Impacts of macrophyte beds on nutrient dynamics and phytoplankton biomass during water level fluctuations

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Abstract:

Phytoplankton blooms have been regarded as a major threat to freshwater ecosystems. Macrophytes play a key role in maintaining a clear water state by competing with phytoplankton for nutrients and light, and/or releasing allelopathic chemicals to inhibit phytoplankton growth. However, macrophytes can become desiccated after severe water level drawdown, subsequently releasing substantial nutrients that stored in their biomass after rewetting. Previous studies have mainly focused on the nutrient uptake by macrophytes, with little attention paid to the potential negative implications of macrophyte loss during water level fluctuations (WLFs). This thesis compared the role of macrophytes as a nutrient sink versus a source during WLFs, and its implications for phytoplankton blooms during WLFs.

The first data chapter of this thesis has examined the nutrient storage capacity of a floating-leaved macrophyte, *Nymphoides indica* in a subtropical reservoir. The nutrient stored in *N. indica* biomass in this reservoir was 30% of total nitrogen (TN) and 150% of total phosphorus (TP) stored in the water column. This species was subsequently used in glasshouse experiments to investigate the impact of different durations of water level drawdown, followed by rewetting on nutrient release from both macrophyte litter and their associated sediment. This study showed that macrophyte beds can shift from a nutrient sink to a source after desiccation, followed by rewetting. This occurred when minimum soil moisture content was reached after 10 weeks of desiccation. This shift also caused an increase in phytoplankton biomass, as nutrients were released.

The effect of WLFs on two submerged macrophyte species, the invasive species *Cabomba caroliniana* (*Cabomba*) and the native species *Hydrilla verticillata* (*Hydrilla*) was also examined. Macrophytes were enriched with stable nitrogen isotope (15N), then
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used to examine the fate of released $^{15}$N from desiccated, and subsequently rewetted macrophytes. The study showed that $^{15}$N released from macrophytes was assimilated by phytoplankton faster than being transferred to other N pools, such as to living macrophytes and sediments. Desiccation of the associated sediment, followed by rewetting, resulted in a greater proportion of the macrophyte-derived $^{15}$N remaining in the water column and assimilated by phytoplankton, compared with the treatments without sediment desiccation (i.e. constantly wet conditions).

Regrowth of macrophytes also changed the fate of nutrients released from macrophyte litter, following rewetting the desiccated sediment. Less $^{15}$N was transferred into the water column, sediments and phytoplankton when macrophytes regrew, compared to the treatment without macrophyte regrowth. As such, 28 d after rewetting, regrown *Cabomba* and *Hydrilla* assimilated 19–100% of released N and 6–42% of released P from macrophyte litter when the biomass ratio of macrophyte litter to regrown macrophytes was 9:1. In addition, regrown *Hydrilla* had significantly higher nutrient uptake rates than *Cabomba*. Despite this, *Cabomba* was more efficient at reducing water column N concentrations, and neither *Cabomba* or *Hydrilla* significantly reduced water column P concentrations. This indicates regrown *Cabomba* and *Hydrilla* differ in their use of water column versus sediment nutrients. This has important implications for the capacity of each species to reduce water column nutrients and control phytoplankton biomass.

This study also showed that litter of the invasive species *Cabomba* decomposed faster and had a higher N and P content, compared with the same amount of *Hydrilla* litter. This resulted in higher water column nutrient and Chlorophyll a concentrations but lower dissolved oxygen concentrations in the *Cabomba* treatments. The *Cabomba* litter also had significantly higher N and P content compared with *N. indica*, indicating
Abstract

*Cabomba* could also have a greater impact on water column nutrients than *N. indica* during decomposition. Overall, both the submerged and floating-leaved macrophyte species in this study had a greater impact on water column P than N, after drying then rewetting. This changed the water column N:P ratio with potential flow-on effects to phytoplankton biomass and species assemblages.

In summary, these findings of the thesis indicate that effective water level management is essential to protect macrophyte beds and reduce the risk of phytoplankton blooms, during drying then rewetting cycles. Management options could include reducing the frequency, amplitude, or duration of water level drawdown to minimize the loss of macrophytes and maximize their regrowth. Since invasive submerged macrophytes have greater potential to increase the phytoplankton biomass during WLFs, more attention should be paid to reservoirs or lakes dominated by invasive submerged macrophytes.

**Keywords:** macrophytes; water level fluctuations; nutrient budgets; stable isotope; phytoplankton blooms; invasive species
Statement of Originality

This work has not previously been submitted for a degree or diploma in any university.

To the best of my knowledge and belief, the thesis contains no material previously
published or written by another person except where due reference is made in the thesis itself.

(Signed)

Jing Lu

June 2017
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<tr>
<td>AFDW</td>
<td>Ash free dry weight</td>
</tr>
<tr>
<td>Al</td>
<td>Aluminium</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>Chl-(a)</td>
<td>Chlorophyll (a)</td>
</tr>
<tr>
<td>DC</td>
<td>Dithionite-citrate</td>
</tr>
<tr>
<td>DIN or DIP</td>
<td>Dissolved inorganic nitrogen or phosphorus</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>DON or DOP</td>
<td>Dissolved organic nitrogen or phosphorus</td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>FSL</td>
<td>Full supply level</td>
</tr>
<tr>
<td>GIS</td>
<td>Geographic information systems</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>ML</td>
<td>Megalitre</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>(\text{NH}_4^+)</td>
<td>Ammonium</td>
</tr>
<tr>
<td>NOx or NO(_2^-)/NO(_3^-)</td>
<td>Nitrate/Nitrite</td>
</tr>
<tr>
<td>(^{15}\text{N})</td>
<td>Stable nitrogen isotope</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>(\text{PO}_4^{3-})</td>
<td>Phosphate</td>
</tr>
<tr>
<td>PON</td>
<td>Particulate organic nitrogen</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetic active radiation</td>
</tr>
<tr>
<td>SEQ</td>
<td>South-east Queensland</td>
</tr>
<tr>
<td>TDN or TDP</td>
<td>Total dissolved nitrogen or phosphorus</td>
</tr>
<tr>
<td>TN or TP</td>
<td>Total nitrogen or phosphorus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>-------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>TOC</td>
<td>Total organic carbon</td>
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Included in this thesis are papers in Chapters 2 and 3, which are co-authored with other researchers. My contribution to each co-authored paper is outlined at the front of the relevant chapter. The bibliographic details/status for these papers including all authors are:

Chapter 2:


Chapter 3:


Chapter 4:


Appropriate acknowledgements of those who contributed to the research but did not qualify as authors are included in each paper.

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Name of Student and corresponding authors for all listed papers: Jing Lu

(Countersigned) _______________ (Date) ________________

Supervisor: Prof. Michele Burford
Chapter One: General introduction

1.1 Freshwater ecosystems and major threats

Freshwater ecosystems include rivers, streams, lakes, ponds, reservoirs, and wetlands occupy < 0.01% of the earth surface (Gleick 1996). Despite the small area, they provide an important habitat fostering biodiversity, e.g. globally 40% fish diversity and 30% vertebrate diversity (Dudgeon et al. 2006). Freshwater ecosystems also provide important functions such as nutrient cycling and renewal, as well as valuable economic goods and services for human activities, e.g. drinking water and food supply, irrigation, flood control, electricity generation, transportation, recreations and pollutant disposal (Wilson et al. 1999).

With the increase of human disturbance, freshwater ecosystems may well be the most endangered ecosystems in the world (Revenga et al. 2005; Dudgeon et al. 2006; David L. Strayer et al. 2010). Dudgeon et al. (2006) grouped the major threats to freshwater diversity into five interacting categories based on previous studies (Naiman and Turner 2000), overexploitation; water pollution; flow modification; destruction or degradation of habitat; and invasion by exotic species. These threats also significantly alter the structure, function, and nutrient dynamics in freshwater ecosystems.

1.2 Eutrophication in freshwater ecosystems

In the early 1900s, researchers started to become aware that nutrients are positively related to the aquatic productivity (Weber 1907; Johnstone 1908). The concept of eutrophication and trophic classification systems were then developed in the following 60 years (Rohlich 1969). Eutrophication refers to the process of natural or artificial nutrient addition into the aquatic ecosystems and their flow-on impacts (Rohlich 1969; Smith et al. 2006). Over the past few decades, eutrophication has emerged as a global
Chapter 1 General Introduction

Concern for freshwater ecosystems. The change from nutrient-poor (oligotrophic) to nutrient-rich (eutrophic) conditions has been proposed as a classic succession pattern due to the natural aging of lake basins (Rodhe 1969). However, the eutrophication process has significantly accelerated due to human activities, especially excessive nutrient inputs.

One of the most consistent problems of eutrophication is that the dominant primary producers in freshwater ecosystems can be shifted to faster-growing nuisance phytoplankton (Scheffer et al. 1993). These nuisance phytoplankton are typically dominated by harmful cyanobacteria (Downing et al. 2001; Huisman et al. 2005). The dominance of nuisance phytoplankton can cause low dissolved oxygen (DO) conditions in the water column and release toxins from toxic algal species (such as Microcystis, Dolicospermum, and Cylindrospermopsis). This can kill or sicken fishes and other aquatic animals in freshwater ecosystems, or threaten human health.

To tackle eutrophication, researchers and managers have tried a range of different ways to restore freshwater ecosystems. Since the nutrient over-enrichment was regarded as the main cause of eutrophication, reducing nutrient levels and increasing water transparency by chemical, physical or biological methods have been tried and tested in the past few decades (Jöbgen et al. 2004; Walpersdorf et al. 2004; Wauer et al. 2005). Starting from the late 1960s, phosphorus (P) was regarded as a more limiting nutrient than nitrogen (N) for phytoplankton growth, as some phytoplankton can fix gaseous N (Kuentzel 1969). Therefore, only P control was recommended at that time. To reduce P level in freshwater ecosystems, chemicals for P precipitation (such as molysite and aluminum sulfate) were added into eutrophic aquatic ecosystems, or eutrophic sediments were dredged out to reduce P release from sediments (Cooke et al. 1982; Murphy et al. 1999; Reddy et al. 2007). However, the negative effects of these
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physical and chemical methods for reducing nutrients are also prominent, such as
destroying aquatic habitats for fish, waterbirds and other trophic levels. With the
increased knowledge of the significance of the N fixation by phytoplankton, as well as
results from several hundreds of bioassays and in situ experiments, N limitation or N
and P co-limitation for phytoplankton growth has been identified in many freshwater
ecosystems (Elser et al. 1990, 2007). Therefore, combined N and P controls are
considered to be equally important to reduce phytoplankton growth.

Besides nutrient control, food-web manipulation (biomanipulation) can also
control the excessive growth of phytoplankton. Biomanipulation theory reveals that
reducing the planktivorous and benthivorous fish abundance, or increasing the piscivore
fish abundance, can increase the zooplankton abundance thus the grazing pressure on
phytoplankton (Shapiro and Wright 1984; Demelo et al. 1992; Horppila et al. 1998).
Although there is no doubt that biomanipulation has been a useful tool for water quality
improvement (Mehner et al. 2002), its long-term effectiveness is still uncertain (Van de
Bund and Van Donk 2002; Ørmond et al. 2007). This is due to the need repeated
apply the biomanipulation to ensure clear water conditions. Biomanipulation is also
more successful in shallow lakes compared to deep stratified lakes, which can be
attributed to the presence of a stable community of macrophytes (Hosper and Meijer
1993; Mehner et al. 2002). Biomanipulation also appears to be more effective in
temperate lakes than in subtropical and tropical lakes. This seems to be due to the higher
fish biomass and higher rates of population recovery in planktivorous fish in tropical
and subtropical systems (Jeppesen et al. 2007).

Macrophytes are vascular aquatic plants that live near or in the water, and most
of them are restricted to the shallow water along the shoreline, due to the limited light
availability in deeper water sites. In shallow freshwater ecosystems, there is an
“alternative stable states” theory, which states that shallow freshwater ecosystems can be shifted from a clear water state dominated by macrophytes to a turbid water state dominated by phytoplankton, with the increase of nutrient concentrations (Scheffer et al. 1993). Therefore, macrophytes play a key role in maintaining the clear water state.

Previous studies have demonstrated that once a system has switched to a turbid state, it can be difficult to switch this system back to a clear state, even though the nutrient levels in the water column have been reduced (Moss 1990; Roberts et al. 2003). This is probably the reason for the unsuccessful trials using physico-chemical methods to restore eutrophic freshwater ecosystems. Therefore, ecological engineering, as a means of using the natural processes and resources of ecosystems to reduce the environmental and economic costs for ecosystem restoration, has been emerged as an alternative to restore ecosystems (Bradshaw 1997; Mitsch 2012). This method can take advantage of ecosystems to generate both ecological and economic benefits (Odum and Odum 2003). In terms of restoring freshwater ecosystems, ecological engineering by using macrophytes can be used to reduce water quality problems.

1.3 The role of macrophytes in freshwater ecosystems

Macrophytes are one of the most important primary producers in freshwater ecosystems, especially in shallow lakes where flat basins can provide abundant suitable habitat for macrophytes (Kimmel and Groeger 1984). There are several life forms of macrophytes growing in the littoral zone of freshwater ecosystems, which are wetland plants, emergent plants, floating-leaved/free-floating plants and submerged plants (Sculthorpe 1967; Boschilia et al. 2012).

Freshwater ecosystems that are dominated by macrophytes usually have a higher overall biodiversity, and lower cell density of phytoplankton (Moss 1990; Van den Berg et al. 1998). Macrophytes can provide ecological niches for higher trophic levels, such
as being refuges for zooplankton, invertebrates, and fishes, which, in turn, can also facilitate their grazing on phytoplankton (Timms and Moss 1984; Lauridsen et al. 1996; Wigand et al. 2000). Macrophytes also can affect the growth and community composition of phytoplankton by competing with them for nutrients and light (Søndergaard and Moss 1998; Van Donk and Van de Bund 2002; Takamura et al. 2003), or by releasing chemical substances (allelopathic effect). These substances negatively affect the photosynthesis of phytoplankton by reducing enzyme activities of superoxide dismutase, or increasing cell membrane permeability, resulting in growth limitation, death or sedimentation of phytoplankton (Nakai et al. 1999; Gross 2003; Li and Hu 2005).

Macrophyte communities also can release oxygen to the water column via photosynthesis and transport oxygen through vascular bundle then release it to the rhizosphere (Sand-Jensen et al. 1982; Reddy et al. 1989a). In addition, macrophyte beds can prevent re-suspension of biological or non-biological particles by trapping phytoplankton and sediment particles, e.g. stands of *Ranunculus circinatus*, *Ceratophyllum demersum*, or *Potamogeton obtusifolius* (Horppila and Nurminen 2003; Meerhoff et al. 2003).

1.4 Nutrient cycling in macrophyte beds in the littoral zone

Macrophytes beds can also affect nutrient dynamics in freshwater ecosystems, especially in the littoral zone. These macrophyte beds typically possess more complex nutrient dynamics compared to the pelagic zones (Barko et al. 1991; Zohary and Ostrovery 2011). Macrophytes can be regarded as a nutrient sink in shallow freshwater ecosystems by taking up excessive nutrients and storing them in their biomass (Brix 1994; Barko and James 1998; Gao et al. 2009). Previous studies have demonstrated that both roots and leaves of macrophytes can take up nutrients from either sediments or the
water column (Carignan 1982; Chambers et al. 1989; Madsen and Cedergreen 2002). This is particularly true for submerged macrophytes, which are rooted in the sediment with their leaves and stems submerged under the water (Barko et al. 1991; Greenway 1997). The epidermal cells of submerged macrophytes can directly take up nutrients from the water column (Rattray et al. 1991).

Quantifying the nutrient utilisation by macrophytes can improve our understanding of nutrient cycling and littoral-pelagic nutrient exchanges (Barko et al. 1991). However, there is a long-term debate on whether the water column or sediment is the main nutrient source for macrophyte growth (Carignan and Kalff 1980; Chambers et al. 1989; Madsen and Cedergreen 2002). Several studies demonstrated that macrophyte shoots without roots could take up enough nutrients for macrophyte growth (Madsen and Cedergreen 2002). In contrast, some experiments have shown that the sediment can provide most of the nutrients for macrophyte growth, rather than the water column (Carignan and Kalff 1980; Chambers et al. 1989). Carignan (1982) developed an empirical model to estimate the relative importance of macrophyte roots and shoots on up-taking P. This empirical model revealed that the ratio of the bioavailable P in the water column versus sediments mainly determines the relative importance of P source for macrophyte growth.

Macrophytes can also mobilize sediment P into the water column by changing the redox and pH conditions of the sediment (Carignan and Kalff 1980; Barko and James 1998). Additionally, macrophytes can increase denitrification rates in the sediment, compared with sites with bare sediments (Caffrey and Kemp 1992; Risgaard-Petersen and Jensen 1997; Forshay and Dodson 2011). This is likely due to the fact that macrophytes can increase coupled nitrification-denitrification in the sediment (Reddy et al. 1989b). Macrophytes can supply organic carbon and oxygen in the rhizosphere,
which provides the energy resource and aerobic conditions for nitrifiers to oxidize ammonium (NH$_4^+$) to nitrite/nitrate (NO$_2^-$/NO$_3^-$) (Christensen and Sorensen 1986; Hernandez and Mitsch 2007). This, in turn, can provide substrates (NO$_2^-$/NO$_3^-$) for anaerobic denitrifiers. Moreover, macrophytes can provide surface areas for biofilms, which may contain high densities of nitrifying and denitrifying bacteria (Körner 1999). Denitrification in biofilms can be as important as sediment denitrification (Eriksson and Weisner 1996, 1999). However, previous studies also revealed that macrophytes can compete with nitrifiers and denitrifiers for N resources (Verhagen et al. 1995; Risgaard-Petersen and Jensen 1997; Bodelier et al. 1998). This might slow down the process of nitrification and denitrification in the sediment (Baldwin and Mitchell 2000; Cavanaugh et al. 2006).

Die-back of macrophytes can also play a major role in nutrient cycling in the littoral zone (Carpenter and Adam 1979; Benfield 1986; Battle and Mihuc 2000; Shilla et al. 2006). With the increase of eutrophication in the past few decades, the abundance of submerged macrophytes has substantially declined or even vanished from some freshwater ecosystems. These dead macrophytes can release nutrients and carbon (C) back into the water column during their decomposition (Rice and Tenore 1981; Chimney and Pietro 2006; Shilla et al. 2006; Kröger et al. 2007). The decomposition process also increased the consumption of DO and reduced the pH in the water column (Twilley et al. 1986). Lower DO and pH levels increase P remineralization from the sediment. The released nutrients from macrophyte decomposition, as well as the loss of competition from macrophytes, might increase the growth of phytoplankton and accelerate the process of eutrophication. A few studies have demonstrated that macrophyte harvesting is an efficient way to prevent the nutrient release and other negative effects caused by the decomposition of macrophytes (Gumbricht 1993; Finnegan et al. 2014; Li et al. 2014). However, the removal of macrophytes might also
cause a short-term nutrient release to the water column because of the disturbance to the sediment.

Invasive macrophyte species (i.e. mostly exotic species) have dominated many freshwater ecosystems, and can also affect the nutrient cycling in those ecosystems. Invasive species usually have a higher biomass compared with other co-occurring native species, due to their greater adaptability to ecological habitats or higher growth rates (Rejmánek and Richardson 1996; Allendorf and Lundquist 2003). A review from Ehrenfeld (2003) showed that invasive plants have the potential to increase the net primary production and N availability of the ecosystem, affect N fixation rates, and produce litter that decay faster than co-occurring native species.

1.5 Major threats to macrophytes in freshwater ecosystems

Eutrophication is one of the most threatening factors to the survival and reproduction of macrophyte communities in freshwater ecosystems, due to the high water column turbidity from phytoplankton blooms. Invasion by exotic macrophytes is another threat to the distribution of native macrophytes, resulted from their more competitive capacity for ecological niches, such as faster growth rates which increases nutrient uptake and occupy habitats (otherwise available to native macrophytes). In addition to eutrophication, water level fluctuations (WLFs) are the other major factor that detrimentally affects the growth and distribution of both native and invasive macrophytes. This is because macrophytes normally could not survive for a long time either during desiccation and when inundated with high volumes of water limiting light for photosynthesis.

