling the specific effects of eutrophication (Baattrup-Pedersen et al., 2016).

There are many factors that potentially confound relationships between water nutrient status and plant biomass. Firstly, spot measurements of water column nutrient concentrations do not integrate potential temporal variations in nutrient levels that the plants have been exposed to prior to the date of sampling. This is particularly problematic when trying to correlate water column nutrient concentrations with biomass *in situ* (Mebane et al., 2014). Most submerged macrophytes can also take up nutrients from the sediment as well as the water column via root uptake (Madsen & Cedergreen, 2002) or have nutrients stored in their tissue from times when nutrient availability was higher (i.e. luxury uptake; Millard, 1988). Thus, studies of plant-nutrient relations should consider these issues in experimental design. For example, measurement of tissue nutrients is a more direct indicator of the supply available for growth than external concentrations (Cavalli et al., 2016; Demars & Edwards, 2007; Duarte, 1992; Gusewell & Koerselman, 2002).

Where occurring, extreme macrophyte proliferation can clog waterways and lead to diverse ecological alterations, including anoxia, altered pH, loss of plant species diversity (Bornette & Puijalon, 2011; Hrivnák et al., 2014; Lacoul & Freedman, 2006; O'hare et al., 2018) and subsequently loss of biodiversity in other trophic levels (Baczyk et al., 2018; Caraco & Cole, 2002; Collier et al., 1999).

Consequently, there is an interest from managers to limit nuisance growths and controlling incoming nutrient loads to streams to prevent prolific macrophyte growth is seen as a tractable option. However, our knowledge on how substantial nutrient reduction needs to be to reverse eutrophication effects and prevent nuisance growth is still lacking (but see O'hare et al., 2018).

Biotic factors, including intrinsic potential of macrophytes, also add complexity to nutrient-plant responses. Large scale studies of lowland streams in Europe suggest that, when plants are no longer competing for nutrients, traits related to enhancing either light interception or light utilisation are dominant in submerged macrophyte communities in eutrophic streams (Baattrup-Pedersen et al., 2015; O'hare et al., 2018). Ellenberg et al. (1991) introduced Ellenberg Indicator Values (EIVs) to vegetation science to classify plant responses to environmental variables. Baattrup-Pedersen et al. (2015) applied the EIV concept to aquatic macrophytes in lowland streams and reported traits of successful taxa in response to eutrophication to include apical and multi-apical growth meristems as strategies for canopy formation at the water surface for better light capture , but also that plants with low EIV(Light) trait scores can be promoted, as these systems are often highly productive and shade-dominated (Baattrup-Pedersen et al., 2015). These traits are commonly found in invasive nuisance species and as such these species often dominate stream sections where light and nutrient levels are high (Mouton et al., 2019). In New Zealand, one such species is *Potamogeton crispus* (Champion et al., 2013), which is also known as a problematic species in the US (Bolduan et al., 1994). However, only few experimental investigations of how the interaction of different levels of light and nutrients affect the performance of aquatic macrophytes exist, although these could shed light on how these abiotic factors facilitate certain species in streams.

To further our understanding of species' response to nutrient increases and how this response is affected by variations in incoming light, we conducted a mesocosm growth experiment of two species occurring in New Zealand from the genus *Potamogeton*: *P. crispus* and *P. ochreatus*. The species were specifically chosen such that we might elucidate how the focal factors and potential interaction of these influenced the response of a native and an alien invasive species with otherwise similar habits of growth. The study used a novel mesocosm setup to mimic slow flowing lowland river conditions, as this is where these two macrophytes often proliferate. The setup consisted of four large (~10 m) flume channels with continuous inflow of purified water dosed with macro- and micronutrients and dissolved inorganic carbon at controlled concentrations. By using clean, low-nutrient, sand as a rooting medium, and providing P and other essential growth elements at non-limiting concentrations in the water column, the effects of N could be isolated. We employed a four-by-four factorial design with four nitrate and four irradiance levels. We used nitrate as a dissolved inorganic N (DIN) source , although ammonium (NH_4^+) is taken up by plants with lower energetic costs, as nitrate (NO_3^-) is the dominant form of DIN in streams, and the one most readily leached from agricultural soils (Kronvang et al., 2005). We hypothesised that (i) internal nutrient status of the plants would scale with water

column nutrient concentration and that (ii) plant performance would reflect the nutrient status of the plant. Furthermore, we hypothesised that (iii) a low irradiance level would negate the effects of an increased nitrate level. In relation to (ii) we hypothesised that (iv) the traits of the alien species would enable it to outperform the native species where both the availability of light and nutrient resources was high. A suite of relevant plant traits was measured to test the above hypotheses.

2.2 Methods

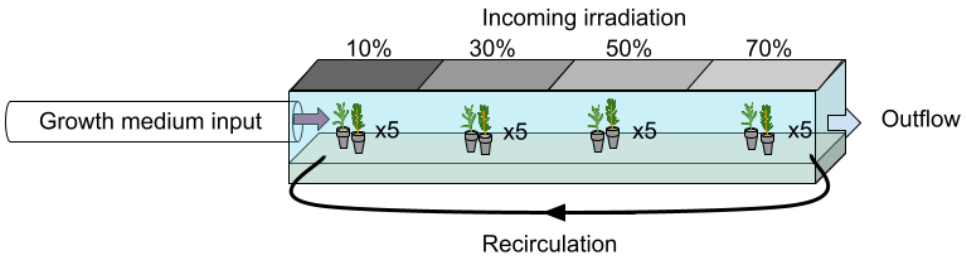
2.2.1 Plants

Potamogeton crispus and *P. ochreatus* were collected from a lowland stream near Morrinsville, New Zealand (37'42''18.59 S; 175'33''4.15 E). *P. crispus* is a naturalised, widely distributed alien species in Aotearoa New Zealand (Figure SI-1), considered native to Eurasia, Africa and Australia (Champion & Hofstra). It is regarded as having a ruderal (R) strategy (sensu Grimes; Riis & Biggs, 2001); characterised by fast growth rates and a high disturbance tolerance (i.e. high stem flexibility and root:shoot ratio). *P. ochreatus* is a native species, however not endemic (De Lange), and is also found nation-wide (Figure SI-1), as well as in Australia and south-east Asia. *P. ochreatus* is classified as a competitive-ruderal (CR) strategist (sensu Grimes; Riis & Biggs, 2001) and thus shares characteristics of *P. crispus* but is less resistant or resilient to disturbance. At low disturbance and high resource availability both species show traits of high stature, dense canopy formation and high morphological plasticity (Riis & Biggs, 2001).

2.2.2 Mesocosms

The experimental setup consisted of four flume mesocosms, each 10 m long and 0.55 m wide, with a water level of 0.35 m and a constant inflow of water at the top (1.5 L min^{-1}) giving a water residence time of ca. 0.8 d. A pump at the lower end of each flume recirculated water to the top end, to increase water velocity, homogenize water column elemental concentrations and prevent stagnation (Figure 2.1). By preventing water stagnation in the high light treatment, this reduced phytoplankton build-up that could affect light climate (e.g. Barker et al., 2008). Water from a groundwater bore was purified using a reverse osmosis system (Ecosoft MO10000) with subsequent addition of micro- and macronutrients to create a growth solution excluding nitrogen, bicarbonate and potassium (cf Michael Smart & Barko, 1985). Bicarbonate and potassium, to complete the growth medium, were added to

Figure 2-1*: Schematic representation of the mesocosm flume experimental setup. See text for details.*



the water via peristaltic pumps immediately before entering the flumes, as well as different amounts of NO_3^- targeting four different N concentrations across the flumes: control (0 µg N L^{-1}), low (50 µg N L^{-1}), medium (500 µg N L^{-1}) and high (5000 µg N L^{-1}). The target P content (as sodium hydrogen phosphate) of the growth medium was 100 µg P L^{-1} and was considered to be in excess in all nitrogen treatments. The flow rates of all pumps were checked at least every three days.

Water samples were taken to verify nutrient concentrations in each treatment. Both top and bottom of the flumes were sampled to test for potential gradients due to nutrient uptake by the plants. Water samples were analysed for DIN (NH_4^+, NO_3^-) and dissolved reactive phosphorus (DRP) on a Lachat flow injection analyser (Quikchem® 8500, Hach, USA), with a detection limit of 1 µg L^{-1}. Trace elements were analysed at Hills laboratories in Hamilton, NZ (see supplementary information for details).

Each flume was covered by shade cloth in four different densities, yielding four light treatments. By measuring incoming irradiation (I) (LI-COR LI-200 sensor) above and below the cloth, the irradiance of each was calculated $(I_{below}) \cdot (I_{above})^{-1}\ X\ 100$ as 70 %, 44 %, 32 %, 8 % of incident. This is very close to the nominated percentages, and for ease of reading we maintain the naming scheme of 70 %, 50 %, 30 %, and 10 % light treatments in which 70 % is highest and 10 % is lowest. Mean daily incoming irradiance for the duration of the experiment was 96.8 ± 29.5 mol photons m^{-2} d^1 (mean ± s.d.), thus the light treatments yielded on average 67.7, 44.5, 30.9, and 7.8 mol photons m^{-2} d^{-1} (Figure SI-2).

2.2.3 Plants

Plants used in experiments comprised 15 cm apical shoots cut from healthy donor plants, potted in 700 mL plastic pots ¾ filled with nutrient poor sand (Particulate carbon, particulate nitrogen, and TRP contents were 0.03 ± 0.006 %, 0.02 ± 0.001 % and 0.01 ± 0.001 %, respectively; Ellawala Kankanamge et al., 2019). Cuttings were set to acclimate for at least 3 weeks in their respective nutrient treatments under 50 % shade cloth, to ensure internal nitrogen was in equilibrium with the external medium and stored N could not affect growth. At experiment start, 15 cm apical shoots taken from recovered plants were transplanted to new pots and 5 replicates of each species were assigned to each of the 16 treatments. From each nutrient treatment, 5 shoots of each species were taken for mean initial biomass (DW_{start}) and internal C and N measurements (20 in total per species). The plants were grown under experimental conditions for ~10 weeks.

2.2.4 Estimation of photosynthetic parameters

Immediately prior to harvest, we used pulse amplitude modulated (PAM) fluorometry (MONI-DA/S, Heinz Walz GmbH, Germany) to create photosynthesis-irradiance (PI) curves of dark acclimated leaves. PAM utilizes the fluorescence of chlorophyll excited by absorption of photons to estimate the

saturation state of photosystem II (PSII) by comparing fluorescence yield under ambient light, when some reaction centres are closed and some open, with that under saturating light (when all reaction centres are closed). Quantum yield of PSII is estimated as Y = (F_m'-F)/F_m', where F is the fluorescence at ambient light and F_m' is fluorescence under saturating light sufficient to close all the reaction centres (Beer & Björk, 2000). The quantum yield of PSII is subsequently used to estimate electron transport rate (ETR) (see below).

The 3rd leaf from the apex was taken from three individuals of each treatment for measurement. If the 3rd leaf was too small to produce viable readings the 4th leaf was taken instead. Leaves were fixed in measuring clips and left in the dark for 30 min before measuring yield at 12 actinic light intensities of increasing magnitude. Subsequently, light absorption factor (AF) of the leaves was determined as the proportion of incident irradiance absorbed by each leaf by measuring irradiance reaching the LiCOR Li200 sensor from a fixed light source (white LED flashlight, similar to the white LED of the PAM instrument) with and without the leaves/top covering the light sensor (Beer & Björk, 2000). Leaves were then frozen for chl. *a* analysis (see below).

ETR of the plants was calculated as $ETR = Y \cdot PAR \cdot AF \cdot 0.5$ where Y is the yield, PAR is the light intensity emitted by the PAM-sensor and AF is the absorption factor of the leaf. ETR was then plotted by the PAR to make the PI-curves. For easier literature comparison we present photosynthesis as mol oxygen produced rather than electrons transferred, we assumed a conversion factor of 0.25 oxygen evolved per electron transferred (Beer & Björk, 2000). To obtain the maximum photosynthetic rate P_{max} and saturation irradiance I_c from the curves, a hyperbolic tangent function $y = P_{max} \tanh(\alpha x \, {P_{max}}^{-1})$ was fitted to each replicate, and I_c was calculated as $I_c = \alpha \, {P_{max}}^{-1}$ (Jassby & Platt, 1976). The parameters P_{max}, I_c and the yield at $PAR_i = 0$ were all chl. *a* corrected.

2.2.5 Harvest

At harvest, for each plant, the length of the main stem and each branch was measured, and biomass was separated into above and below ground fractions, then dried for 48 h at 60 °C to obtain dry weight (DW). Internal tissue C and N concentrations were obtained from ~2 mg subsamples of finely ground dried biomass, by flash combustion followed by separation of the gaseous products and subsequent mass spectroscopy (Vario EL cube, Elementar, Germany; < 0.1 % absolute precision). Ratios of tissue C and N are given by weight.

Chlorophyll and carotenoid content of the leaves from PAM measurements was determined by UV-VIS spectroscopy of ethanol extracts. Plant material was submerged in liquid nitrogen and left to lyophilise in a freeze drier after which ~5 mg (when possible) was weighed and transferred to a test tube and rehydrated with 0.1 ml Milli-Q water. Some leaves were too small for accurate analysis and thus three leaves of a treatment were pooled together as one replicate. Ethanol (4 ml) was added, and

samples were left to extract overnight, in the dark at 4°C. Absorbance was subsequently measured at 470, 648 and 664 nm on a UV-1800 spectrophotometer (Shimadzu). An additional measurement was taken at 750 nm to be able to correct for impurities. Where a measurable absorbance was measured at 750 nm this was subtracted from that at all other wavelengths.

From the lengths and weights, branching degree (number of branches · main stem length^{-1}), lateral spread (total length · main stem length^{-1}), density (DW · total length^{-1}), root to shoot ratio and relative growth rate (RGR) were calculated. RGR was calculated (cf Manolaki et al., 2020) as

$$RGR\ (d^{-1}) = \frac{\ln(DW_{end}) - \ln(DW_{start})}{time} \text{ (I)}$$

From the UV-VIS spectroscopic absorbances, chlorophyll a (C_a), b (C_b), total (C_{a+b}) and total carotenoids (C_{x+c}; all in mg g^{-1} dw) were calculated as follows (Lichtenthaler, 1987):

$$C_a = (13.36A_{664} - 5.19A_{648}) \cdot 4.1 \cdot DW^{-1} \text{ (II)}$$

$$C_b = (27.43A_{648} - 8.12A_{664}) \cdot 4.1 \cdot DW^{-1} \text{ (III)}$$

$$C_{a+b} = (5.24A_{664} + 22.24A_{648}) \cdot 4.1 \cdot DW^{-1} \text{ (IV)}$$

$$C_{x+c} = (1000A_{470} - 2.13C_a - 97.64C_b) \cdot 4.1 \cdot DW^{-1} \cdot 209^{-1} \text{ (V)}$$

Where A_{648}, A_{664} and A_{470} is absorbance at 648, 664 and 470 nm respectively and 4.1 is the volume of liquid added to the sample.

2.2.6 Data analysis

To investigate differences of internal nitrogen (%N), carbon (%C) content and C:N ratio, amongst nutrient treatments, species, and light, we performed Wilcoxon tests between species grouped by nutrient and light treatments, as well as Kruskal-Wallis tests between light and nutrient treatments, grouped by species and nutrient and species and light respectively. Following the Kruskal-Wallis tests, we conducted post hoc pairwise Wilcoxon tests on individual group pairings of light and nutrient treatments. Non-parametric statistics were used as data failed tests of normality and heteroskedasticity.

To evaluate effects of nutrient and light on response parameters, two-way ANOVA tests were attempted, but all parameters failed either or both of Shapiro-Wilk test of normality ($p < 0.05$) or Levene's test of variance homogeneity ($p < 0.05$), even after log transformation of data. Thus, non-parametric Wilcoxon rank-sum tests were employed to ascertain differences between species within light and N groups and between N groups within light treatments and species. Kruskal-Wallis tests

were carried out on light treatments within N group and species, with post hoc pairwise Wilcoxon tests (with Bonferroni-Holm p adjustment method) between specific light levels.

2.3 Results

2.3.1 Nutrient concentrations in water and plants

Dissolved reactive phosphorous concentrations were similar amongst flumes, whereas a clear distinction was evident between DIN concentrations of the four nutrient treatments (Table 2.1). Differences between inflow and outflow were negligible except for the control flume which had more

Table 2.1: *Resulting nutrient concentrations of the water samples taken at top end (upstream) and lower end (downstream) of the mesocosm flumes (n = 1).*

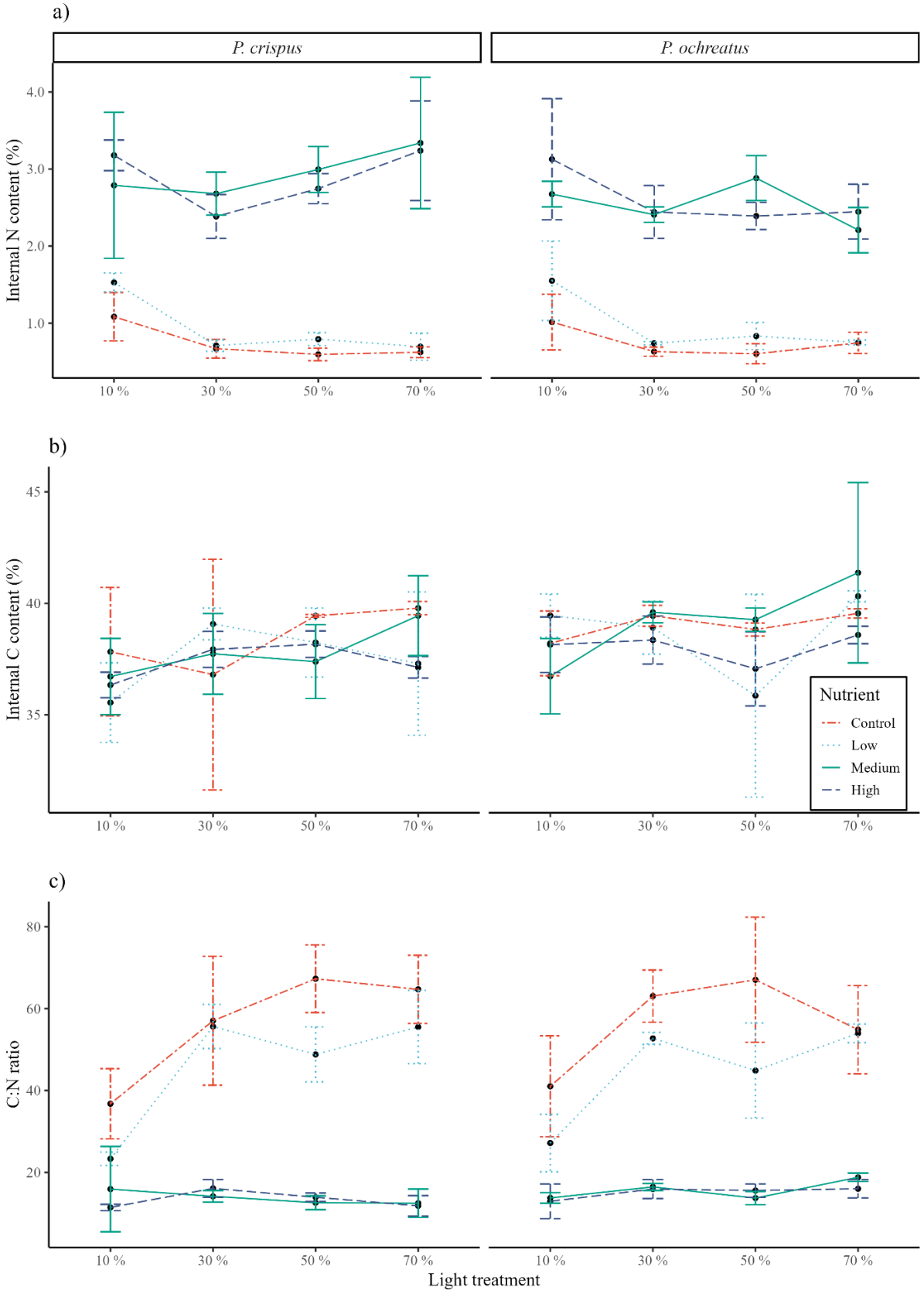
		DRP µg L^{-1}	NH_4^+ µg L^{-1}	NO_3^- µg L^{-1}	DIN µg L^{-1}	DIN:DRP
Control	Upstream	52	20	1	21	0.4 : 1
	Downstream	57	1	<1	<2	<0.04 : 1
Low	Upstream	63	8	21	29	0.5 : 1
	Downstream	71	7	17	24	0.3 : 1
Medium	Upstream	49	23	264	287	5.9 : 1
	Downstream	54	62	252	314	5.8 : 1
High	Upstream	60	15	5470	5485	91 : 1
	Downstream	49	20	5580	5600	114 : 1

ammonium (NH_4^+) in the upstream part. DIN:DRP ratios ranged from <0.04:1 in the control flume to as much as 114:1 in the high DIN flume.

Both species within light treatments showed significant differences in tissue % N content amongst nutrient treatments, whereas in only two instances difference in tissue % C were significant (Kruskal-Wallis rank-sum test $p < 0.05$; Table 2.2, Figure 2.2). Similarly, both species mostly differed in % N content amongst the light treatments and less so for % C content (Table 2.3, Figure 2.2). C:N ratios tended to follow % N, given the greater between-treatment variation in %N than %C. As the number of post hoc Wilcoxon tests between % N, % C, and C:N ratios, within light and nutrient treatments are very high (288 tests in total), the results of the individual tests are omitted here but in summary the results showed two consistent patterns: 1) Within nutrient treatments, there were more significant differences found between control or low and medium or high nutrient treatments than between control and low, and medium and high. Out of 24 comparisons, control differed from low only 6 times, whereas control differed from medium and high 15 and 19 times, respectively, and low differed from medium and high 14 and 15 times, respectively. Medium and high differed in only 3 out of 24 comparisons. 2) Within light treatments, there were more differences between the 10 % light treatment and any other light treatment than amongst the 70, 50, and 30 % light treatments. Out of 72

comparisons involving the 10 % light treatment 27 of these were significantly different, while of the 72 comparisons involving the other treatments only 6 were significantly different. Thus, internal tissue N content was broadly similar in the two higher and the two lower nutrient treatments in both species (Figure 2.2a). Consequently, in terms of internal N status, the four N treatments collapsed into

Figure 2-2: *Internal a) tissue % nitrogen (N), b) % carbon (C), and c) C:N ratio of Potamogeton crispus and P. ochreatus grown at four nutrient and light levels. Lines connect mean values between light treatments and errorbars denote standard deviation.*



high (High and Medium) and low (Low and Control) N-groups (Figure 2.2c). For further analyses, we therefore combined high and medium N treatments, and low and control N treatments as high-N and low-N, respectively, as well as individuals from the high-N and low-N treatments will be referred to as high-N individuals and low-N individuals, respectively.

Table 2.2: *Results of Kruskal-Wallis tests of internal tissue carbon and nitrogen content difference amongst nutrient treatments for each of the two Potamogeton species within light treatments. P < 0.05 denoted by '*', P < 0.01 by '**'.*

Species	Light treatment	Response parameter	n	Kruskal-Wallis rank-sum statistic	P	
P. crispus	70 %	% N	20	14.3	0.002	**
P. crispus	50 %	% N	20	16.1	0.001	**
P. crispus	30 %	% N	20	14.8	0.002	**
P. crispus	10 %	% N	18	10.8	0.013	*
P. ochreatus	70 %	% N	20	14.7	0.002	**
P. ochreatus	50 %	% N	20	16.2	0.001	**
P. ochreatus	30 %	% N	20	16.1	0.001	**
P. ochreatus	10 %	% N	18	13.4	0.004	**
P. crispus	70 %	% C	20	6.9	0.075	
P. crispus	50 %	% C	20	9.2	0.027	*
P. crispus	30 %	% C	20	3.4	0.34	
P. crispus	10 %	% C	18	1.7	0.64	
P. ochreatus	70 %	% C	20	14.4	0.002	**
P. ochreatus	50 %	% C	20	6.4	0.093	
P. ochreatus	30 %	% C	20	4.3	0.23	
P. ochreatus	10 %	% C	18	6.2	0.10	
P. crispus	70 %	C:N ratio	20	14.6	0.002	**
P. crispus	50 %	C:N ratio	20	16.1	0.001	**
P. crispus	30 %	C:N ratio	20	14.9	0.002	**
P. crispus	10 %	C:N ratio	18	11.0	0.012	*
P. ochreatus	70 %	C:N ratio	20	15.6	0.001	**
P. ochreatus	50 %	C:N ratio	20	16.4	0.001	**
P. ochreatus	30 %	C:N ratio	20	16.2	0.001	**
P. ochreatus	10 %	C:N ratio	18	13.4	0.004	**

The only noteworthy difference between species was that *P. crispus* incorporated more N in the 70 % and 50 % light treatment than *P. ochreatus*, but only at 70 % light did it lead to a difference in C:N ratio (Chapter II Appendix Table A.2.3). No obvious differences were seen in the C content of the two plants. Although *P. ochreatus* tended to have slightly higher C content than *P. crispus* (Figure 2.2b), this did not give rise to differences in C:N ratio (Figure 2.2c).

***Table 2.3**: Results of Kruskal-Wallis tests of internal tissue carbon and nitrogen content difference amongst light treatments for each of the two Potamogeton species within nutrient treatments. P < 0.05 denoted by '*' , P < 0.01 by '**'.*

Species	Nutrient treatment	Response parameter	n	Kruskal-Wallis rank-sum statistic	P	
P. crispus	Control	% N	19	9.4	0.02	*
P. crispus	Low	% N	19	10.8	0.01	*
P. crispus	Medium	% N	20	3.8	0.29	
P. crispus	High	% N	20	11.0	0.01	*
P. ochreatus	Control	% N	20	8.0	0.05	*
P. ochreatus	Low	% N	19	9.9	0.02	*
P. ochreatus	Medium	% N	20	11.5	0.009	**
P. ochreatus	High	% N	19	2.6	0.46	
P. crispus	Control	% C	19	4.7	0.20	
P. crispus	Low	% C	19	6.2	0.10	
P. crispus	Medium	% C	20	4.5	0.22	
P. crispus	High	% C	20	13.3	0.004	**
P. ochreatus	Control	% C	20	8.9	0.03	*
P. ochreatus	Low	% C	19	7.7	0.05	
P. ochreatus	Medium	% C	20	12.0	0.007	**
P. ochreatus	High	% C	19	2.5	0.47	
P. crispus	Control	C:N ratio	19	9.4	0.02	*
P. crispus	Low	C:N ratio	19	10.4	0.02	*
P. crispus	Medium	C:N ratio	20	3.3	0.35	
P. crispus	High	C:N ratio	20	11.3	0.01	*
P. ochreatus	Control	C:N ratio	20	8.9	0.03	*
P. ochreatus	Low	C:N ratio	19	8.8	0.03	*
P. ochreatus	Medium	C:N ratio	20	15.3	0.002	**
P. ochreatus	High	C:N ratio	19	2.8	0.43	

2.3.2 Growth and morphology

High-N individuals had higher RGR than low-N ones regardless of species or light treatment save for two instances (Table 2.4), although *P. crispus* at 70 % light was close to the 0.05 mark (p = 0.08). Generally, *P. crispus* had higher variation than *P. ochreatus* meaning that some individuals achieved much higher RGR's than *P. ochreatus* at high-N, the highest (0.06 d^{-1}) found under high irradiance (70%), while many individuals at low-N had negative RGR's regardless of light treatment. However, a significant difference between species was only found between means of the low-N individuals at the lowest irradiance (10 % - W = 14, p = 0.04; Table 4), which was the only treatment where *P. ochreatus* individuals had negative RGR's. Both species showed a similar response trend to changes

in light, by having reduced RGR's at the lowest light treatment (10 %), but for the high-N plants, the significant difference was for *P. ochreatus*, while for low-N plants it was for *P. crispus* (Table 2.4).

P. crispus showed a significant difference between high-N and low-N in branching degree and lateral spread at the highest light treatment only (Table 2.5). Generally, *P. crispus* responded to increased N content with main shoot length increases (Table 2.5), while *P. ochreatus* responded with increases in branching degree and lateral spread (Table 2.5). Both species responded to increase in N content by increased root extension (Table 2.5) and increase in below-ground/above-ground biomass ratio (Table 2.5). For all traits but the below-ground/above-ground biomass ratio, no difference between high-N and low-N plants were found at the 10 % light treatment. For high-N *P. crispus* plants decreasing irradiation intensity tended to decrease branching degree and lateral spread and high-N *P. ochreatus* plants had the lowest main shoot length at 10 % light (Table 2.5). *P. ochreatus* achieved longer shoot lengths than *P. crispus* at low-N, and higher densities overall (Table 2.5), while *P. crispus* had generally higher branching degree and lateral spread than *P. ochreatus*, regardless of light treatment and N group (Table 2.5).

***Table 2.4**: RGR mean (± SD) values of High-N and Low-N groups of Potamogeton crispus and P. ochreatus at four different light treatments. Difference between N groups for each species are reported below. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxson rank sum test: W stat < 10, p < 0.05). Differences of the means between light treatments within species and N-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.*

Parameter	N-group	Species	Light treatment			
			70%	50%	30%	10%
RGR (d^{-1})	High-N	*P. crispus*	0.021^{a}	0.019^{a}	0.025^{a}	0.006^{a}
			±0.018	±0.011	±0.013	±0.011
		P. ochreatus	0.018^{a}	0.021^{a}	0.017^{a}	0.005^{b}
			±0.006	±0.006	±0.007	±0.002
	Low-N	*P. crispus*	0.003^{a}	0.008^{a}	0.005^{ab}	-0.006^{b}
			±0.009	±0.004	±0.011	±0.005
		P. ochreatus	0.006^{ab}	0.009^{a}	0.008^{a}	**0.000^{b}**
			±0.004	±0.004	±0.004	±0.006
Difference between N groups		*P. crispus*	W = 74	**W = 84**	**W = 65**	**W = 89**
		P. ochreatus	**W = 98**	**W = 88**	**W = 93**	W = 53

Table 2.5: *Mean (+- SD) values for growth responses of High-N and Low-N groups of Potamogeton crispus and P. ochreatus at four different light treatments. Difference between N groups for each species are reported below each parameter. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxson rank sum test: W stat < 10, p < 0.05). Differences of the means between light treatments within species and N-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.*

Parameter	N-group	Species	% Incoming irradiation			
			70%	50%	30%	10%
Branching degree (cm^{-1})	High-N	*P. crispus*	**1.3**^{a} ±0.5	0.8^{b} ±0.4	**1.1**^{ab} ±1.1	**0.4**^{c} ±0.2
		P. ochreatus	0.6^{ab} ±0.6	1.0^{a} ±0.8	0.4^{ab} ±0.5	0.2^{b} ±0.1
	Low-N	*P. crispus*	**0.6**^{a} ±0.2	**1.1**^{a} ±0.9	**0.7**^{a} ±0.3	**0.4**^{a} ±0.2
		P. ochreatus	0.2^{a} ±0.1	0.4^{a} ±0.4	0.1^{a} ±0.0	0.1^{a} ±0.1
Difference between N groups		*P. crispus*	**W = 94**	W = 94	W = 47	W = 66
		P. ochreatus	**W = 81**	**W = 77**	W = 67	W = 48
Lateral spread (cm cm^{-1})	High-N	*P. crispus*	**6.6**^{a} ±2.6	3.9^{b} ±1.3	**4.3**^{b} ±1.5	2.5^{c} ±1.3
		P. ochreatus	3.2^{ab} ±2.2	4.8^{a} ±2.7	2.8^{ab} ±2.3	1.8^{b} ±0.6
	Low-N	*P. crispus*	**3.1**^{a} ±1.0	4.2^{a} ±2.7	**3.5**^{ab} ±1.3	2.1^{a} ±0.7
		P. ochreatus	1.6^{a} ±0.5	2.5^{a} ±1.5	1.4^{ab} ±0.4	1.8^{a} ±0.9
Difference between N groups		*P. crispus*	**W = 96**	W = 96	W = 51	W = 63
		P. ochreatus	W = 71	**W = 81**	**W = 79**	W = 39
Main shoot length (cm^{-1})	High-N	*P. crispus*	11.4^{a} ±6.7	14.9^{a} ±11.7	20.1^{a} ±12.3	9.1^{a} ±4.6
		P. ochreatus	13.0^{a} ±3.3	11.6^{a} ±1.8	14.0^{a} ±3.4	9.4^{b} ±1.6
	Low-N	*P. crispus*	7.5^{a} ±1.9	6.1^{a} ±2.0	7.2^{a} ±2.4	7.0^{a} ±3.0
		P. ochreatus	**10.6**^{a} ±1.9	**9.7**^{a} ±3.5	**11.8**^{a} ±1.0	**10.1**^{a} ±1.8
Difference between N groups		*P. crispus*	W = 72	**W = 72**	**W = 82**	W = 88
		P. ochreatus	W = 74	W = 67	W = 74	W = 25

Root length (cm)	High-N	*P. crispus*	9.6[a] ±4.4	10.6[a] ±5.8	13.3[a] ±7.4	5.6[a] ±4.1
		P. ochreatus	7.7[ab] ±6.6	13.9[a] ±6.1	9.6[ab] ±6.6	5.6[b] ±4.5
	Low-N	*P. crispus*	4.1 ±2.5	4.8 ±3.6	3.6 ±3.4	3.4 ±3.4
		P. ochreatus	4.3 ±3.0	4.7 ±3.1	4.1 ±4.5	6.2 ±4.3
Difference between N groups		*P. crispus*	W = **88**	W = 88	W = **76**	W = 91
		P. ochreatus	W = 64	**W = 88**	**W = 77**	W = 39
Density (mg cm^{-1})	High-N	*P. crispus*	0.003[a] ±0.002	0.002[a] ±0.001	0.002[a] ±0.001	0.003[a] ±0.002
		P. ochreatus	**0.004**[a] ±0.001	**0.004**[a] ±0.001	**0.004**[a] ±0.001	0.004[a] ±0.001
	Low-N	*P. crispus*	0.003[a] ±0.001	0.003[a] ±0.001	0.003[a] ±0.001	0.003[a] ±0.005
		P. ochreatus	**0.004**[a] ±0.002	0.004[a] ±0.001	**0.005**[a] ±0.001	**0.003**[a] ±0.001
Difference between N groups		*P. crispus*	W = 45	**W = 45**	W = 20	W = 49
		P. ochreatus	W = 43	W = 46	W = 38	W = 52
Below-/aboveground biomass ratio	High-N	*P. crispus*	0.63[a] ±0.57	0.22[a] ±0.09	0.36[a] ±0.55	0.20[a] ±0.23
		P. ochreatus	0.23[a] ±0.26	0.21[a] ±0.10	0.18[a] ±0.16	0.27[a] ±0.28
	Low-N	*P. crispus*	0.07[a] ±0.09	0.05[a] ±0.06	0.01[a] ±0.02	0.01[a] ±0.01
		P. ochreatus	0.05[a] ±0.06	0.06[a] ±0.08	0.02[a] ±0.02	0.11[a] ±0.19
Difference between N groups		*P. crispus*	**W = 84**	**W = 84**	**W = 76**	**W = 80**
		P. ochreatus	**W = 53**	**W = 79**	**W = 49**	**W = 53**

2.3.3 Photosynthetic parameters

No clear patterns emerged from the photosynthetic parameters, although in all cases of statistical difference, high-N plants had the higher trait score (Table 2.6). Two trends can be noticed, however; 1) high-N plants of both species tended to have higher saturation irradiance (E_k) than low-N plants, and for *P. ochreatus* at 70 and 30 % light, this was reflected in a trend of a lower quantum yield (Φ); 2) high-N plants had higher maximum photosynthetic rate (P_{max}) at 10 % light. Significant differences

***Table 2.6**: Mean (+- SD) values of photosynthetic parameters from High-N and Low-N groups of Potamogeton crispus and P. ochreatus grown at four different light treatments. Difference between N groups for each species are reported below each parameter. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxson rank sum test: W stat < 10, p < 0.05). Differences of the means between light treatments within species and N-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.*

Parameter	N-group	Species	% Incoming irradiation			
			70%	50%	30%	10%
Quantum yield Φ	High-N	*P. crispus*	0.013^{a} ±0.008	0.017^{a} ±0.013	0.013^{a} ±0.009	0.019^{a} ±0.015
		P. ochreatus	0.015^{a} ±0.010	0.014^{a} ±0.010	0.007^{a} ±0.007	0.014^{b} ±0.010
	Low-N	*P. crispus*	0.017^{a} ±0.000	0.009^{a} ±0.007	0.009^{a} ±0.010	0.012^{a} ±0.007
		P. ochreatus	0.028^{a} ±0.018	0.012^{a} ±0.006	0.015^{a} ±0.007	0.006^{a} ±0.005
Difference between N groups		*P. crispus*	W = 3	W = 18	W = 16	W = 25
		P. ochreatus	W = 8	W = 17	W = 7	W = 28
E_k (mmol photons $m^{-2}s^{-1}$)	High-N	P. crispus	51^{a} ±37	62^{a} ±43	66^{a} ±22	34^{a} ±13
		P. ochreatus	88^{a} ±19	53^{ab} ±27	66^{ab} ±15	42^{b} ±14
	Low-N	P. crispus	29^{a} ±0	13^{a} ±3	49^{a} ±27	20^{a} ±3
		P. ochreatus	33^{a} ±8	**41**^{a} ±23	47^{a} ±30	18^{a} ±7
Difference between N groups		*P. crispus*	W = 4	**W = 24**	W = 16	**W = 32**
		P. ochreatus	**W = 24**	W = 19	W = 20	**W = 35**
P_{max} (mmol O_2 $m^{-2}s^{-1}$)	High-N	P. crispus	0.6^{a} ±0.4	1.0^{a} ±1.0	0.8^{a} ±0.5	0.6^{a} ±0.5
		P. ochreatus	1.2^{a} ±0.8	0.7^{a} ±0.5	0.4^{a} ±0.3	0.6^{b} ±0.5
	Low-N	P. crispus	0.5^{a} ±0.0	0.1^{a} ±0.1	0.6^{a} ±0.9	0.2^{a} ±0.1
		P. ochreatus	0.8^{a} ±0.4	**0.4**^{a} ±0.3	0.8^{a} ±0.7	0.1^{b} ±0.1
Difference between N groups		*P. crispus*	W = 3	W = 22	W = 14	W = 26
		P. ochreatus	W = 16	W = 22	W = 9	**W = 32**

Yield (PS II) at PAR = 0	High-N	P. crispus	0.6[a] ±0.1	0.6[a] ±0.1	0.7[a] ±0.1	0.6[a] ±0.1
		P. ochreatus	0.6[a] ±0.1	0.7[a] ±0.0	0.5[a] ±0.2	0.6[b] ±0.1
	Low-N	P. crispus	0.7[a] ±0.0	0.7[a] ±0.0	0.5[a] ±0.1	0.6[a] ±0.0
		P. ochreatus	0.5[a] ±0.0	**0.5**[a] ±0.1	0.5[a] ±0.0	0.6[b] ±0.0
Difference between N groups		*P. crispus*	W = 5	W = 10	**W = 24**	W = 15
		P. ochreatus	W = 19	**W = 30**	W = 24	W = 15

between species were only found for low-N individuals at 50 % light, where *P. ochreatus* had higher P_{max} and E_k, and *P. crispus* had higher yield at PAR = 0 (Table 2.6).

Significant differences between light treatments were found for low-N individuals of *P. ochreatus*, that had lower P_{max} and higher Yield at PAR = 0 at 10 % light compared to all other shade treatments (Table 2.6). *P. ochreatus* also showed difference between E_k at 70 % and 10 % light for high-N plants (Table 2.6). Kruskal-Wallis test results showed differences amongst light treatments in low-N *P. crispus* plants for E_k and Yield at PAR = 0 (P = 0.02), but none of the pairwise Wilcoxon tests were significant (Table 2.6).

As pigment samples had to be pooled across replicates to obtain sufficient for analysis, robust statistical comparisons were not possible. However, it was evident that the content chlorophyll-a, chlorophyl-b and total carotenoids increased with N content for all light treatments in both species (Chaper II Appendix Figure A.2.3). The range of content of each pigment showed no difference between species.

2.4 Discussion

We tested differences between growth rates, morphological traits, and photosynthetic performance parameters of two species of *Potamogeton,* the naturalised *P. crispus* and the native *P. ochreatus,* grown under four N concentrations then split into two N-groups based on internal N content in combination with four irradiances.

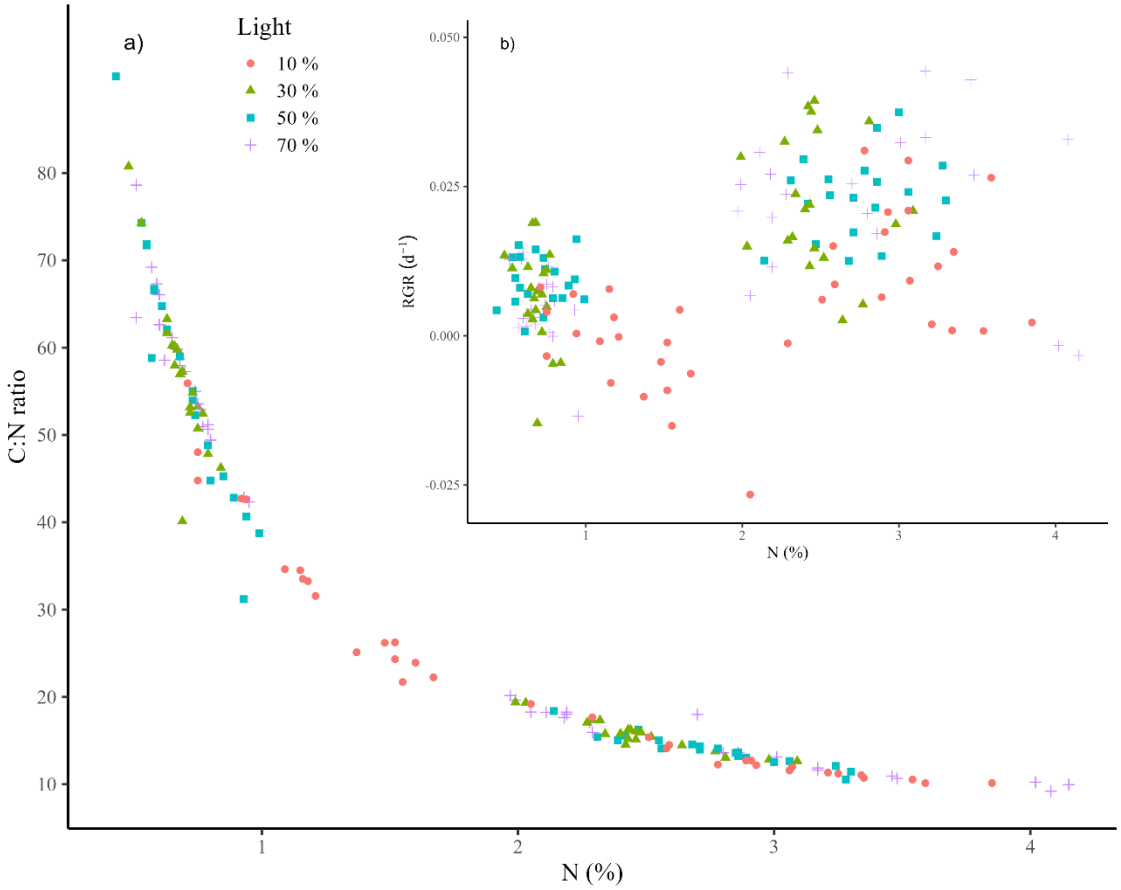
2.4.1 Factors controlling rate of growth - interplay of light and nutrients.