1.6 Causes of water level fluctuations in freshwater ecosystems

WLFs can change both the physico-chemical and biological attributes of freshwater...
ecosystems, such as water quality, the flora and fauna biodiversity (Furey et al. 2004; Geraldes and Geraldes 2005; Zohary and Ostrovsky 2011), and the structure of food-webs (Naselli-Flores and Barone 1997; Baschuk 2010; Baschuk et al. 2012).

Natural WLFs reflect changes in the balance between seasonal rainfall and evaporation (Naselli-Flores and Barone 2005; Zohary and Ostrovsky 2011). Therefore, WLFs in arid and semi-arid areas or irregular rainfall areas are typically more frequent or unpredictable than areas with stable humid climates. Global climate changes can also exacerbate the frequency or amplitude of droughts and floods, thus increasing the impacts of WLFs on freshwater ecosystems (Hirabayashi et al. 2008; Wantzen et al. 2008; Dai 2013). Other anthropogenic causes of WLFs are mainly due to human activities, such as dam construction for drinking water and irrigation needs, as well as water management for electricity generation and flood control (Geraldes and Geraldes 2005). Therefore, reservoirs usually undergo more frequent WLFs than natural lakes, and as such are likely to be more severely impacted than other freshwater ecosystems.

1.6.1 Impacts of WLFs on macrophytes

The amplitude and duration of WLFs, as well as the timing and rates of water level increase and decline, are important factors determining the effect of WLFs on macrophytes (Wantzen et al. 2008). Natural WLFs are inherent features of freshwater ecosystems. They are essential for the survival of some macrophytes that have developed a life cycle to suit these fluctuations, such as rapid germination, growth and blooming when desiccated. Natural WLFs can also be necessary for ecosystem services, such as boosting the nutrient cycling for biogeochemistry, and providing hydropower for human needs (Wantzen et al. 2008; Zohary and Ostrovsky 2011).

The appropriate water level drawdown in highly turbid lakes can increase the light availability to the lake bottom, thus benefitting the growth of submerged
macrophytes (Bucak et al. 2012). This is because macrophytes are usually restricted to the shallow water zones because of the light attenuation in deeper water sites (Middelboe and Markager 1997; Krolová et al. 2012), and many emergent plants could not germinate or survive when deeply submerged (Valk and Davis 1978; Squires and Vandervalk 1992). A shorter period of water level drawdown can also promote seed germination of some macrophytes through sediment exposure (Keddy and Reznicek 1986; Murphy et al. 1990; Brock and Rogers 1998). Therefore, macrophytes can expand their coverage from shallower to deeper water sites after water level drawdown, as long as valid propagules or lateral spreading of macrophytes are remaining in deeper water sites (Thomaz et al. 2006).

Van Geest et al. (2007) investigated ecosystem dynamics of 70 lakes in the floodplains of the Lower Rhine in The Netherlands from 1999 to 2004. This study showed that water level drawdown in these shallow lakes appeared to be the main driver for the water state shifting from a turbid to a macrophyte-dominated state. A shorter period of water level drawdown followed by rewetting can increase the diversity of macrophytes in freshwater ecosystems (Rørslett 1991; Van Geest et al. 2007; Leira and Cantonati 2008), which is in accordance with the Intermediate-Disturbance Hypothesis (Mjelde et al. 2013). The Intermediate-Disturbance Hypothesis predicts that the species diversity within one system will be maximal at moderate scales or intensities of disturbances (Wilson 1994). This is because more spatial and temporal variability in resources and environmental conditions are available for more species to colonize during intervals of intermediate disturbances (Connell 1978; Roxburgh et al. 2004).

In contrast, extreme or untimely WLFs can be detrimental to the survival of macrophytes, thus impairing their role on freshwater ecosystems which includes acting as habitats and food resources for other trophic levels. The rapid and large increase in
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Water level due to extreme floods or human manipulations can significantly increase the water column turbidity and decrease the light availability for macrophytes at the lake bottom (Madsen et al. 2001). This light deficiency for submerged macrophytes, or the oxygen deficiency for emergent macrophytes under deep and turbid water, can result in macrophytes declining their distribution or vanishing from freshwater ecosystems (Van Geest et al. 2005; Deegan et al. 2007).

An extended period of water level drawdown also threatens the survival of macrophytes. The tolerance, resistance, and resilience of macrophyte species to desiccation are crucial factors affecting macrophyte assemblages and diversity after WLFs (Keddy and Reznicek 1986). Recovery of both macrophytes and their propagules might not be possible during extreme droughts (Thomaz et al. 2006) because they might not be able to survive or re-germinate after persistent desiccation. For instance, macrophytes in Itapúa reservoir of Brazil could not recover from a four-month water level drawdown (5 m depth change), even 14 months after the water level had returned to normal levels (Thomaz et al. 2006). Most submerged macrophytes can only survive for several days when desiccated (maximum 2 to 3 weeks) (Van Wijck and De Groot 1993; Adams and Bate 1994). However, propagules in the sediment, especially the seeds, can be viable for several months (Van Wijk 1989), even for years (Valk and Davis 1978; Lu et al. 2012). These propagules in the sediment could become the main source of recruitment if major disturbance removes the aboveground vegetation (Grillas et al. 1993; Thomaz et al. 2006).

Newly exposed habitats provided by water level drawdown could be easily occupied by invasive species. Invasive species may out-compete native vegetation by quickly colonizing new habitats followed by rapid proliferating, resulting in the homogenization of species and loss of macrophyte biodiversity in freshwater.
ecosystems (Zohary and Ostrovsky 2011). Lowering water levels can be used to control the excessive growth of undesirable or invasive submerged species, depending on their different levels of tolerance to desiccation (Ibáñez et al. 2012) and re-germination after direct removal (Cooke 1980; Van Wijk 1989; Sharip et al. 2012; Wilcox 2012).

The effect of WLFs on macrophyte responses might also depend on seasons (Coops et al. 2003). Low water levels in spring and summer may facilitate the expansion of submerged macrophytes by increasing water column temperatures and light availability to the lake bottom, but also could favor the colonization of invasive species. Water level drawdown in winter may have much smaller impacts on submerged macrophytes since no invasion by terrestrial or emergent plants would occur due to their slower growth rates during winter, especially in temperate areas (Cooke 1980). However, extreme conditions, such as ice on lakes and reservoirs in temperate regions, damage plants and impact their re-germination in the next spring (Blindow 1992).

1.6.2 Impacts of WLFs on nutrient dynamics

Water level drawdown, followed by rewetting, has been proved to significantly affect nutrient dynamics in freshwater ecosystems (West et al. 1988; Qiu and McComb 1996; Watchorn 2011). The mineralization of organic matter in sediments can be accelerated by desiccation. Therefore, a significant amount of carbon dioxide and mineral N can be released from sediments after drying then rewetting, which has been known as the “Birch” effect (Birch 1958). Persistent water inundation of sediment may also cause NH$_4^+$ liberation and increased denitrification due to enhanced anoxic conditions (Austin and Lee 1973; Qiu and McComb 1996; Baldwin and Mitchell 2000).

The C and N (e.g. NH$_4^+$) released from sediments after drying then rewetting can provide nitrifiers the energy and substrates required for nitrification, oxidizing NH$_4^+$
to NO$_3^-$ (Stanley and Boulton 1995; Qiu and McComb 1996; Baldwin and Mitchell 2000). The increased NO$_3^-$ concentrations from nitrification, in turn, can promote denitrification (NO$_3^-$ to N$_2$ or N$_2$O) after rewetting. This increased denitrification is likely due to the activation of denitrifying enzymes by oxygen depletion resulting from the flush of microbial respiration associated with macrophyte decomposition (Smith and Parsons 1985; Kern et al. 1996). The increased coupled nitrification-denitrification after drying, then rewetting, has been found in several previous studies (e.g. Smith and Parsons 1985; Groffman and Tiedje 1988; Fromin et al. 2010). However, there could also be lower (e.g. Cavanaugh et al. 2006; Austin and Strauss 2011) or similar (Mitchell and Baldwin 1999) rates of denitrification in the sediment after drying then rewetting, compared to constantly wet conditions. Those studies attributed this to the death of microbes by desiccation (Qiu and McComb 1996).

The sediment P release after drying then rewetting has also been found in several case studies (Watchorn 2011). However, the P release from sediments is more unpredictable than the N release during WLFs. This is because P can be bound to soil particles that are rich in iron, aluminum or calcium (Reddy et al. 1993; Rydin et al. 2000; Geurts et al. 2010). The P adsorption capacity of sediment can also be affected by WLFs as it associates with the detox condition of iron (Fe) in the sediment (Baldwin 1996). The oxidized Fe (III) has a greater P adsorption capacity than the reduced Fe (II) (Baldwin and Mitchell 2000). Kerr’s (2010) study on drying river sediment in Upper Brisbane River showed that P release from sediment was caused predominantly by desiccation time rather than physico-chemical properties or total phosphorus (TP) content of the sediment.

Detrital organic matter derived from desiccated macrophytes can also release nutrients after rewetting, directly via organic N and P decomposition, and indirectly by
the supply of C sources for microbial activities (Baldwin et al. 2008). This, in turn, can cause water quality deterioration in freshwater ecosystems (Bond et al. 2008; Zohary and Ostrovsky 2011). These released nutrients may even induce a shift of the dominant primary producer from macrophytes to phytoplankton (Parparov 1990; Coops et al. 2003; Beklioğlu et al. 2007). Since decomposition of desiccated macrophytes can have negative effects on water quality (Shilla et al. 2006), removing them after drying might be a crucial method for water quality management. However, propagules (e.g. roots, bulbs or seeds) stored in sediments are essential to the recovery of macrophytes after refilling events. The newly germinated macrophytes can improve water quality by taking up nutrients, inhibiting phytoplankton growth, and trapping particles or preventing re-suspension after refilling (Naselli-Flores and Barone 2005). However, the impacts of macrophyte decomposition versus recovery on water quality of lakes or reservoirs during WLFs have not been fully quantified.

1.7 Research aims and thesis structure

Impacts of WLFs on water quality in lakes or reservoirs depend on a range of factors, such as the duration and amplitude of WLFs, physico-chemical characteristics of reservoirs, and also the response of macrophytes during WLFs. The comprehensive analysis of how WLFs can impact water quality under a combination of all these factors still needs more research. Although there have been studies of WLFs in temperate, tropical and subtropical areas, research in areas with more unpredictable droughts and floods (e.g. Australia) are still limited.

Extreme climate conditions, such as droughts and floods, can enhance the negative effects of WLFs on freshwater ecosystems. Australia is a more drought- and flood-prone country than much of the world (Quiggin 2007). Australian reservoirs in the arid, subtropical and tropical regions usually suffer from extremely arid and semi-arid
climatic conditions, and unpredictable rapid refilling events (Watts 2000a). The reservoir volumes can be more easily changed from near-empty to full, or full to near-empty. Therefore, reservoirs in many parts of Australia with more extreme variability in climate conditions are likely to suffer greater impacts from WLFs compared with temperate areas of the world. The south-east Queensland (SEQ) region in Australia has been identified as one of six climate change ‘vulnerability hotspots’ in Australia (Hennessy et al. 2007; McDonald et al. 2010). From 2001 to 2008, SEQ had experienced one of the worst droughts recorded in the past 200 years (Bond et al. 2008). Afterwards, two severe floods followed in both 2011 and 2012. However, the effect of WLFs on water quality is still poorly understood in Australian lakes and reservoirs.

Despite the significant role of macrophytes on changing water quality of reservoirs and lakes, previous studies have been mainly focused on the contribution of sediments to water quality during WLFs. The effect of macrophytes, and the interaction between macrophytes and sediments in macrophyte beds, on water quality during WLFs have not been well quantified. Therefore, the overall aim of this thesis is to investigate the role of macrophyte beds on nutrient dynamics, and their impacts on phytoplankton blooms during WLFs in SEQ, Australia. The study will explore whether macrophytes are nutrient sinks or sources to the overlying water column during WLFs. To be more specific, this thesis includes chapters (Fig. 1-1):

1. Investigating how the duration of water level drawdown affects macrophyte responses, if their response (nutrient-sink vs. nutrient-source) can impact water quality in reservoirs, and the interaction between macrophytes and sediments on nutrient dynamics (Chapter 2);

2. Quantifying the fate of released nutrients from macrophytes during WLFs using stable nitrogen isotope (\(^{15}\)N) tracking techniques, including how much of these
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N are utilized by phytoplankton, and if the sediment desiccation can impact the distribution of these macrophyte-derived N (Chapter 3);

3. Estimating the role of macrophyte regrowth on assimilating the nutrients released from rewetted macrophyte litter. The difference between an invasive and a native submerged macrophyte species will also be compared in this study (Chapter 4).

The main findings from the above Chapters will be summarized and synthesized in Chapter 5. I will also estimate the role of macrophytes as a nutrient sink versus a source during drying then rewetting processes, and explore the impacts of littoral macrophytes on nutrient dynamics and phytoplankton growth in lakes/reservoirs during WLFs in Chapter 5.
1.8 Research approaches

The thesis involves a range of methods to address my research questions, i.e. field surveys, spatial analysis using geographic information systems (GIS), controlled glasshouse experiments; stable isotope tracing techniques, analytical measurements, and data analyses.

1. Field surveys – Types, areal extent, and the biomass of macrophytes were surveyed before and after water level drawdown using multiple transects in the target reservoir of SEQ, Australia.

2. Spatial analyses – Areal extent of macrophytes before and after water level drawdown was estimated from satellite images and calculated by spatial
measurements. These values were validated with the field measurements (i.e. ground-truthing).

3. Controlled experiments – Controlled experiments were conducted in the glasshouse to estimate the resilience of macrophytes to water level drawdown, and the effect of desiccation and rewetting on nutrient release and uptake by macrophytes during this process.

4. Stable isotope tracing techniques: $^{15}$N was used to label macrophyte litter and trace the fate of the litter-derived N after drying then rewetting in the presence of sediment and regrown macrophytes.

5. Analytical measurements and data analyses – Water, macrophyte, and sediment samples for nutrient and $^{15}$N measurements were analysed in the laboratory. All data in this study were statistically analysed using R software (version 3.12).
Chapter Two: Macrophyte beds in a subtropical reservoir shifted from a nutrient sink to a source after drying then rewetting

Changes of macrophyte beds before and after water level drawdown in Tingalpa Reservoir, south-east Queensland, Australia.
Chapter 2 – Macrophyte Beds as a Nutrient Source during WLFs

This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors, are:


My contribution to the paper involved designing the experiment, collecting and analysing the data, providing direction on the structure of the result analysis, writing the manuscript, and addressing the comments from reviewers. Stephen Faggotter helped the sampling in the field and the experiment set-up, and also provided some useful comments to improve the manuscript. Michele Burford is the principle supervisor of this project, contributing to developing the ideas of the experiment, providing direction on the structure of the result analysis and revising the manuscript. Stuart Bunn is the associated supervisor of this project, contributing to providing valuable comments to improve the manuscript.

(Signed) _________________________________ (Date) ________________

Name of Student and corresponding author of the paper: Jing Lu

(Countersigned) _________________________________ (Date) ________________

Supervisor: Prof. Michele Burford
Chapter 2 – Macrophyte Beds as a Nutrient Source during WLFs

2.1 Introduction

Compared with natural lakes, reservoirs can suffer from more frequent and severe WLFs due to human water demands and water level management strategies. WLFs are likely to be greater in arid and semi-arid areas where the climate is more variable, i.e. unpredictable droughts and floods (Heathcote 1969; Mpelasoka et al. 2008). Nutrient cycling processes, biodiversity of flora and fauna and phytoplankton bloom development in reservoirs can be significantly affected by WLFs (Baschuk 2010; Zohary and Ostrovsky 2011; Rangel et al. 2012).

Littoral margins with macrophyte beds in reservoirs or lakes are particularly sensitive or vulnerable to WLFs (Coops et al. 2003; Keitel et al. 2016). Rewetting of the desiccated sediments can result in the release of nutrients, which is also known as the “Birch” effect. The “Birch effect” describes a rapid release of inorganic nitrogen and carbon dioxide after rewetting desiccated sediments (Birch 1958; Wilson and Baldwin 2008). Previous studies have shown that most of the nutrient release occurs in the littoral zone, where there is higher organic matter content in sediments (Steinman et al. 2014). Submerged macrophytes represent an important pool of organic matter and nutrients in the littoral zone. They are also considered to be a key contributor to maintaining the clear water state in shallow lakes or reservoirs (Scheffer 1990). However, when macrophytes become desiccated, they can also leach nutrients into the water column following re-filling events. Most previous studies have focused on the nutrient release capacity of desiccated sediments during WLFs (Bostic and White 2007; Kerr et al. 2010). However, the contribution of macrophyte beds to nutrient fluxes during WLFs is less well understood.

In highly turbid lakes or reservoirs, lowering water levels has been regarded as a potential tool to improve the recolonization/restoration of macrophytes (e.g. Coops and
Chapter 2 – Macrophyte Beds as a Nutrient Source during WLFs

Hosper 2002; Van Geest et al. 2007; Bucak et al. 2012), by increasing light penetration to the lake bottom. However, the impacts of WLFs on macrophytes may depend on the length of WLFs duration (Wantzen et al. 2008). When water level drawdown occurs for a short period, the growth of macrophytes may be promoted due to the greater light transmittance from the shallower water depth (Keddy and Reznicek 1986; Brock and Rogers 1998). Macrophytes can also expand their coverage from shallower to deeper water sites after drawdown, as long as there are viable propagules, or via lateral spreading into deeper water sites (Thomaz et al. 2006).

Conversely, severe WLFs can negatively affect the growth and distribution of macrophytes (Boschilia et al. 2012). Most macrophytes may not survive a long period of water level drawdown and the recovery from their vegetative propagules may not be possible (Thomaz et al. 2006). Macrophytes may then become a source of nutrients and organic matter to adjacent sediments or to the water column after rewetting. Therefore, the duration of water level drawdown and resistance/resilience of macrophytes can be major factors that determine the role of macrophytes on nutrient dynamics during WLFs.

This study used both manipulative experiments and field survey data to examine the nutrient release capacity of a common macrophyte species, the native waterlily Nymphaoides indica (L.) Kuntze (Menyanthaceae), after water level drawdown followed by rewetting. This study aimed to assess: 1) the effect of different drying periods on survival of N. indica; 2) the effect of rewetting on nutrient release from desiccated N. indica and its macrophyte bed, and the implications for water quality; and 3) the effect of the interaction between macrophytes and sediments in macrophyte beds on nutrient release following rewetting after drying.
Chapter 2 – Macrophyte Beds as a Nutrient Source during WLFs

2.2 Methods

2.2.1 Study site

Tingalpa reservoir (27.5306° S, 153.1790° E; Fig. 2-1) is located on the border of the cities of Brisbane and Redlands, SEQ, Australia. In September 2013, the reservoir was slowly drawn down from 100% full supply level (FSL) to 53% FSL in August 2014, with dam wall repairs planned for the future. Before water level drawdown, the reservoir capacity was 24,870 ML at 100% FSL (Seqwater 2013) with a surface area of 4.79 km² and a mean depth of 11 m. After drawdown, the calculated reservoir capacity was 13,200 ML (53% FSL). The water lily, *N. indica*, which is a floating-leaved but sediment-rooted species, was the dominant macrophyte in Tingalpa reservoir before water level drawdown.

2.2.2 Manipulative experiments

*Macrophyte and sediment sampling*

A manipulative experiment was conducted to determine the effect of drying then rewetting on *N. indica* plants and nutrient release from plants and their beds. Samples of *N. indica* and *N. indica* beds were collected at the northern shoreline of Tingalpa reservoir (Fig. 2-1). Sample sites with homogeneous medium-sized living *N. indica* plants (taproot length: approximately 10 cm) were selected along the shoreline on 25th July 2014. At each site, a large PVC pipe (internal diameter: 24.3 cm; depth: 10 cm) was used as a corer to retrieve the *N. indica* samples. The corer was placed over one *N. indica* plant, ensuring all leaves and stems were inside the corer. It was then pushed into the sediment to a depth of 10 cm. A shovel was then used to slowly retrieve the corer with one plant and its accompanying sediment intact inside.
Figure 2-1. Location of sampling sites for macrophyte and sediment-sampling (the concentric circle) and field survey (black dots) in Tingalpa reservoir, Queensland, Australia. (Lu et al. 2017).

Half of the *N. indica* plants with sediments (*N.i.+Sed*) were placed individually into 15 L plastic buckets (bottom diameter: 26 cm; height 23 cm). The other half of the *N. indica* samples were gently washed *in situ* to remove all surrounding sediment. Each *N. indica* plant (*N.i.–Sed*) was then put into a plastic mesh bag (length 43 cm; width: 25 cm; mesh size 2 mm). These litter bags (mesh bags with plants) were sealed with cable ties and transferred into similar buckets used for the *N.i.+Sed* treatment. The
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*N.i.–Sed* treatment was designed to differentiate the role of *N. indica* plants from the interaction of plants with sediments in *N. indica* beds on nutrient release.

**Drying regimes**

Both *N. indica* (*N.i.–Sed*) and *N. indica* bed (*N.i.+Sed*) samples were randomly divided into groups for five drying treatments; 0- (the control treatment without desiccation), 4-, 6-, 10- and 20-week desiccation. Each of the two treatments (*N.i.+Sed* vs. *N.i.–Sed*) had 5 replicates; this resulted in a total of 50 samples (2 treatments × 5 replicates × 5 drying periods). The experiment commenced in winter in a glasshouse with full sunlight, and then was moved outdoors during summer when the glasshouse temperatures became too high, i.e. above 40°C (after 20 weeks of desiccation). In addition, for each drying period, another six buckets (three each with and without an empty mesh bag) were also set up which contained only distilled water as a water control, in order to monitor the effect of other uncontrolled factors to the experiment, such as atmospheric deposition of nutrients.

Ambient light intensity and water temperatures were logged every 30 minutes throughout the experiment using a photosynthetic active radiation (PAR) light logger (LI-1400, Nebraska, USA) and temperature loggers (Thermocron iButton, Maxim Integrated, California, USA), respectively. The moisture content in the sediment after each period of drying was also measured by measuring the weight loss after drying sediment samples to a constant weight at 105°C in an oven.

**Rewetting treatments and sampling protocols after desiccation**

After each period of drying, distilled water (10 L) was gently added (to minimize the disturbance of the sediment) into each bucket without aeration. Distilled water, rather than reservoir water, was used to rewet the treatments to simulate the rewetting impacts
of rainfall (storm water) runoff and also to reduce potential confounding biological
effects from reservoir water, such as the initial nutrient uptake by phytoplankton. The
overlying water was sampled immediately (day 0) and then on days 1, 4, 7, and 14 after
rewetting for the analysis of total dissolved nitrogen (TDN), total dissolved phosphorus
(TDP), NO$_2^-$/NO$_3^-$, NH$_4^+$, phosphate (PO$_4^{3-}$) and total dissolved organic carbon (DOC).
Water samples were filtered through membrane filters (0.45 $\mu$m pore size) and frozen
until analysed. Dissolved nutrients were analysed by colorimetric methods, using a
discrete chemistry analyser (DCA, SmartChem 200, WESTCO Scientific Instruments,
Brookfield, USA; APHA, 2005). DOC was analysed using a total organic carbon (TOC)
analyser (TOC-L, Shimadzu, Japan). Detection limits for PO$_4^{3-}$, NO$_2^-$/NO$_3^-$, NH$_4^+$ and
DOC were 0.01 mg L$^{-1}$, 0.02 mg L$^{-1}$, 0.01 mg L$^{-1}$ and 0.01 mg L$^{-1}$, respectively. Filtered
water samples for TDN and TDP analyses were digested using a simultaneous persulfate
digestion method for N and P (Hosomi and Sudo 1986) before being analysed on the
DCA. Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were
calculated by subtracting inorganic forms of N (NO$_2^-$/NO$_3^-$ and NH$_4^+$) and P (PO$_4^{3-}$)
from TDN and TDP, respectively.

Samples for chlorophyll $a$ (Chl-$a$) were taken only at the end of each rewetting
event (14 days after rewetting). Water samples (80−200 mL) were filtered through glass
fibre filters (GF75, Advantec, Japan). The filters were frozen at −80°C until analysed.
Filters were cut into small pieces first then sonicated in 90% acetone for one minute
with a probe sonicator (Branson 450, USA). The sonicated extract was kept at −20°C
overnight to ensure a fuller extraction of pigments. After extraction, the samples were
centrifuged for 5 min at 3000 rpm (1828 × g RCF; Eppendorf 5810R, Germany), then
filtered through a glass fibre filter (GF75, Advantec, Japan) to remove any additional
particulate matter. Concentrations of Chl-$a$ were calculated by using the trichromatic
equations of Jeffrey (1997) after measuring the absorbance of the extract at 750, 665,
664, 647 and 630 nm with a spectrophotometer (UV-2450 Shimadzu, Japan).

Water temperature, specific conductivity, DO and pH were also measured by a calibrated Hydro-lab logger (Quanta, USA) at each sampling time before sampling water. After water sampling, a recorded volume of distilled water was added into each bucket to compensate for water loss from sampling and evaporation.

Recovery of *N. indica* after drying/rewetting process

The regeneration capacity of *N. indica* plants from its vegetative propagules (roots of *N. indica*) as well as its seed germination capacity in the *N.i.+Sed* treatment was recorded by observing any new shoots/leaves every week for 6 months after each rewetting event.

2.2.3 Field survey and measurements

*Estimation of areal extent and total C, N, P storage of N. indica in Tingalpa reservoir*

Field surveys and satellite images accessed from Google Earth (Google Inc., 2015) were used to estimate the areal extent of *N. indica* before (Oct. 2013) and after (Oct. 2014) water level drawdown in Tingalpa reservoir. Satellite images (Google Inc., 2015) images were used as a first estimation for the areal extent. Field measurement data were used to ground-truth this estimation, then Google Earth Pro (Google Inc., 2015) was used to calculate the areal extent of *N. indica*. The main belt transect method was used for the ground-truthing. Twenty-one transects were chosen parallel to the shoreline of the reservoir, each 2 km long and 10–70 m apart from the shoreline. A water depth of 2–3 m was used for the transect, as this is the typical maximum water depth for this species in this reservoir. Coverage, width and depth of *N. indica* beds were recorded at forty-one sites along transects (Fig. 2-1).
The biomass of *N. indica* per unit area (m²) was estimated from ten 1m × 1m quadrats, which were randomly located at the northern shoreline of the reservoir before water level drawdown (Fig. 2-1). The *N. indica* biomass was estimated only once in this study, since *N. indica* typically retain their coverage (biomass) throughout the year in Tingalpa reservoir. This was verified by comparing the coverage of *N. indica* between summer (December 2012) and winter (July 2012) using Google Earth (Google Inc., 2015) images, before water level drawdown. All *N. indica* plants, including roots, in each quadrat were harvested and rinsed using reservoir water *in situ*. Leaves, stems and roots of *N. indica* were separated in the laboratory and dried in an oven at 60°C to a constant weight. Subsamples of *N. indica* were analysed for total nitrogen (TN) and TP content in the biomass by a segmented flow analyser (Bran + Luebbe AA3 HR, SEAL, UK), following spectrophotometric methods (Rayment and Lyons 2011), after a block digestion with concentrated sulfuric acid (as well as Kjeldahl catalyst tablets, sodium sulfate and selenium; Bremner and Mulvaney 1982).

The total nutrient storage in *N. indica* biomass in the reservoir was calculated using the equation below (2.1):

\[
\text{Nutrient storage (kg)} = \text{biomass per unit area (kg m}^{-2}\text{) } \times \text{areal extent (m}^2\text{)} \times \text{nutrient content per unit biomass (g kg}^{-1}\text{)/1000} \tag{2.1}
\]

*Estimation of TN and TP mass storage in the water column of Tingalpa reservoir*

The TN and TP mass in the water column of Tingalpa reservoir (at 100% FSL) was estimated by the multiplication of the reservoir volume (24,870 ML at 100% FSL) and the mean nutrient concentration data (TN: 0.50 mg L⁻¹; TP: 0.20 mg L⁻¹) in the water column measured in a previous study (late summer 2009; Leigh et al. 2010).
Sediment analyses in Tingalpa reservoir

Five sediment samples (surface layer of 10 cm) without *N. indica* plants were randomly sampled at the same site of *N. indica* beds for the manipulative experiment before water level drawdown (Fig. 2-1), using the same method for *N. indica* bed sampling. Each sediment sample was well mixed (living plants and stones removed), subsampled, oven-dried (40°C) and ground to < 0.5 mm for further analyses. The total organic matter content in the sediment was estimated by measuring the ash free dry weight (AFDW) loss in the desiccated sediment (105°C oven-dried) after 4 h of 500°C treatment in a muffle furnace. TN and TP concentrations in the sediment were analysed using the same method for plant TN and TP analyses. The dithionite-citrate (DC) extractable iron (Fe), aluminium (Al) and total acid digested Fe and Al in the sediment were also analysed. Extractable Fe and Al from sediments was extracted by DC solution (1:50 soil:extractant, 22% sodium citrate solution plus 1g sodium dithionite; Rayment & Lyons, 2011). Total Fe and Al from sediments were determined using a reverse aqua regia microwave digest method (Rayment & Lyons, 2011). The samples for extractable and total Fe and Al were subsequently measured using an inductively-coupled plasma optical emission spectrometry system (ICP-OES; Spectro Analytical Instruments, Kleve, Germany).

### 2.3 Data analyses

All statistical analyses were conducted using R software (version 3.12, R Core Team 2014). The concentrations of nutrients and carbon released from *N.i.+Sed* and *N.i.–Sed* treatments were compared by converting their units to mg g⁻¹ m⁻². This equates to milligram of C, N or P released from per gram of *N. indica* dry biomass in per square metre sediment. To test the effect of two treatments (*N.i.+Sed* vs. *N.i.–Sed*) and five drying periods on changes of dissolved nutrients/carbon in the overlying water over five
rewetting periods, the mixed effect model was used (REML, nlme package in R; Pinheiro et al. 2016). One-way ANOVA, followed by Tukey’s *post-hoc* test, was used to test for differences between the five drying periods on soil moisture content and the survival of *N. indica* plants in the *N.i.+Sed* treatment before rewetting, and on Chl-a concentrations in both *N.i.+Sed* and *N.i.–Sed* treatments, 14 days after rewetting. A t-test was used to test for differences between the two treatments, i.e. *N.i.+Sed* vs. *N.i.–Sed*, on Chl-a concentrations 14 days after rewetting in each drying period treatment. Data were tested for normality, and either log<sub>10</sub> or square-root transformed as required.

### 2.4 Results

#### 2.4.1 Manipulative experiments

The mean water temperature in the buckets in the manipulative experiment increased gradually over the 20-week experiment as the season changed from winter to summer (Table 2-1). The mean water temperature increased by 2°C between each rewetting period, but the maximum temperature did not exceed 32°C during the experiment. Mean daily-accumulated PAR light quanta also increased from 15.8 × 10<sup>6</sup> to 41.3 × 10<sup>6</sup> µmol m<sup>-2</sup> during this period (Table 2-1).
Table 2- 1. Mean (SD) daily-accumulated (0600 to 1830 h) ambient light quanta (µmol m\(^{-2}\)), and mean (SD) daily logged water temperature (°C) in buckets in the manipulative experiment during the 14-day rewetting period, following five drying periods. (Lu et al. 2017)

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<td>17.3</td>
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Recovery of *N. indica* plants after rewetting

The soil moisture content in the *N.i.+Sed* treatment decreased significantly between the different drying periods up to week 6 (One-way ANOVA, Tukey’s *post-hoc* test, \( P < 0.05 \); Fig. 2- 2a), reaching a minimum of 3.5 ± 0.3% (mean ± SD) after 10 weeks of desiccation. After week 6, the soil moisture content in the desiccated sediment was 4.5 ± 0.2%, which was not significantly different compared to week 10. There was also no significant difference in soil moisture content between the 10 and 20-week drying treatments.

During the study, in the *N.i.+Sed* treatment, rewetting for less than 6 weeks of desiccation (i.e. 0 and 4 weeks) resulted in 100% of the *N. indica* plants recovering (regenerated from roots after rewetting). However, for more than 6 weeks of drying, followed by rewetting, only 40% of *N. indica* plants recovered, and 0% survived after the 10 and 20-week desiccation in the *N.i.+Sed* treatment (Fig. 2- 2a). In contrast, none of the *N. indica* plants survived in the *N.i.–Sed* treatment after a 4-week desiccation.

The recovery capacity of *N. indica* from seed germination was higher than that from roots because their seeds still germinated after 20-week desiccation then rewetting.
Figure 2-2. (a) Mean (+ SD) values of soil moisture content (%) in the sediment and recovery percentage (%) of *N. indica* plants in the N.i.+Sed treatment after different drying periods throughout the experiment. (b) Mean (+ SD) concentrations of Chl-a (µg L⁻¹) in the water column of two treatments (N.i.+Sed and N.i.−Sed) 14 d after rewetting, following five drying periods. Different lowercase letters (a, b, c or d) above bars indicate significant differences at $P < 0.05$ level between five drying periods for the N.i.+Sed treatment (One-way ANOVA and Tukey's post-hoc test), and uppercase letters (A, B, C or D) for the N.i.−Sed treatment. Treatments which share a letter in common were not significantly different. An asterisk (*) indicates significant differences at the $P < 0.05$ level between the N.i.+Sed and N.i.−Sed treatments under each drying period (t-test). (Lu et al. 2017)
Table 2-2. Summary of statistical differences (Mixed-effect model) for water quality parameters comparing five drying periods, five rewetting periods and two treatments (N.i.+Sed vs. N.i.–Sed). “+Sed” and “–Sed” in the table refer to N.i.+Sed and N.i.–Sed treatments, respectively.

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<th>Chl-$a$</th>
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$\text{NO}_x$-$\text{N}$: $\text{NO}_2$/$\text{NO}_3$-$\text{N}$. SP. Cond.: specific conductivity. ns: not significant. NA: not available. The asterisk (*) besides “+Sed” or “–Sed” treatments indicate that the parameter in the * labelled treatment was significantly higher than the other unlabeled treatment ($P < 0.05$). Different lowercase letters (a, b, c or d) indicate significant differences at $P < 0.05$ level between five drying periods or between five rewetting periods. From “a” to “d” presents the highest to the lowest treatment. Treatments which share a letter in common were not significantly different. (Lu et al. 2017)
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C, N, and P release from N.i.–Sed and N.i.+Sed treatments after drying/rewetting

Carbon and nutrient concentrations in the water control (in the bucket with empty mesh bags) were close to detection limits. The treatment without desiccation (0-week) was used as the control.

The mean concentrations of DOC in the water column were typically higher (1.5–2 times) in the N.i.+Sed treatment than that in the N.i.–Sed treatment for the 20-week drying treatment (Mixed-effect model, $P < 0.05$), but not for the 4-week drying treatment. The DOC concentrations increased significantly during the first day for the N.i.–Sed treatment, and during the first 4 days for the N.i.+Sed treatment after rewetting. DOC concentrations were then stable in subsequent rewetting periods (Mixed-effect model; Table 2- 2; Fig. 2- 3a, b). For the N.i.+Sed treatment, DOC concentrations were not statistically different between the 0 (control) and 4-week drying treatments. However, in the periods after 6 weeks of desiccation, the DOC concentrations had increased significantly, i.e. treatment 0-week = 4 < 6 < 10 < 20-week (Table 2- 2; Fig. 2- 3a). For the N.i.–Sed treatment, the DOC concentrations were significantly higher in all drying treatments (4, 6, 10 and 20-week) compared to the control (0-week), but there was no statistical difference among drying treatments in weeks 4, 6, 10 and 20 (Table 2- 2; Fig. 2- 3b).

The mean concentrations of DON were significantly higher (1.5–3 times) in the N.i.+Sed treatment compared with the N.i.–Sed treatment in the 10 and 20-week drying periods (Mixed-effect model, $P < 0.05$). In general, for all drying treatments of the N.i.+Sed treatment after rewetting, the DON concentrations kept increasing significantly in the first week (7 d) of rewetting, and then stabilized at the same concentration for the second week of rewetting (Table 2- 2; Fig. 2- 3c). For all drying treatments of the N.i.–Sed treatment, increasing the rewetting period resulted in
significantly higher DON concentrations, i.e. rewetting period on day 14 > 7 > 4 = 1 > 0 (Table 2-2; Fig. 2-3d).

The changes of DON in the N.i.+Sed treatment showed a similar trend between the five drying periods to that of DOC, increasing with drying periods longer than 4 weeks, i.e. the drying treatment 0-week = 4 < 6 < 10 < 20-week (Table 2-2; Fig. 2-3c). The mean concentrations of DON in the N.i.–Sed treatment increased statistically in all the drying treatments compared to the control, but there was no significant difference between drying treatments at 6, 10 and 20 weeks (Table 2-2; Fig. 2-3d).

Similar to DON, the mean concentrations of NH₄⁺ were significantly higher (more than 10 times) in the N.i.+Sed treatment than in the N.i.–Sed treatment for all drying treatments (Mixed-effect model, \( P < 0.05 \); Table 2-2). The concentrations of NH₄⁺ for all drying treatments in the N.i.+Sed treatment did not increase significantly until 7 days after rewetting, then concentrations were stable for the remaining rewetting periods (Table 2-2; Fig. 2-3e). However, the NH₄⁺ release from the N.i.–Sed treatment was not significantly higher compared to the control, except for the 4-week drying treatment after 4 d rewetting (Table 2-2; Fig. 2-3f). Similarly, NH₄⁺ concentrations in the N.i.+Sed treatment did not increase significantly in the 4 and 6-week drying treatments compared to the control. However, after 6 weeks of desiccation, NH₄⁺ concentrations increased as drying periods increased, with the 20-week drying treatment significantly higher than the 10-week, which in turn was significantly higher than the 0 and 4-week drying treatments (Table 2-2; Fig. 2-3e).
Figure 2-3. Mean (+ SD) concentrations (mg g⁻¹ m⁻²) of DOC (a & b), DON (c & d), NH₄⁺ (e & f), DOP (g & h) and PO₄³⁻ (i & j) released from the N.i.+Sed and N.i.–Sed treatment during the 14-day rewetting period, following five drying periods. (Lu et al. 2017)
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In contrast to DON and NH$_4$+, the mean concentrations of PO$_4^{3-}$ were more than 20 times lower in the N.i.+Sed than in the N.i.–Sed treatment for all drying treatments (Mixed-effect model, $P < 0.05$; Table 2-2). The PO$_4^{3-}$ release from the N.i.+Sed treatment was not significantly higher than the control after rewetting, except for one day after rewetting in the 10-week drying treatment (Table 2-2; Fig. 2-3i). In contrast, PO$_4^{3-}$ concentrations in the N.i.–Sed treatment increased significantly after 7 days of rewetting (Table 2-2; Fig. 2-3j). The concentrations of PO$_4^{3-}$ in the N.i.–Sed treatment also increased as drying periods increased, with the 10 and 20-week drying treatments significantly higher than the 4 and 6-week, while the 6-week drying treatment was significantly higher than the control (Table 2-2; Fig. 2-3j).

There was no significant difference in DOP concentrations between N.i.+Sed and N.i.–Sed treatments (Mixed-effect model, $P > 0.05$; Table 2-2). For both N.i.+Sed and N.i.–Sed treatments, the DOP concentrations in all drying treatments increased significantly with increasing rewetting periods within the 14 days of rewetting (Table 2-2, Fig. 2-3g, h). In the case of the N.i.+Sed treatment, the DOP concentrations increased significantly as drying period increased, although there was no significant difference between the 0 and 4-week treatments, or between the 6 and 10-week drying treatments (Table 2-2; Fig. 2-3g). In contrast, DOP was not significantly different in the N.i.–Sed treatment between the 4, 6, 10 and 20-week drying treatments, but they were all statistically higher than the control (Table 2-2; Fig. 2-3h). Overall, concentrations of NO$_2^-/NO_3^-$ in all treatments were close to the analytical detection limit (0.02 mg L$^{-1}$), with no significant differences between treatments (Mixed-effect model, $P > 0.05$; Table 2-2).

The highest proportion of nutrients released from the two treatments (N.i.+Sed and N.i.–Sed) were in organic forms. Specifically, more than 98% of TDN and 40–60%
of TDP released from the *N.i.*−Sed treatment after rewetting were in organic forms (Fig. 2-4). There was a lower proportion (70%) of DON, but higher proportion of DOP (more than 90%) released from the *N.i.*+Sed treatment compared with the *N.i.*−Sed treatment.