Our hypotheses were partly supported; internal N content increased with N treatment, but this did not directly correspond to external concentration; the four nitrogen treatments appeared to collapse to two. Except at the lowest irradiance, the two lowest N treatments in both species yielded % N contents of less than 1%, while at all irradiances the two highest N treatments yielded similar N contents between 2 and 3%. The internal nutrient content of a plant is widely recognised as an indicator of its growth potential, as this represents the nutrients available for production of biomass; below certain %N thresholds, the growth of the plant will be limited, or the plant will senesce (Hutchinson, 1975). These thresholds can vary between species (Gerloff, 1975), but a mean N content for 95% maximum growth and 95% maximum quantum yield for aquatic macrophytes is reported as 1.82 % N and 1.14 % N, respectively (see Demars & Edwards, 2007). Minimum macrophyte content of N to permit growth is reported to be 0.6% (Hutchinson, 1975) and in general, N contents of <1% are rare in natural freshwater macrophytes (e.g. Demars & Edwards, 2007). Internal tissue concentrations above 3 % is considered high for *P. crispus* (Bolduan et al., 1994) though Cavalli et al. (2016) reports concentrations as high as 4.7 % N. Furthermore, N limitation thresholds can be approximated by plotting N content against C:N ratios, and estimating when % N starts changing more so than the C:N ratio (Duarte, 1990). Using this approach, Cavalli et al. (2016) suggests a limitation threshold of <2% for tissue N which corresponds reasonably well with our data when we plot our C:N ratios against % N (Figure 2.3a) and look at the correlation between RGR and % N overall (Figure 2.3b).

Large scale surveys have found that *in situ* freshwater angiosperms normally contain between 2 and 3 % tissue N (Duarte, 1992), and Demars and Edwards (2007) confirmed this, citing a median N content of 2.64% in a dataset of 268 samples across 765 sites. Higher N content (and consequently lower C:N ratio) in plants grown at low light is probably due to lower nutrient demand from growth. Similar effects of other abiotic factors influencing N limitation have been found by Madsen et al. (1998), who finds that low inorganic carbon concentrations lower the growth rates of two aquatic macrophytes and consequently allows an increase in their N content due to lower demand. Adding to the complexity of nutrient dynamics is the fact that the ability to maintain a high N content during growth will vary between species as they inherently vary in nutrient uptake rates, and that these rates differ for NO_3^- and NH_4^+ (Manolaki et al., 2020). Nevertheless, we have effectively created eutrophic

and oligotrophic environments, which yield the high-N and low-N groups, and plants that are at growth saturating and extreme growth limiting nitrogen content. At water column concentrations of

Figure 2-3: *a) % N content of all plants from the experiment plotted against C:N ratio for threshold analysis. The inflexion point of the curve indicates onset of N saturation. And b) the relationship between % N content and RGR - a clear threshold can be witnessed at the 2 % N mark.*



more than ~290 µg NO_3^- L^{-1} internal N content of our plants did not scale with higher water column N and similarly, plants from ~30 µg NO_3^- L^{-1} achieved minimum N content as well as those with no N supply (Figure 2.2).

There was an obvious effect of nutrient content on the response parameters of the plants, in support of our second hypothesis; plant traits were generally higher for high-N plants, as were the contents of pigments. Still, we expected a larger difference between the high-N and low-N plants, particularly at the higher irradiances. We have no good explanation as to why so many of the *P. crispus* high-N plants did not attain better RGR's. Manolaki et al. (2020) finds for *P. crispus* a mean RGR of 0.06 day^{-1} under non-limiting light and nutrient conditions, suggesting an inherent ability of this species to grow even faster than what was attained here. Not much research has been done on growth of *P. ochreatus*, but Cenzato and Ganf (2001) finds maximum RGR of *P. ochreatus* grown in eutrophic sediment to be around 0.02 d^{-1}, comparable to our results. Both species are classified as N-loving

species (Riis & Biggs, 2001) and as such are well adapted to be able to utilize a high resource availability for growth as we see in our experiment. Indeed, pigment content for both species were high compared to previously reported values for similar plants (Huang et al., 2019; Jana & Choudhuri, 1982; Su et al., 2010), suggesting ample capability to upscale the photosynthetic apparatus when resource supply is high. Thus, even though evidently, our plants have not been able to perform to their maximum potential (see supplementary info for details), from the increased growth rates we can see that ecological issues like nuisance biomass will most likely occur when available N concentrations are higher, even when light is quite reduced. We find it interesting that the largest main shoot length difference was found between N groups for *P. crispus*, while *P. ochreatus* differed more in branching degree and lateral spread, when main shoot length is classified as a C-strategist trait, while the others are R-strategist traits (Riis & Biggs, 2001) and thus reversed *cf.* the species Grime's classifications. Possibly, this is an evolutionary strategy of the plants to allocate relatively more resources into traits that fall within their Grime's classification category when resources are scarce and when resources are plenty, there is a surplus to allocate to additional traits. We had anticipated the low-N plants to increase root length more than the high-N plants, to explore the sediment as a nutrient source. A possible explanation for longer roots at high N is flow induced root biomass allocation (Puijalon et al., 2007).

Our third hypothesis was mostly supported, as we see no difference between N groups for most parameters at the lowest irradiance and most of the differences between irradiance levels was between 10 % light and the other levels. Still, we did not expect a difference in RGR between low-N and high-N plants at this irradiance level (*P. crispus* Table 4). Although certainly light limited when compared to the other irradiance levels,10 % light high-N plants were still able to grow. Compensation irradiance points for the plants are reported as 152 ± 23 lux (~2.5 µmol photons m^{-2} s^{-1}) at 15°C for *P. crispus* (Zhou et al., 1997) and 37 µmol photons m^{-2} s^{-1} (Cenzato & Ganf, 2001) for *P. ochreatus* (daily average), both of which are lower than what the plants are exposed to in our experiment. We note however, that the compensation point irradiance of the *P. ochreatus* is the ecological, while for *P. crispus* it is the instantaneous and the ecological is expected to be higher (Givnish, 1988). Furthermore, the conversion from lux to mol photons depends on the type of light source used and this is not stated in the experiment (Zhou et al., 1997), thus the conversion made here can only ever be approximate. Thus, even at these low irradiances both species can maintain positive growth rates, but evidently shading is a constraining factor. Low irradiance has recently been shown to be important in restricting the presence and growth of invasive species (Mouton et al., 2019) and can influence the plants ability to cope with adverse conditions (Cao et al., 2009). The results of our study further exemplify the importance of the light environment and highlights the need for this to be considered when investigating the effects of nutrients. Successful strategies of plants in eutrophic streams are either to grow to the water surface for better light capture or better light utilisation for tolerance of out

shading (Baattrup-Pedersen et al., 2015). As neither of the species exhibited signs of acclimation to shading at the lowest irradiance, i.e. increased quantum yield (α^B) and lower P_{max}, we see no evidence that either of the tested species were opting for the light utilisation strategy although we did not test other parameters that respond to irradiance such as reduced specific leaf area (Wang et al., 2021).

We see some evidence in support of our fourth hypothesis, partly due to *P. crispus* having higher maximum RGR's and thus have the potential to outgrow *P. ochreatus*. Moreover, *P. crispus* had increases in branching degree and lateral spread only in the high light treatment when the N content was high. Furthermore, when the light was only at an intermediate level, the increase in lateral spread and branching degree was not significantly different between N groups but was different for main shoot length. Thus, although growth rates are similar, when resources are high, as we would expect to see in streams in an anthropogenic setting, we see a response of extending laterally with many branches rather than vertically, which is beneficial for dispersal (i.e. increases likelihood of fragmentation), as *P. crispus* spreads by vegetative formation in New Zealand (Hofstra et al., 1995). This response is virtually absent *in P. ochreatus* which generally show the same responses to increases in N content in all but the lowest irradiance treatment. Certainly, the species that suffered the most under low light and low nutrient levels was *P. crispus*, showing the importance of ample resource supply for this species.

2.4.2 Implications for thresholds and management perspective

Eutrophication of stream ecosystems have consequences for macrophyte communities that then propagate through the higher trophic levels and adversely affect ecosystem services. In this study we present results showing how low NO_3-N concentrations in the water column can significantly reduce internal N content and growth rates of plants and how this can be seen across a wide range of irradiance but was less evident below 10% incident. It is important to acknowledge that the mesocosm environment does not mirror a natural setting to perfection. In our case, the plants were exposed to a continuous, stable supply of nutrients and flow rate was kept at a constant low level, creating a constant flux of nutrients from the water to the plants. In a natural setting, precipitation events will likely play an important, pulsed role in supplying N to stream plants, by washing out N from soils and diminishing boundary layer thickness. Especially for oligotrophic streams, this will, at least temporarily, provide a boost of N that can be stored in the plants and help maintain growth even though consequent stream conditions are yet again oligotrophic. In addition, internal N content of the pre experiment plants were mostly above the 1.82 % threshold for growth as determined by Demars and Edwards (2007) and thus it must be assumed that this stored N has in part been available to support growth during the experiment. N uptake rates vary little between *Potamogeton* species (Manolaki et al., 2020), thus utilisation might be the key differentiator. Further studies are required in

the field of temporarily elevated N concentrations and the legacy effect they can have on plant communities.

Despite these experimental limitations, our hypotheses were able to be tested and were partly supported. Most importantly, plants grown under higher nitrate conditions sustained high % N, had higher growth rates, and higher trait scores than plants grown under lower nitrate conditions, suggesting eutrophication thresholds for nitrate-N lie somewhere between the concentrations of the low (47 ug N L^{-1}) and medium (290 µg N L^{-1}) nutrient treatments in our experiment. The current guideline trigger value for nitrate concentration in lowland streams in New Zealand is 444 µg N L^{-1}, a concentration which would offer little protection from macrophyte proliferation Mean concentrations of nitrate-N in lowland streams draining pastoral and urban areas in New Zealand are above these levels (1102 and 705 µg N L^{-1} respectively), while that of streams draining natural catchments is 78 µg N L^{-1} (Larned et al., 2016). Pastoral and urban streams also had much lower ecological health scores (Larned et al., 2019), consistent with eutrophication loss of ecosystem values. There is thus little expectation that growth of existing populations of either species in lowland rivers of Aotearoa New Zealand, of which only 5% of those in Larned et al. (2016)were in natural catchments, are nutrient limited.

Under conditions prevailing in the few lowland streams of Aotearoa New Zealand with minimal anthropogenic change to their catchments, water nitrate concentrations may be sufficiently low to limit the rate of growth of the two *Potamogeton* spp investigated here. In addition, lowland forest provides shading and under the combination of low nitrogen supply with a high degree of shading the native *P. ochreatus* did exhibit a higher RGR than the alien *P. crispus*. It was generally better and maintaining positive growth rates under combined nutrient and light limiting conditions. The suggestion in our results is that there is potential for the alien *P. crispus* to out-grow the native *P. ochreatus* in more eutrophic, brightly lit systems and informs the "phenotypic convergence – phenotypic divergence" paradigm in invasive species theory (Ordonez et al., 2010). Our example suggests that the non-native species may be successful because it is pre-adapted to better exploit conditions prevailing in newly modified catchments, specifically increased resource supply, than pre-existing native organisms.

Our results provide some insight into setting limits to manage macrophyte proliferation in streams of Aotearoa New Zealand. They suggest that reducing resource supply could be an effective management tool to both reverse the advantage from *P. crispus* back to *P. ochreatus*, and also reduce growth rates, as could the restoration of riparian shade. However, a substantial reduction, up to tenfold in m any cases (Larned et al., 2016)in nitrate may be needed that would require significant changes to lowland catchment land use. Such socioeconomic challenges of lowland stream restoration are persistent, and a recent review of forty years of effort in the Netherlands concludes "*biophysical*

objectives and restoration measures, a monitoring deficiency and restoration plans neglecting large scale catchment wide effects hampered the success of ecological stream restoration. It is therefore recommended to improve the monitoring programmes accompanying restoration projects by applying a proper design, matching the relevant spatiotemporal dimensions for the ecosystem under study" (Dos Reis Oliveira et al., 2020). Our results support the need for large-scale catchment consideration if the underlying cases of eutrophication, macrophyte proliferation and species spread are to be addressed.

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References

Baczyk, A., Wagner, M., Okruszko, T., & Grygoruk, M. (2018). Influence of technical maintenance measures on ecological status of agricultural lowland rivers - Systematic review and implications for river management. *Sci Total Environ, 627*, 189-199. DOI:10.1016/j.scitotenv.2018.01.235

Barker, T., Hatton, K. O. C., Connor, L., & Moss, B. (2008). Effects of nitrate load on submerged plant biomass and species richness: results of a mesocosm experiment. *Fundamental and Applied Limnology, 173*(2), 89-100. DOI:10.1127/1863-9135/2008/0173-0089

Beer, S. & Björk, M. (2000). Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. *Aquatic Botany, 66*, 69-76.

Bolduan, B. R., Van Eeckhout, G. C., Quade, H. W., & Gannon, J. E. (1994). Potamogeton crispus–The Other Invader. *Lake and Reservoir Management, 10*(2), 113-125. DOI:10.1080/07438149409354182

Bornette, G. & Puijalon, S. (2011). Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences, 73*(1), 1-14. DOI:10.1007/s00027-010-0162-7

Baattrup-Pedersen, A., Gothe, E., Larsen, S. E., O'Hare, M., Birk, S., Riis, T., & Friberg, N. (2015). Plant trait characteristics vary with size and eutrophication in European lowland streams. *J Appl Ecol, 52*(6), 1617-1628. DOI:10.1111/1365-2664.12509

Baattrup-Pedersen, A., Gothe, E., Riis, T., & O'Hare, M. T. (2016). Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams. *Sci Total Environ, 543*(Pt A), 230-238. DOI:10.1016/j.scitotenv.2015.11.027

Canfield, D. E. & Hoyer, M. V. (1988). Infuence of Nutrient Enrichment and Light Availability on the Abundance of Aquatic Macrophytes in Florida Streams. *Canadian journal of fisheries and aquatic sciences, 45*, 1467-1472.

Cao, T., Xie, P., Ni, L. Y., Zhang, M., & Xu, J. (2009). Carbon and nitrogen metabolism of an eutrophication tolerative macrophyte, Potamogeton crispus, under NH4+ stress and low light availability. *Environmental and Experimental Botany, 66*(1), 74-78. DOI:10.1016/j.envexpbot.2008.10.004

Caraco, N. F. & Cole, J. J. (2002). Contrasting Impacts of a Native and Alien Macrophyte on Dissolved Oxygen in a Large River. *Ecological Applications, 12*(5), 1496-1509. DOI:10.2307/3099987

Carr, G. M., Bod, S. A. E., Duthie, H. C., & Taylor, W. D. (2003). Macrophyte biomass and water quality in Ontario rivers. *Journal of the North American Benthological Society, 22*(2), 182-193. DOI:10.2307/1467991

Cavalli, G., Baattrup-Pedersen, A., & Riis, T. (2016). Nutrient availability and nutrient use efficiency in plants growing in the transition zone between land and water. *Plant Biol (Stuttg), 18*(2), 301-306. DOI:10.1111/plb.12397

Cenzato, D. & Ganf, G. (2001). A comparison of growth responses between two species of Potamogeton with contrasting canopy architecture. *Aquatic Botany, 70*(1), 53-66. DOI:10.1016/S0304-3770(00)00143-1

Champion, P. & Hofstra, D. Potamogeton crispus Fact Sheet (continuously updated). New Zealand Plant Conservation Network. . Retrieved from https://www.nzpcn.org.nz/flora/species/potamogeton-crispus/

Champion, P., Rowe, D., Smith, B., Wells, R., Kilroy, C., & de Winton, M. (2013). *Freshwater pests of New Zealand*. Retrieved from NIWA, Wellington:

Collier, K. J., Champion, P. D., & Croker, G. F. (1999). Patch- and reach-scale dynamics of a macrophyte-invertebrate system in a New Zealand lowland stream. *Hydrobiologia, 392*(2), 89-97. DOI:10.1023/A:1003653717805

de Lange, P. J. Potamogeton ochreatus Fact Sheet (content continuously updated). New Zealand Plant Conservation Network. . Retrieved from https://www.nzpcn.org.nz/flora/species/potamogeton-ochreatus/

Demars, B. O. L. & Edwards, A. C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. *Freshwater Biology, 52*(11), 2073-2086. DOI:10.1111/j.1365-2427.2007.01817.x

Dos Reis Oliveira, P. C., van der Geest, H. G., Kraak, M. H. S., Westveer, J. J., Verdonschot, R. C. M., & Verdonschot, P. F. M. (2020). Over forty years of lowland stream restoration: Lessons learned? *J Environ Manage, 264*, 110417. DOI:10.1016/j.jenvman.2020.110417

Duarte, C. M. (1990). Seagrass Nutrient Content. *Marine Ecology Progress Series, 67*(2), 201-207. DOI:10.3354/meps067201

Duarte, C. M. (1992). Nutrient Concentration of Aquatic Plants - Patterns across Species. *Limnology and Oceanography, 37*(4), 882-889. DOI:10.4319/lo.1992.37.4.0882

Ellawala Kankanamge, C., Matheson, F. E., & Riis, T. (2019). Shading constrains the growth of invasive submerged macrophytes in streams. *Aquatic Botany, 158*. DOI:10.1016/j.aquabot.2019.06.004

Ellenberg, H., Arndt, U., Bretthauer, R., Ruthsatz, B., & Steubing, L. (1991). Biological monitoring. Signals from the environment.

Garbey, C., Thiebaut, G., & Muller, S. (2004). Morphological plasticity of a spreading aquatic macrophyte, Ranunculus peltatus, in response to environmental variables. *Plant Ecology, 173*(1), 125-137. DOI:10.1023/B:VEGE.0000026336.44576.ff

Gerloff, G. C. (1975). *Nutritional ecology of nuisance aquatic plants*: National Environmental Research Center, Office of Research and Development ….

Giorgi, A., Feijoó, C., & Tell, G. (2005). Primary producers in a Pampean stream: temporal variation and structuring role. *Biodiversity and Conservation, 14*(7), 1699-1718. DOI:10.1007/s10531-004-0694-z

Givnish, T. J. (1988). Adaptation to Sun and Shade - a Whole-Plant Perspective. *Australian Journal of Plant Physiology, 15*(1-2), 63-92. DOI:10.1071/Pp9880063

Gusewell, S. & Koerselman, M. (2002). Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspectives in Plant Ecology Evolution and Systematics, 5*(1), 37-61. DOI:10.1078/1433-8319-0000022

Haslam, S. M. (1978). River plants; the macrophytic vegetation of watercourses.

Heaney, S. I., Foy, R. H., Kennedy, G. J. A., Crozier, W. W., & O'Connor, W. C. K. (2001). Impacts of agriculture on aquatic systems: lessons learnt and new unknowns in Northern Ireland. *Marine and Freshwater Research, 52*(1), 151-163. DOI:10.1071/Mf00055

Hilton, J., O'Hare, M., Bowes, M. J., & Jones, J. I. (2006). How green is my river? A new paradigm of eutrophication in rivers. *Sci Total Environ, 365*(1-3), 66-83. DOI:10.1016/j.scitotenv.2006.02.055

Hofstra, D. E., Adam, K. D., & Clayton, J. S. (1995). Isozyme Variation in New-Zealand Populations of Myriophyllum and Potamogeton Species. *Aquatic Botany, 52*(1-2), 121-131. DOI:10.1016/0304-3770(95)00494-K

Hrivnák, R., Kochjarová, J., Oťaheľová, H., Paľove-Balang, P., Slezák, M., & Slezák, P. (2014). Environmental drivers of macrophyte species richness in artificial and natural aquatic water bodies – comparative approach from two central European regions. *Annales de Limnologie - International Journal of Limnology, 50*(4), 269-278. DOI:10.1051/limn/2014020

Huang, W., Han, S., Zhou, Q., Li, W., & Xing, W. (2019). Assessing interactions between environmental factors and aquatic toxicity: Influences of dissolved CO(2) and light on Cd toxicity in the aquatic macrophyte Potamogeton crispus. *Aquat Toxicol, 212*, 247-258. DOI:10.1016/j.aquatox.2019.05.005

Hutchinson, G. E. (1975). *A treatise on limnology: limnological botany* (Vol. 3): Wiley-Interscience.

Jana, S. & Choudhuri, M. A. (1982). Changes Occurring during Aging and Senescence in a Submerged Aquatic Angiosperm (Potamogeton-Pectinatus). *Physiologia Plantarum, 55*(3), 356-360. DOI:10.1111/j.1399-3054.1982.tb00305.x

Jassby, A. D. & Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography, 21*(4), 540-547.

Kern-Hansen, U. & Dawson, F. H. (1978). *The Standing Crop of Aquatic Plants in Lowland Streams in Denmark and the Interrelationships of Nutrients in Plant, Sediment, and Water*. Paper presented at the Proc. EWRS 5th Symp. on Aquatic Weeds.

Kouwe, F. A. (1983). Phosphorous removal in a sewage treatment plant and plant biomass in an effluent recieving lowland stream. *Hydrological Bulletin, 17*(2), 143-156.
Kronvang, B., Jeppesen, E., Conley, D. J., Sondergaard, M., Larsen, S. E., Ovesen, N. B., & Carstensen, J. (2005). Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and coastal waters. *Journal of Hydrology, 304*(1-4), 274-288. DOI:10.1016/j.jhydrol.2004.07.035
Lacoul, P. & Freedman, B. (2006). Environmental influences on aquatic plants in freshwater ecosystems. *Environmental Reviews, 14*(2), 89-136. DOI:10.1139/A06-001
Larned, S. T., Moores, J., Gadd, J., Baillie, B., & Schallenberg, M. (2019). Evidence for the effects of land use on freshwater ecosystems in New Zealand. *New Zealand Journal of Marine and Freshwater Research, 54*(3), 551-591. DOI:10.1080/00288330.2019.1695634
Larned, S. T., Snelder, T., Unwin, M. J., & McBride, G. B. (2016). Water quality in New Zealand rivers: current state and trends. *New Zealand Journal of Marine and Freshwater Research, 50*(3), 389-417. DOI:10.1080/00288330.2016.1150309
Lévesque, D., Hudon, C., James, P. M. A., & Legendre, P. (2016). Environmental factors structuring benthic primary producers at different spatial scales in the St. Lawrence River (Canada). *Aquatic Sciences, 79*(2), 345-356. DOI:10.1007/s00027-016-0501-4
Lichtenthaler, H. K. (1987). Chlorophylls and Carotenoids - Pigments of Photosynthetic Biomembranes. *Methods in Enzymology, 148*, 350-382.
Madsen, T. V. & Cedergreen, N. (2002). Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology, 47*(2), 283-291. DOI:10.1046/j.1365-2427.2002.00802.x
Madsen, T. V., Hahn, P., & Johansen, J. (1998). Effects of inorganic carbon supply on the nitrogen requirement of two submerged macrophytes, Elodea canadensis and Callitriche cophocarpa. *Aquatic Botany, 62*(2), 95-106. DOI:10.1016/S0304-3770(98)00087-4
Manolaki, P., Mouridsen, M. B., Nielsen, E., Olesen, A., Jensen, S. M., Lauridsen, T. L., . . . Riis, T. (2020). A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms. *J Environ Manage, 274*, 111181. DOI:10.1016/j.jenvman.2020.111181
Matheson, F. E., Quinn, J., & Hickey, C. (2012). *Review of the New Zealand instream plant and nutrient guidelines and development of an extended decision making framework: Phases 1 and 2 final report* (HAM2012-081). Retrieved from
Mebane, C. A., Simon, N. S., & Maret, T. R. (2014). Linking nutrient enrichment and streamflow to macrophytes in agricultural streams. *Hydrobiologia, 722*(1), 143-158. DOI:10.1007/s10750-013-1693-4
Michael Smart, R. & Barko, J. W. (1985). Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquatic Botany, 21*(3), 251-263. DOI:10.1016/0304-3770(85)90053-1
Millard, P. (1988). The Accumulation and Storage of Nitrogen by Herbaceous Plants. *Plant Cell and Environment, 11*(1), 1-8. DOI:10.1111/j.1365-3040.1988.tb01769.x
Mouton, T. L., Matheson, F. E., Stephenson, F., Champion, P. D., Wadhwa, S., Hamer, M. P., . . . Riis, T. (2019). Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape. *Sci Total Environ, 659*, 1370-1381. DOI:10.1016/j.scitotenv.2018.12.277
O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lazar, A. N., . . . Bowes, M. J. (2018). Responses of Aquatic Plants to Eutrophication in Rivers: A Revised Conceptual Model. *Front Plant Sci, 9*, 451. DOI:10.3389/fpls.2018.00451
O'Hare, M. T., Clarke, R. T., Bowes, M. J., Cailes, C., Henville, P., Bissett, N., . . . Neal, M. (2010). Eutrophication impacts on a river macrophyte. *Aquatic Botany, 92*(3), 173-178. DOI:10.1016/j.aquabot.2009.11.001
Ordonez, A., Wright, I. J., & Olff, H. (2010). Functional differences between native and alien species: a global-scale comparison. *Functional Ecology, 24*(6), 1353-1361. DOI:10.1111/j.1365-2435.2010.01739.x
Paice, R. L., Chambers, J. M., & Robson, B. J. (2017). Native submerged macrophyte distribution in seasonally-flowing, south-western Australian streams in relation to stream condition. *Aquatic Sciences, 79*(1), 171-185. DOI:10.1007/s00027-016-0488-x

Puijalon, S., Lena, J. P., & Bornette, G. (2007). Interactive effects of nutrient and mechanical stresses on plant morphology. *Ann Bot, 100*(6), 1297-1305. DOI:10.1093/aob/mcm226
Quirino, B. A., Carniatto, N., Thomaz, S. M., Aleixo, M. H. F., & Fugi, R. (2019). The amphibian macrophyte Polygonum punctatum as a temporary habitat and feeding ground for fish. *Aquatic Ecology, 53*(3), 441-452. DOI:10.1007/s10452-019-09700-9
Riis, T. & Biggs, B. J. F. (2001). Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply - a synthesis and conceptual model. *New Zealand Journal of Marine and Freshwater Research, 35*(2), 255-267. DOI:10.1080/00288330.2001.9516996
Riis, T. & Biggs, B. J. F. (2003). Hydrologic and hydraulic control of macrophyte establishment and performance in streams. *Limnology and Oceanography, 48*(4), 1488-1497. DOI:10.4319/lo.2003.48.4.1488
Riis, T., Lambertini, C., Olesen, B., Clayton, J. S., Brix, H., & Sorrell, B. K. (2010). Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation? *Ann Bot, 106*(5), 813-822. DOI:10.1093/aob/mcq176
Riis, T., Tank, J. L., Reisinger, A. J., Aubenau, A., Roche, K. R., Levi, P. S., . . . Bolster, D. (2019). Riverine macrophytes control seasonal nutrient uptake via both physical and biological pathways. *Freshwater Biology, 65*(2), 178-192. DOI:10.1111/fwb.13412
Sand-Jensen, K. (1998). Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology, 39*(4), 663-679. DOI:10.1046/j.1365-2427.1998.00316.x
Sand-Jensen, K., Riis, T., Vestergaard, O., & Larsen, S. E. (2000). Macrophyte decline in Danish lakes and streams over the past 100 years. *Journal of Ecology, 88*(6), 1030-1040. DOI:10.1046/j.1365-2745.2000.00519.x
Sosiak, A. (2002). Long-term response of periphyton and macrophytes to reduced municipal nutrient loading to the Bow River (Alberta, Canada). *Canadian journal of fisheries and aquatic sciences, 59*(6), 987-1001. DOI:10.1139/F02-071
Steffen, K., Becker, T., Herr, W., & Leuschner, C. (2013). Diversity loss in the macrophyte vegetation of northwest German streams and rivers between the 1950s and 2010. *Hydrobiologia, 713*(1), 1-17. DOI:10.1007/s10750-013-1472-2
Su, S. Q., Zhou, Y. M., Qin, J. G., Yao, W. Z., & Ma, Z. H. (2010). Optimisation of the Method for Chlorophyll Extraction in Aquatic Plants. *Journal of Freshwater Ecology, 25*(4), 531-538. DOI:10.1080/02705060.2010.9664402
Vilmin, L., Mogollon, J. M., Beusen, A. H. W., & Bouwman, A. F. (2018). Forms and subannual variability of nitrogen and phosphorus loading to global river networks over the 20th century. *Global and Planetary Change, 163*, 67-85. DOI:10.1016/j.gloplacha.2018.02.007
Wang, L., Wang, X., Han, X., Gao, Y., Liu, B., Zhang, X., & Wang, G. (2021). Potamogeton crispus responses to varying water depth in morphological plasticity and physiological traits. *Environ Sci Pollut Res Int, 28*(4), 4253-4261. DOI:10.1007/s11356-020-10806-z
Zhou, H., Ren, J., & Cai, X. (1997). The concept and measurement of diurnal light compensation point of submerged macrophyte. *Acta Scientiae Circumstantiae, 17*, 256-258.

Chapter III

Direct competition between an alien and a native Aotearoa New Zealand macrophyte: Stronger effects of inherent trait expression than nutrient level.

3.1 Abstract

Where growth conditions are suitable macrophytes are an important component of stream ecosystems, as they provide shelter and substrate, and alter the physiochemical environment. Macrophytes communities are susceptible to eutrophication, which causes extreme biomass accrual that can lead to competitive exclusion of non-competitive species. Especially non-native species can have strong competitive traits and are a threat to freshwater ecosystems worldwide. In this chapter, I will investigate the effects of variability of nutrients and light on competition in the establishment phase between two species common in Aotearoa New Zealand. I will test the performance and competitive outcomes of the non-native *Potamogeton crispus*, and the native *P. ochreatus* in direct competition with each other in a full factorial mesocosm experiment, comprising four nutrient (as NO_3^--N) and two light treatments. Competitive strength was assessed as the difference in relative growth rates and plant growth traits between individuals grown in mixed and monocultures and the relative performance between individuals in the mixed cultures. I saw no effect of nutrients on the ability of either species to supress growth or trait expression of the other species. Both nutrients and light influenced the relative performance of the species. *P. crispus* had greater growth and trait expression than *P. ochreatus* when nutrients were low and light was high, whereas the opposite was the case or there were no differences between the species when nutrients were high, regardless of light level. These responses indicate that, under our experimental conditions, competition has little importance in the establishment phase regardless of eutrophication status, but that trait expression can vary with resource level. Relative trait expression will most likely be the deciding factor for which species will be the dominant one in a stream, however since an unknown factor appeared to have caused an overall growth limitation in this experiment, it is hard to extrapolate results and make predictions as to which of the two investigated species will perform best under field conditions. Furthermore, species interactions depend on more traits and factors than I have investigated here.

3.2 Introduction

3.2.1 Macrophyte importance, diversity decline, native species decline.

Stream ecosystem health is positively affected by a flourishing macrophyte community, providing substrate, food, and shelter for other trophic levels (Bowden et al., 2017). However, anthropogenic induced eutrophication of lotic ecosystems has led to degraded systems that do not provide the same services as pristine systems and lack native plant diversity (Quinn et al., 2011). Increases in nitrogen (N) and phosphorous (P) levels can lead to excessive proliferation of macrophytes that can fill up, or 'clog', a stream channel with biomass which can then lead to increases in sedimentation rates and anoxia below the macrophyte beds (Caraco & Cole, 2002). These changes can then propagate to other trophic levels, affecting them negatively (Schultz & Dibble, 2011). Investigations into eutrophication effects have been more prolific now than 30 years ago, but much remains to be investigated (O'hare et al., 2018). Threshold concentrations for when ecological effects start to appear are still not fully elucidated, nor are competitive interactions at different nutrient levels.

As nutrient concentrations increase, competition for light is increasingly important in determining macrophyte community structure (O'hare et al., 2018). Under eutrophic conditions, the dominating species will thus be those that can compete effectively for incoming irradiation. Trait-based analyses of community structure in streams of varying states of eutrophication in Europe shows that successful macrophyte species under nutrient sufficiency, prove effective in intercepting incoming irradiation, by having fast growth rates and the ability to grow to or out of the water surface to out-shade underlying species (Baattrup-Pedersen et al., 2015; Baattrup-Pedersen et al., 2016). However, these analyses also show that efficient utilisation of light can be an effective strategy in eutrophic streams, as light levels decrease markedly below the large species occupying the water surface (Baattrup-Pedersen et al., 2015; Baattrup-Pedersen et al., 2016). In Aotearoa New Zealand, field investigations also point out that the effect of eutrophication can be dampened by shading (Mouton et al., 2019), exemplifying the importance of the nutrient-light interaction.

In an anthropomorphically impacted river, usually conditions are such that C or R species (*cf* Grime 2008) have a competitive advantage over other species as they are in possession of the traits that make them successful in that particular niche (Quinn et al., 2011). These species are often non-native species that have been successful in colonizing a new area and sometimes have become invasive, typically because they have fast growth rates and are large so they smother other species (Daehler, 2003; Fleming & Dibble, 2014; Van Kleunen et al., 2010). Other traits of successful non-native species are the capacity to incorporate a lot of nutrients into the tissue, also termed luxury uptake (Fogg, 1973). This enables an individual to maintain growth even under fluctuation in nutrient supply. Species that are invading a new area must overcome both abiotic and biotic filters that operate on coarse and fine spatial scales, respectively (Pulzatto et al., 2019). The traits of a species are important

in that they determine how well the species can overcome the filtering. There exists a paucity of data, however, on how macrophytes compete on a fine scale with species of a similar growth form and trait set.

Several experiments have been conducted that compared traits of native and non-native species, however with the caveat that the authors compared species of different genera or classes. (Gufu et al., 2018), found growth rates to be higher at high nutrient supply for non-natives *Egeria densa* and *Salvinia molesta* compared to the native *Vallisneria spiralis*. Ellawala Kankanamge et al. (2019) compared growth rates of multiple non-native species with a charophyte native to Aotearoa New Zealand and found that the charophyte had a much lower growth rate than the non-natives at high light levels. (Kennedy et al., 2009) compared the growth rate of *Hydrilla verticillate* with that of the two natives *Sagittaria kurziana* and *Vallisneria americana* at different nitrate concentrations and found the non-native to be superior and the only species responding to additional nitrate. To be able to conclude on the general competitive dynamic of alien versus native species, one needs to test closely related species, otherwise one concludes rather on differences in growth form than on a general native versus non-native trend (Vilà & Weiner, 2004). Gérard et al. (2014), grew two species of plants at two sediment nutrient levels, eu- and meso-trophic conditions. They used two similar species from the same genus (*Ludwigia*) and could thus conclude that the results are not just from difference in morphotype. They found that one species performed better in direct competition than the other and that this was the species that had become invasive in its new introduced range. They did not however include light as a factor, or control nutrient levels in the water column. Furthermore, both species were non-native, so no comparison to native species could be drawn. As such, there is a general trend of non-native species outperforming natives, and some evidence for competitive strength as a factor that promotes invasiveness. Still, more experimental evidence is needed, specifically experiments using closely related species.

To further elucidate the effect on varying levels of nutrients and light on the competitive interaction between native and non-native species, we set up a mesocosm experiment of *Potamogeton crispus* and *P. ochreatus* grown together in an experimental setup mimicking a slow flowing stream. Based on the results of the studies reviewed above and Chapter II of this thesis, we hypothesised that the alien *P. crispus* was the better competitor at high nutrient levels and high light and that the native *P. ochreatus* was better at low nutrient and light levels.

3.3 Methods:

3.3.1 Experimental procedure.

The experiment was undertaken between 15th October and 15th December 2021, in four outdoor flume mesocosms (length ca. 10 m), with a continuous water supply of 1.5 L min^{-1} and internal recirculation. Phosphorous (P), bicarbonate and trace elements were added in non-limiting concentrations (Smart &

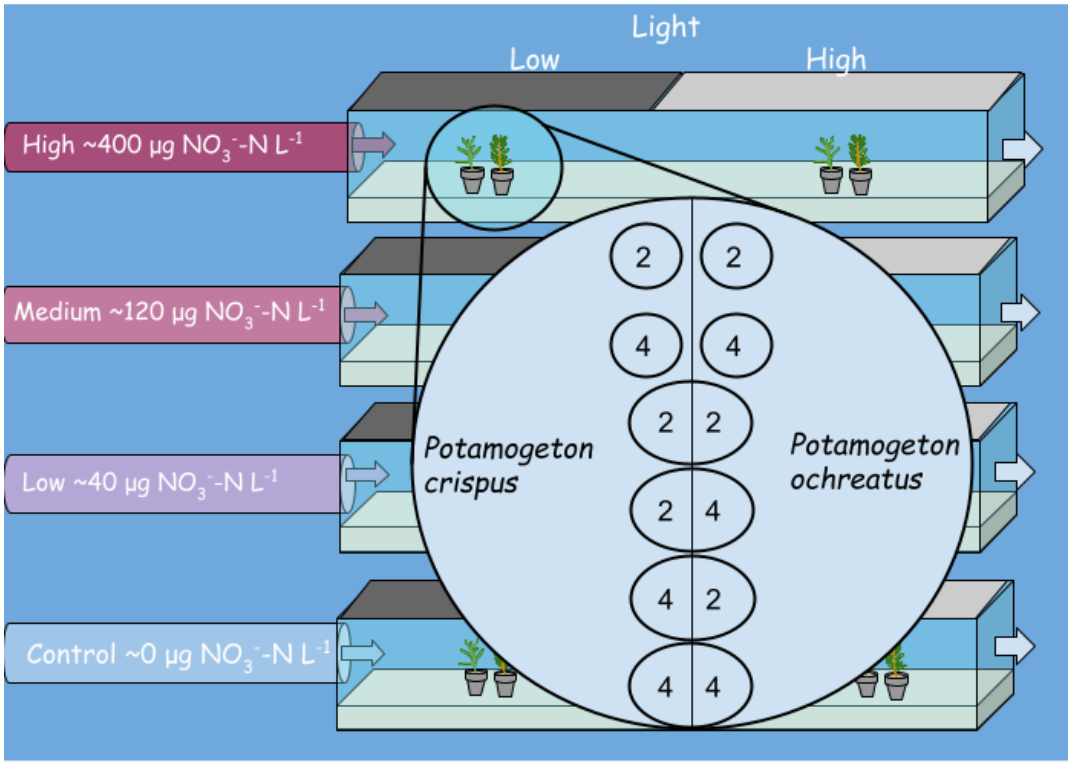
Barko, 1985). We aimed for P concentrations of 100 µg L^{-1} and dissolved inorganic carbon concentrations of 10 mg L^{-1}. Each flume had a target nitrate concentration for each N treatment: 0 N ~0 µg NO_3^--N L^{-1}, 40 N ~40 µg NO_3^--N L^{-1}, 120 N ~120 µg NO_3^--N L^{-1}, 400 N ~400 µg NO_3^--N L^{-1} (Figure 3.1). To include light as a second variable, we covered the upper and lower half with different densities of shade cloth to make two light treatments, High and Low. We used 92 % shade for a light limited environment and 56 % as a non-limiting one (Skovsholt et al., 2023). Average incoming photosynthetically active radiation (PAR) based on hourly measurements for the experimental period was 41 mol m^{-2} d^{-1}. Thus, our treatments yielded 18 mol photons m^{-2} d^{-1} and 3 mol photons m^{-2} d^{-1} on average.

Plants were collected from a small stream south of Morrinsville, Aotearoa New Zealand (37’42’’18.59 S; 175’33’’4.15 E). Before experiment start, plants were acclimated by growing them in their respective nutrient concentration for three weeks under 56 % shade cloth. 10 cm apical shoots of the acclimated plants were transplanted into 700 mL plastic cups (ø 10 cm), filled with hyper oligotrophic sand (Ellawala Kankanamge et al., 2019), in mono and mixed species cups, yielding eight competition treatments: Four single species pots, each species in densities of two (255 individuals m^{-2}) and four (509 individuals m^{-2}), and four mixed pots; 2v2, 2v4, 4v2 and 4v4 (Figure 1). Five replicates of each competition treatment were planted in each of the eight environmental treatments. 1440 plants were planted in total. Five replicate shoots of each nitrogen treatment were set aside at the beginning of the experiment as T_0 plants for subsequent estimation of growth rates.

During the experiment, we collected water samples two to three times a week from each flume to ascertain the long-time stability of the nitrate concentrations in the flumes. Samples were frozen before analysis. Additionally, to investigate diel fluctuations of nitrate concentrations, two OPUS nitrate probes (Trios, Germany) were deployed in the upstream end of the flumes to continuously log nitrate concentrations. The probes were first calibrated using two standards (50 and 500 µg NO_3^--N L^{-1}).

At the end of the incubations, the plants were harvested, and the length of the plants, number and length of side shoots, and roots were measured using a ruler. Plants were subsequently placed in plastic zip lock bags and frozen until laboratory analysis.

***Figure 3-1**: Schematic representation of the mesocosm setup with the four nutrient and two light levels. Within each nutrient and light treatment, we tested morphology, growth, and internal nutrient content of two plant species: non-native species Potamogeton crispus and native species P ochreatus, in 8 density treatments comprising 4 mono species pots and 4 mixed species (black circles).*



3.3.2 Laboratory procedures

Water samples were analysed for nitrate + nitrite-nitrogen (NO_3^--N), ammonium-nitrogen (NH_4^+-N), and dissolved reactive phosphorous (DRP) using a flow injection analyser (FIA; Lachat Quikchem, USA - detection limit: 1 µg L^{-1}).

To obtain dry weight (DW), the entire frozen plants were put in a vacuum drier (CRIST, Germany) for at least 24 hours and then weighed. The plants were then ground to a powder with a mortar and pestle and subsamples taken for C and N analysis, and extraction of chlorophylls. For grinding, we pooled individuals by pot and by species, so that a single measurement of tissue C and N % was obtained for each species in each pot.

Carbon and nitrogen tissue content were analysed by combustion gas analysis on a Vario EL-cube (Elementar, Germany - precision: < 0.01 %).

3.3.3 Data treatment.

Relative growth rate (RGR) was estimated as $(\log(DW\_end) - \log(DW\_start))/(days)$. To quantify the morphological difference between plants we used main stem length and the number and length of the branches to calculate several morphological indices. These were branching degree (number of branches per main stem length), and lateral spread (total length / main stem length). We also report the maximum root length. We used the average value from all the individuals of one species within a pot for further analysis.