**Figure 2-4. The percentage of inorganic and organic forms of dissolved N and P, compared with the total dissolved N and P, released from two treatments (*N.i.*+Sed and *N.i.*−Sed) after five drying periods followed by rewetting. (Lu et al. 2017)**

**Changes in physico-chemical parameters after rewetting**

Changes in DO and pH in the water column were not significantly different between the five drying periods (Mixed-effect model, \( P > 0.05 \), Table 2-2), so data from the different drying periods were combined for each rewetting period. Concentrations of DO decreased significantly from 0 to 24 h after rewetting, and continued to decline to
2.6 ± 1.5 mg L\(^{-1}\) in the *N.i.*−Sed treatment, and to 3.2 ± 1.5 mg L\(^{-1}\) in the *N.i.*+Sed treatment by day 4 (Fig. 2- 5a). Then DO concentrations started to increase gradually over the next 10 days, and reached 3.2 ± 1.6 mg L\(^{-1}\) in the *N.i.*−Sed treatment, and 4.9 ± 1.7 mg L\(^{-1}\) in the *N.i.*+Sed treatment by day 14 (Table 2- 2; Fig. 2- 5a). The level of pH decreased significantly within the first 24 h after rewetting in both two treatments (Mixed-effect model, *P* < 0.05; Table 2- 2), and then gradually increased to values equivalent to day 0, 14 days after rewetting (Fig. 2- 5b). Compared with the *N.i.*+Sed treatment, the concentrations of DO in the *N.i.*−Sed treatment were statistically lower (t-test, *P* < 0.05), while pH levels were statistically higher (t-test, *P* < 0.05) in the water column 7 days after rewetting.

The specific conductivity values were significantly higher in the *N.i.*+Sed treatment compared with the *N.i.*−Sed treatment after the same period of drying, followed by rewetting (Mixed-effect model, *P* < 0.05; Table 2- 2). The specific conductivity values increased significantly with longer drying periods for the *N.i.*+Sed treatment, with the 4-week drying treatment significantly higher than the control, but significantly lower than the 6 and 10-week drying treatments (Fig. 2- 5c). There was no difference between the 6 and 10-week drying treatments, but they were significantly lower than the 20-week drying treatment (Fig. 2- 5c). For the *N.i.*−Sed treatment, the specific conductivity increased significantly after 4 weeks of desiccation followed by rewetting. However, there was no further significant increase specific conductivity values between drying treatments after more than 4 weeks of desiccation, i.e. the 4-, 6-, 10- and 20-week drying treatments were all the same, but they were all significantly higher than the control (Fig. 2- 5d).
Chlorophyll a concentrations after drying/rewetting

For the N.i.+Sed treatment, the Chl-a concentrations in the water column 14 days after rewetting did not increase significantly until the drying period prior to rewetting was greater than 10 weeks (One-way ANOVA, Tukey’s post-hoc test, \( P < 0.05 \), Fig. 2- 2b). This coincided with the time when \textit{N. indica} plants and the sediment were all completely desiccated (Fig. 2- 2a). Compared to the N.i.+Sed treatment, there was significantly less Chl-a in the N.i.–Sed treatment 10 weeks after drying then rewetting (t-test, \( P < 0.05 \)). For the N.i.–Sed treatment, the only significant increase in Chl-a concentrations occurred in the 4-week drying treatment, compared with other drying
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2.4.2 Estimation of nutrient release from *N. indica* beds on a whole reservoir basis

At the reservoir scale, the biomass of *N. indica* per unit area in the macrophyte beds was measured as 1.01 ± 0.17 kg DW m\(^{-2}\) (dry weight m\(^{-2}\)), and the areal extent of *N. indica* was 0.37 km\(^2\) before water level drawdown. Using data on TN (1.06 ± 0.14%) and TP (0.20 ± 0.04%) content of *N. indica*, the TN and TP mass of *N. indica* on a whole reservoir basis was calculated to be 3,870 and 660 kg, respectively.

TN and TP mass (storage) in the water column of the reservoir from which *N. indica* was collected was estimated to be 12,400 kg and 420 kg, respectively, before water level drawdown (at 100% FSL). The content of organic matter (measured by AFDW), TN and TP in sediments (surface layer of 10 cm) of Tingalpa reservoir were 90 ± 20 g kg\(^{-1}\), 2.90 ± 2.00 g kg\(^{-1}\) and 0.23 ± 0.15 g kg\(^{-1}\) respectively, before water level drawdown. Thus, the estimated nutrient storage in the sediment (the area rooted by macrophytes) was 180,000 kg TN and 15,000 kg TP.

After water level drawdown, the areal extent of *N. indica* in Tingalpa reservoir decreased from 0.37 km\(^2\) to 0.07 km\(^2\), with the associated dry biomass decreasing from 370 to 70 tonnes. Based on the data from our manipulative experiment, the TDN and TDP released from *N. indica* in litter bags was 0.21 ± 0.04 mg N g\(^{-1}\) DW and 0.40 ± 0.16 mg P g\(^{-1}\) DW, respectively, 14 days after rewetting. This was used to calculate the total amount of TDN and TDP that would be released from desiccated *N. indica* on a whole reservoir basis. This was estimated to be 60 kg N and 120 kg P. This amount of released nutrients would account for 0.5% of TN and 29% of TP in the storage of the water column in the whole reservoir at 100% FSL. In contrast, the total amount of TDN and TDP released from *N. indica* beds (same area as *N. indica*) would account for more TN (4.3%) and less TP (0.3%) of the reservoir water nutrient storage.
Since Fe and Al in the sediment can contribute to sediment P sorption capacity (Nowlin, Evarts & Vanni, 2005), the DC extractable Fe and Al, and total Fe and Al in the sediment of Tingalpa reservoir were measured as 1.40 ± 0.50%, 0.20 ± 0.05%, 1.60 ± 0.60% and 1.30 ± 0.05%, respectively.

### 2.5 Discussion

Our study showed that *N. indica* biomass was a substantial nutrient sink in Tingalpa reservoir before water level drawdown, compared with the nutrient mass in the water column. However, after a long period of water level drawdown (10 weeks or more in our study) followed by rewetting, the desiccated *N. indica* could turn into a substantial source of nutrients to the water column. Indeed, after 14 days of rewetting, 7% of TN and 52% of TP in the dry biomass of *N. indica* plants were released into the water column. This released nutrient would theoretically account for 0.5% of TN and 29% of TP in the whole reservoir nutrient storage in the water column when the reservoir is full, without considering nutrient processing within the water column. This indicates that decayed *N. indica* might be a relatively more important P source, compared with N, during water level drawdown then rewetting. This difference in N and P release was also noted by Landers et al. (1982), who investigated the nutrient release from the senescing submerged macrophyte *Myriophyllum spicatum* in a subtropical reservoir. Landers et al. (1982) found that the N input to the reservoir from decayed *M. spicatum* was less important than P (2.2% and 18% respectively).
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Table 2-3. Comparison of the percentage (%) of released nutrients relative to the total nutrient content of a range of macrophyte species upon rewetting in this study and that estimated in several previous studies, 14 days after decomposition. (Lu et al. 2017)

<table>
<thead>
<tr>
<th>Macrophyte life forms</th>
<th>Species</th>
<th>Nutrient released (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>Submerged</td>
<td>Potamogeton pectinatus</td>
<td>20%</td>
<td>↑60%</td>
</tr>
<tr>
<td></td>
<td>Hydrilla verticillata</td>
<td>30%</td>
<td>↑70%</td>
</tr>
<tr>
<td></td>
<td>Najas guadalupensis</td>
<td>30%</td>
<td>↑75%</td>
</tr>
<tr>
<td>Floating/float-Leaved</td>
<td>Nymphoides indica</td>
<td></td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>Salvinia natans</td>
<td></td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>Eichhornia crassipes</td>
<td>20%</td>
<td>↑55%</td>
</tr>
<tr>
<td></td>
<td>Pistia stratiotes</td>
<td>50%</td>
<td>↑70%</td>
</tr>
<tr>
<td>Emergent</td>
<td>Carex riparia</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>Typha domingensis</td>
<td>15%</td>
<td>↑20%</td>
</tr>
</tbody>
</table>

↑, − and ↓ stand for higher, similar and lower nutrient release, respectively, compared with N. indica in this study.
The P release proportion from desiccated *N. indica* after 14 days of decomposition in our study was similar to previous studies (Table 2-3; Asaeda et al. 2000; Chimney and Pietro 2006; Longhi et al. 2008; Li et al. 2014). This substantial P release from desiccated *N. indica* in our study is also consistent with Bostic and White’s (2007) study, which showed that the vegetated sediment was able to release five times more P than non-vegetated sediment after a single drawdown (142 d) and re-flooding event.

If *N. indica* is considered in the context of macrophyte beds (*N.i.+Sed*), rather than by just considering the plant, our study showed that desiccated *N. indica* beds released similar amount of DOP as the *N. indica* alone, indicating that desiccated *N. indica* was the main source of DOP release in *N. indica* beds. However, the release of PO$_4^{3-}$ from *N.i.+Sed* was more than 10 times lower than the *N.i.–Sed* treatment. This result conflicts with some previous studies, which showed that air-dried sediments can release much more PO$_4^{3-}$ after rewetting than non-dried sediment (Baldwin et al. 2000; Watts 2000b; Kerr et al. 2010). The lower PO$_4^{3-}$ release in our study was likely due to rapid PO$_4^{3-}$ assimilation by phytoplankton, since phytoplankton biomass in the *N.i.+Sed* treatments was significantly higher than that in *N.i.–Sed* treatment. We calculated that the potential P uptake by phytoplankton in our study based on the literature values for carbon:Chl-*a* ratios ranging from 27–68:1 (Gosselain et al. 2000; Barbosa et al. 2001) and Redfield (1958) C:P molar ratios (106:1; mass ratio 40:1) for phytoplankton. Therefore, the calculated mass ratio of P:Chl-*a* ranged from 0.7–1.7:1 (27–68:1/40:1). Based on this P:Chl-*a* mass ratio, and the concentration of Chl-*a* (320 μg L$^{-1}$) in our *N.i.+Sed* treatment, the maximum PO$_4^{3-}$–P taken up by phytoplankton would be 550 μg L$^{-1}$, which is less than 10% of the released PO$_4^{3-}$ (7000 μg L$^{-1}$) from our *N.i.+Sed* treatment.
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Adsorption of PO$_4^{3-}$ by soil particles in macrophyte beds is another explanation for the lower PO$_4^{3-}$ concentrations in the *N. indica*+Sed treatment compared to the *N. indica*–Sed treatment. This PO$_4^{3-}$ adsorption has also been found in several previous studies of WLFs (Nowlin et al. 2005; Tang et al. 2014; Keitel et al. 2016). Fe and Al in soil particles can contribute to soil P sorption capacity (Nowlin et al. 2005). Our sediment had high extractable Al:TP and Fe:TP ratios (> molar ratio of Al$_{NaOH}$ extractable:P$_{BD}$ extractable = 25), indicating the P adsorption rather than desorption capacity of our sediments as found in other studies (Kopáček et al. 2005; Burford et al. 2012). Additionally, based on previous studies, sediment P influx rates tested in P-enriched water (2 mg L$^{-1}$) was over 390 mg P d$^{-1}$ kg$^{-1}$ for dry sediment (Kerr et al. 2010), or over 50 mg P d$^{-1}$ m$^{-2}$ for wet sediment (Dieter et al. 2015). This is much higher than the maximum PO$_4^{3-}$ release from *N. indica* plants in our study, which had a maximum of 10 mg P d$^{-1}$ kg$^{-1}$ for dry sediment, or 20 mg P d$^{-1}$ m$^{-2}$ for wet sediment after adsorption. Therefore, in the long-term, the storage of P transferred from macrophytes into sediments may become a potential P-source to the water column in anoxic conditions (e.g. Furumai and Ohgaki 1989; Nowlin et al. 2005; Burford et al. 2012). This could result from the reduction process of amorphous hydrous ferric oxide to soluble Fe (II) and subsequent mobilization of P under anoxic conditions.

The N release proportion from rewetting desiccated *N. indica* was nearly 2–3 times lower compared with studies of other macrophyte species after 14 days of decomposition (Table 2-3; Asaeda et al. 2000; Chimney and Pietro 2006; Longhi et al. 2008; Li et al. 2014). This could be due to the fact that we only measured TDN, rather than TN in the water column, in our study. Furthermore, the plant-only treatment in our study might have decomposed more slowly due to the lack of soil microbial activity after drying then rewetting.
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The treatments simulating macrophyte beds in our study had threefold higher TDN release compared with *N. indica* alone. More than 75% of this released TDN was in organic forms, indicating that soil organic matter was the major N source to the water column when rewetting the desiccated macrophyte beds. The decomposition of organic matter in the sediment can be accelerated by sediment exposure to the air as a result of water level drawdown (Aller 1994). Decayed macrophytes are likely to be the major source of organic matter to soil in macrophyte beds. Water level drawdown therefore has the potential to replenish soil organic matter in macrophyte beds (Kleeberg and Heidenreich 2004; Palomo et al. 2004). The replenished soil organic matter from decayed macrophytes may also serve as a continuous slow-release nutrient source to the water column after rewetting. This high organic matter content in sediments might also impede macrophyte recovery due to the high oxygen demand of microbes creating anoxic conditions (Raun et al. 2010). Previous studies also demonstrated that inorganic carbon could accelerate microbial activity, increasing nutrient release rates after rewetting the desiccated sediments and macrophytes (Baldwin et al. 1997; Watts 2000a).

Our study also measured higher phytoplankton biomass in the water column when nutrient release was higher. It is well established that phytoplankton need inorganic N and P for growth, however, they can also use some organic forms of nutrients for growth (e.g. Berman and Chava 1999; Doblin et al. 1999; Burford et al. 2014). Cymbola et al. (2008) demonstrated that the sediment-derived nutrients not only stimulated the growth of phytoplankton, but its interaction with light also changed the phytoplankton community structure, although this was not examined in our study.

The increased phytoplankton biomass in our study also coincided with the timing of macrophyte death. This phytoplankton biomass increase is likely due to the loss of
competition for nutrients and light with macrophytes (Blindow 1992; Coops et al. 2003). Water quality deterioration in lakes or reservoirs after water level drawdown, followed by re-filling events, has also been found in previous studies (e.g. Beklioğlu and Tan 2008; Zohary and Ostrovsky 2011; Callieri et al. 2014). However, the survey of Teferi et al. (2014) on re-filling thirteen completely dried reservoirs in tropical areas (Sudano–Sahelian region, Africa) found an improvement in water quality and less phytoplankton blooms in those reservoirs. This was probably due to the high dilution of nutrients and phytoplankton biomass by flushing of inflow water (or rainfall), as well as the fish mortality during desiccation. Fish removal by mechanisms such as mortality was proposed to increase zooplankton densities, which in turn increased grazing pressure on phytoplankton (biomanipulation).

One limitation of our study is that the temperature and daily-accumulated light intensity increased during the study. Despite this, there was no significant increase in nutrient release between drying periods for the N.i.–Sed treatment after N. indica was completely desiccated, indicating that drying period duration was the main cause, rather than changes in temperature and daily light intensity over the study. The use of distilled water rather than reservoir water to rewet our treatments may also have affected the nutrient concentrations of our study, although a similar rewetting method has been used in previous studies (Fierer and Schimel 2002; Yanai et al. 2007). Distilled water might result in a greater diffusion of nutrients from sediments/macrophytes to the water column due to a steep concentration gradient. However, the control treatment (0-week desiccation) in our study did not result in unrealistic nutrient release from rewetting by distilled water (TDN < 0.2 mg L\(^{-1}\), TDP < 0.009 mg L\(^{-1}\)), compared to the 20-week drying treatment (TDN > 2.0 mg L\(^{-1}\), TDP > 0.6 mg L\(^{-1}\)). This suggests that the use of distilled water was not
a significant factor affecting our results.

Overall, this study showed that rewetting of desiccated macrophyte beds after long drying periods can cause significant nutrient release from both macrophytes and macrophyte beds, which in turn can increase phytoplankton biomass. This suggests that lowering water levels, which have previously been regarded as a potential tool for macrophyte restoration, also has the potential to shift macrophyte beds from a nutrient sink to a source. Additionally, drying then rewetting processes may replenish soil organic matter and increase P storage in macrophyte beds from the decomposition of decayed macrophytes, resulting in the increase of internal nutrient storage, at least in lentic waterbodies. This internal nutrient source in lakes or reservoirs has the potential for a delayed effect on phytoplankton growth (Fisher and Reddy 2001; Bostic and White 2007). The impact of the interaction between macrophytes and sediments on the forms and amount of nutrient release in our study also provides new insights into the nutrient cycling process during WLFs, since a number of previous studies have oversimplified the nutrient response to rewetting by focussing simply on macrophytes or sediments alone.
Chapter Three: Effects of water level fluctuations on nitrogen dynamics in littoral macrophytes

Possible fate for the macrophyte-derived N (from macrophyte litter) after drying then rewetting.

Chapter 2 found that macrophytes could be an important nutrient sink in shallow freshwater ecosystems. However, their role as a nutrient sink and a competitor with phytoplankton can be reversed if they die and subsequently release nutrients.

In this Chapter, I will investigate the fate of the released N from macrophyte decomposition after drying then rewetting.
This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors, are:


My contribution to the paper involved designing the experiment, collecting and analysing the data, providing direction on the structure of the result analysis, writing the manuscript, and addressing the comments from reviewers. Michele Burford is the principle supervisor of this project, contributing to developing the ideas of the experiment, providing direction on the structure of the result analysis and revising the manuscript. Stuart Bunn is the associated supervisor of this project, contributing to providing comments to improve the manuscript and data analyses.

(Signed) _________________________________ (Date)______________

Name of Student and corresponding author of the paper: Jing Lu

(Countersigned) ___________________________ (Date)______________

Supervisor: Prof. Michele Burford
3.1 Introduction

Submerged macrophytes play a key role in assimilating and storing nutrients and controlling phytoplankton blooms as they compete with phytoplankton for nutrients and light (van Donk et al. 1993; Van Donk and Van de Bund 2002). However, this role of macrophytes could be offset during periods of plant die-back when decomposition processes result in nutrient release (Li et al. 2014).

Water level fluctuations (WLFs) can lead to water quality deterioration through the nutrient release from rewetting the desiccated sediments and macrophytes in the littoral zone. As discussed in earlier sections, rewetting after drying can result in significant nitrogen and carbon dioxide release from desiccated sediments, a process known as the “Birch effect” (Birch 1958). We have also demonstrated that water level drawdown has the potential to shift macrophyte beds from a nutrient sink to a source after rewetting in Chapter 2 (Lu et al. 2017). Nutrients released from sediments or decayed macrophytes might become a direct nutrient source for phytoplankton growth. This water quality deterioration during WLFs is a particular issue in areas where frequent hydrological changes occur due to droughts or floods (Beklioğlu and Tan 2008; Romo et al. 2013), and in reservoirs where water levels are affected by water demand for human uses (Zohary and Ostrovsky 2011; Rangel et al. 2012). WLFs are likely to become more extreme with global climate change affecting weather conditions, e.g. more frequent droughts and floods (Hirabayashi et al. 2008; Dai 2011; Callieri et al. 2014).

Macrophyte species may vary in their impacts on water quality during WLFs due to various nutrient uptake/release capabilities and the density of canopy they can form (Frodge et al. 1990). Invasive species, for example, are more likely to form a dense canopy that
reduces oxygen exchange and biodiversity, compared to native species (Schooler et al. 2006; Schooler 2009). These invasive species typically have rapid growth and high adaptability to a range of environmental conditions so that they can out-compete native species (Blumenthal and Hufbauer 2007). Therefore, invasive species are expected to have more nutrient storage than native species, but also release more nutrients when drying and rewetting events occur.

Desiccated macrophyte beds are also capable of recovering after rewetting, mainly by the germination from roots or seeds, or by the growth of shoot fragments in the water column (Thomaz et al. 2006). These regrown submerged macrophytes can utilize nutrients from both the water column and sediments (Carignan and Kalff 1982; Chambers et al. 1989; Madsen and Cedergreen 2002), and thus might reduce the amount of nutrients available for the uptake by phytoplankton. However, the fate of nutrients released from desiccated macrophytes as a result of WLFs, as well as the impact of regrown macrophytes on the availability of nutrients are poorly understood. For example, it is unclear whether these released nutrients are stored in the sediment, re-assimilated by regrown macrophytes, cycled through the water column or released to the atmosphere, and whether these nutrients become available to phytoplankton.