To assess nutrient limitation, we plotted internal C:N ratios against N (%) to see if the relationship followed an idealized hyperbolic relationship (cf. Cavalli et al., 2016). We noticed a lot of individuals fell below the general relationship (Figure 3.2) and found that the low C:N ratio of these plants was due to a low C content. We thus devised a cut-off carbon content of 35 % so that plants with less than this were excluded from the analysis. These individuals can be seen as the lowermost green points on Figure 3.2 and correlated well with individuals that were in poor condition when the experiment ended. All were from the 0 N treatment.

To quantify the competitive interactions of the two species, we used two metrics; competitive ratio (CR) and relative dominance (RD) (Gérard et al., 2014; Olde Venterink & Güsewell, 2010). CR is a response parameter value derived from comparing the trait score of a species in a mixed species pot with the corresponding score from that species in a single species pot. A higher trait score in the mono pot ($CR < 1$) signifies that this species is supressed by the competing species, whereas a higher trait score in the mixed pot ($CR > 1$) means that this species is the superior competitor (Olde Venterink & Güsewell, 2010). Relative dominance (RD) is another way of assessing competitive outcome. It is calculated as the ratio of the trait scores between the two species in the same pot (Olde Venterink & Güsewell, 2010); if one species has higher trait score than the other ($RD > 1$), this is deemed the dominant species. For this paper, RD is calculated as the trait score of *P. crispus/P. ochreatus*, so that a score of >1 returns *P. crispus* as the dominant species, whereas if the score is < 1 then *P. ochreatus* is the dominant species. In all cases (CR's and RD's), significance was determined by Wilcoxon rank sum test ($\alpha = 0.05$).

We found no effect of the different densities of plants within the pots on any of the trait scores (one-way ANOVA, $p > 0.05$), so we pooled all the density treatments together to increase replication in statistical analyses. Thus for each trait, for each species, analyses included values from mono and mixed species pots at different densities (Gérard et al., 2014).

Statistical analysis and figure creation was performed in the R environment ver. 4.2.1 (R Core Team, 2022).

3.4 Results:

The experimental design didn't result in any competitive suppression between species, but difference in trait expression was evident between treatments as *P. crispus* had higher trait scores at low nutrient and high light, while *P. ochreatus* showed greater scores at high nutrient, low light. Flume experimental conditions were stable and although the higher N treatments didn't quite reach the planned concentrations there was still a clear distinction between each N treatment.

3.4.1 Nutrients in water and plants

Aside from a drop toward the end of the experiment (see Figure A.3.1 for details), water column nutrient concentrations in the four experimental flumes were stable, and the four treatments were mostly below, but close to their target concentrations of DRP and NO3-N (Table 3.1). High temporal resolution data from the deployed nitrate probes showed little to no diel variations in the 40 and 120 N treatment, and some diel variation for the 400 N treatment, but this was small relative to the total concentration (Figure A.3.2).

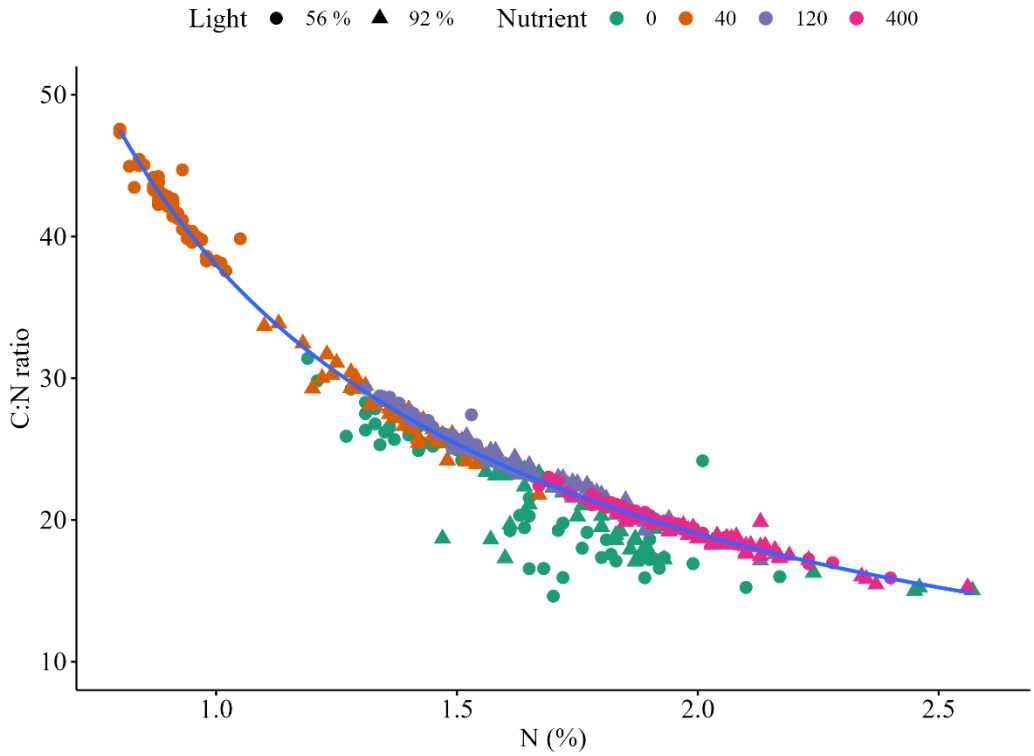
Table 3.1: *Mean concentrations (±SD) of dissolved reactive phosphorous (DRP), ammonium-nitrogen (NH_4^+-N), nitrate + nitrite-nitrogen (NO_3^--N), and dissolved inorganic nitrogen (NH_4^+-N + NO_3^--N) in the four N treatments for the duration of the experiment. n = 9, except 120 N where n = 8.*

	N treatment			
	0	**40**	**120**	**400**
DRP (µg L^{-1})	86 ±32	70 ±20	78 ±21	67 ±18
NH_4^+-N (µg L^{-1})	6 ±3	7 ±4	7 ±2	10 ±6
NO_3^--N (µg L^{-1})	4 ±4	29 ±17	80 ±43	298 ±147
DIN (µg L^{-1})	10 ±3	36 ±15	87 ±42	309 ±146
DIN:DRP ratio	0.1 ±0.1	0.6 ±0.3	1.2 ±0.8	4.8 ±2.6

Plants in treatments with higher N supply incorporated more N in tissues, except for 0 N. More N was incorporated at lower light than high light (Figure 3.2). Internal N content varied between 0.8 % to 2.6 % and showed a consistent hyperbolic pattern of decrease in C:N ratio with increasing N % (Cavalli et al., 2016). Several individuals from the 0 N treatment senesced during the experiment (translucent brown, lacking structural integrity), dominantly of *P. crispus* at high light. These were the plants with carbon content < 35 % and were the ones furthest below the C:N to N hyperbolic relationship and excluded from analysis (lowest green points on Figure 3.2).

P. crispus specimens had higher N content than *P. ochreatus* at high light availability, but *P. ochreatus* had higher C content than *P. crispus* at low light availability. N content was higher for individuals in mixed species pots than those in mono pots (CR > 1) at low light (Table 3.2), however

Figure 3-2: *Relationship between N content (%) and C:N ratio for all individuals of Potamogeton crispus and P. ochreatus grown at four different nutrient (as nitrate-N; green, red, blue, purple) levels and two light levels (high and low; circles or triangulars).*



the real difference was miniscule (Figure 3.3a). *P. crispus* mostly had higher N content than *P. ochreatus* (RD >1) across light treatments (Table 3.3) but difference between species were less pronounced than those between N treatments (Figure 3.3a). C content was similar between mixed and mono pots (CR =1) but *P. ochreatus* had a higher C content than *P. crispus* at low light (RD < 1, Table 3.3). Some significant differences in C content were found at high light but with little real difference between species (Figure 3.3b).

3.4.2 Growth rates and morphology.

CR's for growth and morphological trait expression of both species was unaffected by differences in N and light (Table 3.2), but RD was affected by both. Both species had a tendency towards highest growth rates in mono pots (CR < 1), but not significant (Table 3.2, Figure 3.4). *P. crispus* grew better than *P. ochreatus* at 40 N (RD > 1; Table 3.3) more so at high light (Figure 3.4). At low light, *P. ochreatus* had higher RGRs than *P. crispus* in the remaining N treatments (RD < 1) (Table 3.3, Figure 3.4).

Figure 3-3: *Internal nitrogen (a) and carbon (b), of Potamogeton crispus (PC) and P. ochreatus (PO) grown at four nutrient (0, 40, 120, 400) and two light levels (high and low), in mixed and monocultures. Significant differences between means are shown as p < 0.05: *, p < 0.01: **, p < 0.001: ***, p < 0.0001: ****.*

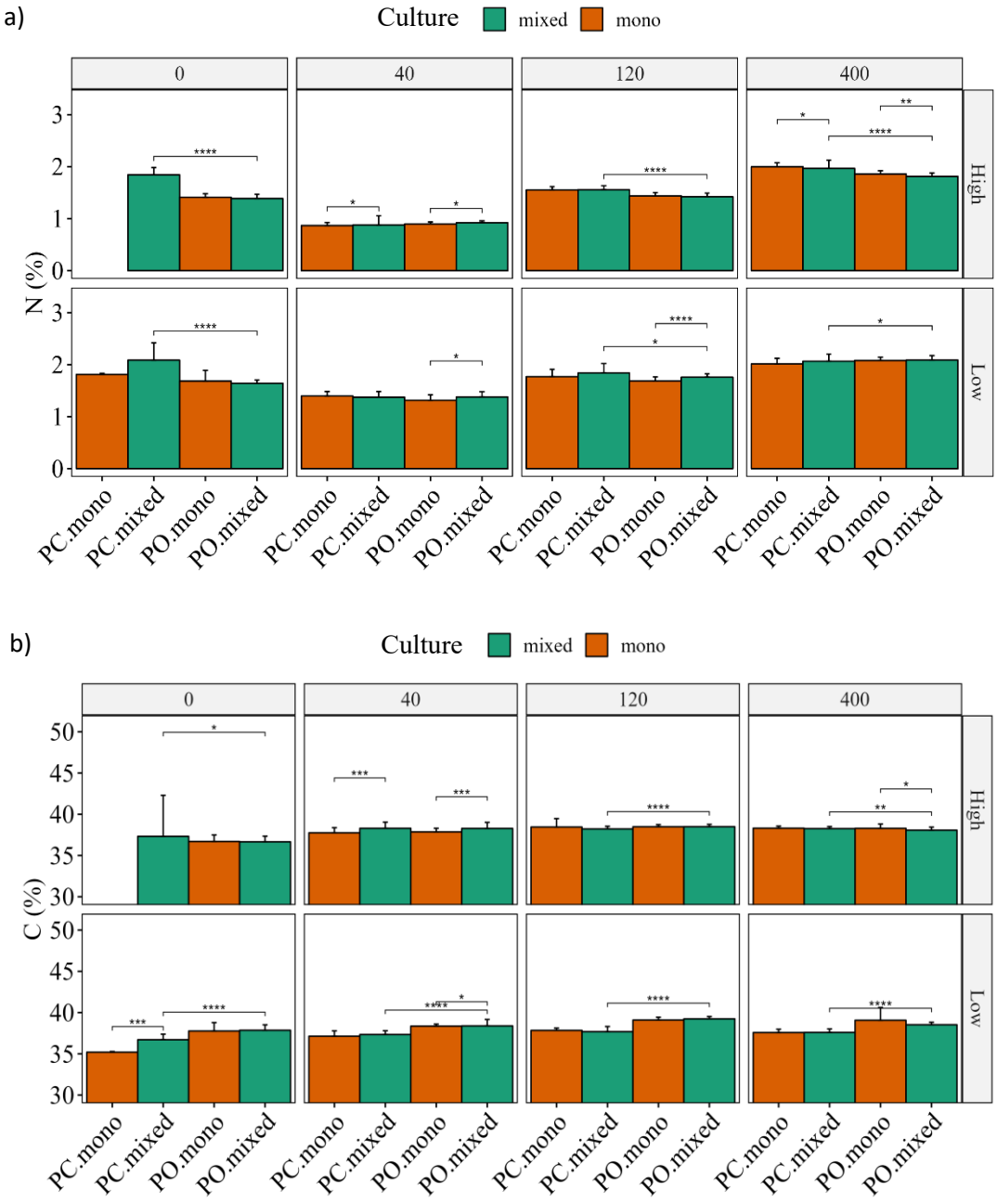


Figure 3-4**: Relative growth rate of Potamogeton crispus (PC) and P. ochreatus (PO) grown at four nutrient (0, 40, 120, 400) and two light levels (high, low), in mixed and monocultures. Significant differences between means are shown as p < 0.05: *, p < 0.01: **, p < 0.001: ***, p < 0.0001: *.*

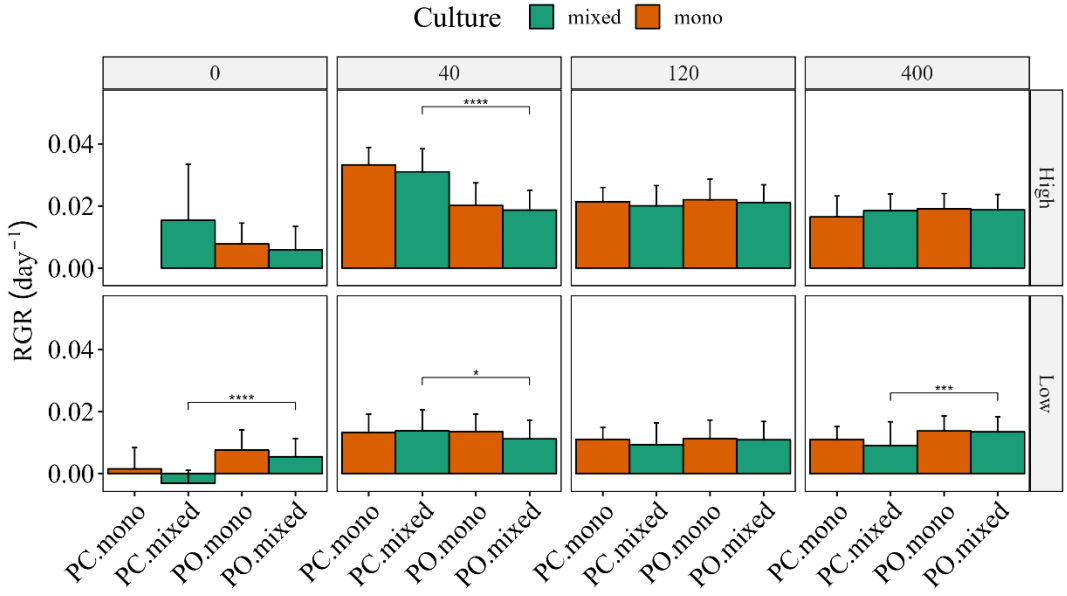


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***Table 3.2**: Competitive ratios (CR's; ratios between mean trait score of mix and monocultures) for internal nitrogen (N) and carbon (C), relative growth rate (RGR), main stem length (MSL), branching degree (BD), lateral spread (LS), and root length (RL), of Potamogeton crispus (PC) and P. ochreatus (PO) grown at four N (0, 40, 120, 400) and two light levels (high and low). nd = no data. Significant differences (Wilcoxon test, p < 0.05) between treatments indicated in bold.*

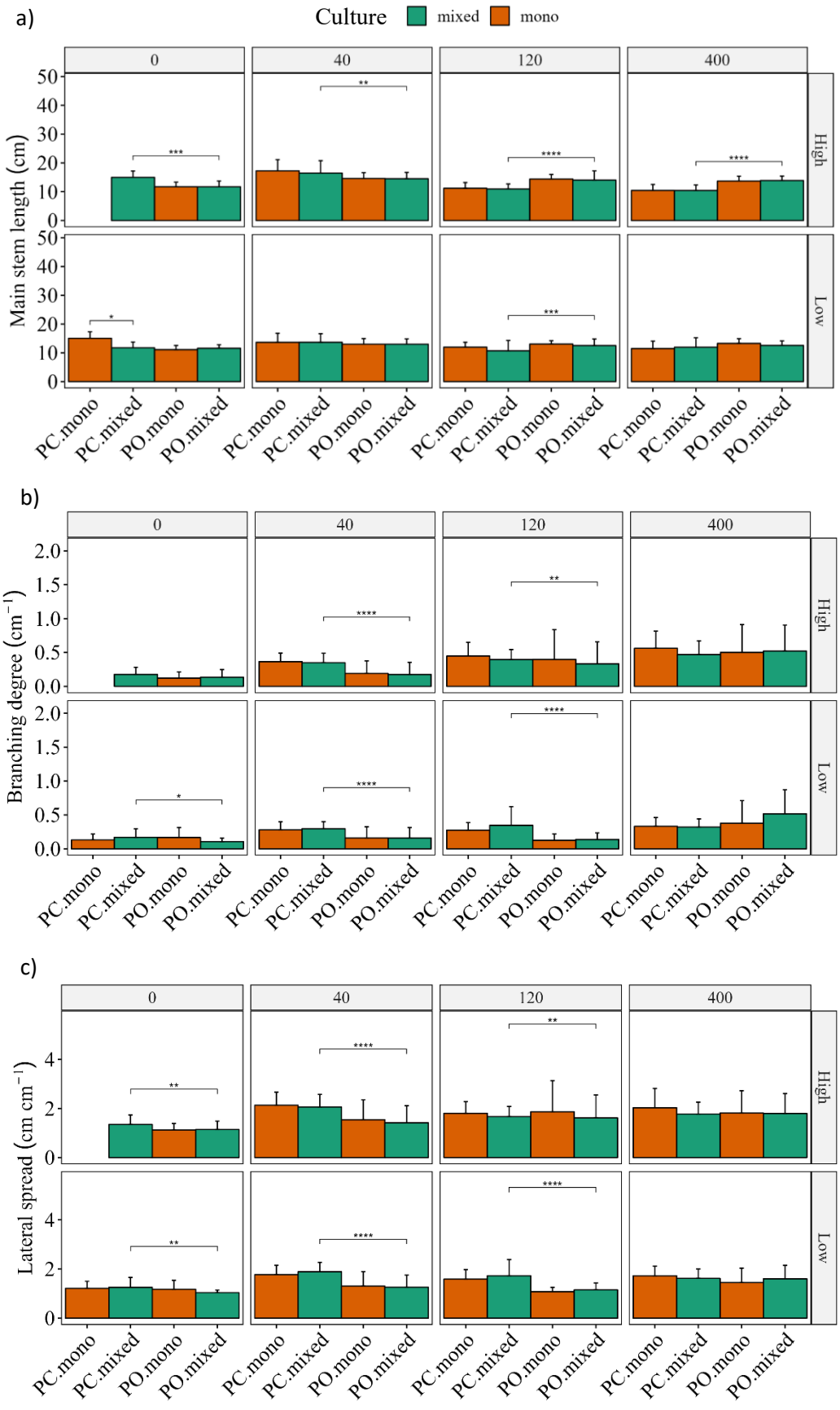
Light	N	Species	N	C	RGR	MSL	BD	LS	RL
high	0	PC	nd	nd	nd	nd	nd	nd	nd
		PO	0.98	1.00	0.75	1.00	1.11	1.02	0.21
	40	PC	**1.01**	**1.01**	0.93	0.96	0.95	0.97	1.16
		PO	**1.03**	**1.01**	0.93	0.99	0.92	0.92	0.64
	120	PC	1.00	0.99	0.94	0.98	0.89	0.92	0.82
		PO	0.99	1.00	0.96	0.97	0.83	0.87	1.15
	400	PC	**0.98**	1.00	1.12	1.00	0.83	0.88	0.93
		PO	**0.98**	**0.99**	0.98	1.01	1.04	0.99	0.60
low	0	PC	1.15	**1.04**	-1.96	**0.78**	1.30	1.04	nd
		PO	0.98	1.00	0.72	1.05	0.64	0.88	nd
	40	PC	0.98	1.01	1.04	1.00	1.06	1.07	**0.26**
		PO	**1.05**	**1.00**	0.83	1.00	0.99	0.97	nd
	120	PC	1.04	1.00	0.85	0.89	1.26	1.09	1.84
		PO	**1.04**	1.00	0.96	0.96	1.10	1.07	2.97
	400	PC	1.02	1.00	0.83	1.04	0.97	0.94	0.47
		PO	1.00	0.99	0.98	0.95	1.37	1.11	**0.16**

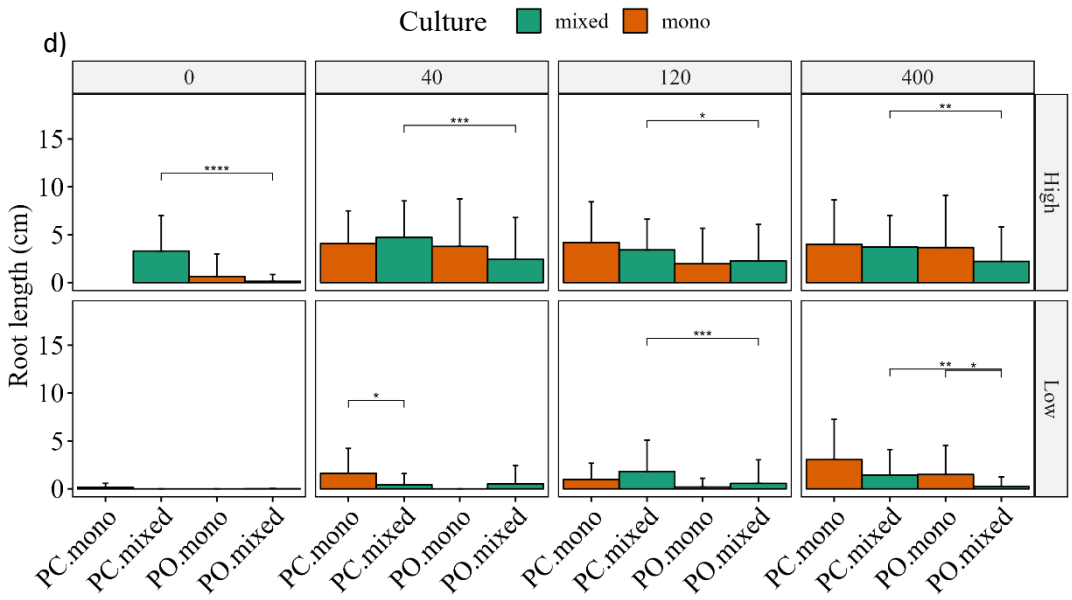
RD for main stem length was positive at low N but negative at high N (Table 3.3),this pattern was less pronounced at low light (Figure 3.5). Branching degree and lateral spread, being related metrics, showed largely the same pattern, increasing with N treatment and light, and mostly highest for *P. crispus* (RD > 1, Table 3.3, Figure 3.5). Root lengths increased with light and was mostly higher for *P. crispus* than *P. ochreatus* (RD > 1, Table 3.3).

Table 3.3: *Relative dominances (RD's; ratio of mean trait scores of Potamogeton crispus (PC) and P. ochreatus (PO) – PC:PO in mixed cultures) for internal nitrogen (N) and carbon (C), relative growth rate (RGR), main stem length (MSL), branching degree (BD), lateral spread (LS), and root length (RL), of individuals grown at four nutrient (0, 40, 120, 400) and two light levels (High and Low). nd = no data. Significant differences (Wilcoxon test, p < 0.05) between treatments indicated in bold.*

Light	Nutrient	N	C	RGR	MSL	BD	LS	RL
High	0	**1.33**	**1.02**	2.62	**1.28**	1.31	**1.18**	**24.51**
	40	0.96	1.00	**1.66**	**1.14**	**1.99**	**1.46**	**1.95**
	120	**1.09**	**0.99**	0.95	**0.78**	**1.19**	**1.03**	**1.50**
	400	**1.09**	**1.00**	0.98	**0.75**	0.90	0.99	**1.68**
Low	0	**1.27**	**0.97**	**-0.56**	1.01	**1.59**	**1.21**	nd
	40	1.00	**0.97**	**1.23**	1.05	**1.85**	**1.50**	0.79
	120	**1.05**	**0.96**	0.86	**0.85**	**2.52**	**1.50**	**3.20**
	400	**0.99**	**0.98**	**0.67**	0.95	0.62	1.01	**5.96**

1 ***Figure 3-5***: *Main stem length (a), branching degree (b), lateral spread (c), and root length (d) of Potamogeton crispus (PC) and P. ochreatus (PO) grown at four N (0, 40, 120, 400) and two light levels (high, low), in mixed and monocultures. Significant differences between means are shown as p < 0.05: *, p < 0.01: **, p < 0.001: ***, p < 0.0001: ****.*




d)
Culture
mixed
mono
0
40
120
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High
Low
Root length (cm)
0
5
10
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*
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PC.mono
PC.mixed
PO.mono
PO.mixed

3.5 Discussion:

We aimed to elucidate how increases in nitrogen and light affects the competitive outcome of *P. crispus* and *P. ochreatus* grown in an experimental setup mimicking a slow flowing stream. We expected that the alien *P. crispus* was the better competitor at high nutrient levels and high light and that the native *P. ochreatus* was better at low nutrient and light levels. To assess the outcome of the competitive interactions between species we looked simultaneously at CR and RD; for a direct competitive interaction to occur and for one species to 'win' that interaction, a species needs to supress the other (CR < 1 for the opponent species) and it needs to dominate in that trait (RD >1). If CR is > 1 this would be considered facilitation, and if CR = 1, there is no effect of the species on each other and any difference in RD will be due to difference in inherent trait expression (Olde Venterink & Güsewell, 2010).

We saw no evidence for a direct competitive effect of one species suppressing the others growth or morphology as the CR was mostly not significantly different to 1 and neither nutrient nor light availability influenced this. Rather we saw evidence for higher trait scores of one of the species relative to the other (RD ≠ 1), and that this was affected by variations in nutrient level and light. Similar studies have also failed to find an effect of nutrient levels on competitive suppression, indeed between morphologically similar species, while finding an effect on trait expression (Gérard et al., 2014). Thus, it seems that the interspecific competition between these species in the field will depend not on one species suppressing the other in the establishment phase, but rather which traits will be most beneficial to express in the environments where the two species co-occur as plants grow. As such, long term competition might still lead to exclusion of one or other species if possession and expression of certain traits will yield a sufficient competitive advantage. This will depend on many factors outside the scope of this study, and so care must be taken when extrapolating our results. Field studies of eutrophic streams has shown that successful species are in possession of certain 'competitive' traits (Baattrup-Pedersen et al., 2015; Baattrup-Pedersen et al., 2016). However, experimental evidence for an effect of nutrient levels on direct competitive suppression between species that both are in possession of these traits but expresses them in different degrees have yet to be presented.

Previous observations of growth responses in a similar setup showed that *P. crispus* increased its RGR with increasing nitrate content (Skovsholt et al., 2023), which is not in line with our observations in the current experiment; we cannot say for sure why this is the case. We note that the daily incoming irradiation was lower for this experiment compared to that in Chapter 2, which could potentially curb growth rate differences between N treatments. *P. crispus* has a low light compensation point and can grow at low light intensities (Bolduan et al., 1994; Su et al., 2001; Zhou et al., 1997), so we had still expected it to grow faster at high light as it attained a higher N content.

We can rule out a competitive suppression since the CR was 1. We did however see that lower light levels negated much of the difference between the two species or even shifted to dominance by *P. ochreatus*, especially at very low N levels, a finding also shared by Skovsholt et al. (2023). *P. ochreatus* thus seems better at maintaining a high or at least positive RGR under resource stress. Invasive species like *P. crispus* usually thrive in high resource environments (Fleming & Dibble, 2014; Quinn et al., 2011), and a shaded stream environment has been shown to contain more native species than a non-shaded one (Mouton et al., 2019). Thus, the success of *P. crispus* in New Zealand streams relative to its native counterpart could well depend on the number of human impacted reaches available.

Which species had the highest morphological trait score was largely in line with what would be expected from the two species considering their strategic alignment (cf. Grime, 1988) but was still affected by light and nutrient levels. We did however, expect *P. crispus*, being a ruderal (Riis & Biggs, 2001), to show higher branching degree as this would aid in dispersal due to it dispersing by fragmentation, and lateral spread for effective light capture. Also root length was highest for *P. crispus*, and this may be due to this species being better adapted to fast flowing water and so needs to have a great anchoring strength (Riis & Biggs, 2001, 2003). *P. crispus* have previously been shown to have faster root growth compared to three declining Danish *Potamogeton* species which could help explain why itself is not in decline (Henriksen et al., 2023). The higher stem length of *P. ochreatus* is in line with its CR classification (Riis & Biggs, 2001) as it is a competitive strategy trait to grow tall (Grime, 1988). The higher stem length of *P. crispus* at 0 and 40 N can probably be attributed to the higher RGR, it's natural that higher growth rates lead to increases in length. The greater length of *P. ochreatus* at 120 N both light levels and 400 N high light must surely be a difference in trait expression as the RGR of both species were equal at these treatments. We did not expect that RD for so many of the traits would become 1 or < 1 at the highest N treatment, as previously it has been demonstrated that *P.* crispus generally show a higher trait scores than *P. ochreatus* under increased resource levels (Skovsholt et al., 2023). We have no good explanation as to why this is the case. However, we still see no evidence of competitive suppression of adjacent species at the highest nutrient levels, which would suggests that the success of *P. crispus* in eutrophic streams is simply due to good utilization of the increased levels of resources made available to it by anthropogenic land use change (Fleming & Dibble, 2014; Vorosmarty et al., 2010).

N content increased with external N and was highest for *P. crispus*, regardless of light level, save for at 40 N. However, at 40 N, where we see no difference in N content between species, the growth rate of *P. crispus* is higher than *P. ochreatus*, so nutrient acquisition must have been higher too to sustain the same level of internal N. A high nutrient storage capacity is particularly an advantage under a fluctuating nutrient supply since stored nutrients, accumulated when ambient nutrient levels are high, can be used for substrate regeneration or production when ambient concentrations decline later. Thus,

storage capacity is considered to be a competitive trait as this as this allows a species to inhabit a wider array of areas and can aid non-native species invasiveness (Davis et al., 2000; Fleming & Dibble, 2014). A high nutrient content is characteristic for submerged angiosperms (Cavalli et al., 2016), and *P. crispus* have been shown to attain as much as 4.7 % tissue N (Manolaki et al., 2020), while field observations also show higher N content of *P. ochreatus* than found in this study (3.63 % - Redekop et al., 2018). Those observations are corroborated by previous results using the same experimental setup, where N content was higher than what we found (Skovsholt et al., 2023). The minimum tissue nitrogen level where growth limitation starts to occur is in the 1.2 – 2.0 % range (Demars & Edwards, 2007; Duarte, 1990; Gerloff & Krombholz, 1966). Our plants can thus be considered as having low N content and it is likely that at least some of the plants are limited by nutrient availability, especially in the two lowest N treatments. We note that the C content of *P. crispus* was much lower than the 43.36 % found by Cavalli et al. (2016), however those of *P. ochreatus* was in line with field observations where C content was 35.82 % (Redekop et al., 2018). While we cannot say for sure that the plants are experiencing nutrient stress, it does seem likely. The stress gradient hypothesis (Maestre et al., 2009) states that along a nutrient gradient, facilitating interactions between species will occur where nutrient levels are low and competitive interactions will occur where nutrients are replete (Maestre et al., 2009). In our experiment, we do not see evidence for either interaction type, as we would need to see CR's < 1 for a competitive interaction and > 1 for both species for a facilitating one.

Allelopathy in *P. crispus* have received attention with evidence both for and against its effectiveness on phytoplankton (Fareed et al., 2008; Haroon & Abdel-Aal, 2016; Nakai et al., 1999; Wu et al., 2007; Yang et al., 2023), while none can be found for *P. ochreatus*. Allelopathic interactions have been found between macrophyte species (Thiebaut et al., 2018), so it cannot be ruled out, however the genus *Potamogeton* is not regarded as having strong allelopathic abilities (Gross, 2003). Lack of allelopathy can help explain why there was no competitive suppression of either species (CR =1).

Density differences in the experiment didn't play a role but as we only had two different densities that were fixed, this does not mean that the situation in the field would be the same. Densities can get high *in situ* when communities are not 'reset' by flushing flows and so this might play a role in facilitating the competitive interactions between the two species. We note here that while we successfully mimicked a slow flowing stream environment, comparisons to a field setting should be made with care. Our setup neither had grazing, fluctuating resource supply, nor flushing flows or lesser variations in flow, all factors that could potentially influence competitive interactions (Bornette & Puijalon, 2011; Fleming & Dibble, 2014). Also, the concentrations of nitrate in the experiment were low compared to heavily impacted streams (Ballantine & Davies-Colley, 2014), and the outcome of the competition between the two species could well be influenced by epiphyte growth on the plants (O'hare et al., 2018). Although not explicitly tested here, most of the factors mentioned here are likely

to be most successfully overcome by the ruderal *P. crispus* rather than the competitive strategist *P. ochreatus* and so we predict that in a real setting, *P. crispus* would be the better competitor overall. Indeed, *P. crispus* have been successful in invading other habitats than streams in Aotearoa New Zealand (Bolduan et al., 1994).

3.5.1 Concluding remarks.

We investigated nutrient and light effects on competitive interactions between two morphologically similar species with different geographical origin. We found no evidence of competitive suppression by either species regardless of resource supply. Rather, we found indications that inherent trait expression related to niche fulfilment strategies (cf. Grime, 1988) affected differences in trait scores between species. Still, the trait scores of each species was influenced by nutrient and light treatments, but growth limitation of an unknown kind diminishes the robustness of the results. We also demonstrated the importance of light as a mitigation measure for the dominance of a non-native over a native one and that it is prudent to include both factors in research on eutrophication effects (Mouton et al., 2019). We did not see the expected effect on growth of an increase in nutrient levels; however, we did see that both species were able to incorporate more N into their tissue with increasing nutrient load. This is particularly important when we put the results into perspective of recent findings from the same experimental setup, as further increases in nitrate concentration does not alter the growth rates nor the trait expression of the two species (Skovsholt et al., 2023). Internal nutrients are the ones ultimately available for growth and maintenance, and if the plants can maintain a non-limiting N content at external DIN concentrations between 120 and 400 µg N l^{-1} this is where the threshold for eutrophication effects will be found. Further increases in nutrient concentrations can be relevant to investigate however, as this can induce luxury uptake and thus be important during fluctuations in nutrient supply and affect the competition between the plants and epiphytic algae (O'hare et al., 2018).

References

Ballantine, D. J. & Davies-Colley, R. J. (2014). Water quality trends in New Zealand rivers: 1989–2009. *Environmental Monitoring and Assessment, 186*(3), 1939-1950. DOI:10.1007/s10661-013-3508-5

Bolduan, B. R., Van Eeckhout, G. C., Quade, H. W., & Gannon, J. E. (1994). Potamogeton crispus–The Other Invader. *Lake and Reservoir Management, 10*(2), 113-125. DOI:10.1080/07438149409354182

Bornette, G. & Puijalon, S. (2011). Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences, 73*(1), 1-14. DOI:10.1007/s00027-010-0162-7

Bowden, W. B., Glime, J. M., & Riis, T. (2017). Macrophytes and bryophytes. In *Methods in Stream Ecology, Volume 1* (pp. 243-271): Elsevier.

Baattrup-Pedersen, A., Gothe, E., Larsen, S. E., O'Hare, M., Birk, S., Riis, T., & Friberg, N. (2015). Plant trait characteristics vary with size and eutrophication in European lowland streams. *J Appl Ecol, 52*(6), 1617-1628. DOI:10.1111/1365-2664.12509

Baattrup-Pedersen, A., Gothe, E., Riis, T., & O'Hare, M. T. (2016). Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams. *Sci Total Environ, 543*(Pt A), 230-238. DOI:10.1016/j.scitotenv.2015.11.027

Caraco, N. F. & Cole, J. J. (2002). Contrasting Impacts of a Native and Alien Macrophyte on Dissolved Oxygen in a Large River. *Ecological Applications, 12*(5), 1496-1509. DOI:10.2307/3099987

Cavalli, G., Baattrup-Pedersen, A., & Riis, T. (2016). Nutrient availability and nutrient use efficiency in plants growing in the transition zone between land and water. *Plant Biol (Stuttg), 18*(2), 301-306. DOI:10.1111/plb.12397

Daehler, C. C. (2003). Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. *Annual Review of Ecology Evolution and Systematics, 34*(1), 183-211. DOI:10.1146/annurev.ecolsys.34.011802.132403

Davis, M. A., Grime, J. P., & Thompson, K. (2000). Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology, 88*(3), 528-534. DOI:https://doi.org/10.1046/j.1365-2745.2000.00473.x

Demars, B. O. L. & Edwards, A. C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. *Freshwater Biology, 52*(11), 2073-2086. DOI:10.1111/j.1365-2427.2007.01817.x

Duarte, C. M. (1990). Seagrass Nutrient Content. *Marine Ecology Progress Series, 67*(2), 201-207. DOI:10.3354/meps067201

Ellawala Kankanamge, C., Matheson, F. E., & Riis, T. (2019). Shading constrains the growth of invasive submerged macrophytes in streams. *Aquatic Botany, 158*. DOI:10.1016/j.aquabot.2019.06.004

Fareed, M. F., Haroon, A. M., & Rabeh, S. A. (2008). Antimicrobial activity of some macrophytes from Lake Manzalah (Egypt). *Pakistan journal of biological sciences : PJBS, 11*(21), 2454-2463. DOI:10.3923/pjbs.2008.2454.2463

Fleming, J. P. & Dibble, E. D. (2014). Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia, 746*(1), 23-37. DOI:10.1007/s10750-014-2026-y

Fogg, G. E. (1973). PHOSPHORUS IN PRIMARY AQUATIC PLANTS. In S. H. Jenkins & K. J. Ives (Eds.), *Phosphorus in Fresh Water and the Marine Environment* (pp. 77-91): Pergamon.

Gérard, J., Brion, N., & Triest, L. (2014). Effect of water column phosphorus reduction on competitive outcome and traits of Ludwigia grandiflora and L. peploides, invasive species in Europe. *Aquatic Invasions, 9*(2), 157-166. DOI:10.3391/ai.2014.9.2.04

Gerloff, G. C. & Krombholz, P. H. (1966). *Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants*. Retrieved from Department of Botany:

Grime, J. P. (1988). The C-S-R model of primary plant strategies — origins, implications and tests. In L. D. Gottlieb & S. K. Jain (Eds.), *Plant Evolutionary Biology* (pp. 371-393). Dordrecht: Springer Netherlands.

Gross, E. M. (2003). Allelopathy of Aquatic Autotrophs. *Critical Reviews in Plant Sciences, 22*(3-4), 313-339. DOI:10.1080/713610859

Gufu, G. D., Manea, A., Vorreiter, L., & Leishman, M. R. (2018). Do invasive exotic and native freshwater plant species respond similarly to low additional nitrate doses? *Aquatic Botany, 151*, 1-8. DOI:10.1016/j.aquabot.2018.07.007

Haroon, A. M. & Abdel-Aal, E. I. (2016). Chemical composition and in vitro anti-algal activity of Potamogeton crispus and Myriophyllum spicatum extracts. *The Egyptian Journal of Aquatic Research, 42*(4), 393-404. DOI:https://doi.org/10.1016/j.ejar.2016.12.002

Henriksen, L. D. R., Larsen, M. B., Johnsen, T. J., Larsen, S. E., Riis, T., & Baattrup-Pedersen, A. (2023). Root development is faster in common species of Potamogeton compared to declining species of Potamogeton in Danish lowland streams. *Aquatic Botany, 189*, 103702. DOI:https://doi.org/10.1016/j.aquabot.2023.103702

Kennedy, T. L., Horth, L. A., & Carr, D. E. (2009). The effects of nitrate loading on the invasive macrophyte Hydrilla verticillata and two common, native macrophytes in Florida. *Aquatic Botany, 91*(3), 253-256. DOI:10.1016/j.aquabot.2009.06.008

Maestre, F. T., Callaway, R. M., Valladares, F., & Lortie, C. J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology, 97*(2), 199-205. DOI:https://doi.org/10.1111/j.1365-2745.2008.01476.x

Manolaki, P., Mouridsen, M. B., Nielsen, E., Olesen, A., Jensen, S. M., Lauridsen, T. L., . . . Riis, T. (2020). A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms. *J Environ Manage, 274*, 111181. DOI:10.1016/j.jenvman.2020.111181

Mouton, T. L., Matheson, F. E., Stephenson, F., Champion, P. D., Wadhwa, S., Hamer, M. P., . . . Riis, T. (2019). Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape. *Sci Total Environ, 659*, 1370-1381. DOI:10.1016/j.scitotenv.2018.12.277

Nakai, S., Inoue, Y., Hosomi, M., & Murakami, A. (1999). Growth inhibition of blue–green algae by allelopathic effects of macrophytes. *Water Science and Technology, 39*(8), 47-53. DOI:https://doi.org/10.1016/S0273-1223(99)00185-7

O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lazar, A. N., . . . Bowes, M. J. (2018). Responses of Aquatic Plants to Eutrophication in Rivers: A Revised Conceptual Model. *Front Plant Sci, 9*, 451. DOI:10.3389/fpls.2018.00451

Olde Venterink, H. & Güsewell, S. (2010). Competitive interactions between two meadow grasses under nitrogen and phosphorus limitation. *Functional Ecology, 24*(4), 877-886. DOI:10.1111/j.1365-2435.2010.01692.x

Pulzatto, M. M., Cunha, E. R., Dainez-Filho, M. S., & Thomaz, S. M. (2019). Association Between the Success of an Invasive Macrophyte, Environmental Variables and Abundance of a Competing Native Macrophyte. *Front Plant Sci, 10*, 514. DOI:10.3389/fpls.2019.00514

Quinn, L. D., Schooler, S. S., & Van Klinken, R. D. (2011). Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions, 17*(1), 132-143. DOI:10.1111/j.1472-4642.2010.00726.x

R Core Team, R. (2022). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Redekop, P., Gross, E. M., Nuttens, A., Hofstra, D. E., Clayton, J. S., & Hussner, A. (2018). Hygraula nitens, the only native aquatic caterpillar in New Zealand, prefers feeding on an alien submerged plant. *Hydrobiologia, 812*, 13-25.

Riis, T. & Biggs, B. J. F. (2001). Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply - a synthesis and conceptual model. *New Zealand Journal of Marine and Freshwater Research, 35*(2), 255-267. DOI:10.1080/00288330.2001.9516996

Riis, T. & Biggs, B. J. F. (2003). Hydrologic and hydraulic control of macrophyte establishment and performance in streams. *Limnology and Oceanography, 48*(4), 1488-1497. DOI:10.4319/lo.2003.48.4.1488

Schultz, R. & Dibble, E. (2011). Effects of invasive macrophytes on freshwater fish and macroinvertebrate communities: the role of invasive plant traits. *Hydrobiologia, 684*(1), 1-14. DOI:10.1007/s10750-011-0978-8

Skovsholt, L. J., Riis, T., Matheson, F., & Hawes, I. (2023). Growth response to nitrate enrichment helps facilitate success of an alien Potamogeton in New Zealand streams. *Heliyon, 9*(4), e15528. DOI:https://doi.org/10.1016/j.heliyon.2023.e15528

Smart, R. M. & Barko, J. W. (1985). Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquatic Botany, 21*, 251-263.

Su, S., Shen, A., Tang, H., & Yao, W. (2001). Effects of temperature, light intensity and pH on photosynthesis in Potamogeton crispus L. *Journal of Southwest Agricultural University, 23*(6), 532-534.

Thiebaut, G., Thouvenot, L., & Rodriguez-Perez, H. (2018). Allelopathic Effect of the Invasive Ludwigia hexapetala on Growth of Three Macrophyte Species. *Front Plant Sci, 9*, 1835. DOI:10.3389/fpls.2018.01835

van Kleunen, M., Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecol Lett, 13*(2), 235-245. DOI:10.1111/j.1461-0248.2009.01418.x

Vilà, M. & Weiner, J. (2004). Are Invasive Plant Species Better Competitors Than Native Plant Species? - Evidence from Pair-Wise Experiments. *Oikos, 105*, 229-238.

Vorosmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., . . . Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature, 467*(7315), 555-561. DOI:10.1038/nature09440

Wu, Z. B., Zhang, S. H., Wu, X. H., Cheng, S. P., & He, F. (2007). Allelopathic interactions between Potamogelon maackianus and Microcystis aeruginosa. *Allelopathy Journal, 20*(2), 327-338.

Yang, C., Shen, X., Shi, X., Cui, Z., Nan, J., Lu, H., . . . Huang, Q. (2023). Impact of submerged macrophytes on growth and 2-MIB release risk of Pseudanabaena sp.: From field monitoringa to cultural experiments. *Journal of Hazardous Materials, 442*, 130052. DOI:https://doi.org/10.1016/j.jhazmat.2022.130052

Zhou, H., Ren, J., & Cai, X. (1997). The concept and measurement of diurnal light compensation point of submerged macrophyte. *Acta Scientiae Circumstantiae, 17*, 256-258.

Chapter IV

Trait-defined groups of aquatic macrophytes respond differently to eutrophication of unshaded lowland streams.

2.1 Abstract

Understanding eutrophication effects on stream macrophytes is key to meaningful environmental management and governance. However, the response of macrophyte communities to eutrophication is complex and often the effect of eutrophication is obscured by habitat variability.

In this study we quantified macrophyte community composition and abundance along a eutrophication gradient in 30 lowland streams. We selected slow flowing, medium sized streams with minimal shading, to focus on examining water quality effects. We measured the internal nutrient status of the plants and the bioavailable nutrient fraction of the sediment and used a national database of modelled stream descriptors to obtain information about land use and other water quality variables. We statistically examined relationships between macrophyte community composition and abundance and nutrient, underwater light, carbon availability and disturbance along the eutrophication gradient.

We found that macrophytes with different growth strategies were related to different aspects of eutrophication. An increased abundance of submerged species was associated with elevated water column phosphorus (P) and alkalinity, while increased abundance of emergent species was associated with high sediment P, nitrate (N) and low disturbance.

We did not find a relationship between nutrient inputs and nutrient content of the macrophytes but did find that sediment P correlated with P inputs to streams. We also observed that non-native taxa dominated eutrophic streams.

Our results indicate that 1) non-native species are common in unshaded lowland streams with low water clarity and high nutrient and bicarbonate availability; 2) emergent species are associated with high nitrogen availability and suspended sediment and 3) submerged species prevail where phosphorus and bicarbonate availability is high. Our findings improve understanding of how stream macrophyte communities respond to changes in water quality related to eutrophication and anthropogenic changes in catchments and will contribute to an informed management of macrophytes in streams.

4.2 Introduction

Macrophytes are important ecological components in many stream ecosystems, as they modify hydrology, channel morphology and element cycling and provide food, shelter and substrate for aquatic life (Carpenter & Lodge, 1986; Chambers et al., 2008; Collier et al., 1999; Dibble et al., 1996; Quirino et al., 2019). In turn, their growth in streams is influenced by resource availability, particularly light, inorganic carbon and nutrients (Bornette & Puijalon, 2011) and flow regime (Riis & Biggs, 2003). Where stream conditions are otherwise suitable, an increased availability of nutrients (i.e. eutrophication) can support proliferation of macrophytes (Mebane et al., 2014). As a result, macrophytes have the potential to accrue large amounts of biomass, which can clog streams, create an aesthetic or recreational nuisance, and impair stream ecological functioning by affecting stream dissolved oxygen and pH (Matheson et al., 2012). Furthermore, as fast-growing species benefit most from surplus nutrient loads, to an extent where competitive exclusion of some species can occur, lowering species richness (Flores-Moreno et al., 2016; Quinn et al., 2011). Consequently, there is considerable interest in how the factors that regulate macrophyte growth and biomass accrual might be managed to avoid the development of problematic proliferations.

Chiefly, eutrophication research focus on phosphorus (P) enrichment as the primary inducer of community changes (Hilton et al., 2006; O'hare et al., 2018). Nevertheless, other field studies have found nitrogen (N) to have a greater effect than P on macrophyte biomass, that both nutrients have a degree of control, or that neither restricts biomass (Demars & Harper, 2005; Feijoo & Lombardo, 2007; Giorgi et al., 2005; Kennedy et al., 2015; Sosiak, 2002). Reconciling this conundrum has led to the suggestion that habitat variability can obscure eutrophication effects as this exerts a higher order control on community structure, and thus effects should be studied within defined habitat templates (O'hare et al., 2018). Research into nutrient affinities and use efficiencies between macrophyte types suggest differences in responses to increases in N and P within plants with different growth strategies (Cavalli et al., 2016; Manolaki et al., 2020). Furthermore, the dominant macrophyte type in eutrophic streams has been shown to depend on carbon availability (Riis et al., 2000; Sand-Jensen et al., 2022). Thus, there is still a need to expand the current eutrophication framework and reconcile it with other factors that determine macrophyte response.

Trait community composition analyses have previously been used to untangle the effects of nutrient from other confounding factors, such as management by weed-cutting, hydro-morphological alterations, and riparian shading (Baattrup-Pedersen et al., 2016; Mouton et al., 2019). These studies show prevalence of submerged macrophytes with traits related to good light interception or light utilisation in communities of eutrophic streams (Baattrup-Pedersen et al., 2015; Baattrup-Pedersen et al., 2016; Mouton et al., 2019). However, the design of those studies may have obscured nuances in the results because of co-variation between stressors. As such, eutrophic streams are often in parts of a

catchment where forest vegetation has been removed and are thus unshaded and hydro-morphologically altered. Trait community analysis exclusively on a gradient of eutrophication has still not been attempted although this may be useful in the untangling of drivers affecting macrophyte communities in stream where problematic proliferations are likely to occur.

To better understand eutrophication effects on stream macrophyte communities, we therefore investigated interrelationships amongst key environmental drivers, species, and trait composition, in susceptible streams on a eutrophication gradient. Using available spatial data and a GIS-based pre-selection procedure we identified streams that were suitable for macrophyte growth and similar in terms of medium size, low gradient slope, minimal shading but differed with respect to nutrient input as predicted by catchment characteristics and land use.

Our specific aims were to explore the expectation that, within unshaded, slow-flowing, lowland streams, eutrophication resulted in an increase in macrophyte biomass (clogginess), selection for species with high-performance-related traits and a corresponding reduction in species diversity. As successful non-native and invasive species typically have higher values of performance-related traits than native species (Van Kleunen et al., 2010), we expected the most impacted streams to be dominated by non-native species (Mouton et al., 2019). We also expected that internal tissue N and P content would increase with eutrophication, and that submerged species would have a higher content than emergent species, but emergent species would respond more strongly to N. sation

4.3 Methods

To investigate the effects of eutrophication on stream macrophyte communities we surveyed 30 stream reaches, in the Waikato and Bay of Plenty regions of New Zealand (Figure 4.1). For each reach, we collected samples to determine the nutrient status of plants, water, and sediment, measured stream shade, channel dimensions and flow attributes, compiled available stream descriptor data from databases, and correlated environmental variables to the macrophyte abundance, nutrient status, community composition, and community trait composition.

Site selection

The 30 sites were selected using a GIS, with spatial layers derived from a national modelled descriptor dataset of stream sub-catchments and reaches, which included channel slope, riparian shading, stream discharge and nitrate-N concentrations (Whitehead, 2018). We aimed for stream reaches that in all aspects other than nitrate-N had optimal conditions for macrophyte growth, to strengthen our confidence in the responses being due to variability in the level of eutrophication. Streams were thus selected with the following criteria: low channel slope (< 2 %); minimal shading (<

25 %); and medium size (a discharge of approximately 1 m^{-3} s^{-1}). From this list, we then selected streams to cover a range of predicted nitrate-N concentrations from 0.28 to 11.17 mg L^{-1}. Selected streams were visually assessed before the survey, to check the reliability of the spatial predictions and to exclude any sites that were too deep for wading, had torrential flow, or exclusively rocky substrate.

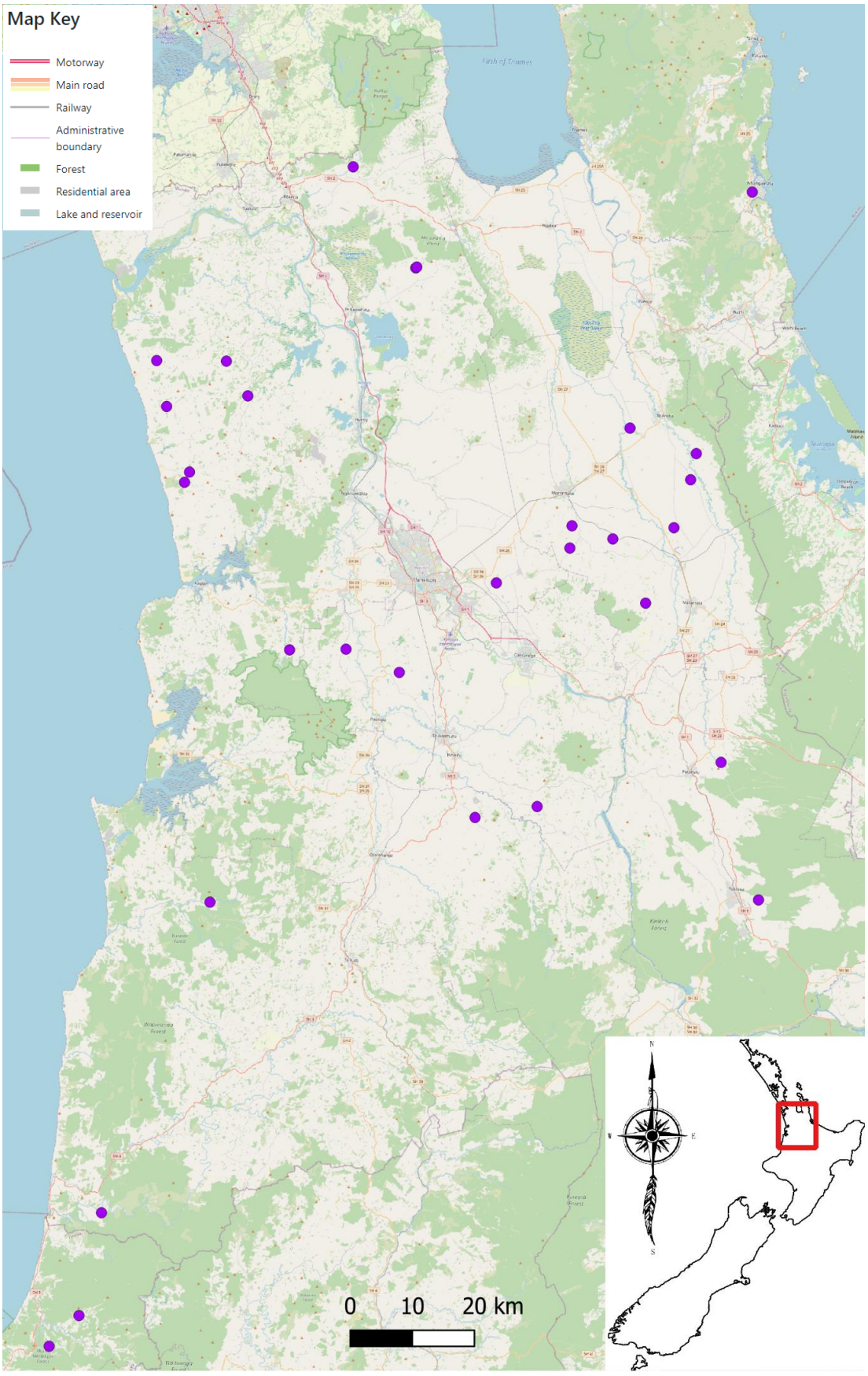
4.3.1 Field procedure

4.3.1.1 Physical characterisation

At each stream a reach of 100 m was targeted, however some reaches were shortened to avoid shade from riparian vegetation (60 m minimum). Five transects were marked out at approximately equidistant intervals within each reach. At each transect, the stream wetted width was measured and

five points across the stream were marked at 10%, 30%, 50%, 70% and 90% of the wetted width. At each point, water depth was measured, and the dominant substrate type was categorically assessed within the area of a 0.25 m^2 square quadrat as clay, mud (black, <0.25 mm^2), sand (0.25 – 3 mm^2), fine gravel (3 – 5 mm^2), coarse gravel (5 – 15 mm^2), small cobbles (15 – 30 mm^2), large cobbles (30 – 60 mm^2), stones (60 – 200 mm^2), rock (> 200 mm^2). Water velocity in the reach was estimated using a salt slug release; 5 L of stream water was mixed with 0.5 kg of table salt and poured into the centre of the channel at the upstream end of the reach while an AquaTROLL 600 (In-Situ, USA) or EXO2 (YSI, USA) multiparameter sonde continuously measured conductivity at the downstream point.

Figure 4-1: *Map of the study area and its location within Aotearoa New Zealand. Purple dots mark the 30 study reaches. Most sites are located within the Waikato region, save for the two southernmost sites which are situated in the Taranaki region. Background map from openstreetmap.org.*



4.3.1.2 Reach physicochemical characteristics.

At each transect point, a 5 cm deep sediment core sample was taken with a cut-off syringe (⌀ = 2cm), for analysis of phosphorus (P) content, unless substrate could not be penetrated at that point (2% of sample points). Oxygen concentration and saturation, pH and temperature were measured by the multiparameter sonde. At the upstream point of the reach, three water samples were taken in 300 mL acid-cleaned plastic bottles in the centre of the channel for determination of nitrate-N + nitrite-N (NO_3^--N), ammonium-N (NH_4^+-N) and dissolved reactive-P (DRP), total-N, total-P and total suspended sediment (TSS) in the laboratory, and a single water sample was taken in a brown opaque glass bottle (>300 mL) for determination of alkalinity.

4.3.1.3 Light environment.

Light availability at the stream water surface was characterized using two methods: 1) estimation of diffuse non-interceptance (DIFN) and 2) shade estimation using a clinometer. DIFN was estimated by measuring diffuse incoming irradiation at each transect point at the stream surface (LAI-2000 plant canopy analyser, LI-COR Biosciences, USA) and subsequently correcting for the ambient irradiance measured by a reference light logger that was set up in an unshaded location near the reach and set to log for the duration of the in-stream procedure (Rutherford et al., 1997). Shading was estimated by clinometer from the centre of the stream channel (50% transect point) and at water level by measuring angles to the top of the surrounding canopy and topography in 8 azimuthal directions with 45° intervals and estimating percentage gap fractions between the two angles. Shading (%) was then calculated based on these measurements and an average value determined for each survey reach (Davies-Colley & Payne, 1998). The two methods differed in that the DIFN included light attenuation from emergent macrophytes present at transect points close to the stream margin while shading measured by clinometer in the centre of the channel was not, although taking measurements in the channel centre neglects any spatial variation in shade across the channel. Shading is often higher close to the channel margins than in the centre. Nevertheless, we considered that shade measured by clinometer likely gave a more precise estimate of the light environment for all macrophytes present in the reach, while DIFN provided an estimate of how much light is reaching the water surface.

4.3.1.4 Aquatic vegetation.

Within a 0.25 m^2 square quadrat at each transect point, the percentage cover of the stream bed and percentage occupancy in the water column ("clogginess") by each macrophyte species was estimated. The maximum height of each species in the water column was measured using a weighted measuring tape. For plants that emerged above the water surface, the height and cover of each species above the water surface were also determined. Additionally, the top 10 cm (occasionally more to ensure presence of leaves) of three individuals of the dominant species in each reach were collected for subsequent laboratory analysis to determine internal N and P content (see below). If a species could not be identified in the field, it was taken back to the laboratory for further taxonomic evaluation.

4.3.2 Laboratory procedure.

Water samples were analysed for NO_3^--N, NH_4^+-N, and DRP on a flow injection analyser (FIA; Lachat Quikchem, USA - detection limit 1 µg L^{-1}). Total nitrogen (TN) was also analysed by FIA, as nitrite-nitrogen after persulphate digestion and cadmium reduction (detection limit 10 µg L^{-1}). Total phosphorus (TP) was estimated as DRP following potassium persulfate digestion (detection limit 1 µg L^{-1}). Suspended solids (SS) were determined gravimetrically after filtration onto pre-weighed Whatman GF/C filters and drying at 104 °C (2540D in Apha, 2017).

Alkalinity was determined by Gran titration (Apha, 2017). 0.2 N HCl was slowly added to 100 ml of sample and the volume of acid needed to reach pH of 4.5, 4.3, and 4.2 was recorded.

For analysis of N and P content in macrophyte samples, plant material was dried at 60 °C for at least 48 hours, before grinding to a fine powder in a hammer mill. Plant samples for each species in a reach were pooled due to low amounts of dry biomass. The sample material was transferred to 50 mL polypropylene test tubes, an alkaline potassium persulphate solution was added along with purified water, and samples were autoclaved for 1 h at 121 °C (Patton & Kryskalla, 2003). This method allows both P and N to be measured from the same digest (Ebina et al., 1983). Standards were created from glycine (N) and glycerophosphate (P) to check digestion efficacy (Patton & Kryskalla, 2003). N and P contents of the samples were then determined as nitrate and phosphate on a microplate reader (BMG Labtech, Germany) following the vanadium reduction method (Schnetger & Lehners, 2014) and a molybdenum blue colorimetric method (Hansen & Koroleff, 1999), respectively.

Sediment P was determined following a modified procedure from Lukkari et al. (2007). As most of the P fraction in sediments is inaccessible to plants and therefore of only minor relevance for plant - sediment correlations (Mebane et al., 2014), only the bioavailable fraction of the sediment P was estimated. 2 g sediment (fresh weight) was incubated by shaking in a sodium dithionite – sodium bicarbonate solution for 1 hour, with two 15 min washing steps. The samples were spun after each step and the supernatants were pooled. The samples were analysed as above on a microplate reader following the molybdenum blue colorimetric method (Hansen & Koroleff, 1999).

4.3.3 Data analysis.

4.3.3.1 Reach physical and physicochemical parameters.

Mean water travel time (in seconds) was taken as half the time it took all the salt to pass through the reach (return to background conductivity levels) (Lamberti & Hauer, 2017). This was then used in combination with the average cross section measured in the transects (m^2) and reach length (m) to calculate a mean discharge for the reach (m^3/s). Oxygen concentration and saturation, pH and

temperature were taken as the average value of all readings taken by the multiparameter sonde. Alkalinity was calculated based on the endpoints of the laboratory titration (Apha, 2017).

4.3.3.2 Predicted physicochemical descriptors from national models.

Recognising that lowland streams are subject to substantial temporal variation in physicochemical attributes, we supplemented spot measurements made at the time of macrophyte sampling with modelled attributes extracted from the NZRiverMaps tool. We also extracted catchment property data for each reach from the freshwater ecosystems of New Zealand (FENZ) database (Gay, 2013). Water physiochemistry modelled data is created from random forest models that uses climatic, geological, topographic, land cover, and hydrological conditions as predictors and water sample results from national monitoring in the period 2016-2020 as 'training' data. The monitoring data originates from 715–973 sites (parameter dependent), of which the Waikato region in which we sample is well represented (Whitehead et al., 2022a). The most important predictors for the modelled nutrient, water clarity and turbidity attributes are stock density, elevation, variability in rainfall and urban landcover in the catchment (Whitehead et al., 2022a). The selected attributes and individual descriptions are described in Table 4.1.

Alkalinity was missing for 8 sites, so we filled the data gaps with model estimates from a linear regression model fitted to the measured alkalinity data. The model predicted alkalinity from the calcium and phosphorus content, and soil hardness of the catchment (Gay, 2013) and was tested significant by linear modelling ($F = 10.45$, p-value = 0.0003) with an R^2 of 0.64.

4.3.3.3 Macrophyte community

Individual species clogginess (estimate of the percent of the water column occupied by plant biomass) was averaged first by transect, then for the entire reach.

To characterise diversity of the macrophyte community we estimated Shannon's diversity index and Pielou's evenness (Ricotta, 2018). For each reach, with a total number of species R with relative abundances p_i, Shannon's diversity was estimated as $H' = -\sum_{i=1}^{R} p_i \ln p_i$, and Pielou's evenness as $J' = H' \ln R^{-1}$ (Pielou, 1966). Relative abundance p_i was taken as the clogginess of species i.

A range of plant traits, growth types, and geographic origins were selected based on previous investigations of relations between traits and environmental parameters (Mouton et al., 2019). This was done for easier comparison and as we expected to discover most of the species encountered by Mouton et al. (2019). The chosen traits cover ecological preference, morphology, dispersal, and colonisation (Table 2). Trait scores for each species were either obtained from Mouton et al. (2019), literature therein, or assigned by expert opinion. In addition, each species was assigned to one of four guilds/lifeforms: emergent, floating, submerged, and terrestrial (Table 1). Bryophytes and filamentous

green algae were excluded from the analysis as we could not confidently assign trait scores to these groups.

4.3.3.4 Statistical approach

All statistical analyses were carried out in the R environment ver. 4.2.2 (R Core Team, 2022).

***Table 4.1**: Summary of the traits used in the analysis.*

Variable (unit where applicable)	Source
Frequency of events 3xMean (FRE3) The average number of events per year that exceed three times the median flow (events/year). Calculated from mean daily flows with no windows applied to account for peaks that occur in quick succession. Provides an estimate of flow flashiness. Lower values mean less frequent events.	Booker (2013)
Mean annual low flow (MALF) The mean of the annual low flow series after having applied a 7-day running average (m^3 s^{-1}). Lower values mean less flow.	Booker and Woods (2014)
Nitrate + Nitrite (Nitrate.Nitrite) Nitrate + nitrite nitrogen (mg/L) as measured using Nitrate-N in filtered samples, ion chromatography, or nitrate-N + nitrite-N (NNN) in filtered samples, cadmium reduction or nitrate-N + nitrite-N - nitrite-N in filtered samples, azo dye colorimetry or optical sensor. Estimate of the median from many samples. Lower values mean less nutrient.	Whitehead et al. (2022)
Ammoniacal nitrogen (NH4) Ammonium nitrogen (mg/L) as measured using filtered samples, phenol/hypochlorite colorimetry and adjusted to account for the pH at the time of sampling. Estimate of the median from many samples. Lower values mean less nutrient.	Whitehead et al. (2022)
Total Nitrogen (TN) Total nitrogen (mg/L) as measured using unfiltered samples, persulphate digestion to nitrate, nitrate-N by cadmium reduction. Estimate of the median from many samples. Lower values mean less nutrient.	Whitehead et al. (2022)
Total Phosphorus (TP) Total phosphorus (mg/L) as measured using unfiltered samples, persulphate digestion to orthophosphate, molybdenum blue colorimetry. Estimate of the median from many samples. Lower values mean less nutrient.	Whitehead et al. (2022)
Dissolved reactive phosphorus (DRP) Dissolved reactive phosphorus (mg/L) as measured using filtered samples, molybdenum blue colorimetry. Estimate of the median from many samples. Lower values mean less nutrient.	Whitehead et al. (2022)
Total Suspended Solids (TSS) Total suspended solids (mg/l). Estimate of the median from many samples. Lower values mean less sediment.	Unwin and Larned (2013)
Visual Clarity (Clarity) Visual clarity using the black disc method (m). The sighting distance of a black disk placed underwater using a periscope-type viewer. Estimate of the median from many samples. Higher values indicate clearer water.	Whitehead et al. (2022)

We investigated relationships between measured and predicted stream physical and chemical parameters using simple linear regression models.

To analyse relationships between environment and plant communities, RLQ analysis (Dray et al., 2014) was used, using the *ade4* package (Dray & Dufour, 2007). RLQ is a multivariate technique that utilizes a three-step approach in which a matrix of environmental characteristics of a set of sites (R) is related to a matrix of plant traits for a set of species (Q) via a matrix of these species abundances at these sites (L). A correspondence analysis (CA) was performed on the L matrix and subsequently, the row and column scores are used as weights in principal component analyses on matrices R and Q, respectively. All ordinations are then combined in a RLQ analysis which "finds linear combinations of environmental variables (i.e., environmental gradient) and of traits (i.e., trait syndrome) such that their squared cross-covariance is maximum" (Dray et al., 2014). The significance of the results was tested by Monte Carlo test with 49999 permutations.

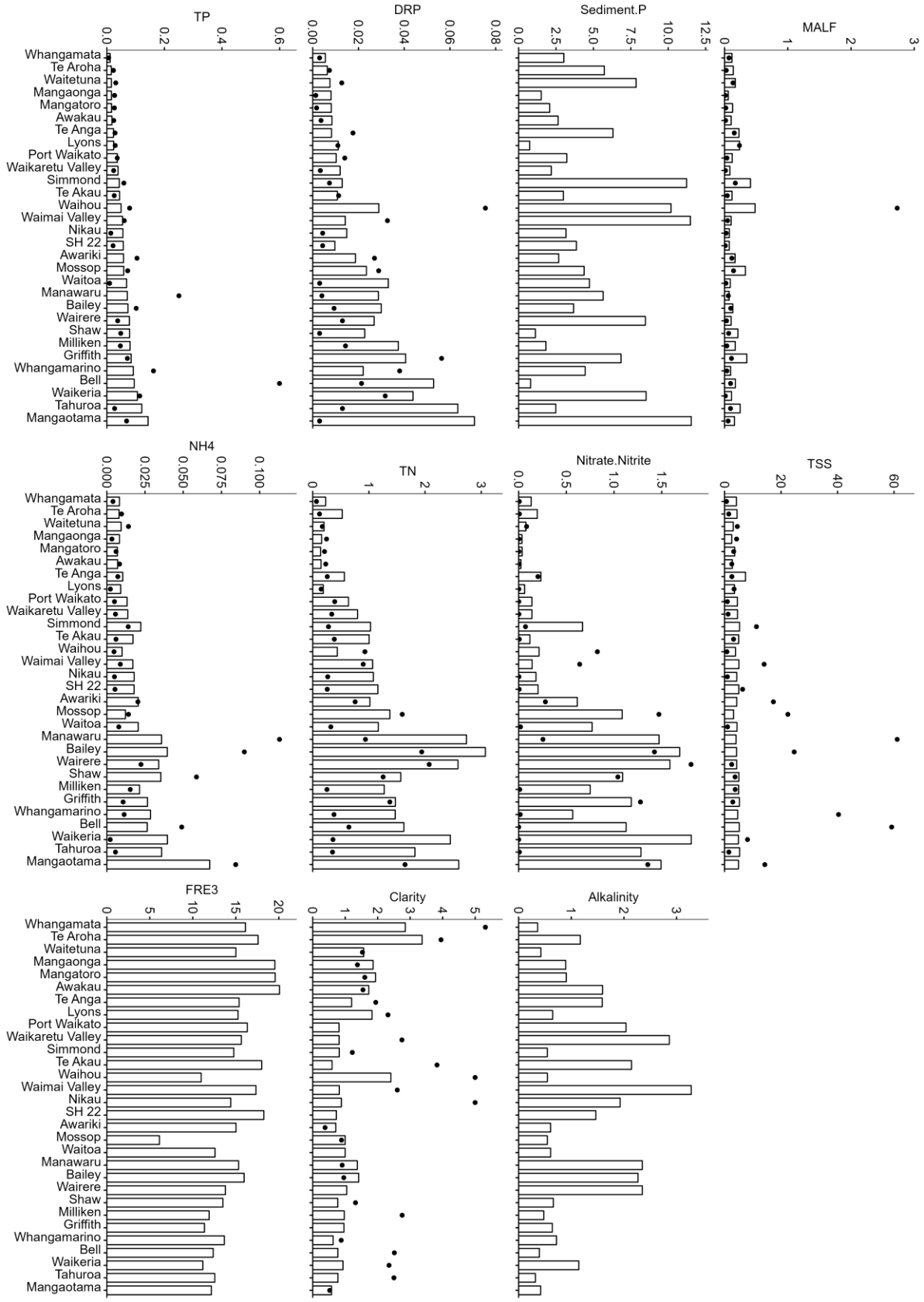
We used predicted physicochemical data in the RLQ analysis, as spot samples correlated rather poorly with these based on linear modelling results (Figure A.4.1), and, of the two, predicted values were considered likely to provide a better indication of the contaminant loading to a stream over time. Prior to RLQ analysis we analysed variables for redundancy using variance inflation factors (VIFs) with *vifstep* function from the R package *usdm* (Naimi et al., 2014). Variables were analysed for co-correlation, and each assigned an inflation score and removed until no variable had an inflation score higher than 10. The variables that were included in the RLQ analysis were: FRE3, MALF, DRP, Nitrate.Nitrite, NH4, Clarity, Alkalinity, and Sediment P.

We performed a hierarchical cluster analysis to analyse groupings of species based on the RLQ results (Mouton et al., 2019). We created a dissimilarity matrix by computing Euclidian distances between first and second axis scores of the species. Then, we calculated 5 groups by creating a dendrogram with unweighted pair group method with an arithmetic mean (UPGMA) and cutting the dendrogram tree at an arbitrary Euclidian distance of 2. We used a combination of base R and the "factoextra" package (Alboukadel & Fabian, 2020).

Table 4.2: *Biological response variables acquired from 30 New Zealand lowland streams.*

Variable	description
H	Shannon's diversity
J	Pielou's evenness
mean.tissue.N	Mean N content of individuals of the dominant species (%)
mean.tissue.P	Mean N content of individuals of the dominant species (%)
tissue.N.P	Ratio of tissue N and P

Figure 4-2: *Environmental parameter scores from each site, ordered by increasing TP. White bars represent the modelled data and sediment samples (Units in table 1 except Average sediment P: µg mg FW^{-1}). Black dots represent the measured values of the given parameter.*



To visualize how diversity, evenness and tissue N and P were correlated with macrophyte communities and environmental drivers, we created contour maps based on site specific averages of the variables in the RLQ space. Contour maps show the z axis score (e.g. diversity) with interpolations on a grid of x and y values, here created by the site scores on the first and second axis of the RLQ, by colour intensity. This way, the contour plots can be overlayed on the RLQ result plots and patterns can be assessed. To visualize correlations with catchment land use cover, the same was done for land cover of intensive agriculture, native forest, exotic forest, and urban areas, extracted the FENZ database (Gay, 2013).

4.4 Results

4.4.1 Stream habitats and macrophytes

The 30 chosen stream reaches were characterized by a mean (±SD) shading of 13 % (±5) with a mean DIFN of 0.26 (±0.17). Mean width was 4.0 m (± 2.1) while mean depth was 53 cm (± 20). Mean discharge was 0.16 m^3 s^{-1} (± 0.49) with a mean water velocity of 0.05 m s^{-1} (± 0.05). The streams varied in TP from 0.01 mg l^{-1} to 0.14 mg l^{-1} (Figure 4.2). Reaches with high TP were characterised by high sediment concentrations and high N and DRP, and lower visual clarity (Figure 4.2).

A total of 71 macrophyte species were identified in the field sampling campaign, varying in occurrence from being present at a single reach (occurrence = 0.03) to 20 reaches (occurrence = 0.67; Table 4.4). The species pool was dominated by non-native species (61 %) and comprised 32 % emergent, 32 % terrestrial species, 27% submerged, 7% floating, and <2% unknown.

RLQ and cluster analysis

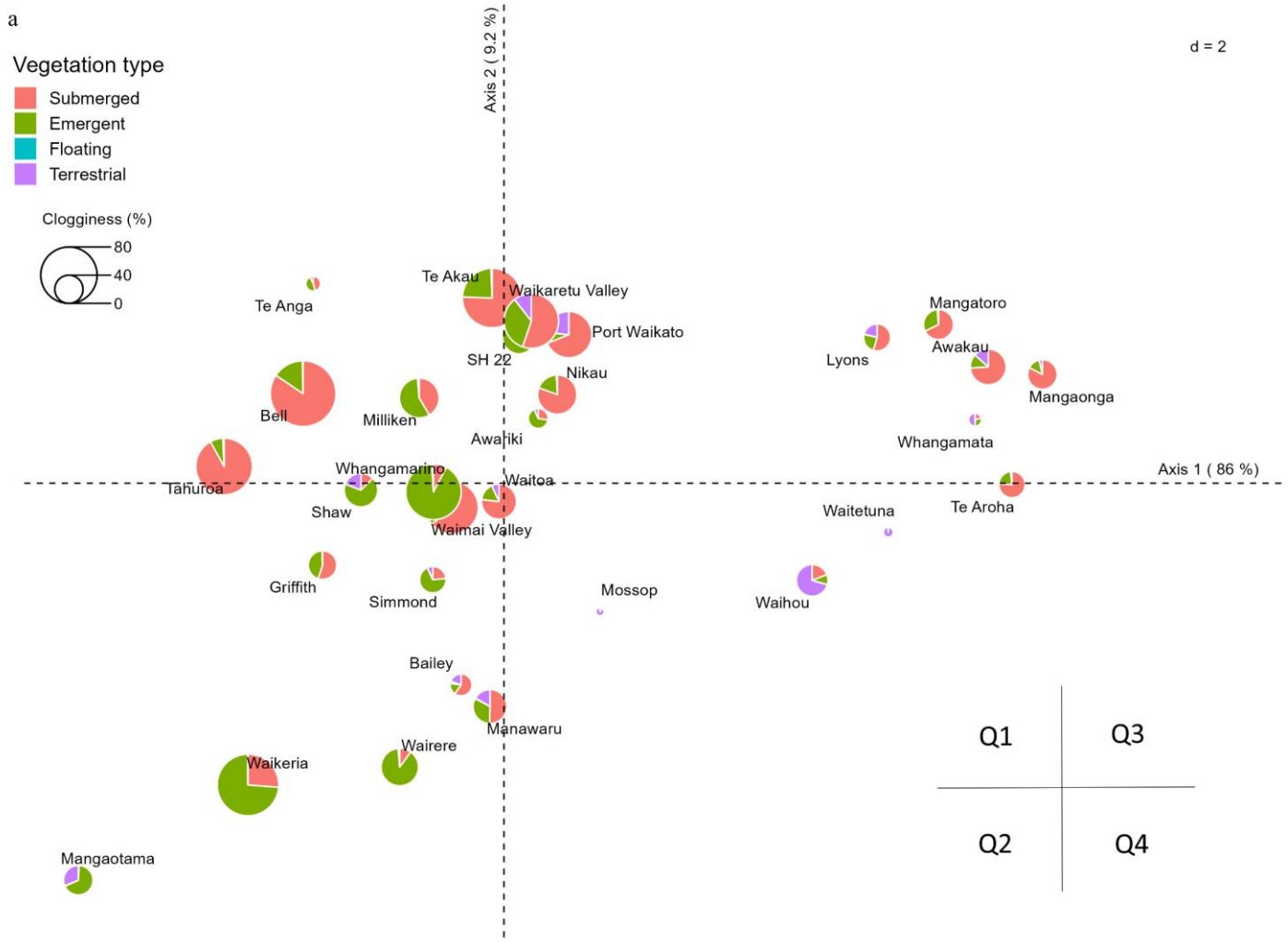
The RLQ analysis resulted in 86 % and 9.2 % variance explained on the first and second axis, respectively (Table 4.5). Our RLQ results were significant as tested by Monte Carlo permutation test: p-value < 0.05.

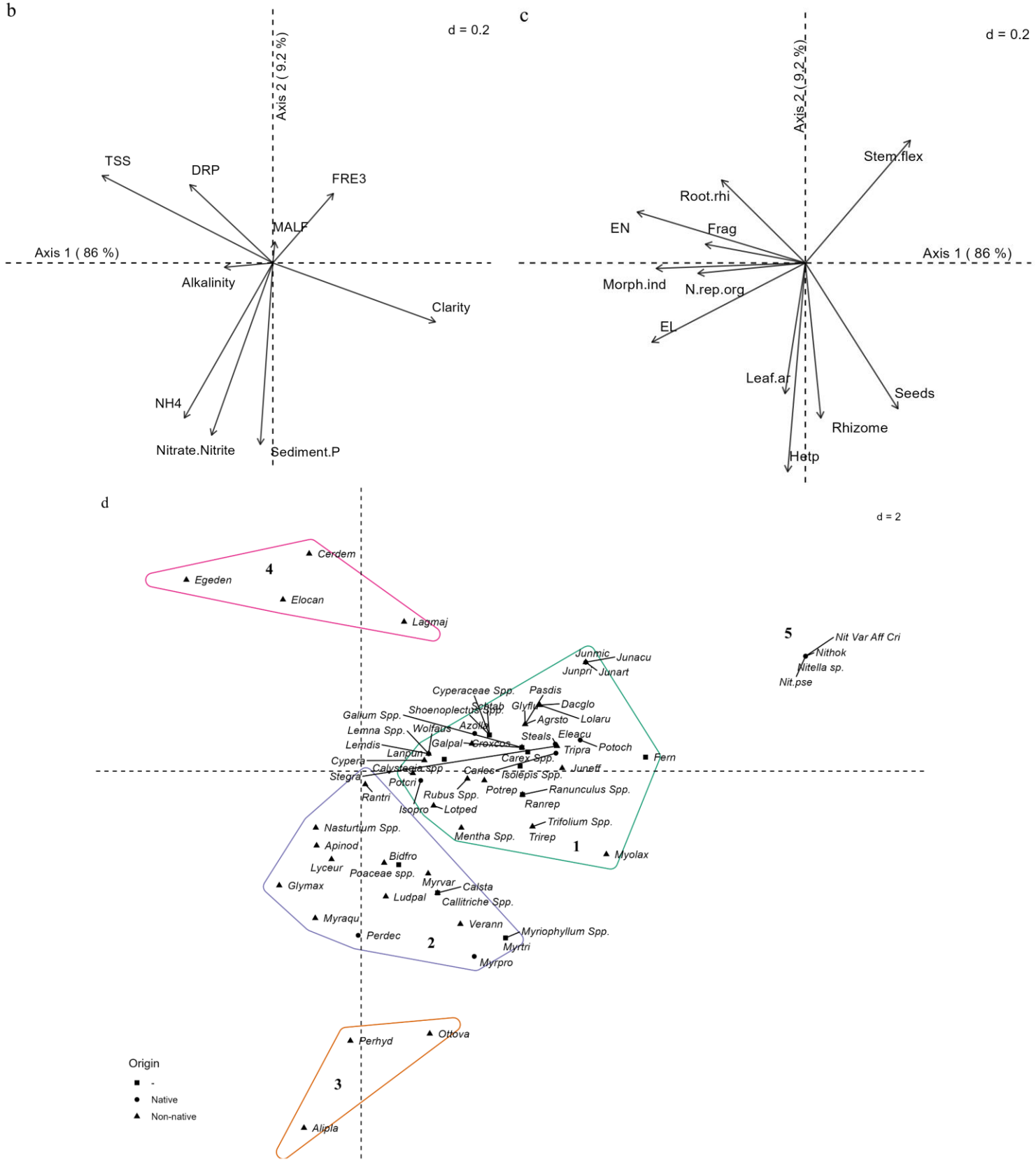
Environmentally, the first RLQ axis was associated with levels of nutrients, particularly phosphate, suspended solids and alkalinity, all increasing towards Q1 and Q2, while low nutrients, high flood frequency and high water clarity tended to be associated with Q3 and Q4. This tends to suggest a eutrophication gradient increasing from right to left in the RLQ plots. Biologically, sites towards the eutrophic end of Axis 1 tended to have highest clogginess (Figure 4.3a, b). The species of plants found at sites towards the eutrophic end were large, effective dispersers, with high affinity for light and nutrients and were predominantly of non-native origin (Figure 4.3c, d). Plants at sites with less degraded water quality were a mixture of species that were flexible and dispersing by seeds, and in

the least degraded sites we saw occurrences of native *Nitella* species (Figure 4.3). RLQ results also showed a variation in vegetation type along Axis 2: sites with primarily submerged vegetation were present in Q1 and Q3, corresponding to high DRP and suspended solid levels, as well as more disturbance, while sites with primarily emergent vegetation (Q2) correlated with high N and sediment P levels (Figure 4.3a, b). This was also evident in trait responses, where stem flexibility was higher for the submerged species, while emergent species were large leaved, had heterophylly, and reproduced by rhizome (Figure 4.3c).