Sediment desiccation has been found to increase the regrowth of macrophytes due to the increased nutrient availability in the system after rewetting, thus impacting the fate of nutrients released from macrophytes decomposition (Barko et al. 1986; James et al. 2004). Sediment desiccation might also affect the nutrient dynamics through interfering microbial activities or redox conditions. For instance, sediment desiccation can increase the nitrification process by providing energy (C), substances (NH$_4^+$) and oxidization conditions.
for nitrifiers after rewetting, or slow down microbial activities after severe drawdown conditions (Qiu and McComb 1996; Baldwin and Mitchell 2000).

There has been increasing evidence of N limitation for phytoplankton growth in freshwater ecosystems (e.g. Cavanaugh et al. 2006; Elser et al. 2007). The substantial N released from macrophyte beds after drying then rewetting, therefore, could be a significant trigger for phytoplankton blooms in N-deficient lakes or reservoirs. Stable nitrogen isotope ($^{15}$N) tracing techniques can be used to investigate N cycling processes in aquatic ecosystems, such as the N assimilation by phytoplankton or periphyton (Neess et al. 1962; Glibert et al. 1982; Axler and Reuter 1996), the transformation of N inputs in food-webs (Hadwen and Bunn 2005), and the denitrification in lake sediments (Nielsen 1992; Risgaard-Petersen and Jensen 1997) or in wetlands (Jahangir et al. 2017). This technique has also been used in forests or farmlands to determine the fate of plant-derived N (Müller and Sundman 1988; Zeller et al. 2000).

In this study, we used $^{15}$N tracing techniques to examine the fate of macrophyte-derived N upon rewetting following a drying event. We aimed to investigate: 1) if the species composition of the litter, i.e. *Cabomba caroliniana* (invasive) and *Hydrilla verticillata* (native), affects N dynamics upon rewetting; 2) the effect of living submerged macrophytes on the fate of N derived from macrophyte litter; and 3) the effect of sediment desiccation history on the fate of N derived from macrophyte litter.

### 3.2 Methods

3.2.1 Labelling of submerged macrophyte with $^{15}$N

Healthy shoots of *C. caroliniana* (*Cabomba*) and *H. verticillata* (*Hydrilla*) were collected
from Ewen Maddock reservoir (26.6808° S, 153.0061° E) and Wyaralong reservoir (27.9092° S, 152.8811° E) respectively in SEQ, Australia. Macrophyte shoots were washed thoroughly with tap water in the laboratory to remove any attached periphyton and were subsequently cultivated in the laboratory in a \(^{15}\text{N}\)-labelled nutrient solution.

A 2000-litre water tank (diameter: 190 cm; height: 90 cm) was used for macrophyte cultivation. The tank was cleaned with sodium dichloroisocyanurate, filled with tap water, and left in natural sunlight for a week to remove the chlorine. The macrophyte shoots (length: 30 cm) were secured on a plastic mesh by cable ties and anchored to the bottom of the tank by stones. A \(^{15}\text{N}\)-labelled nutrient solution was made up according to the modified one-fifth strength standard Hoagland nutrient solution (Hoagland 1937), including N (from sodium nitrate: \(\text{Na}^{15}\text{NO}_3\); \(^{15}\text{N}\): 99.9%, 0.42 mg \(^{15}\text{N}\) L\(^{-1}\)), P (0.06 mg P L\(^{-1}\)), potassium, magnesium, iron, and other micronutrients. The nutrient solution was added to the tank every week to ensure macrophytes had sufficient nutrients for growth, and to achieve the desired level of \(^{15}\text{N}\) enrichment. Each species was cultivated separately in the tank for two weeks. The final \(^{15}\text{N}\) ratio in macrophyte biomass was 2.5 atom% for \textit{Cabomba} and 4.0 atom% for \textit{Hydrilla}.

After two weeks of cultivation, the \(^{15}\text{N}\)-labelled \textit{Cabomba} and \textit{Hydrilla} were harvested, cut into 10 cm fragments and mixed well. Subsamples of each species were oven-dried at 50°C to a constant weight, and 2.5 g of dry biomass was packed into each litter bag (width: 5 cm; length: 10 cm; pore size: 2 mm). Total carbon (TC) and TN content in \textit{Cabomba} and \textit{Hydrilla} litter was measured using a stable isotope ratio mass spectrometer (Sercon Hydra 20–22, Sercon Ltd, UK), with a front sample combustion system (Euro EA-GLS elemental analyser, Sercon Ltd, UK). The C:N ratio was then
calculated in macrophyte litter.

3.2.2 Experimental design for sediment desiccation

The litter bags were placed in mesocosms with two sediment treatments prior to commencing the experiment, a dried then rewetted sediment treatment and a constantly wet sediment treatment. The dried then rewetted treatment was to simulate the effect of water level drawdown followed by rewetting. Fresh sediment was collected from the surface (10 cm) of the littoral zone (water depth 5–10 cm) of Tingalpa Reservoir (27.5281° S, 153.1803° E) in SEQ using a spade. The sediment was well mixed to homogenize and remove stones and plant roots, then sediment samples (800 g wet weight each) was placed in the bottom of 128 plastic non-transparent mesocosms (5 L, diameter: 18 cm, height 20 cm). Half of the mesocosms (n = 64) were dried for 10 weeks in a glasshouse (mean temperature 30 ± 4°C) to reach a minimum soil moisture around 1.1%. The remaining mesocosms (n = 64) were kept moist by adding 300 mL deionized water weekly (water depth < 0.5 cm). After this 10-week period, a litter bag with either $^{15}$N-labelled *Cabomba* (C) or *Hydrilla* (H) was added to each mesocosm and anchored to the sediment with stones. Therefore, the treatments consisted of “constantly wet” (WC or WH) and “previously dried” (DC or DH) sediment, containing either *Cabomba* or *Hydrilla* litter.

3.2.3 Mesocosm set-up and sampling methods

One litre of surface reservoir water, sampled from Tingalpa Reservoir (27.5281° S,153.1803° E), was mixed with 3 L of deionized water and added to each mesocosm to rewet all treatments. To investigate the impact of macrophyte regrowth on the fate of $^{15}$N, half of the treatments had 6 g (wet biomass) of healthy living *Cabomba* or
Hydrilla shoot fragments added (Cs or Hs) into the water column. Therefore, the experiment had three main treatments, i.e. treatment 1 (T1): different species in the litter (Cabomba versus Hydrilla); T2: sediment desiccation history (“dried then rewetted” versus “constantly wet”); T3: living macrophytes (presence versus absence). The three main treatments consisted of eight specific treatments, WC, WCCs, DC, and DCCs for Cabomba and WH, WHHs, DH, DHHs for Hydrilla, each with four replicates for four rewetting periods respectively (Table 3-1).

Table 3-1. Experimental design. Sampling occasions for each treatment were 3, 7, 14, and 28 d after rewetting. Four replicates were used for each specific treatment. (Lu et al. 2017a)

<table>
<thead>
<tr>
<th>Codes for the eight specific treatments</th>
<th>Species in the litter (C or H)</th>
<th>Sediment desiccation (D or W)</th>
<th>Living macrophytes (Cs or Hs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>Cabomba</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DCCs</td>
<td>Cabomba</td>
<td>+</td>
<td>+</td>
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<td>Hydrilla</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>WHHs</td>
<td>Hydrilla</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

Samples of five major N pools from each mesocosm were taken from four replicates on day 3, 7, 14 and 28 after rewetting. These N pools were: particulate organic N (PON) in the water column; the total dissolved water column N without PON (dissolved fraction thereafter); the entire sediment in the mesocosm (well mixed); the living macrophyte regrown from fragments (regrown macrophytes thereafter); and the remaining macrophyte litter. PON samples were assumed to represent the phytoplankton N pool (Del Giorgio and
France 1996). In addition, samples for $^{15}$N-natural abundance measurement in each pool were taken before rewetting the mesocosms when $^{15}$N-labelled litter was not added.

Chlorophyll $a$ concentrations and $^{15}$N isotope ratios for PON samples were measured by filtering 100 mL of water samples onto pre-combusted (450°C, 4 h) glass fiber filters (47 mm diameter; GF75, Advantec, Japan). Filters for Chl-$a$ were frozen at –80°C until analysed, while those for isotope analysis were oven-dried at 50°C to a constant weight. The supernatant from filtration (500 mL), which represented for the dissolved fraction of the water column, was frozen in wide mouth high-density polyethylene jars for subsequent freeze drying (VirTis-wizard 2.0, VWR, USA). The macrophyte litter in litter bags and the living macrophytes in each mesocosm were rinsed with deionized water and dried in an oven at 50°C to a constant weight. At the end of sampling, the entire sediment in each mesocosm was well mixed, subsampled, freeze-dried and ground for $^{15}$N analysis.

Water samples for TDN and TDP concentrations were processed by filtering through membrane filters (0.45 $\mu$m pore size, Whatman). TDN and TDP were analysed colorimetrically using Continuous Segmented Flow Analyser (SEAL Auto Analyser 3 HR, SEAL Analytical Limited, UK). Concentrations of Chl-$a$ were measured and calculated using the same method listed in Chapter 2.

Total dry weights or volumes of each N pool were recorded for subsequent $^{15}$N mass balance calculations. The water temperature in representative mesocosms and the ambient light intensity above mesocosms were recorded at a frequency of 30 mins during the experiment using temperature loggers (Thermocron iButton, Maxim Integrated, California, USA) and a PAR light logger (LI-1400, Nebraska, USA), respectively. The specific conductivity, DO, pH and turbidity in the water column in each mesocosm were measured.
around 17:00 and 18:00 h on each sampling occasion using a calibrated Hydro-lab logger (Quanta, USA).

3.2.4 Isotope $^{15}$N analyses

Dried macrophyte and sediment samples were ground into fine ($< 0.1$ mm) homogenized powder using a RETSCH Mixer Mill (MM 400, GENEQ Inc., Canada). Sub-samples (2–3 mg; approximately $50 \mu$g N in each sample) of plant material, sediment, and freeze dried water samples, and one-eighth of each PON filter were weighed and folded into tin capsules for subsequent isotope analyses. TN content and $^{15}$N/$^{14}$N ratios of all samples from treatments before and after rewetting were measured using a stable isotope ratio mass spectrometer (Sercon Hydra 20–22, Sercon Ltd, UK), with a front sample combustion system (Euro EA-GLS elemental analyser, Sercon Ltd, UK).

The mass balance for $^{15}$N in each mesocosm was calculated using the amount of added $^{15}$N, which was the subtraction between the total $^{15}$N and the naturally occurring $^{15}$N (calculated from $^{15}$N-natural abundance) in each pool. The $^{15}$N-natural abundance in each N pool was measured from the samples before $^{15}$N-labelled litter was added. Since Cabomba and Hydrilla litter had different $^{15}$N enrichment levels after cultivation, the $^{15}$N mass in each N pool in Cabomba or Hydrilla litter treatments were standardized to a percentage relative to the initial $^{15}$N mass input (3.1):

\[
\text{\% of added } ^{15}\text{N (in each pool)} = \frac{\text{Additional } ^{15}\text{N mass in each pool}}{\text{Total input of additional } ^{15}\text{N mass in Cabomba or Hydrilla treatments}} \times 100\% \tag{3.1}
\]

Therefore, the relative importance of each N pool in the two macrophyte litter treatments
can be compared using the standardized added $^{15}$N percentage ($%^{15}$N thereafter). The recovery of $^{15}$N for each mesocosm was the total $%^{15}$N of all quantified N pools. The residual $^{15}$N was the total of unquantified N pools, i.e. the subtraction between 100% and the recovered $%^{15}$N.

### 3.3 Data analyses

Data were analysed using R software (version 3.12, R Core Team 2014). A General Linear Model (GLM) was used to compare the differences of standardized added $^{15}$N percentage ($%^{15}$N) transferred to each N pool and the total recovery of $^{15}$N in each mesocosm amongst the eight specific treatments (DC, DCCs, WC, WCCs, DH, DHHs, WH, and WHHs). For each parameter, four fixed variables were applied in the linear model, i.e. T1: macrophyte species in the litter (*Cabomba* versus *Hydrilla*); T2: sediment desiccation history (“dried then rewetted” versus “constantly wet”), T3: living macrophytes (presence versus absence), T4: rewetting periods. Sum of squares for T1, T2, and T3 in each model were calculated to compare the relative magnitude of the significant differences among the three treatments. The interactions between T1, T2, and T3 and their interactions with rewetting periods were also applied in the linear model. However, the interaction terms amongst T1, T2, and T3 were dropped from the model for the N pool of dissolved fraction, PON, sediment and the litter as they did not significantly improve the model. The differences amongst T1, T2, and T3 for each N pool after each rewetting period were also compared using GLM.

GLM was also used to compare physio-chemical variables (specific conductivity, pH, DO and turbidity), water column Chl-$a$ concentrations, water column TDN and TDP concentrations, and TN and total biomass lost from *Cabomba* and *Hydrilla* litter amongst the eight specific treatments. The comparison of nutrient concentrations in the water
column was based on nutrients leaching from the same dry biomass of *Cabomba* and *Hydrilla* litter. A two-way ANOVA was used to determine the effect of macrophyte species and the sediment desiccation process on the biomass increase of living macrophytes after each rewetting period. Data were tested for normality and natural log-transformed as required.

### 3.4 Results

#### 3.4.1 Physio-chemical and nutrient variables after rewetting

The mean daily temperature and daily-accumulated light quanta were 24.1 ± 3.1°C and 11.7 ± 3.9 mol m⁻² d⁻¹, respectively, during the experiment. The C:N ratio in the *Cabomba* litter was 11 ± 2, which was significantly lower than the *Hydrilla* litter (17 ± 5). The TN content in *Cabomba* litter (2.8%) was significantly higher than the *Hydrilla* litter (1.7%).

Across treatments, the sediment desiccation, macrophyte species in the litter, and the addition of living macrophytes (regrown macrophytes) significantly affected the physio-chemical and nutrient variables in the water column after rewetting (Table 3-2). Specifically, the dried then rewetted sediment treatment significantly increased the water column specific conductivity, but significantly decreased turbidity after rewetting, compared to the constantly wet sediment treatment for both *Cabomba* and *Hydrilla* treatments (GLM, *P* < 0.05; Table 3-2). The decomposition of *Cabomba* litter resulted in significantly lower DO concentrations and lower specific conductivity values, but higher turbidity, compared to that of the *Hydrilla* litter treatment. The regrowth of living *Cabomba* and *Hydrilla* significantly decreased specific conductivity and turbidity, but significantly increased DO concentrations (measurements were only done during the daytime) in the
water column. The value of pH was not significantly different among the eight specific treatments.

Table 3-2. The mean value (± SD) of physio-chemical parameters, and TDP concentrations in the water column of the eight specific treatments after rewetting (on days 0, 3, 7, 14, 28). Codes for treatment abbreviations explained in Table 3-1. (Lu et al. 2017a)

<table>
<thead>
<tr>
<th>Codes</th>
<th>Days after rewetting</th>
<th>SP. Cond. (mS cm⁻¹)</th>
<th>DO (mg L⁻¹)</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
<th>TDP (mg P L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>0 d</td>
<td>0.05 ± 0.01</td>
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<td>6.43 ± 0.02</td>
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<td>0.01 ± 0.00</td>
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<tr>
<td></td>
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<td>50.55 ± 34.81</td>
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</tr>
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<td>7.32 ± 0.02</td>
<td>126.35 ± 58.92</td>
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<td>6.43 ± 0.02</td>
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<td>0.01 ± 0.00</td>
</tr>
<tr>
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<td>7.05 ± 0.02</td>
<td>22.80 ± 6.79</td>
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</tbody>
</table>
### Codes

<table>
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<tr>
<th>Days after rewetting</th>
<th>SP. Cond. (mS cm(^{-1}))</th>
<th>DO (mg L(^{-1}))</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
<th>TDP (mg P L(^{-1}))</th>
</tr>
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<tbody>
<tr>
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<td>28 d</td>
<td>0.26 ± 0.02</td>
<td>6.35 ± 0.47</td>
<td>7.80 ± 0.47</td>
<td>68.23 ± 45.86</td>
<td>0.01 ± 0.00</td>
</tr>
</tbody>
</table>

*SP: Cond.: specific conductivity. NTU: nephelometric turbidity unit.*

In general, the dried then rewetted sediment treatment had significantly higher TDN and TDP concentrations in the water column, compared to the constantly wet sediment treatment for both *Cabomba* and *Hydrilla* (GLM, \(P < 0.05\); Fig. 3-1; Table 3-2). The TDN and TDP concentrations in the water column of *Hydrilla* treatments were significantly lower than that in *Cabomba* treatments, 14 and 28 d after rewetting. The presence of regrown *Cabomba* significantly decreased water column TDN concentrations across the rewetting period, compared to the control with the absence of living *Cabomba*. In contrast, the presence of regrown *Hydrilla* only significantly reduced water column TDN and TDP concentrations 28 d after rewetting, compared to the control without living *Hydrilla*. 
Figure 3-1. The comparison of water column TDN concentrations between different litter species and the presence of living macrophytes in dried then rewetted (a) and constantly wet treatments (b), respectively. (Lu et al. 2017a)

3.4.2 Time effects of rewetting on the fate of macrophyte-derived $^{15}$N

After rewetting, the $^{15}$N distribution among N pools changed over time. Generally, the $\%^{15}$N of macrophyte-derived $^{15}$N released into the dissolved fraction of the water column (dissolved fraction) and stored in the PON was highest 3–7 d after rewetting for both Cabomba and Hydrilla treatments (Table 3-3). The $\%^{15}$N then decreased with longer rewetting periods. In contrast, the $\%^{15}$N transferred to the sediment and regrown macrophytes increased gradually during the 28 d of rewetting.
3.4.3 Species differences in the litter on the fate of macrophyte-derived $^{15}$N

The treatment of “species differences in the litter” explained more variation in the $^{15}$N distribution in the pools of dissolved fraction, PON, and the residual N, compared to the other two treatments, i.e. “sediment desiccation history” and the “presence of living macrophytes” (Table 3-3). For instance, 28 d after rewetting, the $\%^{15}$N released from *Cabomba* ($57.6 \pm 9.1\%$) and *Hydrilla* litter ($54.1 \pm 3.0\%$) in the dried then rewetted sediment treatment (i.e. DH and DC) was not significantly different (GLM, $P > 0.05$; Fig. 3-2a, b). Although the same $\%^{15}$N was released from each species, a significantly higher $\%^{15}$N from *Cabomba* litter was transferred into the dissolved fraction ($3.9 \pm 1.3\%$) and PON ($3.5 \pm 3.8\%$) of the water column, compared to *Hydrilla* litter ($2.3 \pm 0.5\%$ and $0.7 \pm 0.4\%$ respectively; Fig. 3-2a, b). The residual $^{15}$N for the DC treatment accounted for $19.4 \pm 5.2\%$ of the total $^{15}$N input 28 d after rewetting, which was significantly lower than $32.4 \pm 3.3\%$ for the DH treatment.

The percentage of released TN (relative to total TN content in the biomass of macrophytes) was also different between *Cabomba* and *Hydrilla* litter after the same period of rewetting. A significantly higher percentage of the plant TN content was released from *Cabomba* litter ($53 \pm 6\%$), compared to *Hydrilla* litter ($36 \pm 10\%$), 28 d after rewetting (GLM, $P < 0.05$). There was also a significantly higher percentage of dry biomass lost from *Cabomba* litter ($60.3 \pm 6.2\%$) than from *Hydrilla* litter ($43.9 \pm 3.8\%$), three weeks after rewetting (GLM, $P < 0.05$).

3.4.4 Effect of sediment desiccation on the fate of macrophyte-derived $^{15}$N

Sediment desiccation did not affect the total $\%^{15}$N released from macrophyte litter 28 d after rewetting. However, sediment desiccation significantly altered the $^{15}$N distribution in different N pools for both *Cabomba* and *Hydrilla* treatments (Table 3-3).
The treatment of “sediment desiccation history” explained more variation in the \(^{15}\)N distribution in the sediment pool compared to the other two treatments, i.e. “species in the litter” and the “presence of living macrophytes” (Table 3-3). In general, the dried then rewetted sediment treatment had a significantly lower \(^{15}\)N in the sediment pool, compared to the constantly wet sediment treatment. Instead, the dried then rewetted sediment treatment significantly increased the released \(^{15}\)N transferring to the dissolved fraction and to the regrown macrophytes, compared to the constantly wet sediment treatment, for both *Cabomba* and *Hydrilla* treatments 28 d after rewetting (Table 3-3, Fig. 3-2c, d). The dried then rewetted sediment treatment also had a significantly higher \(^{15}\)N transferred into the PON compared to the constantly wet sediment in the *Hydrilla* treatment.