Hierarchical clustering (dendrogram in supp inf) showed five groupings of species: (1) A broad mix of species, including many terrestrial species and sedges occupied the centre of Q3 and Q4; (2) Large leaved, emergent species, in Q2 and Q4, with *Glyceria maxima* at the most eutrophic part and

Figure 4-3*: Visual output of RLQ analysis of environment-distribution-trait relationships for aquatic vegetation in 30 New Zealand lowland streams. Relationships with Axis 1 and 2 of: (a) Sites in which pie-size denotes total clogginess and colours proportional occurrence of each of the four vegetation classes, (b) Environmental variables, (c) Plant traits, (d) Species distribution in the RLQ space, with origins, circled by group belongings (1-5) as determined by hierarchical clustering - note that the Nitella species constitute a group.*





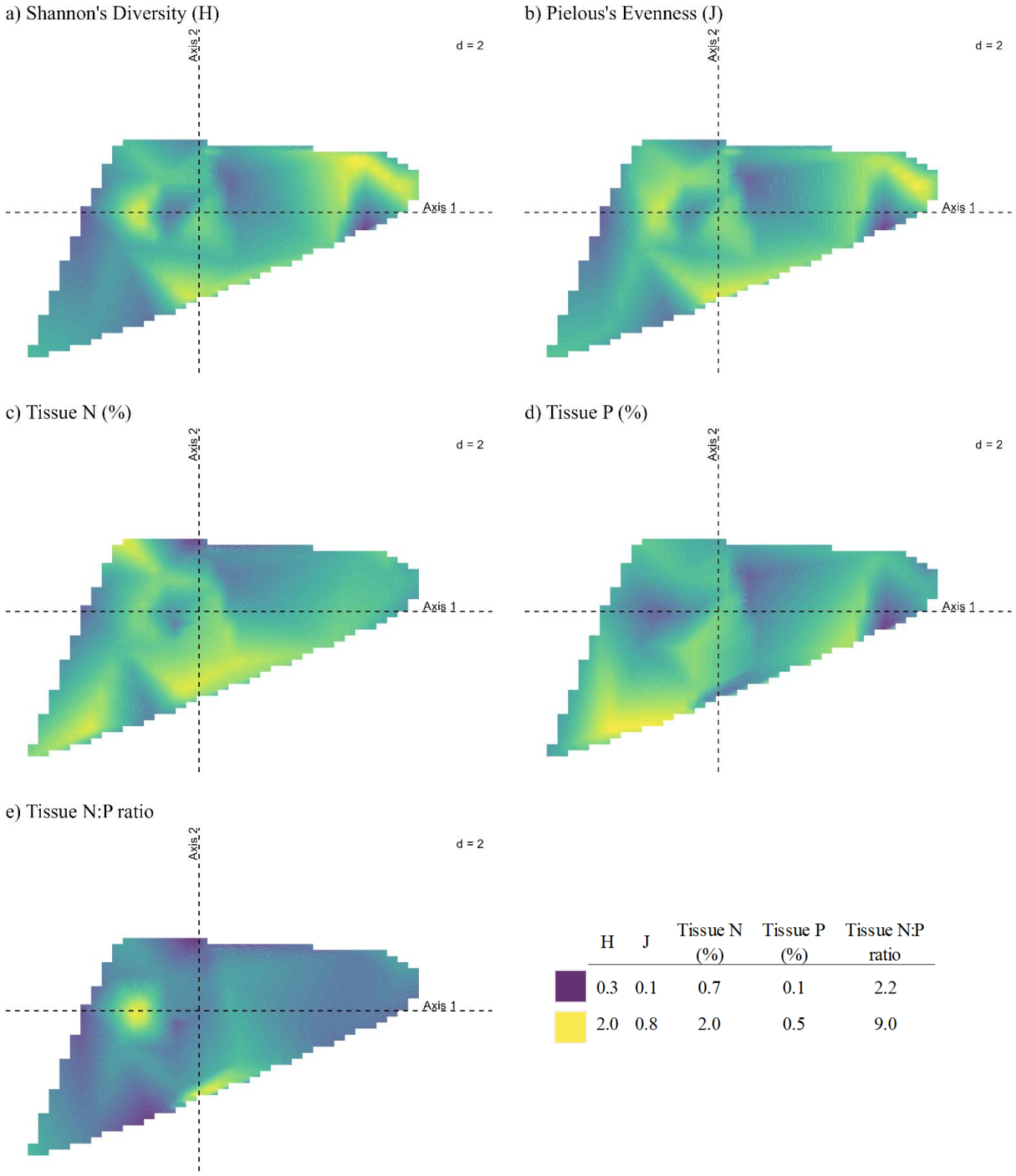
Myriophyllum trifolium, and *M. propinquum* at the least; (3) Emergent species that correlated most with N and Sediment P were *Alisma plantago-aquatica*, *Persicaria hydropiper*, and *Ottelia ovalifolia*; (4) The submerged "oxygen-weeds" *Egeria densa, Lagarosiphon major* and *Elodea canadensis,* together with *Ceratophyllum demersum* that mainly disperse by fragmentation, were dominant on

sites with high phosphorus and to a degree, high alkalinity; and, (5) the native *Nitella* species, with generally low trait scores, but with a high stem flexibility, occurring in clear, least disturbed waters.

Contour plots showed that diversity (median = 1.4) and evenness (median = 0.6) of communities were often highest when clogginess was low (Q3, Q4), but otherwise did not show a clear pattern, although the highest diversity score did occur at low impacted sites in Q3 (Figure 4.4a, b). Average tissue N content had no obvious pattern and was generally low (median = 1.5 %) with highest values in Q1, Q2, and Q4 (Figure 4.4c). It was, however, always low at less impacted sites (Q3). Average tissue P content (median = 0.3) did not follow the pattern for tissue N, rather, tissue P was highest in but a few sites in Q2 (Figure 4d). Tissue N:P ratio (median = 4.5) was high at select sites where tissue P was low (Figure 4.4e).

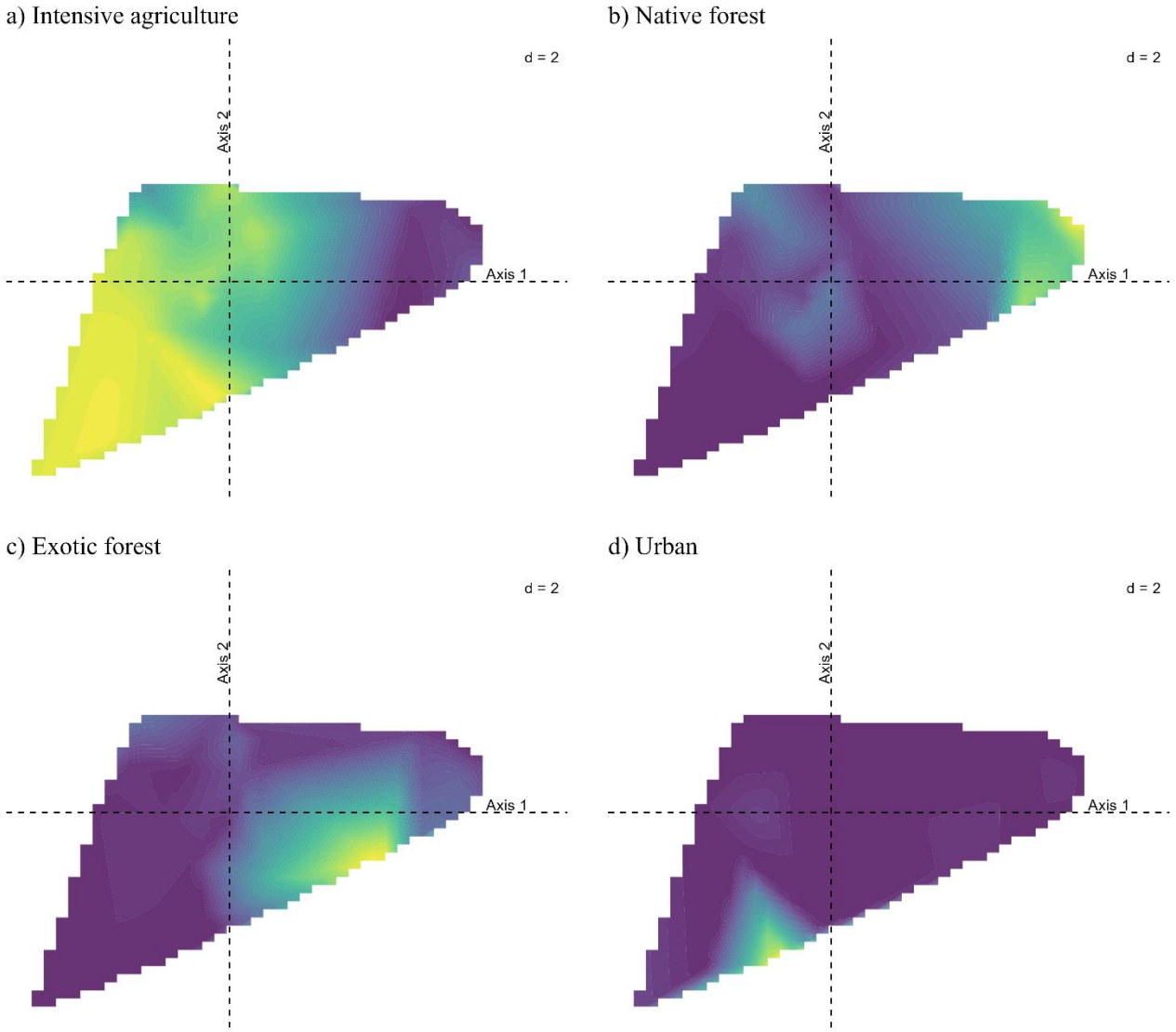
Land use also showed obvious patterns corresponding to environmental variables (Figure 4.3b) with agriculture and urban areas favoured in Q1 and Q2, and forestry in Q3 and Q4, although with little overlap of native and exotic forest (Figure 4.5).

***Figure 4-4**: Spatial distribution of biological response parameters in the RLQ space based on site specific values shown by contour mapping. Minimum and maximum values are shown in the lower right table and corresponds to the colour extremes.*



	H	J	Tissue N (%)	Tissue P (%)	Tissue N:P ratio
	0.3	0.1	0.7	0.1	2.2
	2.0	0.8	2.0	0.5	9.0

Figure 4-5: *Spatial distribution of catchment land use in the RLQ space based on site specific values shown by contour mapping. Minimum and maximum values are shown by the colour extremes, blue = low, yellow = high.*



4.5 Discussion

In this chapter we investigated the effect of eutrophication and hydrology on the macrophyte compartment of 30 minimally shaded lowland streams in Aotearoa New Zealand on a eutrophication gradient. We found that eutrophic sites were characterized by increased clogginess and dominance of larger, more productive non-native species. We also found that submerged species were associated with sites high in water column P while emergent species associated with those with high water column N and sediment P. Vegetation-environment interactions, even in similar streams with few limitations on growth factors, remain complex, however, and community dynamics are most likely a product of multiple interacting factors.

4.5.1 Eutrophication gradient

We argue here that we have successfully found streams located on a eutrophication gradient. The first RLQ axis, which explained most (86%) of the variation, showed a clear gradient of the stressors typically associated with eutrophication from increased human catchment impacts (i.e. P, suspended solids, and low clarity). There was also a clear distinction between different land use types, that separated along the first RLQ axis, indicative of human influence especially in Q1 and Q2, with activities consistent with the eutrophication effects discussed below. We selected the streams based on estimates of in-stream NO_3^- concentrations from the CLUES model (Leathwick et al., 2008), which has land use as one of the main predicting factors, and so the correlations with land use was to be expected. However, the Random Forest-derived stream chemistry model that was ultimately used to derive the variables used in the RLQ analysis uses a wider range of catchment, geological, climate and land use predictors, and is trained and validated using a large number of in-stream concentrations (Whitehead et al., 2022a). We consider the gradients indicated by the RLQ, supported by the consistent biological response in terms of clogginess and species composition, indicates that while the apparent relationships need to be interpreted with care, they can be interpreted.

4.5.2 Clogginess/abundance

Within the above caveats, our expectations about increases in abundance with eutrophication were met; communities in eutrophic streams were characterized by high clogginess. This has been found by others before (e.g. Quinn et al., 2011), but the relationship between biomass and different nutrient species (N, P) in different phases (water, sediment) are complex (Mebane et al., 2014) so results are not always uniform (e.g. Carr & Chambers, 1998). This suggests that our site selection process found streams that are not confounded by other limiting factors for growth, a critical requirement for investigating eutrophication effects (O'hare et al., 2018). Our results are not surprising, but neither are they trivial. Eutrophication threatens freshwater ecosystems globally (Vorosmarty et al., 2010), and our results add to the body of evidence that eutrophication needs to be mitigated if problematic proliferations are to be reduced and systems are to be restored.

Still, other factors than difference in nutrient levels could influence clogginess. For instance, disturbance (as FRE 3) was higher in the more pristine sites, which could keep clogginess at lower levels (Riis & Biggs, 2001). Water clarity could theoretically contribute to lower submerged species abundance due to light limitation, but this seems not to be the case. Rather submerged species in our study are most frequent in low clarity streams, potentially confounded by other correlates, particularly nutrients which also tend to be high in nutrients and relatively shallow depth. One thing we could not account for was potential mechanical removal or spraying for removal of macrophyte proliferations, a common management practice in Aotearoa New Zealand streams (Collins et al., 2018). However, these practices would most likely be used in streams in areas with agriculture and thus if these practices were controlling abundance, we would not see high abundance levels in Q1 and Q2.

Possibly, some of the sites in those quadrants are influenced by removal and this is why we do not see high abundances at these sites. Nevertheless, in summation, we believe increased resource availability (alkalinity also increased alongside eutrophication) is more important for the observed abundance pattern than a lack of disturbance.

4.5.3 Species distribution

We saw a clear dominance of non-native species in eutrophic reaches. Furthermore, we saw a partitioning of submerged and emergent species in the impacted reaches, where submerged species seemed to respond stronger to TSS, DRP and Clarity, and emergent species responded to FRE3, N and sediment P. We suggest multiple factors contribute to this pattern.

As expected, eutrophication led to dominance of non-native species in the impacted reaches, indeed group 3 and 4 at the extreme ends of the aforementioned gradients comprised purely non-native species. Several studies have found non-native dominance in streams that have a high degree of human impact which can lead to the exclusion of native species (Quinn et al., 2011), or loss of functional diversity (Mouton et al., 2019). We cannot conclude that the non-native species in the study area have met their full dispersion potential, and we acknowledge that our study contains a bias, as the streams located in areas of high human impact are also the streams most likely to receive introduced species. It is clear however, that the non-native species have displaced native species in the high impacted stream reaches. Managing actions to mitigate invasive species pressure should therefore include constraints on nutrient inputs to streams.

Non-native species are not a taxonomic group, but those that are also invasive often share common phenological aspects that make them particularly good at dispersing and dominating in high resource environments (Daehler, 2003; Van Kleunen et al., 2010; Vilà & Weiner, 2004). This corroborates our results; traits dominating in eutrophic environments are characteristic of large productive species, with effective dispersal and overwintering capabilities. Previous trait-based studies have found that productivity-related traits do not necessarily correlate with eutrophication, and that eutrophication can even lead to low Ellenberg L community trait scores due to shading by canopy-forming species (Baattrup-Pedersen et al., 2015), a result not shared by our study. We suggest the reason we do not find these traits is due to the strict selection of streams; the benign conditions for growth in general make the competitive pressure on less productive species extremely high, not only from the most productive species, but also by epiphytes (Hilton et al., 2006) and this lead to a competitive exclusion. Our results could be used to strengthen assessments of invasion probability of problematic species by looking at species-specific trait values.

Several factors may contribute to the submerged/emergent partitioning. Submerged species typically respond to phosphorus (O'hare et al., 2010) and have the benefit of nutrient uptake from the water column (Madsen & Cedergreen, 2002). Furthermore, the submerged species of group 4 all have the

capability to grow to the surface to establish a canopy, a key trait in eutrophic streams as this lets them bypass light limitation (Baattrup-Pedersen et al., 2015), in our study illustrated by high TSS and low clarity. Lastly, bicarbonate levels have been found to influence species distribution in eutrophic streams (Riis et al., 2000; Sand-Jensen et al., 2022), and we do see the species of group 4 at high alkalinity. Bicarbonate users in streams are not as ubiquitous as in lakes, nevertheless this ability likely incurs a competitive advantage in lotic waters (Iversen et al., 2019), especially if the reaeration quotient is lowered by high clogginess.

Emergent species differ from submerged species in their nutrient uptake capacity (Manolaki et al., 2020) and are particularly effective in using nitrogen and are thus better at utilizing surplus nitrogen (Cavalli et al., 2016). This could explain their strong response to the N species. Furthermore, the response to FRE3 and sediment P makes sense as the less flexible stems and large root structures (Haslam, 1978) make emergent species well suited for low disturbance and high sediment P environments. A lower MALF may also help emergent species to dominate a reach, as the reach is bound to be either narrower or shallower, providing more suitable conditions for emergent species to spread across the channel.

4.5.4 Internal N and P

We did not find the expected relationship between internal nutrient status and eutrophication. While plants at sites on the pristine end of the eutrophication spectrum mostly had a low internal nutrient content, there was no unequivocal pattern. This might be because the plants have an internal homeostasis that is stronger than the response to the difference in nutrient levels between sites (Demars & Edwards, 2007). But it might also be because the plants from high nutrient sites have attained such a high abundance that the nutrient content have been diluted over a large amount of biomass, or that the movement of water has been slowed to a point were diffusive boundary layer thickness limits nutrient uptake (Sand-Jensen, 1998). Furthermore, the massive macrophyte proliferations of submerged species has their biomass concentrated at the stream surface which forces the water flow underneath the beds; away from where the nutrients are taken up and are most needed (Madsen & Cedergreen, 2002). Indeed, the sites from Q1 and Q2 with low internal N are characterized by a high clogginess. The P content of plants was highest sites dominated by emergent species, possibly because these sites were characterized by high sediment P. As internal N:P ratios did not show any meaningful patterns either, we do not recommend using internal N and P status as a viable indicator of eutrophication.

4.5.5 Concluding remarks

In this chapter we present additional evidence for the degradation of stream ecosystems by human induced eutrophication, here investigated as effects on the macrophyte compartment. By selecting our study sites on the basis of different nutrient regimes but otherwise similar in properties, we were able to focus on the direct effects of eutrophication on macrophyte communities. The eutrophic streams in

our survey were characterized by proliferations of fast growing, highly productive species. Furthermore, the species of the eutrophic reaches were all non-native, illustrating how eutrophication in Aotearoa New Zealand appears to have led to native species decline. We found that species traits tended to correlate with different environmental variables. Submerged species were most strongly associated with high water column P while emergent species were more so with high water column N and sediment P. While other factors influenced this pattern, we believe that a framework of eutrophication effects on plants could usefully acknowledge the physiological differences between submerged and emergent species. We also stress that management of stream ecosystems needs to consider that threshold nutrient levels are potentially quite low but would do well to bear in mind that areas with a low degree of agriculture and a high degree of native forest cover in the catchment appear to have healthy communities.

References

Alboukadel, K. & Fabian, M. (2020). Extract and Visualize the Results of Multivariate Data Analyses. Retrieved from https://github.com/kassambara/factoextra

APHA. (2017). *Standard methods for the examination of water and wastewater* (23 ed.).

Booker, D. J. (2013). Spatial and temporal patterns in the frequency of events exceeding three times the median flow (FRE3) across New Zealand. *Journal of Hydrology, 52*, 1-25.

Booker, D. J. & Woods, R. A. (2014). Comparing and combining physically-based and empirically-based approaches for estimating the hydrology of ungauged catchments. *Journal of Hydrology, 508*, 227-239.

Bornette, G. & Puijalon, S. (2011). Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences, 73*(1), 1-14. DOI:10.1007/s00027-010-0162-7

Baattrup-Pedersen, A., Gothe, E., Larsen, S. E., O'Hare, M., Birk, S., Riis, T., & Friberg, N. (2015). Plant trait characteristics vary with size and eutrophication in European lowland streams. *J Appl Ecol, 52*(6), 1617-1628. DOI:10.1111/1365-2664.12509

Baattrup-Pedersen, A., Gothe, E., Riis, T., & O'Hare, M. T. (2016). Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams. *Sci Total Environ, 543*(Pt A), 230-238. DOI:10.1016/j.scitotenv.2015.11.027

Caraco, N. F. & Cole, J. J. (2002). Contrasting Impacts of a Native and Alien Macrophyte on Dissolved Oxygen in a Large River. *Ecological Applications, 12*(5), 1496-1509. DOI:10.2307/3099987

Carpenter, S. R. & Lodge, D. M. (1986). Effects of Submersed Macrophytes on Ecosystem Processes. *Aquatic Botany, 26*(3-4), 341-370. DOI:Doi 10.1016/0304-3770(86)90031-8

Carr, G. M. & Chambers, P. A. (1998). Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. *Freshwater Biology, 39*(3), 525-536. DOI:10.1046/j.1365-2427.1998.00300.x

Cavalli, G., Baattrup-Pedersen, A., & Riis, T. (2016). Nutrient availability and nutrient use efficiency in plants growing in the transition zone between land and water. *Plant Biol (Stuttg), 18*(2), 301-306. DOI:10.1111/plb.12397

Chambers, P., Lacoul, P., Murphy, K., & Thomaz, S. (2008). Global diversity of aquatic macrophytes in freshwater. *Freshwater animal diversity assessment*, 9-26.

Collier, K. J., Champion, P. D., & Croker, G. F. (1999). Patch- and reach-scale dynamics of a macrophyte-invertebrate system in a New Zealand lowland stream. *Hydrobiologia, 392*(2), 89-97. DOI:10.1023/A:1003653717805

Collins, K. E., Febria, C. M., Warburton, H. J., Devlin, H. S., Hogsden, K. L., Goeller, B. C., . . . Harding, J. S. (2018). Evaluating practical macrophyte control tools on small agricultural waterways in Canterbury, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 53*(2), 182-200. DOI:10.1080/00288330.2018.1487454

Daehler, C. C. (2003). Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. *Annual Review of Ecology Evolution and Systematics, 34*(1), 183-211. DOI:10.1146/annurev.ecolsys.34.011802.132403

Davies-Colley, R. J. & Payne, G. W. (1998). Measuring Stream Shade. *Journal of the North American Benthological Society, 17*(2), 250-260. DOI:10.2307/1467966

Demars, B. O. L. & Edwards, A. C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. *Freshwater Biology, 52*(11), 2073-2086. DOI:10.1111/j.1365-2427.2007.01817.x

Demars, B. O. L. & Harper, D. M. (2005). Distribution of aquatic vascular plants in lowland rivers: separating the effects of local environmental conditions, longitudinal connectivity and river basin isolation. *Freshwater Biology, 50*(3), 418-437. DOI:10.1111/j.1365-2427.2004.01329.x

Dibble, E. D., Killgore, K. J., & Harrel, S. L. (1996). Assessment of fish-plant interactions. *Multidimensional Approaches to Reservoir Fisheries Management, 16*, 357-372.

Dray, S., Choler, P., Dolédec, S., Peres-Neto, P. R., Thuiller, W., Pavoine, S., & ter Braak, C. J. F. (2014). Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology, 95*(1), 14-21. DOI:https://doi.org/10.1890/13-0196.1

Dray, S. & Dufour, A.-B. (2007). The ade4 Package: Implementing the Duality Diagram for Ecologists. *Journal of Statistical Software, 22*(4), 1 - 20. DOI:10.18637/jss.v022.i04

Ebina, J., Tsutsui, T., & Shirai, T. (1983). Simultaneous determination of total nitrogen and total phosphorous in water using peroxodisulfate oxidation. *Water Res, 12*, 1721-1726.

Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., . . . Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett, 10*(12), 1135-1142. DOI:10.1111/j.1461-0248.2007.01113.x

Elser, J. J., Fagan, W. F., Kerkhoff, A. J., Swenson, N. G., & Enquist, B. J. (2010). Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytol, 186*(3), 593-608. DOI:10.1111/j.1469-8137.2010.03214.x

Feijoo, C. S. & Lombardo, R. J. (2007). Baseline water quality and macrophyte assemblages in Pampean streams: a regional approach. *Water Res, 41*(7), 1399-1410. DOI:10.1016/j.watres.2006.08.026

Fleming, J. P. & Dibble, E. D. (2014). Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia, 746*(1), 23-37. DOI:10.1007/s10750-014-2026-y

Flores-Moreno, H., Reich, P. B., Lind, E. M., Sullivan, L. L., Seabloom, E. W., Yahdjian, L., . . . Borer, E. T. (2016). Climate modifies response of non-native and native species richness to nutrient enrichment. *Philos Trans R Soc Lond B Biol Sci, 371*(1694). DOI:10.1098/rstb.2015.0273

Gay, B. (2013). *Freshwater Ecosystems of New Zealand (FENZ) Development Recommendations.* Retrieved from EOS Ecological Report No. 12082-DEP01-01. Prepared for Department of Conversation.:

Gerloff, G. C. & Krombholz, P. H. (1966). *Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants*. Retrieved from Department of Botany:

Giorgi, A., Feijoó, C., & Tell, G. (2005). Primary producers in a Pampean stream: temporal variation and structuring role. *Biodiversity and Conservation, 14*(7), 1699-1718. DOI:10.1007/s10531-004-0694-z

Gusewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytol, 164*(2), 243-266. DOI:10.1111/j.1469-8137.2004.01192.x

Hansen, H. P. & Koroleff, F. (1999). Determination of nutrients. *Methods of seawater analysis*, 159-228.

Haslam, S. M. (1978). River plants; the macrophytic vegetation of watercourses.

Hilton, J., O'Hare, M., Bowes, M. J., & Jones, J. I. (2006). How green is my river? A new paradigm of eutrophication in rivers. *Sci Total Environ, 365*(1-3), 66-83. DOI:10.1016/j.scitotenv.2006.02.055

Iversen, L. L., Winkel, A., Baastrup-Spohr, L., Hinke, A. B., Alahuhta, J., Baattrup-Pedersen, A., . . . Pedersen, O. (2019). Catchment properties and the photosynthetic trait composition of freshwater plant communities. *Science, 366*(6467), 878-881. DOI:10.1126/science.aay5945

Julian, J. P., Seegert, S. Z., Powers, S. M., Stanley, E. H., & Doyle, M. W. (2011). Light as a first-order control on ecosystem structure in a temperate stream. *Ecohydrology, 4*(3), 422-432. DOI:10.1002/eco.144

Kennedy, M. P., Lang, P., Grimaldo, J. T., Martins, S. V., Bruce, A., Hastie, A., . . . Murphy, K. J. (2015). Environmental drivers of aquatic macrophyte communities in southern tropical African rivers: Zambia as a case study. *Aquatic Botany, 124*, 19-28. DOI:10.1016/j.aquabot.2015.03.002

Kennedy, T. L., Horth, L. A., & Carr, D. E. (2009). The effects of nitrate loading on the invasive macrophyte Hydrilla verticillata and two common, native macrophytes in Florida. *Aquatic Botany, 91*(3), 253-256. DOI:10.1016/j.aquabot.2009.06.008

Lacoul, P. & Freedman, B. (2006). Environmental influences on aquatic plants in freshwater ecosystems. *Environmental Reviews, 14*(2), 89-136. DOI:10.1139/A06-001

Lamberti, G. A. & Hauer, F. R. (2017). *Methods in stream ecology: volume 2: ecosystem function*: Academic Press.

Leathwick, J., Julian, K., Elith, J., Chadderton, L., Ferrier, S., & Snelder, T. (2008). *A biologically-optimised environmental classification of New Zealand rivers and streams: reanalysis excluding human impacts variables.* Retrieved from NIWA, Hamilton. :

Loladze, I. & Elser, J. J. (2011). The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio. *Ecol Lett, 14*(3), 244-250. DOI:10.1111/j.1461-0248.2010.01577.x

Lukkari, K., Hartikainen, H., & Leivuori, M. (2007). Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. *Limnology and Oceanography-Methods, 5*, 433-444. DOI:DOI 10.4319/lom.2007.5.433

Madsen, T. V. & Cedergreen, N. (2002). Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology, 47*(2), 283-291. DOI:10.1046/j.1365-2427.2002.00802.x

Manolaki, P., Mouridsen, M. B., Nielsen, E., Olesen, A., Jensen, S. M., Lauridsen, T. L., . . . Riis, T. (2020). A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms. *J Environ Manage, 274*, 111181. DOI:10.1016/j.jenvman.2020.111181

Matheson, F. E., Quinn, J., & Hickey, C. (2012). *Review of the New Zealand instream plant and nutrient guidelines and development of an extended decision making framework: Phases 1 and 2 final report* (HAM2012-081). Retrieved from

Mebane, C. A., Simon, N. S., & Maret, T. R. (2014). Linking nutrient enrichment and streamflow to macrophytes in agricultural streams. *Hydrobiologia, 722*(1), 143-158. DOI:10.1007/s10750-013-1693-4

Mouton, T. L., Matheson, F. E., Stephenson, F., Champion, P. D., Wadhwa, S., Hamer, M. P., . . . Riis, T. (2019). Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape. *Sci Total Environ, 659*, 1370-1381. DOI:10.1016/j.scitotenv.2018.12.277

Naimi, B., Hamm, N. A. S., Groen, T. A., Skidmore, A. K., & Toxopeus, A. G. (2014). Where is positional uncertainty a problem for species distribution modelling? *Ecography, 37*(2), 191-203. DOI:https://doi.org/10.1111/j.1600-0587.2013.00205.x

O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lazar, A. N., . . . Bowes, M. J. (2018). Responses of Aquatic Plants to Eutrophication in Rivers: A Revised Conceptual Model. *Front Plant Sci, 9*, 451. DOI:10.3389/fpls.2018.00451

O'Hare, M. T., Clarke, R. T., Bowes, M. J., Cailes, C., Henville, P., Bissett, N., . . . Neal, M. (2010). Eutrophication impacts on a river macrophyte. *Aquatic Botany, 92*(3), 173-178. DOI:10.1016/j.aquabot.2009.11.001

Patton, C. J. & Kryskalla, J. R. (2003). *Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water* (03-4174). Retrieved from

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., . . . Janssens, I. A. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat Commun, 4*, 2934. DOI:10.1038/ncomms3934

Quinn, L. D., Schooler, S. S., & Van Klinken, R. D. (2011). Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions, 17*(1), 132-143. DOI:10.1111/j.1472-4642.2010.00726.x

Quirino, B. A., Carniatto, N., Thomaz, S. M., Aleixo, M. H. F., & Fugi, R. (2019). The amphibian macrophyte Polygonum punctatum as a temporary habitat and feeding ground for fish. *Aquatic Ecology, 53*(3), 441-452. DOI:10.1007/s10452-019-09700-9

R Core Team, R. (2022). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Reich, P. B., Oleksyn, J., & Wright, I. J. (2009). Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. *Oecologia, 160*(2), 207-212. DOI:10.1007/s00442-009-1291-3

Riis, T. & Biggs, B. J. F. (2001). Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply - a synthesis and conceptual model.

New Zealand Journal of Marine and Freshwater Research, 35(2), 255-267. DOI:10.1080/00288330.2001.9516996

Riis, T. & Biggs, B. J. F. (2003). Hydrologic and hydraulic control of macrophyte establishment and performance in streams. *Limnology and Oceanography, 48*(4), 1488-1497. DOI:10.4319/lo.2003.48.4.1488

Riis, T., Sand-Jensen, K., & Vestergaard, O. (2000). Plant communities in lowland Danish streams: species composition and environmental factors. *Aquatic Botany, 66*(4), 255-272. DOI:Doi 10.1016/S0304-3770(99)00079-0

Rutherford, K., Davies-Colley, R. J., Quinn, J. M., Stroud, M. J., & Cooper, A. B. (1997). *Stream Shade: Towards a Restoration Strategy*. Retrieved from NIWA, Hamilton:

Sand-Jensen, K. (1998). Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology, 39*(4), 663-679. DOI:10.1046/j.1365-2427.1998.00316.x

Sand-Jensen, K., Jensen, R. S., Gomes, M., Kristensen, E., Martinsen, K. T., Kragh, T., . . . Borum, J. (2018). Photosynthesis and calcification of charophytes. *Aquatic Botany, 149*, 46-51. DOI:https://doi.org/10.1016/j.aquabot.2018.05.005

Sand-Jensen, K., Riis, T., & Martinsen, K. T. (2022). Photosynthesis, growth, and distribution of plants in lowland streams—A synthesis and new data analyses of 40 years research. *Freshwater Biology, 67*(7), 1255-1271. DOI:https://doi.org/10.1111/fwb.13915

Schnetger, B. & Lehners, C. (2014). Determination of nitrate plus nitrite in small volume marine water samples using vanadium(III)chloride as a reduction agent. *Marine Chemistry, 160*, 91-98. DOI:10.1016/j.marchem.2014.01.010

Sosiak, A. (2002). Long-term response of periphyton and macrophytes to reduced municipal nutrient loading to the Bow River (Alberta, Canada). *Canadian journal of fisheries and aquatic sciences, 59*(6), 987-1001. DOI:10.1139/F02-071

Unwin, M. J. & Larned, S. T. (2013). *Statistical models, indicators and trend analyses for reporting national-scale river water quality.* Retrieved from

van Kleunen, M., Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecol Lett, 13*(2), 235-245. DOI:10.1111/j.1461-0248.2009.01418.x

Vilà, M. & Weiner, J. (2004). Are Invasive Plant Species Better Competitors Than Native Plant Species? - Evidence from Pair-Wise Experiments. *Oikos, 105*, 229-238.

Vilmin, L., Mogollon, J. M., Beusen, A. H. W., & Bouwman, A. F. (2018). Forms and subannual variability of nitrogen and phosphorus loading to global river networks over the 20th century. *Global and Planetary Change, 163*, 67-85. DOI:10.1016/j.gloplacha.2018.02.007

Vorosmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., . . . Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature, 467*(7315), 555-561. DOI:10.1038/nature09440

Wheal, M. S., Fowles, T. O., & Palmer, L. T. (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. *Analytical Methods, 3*(12), 2854-2863. DOI:10.1039/c1ay05430a

Whitehead, A. (2018). *Spatial modelling of river water-quality state. Incorporating monitoring data from 2013 to 2017.* Retrieved from

Whitehead, A. & Booker, D. J. (2020). NZ River Maps: An interactive online tool for mapping predicted freshwater variables across New Zealand. Retrieved from https://shiny.niwa.co.nz/nzrivermaps/

Whitehead, A., Fraser, C., & Snelder, T. H. (2022a). *Spatial modelling of river water-quality state: incorporating monitoring data from 2016 to 2020.* Retrieved from NIWA, Christchurch:

Whitehead, A. L., Fraser, C., & Snelder, T. H. (2022b). *Spatial modelling of river water-quality state: incorporating monitoring data from 2016 to 2020.* Retrieved from

Young, R. G. & Collier, K. J. (2009). Contrasting responses to catchment modification among a range of functional and structural indicators of river ecosystem health. *Freshwater Biology, 54*(10), 2155-2170.

Chapter V
New insights on macrophyte dynamics in eutrophic streams

5.1 Background and logical structure of this thesis

Research on eutrophication effects on macrophytes in streams has been increasingly focused on reconciling concepts from lake ecology, which was extensively studied in the 80's and 90's, with stream ecology. Starting with Hilton et al. (2006), river eutrophication science has taken a great step forward, culminating in the revised conceptual model of O'hare et al. (2018), that conceptualizes that the dominance of different autotrophic groups shifts with increasing levels of P (probably) dependent on the slope and size of the stream. More specifically, O'Hare et al. (2018) predicts that:

1. Phytoplankton can dominate in large slow flowing rivers where the residence time is high.
2. Epiphytes, being able to grow on the surface of both plants and the stream bed are always strong competitors in eutrophic streams as they have good access to light, though they are susceptible to sloughing and can be shaded by emergent or floating vegetation.
3. Emergent species have the advantage of being able to grow out of the water and reach not only light but also CO_2, however these are restricted to margins and slower flowing waters, and the same goes for floating-leaved species (e.g., water lilies), and these also require still water.
4. Submerged species perform well in streams with a medium slope, but in slower flowing streams they will be out shaded by phytoplankton/epiphytes or emergent or floating-leaved species.

Thus, eutrophication effects in streams will be dependent on several other factors than just the amount of nutrients. Following O'Hare et al. 2018, the threshold concentration for when eutrophication effects start to occur is deemed to be around 100 µg P L^{-1}. Above this concentration, periphyton always has the potential to outcompete the macrophytes in the water (O'hare et al., 2018).

What is less known however is whether this conceptual framework holds when considering N along with P instead of only P. Also, there is less information about how species of macrophytes compete with species of similar growth forms. Additionally, the threshold still needs empirical confirmation, and it may well be different for N than for P. Another question is how light interacts with the

eutrophication framework. Eutrophic streams as a rule only stay eutrophic when the incoming energy levels are high, otherwise the production levels will fall below what we can classify as a eutrophic system. Eutrophication thus also depends on the light availability. This thesis sought to address the uncertainties still prevailing in this conceptual stream eutrophication framework and create an improved understanding of macrophyte responses to eutrophication in streams and rivers.

The findings of this thesis have obvious management implications, and, in this chapter, I will endeavor to show how the newfound knowledge on biological drivers and responses can be utilized in a management context. Knowledge on which streams that are susceptible to adverse effects of plant proliferation is very important, as is knowledge on factors that can help manage this issue.

In this chapter, I will also make an overall conclusion on eutrophication effects on stream macrophytes based on the results from chapters II, III, and IV (Figure 5.1).

The combined results of these chapters are then used to revise the conceptual framework on threshold concentrations for eutrophication effects on stream macrophytes. Following this, I will suggest new avenues of research that could further improve the knowledge on eutrophication effects.

5.2 New ecological perspectives on eutrophication effects on stream macrophytes.

5.2.1 Responses of stream communities to eutrophication.

I have gained new insights into interactions between community dynamics and stream nutrient status (Figure 5.1), and whether existing paradigms prevail in a lesser explored part of the world. I have found that in Aotearoa New Zealand when nutrient inputs to streams are high, there is dominance of fast growing, competitive species (see 1.1.6.2), and these are predominantly non-native, often invasive species. Additionally, I have shown that in eutrophic streams, submerged species dominate at high water column P and emergent species dominate at high water column N and sediment P..

Other studies have found that species with low light affinity (low Ellenberg L) prevailed in eutrophic streams, as they utilized the niche created by the shading from canopy forming species with high biomass accrual rates and apical growth meristems (Baattrup-Pedersen et al., 2015). Presently, species with low light affinity in Aotearoa New Zealand are mostly native species (Ellawala Kankanamge et al., 2019; Mouton et al., 2019) and I see these species excluded from the eutrophic streams I surveyed (chapter IV). Additionally, community trait analysis showed high Ellenberg L trait scores in communities in eutrophic streams. I attribute the differences between Baattrup-Pedersen et al. (2015) and my study to the selection of streams for my field survey; I specifically targeted similar sized streams with few limiting conditions for macrophyte growth while they had a broader range of conditions which can have obscured eutrophication effects. This suggests that lowland New Zealand streams are indeed susceptible to eutrophication effects and that pressure from fast growing species (and potentially epiphytes) under nutrient surplus can lead to exclusion of even shade tolerant species.

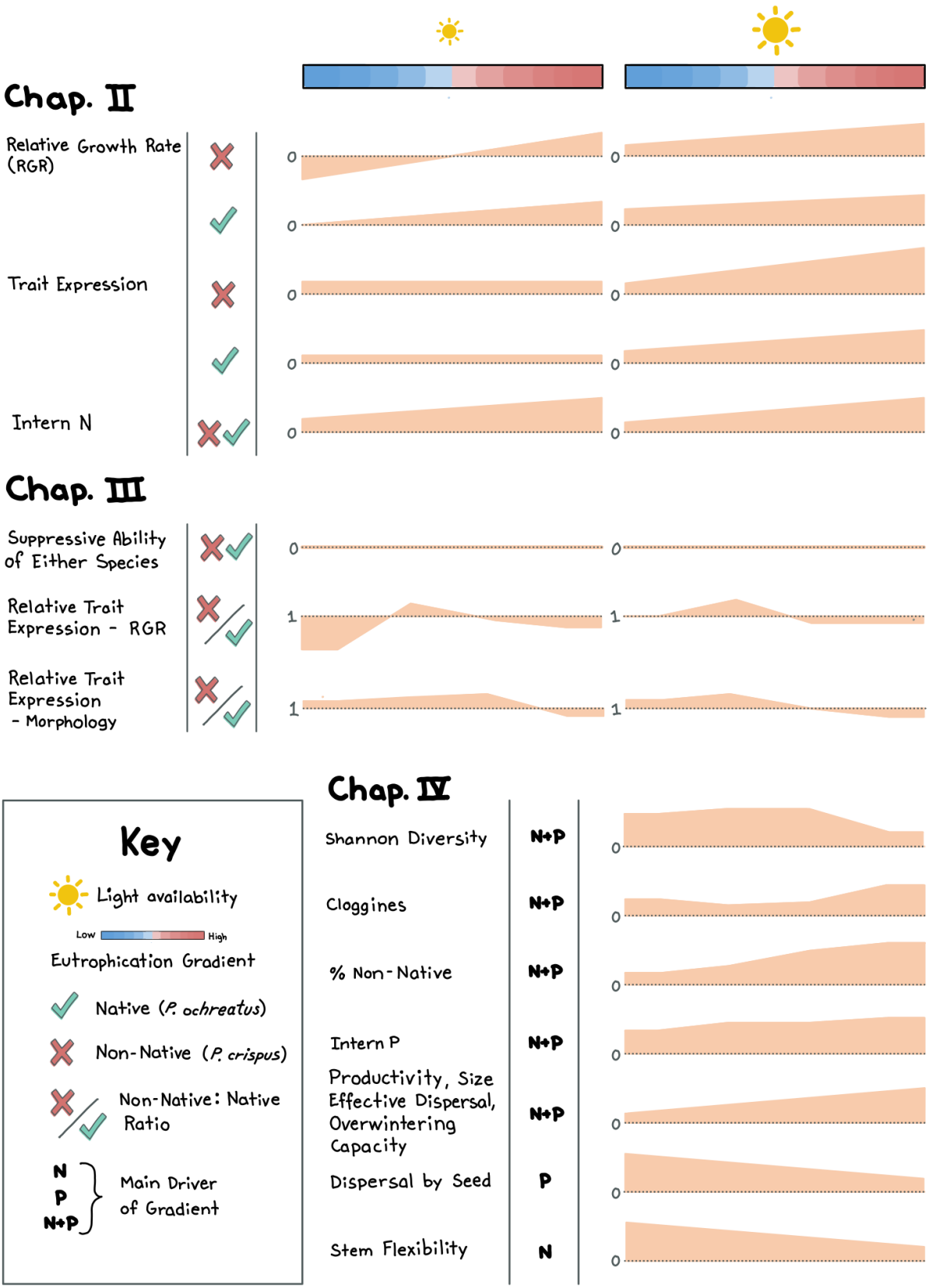


***Figure 5-1**: Responses of macrophytes to eutrophication in streams based on two mesocosm experiments (chapters II and III) and a field survey (chapter IV). Responses are shown as changing on a eutrophication gradient at low (left column) or high (right column) light availability. Trait expression is an aggregate parameter of the height, width, branching, and root length described in chapters II and III. See key for symbology. Made by Eya Esmaralda Lykke Sandvej-Larsen.*

The current framework for eutrophication response proposes that dominance by different species groups (emergent, submerged, floating etc) is driven by differences in stream hydrology (O'hare et al., 2018). I show here that in streams with similar hydrology, different species groups can be dominating (or indeed, co-occurring) along nutrient gradients. This is represented by the dominance of submerged exotic taxa in high nutrient (particularly phosphorus), high hardness streams, and dominance of emergent species in high nutrient (particularly nitrogen), high turbidity streams. I cannot refute that the reason for this distribution pattern could be due to the as yet patchy distribution of theses non-native species, but other arguments for this pattern are compelling. Carbon source has recently been linked to distribution of macrophytes in lowland streams in Denmark, whose waterways are generally eutrophic (Sand-Jensen et al., 2000; Sand-Jensen et al., 2022). Furthermore, nitrogen affinity has been found to increase in species on a water to land ecotone, making nitrogen increases relatively more beneficial for emergent and terrestrial species than submerged ones (Cavalli et al., 2016). Thus, there is reason to include carbon source and different nutrient species into the current eutrophication framework.

P nutrient dynamics have also been further elucidated. I have shown that clogginess in streams correlated with bioavailable sediment P. This has been found by others (Mebane et al., 2014), but the current eutrophication framework is largely built around correlations with open water nutrient concentrations (O'hare et al., 2018). Spot measurements of water samples are not always an accurate measure for nutrient availability over time, however, and while nutrient content of the plants is expected to be a more reliable time-integrated measure, this is not always the case (Demars & Edwards, 2007, 2008) and was not found in my field survey. So, the sediment nutrients seem like a good candidate for a stream compartment that integrates nutrient input over time. Further insight into how sediment nutrient content can be correlated with macrophyte communities could still be investigated, for example by correcting total sediment P with Fe content, as this exerts control over bioavailability (Mebane et al., 2014).

I found that diversity of the macrophyte communities was lowest in the eutrophic streams (Figure 5.1). Diversity was highest in mesotrophic streams and slightly less in the oligotrophic ones. While diversity decline with eutrophication is not a novel concept (Bornette & Puijalon, 2011), many studies have failed to show this relationship in the field (Giorgi et al., 2005; Lévesque et al., 2016; Son et al., 2018). As mentioned previously, the reason that this trend is evident in my study is likely due to the selection of a specific type of stream. Notably, a large part of the biodiversity recorded in this study owes to terrestrial species being present in the sample plots. Thus, the diversity metric needs to be used with care, and a more useful metric for assessing stream ecosystem quality could be that species are wanted or unwanted in the streams, e.g., decrease in native species richness (Quinn et al., 2011).

5.2.2 Drivers of ecosystem change; responses of growth and trait expression.

By conducting two controlled environment experiments, I have strengthened our knowledge of macrophyte growth response to different nutrient concentrations. Specifically, we are closer to identifying a nitrogen threshold concentration below which nutrient supply may limit growth. In the first experiment, plants of the species *Potamogeton crispus* and *P. ochreatus* both achieved their highest growth rates at approximately 250 µg N L^{-1} (as NO_3^--N), by when both species had reached a high internal N concentration, which did not increase when more N was supplied. Interestingly, light levels as low as 30 % of incoming daylight (30.9 mol photons m^{-2} d^{-1}) yielded a higher growth rate in individuals grown at >250 µg N L^{-1} compared to plants grown at lower concentrations. Only at the lowest light level (7.8 mol photons m^{-2} d^{-1}) did we see a decrease in growth rate compared to higher light levels and this was the only light treatment that yielded no differences in growth rates between the different nutrient treatments. We also saw an interaction between light and nitrate levels when comparing growth of the two species. *P. ochreatus* had higher growth rates than *P. crispus* only when both light and nutrient were low and highest resource availability led to the alien *P. crispus* achieving the maximum growth rates. This corroborates previous findings in which nutrient and light levels have found to interact in the eutrophication paradigm (Baattrup-Pedersen et al., 2016; Mouton et al., 2019; Quinn et al., 2011). Furthermore, it adds to the evidence of high resource environments being more susceptible to invasion (Fleming & Dibble, 2014).

I have found that expression of morphological traits increases with nutrient levels (Figure 5.1) and probably also determines competitive performance a result shared by others (e.g. Cronin & Lodge, 2003; Gérard et al., 2014). Trait expression in experimental eutrophic settings yielded taller, wider plants, with more tissue N and this was enhanced with increases in light for *P. crispus* especially. These traits are all characteristic of successful invaders (Fleming & Dibble, 2014), hence the high probability of eutrophication aiding *P. crispus* in becoming invasive. Still, the traits chosen for investigation in the experimental part of the thesis of course also had to be restricted due to time constraints. As such, the response to nutrient increases of many traits that could be relevant for persistence and competitive ability have not been experimentally tested. I can see from the field survey however, that the trait composition of the community changes and so the traits of the species must matter for their competitive performance. The possession of a trait is not the same as the relative expression of it on a nutrient gradient and species that become invasive have shown to have different degrees of trait expression and plasticity compared to native populations (Hyldgaard & Brix, 2012; Hyldgaard et al., 2012). What might be the case then, is that species must have the ability to increase trait expression with increases in nutrient levels in addition to possessing a certain trait for it to be competitively viable in a eutrophic environment. Most likely, this is what is the case for invasive strains of certain species. There is potential for further investigations of this, especially in traits related to dispersal and bicarbonate use, which have not been extensively covered by this thesis.

Growth responses from the first experiment did not transfer to a competitive response in the second experiment. Indeed, we saw no evidence of one species exerting a negative effect on either the growth rate or the morphology of the other species (Figure 5.1) as otherwise seen by others (Gérard et al., 2014). Difference between growth responses and direct competitive outcomes on a nutrient gradient has been seen before in investigations of the invasive *Hydrilla verticillata* in North America; the species showed higher growth rates across the gradient (Kennedy et al., 2009), but was outcompeted at low nutrient levels (Van et al., 1999). This well exemplifies the complexity of factors structuring macrophyte communities in streams. Nevertheless, it seems that, at least for these two species under our experimental conditions, the outcome of the competition between them depends on differences in trait expression. My experimental evidence points to *P. crispus* being better at expressing competitive traits and in the field survey this species is indeed positioned higher on the eutrophication gradient than *P. ochreatus*. While our experiments cannot conclude whether these growth responses are a general trend of native versus non-native species, as two species are too few to draw such conclusions, in our observational study of many streams we did see evidence that non-native species were more prevalent in eutrophic streams than were the native ones.

5.3 Management perspectives. Resource reduction as a management tool?

Regionally, from my field survey I see that non-native species with fast growth are dominant in the low-lying eutrophic streams of Aotearoa New Zealand's North Island (Figure 5.1). Specifically, these are the submerged species *Egeria densa, Lagarosiphon major, Elodea canadensis*, and C*eratophyllum demersum.* These species seem to have outcompeted the native charophytes, e.g., of the *Chara* and *Nitella* genera, which we assume would have prevailed in many streams prior to these introductions. The non-native, emergent species *Glyceria maxima*, and *Alisma-plantago aquatica* are also dominating the eutrophic streams. These apparent changes show how high resource supply can lead to impoverished macrophyte communities via a competitive exclusion mechanism, which has particular impact on native taxa. The highest diversity of plants *in situ* was found at intermediate nutrient levels. This is in line with growth responses from the mesocosm experiments, as we see some species performing poorly when nutrient supply is very low. The stress gradient hypothesis states that on a resource gradient, competition will dominate on the high resource end, and facilitation will dominate on the low resource end (Maestre et al., 2009). Where competition dominates, the few species that excel will exclude others and where facilitation dominates only the most stress tolerant species can persist. Thus, in the middle, there is room for species that are not the best at doing either thing. This group comprises most species and thus gives rise to the highest diversity. This will however also depend on other factors influencing macrophyte growth, such as disturbance.

This thesis has sought to answer the question: Can management of resource input to streams be a viable control mechanism on macrophyte proliferation? Macrophyte proliferation is detrimental to

stream health and the knowledge on threshold concentrations below which nutrients can limit growth can be used to predict at-risk areas. Several aspects of eutrophication need to be considered to fully answer this question. Based on growth responses in an experimental setup, the threshold concentration for N seems rather low. Effective reduction of nutrients down to mean concentrations of <100 µg NO_3^--N L^{-1} is unlikely to be feasible in areas with a large proportion of agriculture in the catchment, as the concentration in these streams can be as much as several mg L^{-1} (Ballantine & Davies-Colley, 2014). If nutrient input is substantially lowered however, we do see an effect on growth response, and if the reduction can be combined with measures to reduce incoming irradiation (e.g., riparian tree planting) this can be an effective measure in reducing problematic macrophyte proliferations. This is, among other things, due to the 'dilution' effect of nutrients into the biota of the streams that we see happening *in situ* when macrophyte biomass increases. And, if multiple types of macrophytes exist in a stream, nutrient uptake is improved and spread out on different growth forms, leaving less for the submerged species that are often the ones causing clogginess (Manolaki et al., 2020). This effect needs to be quite substantial, as submerged species are often effective in taking up nutrients (Manolaki et al., 2020), as I also show in this thesis. With regards to P inputs, we can provide less certain answers, but it is evident from the field survey that streams with lower P-levels (< 50 µg L^{-1}) have lower clogginess than streams with high P levels.

5.4 Concluding remarks.

In this thesis, I have expanded our knowledge on the effect of eutrophication on macrophytes in streams. I can extend the framework of O'hare et al. (2018), by adding that in the medium sized streams, competition between growth forms is influenced by several factors including different nutrient species. I found that there is a strong coupling of expression of certain traits and community composition in eutrophic areas. Furthermore, I found that this leads to low diversity, high biomass communities dominated by non-native invasive species. Due to invasive species being a global issue, I see no reason why the results of this thesis, while performed in Aotearoa New Zealand, cannot be extrapolated to other regions of the world.

Rivers and streams are fantastic and unique ecosystems and exploring them is filled with wonder and joyous experiences. I hope that this work will inspire more research in the field of eutrophication effects on riverine communities as these areas are clearly in danger of degrading and must rely on us to save them.

5.5 Future research

Several factors could be improved when investigating eutrophication effects in the future. And key driver-response dynamics could be further investigated.

1) The experimental setup needs to be improved in future experiments. The tubes feeding the growth medium to the flumes were prone to clogging and so needed to be checked regularly, which was time consuming. Additionally, some elements (the Fe, particularly) tended to precipitate before reaching the flumes, and hence, the plants.

2) Our experiments only included nitrate as a factor for responses. Future work should include both N and P to find the threshold concentrations for both nutrient species. This would be relevant to test in both the sediment and water phase.

3) Direct competition did not seem to be affected by nutrient levels; however, this could be the case for other species sets. Future research could include a variety of species, that were ecologically relevant for the stream of Aotearoa New Zealand. Additionally, competitive interactions could be investigated over a longer timeframe.

4) Both species used in the mesocosm experiments produce tubers as an overwintering strategy. Future research could include differences in tuber production and germination success at different nutrient levels.

5) The field survey only included P in sediments. Another field survey would benefit from the inclusion of N availability in sediments. Furthermore, the procedure of sediment handling can be improved upon for more precise results. P dynamics of sediments are especially complex and further studies would benefit from a cross collaboration with experts on sediment chemistry.

6) Photosynthesis dynamics were not affected by nutrient levels in chapter III. This seems unrealistic, as this provides the basis for the growth response that is evident. Further studies should include more or better estimates of plant production, to better understand the mechanistic responses to variations in nutrient levels.

7) The effects of epiphytes on macrophyte community structure have potential to be further explored. In the size of streams that I have been exploring, epiphytes pressure has not been so great as to exclude submerged macrophytes altogether, but they might have played a role in excluding some species and this dynamic could be investigated.

References

Ballantine, D. J. & Davies-Colley, R. J. (2014). Water quality trends in New Zealand rivers: 1989–2009. *Environmental Monitoring and Assessment, 186*(3), 1939-1950. DOI:10.1007/s10661-013-3508-5

Bornette, G. & Puijalon, S. (2011). Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences, 73*(1), 1-14. DOI:10.1007/s00027-010-0162-7

Baattrup-Pedersen, A., Gothe, E., Larsen, S. E., O'Hare, M., Birk, S., Riis, T., & Friberg, N. (2015). Plant trait characteristics vary with size and eutrophication in European lowland streams. *J Appl Ecol, 52*(6), 1617-1628. DOI:10.1111/1365-2664.12509

Baattrup-Pedersen, A., Gothe, E., Riis, T., & O'Hare, M. T. (2016). Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams. *Sci Total Environ, 543*(Pt A), 230-238. DOI:10.1016/j.scitotenv.2015.11.027

Cavalli, G., Baattrup-Pedersen, A., & Riis, T. (2016). Nutrient availability and nutrient use efficiency in plants growing in the transition zone between land and water. *Plant Biol (Stuttg), 18*(2), 301-306. DOI:10.1111/plb.12397

Cronin, G. & Lodge, D. M. (2003). Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia, 137*(1), 32-41. DOI:10.1007/s00442-003-1315-3

Demars, B. O. L. & Edwards, A. C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. *Freshwater Biology, 52*(11), 2073-2086. DOI:10.1111/j.1365-2427.2007.01817.x

Demars, B. O. L. & Edwards, A. C. (2008). Tissue nutrient concentrations in aquatic macrophytes: comparison across biophysical zones, surface water habitats and plant life forms. *Chemistry and Ecology, 24*(6), 413-422. DOI:Pii 905940730 10.1080/02757540802534533

Ellawala Kankanamge, C., Matheson, F. E., & Riis, T. (2019). Shading constrains the growth of invasive submerged macrophytes in streams. *Aquatic Botany, 158*. DOI:10.1016/j.aquabot.2019.06.004

Fleming, J. P. & Dibble, E. D. (2014). Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia, 746*(1), 23-37. DOI:10.1007/s10750-014-2026-y

Gérard, J., Brion, N., & Triest, L. (2014). Effect of water column phosphorus reduction on competitive outcome and traits of Ludwigia grandiflora and L. peploides, invasive species in Europe. *Aquatic Invasions, 9*(2), 157-166. DOI:10.3391/ai.2014.9.2.04

Giorgi, A., Feijoó, C., & Tell, G. (2005). Primary producers in a Pampean stream: temporal variation and structuring role. *Biodiversity and Conservation, 14*(7), 1699-1718. DOI:10.1007/s10531-004-0694-z

Hilton, J., O'Hare, M., Bowes, M. J., & Jones, J. I. (2006). How green is my river? A new paradigm of eutrophication in rivers. *Sci Total Environ, 365*(1-3), 66-83. DOI:10.1016/j.scitotenv.2006.02.055

Hyldgaard, B. & Brix, H. (2012). Intraspecies differences in phenotypic plasticity: Invasive versus non-invasive populations of Ceratophyllum demersum. *Aquatic Botany, 97*(1), 49-56. DOI:10.1016/j.aquabot.2011.11.004

Hyldgaard, B., Sorrell, B., Olesen, B., Riis, T., & Brix, H. (2012). Geographically distinct Ceratophyllum demersum populations differ in growth, photosynthetic responses and phenotypic plasticity to nitrogen availability. *Funct Plant Biol, 39*(9), 774-783. DOI:10.1071/FP12068

Kennedy, T. L., Horth, L. A., & Carr, D. E. (2009). The effects of nitrate loading on the invasive macrophyte Hydrilla verticillata and two common, native macrophytes in Florida. *Aquatic Botany, 91*(3), 253-256. DOI:10.1016/j.aquabot.2009.06.008

Lévesque, D., Hudon, C., James, P. M. A., & Legendre, P. (2016). Environmental factors structuring benthic primary producers at different spatial scales in the St. Lawrence River (Canada). *Aquatic Sciences, 79*(2), 345-356. DOI:10.1007/s00027-016-0501-4

Maestre, F. T., Callaway, R. M., Valladares, F., & Lortie, C. J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology, 97*(2), 199-205. DOI:https://doi.org/10.1111/j.1365-2745.2008.01476.x

Manolaki, P., Mouridsen, M. B., Nielsen, E., Olesen, A., Jensen, S. M., Lauridsen, T. L., . . . Riis, T. (2020). A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms. *J Environ Manage, 274*, 111181. DOI:10.1016/j.jenvman.2020.111181

Mebane, C. A., Simon, N. S., & Maret, T. R. (2014). Linking nutrient enrichment and streamflow to macrophytes in agricultural streams. *Hydrobiologia, 722*(1), 143-158. DOI:10.1007/s10750-013-1693-4

Mouton, T. L., Matheson, F. E., Stephenson, F., Champion, P. D., Wadhwa, S., Hamer, M. P., . . . Riis, T. (2019). Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape. *Sci Total Environ, 659*, 1370-1381. DOI:10.1016/j.scitotenv.2018.12.277

O'Hare, M. T., Baattrup-Pedersen, A., Baumgarte, I., Freeman, A., Gunn, I. D. M., Lazar, A. N., . . . Bowes, M. J. (2018). Responses of Aquatic Plants to Eutrophication in Rivers: A Revised Conceptual Model. *Front Plant Sci, 9*, 451. DOI:10.3389/fpls.2018.00451

Quinn, L. D., Schooler, S. S., & Van Klinken, R. D. (2011). Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions, 17*(1), 132-143. DOI:10.1111/j.1472-4642.2010.00726.x

Sand-Jensen, K., Riis, T., Vestergaard, O., & Larsen, S. E. (2000). Macrophyte decline in Danish lakes and streams over the past 100 years. *Journal of Ecology, 88*(6), 1030-1040. DOI:10.1046/j.1365-2745.2000.00519.x

Sand‐Jensen, K., Riis, T., & Martinsen, K. T. (2022). Photosynthesis, growth, and distribution of plants in lowland streams—A synthesis and new data analyses of 40 years research. *Freshwater Biology*.

Son, D., Cho, H., & Lee, E. J. (2018). Determining factors for the occurrence and richness of submerged macrophytes in major Korean rivers. *Aquatic Botany, 150*, 82-88. DOI:10.1016/j.aquabot.2018.07.003

Van, T. K., Wheeler, G. S., & Center, T. D. (1999). Competition between Hydrilla verticillata and Vallisneria americana as influenced by soil fertility. *Aquatic Botany, 62*(4), 225-233. DOI:Doi 10.1016/S0304-3770(98)00100-4

Appendix I

Table A.1.1 Simplified overview of studies investigating macrophyte-nutrient relations in streams. References can be found in chapter I.
Response symbols: ↑ increasing, ↓ decreasing, ∩ unimodal, and − no response

Reference	Type	Nutrient	Water column or sediment focus	Response	Notes
Responses of biomass					
(Barker et al., 2008)	Mesocosm	N	Water	∩, − and ↓	Species specific response. Influenced by shading from phytoplankton.
(Angelstein et al., 2009)	Mesocosm	N+P	Sediment	↑	
(Baattrup-Pedersen et al., 2016)	Field survey	N+P	Water	↑	
(Barko et al., 1991)	Review	N	Sediment	↑	Lake influence.
		P	”	−	
(Boedeltje et al., 2005)	Mesocosm	N	Water	↓	N as NO_3^- on *Potamogeton alpinus.*
(Canfield Jr. and Hoyer, 1988)	Field survey	N+P	Water	−	
(Carr and Chambers, 1998)	Field survey + mesocosm	N	Sediment	−	N+P had greater effect than individual N and P.
		P	”	↑	
		N+P	”	↑	
(Carr et al., 2003)	Field survey	N+P	Water	↑	Weak correlation, only explaining ~27 % of variability in biomass.
(Carr et al., 1997)	Review	N+P	Both	↑	Results carry some uncertainties due to lack of knowledge in root/shoot uptake partitioning.
(Chambers et al., 1989)	Field manipulation	N+P	Sediment	↑	
(Cronin and Lodge, 2003)	Field manipulation	N+P	Sediment	↑	Lake study. High nutrient treatment killed *Nuphar adventea*.
(Elser et al., 2007)	Meta study	N	Water	↑	N+P had greater effect than individual N and P.
		P		↑	
		N+P		↑	Results are mostly from algae studies.
(Garbey et al., 2004)	Field survey	N+P	Water	↑	N as NH_4^+. Specific to *Ranunculus peltatus.* Additional effects on morphology.
(Gérard et al., 2014)	Mesocosm	P	Water	↑	Additional effects on morphology.
(Gufu et al., 2018)	Mesocosm	N	Water	↑ and ∩	N as NO_3^-.

					↑ for *Egeria densa and Vallisneria spiralis*, ∩ for *Salvinia molesta*.
(Heaney et al., 2001)	Field survey	P	Water	↑	Specifically, *Ranunculus penicillatus*. Somewhat speculative.
(Hilton et al., 2006)	Review	(P)	Water	∩	From conceptual model. Some uncertainty in P vs N. Decrease in biomass at high nutrients due to shading by algae.
(James et al., 2006)	Mesocosm	N+P	Water	↓	Decrease in root/shoot ratio.
(Kennedy et al., 2009)	Mesocosm	N	Water	↑ and −	Effect on *Hydrilla verticillata,* but none on *Vallisneria americana* and *Sagittaria kurziana*
(Kern-Hansen and Dawson, 1978)	Field survey	N+P	Water	−	
(Kouwe, 1983)	Field survey	P	Water	−	No effect in part due to high natural levels.
(Lévesque et al., 2017)	Field survey	N+P	Water	−	Biomass was positively related to conductivity.
(Matheson et al., 2012)	Review	N+P	Both	↑	More often N than P limitation.
(Mebane et al., 2014)	Field survey	N	Both	↑	
		P	Sediment	↑	
(Njambuya et al., 2011)	Laboratory bioassay	N+P	Water	↑ and −	Effect on *Lemna minuta*, none on *L. minor.*
(O'Hare et al., 2018)	Review	(P)	Water	↑ and ∩	Very much dependant on physical habitat. Reduction in biomass due to out-shading by algae in high nutrient settings.
(O'Hare et al., 2010)	Field survey	P	Water	↑	Further increases with elevated HCO_3^-.
(Ray et al., 2014)	Laboratory bioassay	P	Water	↑	*Lemna minor*.
(Riis et al., 2010)	Field survey	N+P	Water	↑ and −	Effect on *Elodea canadensis* and *Lagarosiphon major*, none on *Egeria major*.
(Ságová-Marečková et al., 2009)	Mesocosm	N+P	Sediment	↑	*Sparganium emersum*.
(Sosiak, 2002)	Field survey	N	Water	↑	*Potamogeton vaginatus* and *Potamogeton pectinatus*. Largest effect of N (as NO_3^-).
		P	”	↑ and −	
(Van et al., 1999)	Mesocosm	N+P	Sediment	↑	Greater effect on *Hydrilla verticillata* than *Vallisneria Americana*.
(Wersal and Madsen, 2011)	Mesocosm	N	Water	↑	*Myriophyllum aquaticum*.
		P	”	↓	Negative relationship with P is probably due to excessive algal growth.
(Xie et al., 2010)	Field manipulation	N+P	Sediment	↑	Greater response of non-native species compared to native.

Responses of species richness					
(Barker et al., 2008)	Mesocosm	N	Water	↓	Influenced by shading from phytoplankton.
(Bornette and Puijalon, 2011)	Review	N	Water	↓	
		N+P	”	∩	
		N+P	Sediment	↓	
(Giorgi et al., 2005)	Field survey	P	Water	↑	Water velocity is more important.
(Lacoul and Freedman, 2006)	Review	N+P	Both	∩	
(Lévesque et al., 2017)	Field survey	N+P	Water	−	
(O’Hare et al., 2018)	Review	(P)	Water	− and ↓	Dependant on hydrological setting, greatest influence in ‘calm’ settings.
(Quinn et al., 2011)	Field survey	N+P	Water	−	
(Son et al., 2018)	Field survey	N+P	Water	−	Only submerged species. Negative correlation with suspended solids and chlorophyll *a*.
(Steffen et al., 2013)	Field survey	N+P	Water	↓	Postulated relationship. Also affected by other disturbing factors, e.g. stream morphologic alterations.
Responses of community composition					
(Baattrup-pedersen et al., 2015)	Field survey	N+P	Water	↑ in species w. apical growth meristems and low light tolerance.	
(Baattrup-Pedersen et al., 2016)	Field survey	N+P	Water	↑ in species w. high Ellenberg N, forming dense stands and w. high overwintering capacity.	
(Bornette and Puijalon, 2011)	Review	N	Water	↑ in competitive free-floating species	
		N+P	Sediment	↑ in competitive floating-leaved species.	
(Carbiener et al., 1990)	Field survey	N+P	Water	Community shifts along a eutrophication gradient.	N as NH_4^+.
(Clarke et al., 2001)	Field survey	N+P	Sediment	Trend towards species specific ranges, especially for P.	No explicit conclusions.
(Demars and Harper, 1998)	Field survey	N+P	Water	Change in cover of preselected indicator species.	
(Demars and Harper, 2005)	Field survey	N+P	Water	No correlation with plant distribution.	

(Feijoó et al., 2007)	Field survey	N	Water	Increase in typical eutrophic associated genera.	N as NO_3^-. Streams were eutrophic with regard to P but meso-eutrophic for N.
(Flores-Moreno et al., 2016)	Field survey	N+P	-	Increase in non-native and decrease in native richness.	Terrestrial grassland.
(Giblin, 2017)	Field survey	N+P	Water	Increases in abundance of submergent, emergent and floating leaved vegetation not correlated with nutrient increases.	No analysis of sediment nutrients.
(Giorgi et al., 2005)	Field survey	N	Water	Positive correlation with evenness.	N as NH_4^+. Velocity is more important.
(Hilton et al., 2006)	Review	(P)	Water	Increase in tall emergent and floating leaved species due to light competition.	Some uncertainty in P vs N. Depending on flow.
(Jones et al., 2012)	Review	P	Sediment	Dominance of competitive species with rapid growth and rank structure.	
(Kennedy et al., 2015)	Field survey	N+P	Water	Drives community structure differences between ecoregions.	Along with other factors. N (NO_3^-) more important than P.
(Lacoul and Freedman, 2006)	Review	N+P	Both	Changes in 'indicator' species.	
(Mebane et al., 2014)	Field survey	N+P	Both	–	Other factors are better determinants of community composition.
(Mouton et al., 2019)	Field survey	N+P	Water	Growth of functionally unique species.	Larger effect of hydrologic disturbances and shading.
(O'Hare et al., 2018)	Review	(P)	Water	Increase in competitive species, especially strong light competitors.	Dependant on hydrological setting, greatest influence in 'calm' settings.
(O'Hare et al., 2011)	Field survey	N+P	Water	High linear emergent occurrence and reduced abundance of submerged *Ranunculus*.	As correlated with specific stream power (SSP Wm^{-2}).
(Quinn et al., 2011)	Field survey	N+P	Water	Increase of non-native species.	General response of non-natives to anthropogenic disturbance.
(Riis and Biggs, 2001)	Review	N+P	Both	Dominance of C or R strategists.	Dependant on disturbance regime.
(Rosso and Fernández Cirelli, 2013)	Field survey	N	Water	Increases in emergent species	N as NO_3^-.

				relative to submerged.	
(Schneider and Melzer, 2004)	Field survey	P	Both	Species specific relations between occurrence and nutrient levels.	Unclear if nutrients levels are merely a product of other controlling habitat factors.
(Steffen et al., 2014)	Field survey	N+P	Both	Hydrochemistry of secondary importance compared to water velocity and river size.	

Appendix II

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Research article

Growth response to nitrate enrichment helps facilitate success of an alien *Potamogeton* in New Zealand streams

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Potamogeton crispus

ABSTRACT

Motivated by stream ecosystem degradation by eutrophication, we mimicked slow flowing lowland stream conditions with a novel experimental setup to further our understanding of aquatic plant responses to increases in nitrate and light. We conducted a mesocosm growth experiment of two species from the genus *Potamogeton*: *P. crispus* (alien) and *P. ochreatus* (native), grown at four nitrate and four light levels. We hypothesised that (i) internal nutrient status of the plants would scale with water column nutrient concentration, and that (ii) plant performance would reflect the nutrient status of the plant. Furthermore, we hypothesised that (iii) a low irradiance level would negate the effects of an increased nitrate level. In relation to (ii) we hypothesised that (iv) the traits of the alien species would enable it to outperform the native species where both the availability of light and nutrient resources was high. Internal tissue N content was broadly similar in the two higher (>250 μg NO_3^- L^{-1}) and the two lower nutrient treatments (<20 μg NO_3^- L^{-1}) in both species and plants were therefore collapsed into high and low *N*-groups. High-N individuals had higher growth rates than low-N ones regardless of species or light treatment and plants had reduced growth rates at the lowest light treatment, however this response was less evident for *P. crispus*. The highest growth rate was found at the high-N individuals of *P. crispus* at the highest light treatment, and correspondingly, in this treatment this species exhibited an increase in branching degree and lateral spread from the low-N plants. As *P. crispus* spreads by fragmentation, our results show it to be a highly effective competitor in anthropogenically impacted areas compared to its native counterpart. Our study exemplifies how light can influence eutrophication responses of plants and how both need to be accounted for in management decisions.

1. Introduction

Aquatic submerged macrophytes support many important services in stream ecosystems. They provide food and habitat for invertebrates and fish [1–4], alter the physico-chemical environment [5] and affect nutrient cycling [6]. However, increased anthropogenic influences have impacted stream macrophyte communities and the services they support [7–9]. Of particular relevance to macrophytes has been the global increase in fertilizer use, which has led to widespread eutrophication across ecosystems [10] and excessive biomass production [11,12], also termed 'nuisance growth' [13,14].

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Nuisance biomass accrual by macrophytes is dependent on the complex interplay of multiple environmental and biotic factors, and is most frequently occurring in lowland reaches, where water velocity is sufficiently low to reduce scouring [15] and light is not limiting macrophyte growth [7]. When these conditions are met, in-stream biomass scales with nutrient levels [16–23]. When these conditions are not met, no direct link between macrophyte biomass and stream nutrient concentrations is observed [24–27], making simple relationships unlikely and underpins the importance of disentangling the specific effects of eutrophication [16].

There are many factors that potentially confound relationships between water nutrient status and plant biomass. Firstly, spot measurements of water column nutrient concentrations do not integrate potential temporal variations in nutrient levels that the plants have been exposed to prior to the date of sampling. This is particularly problematic when trying to correlate water column nutrient concentrations with biomass *in situ* [20]. Most submerged macrophytes can also take up nutrients from the sediment as well as the water column via root uptake [28] or have nutrients stored in their tissue from times when nutrient availability was higher [i.e. luxury uptake; [29]]. Thus, studies of plant-nutrient relations should consider these issues in experimental design. For example, measurement of tissue nutrients is a more direct indicator of the supply available for growth than external concentrations [30–33].

Where occurring, extreme macrophyte proliferation can clog waterways and lead to diverse ecological alterations, including anoxia, altered pH, loss of plant species diversity [12,34–36] and subsequently loss of biodiversity in other trophic levels [1,37,38]. Consequently, there is an interest from managers to limit nuisance growths and controlling incoming nutrient loads to streams to prevent prolific macrophyte growth is seen as a tractable option. However, our knowledge on how substantial nutrient reduction needs to be to reverse eutrophication effects and prevent nuisance growth is still lacking [but see Ref. [12]].

Biotic factors, including intrinsic potential of macrophytes, also add complexity to nutrient-plant responses. Large scale studies of lowland streams in Europe suggest that, when plants are no longer competing for nutrients, traits related to enhancing either light interception or light utilization are dominant in submerged macrophyte communities in eutrophic streams [12,39]. Ellenberg et al. [40] introduced Ellenberg Indicator Values (EIVs) to vegetation science to classify plant responses to environmental variables. Baattrup-Pedersen et al. [39] applied the EIV concept to aquatic macrophytes in lowland streams and reported traits of successful taxa in response to eutrophication to include apical and multi-apical growth meristems as strategies for canopy formation at the water surface for better light capture, but also that plants with low EIV(Light) trait scores can be promoted, as these systems are often highly productive and shade-dominated [39]. These traits are commonly found in invasive nuisance species and as such these species often dominate stream sections where light and nutrient levels are high [7]. In New Zealand, one such species is *Potamogeton crispus* [41], which is also known as a problematic species in the US [42]. However, only few experimental investigations of how the interaction of different levels of light and nutrients affect the performance of aquatic macrophytes exist, although these could shed light on how these abiotic factors facilitate certain species in streams.

To further our understanding of species' response to nutrient increases and how this response is affected by variations in incoming light, we conducted a mesocosm growth experiment of two species occurring in New Zealand from the genus *Potamogeton*: *P. crispus* and *P. ochreatus*. The species were specifically chosen such that we might elucidate how the focal factors and potential interaction of these influenced the response of a native and an alien invasive species with otherwise similar habits of growth. The study used a novel mesocosm setup to mimic slow flowing lowland river conditions, as this is where these two macrophytes often proliferate. The setup consisted of four large (~10 m) flume channels with continuous inflow of purified water dosed with macro- and micronutrients and dissolved inorganic carbon at controlled concentrations. By using clean, low-nutrient, sand as a rooting medium, and providing P and other essential growth elements at non-limiting concentrations in the water column, the effects of N could be isolated. We employed a four-by-four factorial design with four nitrate and four irradiance levels. We used nitrate as a dissolved inorganic N (DIN) source, although ammonium (NH_4^+) is taken up by plants with lower energetic costs, as nitrate (NO_3^-) is the dominant form of DIN in streams, and the one most readily leached from agricultural soils [43]. We hypothesised that (i) internal nutrient status of the plants would scale with water column nutrient concentration and that (ii) plant performance would reflect the nutrient status of the plant. Furthermore, we hypothesised that (iii) a low irradiance level would negate the effects of an increased nitrate level. In relation to (ii) we hypothesised that (iv) the traits of the alien species would enable it to outperform the native species where both the availability of light and nutrient resources was high. A suite of relevant plant traits was measured to test the above hypotheses.

2. Methods

2.1. Potamogeton crispus and P. Ochreatus

Were collected from a lowland stream near Morrinsville, New Zealand (37′42″18.59 S; 175′33″4.15 E). *P. crispus* is a naturalised, widely distributed alien species in Aotearoa New Zealand (Figure SI-1), considered native to Eurasia, Africa and Australia [44]. It is regarded as having a ruderal (R) strategy [sensu Grimes [45]]; characterised by fast growth rates and a high disturbance tolerance (i.e. high stem flexibility and root:shoot ratio). *P. ochreatus* is a native species, however not endemic [46], and is also found nation-wide (Figure SI-1), as well as in Australia and south-east Asia. *P. ochreatus* is classified as a competitive-ruderal (CR) strategist [sensu Grimes; 45] and thus shares characteristics of *P. crispus* but is less resistant or resilient to disturbance. At low disturbance and high resource availability both species show traits of high stature, dense canopy formation and high morphological plasticity [45].

2.2. Mesocosms

The experimental setup consisted of four flume mesocosms, each 10 m long and 0.55 m wide, with a water level of 0.35 m and a constant inflow of water at the top (1.5 L min^{-1}) giving a water residence time of ca. 0.8 d. A pump at the lower end of each flume

recirculated water to the top end, to increase water velocity, homogenize water column elemental concentrations and prevent stagnation (Fig. 1). By preventing water stagnation in the high light treatment, this reduced phytoplankton build-up that could affect light climate [e.g. Ref. [47]]. Water from a groundwater bore was purified using a reverse osmosis system (Ecosoft MO10000) with subsequent addition of micro- and macronutrients to create a growth solution excluding nitrogen, bicarbonate and potassium [cf [48]]. Bicarbonate and potassium, to complete the growth medium, were added to the water via peristaltic pumps immediately before entering the flumes, as well as different amounts of NO_3 targeting four different N concentrations across the flumes: control (0 μg N L^{-1}), low (50 μg N L^{-1}), medium (500 μg N L^{-1}) and high (5000 μg N L^{-1}). The target P content (as sodium hydrogen phosphate) of the growth medium was 100 μg P L^{-1} and was considered to be in excess in all nitrogen treatments. The flow rates of all pumps were checked at least every three days.

Water samples were taken to verify nutrient concentrations in each treatment. Both top and bottom of the flumes were sampled to test for potential gradients due to nutrient uptake by the plants. Each flume was covered by shade cloth in four different densities, yielding four light treatments. By measuring incoming irradiation (I) (LI-COR LI-200 sensor) above and below the cloth, the irradiance of each was calculated $(I_{below}) \cdot (I_{above})^{-1} X\ 100$ as 70%, 44%, 32%, 8% of incident. This is very close to the nominated percentages, and for ease of reading we maintain the naming scheme of 70%, 50%, 30%, and 10% light treatments in which 70% is highest and 10% is lowest. Mean daily incoming irradiance for the duration of the experiment was 96.8 ± 29.5 mol photons $m^{-2} d^{-1}$ (mean ± s.d.), thus the light treatments yielded on average 67.7, 44.5, 30.9, and 7.8 mol photons $m^{-2} d^{-1}$ (Figure SI-2).

2.3. Plants

Plants used in experiments comprised 15 cm apical shoots cut from healthy donor plants, potted in 700 ml plastic pots ¾ filled with nutrient poor sand [Particulate carbon, particulate nitrogen, and TRP contents were 0.03 ± 0.006%, 0.02 ± 0.001% and 0.01 ± 0.001%, respectively; [49]]. Cuttings were set to acclimate for at least 3 weeks in their respective nutrient treatments under 50% shade cloth, to ensure internal nitrogen was in equilibrium with the external medium and stored N could not affect growth. At experiment start, 15 cm apical shoots taken from recovered plants were transplanted to new pots and 5 replicates of each species were assigned to each of the 16 treatments. From each nutrient treatment, 5 shoots of each species were taken for mean initial biomass (DW_{start}) and internal C and N measurements (20 in total per species). The plants were grown under experimental conditions for ~10 weeks.

2.4. Estimation of photosynthetic parameters

Immediately prior to harvest, we used pulse amplitude modulated (PAM) fluorometry (MONI-DA/S, Heinz Walz GmbH, Germany) to create photosynthesis-irradiance (PI) curves of dark acclimated leaves. PAM utilizes the fluorescence of chlorophyll excited by absorption of photons to estimate the saturation state of photosystem II (PSII) by comparing fluorescence yield under ambient light, when some reaction centres are closed and some open, with that under saturating light (when all reaction centres are closed). Quantum yield of PSII is estimated as $Y = (F_m'\text{-}F)/F_m'$, where F is the fluorescence at ambient light and F_m' is fluorescence under saturating light sufficient to close all the reaction centres [50]. The quantum yield of PSII is subsequently used to estimate electron transport rate (ETR) (see below).

The 3rd leaf from the apex was taken from three individuals of each treatment for measurement. If the 3rd leaf was too small to produce viable readings the 4th leaf was taken instead. Leaves were fixed in measuring clips and left in the dark for 30 min before measuring yield at 12 actinic light intensities of increasing magnitude. Subsequently, light absorption factor (AF) of the leaves was determined as the proportion of incident irradiance absorbed by each leaf by measuring irradiance reaching the LiCOR Li200 sensor from a fixed light source (white LED flashlight, similar to the white LED of the PAM instrument) with and without the leaves/top covering the light sensor [50]. Leaves were then frozen for chl. *an* analysis (see below).

ETR of the plants was calculated as $ETR = Y \bullet PAR \bullet AF \bullet 0.5$ where Y is the yield, PAR is the light intensity emitted by the PAM-sensor and AF is the absorption factor of the leaf. ETR was then plotted by the PAR to make the PI-curves. For easier literature comparison we present photosynthesis as mol oxygen produced rather than electrons transferred, we assumed a conversion factor of 0.25 oxygen evolved per electron transferred [50]. To obtain the maximum photosynthetic rate P_{max} and saturation irradiance I_c from the curves, a hyperbolic tangent function $y = P_{max} \tanh(\alpha x P_{max}^{-1})$ was fitted to each replicate, and I_c was calculated as $I_c = \alpha P_{max}^{-1}$ [51]. The parameters P_{max}, I_c and the yield at $PAR_i = 0$ were all chl. *A* corrected.

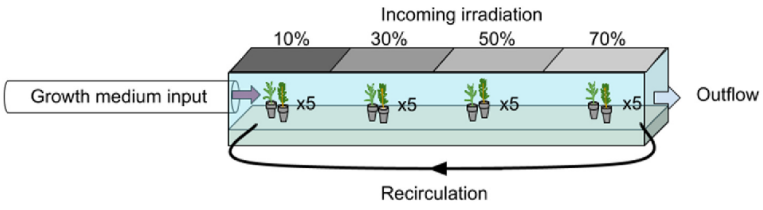

Fig. 1. Schematic representation of the mesocosm flume experimental setup. See text for details.

2.5. Harvest

At harvest, for each plant, the length of the main stem and each branch was measured, and biomass was separated into above and below ground fractions, then dried for 48 h at 60 °C to obtain dry weight (DW). Internal tissue C and N concentrations were obtained from ~2 mg subsamples of finely ground dried biomass, by flash combustion followed by separation of the gaseous products and subsequent mass spectroscopy (Vario EL cube, Elementar, Germany; <0.1% absolute precision). Ratios of tissue C and N are given by weight. Water samples were analyzed for DIN (NH_4^+, NO_3) and dissolved reactive phosphorus (DRP) on a Lachat flow injection analyser (Quikchem® 8500, Hach, USA), with a detection limit of 1 μg L^{-1}. Trace elements were analyzed at Hills laboratories in Hamilton, NZ (see supplementary information for details).

Chlorophyll and carotenoid content of the leaves from PAM measurements was determined by UV-VIS spectroscopy of ethanol extracts. Plant material was submerged in liquid nitrogen and left to lyophilise in a freeze drier after which ~5 mg (when possible) was weighed and transferred to a test tube and rehydrated with 0.1 ml Milli-Q water. Some leaves were too small for accurate analysis and thus three leaves of a treatment were pooled together as one replicate. Ethanol (4 ml) was added, and samples were left to extract overnight, in the dark at 4 °C. Absorbance was subsequently measured at 470, 648 and 664 nm on a UV-1800 spectrophotometer (Shimadzu). An additional measurement was taken at 750 nm to be able to correct for impurities. Where a measurable absorbance was measured at 750 nm this was subtracted from that at all other wavelengths.

From the lengths and weights, branching degree (number of branches · main stem length $^{-1}$), lateral spread (total length · main stem length $^{-1}$), density (DW · total length $^{-1}$), root to shoot ratio and relative growth rate (RGR) were calculated. RGR was calculated [cf [52]] as

$$RGR\left(d^{-1}\right) = \frac{\ln\left(DW_{end}\right) - \ln(DW_{start})}{time} \quad (1)$$

From the UV-VIS spectroscopic absorbances, chlorophyll *a* (C_a), b (C_b), total (C_{a+b}) and total carotenoids (C_{x+c}; all in mg g^{-1} dw) were calculated as follows [53]:

$$C_a = \left(13.36A_{664} - 5.19A_{648}\right) \bullet 4.1 \bullet DW^{-1} \quad (2)$$

$$C_b = \left(27.43A_{648} - 8.12A_{664}\right) \bullet 4.1 \bullet DW^{-1} \quad (3)$$

$$C_{a+b} = \left(5.24A_{664} + 22.24A_{648}\right) \bullet 4.1 \bullet DW^{-1} \quad (4)$$

$$C_{x+c} = \left(1000A_{470} - 2.13C_a - 97.64C_b\right) \bullet 4.1 \bullet DW^{-1} \bullet 209^{-1} \quad (5)$$

Where A_{648}, A_{664} and A_{470} is absorbance at 648, 664 and 470 nm respectively and 4.1 is the volume of liquid added to the sample.

2.6. Data analysis

To investigate differences of internal nitrogen (%N), carbon (%C) content and C:N ratio, amongst nutrient treatments, species, and light, we performed Wilcoxon tests between species grouped by nutrient and light treatments, as well as Kruskal-Wallis tests between light and nutrient treatments, grouped by species and nutrient and species and light respectively. Following the Kruskal-Wallis tests, we conducted post hoc pairwise Wilcoxon tests on individual group pairings of light and nutrient treatments. Non-parametric statistics were used as data failed tests of normality and heteroskedasticity.

To evaluate effects of nutrient and light on response parameters, two-way ANOVA tests were attempted, but all parameters failed either or both of Shapiro-Wilk test of normality ($p < 0.05$) or Levene's test of variance homogeneity ($p < 0.05$), even after log transformation of data. Thus, non-parametric Wilcoxon rank-sum tests were employed to ascertain differences between species within light and N groups and between N groups within light treatments and species. Kruskal-Wallis tests were carried out on light treatments within N group and species, with post hoc pairwise Wilcoxon tests (with Bonferroni-Holm p adjustment method) between specific light levels.

Table 1
Resulting nutrient concentrations of the water samples taken at top end (upstream) and lower end (downstream) of the mesocosm flumes (n 1).