Specifically, the dried then rewetted sediment treatment had 11.9\% (*Cabomba*, DC) and 18.6\% (*Hydrilla*, DH) less \(^{15}\)N transferred to the sediment pool, compared to the constantly wet sediment treatment (WC and WH), 28 d after rewetting, but 1–2\% more into the dissolved fraction of the water column (Fig. 3-2a, b, c, d). The regrown macrophytes in the dried then rewetted sediment treatment stored 3.2 ± 0.6\% (DCCs) and 5.1 ± 3.4\% (DHHs) more \(^{15}\)N than the constantly wet sediment treatments for *Cabomba* (WCCs) and *Hydrilla* (WHHs) treatments respectively (Fig. 3-2e, f, g, h). For residual \(^{15}\)N in mesocosms, there was an interaction between sediment desiccation history and species differences in the litter. For *Hydrilla* treatments, the residual \(^{15}\)N was the highest in the dried then rewetted sediment treatment without living *Hydrilla* (DH), compared to all other treatments 28 d after rewetting (GLM, \(P < 0.05\)). The dried then rewetted sediment treatment for *Cabomba* also had a relatively higher residual \(^{15}\)N, compared to all other treatments but not significantly different (GLM, \(P > 0.05\)).
Table 3- 3. Summary of statistical results (F values and significances) for the effect of different treatments on $^{15}$N distribution in different N pools and water column TDN concentrations during rewetting periods. (Lu et al. 2017a)

<table>
<thead>
<tr>
<th>Treatments (T)</th>
<th>$^{15}$N pools</th>
<th>Water column TDN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissolved fraction</td>
<td>PON</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$ for the linear model</td>
<td>0.97</td>
<td>0.84</td>
</tr>
<tr>
<td>T1: Species in the litter</td>
<td>140.98**</td>
<td>63.01**</td>
</tr>
<tr>
<td>T2: Sediment history</td>
<td>46.23**</td>
<td>3.84'</td>
</tr>
<tr>
<td>T3: Living macrophytes</td>
<td>2.71'</td>
<td>2.90'</td>
</tr>
<tr>
<td>Sum of squares</td>
<td>T1&gt;T2&gt;T3</td>
<td>T1&gt;T2&gt;T3</td>
</tr>
<tr>
<td>0 d</td>
<td>d</td>
<td>c</td>
</tr>
<tr>
<td>3 d</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>7 d</td>
<td>ab</td>
<td>a</td>
</tr>
<tr>
<td>14 d</td>
<td>bc</td>
<td>ab</td>
</tr>
<tr>
<td>28 d</td>
<td>c</td>
<td>b</td>
</tr>
</tbody>
</table>

NA: not available. The * and ** indicate significant differences at P < 0.05 and P < 0.01 levels. The ´indicates significant interaction between the treatment and rewetting periods at P < 0.05 level. Different lowercase letters (a, b, c, or d) indicate significant differences at P < 0.05 level between the five rewetting periods, ordered from “a” to “d” presenting the highest to the lowest treatment. Treatments which share a letter in common were not significantly different.
Figure 3-2. The fate of $^{15}$N from eight Cabomba and Hydrilla treatments 28 d after rewetting, presenting as a standardized added $^{15}$N percentage ($\%^{15}$N) relative to the initial input of $^{15}$N in each treatment. The thickness of arrows shows the relative importance. (Lu et al. 2017a)
3.4.5 Effect of macrophyte regrowth on the fate of macrophyte-derived $^{15}$N

The regrown macrophytes were a significant $^{15}$N pool in both *Cabomba* and *Hydrilla* litter treatments. The treatment of “sediment desiccation history” explained more variation in the $^{15}$N distribution in the pool of regrown macrophytes, compared to the treatment of “species differences in the litter” (Table 3-3). The dried then rewetted sediment treatment had a significantly higher percentage of $^{15}$N in the regrown macrophytes, compared to the constantly wet sediment treatment for both *Cabomba* and *Hydrilla* (GLM, $P < 0.05$). The $^{15}$N taken up by regrown *Cabomba* accounted for 11.6 ± 2.2% and 8.4 ± 2.8% of the total $^{15}$N input for DCCs and WCCs treatments respectively, 28 d after rewetting (Fig. 3-2e, g). The $^{15}$N assimilated by regrown *Hydrilla* took up 8.2 ± 4.1% of added $^{15}$N for the DHHs treatment, and 3.1 ± 0.7% for the WHHs treatment 28 d after rewetting (Fig. 3-2f, h).

The presence of regrown macrophytes significantly decreased the $^{15}$N distribution in other N pools, i.e. the sediment, the dissolved fraction, and PON. In addition, the effect of regrown macrophytes varied between species. Specifically, the regrown *Hydrilla* significantly reduced the %$^{15}$N in the sediment pool, 7 d and 14 d after rewetting, compared to the control with absence of living *Hydrilla* (GLM, $P < 0.05$; Table 3-3). In contrast, the regrown *Cabomba* only significantly reduced the sediment $^{15}$N pool at the rewetting period of 14 d, compared to other periods of rewetting. The regrown *Cabomba* significantly reduced the %$^{15}$N stored by the PON, 3 d after rewetting, and the dissolved $^{15}$N fraction, 7 d after rewetting, compared to the control with the absence of living *Cabomba*. In contrast, the regrown *Hydrilla* did not significantly affect the $^{15}$N distribution in the dissolved fraction or the PON during the entire rewetting period.
Figure 3-3. The mean (+ SD) percentage increase in biomass (a) of regrown macrophytes in the four treatments containing living macrophytes (DCCs, WCCs, DHHs, and WHHs). The mean (+ SD) concentrations of water column Chl-a in the treatments with the absence (b) and presence (c) of living macrophytes after rewetting. The Spe.* and Sed.* marked above bars refer to the significant differences between litter species (Cabomba and Hydrilla) and sediment history treatments (dried then rewetted and constantly wet) at $P < 0.05$ level, respectively, and ** indicates significant differences at $P < 0.01$ level. (Lu et al. 2017a)
The percentage increase in biomass of regrown *Cabomba* and *Hydrilla* were not significantly different, except for 7 d after rewetting, when the percentage increase in regrown *Cabomba* biomass was significantly greater than *Hydrilla* ($F_{1,13} = 20.95$, $P < 0.05$, ANOVA; Fig. 3-3a).

### 3.4.6 Changes of Chl-α in the water column after rewetting

The mean concentrations of Chl-α in the water column was significantly higher after one week of rewetting, compared to day 0 (GLM, $P < 0.05$, Fig. 3-3b, c). The dried then rewetted sediment treatment had significantly higher Chl-α concentrations, compared to the constantly wet sediment treatment with the presence of living macrophytes after two weeks of rewetting. The *Cabomba* litter treatment had significantly higher Chl-α concentrations, compared to the *Hydrilla* litter treatment after two weeks of rewetting, especially in the dried then rewetted sediment treatment.

### 3.5 Discussion

This study is the first to investigate the fate of N released from the litter of submerged macrophytes during a cycle of drying then rewetting. Our results showed that the fate of released N from desiccated macrophytes (macrophyte-derived N) after rewetting was impacted by 1. the sediment desiccation process (from water level drawdown); 2. macrophyte species; and 3. the presence of living macrophytes (Fig. 3-4).
Figure 3-4. Synthesis of experimental findings highlighting the comparison of the fate of macrophyte-derived $^{15}$N between constantly wet (a) and dried then rewetted sediment (b) treatments and between Cabomba (c) and Hydrilla (d) treatments. The rectangle refers to different $^{15}$N pools in the mesocosm. The shaded rectangles refer to the three main treatments in our study, i.e. T1. species differences in the litter, T2. sediment desiccation history, and T3. the presence of living macrophytes. The direction of arrows show the $^{15}$N flow from $^{15}$N-labelled macrophyte litter to other N pools. The thickness of arrows and box outlines presented the relative importance of each N pool compared between the Cabomba (a) and Hydrilla (b) treatments, or between the constantly wet (c) and dried then rewetted sediment (d) treatments. (Lu et al. 2017a)
3.5.1 Transformation of macrophyte-derived N between N pools after drying/rewetting

Our study showed that the $^{15}$N released from macrophyte decomposition after drying then rewetting was rapidly transferred to the dissolved fraction of the water column, and quickly assimilated by PON, which is likely dominated by phytoplankton (Del Giorgio and France 1996). The $^{15}$N in these fractions was then gradually transferred into the sediment and, in the case of treatments with living macrophytes, incorporated into the biomass of regrown macrophytes. This time difference between phytoplankton and regrown macrophytes for $^{15}$N accumulation indicates that regrown macrophytes had a lower N uptake rate than phytoplankton in the water column. This rapid $^{15}$N assimilation of phytoplankton also coincided with higher water column Chl-$a$ concentrations.

Phytoplankton are capable of rapid N uptake with dissolved N turnover rates from minutes to hours (Lehman 1980; Glibert and Goldman 1981). Therefore, our results indicate that even though sediments are a long-term store of much of the N lost from macrophyte decomposition, the short-term (approximately the first week in our study) N flushed into the water column upon rewetting might become an important trigger for phytoplankton blooms.

Sediment desiccation and rewetting also have impacts on nutrient availability for phytoplankton in the water column. Previous studies have demonstrated that a N flush from dried sediment into the water column could occur upon rewetting. This N flush is mainly due to the increased mineralization of organic matter in the previously dried sediment after rewetting (known as “Birch effect”; Birch 1958; Qiu and McComb 1996). However, our study showed that the sediment desiccation can also cause a N flush from decayed macrophytes into the water column (Fig. 3- 4a, b), compared to constantly wet conditions. Sediment desiccation in our study significantly increased the percentage of macrophyte-derived $^{15}$N remaining in the dissolved fraction of the water.
column and transferred into regrown macrophytes, compared to the constantly wet conditions. In contrast, more macrophyte-derived $^{15}$N was stored into the sediment when desiccation did not occur. The macrophyte-derived N remained in the water column, rather than stored in the sediment, is more likely to cause severe water quality deterioration in aquatic ecosystems, such as promoting phytoplankton blooms.

There are three possible reasons for the difference between our sediment desiccation treatments. Firstly, the increased mineral N content in the dried then rewetted sediment might slow down the $^{15}$N diffusion from the water column to the sediment, which in turn increases the N diffusion from the latter to the former, due to a steeper concentration gradient at the sediment-water interface. Secondly, the sediment bulk density in our mesocosm increased, at least, 30–40% after complete desiccation. The compaction of sediment after desiccation might also result in a slower $^{15}$N diffusion from the water column into the previously dried sediment (Barko et al. 1986). Thirdly, the complete desiccation (soil moisture content 1.1%) in our study is also likely to reduce the microbial activities in the dried then rewetted sediment, resulting in less $^{15}$N being processed by microbes into the sediment. Additionally, the constantly wet treatments could have more benthic algae growing on the surface of the sediment compared with the dried then rewetting treatment. This could further increase the transportation of $^{15}$N into the sediment pool.

3.5.2 Comparison of species differences in the litter on fate of macrophyte-derived N

Previous macrophyte decomposition studies have typically compared the N release from the equivalent biomass between different macrophyte species (e.g. Battle and Mihuc 2000; Chimney and Pietro 2006; Lan et al. 2012). However, our study was able to compare the fate of released N relative to the total N content of the two species, using data from the $^{15}$N-labelled macrophyte litter. Our standardized results showed that if
*Cabomba* and *Hydrilla* having the same amount of $^{15}$N in their biomass, significantly more proportion of the $^{15}$N released from *Cabomba* litter was transferred to the dissolved fraction of the water column and assimilated by phytoplankton, compared to the *Hydrilla* litter (Fig. 3-4c, d). Moreover, *Cabomba* litter had 1.6 times the TN content of *Hydrilla* litter in our study, indicating that a lower biomass of *Cabomba* litter (70% of equivalent *Hydrilla* biomass) can release the same amount of N as *Hydrilla* litter.

The higher percentage of released N remaining in the water column for the *Cabomba* litter treatment, compared to the *Hydrilla* litter in our study, is likely due to a faster decomposition rate for the *Cabomba* litter. We concluded this from the fact that a greater TN and biomass loss was measured in *Cabomba* litter than in *Hydrilla* litter after the same period of rewetting. *Cabomba* litter also had a significantly lower C:N ratio compared with *Hydrilla* litter, indicating a faster net decomposition rate and faster N release rate for *Cabomba* litter (Geurts et al. 2010).

The *Cabomba* treatment also resulted in higher $^{15}$N storage in the phytoplankton, as well as higher TDN and TDP concentrations, and lower DO concentrations in the water column, compared to the *Hydrilla* treatment after rewetting. This indicates that the invasive *Cabomba* is more likely to cause more serious water quality deterioration after drying then rewetting, compared to native *Hydrilla*.

3.5.3 The effect of regrowth of submerged macrophytes on macrophyte-derived N

Our study also demonstrated the effect of macrophyte regrowth on re-distributing macrophyte litter-derived N within different N pools. The regrown macrophytes were the second largest $^{15}$N pool (smaller than the sediment pool) for the released N from macrophyte decomposition. The $^{15}$N assimilated by regrown *Cabomba* and *Hydrilla*
were 30 ± 7% and 20 ± 5% of total released $^{15}\text{N}$ in dried then rewetted treatments, and 18 ± 10% and 6 ± 3% of total released $^{15}\text{N}$ in constantly wet treatments, 28 d after rewetting. The regrowth of *Hydrilla* also significantly reduced the sediment $^{15}\text{N}$, while regrown *Cabomba* significantly reduced the $^{15}\text{N}$ in the water column and PON (Fig. 3-4c, d). This indicates that regrown *Hydrilla* might assimilate a greater proportion of assimilated N from the sediment than from the water column, compared with regrown *Cabomba*. However, both regrown species in our study (i.e. *Cabomba* and *Hydrilla*) significantly reduced water column TDN concentrations 28 d after rewetting, by which time the plant biomass had reached approximately 4.5 kg m$^{-3}$ in the mesocosm. This indicates a time-dependent and biomass-dependent effect of regrown submerged macrophytes on reducing water column N. Therefore, maintaining a certain amount of submerged macrophytes could be a key factor on alleviating the nutrient increase thus phytoplankton growth in the water column during WLFs.

3.5.4 Potential N removal from the system by desiccation and macrophyte regeneration

The mean recovery of $^{15}\text{N}$ in our study (90 ± 12%) was comparable to other plant-derived $^{15}\text{N}$ tracing studies in forest or farmland settings (Müller and Sundman 1988; Zeller et al. 2000, 2001), which typically had values ranging from 89% to 102%. Sediment desiccation in our study reduced the $^{15}\text{N}$ recovery, compared to the constantly wet sediment treatment and/or the dried then rewetted sediment treatment with living macrophytes. One possible explanation for these differences is that more N from macrophyte decomposition in the dried then rewetted treatment was converted to gaseous N, and removed from the system by denitrification. Increased denitrification after drying then rewetting, compared with constantly wet conditions, has also been determined in several previous studies (Smith and Parsons 1985; Groffman and Tiedje 1988; Fromin et al. 2010). This increased denitrification is likely due to increased C and
Chapter 3 – Nitrogen Dynamics in Littoral Macrophytes during WLFs

N mineralization during the drying and rewetting cycle, resulting in the increased coupled nitrification-denitrification. Specifically, the C and N (e.g. NH$_4^+$) released from drying then rewetting can provide nitrifiers the energy and substrates that required for nitrification, which oxidized NH$_4^+$ to NO$_3^-$ (Stanley and Boulton 1995; Qiu and McComb 1996; Baldwin and Mitchell 2000). Increased NO$_3^-$ concentrations from nitrification, in turn, can provide substrates and electron acceptors thus promote denitrification (NO$_3^-$ to N$_2$ or N$_2$O) after rewetting, especially when anaerobic conditions occur after drying then rewetting. This is because the faster activation of denitrifying enzymes by oxygen depletion resulted from the flush of microbial respiration and macrophytes decomposition (Smith and Parsons 1985; Kern et al. 1996).

However, when submerged macrophytes regrow after rewetting they may compete with nitrifiers for the available mineral N (Bodelier et al. 1998), which may limit subsequent nitrification-denitrification (Baldwin and Mitchell 2000; Cavanaugh et al. 2006).

In summary, our findings indicate that the water level drawdown followed by rewetting can cause a flush of macrophyte-derived N transferring into the water column and assimilated by phytoplankton, increasing impacts on water quality, compared to constantly wet conditions. Water level drawdown followed by rewetting is also an important trigger for macrophytes loss and subsequent decomposition. The invasive species *Cabomba* can cause more serious water quality deterioration (e.g. higher TDN and TDP, higher Chl-a and lower DO concentrations) after rewetting, compared to the native *Hydrilla*. This is a result of the higher TN content and faster decomposition rate of the *Cabomba* litter, relative to *Hydrilla* litter. The regrowth of submerged macrophytes is able to reduce the N content in the sediment and/or in the water column pool, playing a positive role in counteracting N release from macrophyte litter. These findings add to our understanding of N dynamics in littoral macrophytes, and key
factors affecting the macrophyte-derived N fate during WLFs in shallow aquatic ecosystems.
Chapter Four: The comparison of nutrient release and uptake by littoral macrophytes during water level fluctuations

Chapter 3 found that macrophyte-derived N after drying then rewetting was utilized by phytoplankton rapidly. Recovered macrophytes played a positive role on assimilating these released N from macrophyte litter decomposition after drying then rewetting.

In this Chapter, I will compare the relative importance of nutrient release (both N and P) from macrophyte litter versus nutrient uptake by recovering macrophytes during WLFs, and the flow-on impacts on nutrient dynamics and phytoplankton biomass. In addition, I will continue to explore the differences between two macrophyte species, the invasive species (Cabomba) and native species (Hydrilla).
Chapter 4 – Nutrient Release vs. Uptake in Littoral Macrophytes during WLFs

This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors, are:

**Lu J., Bunn S.E., Burford M.A. 2017.** The comparison of nutrient release and uptake by littoral macrophytes during water level fluctuations. *Science of the Total Environment.* (Under review)

My contribution to the paper involved designing the experiment, collecting and analysing the data, providing direction on the structure of the result analysis, writing the manuscript, and addressing the comments from reviewers. Michele Burford is the principle supervisor of this project, contributing to developing the ideas of the experiment, providing direction on the structure of the result analysis and revising the manuscript. Stuart Bunn is the associated supervisor of this project, contributing to providing comments to improve the manuscript and data analyses.

(Signed) __________________________ (Date) 22 June 2017

Name of Student and corresponding authors for all listed papers: Jing Lu

(Countersigned) __________________________ (Date) 23 June 2017

Supervisor: Prof. Michele Burford
4.1 Introduction

In contrast to the macrophyte die-back during WLFs, submerged macrophytes can recover after rewetting depending on the duration of water level drawdown. Re-germination from seeds or dormant propagules provide an important recovery mechanism after severe desiccation, as these propagules are more drought resistant than the aboveground biomass and other vegetative propagules (Liu et al. 2006; Bornette and Puijalon 2011). However, the shoot fragments carried by the water column might be a more rapid way for macrophytes to recover (Barrat-Segretain and Bornette 2000). For some submerged macrophytes, the vegetative propagules might be the main propagation method (Barrat-Segretain and Cellot 2007). For example, the macrophyte *Cabomba caroliniana*, typically propagates mainly through vegetative propagules where it has been an invasive plant in Australia and China (Jin et al. 2005; Schooler 2009), and only one leaf node is needed to grow a new plant (Schooler 2009).

Determining the relative importance of macrophytes as a nutrient sink or source in shallow freshwater ecosystems is an important step to predict the effect of WLFs on water quality. However, this has not been well quantified. In this Chapter, I will investigate the relative importance of the nutrient release from macrophyte litter versus nutrient uptake by living macrophytes (regrown from shoot fragments), and the flow-on impacts on water quality during a cycle of drying then rewetting. I will compare these impacts between the same invasive species (*Cabomba*) and native species (*Hydrilla*) as in Chapter 3. I will also examine the impacts of macrophyte bed desiccation on water quality upon rewetting, compared with a bare sediment treatment.
4.2 Methods

4.2.1 Mesocosm set-up

The experiment set-up for this Chapter was the same as in Chapter 3 (refer to section 3.2.3 Mesocosm set-up and sampling methods), including three main treatments, i.e. treatment 1 (T1): different species in the litter (*Cabomba* versus *Hydrilla* litter; i.e. C or H); T2: sediment desiccation history (“dried then rewetted” versus “constantly wet”; i.e. D or W); T3: living macrophytes (presence versus absence; i.e. Cs, Hs or none). The three main treatments consisted of eight specific treatments, WC, WCCs, DC, and DCCs for *Cabomba* and WH, WHHs, DH, DHHs for *Hydrilla*, with 4 replicates for each sampling occasion (Table 3-1).

Six extra mesocosms with no macrophyte litter or living macrophyte shoots were also set up with bare sediment as the control treatments. Therefore, these eight control treatments contained either 1. “dried then rewetted” sediment (CK_D), or 2. “constantly wet” sediment (CK_W) with four replicates each.

4.2.2 Sampling and analysis methods

As detailed in section 3.2.3, samples for each mesocosm were taken 3, 7, 14, and 28 d after rewetting. For each sampling occasion, four replicates of each treatment (except the control) were harvested. Water samples for the control treatment were repeatedly collected 3, 7, 14, and 28 d after rewetting. The control treatment was only harvested at the end of the experiment (28 d after rewetting) for the analysis of Chl-α concentrations.

Except for TDN and TDP samples in section 3.2.3, water samples for NH₄⁺, NO₃⁻/NO₂⁻, and PO₄³⁻ concentrations were also filtered through membrane filters (0.45 µm pore size). These dissolved nutrients were analysed colorimetrically using
Continuous Segmented Flow Analyser (SEAL Auto Analyser 3 HR, SEAL Analytical Limited, UK). Water column Chl-\(\alpha\) samples (100 mL) were filtered onto glass fibre filters (47 mm diameter; GF75, Advantec, Japan) and frozen at –80°C until analysed. Concentrations of Chl-\(\alpha\) were measured and calculated using the same method listed in Chapter 2.

The remaining macrophyte litter and living macrophytes in each mesocosm were rinsed with deionized water, dried in an oven at 50°C to a constant weight and dry weight recorded. The TN and TP content in the remaining litter and living macrophytes was analysed by a segmented flow analyser (Bran + Luebbe AA3 HR, SEAL, UK), following spectrophotometric methods (Rayment and Lyons 2011), after a block digestion with concentrated sulfuric acid (as well as Kjeldahl catalyst tablets, sodium sulfate and selenium; Bremner & Mulvaney 1982). The TC content in the remaining litter and living macrophytes was analysed using a mass spectrometer (Sercon Hydra 20–22, Sercon Ltd, UK), with a front sample combustion system (Euro EA-GLS elemental analyser, Sercon Ltd, UK). The C, N, and P mass content in the litter was used to calculate the C:N:P ratios.

The floating macrophyte shoots started to grow new roots 3 to 7 d after rewetting and then gradually anchored into the sediment. The number and length of roots for living Cabomba and Hydrilla were recorded on each sampling occasion.

4.2.3 Nutrient release and uptake rate calculations

The N and P release rate from macrophyte litter, and the N and P uptake rate for living macrophytes were estimated using exponential equations (4.1, 4.2, 4.3, & 4.4; Olson 1963):
Chapter 4 – Nutrient Release vs. Uptake in Littoral Macrophytes during WLFs

Nutrient release rates:

\[ W_t = W_0 e^{-k_{N,R} t} \] …………………………………………………………………………………………..(4.1)

\[ W_t = W_0 e^{-k_{P,R} t} \] …………………………………………………………………………………………..(4.2)

Nutrient uptake rates:

\[ W'_t = W'_0 e^{-k_{N,U} t} \] …………………………………………………………………………………………..(4.3)

\[ W'_t = W'_0 e^{-k_{P,U} t} \] …………………………………………………………………………………………..(4.4)

Where:

- \( W_t \) is the remaining N or P mass in the litter;
- \( W'_t \) is the accumulated N or P mass in living macrophytes;
- \( W_0 \) and \( W'_0 \) are the initial N or P mass in the litter or in living macrophytes, respectively;
- \( t \) is the time of decomposition or rewetting (d);
- \( k_{N,R} \), \( k_{P,R} \), \( k_{N,U} \) and \( k_{P,U} \) are the decomposition or uptake rate coefficients (d\(^{-1}\));
  - \( k_{N,R} \) and \( k_{P,R} \) refer to the decomposition rates of N and P in macrophyte litter;
  - \( k_{N,U} \) and \( k_{P,U} \) refer to the uptake rates of N and P for living macrophytes.

4.2.4 Accumulative nutrient uptake rate calculations for each sampling occasion

Living Cabomba and Hydrilla were grown under different litter treatments which had different available nutrients in the water column after rewetting. The nutrient concentrations in the water column could impact nutrient uptake rates for macrophytes, and the interaction between them followed saturation kinetics or linear correlation (Cedergreen and Madsen 2002). This means higher water column nutrients could increase nutrient uptake rates of macrophytes at least before the required nutrients for
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Macrophyte growth were saturated. Therefore, in order to compare the nutrient uptake rates between the two species after each sampling occasion, the accumulative nutrient uptake rates for living Cabomba and Hydrilla on each sampling occasion for living Cabomba and Hydrilla were standardized to per unit bioavailable nutrients below (4.5):

Accumulative nutrient uptake rates = increased N or P (mg) in regrown macrophytes/ increased dry biomass (g)/ rewetting periods (d)/ average dissolved inorganic N (DIN = NH₄⁺ + NO₃⁻/NO₂⁻) or P (DIP: PO₄³⁻) concentrations during the rewetting period…………………………………………………………………………………………………(4.5)

The nutrient uptake to release percentage was defined as the percentage of the total amount of assimilated nutrients in living macrophytes compared with the total released nutrients from macrophyte litter in the same mesocosm. This percentage was calculated for all mesocosms containing both macrophyte litter and living macrophytes (i.e. treatments of DCCs, WCCs, DHHs, and WHHs).

4.3 Data analyses

Data were analysed using R software (version 3.12, R Core Team 2014). Linear regressions were used to compare the differences of water column nutrients amongst treatments and the nutrient uptake rates between Cabomba and Hydrilla. Instead of using the P value as the standard for significant differences amongst treatments, we used the 95% confidence levels that calculated in the model predictions based on linear regressions and visualized the predictions in figures. The water column nutrient differences amongst Cabomba and Hydrilla litter, and the control treatment without litter were compared under both 1. dried then rewetted and 2. constantly wet conditions. The effect of living macrophytes and the control (i.e. absence of living macrophyte shoots) on water column nutrients was also compared under both 1. dried then rewetted and 2. constantly wet conditions. The accumulative nutrient uptake rates were
standardized to per unit DIN or DIP concentration, for each sampling occasion, then compared between the living *Cabomba* and *Hydrilla*.

General linear models (GLM) were used to determine the differences of C:N:P ratios in *Cabomba* and *Hydrilla* litter amongst treatments with four fixed variables and their interactions in the linear model: T1: macrophyte litter species (*Cabomba* versus *Hydrilla*); T2: sediment desiccation history (“dried then rewetted” versus “constantly wet”); T3: living macrophytes (presence versus absence); and T4: rewetting periods. General linear models were also used to determine the differences of N and P release rates amongst treatments.

The nutrient uptake and release rates of macrophytes in the same mesocosm were compared using t-test. The nutrient uptake differences between the “dried then rewetted” and “constantly wet” conditions for the same species were also compared using t-test. All data were tested for normality and natural log-transformed as required.

### 4.4 Results

#### 4.4.1 C:N:P ratios in macrophyte litter

*Cabomba* litter had significantly lower C:N and C:P ratios (C:N = 11 ± 2; C:P = 309 ± 34) than *Hydrilla* litter (C:N = 17 ± 5; C:P = 547 ± 80) before and during rewetting periods (GLM, *P* < 0.05). The C:N ratio in the remaining *Cabomba* and *Hydrilla* litter and the C:P ratio in the remaining *Hydrilla* litter decreased significantly with a longer period of rewetting (GLM, *P* < 0.05). The N:P ratios were not significantly different between *Cabomba* and *Hydrilla* litter, but in both cases increased significantly with longer rewetting periods (GLM, *P* < 0.05).
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4.4.2 N and P release rates by macrophyte litter

The sediment desiccation history did not significantly affect the N and P release rates from macrophyte litter, but there were differences between species, and between N versus P. *Cabomba* litter had a significantly higher N and P release rates (*k*<sub>N,R</sub> and *k*<sub>P,R</sub>) compared to *Hydrilla* litter (GLM, *P* < 0.05; Fig. 4-1). The *k*<sub>N,R</sub> and *k*<sub>P,R</sub> for *Cabomba* litter were 0.024–0.032 and 0.056–0.067, respectively, compared with 0.010–0.013 and 0.040–0.041 for *Hydrilla* litter. The P release rate was also significantly greater than the N release rate for both *Cabomba* and *Hydrilla* litter, coinciding with an increased N:P ratio in the remaining litter with longer rewetting periods. The presence of living *Cabomba* significantly increased the *k*<sub>P,R</sub> of *Cabomba* litter by 0.011.

![Figure 4-1](image)

*Figure 4-1. The mean (+ SD) N and P release rate (k, d⁻¹) of Cabomba and Hydrilla litter during 28 d of decomposition. ** refer to the significant difference between treatments at *P* < 0.01 level. Codes for treatment abbreviations explained in Table 3-1.*

4.4.3 Effects of litter decomposition on water column nutrients

Water column TDN, TDP, and PO₄³⁻ concentrations were significantly higher in the treatments with *Cabomba* litter, compared to the *Hydrilla* litter. Furthermore, these
concentrations and DON concentrations were all significantly higher in the litter treatments from both species, compared to the control which had no macrophyte litter (linear model predictions; Table S4-1; Fig. 4-2). However, water column DON concentrations were significantly higher in the *Hydrilla* litter treatments than the *Cabomba* treatments (linear model predictions; Fig 4-2a & b). Water column DOP concentrations in the *Hydrilla* litter treatments (rather than *Cabomba* litter) were significantly higher than the control (linear model predictions; Fig 4-2c & d).

The treatments with *Cabomba* litter slightly increased the water column NH$_4^+$ concentrations compared to the control lacking *Cabomba* litter, while the addition of *Hydrilla* litter did not significantly increase the water column NH$_4^+$ concentrations compared to the control (linear model predictions; Fig. 4-2e & f). Although the *Cabomba* litter treatments had significantly higher NO$_3^-/NO_2^-$ concentrations than the *Hydrilla* treatments, the water column NO$_3^-/NO_2^-$ concentrations in both *Cabomba* and *Hydrilla* litter treatments were significantly lower compared to the control (linear model predictions; Fig. 4-2g & h).

The sediment desiccation history (i.e. dried then rewetted treatments) resulted in a significant increase in the water column TDN, TDP, DON, DOP, NH$_4^+$, and NO$_3^-/NO_2^-$ concentrations after rewetting, but it did not significantly impact the PO$_4^{3-}$ concentrations in the water column, compared to the constantly wet treatments (Table S4-1; Fig. 4-2). Additionally, water column TDN, DON, and NO$_3^-/NO_2^-$ concentrations increased with longer rewetting periods, and were significantly higher 14 d and 28 d after rewetting (Fig. 4-2). However, the water column TDP and PO$_4^{3-}$ concentrations decreased with longer rewetting periods 3 d after rewetting, and DOP concentrations were also significantly lower 28 d after rewetting, compared with the beginning of the rewetting period (Fig. 4-2).
Figure 4-2. The linear regression model predictions (95% confidence) for the changes of water column DON (a, b), DOP (c, d), NH$_4^+$ (e, f), NOx (NO$_3^-/NO_2^-$; g, h) and PO$_4^{3-}$ (i, j) concentrations with the addition of Cabomba and Hydrilla litter in the "constantly wet" (grey symbols) and "dried then rewetted" (black symbols) treatments with the absence and presence of living macrophytes.
4.4.4 Comparison of nutrient release and uptake by macrophytes

The total amount of N and P released from Cabomba or Hydrilla litter was significantly higher than the total amount of N and P assimilated by living Cabomba or Hydrilla, on most of the sampling occasions (Fig. 4-3). However, there are three exceptions where there was no difference, i.e. N release and uptake for Cabomba treatments 3 d after rewetting (Fig. 4-3a), and the N release and uptake for Hydrilla treatments 14 d and 28 d after rewetting (Fig. 4-3b) in all the dried then rewetted treatments.

The percentage of the total amount of N or P assimilated by living Cabomba or Hydrilla, relative to the total amount of released N or P from macrophyte litter, was defined as the N or P uptake to release percentages. Overall, the N uptake to release percentage was significantly higher than that for P (Fig. 4-3; Table 4-1). The dried then rewetted treatments also had significantly higher N and P uptake to release percentages for both Cabomba and Hydrilla treatments compared to the constantly wet treatments. Specifically, after 28 d of rewetting, the P uptake to release percentages in the Cabomba and Hydrilla litter treatments were only 6 ± 4% and 14 ± 5%, respectively, in the constantly wet conditions (Table 4-1). In contrast, in the dried then rewetted conditions, these P uptake to release percentages increased to 15 ± 8% and 42 ± 10% for Cabomba and Hydrilla respectively. The N uptake to release percentages for Cabomba and Hydrilla were 26 ± 9% and 19 ± 10% in the constantly wet conditions, and 55 ± 1% and 100 ± 27%, respectively, in the dried then rewetted conditions.
Figure 4-3. The comparison between the total amount (mg) of released nutrients from macrophyte litter in each mesocosm and the total amount of assimilated nutrients by living macrophytes in the same mesocosm. a, b, c, and d showed the N results in DCCs, DHHs, WCCs, and WHHs treatments, respectively; e, f, g, and h showed the P results in DCCs, DHHs, WCCs, and WHHs treatments, respectively.
Table 4-1. The comparison of the nutrient uptake to release percentage (the percentage of total assimilated nutrients by living macrophytes accounted for the total released nutrients from macrophyte litter) between two methods calculated from the labelled $^{15}$N and the nutrient mass changes in the biomass for Cabomba and Hydrilla with different sediment history, 28 d after rewetting.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N uptake to release percentage</th>
<th>P uptake to release percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labelled $^{15}$N calculation</td>
<td>Nutrient mass calculation</td>
</tr>
<tr>
<td>Cabomba</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried/rewetted</td>
<td>30 ± 7%</td>
<td>55 ± 1%</td>
</tr>
<tr>
<td>Constantly wet</td>
<td>18 ± 10%</td>
<td>26 ± 9%</td>
</tr>
<tr>
<td>Hydrilla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried/rewetted</td>
<td>20 ± 5%</td>
<td>100 ± 27%</td>
</tr>
<tr>
<td>Constantly wet</td>
<td>6 ± 3%</td>
<td>19 ± 10%</td>
</tr>
</tbody>
</table>

The P uptake rates ($k; \text{d}^{-1}$) of living *Cabomba* or *Hydrilla* were significantly lower than the P release rates ($k; \text{d}^{-1}$) from *Cabomba* or *Hydrilla* litter in either dried then rewetted or constantly wet conditions (t-test, $p < 0.05$; Fig. 4-4a). In contrast, the N uptake rates of living *Hydrilla* were significantly higher than the N release rates of *Hydrilla* litter in the dried then rewetted conditions (t-test, $p < 0.05$; Fig. 4-4b). However, there was no significant difference between the N uptake rates and release rates for *Hydrilla* treatments in constantly wet conditions or for all *Cabomba* treatments (Fig. 4-4b).

The N uptake rates in dried then rewetted treatments were significantly higher compared to those in constantly wet treatments (t-test, $p < 0.05$; Fig. 4-4b). However, there was no significant difference in P uptake rates for macrophytes between dried then rewetted and constantly wet treatments (t-test, $p > 0.05$; Fig. 4-4b). Living *Hydrilla* had significantly higher accumulative N and P uptake rates when standardized to per unit DIN or DIP concentration (as outlined in the Methods, equation 4.5), compared to living
*Cabomba* in both dried then rewetted treatments (Fig. 4-5 a & c) and constantly wet treatments (Fig. 4-5 b & d).

**Figure 4-4.** The comparison of P (a) uptake rates of living macrophytes versus P release rates of macrophyte litter in the same mesocosm for different treatments, and the comparison of N (b) uptake rates of living macrophytes versus N release rates of macrophyte litter in the same mesocosm for different treatments.
Figure 4-5. The comparison of linear regression model predictions (95% confidence) for the standardized accumulative N and P uptake rates (mg N g⁻¹ dry biomass d⁻¹ unit⁻¹ DIN or DIP) between living Cabomba and Hydrilla after each sampling occasion in the "dried then rewetted" (a, c) and "constantly wet" treatments (b, d).
Figure 4-6. The linear regression model predictions (95% confidence) for the changes of water column DON (a, b), NOx (NO\textsubscript{3}/NO\textsubscript{2}; c, d), NH\textsubscript{4}\textsuperscript{+} (e, f), DOP (g, h), and PO\textsubscript{4}\textsuperscript{3-} (i, j) concentrations in the presence of living Cabomba and Hydrilla in the “dried then rewetted” and “constantly wet” treatments.
4.4.5 Effects of living macrophytes on dissolved nutrients

The presence of living *Cabomba* significantly reduced the water column DON concentrations in the first three weeks of rewetting in the dried then rewetted treatments, and in the last two or three weeks of rewetting in the constantly wet treatments, compared to the control (Table S4-1; Fig. 4-6a & b). In contrast, living *Hydrilla* only significantly reduced the water column DON, 14 d after rewetting, in the dried then rewetted treatments rather than in constantly wet conditions. Both living *Cabomba* and *Hydrilla* significantly reduced the NH$_4^+$ concentrations, 14 d after rewetting in the dried then rewetted treatments, whereas only living *Cabomba* significantly reduced NH$_4^+$ concentrations in the constantly wet treatments, 28 d after rewetting, compared to the control (Table S4-1; Fig. 4-6c & d). However, the presence of living *Cabomba* slightly increased the water column NO$_3^-$/NO$_2^-$ and PO$_4^{3-}$ concentrations in the first two weeks of rewetting in the dried then rewetted treatments, compared to the control (Fig. 4-6e, f, i & j). In contrast, the living *Hydrilla* did not significantly affect the NO$_3^-$ /NO$_2^-$ and PO$_4^{3-}$ concentrations in the water column. There was no significant difference in water column DOP concentrations on most sampling occasions amongst living *Cabomba*, *Hydrilla* and the control treatments, except the living *Cabomba* significantly reduced the water column DOP concentrations compared with the control in constantly wet treatments, 28 d after rewetting (Fig. 4-6g & h).

4.4.6 Water column N:P ratios and Chl-a concentrations

Water column molar N:P ratios (DIN:DIP) were significantly lower in the *Cabomba* and *Hydrilla* litter treatments, compared to the control treatment without litter (i.e. bare sediment treatments), in the first three weeks of rewetting (GLM, $P < 0.05$; Fig. 4-7a, b, c & d). The *Hydrilla* treatments had a significantly higher water column N:P ratio in the first two weeks after rewetting, but a significantly lower N:P ratio 28 d
after rewetting, compared to the *Cabomba* treatments (GLM, *P* < 0.05; Fig. 4- 7a, b, c & d). The water column N:P ratios for the *Hydrilla* treatments started around 40:1 (39 ± 7:1) on day 0 and decreased to between 10:1 and 21:1 over the 28 d rewetting period, except for the N:P ratio in the dried then rewetted treatments, 7 d after rewetting. The N:P ratio in the dried then rewetted treatments for *Hydrilla* increased significantly to higher than 40:1, 7 d after rewetting.

The N:P ratio for the *Cabomba* treatments also started around 40:1 on day 0, but decreased significantly to less than 10:1 in the first 7 d after rewetting. Then the N:P ratio in the *Cabomba* treatments increased with longer rewetting periods. However, it was not significantly greater than 21:1 until 14 d or 28 d after rewetting for the dried then rewetted and the constantly wet treatments, respectively.