		DRP μg L^{-1}	NH_4^+ μg L^{-1}	NO_3 μg L^{-1}	DIN μg L^{-1}	DIN:DRP
Control	Upstream	52	20	1	21	0.4 : 1
	Downstream	57	1	<1	<2	<0.04 : 1
Low	Upstream	63	8	21	29	0.5 : 1
	Downstream	71	7	17	24	0.3 : 1
Medium	Upstream	49	23	264	287	5.9 : 1
	Downstream	54	62	252	314	5.8 : 1
High	Upstream	60	15	5470	5485	91 : 1
	Downstream	49	20	5580	5600	114 : 1

3. Results

3.1. Nutrient concentrations in water and plants

Dissolved reactive phosphorous concentrations were similar amongst flumes, whereas a clear distinction was evident between DIN concentrations of the four nutrient treatments (Table 1). Differences between inflow and outflow were negligible except for the control flume which had more ammonium (NH_4^+) in the upstream part. DIN:DRP ratios ranged from <0.04:1 in the control flume to as much as 114:1 in the high DIN flume.

Both species within light treatments showed significant differences in tissue %N content amongst nutrient treatments, whereas in only two instances difference in tissue %C were significant (Kruskal-Wallis rank-sum test $p < 0.05$; Table 2, Fig. 2). Similarly, both species mostly differed in %N content amongst the light treatments and less so for %C content (Table 3, Fig. 2). C:N ratios tended to follow %N, given the greater between-treatment variation in %N than %C. As the number of post hoc Wilcoxon tests between % N, % C, and C:N ratios, within light and nutrient treatments are very high (288 tests in total), the results of the individual tests are omitted here but in summary the results showed two consistent patterns: 1) Within nutrient treatments, there were more significant differences found between control or low and medium or high nutrient treatments than between control and low, and medium and high. Out of 24 comparisons, control differed from low only 6 times, whereas control differed from medium and high 15 and 19 times, respectively, and low differed from medium and high 14 and 15 times, respectively. Medium and high differed in only 3 out of 24 comparisons. 2) Within light treatments, there were more differences between the 10% light treatment and any other light treatment than amongst the 70, 50, and 30% light treatments. Out of 72 comparisons involving the 10% light treatment 27 of these were significantly different, while of the 72 comparisons involving the other treatments only 6 were significantly different.

Thus, internal tissue N content was broadly similar in the two higher and the two lower nutrient treatments in both species (Fig. 2a). Consequently, in terms of internal N status, the four N treatments collapsed into high (High and Medium) and low (Low and Control) *N*-groups (Fig. 2c). For further analyses, we therefore combined high and medium N treatments, and low and control N treatments as high-N and low-N, respectively, as well as individuals from the high-N and low-N treatments will be referred to as high-N individuals and low-N individuals, respectively.

The only noteworthy difference between species was that *P. crispus* incorporated more N in the 70% and 50% light treatment than *P. ochreatus*, but only at 70% light did it lead to a difference in C:N ratio (Supplementary Table 3). No obvious differences were seen in the C content of the two plants. Although *P. ochreatus* tended to have slightly higher C content than *P. crispus* (Fig. 2b), this did not give rise to differences in C:N ratio (Fig. 2c).

3.2. Growth and morphology

High-N individuals had higher RGR than low-N ones regardless of species or light treatment save for two instances (Table 4), although *P. crispus* at 70% light was close to the 0.05 mark ($p = 0.08$). Generally, *P. crispus* had higher variation than *P.* ochreatus meaning that some individuals achieved much higher RGR's than *P. ochreatus* at high-N, the highest (0.06 d^{-1}) found under high

Table 2
Results of Kruskal-Wallis tests of internal tissue carbon and nitrogen content difference amongst nutrient treatments for each of the two *Potamogeton* species within light treatments. $P < 0.05$ denoted by '*', $P < 0.01$ by '**'.

Species	Light treatment	Response parameter	n	Kruskal-Wallis rank-sum statistic	P
P. crispus	70%	% N	20	14.3	0.002 **
P. crispus	50%	% N	20	16.1	0.001 **
P. crispus	30%	% N	20	14.8	0.002 **
P. crispus	10%	% N	18	10.8	0.013 *
P. ochreatus	70%	% N	20	14.7	0.002 **
P. ochreatus	50%	% N	20	16.2	0.001 **
P. ochreatus	30%	% N	20	16.1	0.001 **
P. ochreatus	10%	% N	18	13.4	0.004 **
P. crispus	70%	% C	20	6.9	0.075
P. crispus	50%	% C	20	9.2	0.027 *
P. crispus	30%	% C	20	3.4	0.34
P. crispus	10%	% C	18	1.7	0.64
P. ochreatus	70%	% C	20	14.4	0.002 **
P. ochreatus	50%	% C	20	6.4	0.093
P. ochreatus	30%	% C	20	4.3	0.23
P. ochreatus	10%	% C	18	6.2	0.10
P. crispus	70%	C:N ratio	20	14.6	0.002 **
P. crispus	50%	C:N ratio	20	16.1	0.001 **
P. crispus	30%	C:N ratio	20	14.9	0.002 **
P. crispus	10%	C:N ratio	18	11.0	0.012 *
P. ochreatus	70%	C:N ratio	20	15.6	0.001 **
P. ochreatus	50%	C:N ratio	20	16.4	0.001 **
P. ochreatus	30%	C:N ratio	20	16.2	0.001 **
P. ochreatus	10%	C:N ratio	18	13.4	0.004 **

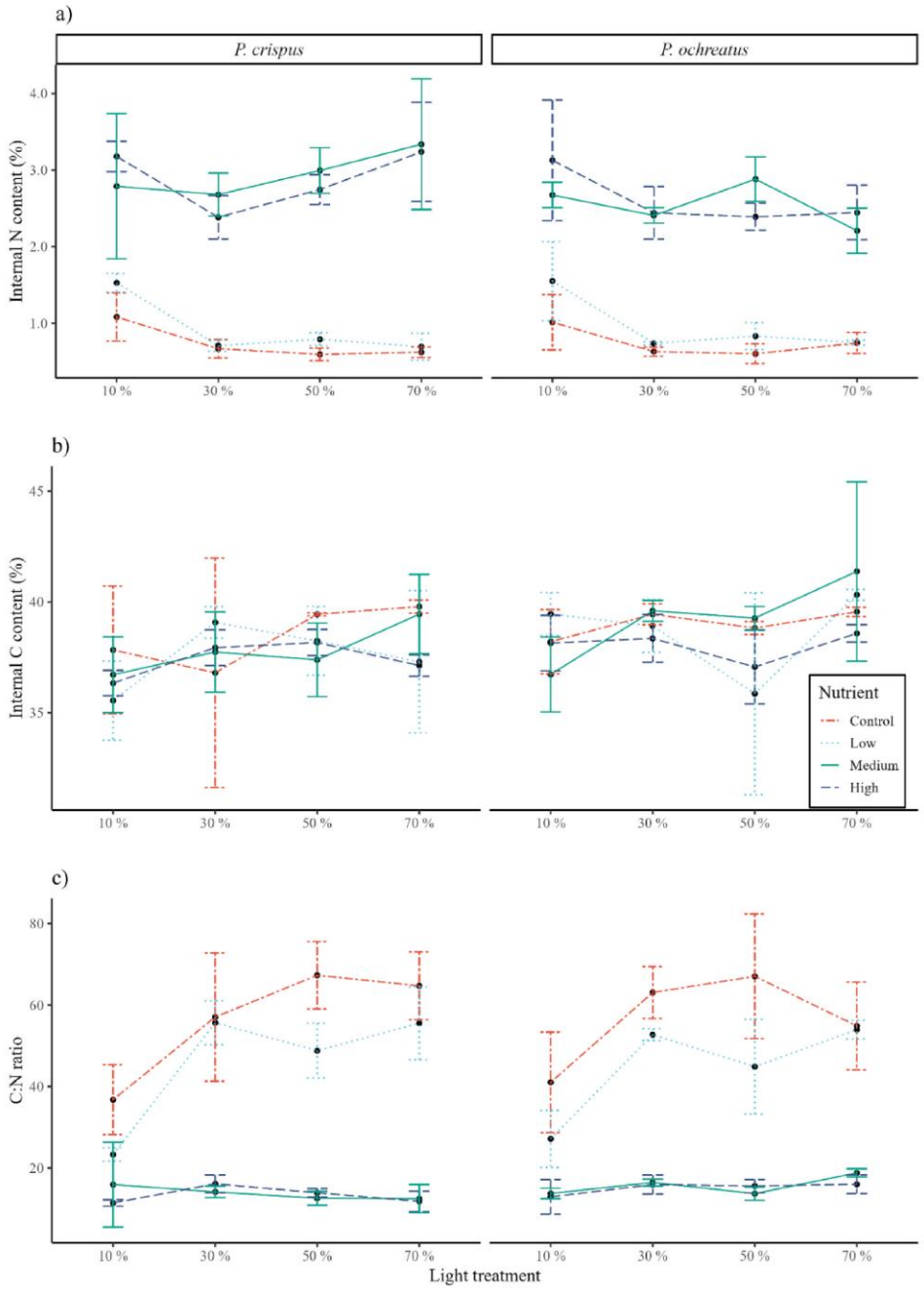

Fig. 2. Internal tissue % nitrogen (N), % carbon (C), and C:N ratio of *Potamogeton crispus* and *P. ochreatus* grown at four nutrient and light levels. Lines connect mean values between light treatments and errorbars denote standard deviation.

Table 3
Results of Kruskal-Wallis tests of internal tissue carbon and nitrogen content difference amongst light treatments for each of the two *Potamogeton* species within nutrient treatments. P < 0.05 denoted by '*', P < 0.01 b y '**'.

Species	Nutrient treatment	Response parameter	n	Kruskal-Wallis rank-sum statistic	P
P. crispus	Control	% N	19	9.4	0.02 *
P. crispus	Low	% N	19	10.8	0.01 *
P. crispus	Medium	% N	20	3.8	0.29
P. crispus	High	% N	20	11.0	0.01 *
P. ochreatus	Control	% N	20	8.0	0.05 *
P. ochreatus	Low	% N	19	9.9	0.02 *
P. ochreatus	Medium	% N	20	11.5	0.009 **
P. ochreatus	High	% N	19	2.6	0.46
P. crispus	Control	% C	19	4.7	0.20
P. crispus	Low	% C	19	6.2	0.10
P. crispus	Medium	% C	20	4.5	0.22
P. crispus	High	% C	20	13.3	0.004 **
P. ochreatus	Control	% C	20	8.9	0.03 *
P. ochreatus	Low	% C	19	7.7	0.05
P. ochreatus	Medium	% C	20	12.0	0.007 **
P. ochreatus	High	% C	19	2.5	0.47
P. crispus	Control	C:N ratio	19	9.4	0.02 *
P. crispus	Low	C:N ratio	19	10.4	0.02 *
P. crispus	Medium	C:N ratio	20	3.3	0.35
P. crispus	High	C:N ratio	20	11.3	0.01 *
P. ochreatus	Control	C:N ratio	20	8.9	0.03 *
P. ochreatus	Low	C:N ratio	19	8.8	0.03 *
P. ochreatus	Medium	C:N ratio	20	15.3	0.002 **
P. ochreatus	High	C:N ratio	19	2.8	0.43

irradiance (70%), while many individuals at low-N had negative RGR's regardless of light treatment. However, a significant difference between species was only found between means of the low-N individuals at the lowest irradiance (10% - W = 14, p = 0.04; Table 4), which was the only treatment where *P. ochreatus* individuals had negative RGR's. Both species showed a similar response trend to changes in light, by having reduced RGR's at the lowest light treatment (10%), but for the high-N plants, the significant difference was for *P. ochreatus*, while for low-N plants it was for *P. crispus* (Table 4).

P. crispus showed a significant difference between high-N and low-N in branching degree and lateral spread at the highest light treatment only (Table 5). Generally, *P. crispus* responded to increased N content with main shoot length increases (Table 5), while *P. ochreatus* responded with increases in branching degree and lateral spread (Table 5). Both species responded to increase in N content by increased root extension (Table 5) and increase in below-ground/above-ground biomass ratio (Table 5). For all traits but the below-ground/above-ground biomass ratio, no difference between high-N and low-N plants were found at the 10% light treatment. For high-N *P. crispus* plants decreasing irradiation intensity tended to decrease branching degree and lateral spread and high-N *P. ochreatus* plants had the lowest main shoot length at 10% light (Table 5). *P. ochreatus* achieved longer shoot lengths than *P. crispus* at low-N, and higher densities overall (Table 5), while *P. crispus* had generally higher branching degree and lateral spread than *P. ochreatus*, regardless of light treatment and N group (Table 5).

Table 4
RGR mean (±SD) values of High-N and Low-N groups of *Potamogeton crispus* and *P. ochreatus* at four different light treatments. Difference between N groups for each species are reported below. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxson rank sum test: W stat <10, p < 0.05). Differences of the means between light treatments within species and *N*-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.

Parameter	*N*-group	Species	Light treatment			
			70%	50%	30%	10%
RGR (d^{-1})	High-N	*P. crispus*	0.021^{a} ±0.018	0.019^{a} ±0.011	0.025^{a} ±0.013	0.006^{a} ±0.011
		P. ochreatus	0.018^{a} ±0.006	0.021^{a} ±0.006	0.017^{a} ±0.007	0.005^{b} ±0.002
	Low-N	*P. crispus*	0.003^{a} ±0.009	0.008^{a} ±0.004	0.005^{ab} ±0.011	-0.006^{b} ±0.005
		P. ochreatus	0.006^{ab} ±0.004	0.009^{a} ±0.004	0.008^{a} ±0.004	**0.000^{b}** ±0.006
Difference between N groups		*P. crispus*	W = 74	**W = 84**	**W = 65**	**W = 89**
		P. ochreatus	**W = 98**	**W = 88**	**W = 93**	W = 53

Table 5
Mean (+- SD) values for growth responses of High-N and Low-N groups of *Potamogeton crispus* and *P. ochreatus* at four different light treatments. Difference between N groups for each species are reported below each parameter. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxson rank sum test: W stat <10, $p < 0.05$). Differences of the means between light treatments within species and *N*-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.

Parameter	*N*-group	Species	% Incoming irradiation			
			70%	50%	30%	10%
Branching degree (cm ^{1})	High-N	*P. crispus*	**1.3**^{a} ±0.5	0.8^{b} ±0.4	**1.1**^{ab} ±1.1	**0.4**^{c} ±0.2
		P. ochreatus	0.6^{ab} ±0.6	1.0^{a} ±0.8	0.4^{ab} ±0.5	0.2^{b} ±0.1
	Low-N	*P. crispus*	**0.6**^{a} ±0.2	**1.1**^{a} ±0.9	**0.7**^{a} ±0.3	**0.4**^{a} ±0.2
		P. ochreatus	0.2^{a} ±0.1	0.4^{a} ±0.4	0.1^{a} ±0.0	0.1^{a} ±0.1
Difference between N groups		*P. crispus*	**W = 94**	W = 94	W = 47	W = 66
		P. ochreatus	**W = 81**	**W = 77**	W = 67	W = 48
Lateral spread (cm cm ^{1})	High-N	*P. crispus*	**6.6**^{a} ±2.6	3.9^{b} ±1.3	**4.3**^{b} ±1.5	2.5^{c} ±1.3
		P. ochreatus	3.2^{ab} ±2.2	4.8^{a} ±2.7	2.8^{ab} ±2.3	1.8^{b} ±0.6
	Low-N	*P. crispus*	**3.1**^{a} ±1.0	4.2^{a} ±2.7	**3.5**^{ab} ±1.3	2.1^{a} ±0.7
		P. ochreatus	1.6^{a} ±0.5	2.5^{a} ±1.5	1.4^{ab} ±0.4	1.8^{a} ±0.9
Difference between N groups		*P. crispus*	**W = 96**	W = 96	W = 51	W = 63
		P. ochreatus	W = 71	**W = 81**	**W = 79**	W = 39
Main shoot length (cm ^{1})	High-N	*P. crispus*	11.4^{a} ±6.7	14.9^{a} ±11.7	20.1^{a} ±12.3	9.1^{a} ±4.6
		P. ochreatus	13.0^{a} ±3.3	11.6^{a} ±1.8	14.0^{a} ±3.4	9.4^{b} ±1.6
	Low-N	*P. crispus*	7.5^{a} ±1.9	6.1^{a} ±2.0	7.2^{a} ±2.4	7.0^{a} ±3.0
		P. ochreatus	**10.6**^{a} ±1.9	**9.7**^{a} ±3.5	**11.8**^{a} ±1.0	**10.1**^{a} ±1.8
Difference between N groups		*P. crispus*	W = 72	**W = 72**	**W = 82**	W = 88
		P. ochreatus	W = 74	W = 67	W = 74	W = 25
Root length (cm)	High-N	*P. crispus*	9.6^{a} ±4.4	10.6^{a} ±5.8	13.3^{a} ±7.4	5.6^{a} ±4.1
		P. ochreatus	7.7^{ab} ±6.6	13.9^{a} ±6.1	9.6^{ab} ±6.6	5.6^{b} ±4.5
	Low-N	*P. crispus*	4.1 ±2.5	4.8 ±3.6	3.6 ±3.4	3.4 ±3.4
		P. ochreatus	4.3 ±3.0	4.7 ±3.1	4.1 ±4.5	6.2 ±4.3
Difference between N groups		*P. crispus*	W = **88**	W = 88	W = **76**	W = 91
		P. ochreatus	W = 64	**W = 88**	**W = 77**	W = 39
Density (mg cm ^{1})	High-N	*P. crispus*	0.003^{a} ±0.002	0.002^{a} ±0.001	0.002^{a} ±0.001	0.003^{a} ±0.002
		P. ochreatus	**0.004**^{a} ±0.001	**0.004**^{a} ±0.001	**0.004**^{a} ±0.001	0.004^{a} ±0.001
	Low-N	*P. crispus*	0.003^{a} ±0.001	0.003^{a} ±0.001	0.003^{a} ±0.001	0.003^{a} ±0.005
		P. ochreatus	**0.004**^{a} ±0.002	0.004^{a} ±0.001	**0.005**^{a} ±0.001	**0.003**^{a} ±0.001
Difference between N groups		*P. crispus*	W = 45	**W = 45**	W = 20	W = 49
		P. ochreatus	W = 43	W = 46	W = 38	W = 52
Below-/aboveground biomass ratio	High-N	*P. crispus*	0.63^{a} ±0.57	0.22^{a} ±0.09	0.36^{a} ±0.55	0.20^{a} ±0.23
		P. ochreatus	0.23^{a} ±0.26	0.21^{a} ±0.10	0.18^{a} ±0.16	0.27^{a} ±0.28
	Low-N	*P. crispus*	0.07^{a} ±0.09	0.05^{a} ±0.06	0.01^{a} ±0.02	0.01^{a} ±0.01
		P. ochreatus	0.05^{a} ±0.06	0.06^{a} ±0.08	0.02^{a} ±0.02	0.11^{a} ±0.19
Difference between N groups		*P. crispus*	**W = 84**	**W = 84**	**W = 76**	**W = 80**
		P. ochreatus	**W = 53**	**W = 79**	**W = 49**	**W = 53**

3.3. Photosynthetic parameters

No clear patterns emerged from the photosynthetic parameters, although in all cases of statistical difference, high-N plants had the higher trait score (Table 6). Two trends can be noticed, however; 1) high-N plants of both species tended to have higher saturation irradiance (E_k) than low-N plants, and for *P. ochreatus* at 70 and 30% light, this was reflected in a trend of a lower quantum yield (Φ); 2) high-N plants had higher maximum photosynthetic rate (P_{max}) at 10% light. Significant differences between species were only found for low-N individuals at 50% light, where *P. ochreatus* had higher P_{max} and E_k, and *P. crispus* had higher yield at PAR = 0 (Table 6).

Significant differences between light treatments were found for low-N individuals of *P. ochreatus*, that had lower P_{max} and higher Yield at PAR = 0 at 10% light compared to all other shade treatments (Table 6). *P. ochreatus* also showed difference between E_k at 70% and 10% light for high-N plants (Table 6). Kruskal-Wallis test results showed differences amongst light treatments in low-N *P. crispus* plants for E_k and Yield at PAR = 0 (P = 0.02), but none of the pairwise Wilcoxon tests were significant (Table 6).

As pigment samples had to be pooled across replicates to obtain sufficient for analysis, robust statistical comparisons were not possible. However it was evident that the content chlorophyll-a, chlorophyl-b and total carotenoids increased with N content for all light treatments in both species (Figure SI-3). The range of content of each pigment showed no difference between species.

4. Discussion

We tested differences between growth rates, morphological traits, and photosynthetic performance parameters of two species of

Table 6
Mean (+- SD) values of photosynthetic parameters from High-N and Low-N groups of *Potamogeton crispus* and *P. ochreatus* grown at four different light treatments. Difference between N groups for each species are reported below each parameter. Difference between species within N group and light treatment are shown as bold text on the highest scoring species if significant (Wilcoxson rank sum test: W stat <10, p < 0.05). Differences of the means between light treatments within species and *N*-group are shown as alphabetical operators after each mean value (i.e. ^{abc}), as tested by pairwise Wilcoxon tests.

Parameter	*N*-group	Species	% Incoming irradiation			
			70%	50%	30%	10%
Quantum yield Φ	High-N	*P. crispus*	0.013^{a} ±0.008	0.017^{a} ±0.013	0.013^{a} ±0.009	0.019^{a} ±0.015
		P. ochreatus	0.015^{a} ±0.010	0.014^{a} ±0.010	0.007^{a} ±0.007	0.014^{b} ±0.010
	Low-N	*P. crispus*	0.017^{a} ±0.000	0.009^{a} ±0.007	0.009^{a} ±0.010	0.012^{a} ±0.007
		P. ochreatus	0.028^{a} ±0.018	0.012^{a} ±0.006	0.015^{a} ±0.007	0.006^{a} ±0.005
Difference between N groups		*P. crispus*	W = 3	W = 18	W = 16	W = 25
		P. ochreatus	W = 8	W = 17	W = 7	W = 28
E_k (mmol photons $m^{-2} s^{-1}$)	High-N	P. crispus	51^{a} ±37	62^{a} ±43	66^{a} ±22	34^{a} ±13
		P. ochreatus	88^{a} ±19	53^{ab} ±27	66^{ab} ±15	42^{b} ±14
	Low-N	P. crispus	29^{a} ±0	13^{a} ±3	49^{a} ±27	20^{a} ±3
		P. ochreatus	33^{a} ±8	**41^{a}** ±23	47^{a} ±30	18^{a} ±7
Difference between N groups		*P. crispus*	W = 4	**W = 24**	W = 16	**W = 32**
		P. ochreatus	**W = 24**	W = 19	W = 20	**W = 35**
P_{max} (mmol O_2 $m^{-2} s^{-1}$)	High-N	P. crispus	0.6^{a} ±0.4	1.0^{a} ±1.0	0.8^{a} ±0.5	0.6^{a} ±0.5
		P. ochreatus	1.2^{a} ±0.8	0.7^{a} ±0.5	0.4^{a} ±0.3	0.6^{b} ±0.5
	Low-N	P. crispus	0.5^{a} ±0.0	0.1^{a} ±0.1	0.6^{a} ±0.9	0.2^{a} ±0.1
		P. ochreatus	0.8^{a} ±0.4	**0.4^{a}** ±0.3	0.8^{a} ±0.7	0.1^{b} ±0.1
Difference between N groups		*P. crispus*	W = 3	W = 22	W = 14	W = 26
		P. ochreatus	W = 16	W = 22	W = 9	**W = 32**
Yield (PS II) at PAR = 0	High-N	P. crispus	0.6^{a} ±0.1	0.6^{a} ±0.1	0.7^{a} ±0.1	0.6^{a} ±0.1
		P. ochreatus	0.6^{a} ±0.1	0.7^{a} ±0.0	0.5^{a} ±0.2	0.6^{b} ±0.1
	Low-N	P. crispus	0.7^{a} ±0.0	0.7^{a} ±0.0	0.5^{a} ±0.1	0.6^{a} ±0.0
		P. ochreatus	0.5^{a} ±0.0	**0.5^{a}** ±0.1	0.5^{a} ±0.0	0.6^{b} ±0.0
Difference between N groups		*P. crispus*	W = 5	W = 10	**W = 24**	W = 15
		P. ochreatus	W = 19	**W = 30**	W = 24	W = 15

Potamogeton, the naturalised *P. crispus* and the native *P. ochreatus,* grown under four N concentrations then split into two *N*-groups based on internal N content in combination with four irradiances.

4.1. Factors controlling rate of growth - interplay of light and nutrients

Our hypotheses were partly supported; internal N content increased with N treatment, but this did not directly correspond to external concentration; the four nitrogen treatments appeared to collapse to two. Except at the lowest irradiance, the two lowest N treatments in both species yielded % N contents of less than 1%, while at all irradiances the two highest N treatments yielded similar N contents between 2 and 3%. The internal nutrient content of a plant is widely recognised as an indicator of its growth potential, as this represents the nutrients available for production of biomass; below certain %N thresholds, the growth of the plant will be limited, or the plant will senesce [54]. These thresholds can vary between species [55], but a mean N content for 95% maximum growth and 95% maximum quantum yield for aquatic macrophytes is reported as 1.82% N and 1.14% N, respectively [see 31]. Minimum macrophyte content of N to permit growth is reported to be 0.6% [54] and in general, N contents of <1% are rare in natural freshwater macrophytes [e.g. 31]. Internal tissue concentrations above 3% is considered high for *P. crispus* [42] though Cavalli et al. [32] reports concentrations as high as 4.7% N. Furthermore, N limitation thresholds can be approximated by plotting N content against C:N ratios, and estimating when % N starts changing more so than the C:N ratio [56]. Using this approach, Cavalli et al. [32] suggests a limitation threshold of <2% for tissue N which corresponds reasonably well with our data when we plot our C:N ratios against % N (Fig. 3a) and look at the correlation between RGR and % N overall (Fig. 3b).

Large scale surveys have found that *in situ* freshwater angiosperms normally contain between 2 and 3% tissue N [33], and Demars and Edwards [31] confirmed this, citing a median N content of 2.64% in a dataset of 268 samples across 765 sites. Higher N content (and consequently lower C:N ratio) in plants grown at low light is probably due to lower nutrient demand from growth. Similar effects of other abiotic factors influencing N limitation have been found by Madsen et al. [57], who finds that low inorganic carbon concentrations lower the growth rates of two aquatic macrophytes and consequently allows an increase in their N content due to lower demand. Adding to the complexity of nutrient dynamics is the fact that the ability to maintain a high N content during growth will vary between species as they inherently vary in nutrient uptake rates, and that these rates differ for NO_3 and NH_4^+ [52]. Nevertheless, we have effectively created eutrophic and oligotrophic environments, which yield the high-N and low-N groups, and plants that are at

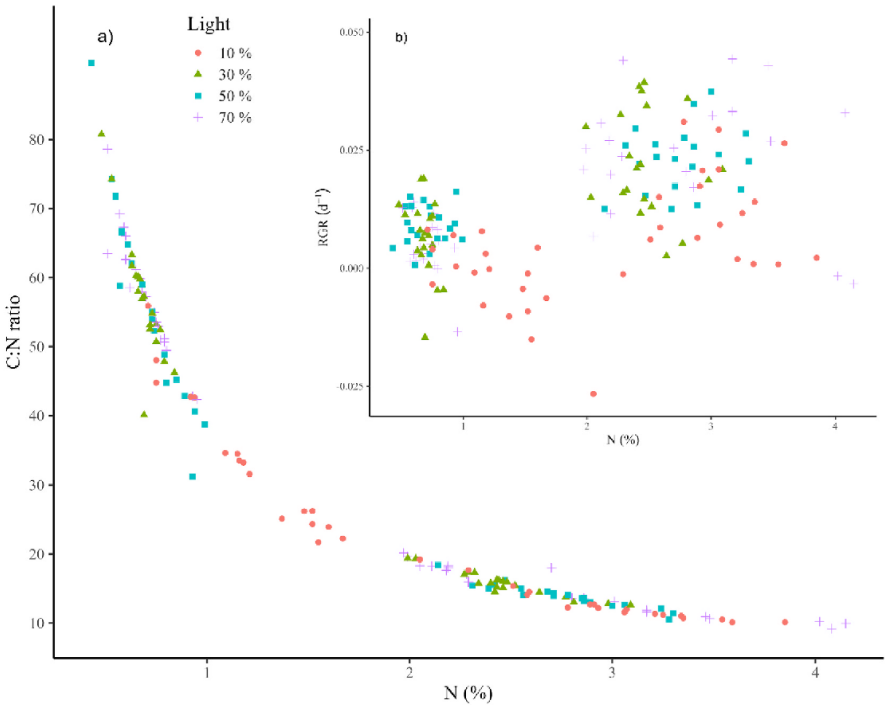

Fig. 3. a) % N content of all plants from the experiment plotted against C:N ratio for threshold analysis [56]. The inflexion point of the curve indicates onset of N saturation. And b) the relationship between % N content and RGR - a clear threshold can be witnessed at the 2% N mark.

growth saturating and extreme growth limiting nitrogen content. At water column concentrations of more than ~290 μg NO_3 L^{-1} internal N content of our plants did not scale with higher water column N and similarly, plants from ~30 μg NO_3 L^{-1} achieved minimum N content as well as those with no N supply (Fig. 2).

There was an obvious effect of nutrient content on the response parameters of the plants, in support of our second hypothesis; plant traits were generally higher for high-N plants, as were the contents of pigments. Still, we expected a larger difference between the high-N and low-N plants, particularly at the higher irradiances. We have no good explanation as to why so many of the *P. crispus* high-N plants did not attain better RGR's. Manolaki et al. [52] finds for *P. crispus* a mean RGR of 0.06 day^{-1} under non-limiting light and nutrient conditions, suggesting an inherent ability of this species to grow even faster than what was attained here. Not much research has been done on growth of *P. ochreatus*, but Cenzato and Ganf [58] finds maximum RGR of *P. ochreatus* grown in eutrophic sediment to be around 0.02 d^{-1}, comparable to our results. Both species are classified as high N loving species [45] and as such are well adapted to be able to utilize a high resource availability for growth as we see in our experiment. Indeed, pigment content for both species were high compared to previously reported values for similar plants [59–61], suggesting ample capability to upscale the photosynthetic apparatus when resource supply is high. Thus, even though evidently, our plants have not been able to perform to their maximum potential (see supplementary info for details), from the increased growth rates we can see that ecological issues like nuisance biomass will most likely occur when available N concentrations are higher, even when light is quite reduced. We find it interesting that the largest main shoot length difference was found between N groups for *P. crispus*, while *P. ochreatus* differed more in branching degree and lateral spread, when main shoot length is classified as a *C*-strategist trait, while the others are R-strategist traits [45] and thus reversed cf. the species Grime's classifications. Possibly, this is an evolutionary strategy of the plants to allocate relatively more resources into traits that fall within their Grime's classification category when resources are scarce and when resources are plenty, there is a surplus to allocate to additional traits. We had anticipated the low-N plants to increase root length more than the high-N plants, to explore the sediment as a nutrient source. A possible explanation for longer roots at high N is flow induced root biomass allocation [62].

Our third hypothesis was mostly supported, as we see no difference between N groups for most parameters at the lowest irradiance and most of the differences between irradiance levels was between 10% light and the other levels. Still, we did not expect a difference in RGR between low-N and high-N plants at this irradiance level (*P. crispus* Table 4). Although certainly light limited when compared to the other irradiance levels, 10% light high-N plants were still able to grow. Compensation irradiance points for the plants are reported as 152 ± 23 lux (~2.5 μmol photons m^{-2} s^{-1}) at 15 °C for *P. crispus* [63] and 37 μmol photons m^{-2} s^{-1} [58] for *P. ochreatus* (daily average), both of which are lower than what the plants are exposed to in our experiment. We note however, that the compensation point irradiance of the *P. ochreatus* is the ecological, while for *P. crispus* it is the instantaneous and the ecological is expected to be higher [64]. Furthermore, the conversion from lux to mol photons depends on the type of light source used and this is not stated in the experiment [63], thus the conversion made here can only ever be approximate. Thus, even at these low irradiances both species can maintain positive growth rates, but evidently shading is a constraining factor. Low irradiance has recently been shown to be important in restricting the presence and growth of invasive species [7] and can influence the plants ability to cope with adverse conditions [65]. The results of our study further exemplify the importance of the light environment and highlights the need for this to be considered when investigating the effects of nutrients. Successful strategies of plants in eutrophic streams are either to grow to the water surface for better light capture or better light utilization for tolerance of out shading [39]. As neither of the species exhibited signs of acclimation to shading at the lowest irradiance, i.e. increased quantum yield (α^B) and lower P_{max}, we see no evidence that either of the tested species were opting for the light utilization strategy although we did not test other parameters that respond to irradiance such as reduced specific leaf area [66].

We see some evidence in support of our fourth hypothesis, partly due to *P. crispus* having higher maximum RGR's and thus have the potential to outgrow *P. ochreatus*. Moreover, *P. crispus* had increases in branching degree and lateral spread only in the high light treatment when the N content was high. Furthermore, when the light was only at an intermediate level, the increase in lateral spread and branching degree was not significantly different between N groups but was different for main shoot length. Thus, although growth rates are similar, when resources are high, as we would expect to see in streams in an anthropogenic setting, we see a response of extending laterally with many branches rather than vertically, which is beneficial for dispersal (i.e. increases likelihood of fragmentation), as *P. crispus* spreads by vegetative formation in New Zealand [67]. This response is virtually absent *in P. ochreatus* which generally show the same responses to increases in N content in all but the lowest irradiance treatment. Certainly, the species that suffered the most under low light and low nutrient levels was *P. crispus*, showing the importance of ample resource supply for this species.

4.2. Implications for thresholds and management perspective

Eutrophication of stream ecosystems have consequences for macrophyte communities that then propagate through the higher trophic levels and adversely affect ecosystem services. In this study we present results showing how low NO_3–N concentrations in the water column can significantly reduce internal N content and growth rates of plants and how this can be seen across a wide range of irradiance but was less evident below 10% incident. It is important to acknowledge that the mesocosm environment does not mirror a natural setting to perfection. In our case, the plants were exposed to a continuous, stable supply of nutrients and flow rate was kept at a constant low level, creating a constant flux of nutrients from the water to the plants. In a natural setting, precipitation events will likely play an important, pulsed role in supplying N to stream plants, by washing out N from soils and diminishing boundary layer thickness. Especially for oligotrophic streams, this will, at least temporarily, provide a boost of N that can be stored in the plants and help maintain growth even though consequent stream conditions are yet again oligotrophic. In addition, internal N content of the pre

experiment plants were mostly above the 1.82% threshold for growth as determined by Demars and Edwards [31] and thus it must be assumed that this stored N has in part been available to support growth during the experiment. N uptake rates vary little between *Potamogeton* species [52], thus utilization might be the key differentiator. Further studies are required in the field of temporarily elevated N concentrations and the legacy effect they can have on plant communities.

Despite these experimental limitations, our hypotheses were able to be tested and were partly supported. Most importantly, plants grown under higher nitrate conditions sustained high % N, had higher growth rates, and higher trait scores than plants grown under lower nitrate conditions, suggesting eutrophication thresholds for nitrate-N lie somewhere between the concentrations of the low (47 $\mu g\ N\ L^{-1}$) and medium (290 $\mu g\ N\ L^{-1}$) nutrient treatments in our experiment. The current guideline trigger value for nitrate concentration in lowland streams in New Zealand is 444 $\mu g\ N\ L^{-1}$, a concentration which would offer little protection from macrophyte proliferation Mean concentrations of nitrate-N in lowland streams draining pastoral and urban areas in New Zealand are above these levels (1102 and 705 $\mu g\ N\ L^{-1}$ respectively), while that of streams draining natural catchments is 78 $\mu g\ N\ L^{-1}$ [68]. Pastoral and urban streams also had much lower ecological health scores [69], consistent with eutrophication loss of ecosystem values. There is thus little expectation that growth of existing populations of either species in lowland rivers of Aotearoa New Zealand, of which only 5% of those in Larned et al. [68]were in natural catchments, are nutrient limited.

Under conditions prevailing in the few lowland streams of Aotearoa New Zealand with minimal anthropogenic change to their catchments, water nitrate concentrations may be sufficiently low to limit the rate of growth of the two *Potamogeton* spp investigated here. In addition, lowland forest provides shading and under the combination of low nitrogen supply with a high degree of shading the native *P. ochreatus* did exhibit a higher RGR than the alien *P. crispus*. It was generally better and maintaining positive growth rates under combined nutrient and light limiting conditions. The suggestion in our results is that there is potential for the alien *P. crispus* to out-grow the native *P. ochreatus* in more eutrophic, brightly lit systems and informs the "phenotypic convergence – phenotypic divergence" paradigm in invasive species theory [70]. Our example suggests that the non-native species may be successful because it is pre-adapted to better exploit conditions prevailing in newly modified catchments, specifically increased resource supply, than pre-existing native organisms.

Our results provide some insight into setting limits to manage macrophyte proliferation in streams of Aotearoa New Zealand. They suggest that reducing resource supply could be an effective management tool to both reverse the advantage from *P. crispus* back to *P. ochreatus*, and also reduce growth rates, as could the restoration of riparian shade. However, a substantial reduction, up to tenfold in m any cases [68]in nitrate may be needed that would require significant changes to lowland catchment land use. Such socioeconomic challenges of lowland stream restoration are persistent, and a recent review of forty years of effort in the Netherlands concludes "*biophysical objectives and restoration measures, a monitoring deficiency and restoration plans neglecting large scale catchment wide effects hampered the success of ecological stream restoration. It is therefore recommended to improve the monitoring programmes accompanying restoration projects by applying a proper design, matching the relevant spatiotemporal dimensions for the ecosystem under study*" [71]. Our results support the need for large-scale catchment consideration if the underlying cases of eutrophication, macrophyte proliferation and species spread are to be addressed.

Author contribution statement

Louis Skovsholt: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tenna Riis: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Fleur Matheson; Ian Hawes: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15528.

References

[1] K.J. Collier, P.D. Champion, G.F. Croker, Patch- and reach-scale dynamics of a macrophyte-invertebrate system in a New Zealand lowland stream, Hydrobiologia 392 (2) (1999) 89–97, https://doi.org/10.1023/A:1003653717805.

[2] A. Giorgi, C. Feijoó, G. Tell, Primary producers in a Pampean stream: temporal variation and structuring role, Biodivers. Conserv. 14 (7) (2005) 1699–1718, https://doi.org/10.1007/s10531-004-0694-z.

[3] R.L. Paice, J.M. Chambers, B.J. Robson, Native submerged macrophyte distribution in seasonally-flowing, south-western Australian streams in relation to stream condition, Aquat. Sci. 79 (1) (2017) 171–185, https://doi.org/10.1007/s00027-016-0488-x.

[4] B.A. Quirino, N. Carniatto, S.M. Thomaz, M.H.F. Aleixo, R. Fugi, The amphibian macrophyte Polygonum punctatum as a temporary habitat and feeding ground for fish, Aquat. Ecol. 53 (3) (2019) 441–452, https://doi.org/10.1007/s10452-019-09700-9.

[5] K. Sand-Jensen, Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams, Freshw. Biol. 39 (4) (1998) 663–679, https://doi.org/10.1046/j.1365-2427.1998.00316.x.

[6] T. Riis, J.L. Tank, A.J. Reisinger, A. Aubenau, K.R. Roche, P.S. Levi, A. Baattrup-Pedersen, A.B. Alnoee, D. Bolster, Riverine macrophytes control seasonal nutrient uptake via both physical and biological pathways, Freshw. Biol. 65 (2) (2019) 178–192, https://doi.org/10.1111/fwb.13412.

[7] T.L. Mouton, F.E. Matheson, F. Stephenson, P.D. Champion, S. Wadhwa, M.P. Hamer, A. Catlin, T. Riis, Environmental filtering of native and non-native stream macrophyte assemblages by habitat disturbances in an agricultural landscape, Sci. Total Environ. 659 (2019) 1370–1381, https://doi.org/10.1016/j.scitotenv.2018.12.277.

[8] K. Sand-Jensen, T. Riis, O. Vestergaard, S.E. Larsen, Macrophyte decline in Danish lakes and streams over the past 100 years, J. Ecol. 88 (6) (2000) 1030–1040, https://doi.org/10.1046/j.1365-2745.2000.00519.x.

[9] K. Steffen, T. Becker, W. Herr, C. Leuschner, Diversity loss in the macrophyte vegetation of northwest German streams and rivers between the 1950s and 2010, Hydrobiologia 713 (1) (2013) 1–17, https://doi.org/10.1007/s10750-013-1472-2.

[10] L. Vilmin, J.M. Mogollon, A.H.W. Beusen, A.F. Bouwman, Forms and subannual variability of nitrogen and phosphorus loading to global river networks over the 20th century, Global Planet. Change 163 (2018) 67–85, https://doi.org/10.1016/j.gloplacha.2018.02.007.

[11] J. Hilton, M. O'Hare, M.J. Bowes, J.I. Jones, How green is my river? A new paradigm of eutrophication in rivers, Sci. Total Environ. 365 (1–3) (2006) 66–83, https://doi.org/10.1016/j.scitotenv.2006.02.055.

[12] M.T. O'Hare, A. Baattrup-Pedersen, I. Baumgarte, A. Freeman, I.D.M. Gunn, A.N. Lazar, R. Sinclair, A.J. Wade, M.J. Bowes, Responses of aquatic plants to eutrophication in rivers: a revised conceptual model, Front. Plant Sci. 9 (2018) 451, https://doi.org/10.3389/fpls.2018.00451.

[13] S.M. Haslam, River Plants; the Macrophytic Vegetation of Watercourses, 1978.

[14] F.E. Matheson, J. Quinn, C. Hickey, Review of the New Zealand Instream Plant and Nutrient Guidelines and Development of an Extended Decision Making Framework: Phases 1 and 2 Final Report, NIWA, 2012.

[15] T. Riis, B.J.F. Biggs, Hydrologic and hydraulic control of macrophyte establishment and performance in streams, Limnol. Oceanogr. 48 (4) (2003) 1488–1497, https://doi.org/10.4319/lo.2003.48.4.1488.

[16] A. Baattrup-Pedersen, E. Gothe, T. Riis, M.T. O'Hare, Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams, Sci. Total Environ. 543 (Pt A) (2016) 230–238, https://doi.org/10.1016/j.scitotenv.2015.11.027.

[17] G.M. Carr, S.A.E. Bod, H.C. Duthie, W.D. Taylor, Macrophyte biomass and water quality in Ontario rivers, J. North Am. Benthol. Soc. 22 (2) (2003) 182–193, https://doi.org/10.2307/1467991.

[18] C. Garbey, G. Thiebaut, S. Muller, Morphological plasticity of a spreading aquatic macrophyte, Ranunculus peltatus, in response to environmental variables, Plant Ecol. 173 (1) (2004) 125–137, https://doi.org/10.1023/B:VEGE.0000026336.44576.ff.

[19] S.I. Heaney, R.H. Foy, G.J.A. Kennedy, W.W. Crozier, W.C.K. O'Connor, Impacts of agriculture on aquatic systems: lessons learnt and new unknowns in Northern Ireland, Mar. Freshw. Res. 52 (1) (2001) 151–163, https://doi.org/10.1071/Mf00055.

[20] C.A. Mebane, N.S. Simon, T.R. Maret, Linking nutrient enrichment and streamflow to macrophytes in agricultural streams, Hydrobiologia 722 (1) (2014) 143–158, https://doi.org/10.1007/s10750-013-1693-4.

[21] M.T. O'Hare, R.T. Clarke, M.J. Bowes, C. Cailes, P. Henville, N. Bissett, C. McGahey, M. Neal, Eutrophication impacts on a river macrophyte, Aquat. Bot. 92 (3) (2010) 173–178, https://doi.org/10.1016/j.aquabot.2009.11.001.

[22] T. Riis, C. Lambertini, B. Olesen, J.S. Clayton, H. Brix, B.K. Sorrell, Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation? Ann. Bot. 106 (5) (2010) 813–822, https://doi.org/10.1093/aob/mcq176.

[23] A. Sosiak, Long-term response of periphyton and macrophytes to reduced municipal nutrient loading to the Bow River (Alberta, Canada), Can. J. Fish. Aquat. Sci. 59 (6) (2002) 987–1001, https://doi.org/10.1139/F02-071.

[24] D.E. Canfield, M.V. Hoyer, Influence of nutrient enrichment and light availability on the abundance of aquatic macrophytes in Florida streams, Can. J. Fish. Aquat. Sci. 45 (1988) 1467–1472.

[25] U. Kern-Hansen, F.H. Dawson, The standing crop of aquatic plants in lowland streams in Denmark and the interrelationships of nutrients in plant, sediment, and water, in: Proc. EWRS 5th Symp, on Aquatic Weeds, 1978.

[26] F.A. Kouwe, Phosphorous removal in a sewage treatment plant and plant biomass in an effluent recieving lowland stream, Hydrol. Bull. 17 (2) (1983) 143–156.

[27] D. Levesque, C. Hudon, P.M.A. James, P. Legendre, Environmental factors structuring benthic primary producers at different spatial scales in the St. Lawrence River (Canada), Aquat. Sci. 79 (2) (2016) 345–356, https://doi.org/10.1007/s00027-016-0501-4.

[28] T.V. Madsen, N. Cedergreen, Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream, Freshw. Biol. 47 (2) (2002) 283–291, https://doi.org/10.1046/j.1365-2427.2002.00802.x.

[29] P. Millard, The accumulation and storage of nitrogen by herbaceous plants, Plant Cell Environ. 11 (1) (1988) 1–8, https://doi.org/10.1111/j.1365-3040.1988.tb01769.x.

[30] S. Gusewell, M. Koerselman, Variation in nitrogen and phosphorus concentrations of wetland plants, Perspect. Plant Ecol. Evol. Systemat. 5 (1) (2002) 37–61, https://doi.org/10.1078/1433-8319-0000022.

[31] B.O.L. Demars, A.C. Edwards, Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply, Freshw. Biol. 52 (11) (2007) 2073–2086, https://doi.org/10.1111/j.1365-2427.2007.01817.x.

[32] G. Cavalli, A. Baattrup-Pedersen, T. Riis, Nutrient availability and nutrient use efficiency in plants growing in the transition zone between land and water, Plant Biol. 18 (2) (2016) 301–306, https://doi.org/10.1111/plb.12397.

[33] C.M. Duarte, Nutrient concentration of aquatic plants - patterns across species, Limnol. Oceanogr. 37 (4) (1992) 882–889, https://doi.org/10.4319/lo.1992.37.4.0882.

[34] P. Lacoul, B. Freedman, Environmental influences on aquatic plants in freshwater ecosystems, Environ. Rev. 14 (2) (2006) 89–136, https://doi.org/10.1139/A06-001.

[35] G. Bornette, S. Puijalon, Response of aquatic plants to abiotic factors: a review, Aquat. Sci. 73 (1) (2010) 1–14, https://doi.org/10.1007/s00027-010-0162-7.

[36] R. Hrivnak, J. Kochjarova, H. Otahelova, P. Palove-Balang, M. Slezak, P. Slezak, Environmental drivers of macrophyte species richness in artificial and natural aquatic water bodies – comparative approach from two central European regions, Annales de Limnologie - Int. J. Limnol. 50 (4) (2014) 269–278, https://doi.org/10.1051/limn/2014020.

[37] N.F. Caraco, J.J. Cole, Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river, Ecol. Appl. 12 (5) (2002) 1496–1509, https://doi.org/10.2307/3099987.

[38] A. Baczyk, M. Wagner, T. Okruszko, M. Grygoruk, Influence of technical maintenance measures on ecological status of agricultural lowland rivers - systematic review and implications for river management, Sci. Total Environ. 627 (2018) 189–199, https://doi.org/10.1016/j.scitotenv.2018.01.235.

[39] A. Baattrup-Pedersen, E. Gothe, S.E. Larsen, M. O'Hare, S. Birk, T. Riis, N. Friberg, Plant trait characteristics vary with size and eutrophication in European lowland streams, J. Appl. Ecol. 52 (6) (2015) 1617–1628, https://doi.org/10.1111/1365-2664.12509.

[40] H. Ellenberg, U. Arndt, R. Bretthauer, B. Ruthsatz, L. Steubing, Biological Monitoring. Signals from the Environment, 1991.
[41] P. Champion, D. Rowe, B. Smith, R. Wells, C. Kilroy, M. de Winton, Freshwater Pests of New Zealand, NIWA, Wellington, 2013.
[42] B.R. Bolduan, G.C. Van Eeckhout, H.W. Quade, J.E. Gannon, Potamogeton crispus– the other invader, Lake Reservoir Manag. 10 (2) (1994) 113–125, https://doi.org/10.1080/07438149409354182.
[43] B. Kronvang, E. Jeppesen, D.J. Conley, M. Sondergaard, S.E. Larsen, N.B. Ovesen, J. Carstensen, Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and coastal waters, J. Hydrol. 304 (1–4) (2005) 274–288, https://doi.org/10.1016/j.jhydrol.2004.07.035.
[44] Champion, P. and D. Hofstra. Potamogeton crispus Fact Sheet (continuously updated). New Zealand Plant Conservation Network. [Web page] [cited 2021 October]; Available from: https://www.nzpcn.org.nz/flora/species/potamogeton-crispus/.
[45] R. Riis, B.J.F. Biggs, Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply - a synthesis and conceptual model, N. Z. J. Mar. Freshw. Res. 35 (2) (2001) 255–267, https://doi.org/10.1080/00288330.2001.9516996.
[46] de Lange, P.J. Potamogeton ochreatus Fact Sheet (content continuously updated). New Zealand Plant Conservation Network. [cited 2021 October]; Available from: https://www.nzpcn.org.nz/flora/species/potamogeton-ochreatus/.
[47] T. Barker, K.O.C. Hatton, L. Connor, B. Moss, Effects of nitrate load on submerged plant biomass and species richness: results of a mesocosm experiment, Fundamental and Applied Limnology 173 (2) (2008) 89–100, https://doi.org/10.1127/1863-9135/2008/0173-0089.
[48] R. Michael Smart, J.W. Barko, Laboratory culture of submersed freshwater macrophytes on natural sediments, Aquat. Bot. 21 (3) (1985) 251–263, https://doi.org/10.1016/0304-3770(85)90053-1.
[49] C. Ellawala Kankanamge, F.E. Matheson, T. Riis, Shading constrains the growth of invasive submerged macrophytes in streams, Aquat. Bot. 158 (2019), https://doi.org/10.1016/j.aquabot.2019.06.004.
[50] S. Beer, M. Bjork, Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry, Aquat. Bot. 66 (2000) 69–76.
[51] A.D. Jassby, T. Platt, Mathematical formulation of the relationship between photosynthesis and light for phytoplankton, Limnol. Oceanogr. 21 (4) (1976) 540–547.
[52] P. Manolaki, M.B. Mouridsen, E. Nielsen, A. Olesen, S.M. Jensen, T.L. Lauridsen, A. Baattrup-Pedersen, B.K. Sorrell, T. Riis, A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms, J. Environ. Manag. 274 (2020), 111181, https://doi.org/10.1016/j.jenvman.2020.111181.
[53] H.K. Lichtenthaler, Chlorophylls and carotenoids - pigments of photosynthetic biomembranes, Methods Enzymol. 148 (1987) 350–382.
[54] G.E. Hutchinson, A Treatise on Limnology: Limnological Botany, vol. 3, Wiley-Interscience, 1975.
[55] G.C. Gerloff, Nutritional Ecology of Nuisance Aquatic Plants, National Environmental Research Center, Office of Research and Development, 1975.
[56] C.M. Duarte, Seagrass nutrient content, Mar. Ecol. Prog. Ser. 67 (2) (1990) 201–207, https://doi.org/10.3354/meps067201.
[57] T.V. Madsen, P. Hahn, J. Johansen, Effects of inorganic carbon supply on the nitrogen requirement of two submerged macrophytes, Elodea canadensis and Callitriche cophocarpa, Aquat. Bot. 62 (2) (1998) 95–106, https://doi.org/10.1016/S0304-3770(98)00087-4.
[58] D. Cenzato, G. Ganf, A comparison of growth responses between two species of Potamogeton with contrasting canopy architecture, Aquat. Bot. 70 (1) (2001) 53–66, https://doi.org/10.1016/S0304-3770(00)00143-1.
[59] W. Huang, S. Han, Q. Zhou, W. Li, W. Xing, Assessing interactions between environmental factors and aquatic toxicity: influences of dissolved CO(2) and light on Cd toxicity in the aquatic macrophyte Potamogeton crispus, Aquat. Toxicol. 212 (2019) 247–258, https://doi.org/10.1016/j.aquatox.2019.05.005.
[60] S. Jana, M.A. Choudhuri, Changes occurring during aging and senescence in a submerged aquatic angiosperm (Potamogeton-Pectinatus), Physiol. Plantarum 55 (3) (1982) 356–360, https://doi.org/10.1111/j.1399-3054.1982.tb00305.x.
[61] S.Q. Su, Y.M. Zhou, J.G. Qin, W.Z. Yao, Z.H. Ma, Optimization of the method for chlorophyll extraction in aquatic plants, J. Freshw. Ecol. 25 (4) (2010) 531–538, https://doi.org/10.1080/02705060.2010.9664402.
[62] S. Puijalon, J.P. Lena, G. Bornette, Interactive effects of nutrient and mechanical stresses on plant morphology, Ann. Bot. 100 (6) (2007) 1297–1305, https://doi.org/10.1093/aob/mcm226.
[63] H. Zhou, J. Ren, X. Cai, The concept and measurement of diurnal light compensation point of submerged macrophyte, Acta Sci. Circumstantiae 17 (1997) 256–258.
[64] T.J. Givnish, Adaptation to sun and shade - a whole-plant perspective, Aust. J. Plant Physiol. 15 (1–2) (1988) 63–92, https://doi.org/10.1071/Pp9880063.
[65] T. Cao, P. Xie, L.Y. Ni, M. Zhang, J. Xu, Carbon and nitrogen metabolism of an eutrophication tolerative macrophyte, Potamogeton crispus, under NH4+ stress and low light availability, Environ. Exp. Bot. 66 (1) (2009) 74–78, https://doi.org/10.1016/j.envexpbot.2008.10.004.
[66] L. Wang, X. Wang, X. Han, Y. Gao, B. Liu, X. Zhang, G. Wang, Potamogeton crispus responses to varying water depth in morphological plasticity and physiological traits, Environ. Sci. Pollut. Res. Int. 28 (4) (2021) 4253–4261, https://doi.org/10.1007/s11356-020-10806-z.
[67] D.E. Hofstra, K.D. Adam, J.S. Clayton, Isozyme variation in new-zealand populations of Myriophyllum and Potamogeton species, Aquat. Bot. 52 (1–2) (1995) 121–131, https://doi.org/10.1016/0304-3770(95)00494-K.
[68] S.T. Larned, T. Snelder, M.J. Unwin, G.B. McBride, Water quality in New Zealand rivers: current state and trends, N. Z. J. Mar. Freshw. Res. 50 (3) (2016) 389–417, https://doi.org/10.1080/00288330.2016.1150309.
[69] S.T. Larned, J. Moores, J. Gadd, B. Baillie, M. Schallenberg, Evidence for the effects of land use on freshwater ecosystems in New Zealand, N. Z. J. Mar. Freshw. Res. 54 (3) (2019) 551–591, https://doi.org/10.1080/00288330.2019.1695634.
[70] A. Ordonez, I.J. Wright, H. Olff, Functional differences between native and alien species: a global-scale comparison, Funct. Ecol. 24 (6) (2010) 1353–1361, https://doi.org/10.1111/j.1365-2435.2010.01739.x.
[71] P.C. Dos Reis Oliveira, H.G. van der Geest, M.H.S. Kraak, J.J. Westveer, R.C.M. Verdonschot, P.F.M. Verdonschot, Over forty years of lowland stream restoration: lessons learned? J. Environ. Manag. 264 (2020), 110417 https://doi.org/10.1016/j.jenvman.2020.110417.

Supplementary information for Growth response to nitrate enrichment helps facilitate success of an alien *Potamogeton* in New Zealand streams.

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To assess trace element concentrations we took water samples from the mesocosm flumes and had them analysed for a range of dissolved metals, chloride, sulphate, and dissolved inorganic carbon. Samples were taken on 28/01/2020 and were shipped to R J Hill Laboratories Ltd in Hamilton, New Zealand for analysis. Procedural details and detection limits can be found in Table 1. Results are reported in Table 2.

A detailed description of the statistics can be found in the main text.

Distribution maps were obtained from Global Biodiversity Information Centre's webpage (gbic.org), accessed on 28/10/2021. We realise this might not cover all observations of the two species, but the maps clearly indicate both species can be found throughout the country, even though local or regional distributions might differ. Maps are shown in Figure 1.

Daily irradiation data was obtained from a climate station located at the experimental facility. Data was obtained as daily MJ m^{-2}, and recalculated to photosynthetically active radiation (PAR) µmol m^{-2} s^{-1} by $\frac{x}{86400\ s\ d^{-1}} * 1000000 * 4.6\ mol\ J^{-1}$. Data is shown in Figure 2.

Table S1: Brief description of the methods used for water samples analysis with detection limits for each test. The detection limits given are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples if insufficient amount of sample was available, or if the matrix required that dilutions be performed during analysis (not reported).

Individual Tests	**Method description**	**Detection limit**
Filtration, Unpreserved	Sample filtration through 0.45µm membrane filter.	
Filtration for dissolved metals analysis	Sample filtration through 0.45µm membrane filter and preservation with nitric acid. APHA 3030 B 23rd ed. 2017.	
Dissolved Boron	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.005 mg l^{-1}
Dissolved Calcium	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.05 mg l^{-1}
Dissolved Copper	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.0005 mg l^{-1}
Dissolved Iron	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.02 mg l^{-1}
Dissolved Magnesium	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.02 mg l^{-1}
Dissolved Manganese	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.0005 mg l^{-1}
Dissolved Molybdenum	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.0002 mg l^{-1}
Dissolved Potassium	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.05 mg l^{-1}
Dissolved Sodium	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.02 mg l^{-1}
Dissolved Zinc	Filtered sample, ICP-MS, trace level. APHA 3125 B 23rd ed. 2017.	0.0010 mg l^{-1}
Chloride	Filtered sample, Ion Chromatography. APHA 4110 B (modified) 23rd ed. 2017.	0.5 mg l^{-1}

Sulphate	Filtered sample, Ion Chromatography. APHA 4110 B (modified) 23rd ed. 2017	0.5 mg l^{-1}
Dissolved Inorganic Carbon	Filtered sample, Supercritical persulphate oxidation, IR detection. APHA 5310 C (modified) 23rd ed. 2017.	0.35 mg l^{-1}

Table S2: Dissolved constituents of water samples taken at both ends of the mesocosm flumes (n = 1).

		Dissolved												
		Boron	Calcium	Copper	Iron	Magnesium	Manganese	Molybdenum	Potassium	Sodium	Zinc	Inorganic Carbon	Chloride	Sulphate
Treatment	**Position in flume**	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
Control	Upstream	0.35	29	0.0012	< 0.02	11.7	0.0086	0.0025	12.5	38	0.0032	22	48	40
Control	Downstream	0.35	28	0.0014	< 0.02	11.4	0.0141	0.0025	12.4	38	0.0027	23	49	40
Low	Upstream	0.36	27	0.0012	< 0.02	11.4	0.032	0.0026	6.2	18.3	0.0072	9.8	46	40
Low	Downstream	0.37	26	0.0013	< 0.02	11.3	0.034	0.0029	6	17.9	0.0075	10.8	46	39
Medium	Upstream	0.37	26	0.0021	< 0.02	11.3	0.045	0.0028	5.3	16.2	0.0066	8.2	46	39
Medium	Downstream	0.37	26	0.0019	< 0.02	11.3	0.047	0.0024	5.1	15.9	0.0069	8.8	46	40
High	Upstream	0.35	25	0.0015	< 0.02	10.9	0.0047	0.0033	6.1	25	0.006	9.6	46	39
High	Downstream	0.37	26	0.0017	< 0.02	11.2	0.0053	0.0027	6.2	26	0.0059	9.7	45	39

Table SI-3: Results of Wilcoxon test of difference in internal carbon and nitrogen response parameters between species, within nutrient and light treatment (see Fig 1 main text). P values < 0.05 denoted by '*', P < 0.01 by '**'.

Nutrient treatment	Shade treatment	Response	n, crispus	n, ochreatus	W statistic	P	
Control	70%	% N	5	5	8	0.42	
Control	50%	% N	5	5	10	0.67	
Control	30%	% N	5	5	16.5	0.46	
Control	10%	% N	4	5	12.5	0.62	
Control	70%	% C	5	5	18	0.31	
Control	50%	% C	5	5	25	0.008	**
Control	30%	% C	5	5	8	0.42	
Control	10%	% C	4	5	11	0.91	
Control	70%	C:N ratio	5	5	17	0.42	
Control	50%	C:N ratio	5	5	15	0.69	
Control	30%	C:N ratio	5	5	9	0.55	
Control	10%	C:N ratio	4	5	7	0.56	
Low	70%	% N	5	5	9.5	0.6	
Low	50%	% N	5	5	9	0.55	
Low	30%	% N	5	5	5	0.14	
Low	10%	% N	4	4	10.5	0.56	
Low	70%	% C	5	5	2.5	0.05	*
Low	50%	% C	5	5	16	0.55	
Low	30%	% C	5	5	15	0.69	
Low	10%	% C	4	4	0	0.03	*
Low	70%	C:N ratio	5	5	15	0.69	
Low	50%	C:N ratio	5	5	17	0.42	
Low	30%	C:N ratio	5	5	20	0.15	
Low	10%	C:N ratio	4	4	4	0.34	

Medium	70%	% N	5	5	22	0.06	
Medium	50%	% N	5	5	15	0.69	
Medium	30%	% N	5	5	20	0.15	
Medium	10%	% N	5	5	20	0.15	
Medium	70%	% C	5	5	10	0.69	
Medium	50%	% C	5	5	1	0.02	*
Medium	30%	% C	5	5	3	0.06	
Medium	10%	% C	5	5	10	0.69	
Medium	70%	C:N ratio	5	5	3	0.06	
Medium	50%	C:N ratio	5	5	8	0.42	
Medium	30%	C:N ratio	5	5	2	0.03	*
Medium	10%	C:N ratio	5	5	5	0.15	
High	70%	% N	5	5	23	0.04	*
High	50%	% N	5	5	23	0.03	*
High	30%	% N	5	5	11.5	0.92	
High	10%	% N	5	4	8	0.73	
High	70%	% C	5	5	0	0.008	**
High	50%	% C	5	5	17	0.42	
High	30%	% C	5	5	10	0.69	
High	10%	% C	5	4	1	0.03	*
High	70%	C:N ratio	5	5	2	0.03	*
High	50%	C:N ratio	5	5	5	0.15	
High	30%	C:N ratio	5	5	12	1	
High	10%	C:N ratio	5	4	11	0.91	

Table SI-4: Kruskal Wallis and post-hoc pairwise Wilcoxon test results of differences of growth, morphological and photosynthetic parameters between light treatments within two species of genus *Potamogeton* and their N group.

	Species	*P. crispus*		*P. ochreatus*	
	N group	Low-N	High-N	Low-N	High-N
RGR	*Kruskal Wallis Chi-squared*	14	6	12	13
	P value	**0.003**	0.09	**0.009**	**0.004**
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.21	0.97	0.16	0.43
70 % - 30 %	*P value*	0.62	0.97	0.51	0.43
70 % - 10 %	*P value*	**0.03**	0.16	0.06	**0.001**
50 % - 30 %	*P value*	0.62	0.24		0.18
50 % - 10 %	*P value*	**0.003**	0.09	**0.02**	**0.001**
30 % - 10 %	*P value*	0.06	0.04	**0.02**	**0.001**
Main shoot length	*Kruskal Wallis Chi-squared*	2.2	5.6	5	17
	P value	0.53	0.1	0.2	**0.001**
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.67	0.62	0.88	0.37
70 % - 30 %	*P value*	0.89	0.36	0.28	0.62
70 % - 10 %	*P value*	0.89	0.49	0.88	**0.007**
50 % - 30 %	*P value*	0.89	0.46	0.28	0.11
50 % - 10 %	*P value*	0.89	0.46	0.97	**0.01**
30 % - 10 %	*P value*	0.89	0.17	0.18	**0.003**
Root length	*Kruskal Wallis Chi-squared*	1.2	7.8	1.4	9.7
	P value	0.76	0.05	0.69	**0.02**

Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.78	0.71	0.85	0.12
70 % - 30 %	*P value*	0.78	0.43	0.78	0.62
70 % - 10 %	*P value*	0.78	0.15	0.78	0.32
50 % - 30 %	*P value*	0.78	0.57	0.78	0.32
50 % - 10 %	*P value*	0.78	0.15	0.78	**0.03**
30 % - 10 %	*P value*	0.79	0.08	0.78	0.14
Branching degree	*Kruskal Wallis Chi-squared*	8.8	20	7.2	11
	P value	**0.03**	**0.0001**	0.07	**0.01**
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.16	**0.05**	0.29	0.18
70 % - 30 %	*P value*	0.39	0.07	0.18	0.37
70 % - 10 %	*P value*	0.25	**0.002**	0.74	0.15
50 % - 30 %	*P value*	0.37	0.68	0.17	0.12
50 % - 10 %	*P value*	0.07	**0.01**	0.29	**0.01**
30 % - 10 %	*P value*	0.16	**0.003**	0.18	1.00
Lateral spread	*Kruskal Wallis Chi-squared*	7.8	19	4.8	9.8
	P value	0.05	**0.0003**	0.2	**0.02**
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.52	**0.02**	0.52	0.24
70 % - 30 %	*P value*	0.69	**0.02**	0.37	0.68
70 % - 10 %	*P value*	0.09	**0.005**	0.90	0.18
50 % - 30 %	*P value*	0.79	0.62	0.33	0.18
50 % - 10 %	*P value*	0.09	**0.02**	0.53	**0.02**
30 % - 10 %	*P value*	0.09	**0.02**	0.33	0.25

Below-/aboveground biomass ratio	*Kruskal Wallis Chi-squared*	1	8.1	0.08	1.1
	P value	0.8	**0.04**	1.0	0.78
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.89	0.09	1.00	0.88
70 % - 30 %	*P value*	0. 89	0.09	1.00	0.88
70 % - 10 %	*P value*	0. 89	0.09	1.00	0.88
50 % - 30 %	*P value*	0. 89	0.57	1.00	0.88
50 % - 10 %	*P value*	0. 89	0.35	1.00	0.88
30 % - 10 %	*P value*	0. 89	0.48	1.00	0.88
Density	*Kruskal Wallis Chi-squared*	8	2	6.1	2.2
	P value	**0.05**	0.6	0.1	0.5
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.17	0.78	0.40	0.75
70 % - 30 %	*P value*	0.72	0.78	0.28	0.89
70 % - 10 %	*P value*	0.11	0.89	0.43	0.75
50 % - 30 %	*P value*	0.88	0.78	0.86	0.75
50 % - 10 %	*P value*	0.08	0.83	0.23	0.75
30 % - 10 %	*P value*	0.15	0.78	0.23	0.75
Maximum photosynthetic rate	*Kruskal Wallis Chi-squared*	3.2	1.1	13	5.6
	P value	0.36	0.77	**0.01**	0.13
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	1	0.96	0.17	0.26
70 % - 30 %	*P value*	1	0.96	0.73	0.26
70 % - 10 %	*P value*	0.99	0.96	**0.02**	0.26
50 % - 30 %	*P value*	1	0.96	0.82	0.33

50 % - 10 %	*P value*	0.86	0.96	**0.01**	0.66
30 % - 10 %	*P value*	1	0.96	**0.02**	0.64
Saturation irradiance	*Kruskal Wallis Chi-squared*	9.3	5.0	5.6	11
	P value	**0.02**	0.17	0.13	**0.01**
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.5	0.66	0.91	0.10
70 % - 30 %	*P value*	0.5	0.66	0.91	0.12
70 % - 10 %	*P value*	0.5	0.78	0.19	**0.01**
50 % - 30 %	*P value*	0.2	0.77	0.91	0.37
50 % - 10 %	*P value*	0.2	0.66	0.19	0.66
30 % - 10 %	*P value*	0.2	**0.04**	0.19	0.09
Yield at PAR = 0	*Kruskal Wallis Chi-squared*	9.5	2.2	12	2.2
	P value	**0.02**	0.5	**0.009**	0.5
Pairwise Wilcoxon test					
70 % - 50 %	*P value*	0.6	1	1	0.93
70 % - 30 %	*P value*	0.6	1	1	0.93
70 % - 10 %	*P value*	0.67	0.67	**0.02**	0.93
50 % - 30 %	*P value*	0.2	1	1	0.57
50 % - 10 %	*P value*	0.11	0.67	**0.02**	0.93
30 % - 10 %	*P value*	0.11	0.67	**0.01**	0.93
Quantum yield	*Kruskal Wallis Chi-squared*	1.3	1.3	7.8	3.3
	P value	0.74	0.72	0.05	0.35
Pairwise Wilcoxon test					

70 % - 50 %	*P value*	0.5	0.96	0.26	1
70 % - 30 %	*P value*	1	0.80	0.26	0.13
70 % - 10 %	*P value*	0.67	0.28	0.04	0.70
50 % - 30 %	*P value*	1	0.51	0.82	0.31
50 % - 10 %	*P value*	0.79	0.69	0.09	0.79
30 % - 10 %	*P value*	1	0.46	**0.04**	0.18

Figure SI-1: Distribution of *Potamogeton crispus* and *P. ochreatus* in Aotearoa New Zealand by observations from Global biodiversity Information centre (gbif.org). Accessed on 28/10/2021.

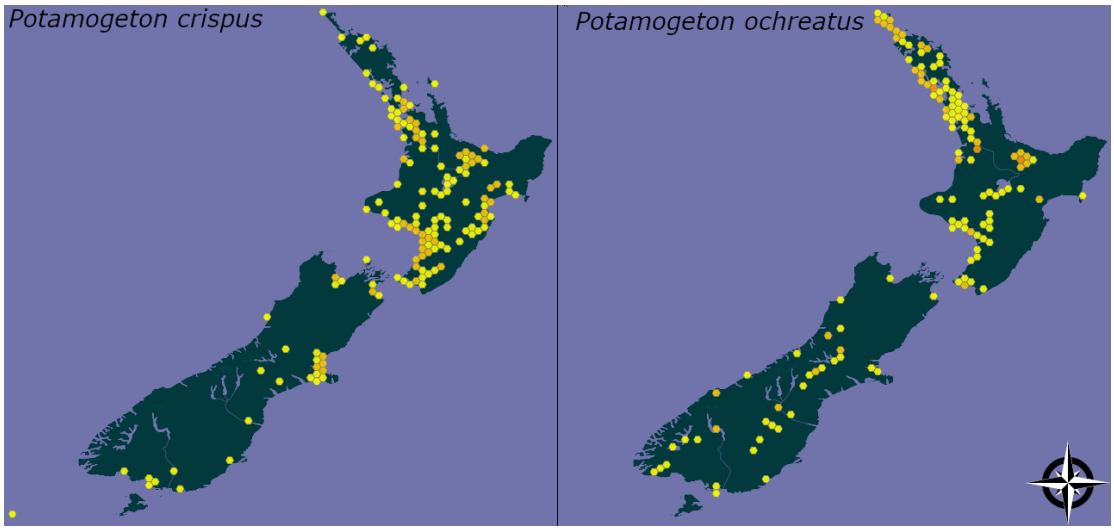


Figure SI-2: Average daily incoming irradiation at the climate station at the experimental facility in the experiment period given as photosynthetically active radiation (PAR). Shading cloth absorption (30 – 90 %) was applied to unshaded values (white bars) and shown by grey intensity with dashed white lines showing the mean.

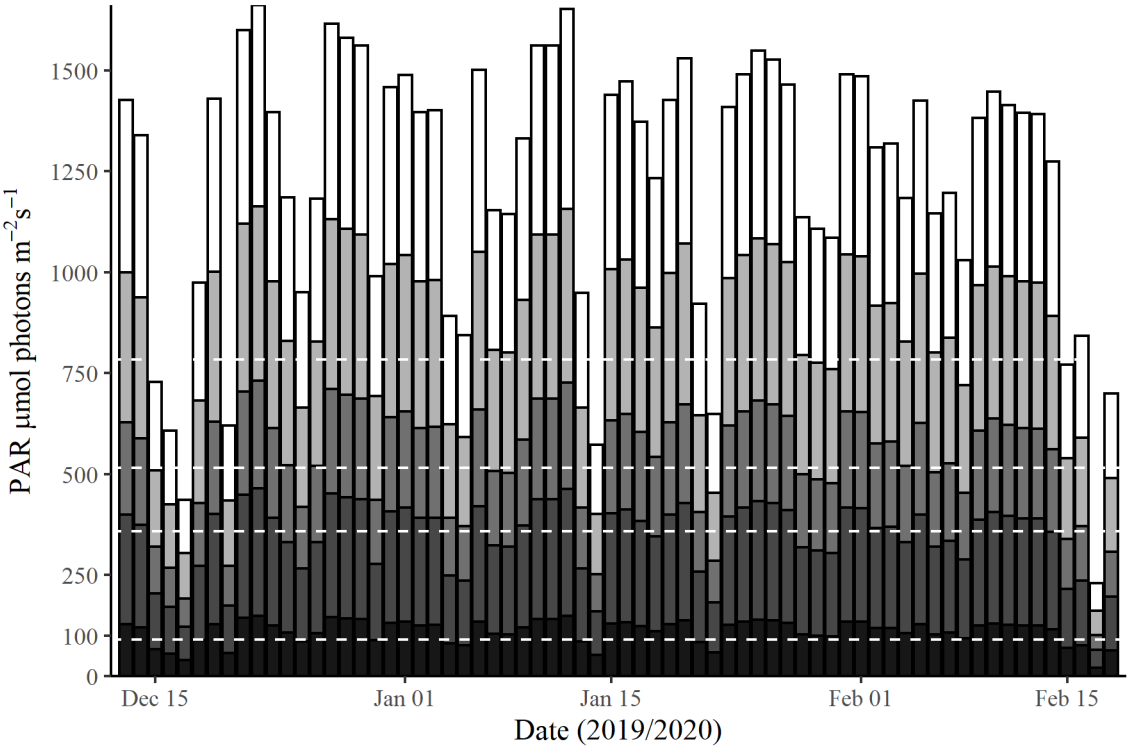
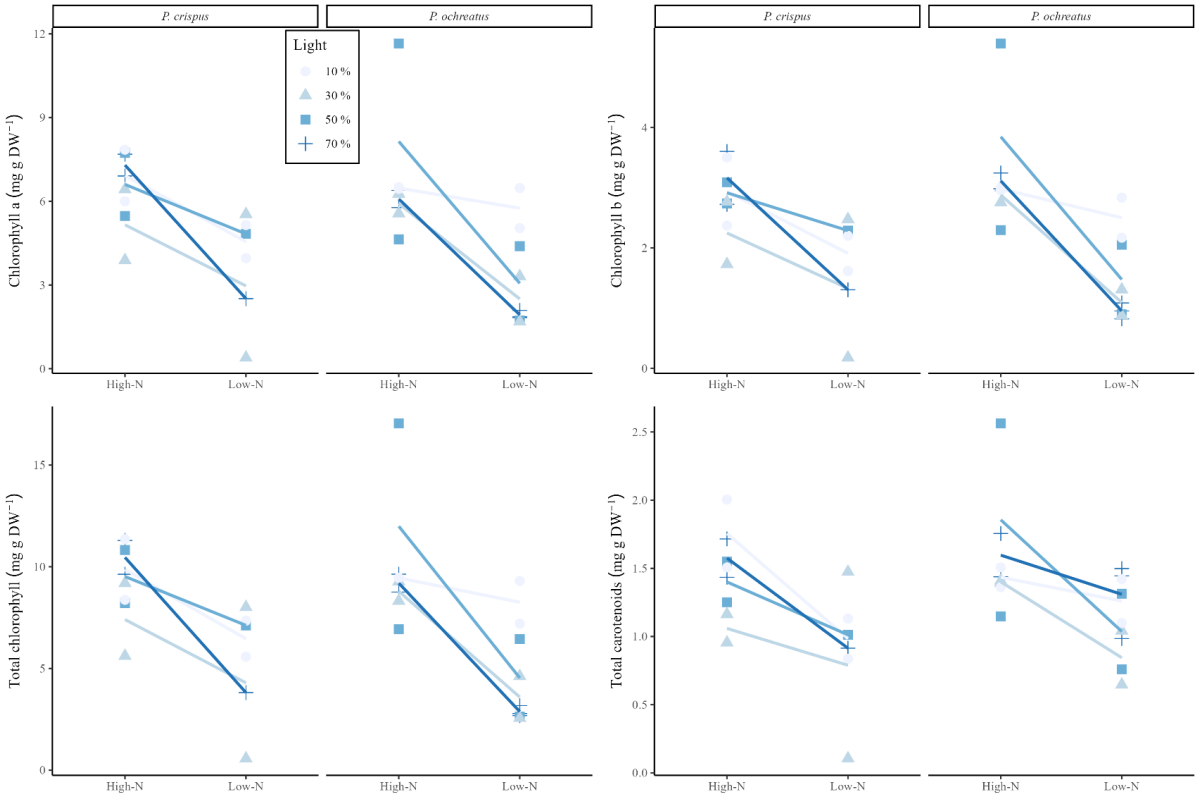


Figure SI-3 – Pigment content of *Potamogeton crispus* and *P. ochreatus* by N content-groups and light treatment. Trendlines depict change in average pigment content between N-groups (n = 2 pr shade treatment, except *P. crispus* at low-N where for 70 % and 50 % n = 1). Note that colour intensity indicates light treatment, where less intense = lower incoming irradiation.



References

Barko, J. W. & James, W. F. (1998). Effects of Submerged Aquatic Macrophytes on Nutrient Dynamics, Sedimentation, and Resuspension. In E. Jeppesen, M. Søndergaard, & K. S. Christoffersen (Eds.), *The Structuring Role of Submerged Macrophytes in Lakes* (Vol. 131): Springer Science & Business Media.

Appendix III

Figure A.3.1 – Time series of concentrations of **a)** dissolved inorganic nitrogen (DIN), and **b)** dissolved reactive phosphorous (DRP) during the experiment in the four flumes. Moving average (sample + 2 previous samples) as broken lines.

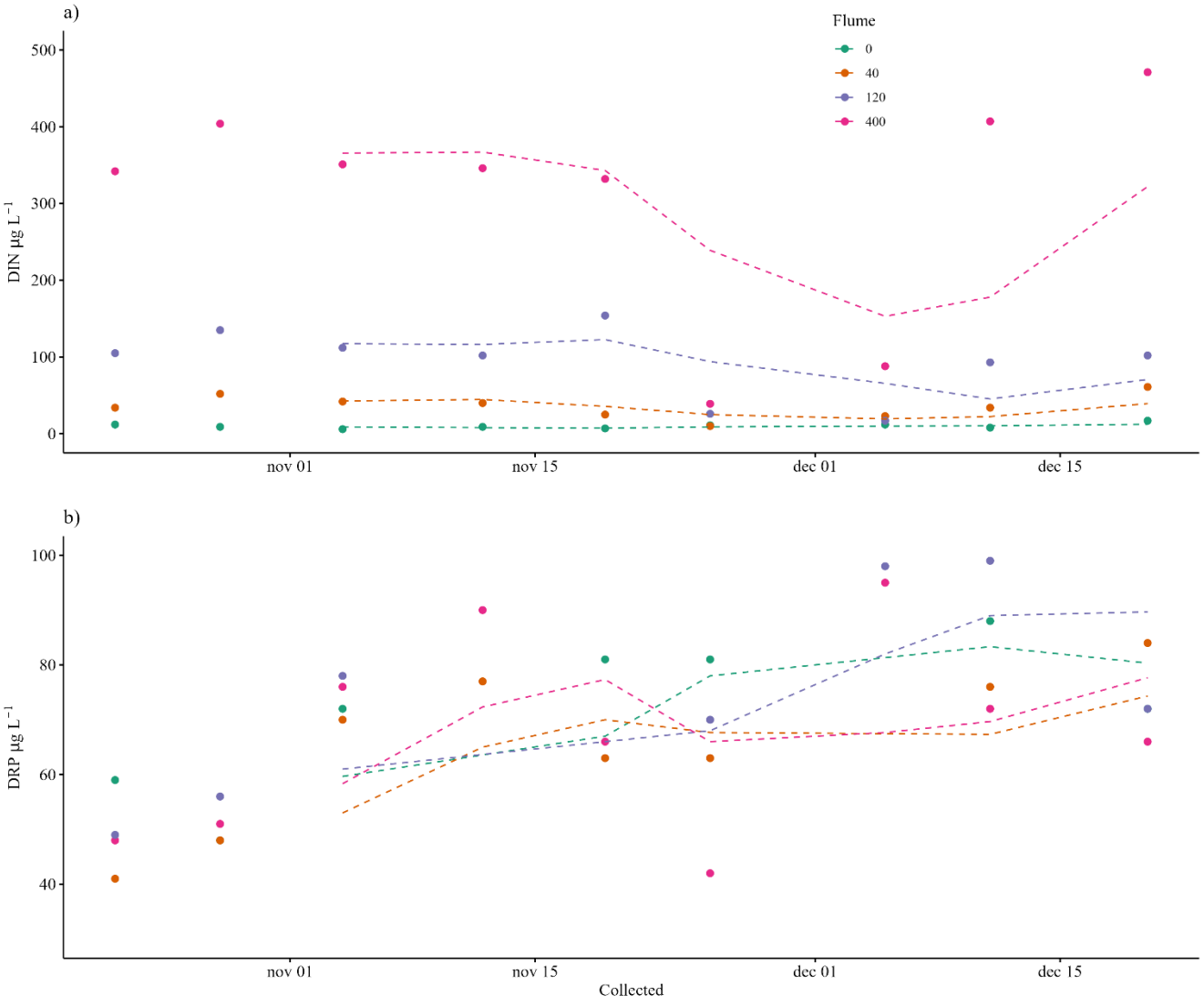
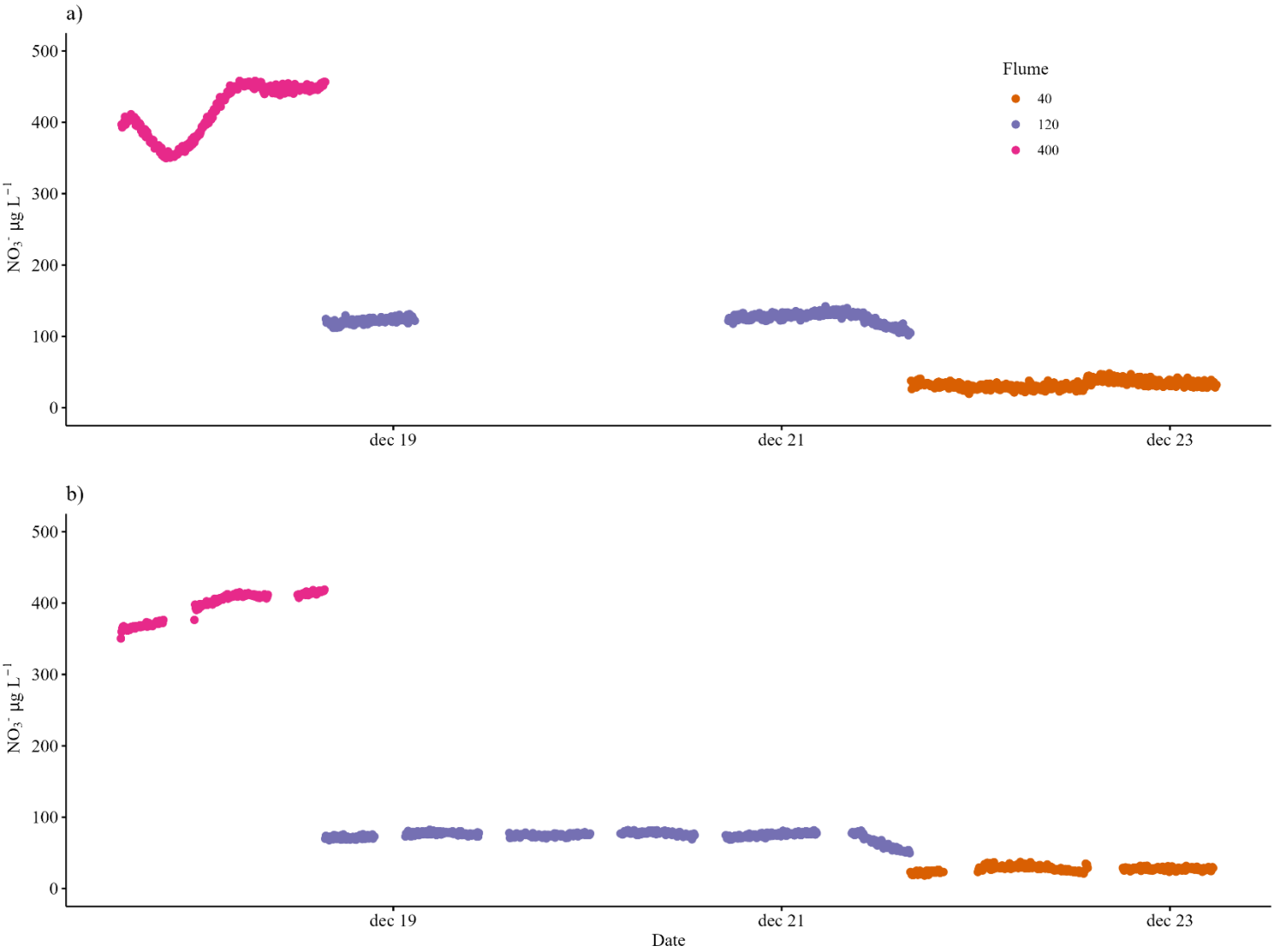


Figure A.3.2 – Estimated NO_3^- concentrations over time from telemetry data from two TriOS probes deployed in the 40 N, 120 N, and 400 N treatment. **a)** probe #1, **b)** probe #2. Interruptions in the sequence are due to connection errors. No reliable signal could be obtained from the 0 N treatment.



Appendix IV

Figure A.4.1 – Linear regressions between modelled and measured physical and chemical attributes of the selected streams. Measured parameters on the Y-axis and modelled on the X-axis. Grey area depicts the standard error of the linear regression line.