Water column Chl-*a* concentrations were very low (< 5 µg L⁻¹) in the control treatment with no litter, 28 d after rewetting, which was significantly lower than that in *Cabomba* and *Hydrilla* treatments (5–30 µg L⁻¹). Between the two species treatments, we found Chl-*a* concentrations for the *Cabomba* treatments were significantly higher than the *Hydrilla* treatments (Fig. 4- 8a, b, c & d). The dried then rewetted treatments also had significantly higher Chl-*a* concentrations than the constantly wet treatments, especially in the presence of living macrophytes. The Chl-*a* concentrations for the *Hydrilla* treatments increased significantly in the first week after rewetting and peaked on day 7, then decreased significantly in the last three weeks after rewetting. In contrast, the Chl-*a* concentrations for the *Cabomba* treatments increased significantly in the first two weeks after rewetting, peaking on day 14, then decreased significantly in the following two weeks of rewetting.
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Figure 4-7. The comparison of the mean (+ SD) natural log-transformed water column molar N:P ratios (DIN:DIP) in the Cabomba litter, Hydrilla litter, and no litter (the control) treatments in the “dried then rewetted” (a & c) and “constantly wet” treatments (b & d) in the absence (DC, DH, WC, and WH) and presence of living macrophytes (DCCs, DHHs, WCCs, and WHHs). The N:P ratios were compared with Sterner (2008) (21:1) and Grayson et al. (1997) (10:1) using dashed and dotted lines, respectively.
4.5 Discussion

This study is the first, to our knowledge, to directly compare the relative importance of nutrient release versus uptake for two species of submerged macrophytes as a result of manipulating WLFs. Our results showed that nutrient release from macrophyte litter could be a significant contributor of water column nutrients, but the scale, relative to macrophyte nutrient uptake, and the following impacts on water quality were dependent on macrophyte species, N versus P differences, and sediment drying-wetting regimes.

Figure 4-8. The comparison of the mean (+ SD) Chl-a concentrations (mg L$^{-1}$) between the Cabomba and Hydrilla litter treatments in the “dried then rewetted” (a & c) and “constantly wet” treatments (b & d) with the absence (DC, DH, WC, and WH) and presence of living macrophytes (DCCs, DHHs, WCCs, and WHHs). The C* marked above bars refer to the parameter in the Cabomba treatments (C) was significantly greater at $P < 0.05$ level than the Hydrilla treatments (H).
4.5.1 N versus P release from macrophyte litter

Our results showed that the P release rate from litter decomposition was significantly higher than the N release for both *Cabomba* and *Hydrilla*. Additionally, living *Cabomba* and *Hydrilla* were more efficient at assimilating the N released from macrophyte litter, compared to P. This was reflected in significantly reduced water column DON and NH$_4^+$ concentrations, but not DOP and PO$_4^{3-}$ concentrations with the presence of living macrophytes. These results indicate that decomposition of macrophyte litter is likely to have a greater impact on water column P than N during WLFs. Our previous study (Chapter 2) also demonstrated a greater contribution of released P to water column TP, compared with the comparable N input using the floating-leaved macrophyte *N. indica* (Lu et al. 2017). Landers (1982) investigated the nutrient release from the submerged macrophyte, *Myriophyllum spicatum* during its annual die-back, and also demonstrated a greater P impact on water column nutrients than N.

This difference in N versus P release from macrophyte litter caused a decrease in water column N:P ratios, indicating that the macrophyte decomposition may not only increase N and P concentrations, but can also alter N:P ratios. This increased water column N and P concentrations, as well as the decreased N:P ratios, could be a particular issue for P-limited lakes for phytoplankton growth as they have the potential to directly boost the phytoplankton growth. The growth of phytoplankton in many lakes worldwide has been regarded as P-limited (Schindler 1977; Correll 1998; Schindler et al. 2008) or N/P co-limited (Elser et al. 1990; Muhid and Burford 2012). Additionally, there is some evidence that shifts in N:P ratios can also shift phytoplankton species composition with flow-on effects to aquatic food webs (Elser et al. 2000).

Released PO$_4^{3-}$ from macrophyte litter can, at least partially, be adsorbed by soil particles after drying then rewetting (Chapter 2; Lu et al. 2017), especially the iron-
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(Geurts et al. 2010), aluminium- (Kopáček et al. 2000; Rydin et al. 2000), or calcium-rich sediments (Reddy et al. 1993). Therefore, in these systems, the effect of litter nutrient release on phytoplankton growth might be partially alleviated in P-deficient lakes or reservoirs during WLFs. However, desiccated sediments also could substantially release DIN after rewetting (Birch 1958). This could, in turn, increase the potential for phytoplankton growth in N-deficient lakes or reservoirs (Beklioğlu and Tan 2008).

4.5.2 Species differences - litter decomposition

Our study also showed species differences in decomposition effects on water quality. The decomposition of the Cabomba litter, compared to the same biomass of Hydrilla litter, resulted in significantly higher N and P concentrations, as well as higher Chl-a concentrations, in the water column. These higher concentrations in the water of the Cabomba litter treatments were likely due to the higher N and P content of this species, and lower C:N and C:P ratios in the litter before and during decomposition, compared to Hydrilla litter. These differences indicate faster N and P release rates in Cabomba litter compared with Hydrilla. Lower C:N and C:P ratios in litter or detritus are an indicator of higher net mineralization rates (Geurts et al. 2010).

The significantly lower water column Chl-a concentrations in the Hydrilla treatments compared to the Cabomba treatments are likely due to significantly lower bioavailable P concentrations (0.001 mg L⁻¹) in the first week of rewetting, and significantly lower bioavailable N concentrations (0.11 mg L⁻¹) in the last two weeks (week 3 & 4) of rewetting, compared with the Cabomba treatments (0.13 mg P L⁻¹ and 0.60 mg N L⁻¹, respectively). Nutrient limitation for phytoplankton growth can be partially assessed by N:P ratios in the water column, e.g. N:P (molar) ratio less than 16:1 (TN:TP, Redfield ratio) is more likely to be N rather than P limited, and vice versa.
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for P limitation (Redfield 1958). More recent studies have revised this ratio, i.e. ratios greater than ~21:1 for P limitation, and less than ~10:1 for N limitation for lakes with low nutrient conditions (Grayson et al. 1997; Sterner 2008). The DIN:DIP ratio has also been used as a useful indicator to assess the nutrient limitation for phytoplankton growth (Rhee 1978; Morris and Lewis Jr 1988). In the present study, we compared the water column DIN:DIP ratio amongst treatments, rather than TN:TP, due to treatment differences in turbidity confounding TN and TP values.

In the present study, the water initially used for rewetting had an N:P ratio higher than 21:1 (39 ± 7:1), indicating the potential for P limitation, relative to N, for phytoplankton growth at the beginning of the experiment. For the *Hydrilla* treatments, the higher water column N:P ratio in the in the first week of rewetting compared to the *Cabomba* treatments, together with the relatively higher N:P ratio on day 0, indicate P limitation relative to N for phytoplankton growth in *Hydrilla* treatments compared with *Cabomba*. In addition, sediment desiccation also resulted in higher water column N:P ratios compared with the constantly wet conditions. This may have exacerbated the P limitation in *Hydrilla* treatments.

In the case of *Cabomba* treatments, they had the same water column N:P ratio (39 ± 7:1) as *Hydrilla* treatments on day 0. However, *Cabomba* treatments had a lower water column N:P ratio (< 10:1) in the first week after rewetting, and a higher N:P (≥ 21:1) ratio in the last two weeks of rewetting, compared to the *Hydrilla* treatments. This suggests that the growth of phytoplankton in the *Cabomba* treatments could shift from P to N limitation early in the experiment, followed by P limitation again later the experiment. Therefore, the decomposition of different macrophyte species, together with the sediment drying-wetting regimes during WLFs, may affect the water column N:P ratios in lakes or reservoirs, with the flow-on effects to nutrient availability and
phytoplankton biomass and their community dynamics.

4.5.3 Species differences - nutrient uptake

Our results showed that when dry biomass of macrophyte litter to living macrophytes = 9:1, the N uptake rates of living macrophytes during 28 d of rewetting were equal or even higher than the N release rates from macrophyte litter. However, the presence of living macrophytes did not significantly reduce the water column N concentrations until 14 d after rewetting when living macrophytes accumulated higher biomass (3.5–4.5 kg m\(^{-3}\) wet weight). In addition, our study demonstrated that living Hydrilla had a significantly higher N and P uptake rates than living Cabomba. However, living Cabomba was more efficient at reducing the water column N compared with Hydrilla. These results indicate that Cabomba and Hydrilla appear to differ in the relative proportion of water column and sediment nutrients assimilated for their growth.

There is a long-standing debate on the relative importance of water column versus sediment nutrients for the growth of submerged macrophytes (Carignan and Kalff 1980; Barko et al. 1986; Chambers et al. 1989; Madsen and Cedergreen 2002). Quantifying the contribution of different nutrient sources required for macrophyte growth can improve our understanding of littoral nutrient cycling and littoral-pelagic nutrient exchanges (Barko et al. 1991).

In order to verify our initial hypothesis that the two macrophyte species utilized sediment and water column nutrients equally, we compared the N uptake to release percentage calculated from 1. the nutrient mass changes in macrophyte biomass in the present study; and 2. the calculated labelled \(^{15}\)N pools from the same experiment set-up (Chapter 3; Lu et al., submitted). Since the \(^{15}\)N method labelled the water column more efficiently than the sediment (Chapter 3; Lu et al., submitted), the \(^{15}\)N pool in the...
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Regrown macrophytes reflected principally water column sources, while the nutrient mass method captured both water column and sediment sources. Our study showed that for the *Cabomba* treatments, the N uptake to release percentage calculated from the $^{15}$N-labelling method accounted for approximately 50% of the nutrient mass method, compared with less than one-third for the *Hydrilla* treatments (Table 4-1). These results indicate that the water column was a significant contributor to the N assimilated by living *Cabomba* in our experiment. In contrast, most of the assimilated N by living *Hydrilla* was likely from the non-$^{15}$N-labelled source, i.e. sediments. In Chapter 3, we also found that the regrowth of *Hydrilla* significantly reduced the sediment $^{15}$N derived from macrophyte litter, while regrown *Cabomba* significantly reduced the $^{15}$N in the water column and PON (Fig. 3-4c, d). Therefore, these results refuted our null hypothesis that living *Cabomba* and *Hydrilla* used the same N source for growth. This species difference on nutrient uptake may be because *Hydrilla* can regrow longer roots (15–25 cm) and be anchored to the sediment more quickly than *Cabomba* (10–15 cm) when they had the same number of regrown roots after two weeks of rewetting. The larger leaf surface area for living *Cabomba* might also increase its nutrient uptake capability from water column sources, compared to living *Hydrilla*.

Neither of the two living macrophytes reduced water column P, indicating that they relied more on sediment, rather than water column P. A study of Carignan and Kalff (1980) showed that nine submerged macrophytes met their P requirements from sediment sources rather than water column sources. Several previous studies characterized the macrophytes as potential P pumps in the sediment to the overlying water column, by mobilizing P from the sediment through metabolic activities that change the sediment pH and redox conditions (Carignan and Kalff 1980; Barko and James 1998). In this study, macrophyte regrowth relying on less proportion of water
column than sediment P is also likely due to the completion with phytoplankton for the water column P. This is because the growth of phytoplankton was likely to be initially limited by P in the mesocosms of this study (initial water column N:P ratio = 39 ± 7:1), resulting in a rapid P utilization by phytoplankton after rewetting.

4.6 Conclusions

In conclusion, this study showed that two macrophyte species could release significant concentrations of nutrients when desiccated then rewetted, but regrowth of macrophytes after rewetting can efficiently utilize these nutrients. There were distinct differences between the species in terms of their effectiveness at nutrient uptake from the water column, which appeared to be related to the relative use of water column versus sediment nutrients for macrophyte growth. Proportionally more P than N was accumulated in the water column, shifting N:P ratios and stimulating phytoplankton biomass, in the desiccated then rewetted macrophytes treatments, compared to the bare sediment treatments without macrophytes. Overall, our study highlights the importance to use macrophytes according to their various nutrient assimilate strategies and their interaction with sediments to reduce nutrient levels and improve water quality during WLFs.
Chapter Five: General discussion

5.1 Key findings of the thesis

This thesis aimed to investigate the impact of macrophytes (nutrient sink versus source) on nutrient dynamics of reservoirs, and implications for phytoplankton blooms during WLFs. The main findings of this thesis are outlined below and summarized in Figure 5-1:

1. Macrophytes are vital nutrient sinks for shallow lakes or reservoirs, storing a comparable amount of nutrients as that in the water column. However, macrophyte beds can be shifted from a nutrient sink to a source after water level drawdown, followed by rewetting. This has flow-on effects that enhance phytoplankton biomass.

2. The invasive submerged macrophyte, *Cabomba* had significantly lower C:N and C:P ratios, therefore faster decomposition rates in its litter, compared to the native submerged macrophyte *Hydrilla*. As a result, there were higher nutrient and Chl-a concentrations, and lower DO concentrations in the water column associated with *Cabomba* decomposition than that of *Hydrilla*. However, litter from both of these submerged macrophyte species had higher N content and lower C:N ratios, when compared to the litter of the floating-leaved macrophyte, *N. indica*. *N. indica* litter also had significantly lower P content than *Cabomba* litter, but slightly higher P content than *Hydrilla* litter.

3. The presence or absence of macrophyte litter in the desiccated and rewetted sediment resulted in different N:P ratios in the water column. Macrophyte litter from both submerged and floating-leaved macrophytes (i.e. *N. indica, Cabomba*, and *Hydrilla*) had a greater impact on water column P than N, upon rewetting. Furthermore, during macrophyte regrowth after rewetting, the capacity of the plants to uptake water column
Chapter 5 – General Discussion

P was proportionately lower than their capacity to uptake N from the water column. This led to a decrease in water column N:P ratios during macrophyte decomposition. Declines in water column N:P ratios, in turn, have the potential to increase phytoplankton biomass in P-deficient lakes or reservoirs. In contrast, bare desiccated sediments without macrophytes had a greater impact on water column N than P, upon rewetting, which could increase phytoplankton biomass in N-deficient lakes or reservoirs.

4. The N and P utilized by regrown macrophytes accounted for 19–100% of N and 6–42% of P released from decaying macrophyte litter when the ratios were 9:1 (litter to regrown biomass). However, regrown *Cabomba* was more efficient than *Hydrilla* at reducing the water column N. In contrast, regrown *Hydrilla* reduced the sediment N content to a greater extent than *Cabomba*. However, neither regrown *Cabomba* or *Hydrilla* significantly reduced the water column PO₄³⁻ concentrations in this study.

5. Sediments in macrophyte beds could act as either an additional nutrient source or sink for nutrient release from macrophyte litter during WLFs. Rewetting the desiccated sediment increased N release from soil organic matter in macrophyte beds, but these same sediments bound mineral P that was released from macrophyte litter. Rewetting the desiccated sediment also resulted in a higher proportion of macrophyte-derived N remaining in the water column compared with the constantly wet conditions. This N was then assimilated by phytoplankton rather than being transferred into the sediment.
Figure 5-1. Conceptual diagram for key findings. Thicker full lines indicate a stronger positive impact from the same upper box to a lower box, compared to the thinner full lines. Thicker dashed lines indicate a stronger negative impact from the same upper box to a lower box, compared to the thinner dashed lines.

5.2 Research implications

My thesis provides new insights into the capacity of macrophytes to alter nutrient dynamics and control phytoplankton blooms during WLFs, through investigating the role of macrophytes as a nutrient sink versus a source. My study demonstrated that extended water level drawdown duration was an important trigger to shift macrophyte beds (both macrophytes and sediments) from a nutrient sink to a source. These findings suggest that the positive role of macrophytes could be maintained through effective
water level management, such as reducing water level drawdown durations or reducing water level drawdown magnitude during WLFs.

The water quality in lakes or reservoirs with submerged macrophytes (e.g. *Cabomba* and *Hydrilla* in this study) are very likely to be more impacted by WLFs, compared with the one dominated by floating-leaved or emergent macrophytes (e.g. *N. indica* in this study). This is because submerged macrophytes tested in this thesis have more rapid nutrient release rates and higher nutrient content in the biomass, compared with floating-leaved macrophytes. This indicates the same amount of the two submerged macrophytes is more likely to release more nutrients than the *N. indica* litter. In addition, drought resilience of macrophytes could also be vital factors minimizing their negative impacts on water quality during WLFs. Macrophyte species with low drought resilience are more easily desiccated than those with high resilience, thus releasing more nutrients after rewetting than those more drought-resilient species. For example, submerged macrophytes are likely to have lower drought resilience, compared with other types of macrophytes (e.g. floating-leaved or emergent species), as submerged macrophytes could not survive during extended desiccation. In contrast, the floating-leaved and emergent macrophytes have drought-resilient above-ground vegetation and also taproots anchored into the sediment. These characteristics increase their survival and recovery capacity after drying then rewetting, compared to submerged macrophytes.

Submerged macrophytes are also more effective at controlling phytoplankton biomass than floating-leaved or emergent species. This is because the epidermal cells of submerged macrophytes can utilize nutrients directly from the water column and compete with phytoplankton. Therefore, these findings suggest that WLFs should be managed more carefully in lakes or reservoirs dominated by submerged macrophytes,
compared to lakes or reservoirs dominated by floating-leaved or emergent macrophytes.

Macrophyte regeneration from seed banks in the sediment is another important survival strategy for macrophytes during severe WLFs. Lakes and reservoirs that have abundant seed banks for macrophyte regeneration, including both vegetative and non-vegetative propagules, could play a key role in reducing the nutrient build-up in the water column during WLFs. However, multiple water level drawdown events might exhaust the macrophyte seed banks by increasing their germination frequency at drawdown duration. This could reduce the capacity of macrophytes to recover during future WLFs. Therefore, improved water level management practices, such as reducing WLFs frequency, could allow macrophytes to replenish their sediment seed banks and increase their resilience to future WLFs.

My thesis also found that desiccated invasive macrophytes could potentially cause more severe water quality deterioration than native macrophytes (e.g. lower water column DO and higher Chl-a concentrations) after rewetting. Invasive species usually have higher N and P content, lower C:N and C:P ratios, and higher biomass than co-occurring native species. This leads to faster litter remineralization rates and greater nutrient release during WLFs. Therefore, careful water level management is required in reservoirs or lakes dominated by invasive macrophytes, in order to control the build-up of invasive species and their negative impacts on aquatic ecosystems. In contrast, invasive species typically recovery faster than native species after drying then rewetting, due to their faster regrowth rates in the new environmental conditions. Or some invasive species are likely to have higher drought resistance, and thus may release less nutrients in the water column after short periods of desiccation followed by rewetting. Therefore, invasive species are very likely to take over from the co-occurring native species during WLFs. This increased invasive biomass could lead to additional negative impacts on
freshwater ecosystems, such as reducing the ecosystem diversity and altering the trophic structure.

Nutrient release from macrophyte decomposition may change the N:P ratio in the water column. Therefore, lakes or reservoirs with varying levels of phytoplankton nutrient limitation could be differentially affected by macrophytes during WLFs. All desiccated macrophytes tested in my thesis (N. indica, Cabomba and Hydrilla) had a bigger impact on water column P than N concentrations after rewetting. This could be a particular issue in lakes or reservoirs where phytoplankton growth is limited by P availability, and the increased P could stimulate phytoplankton blooms. However, the bare desiccated sediments without macrophytes had a greater impact on water column N than P after rewetting, indicating bare sediments are potentially a higher risk in boosting phytoplankton biomass in N-deficient lakes. Therefore, it is important to consider the background nutrient status in lakes and reservoirs, and the impacts of macrophyte beds on water column nutrients when predicting phytoplankton responses to WLFs.

Overall, this thesis suggests that reducing macrophyte biomass loss and increasing their recovery during WLFs can reduce the potential of phytoplankton blooms. Other important factors to consider when managing WLFs to control phytoplankton blooms include: 1) the drought resilience of macrophytes dominating the system; 2) the presence of invasive species; and 3) the nutrient status and implications for changes in concentrations and ratios for primary producers (Fig. 5-1).

The management of WLFs, macrophytes, or other trophic levels could also reduce the risk of phytoplankton blooms. For example, shorter water level drawdown periods followed by rewetting can cause less damage to macrophyte beds and thus reduce the risk of phytoplankton blooms, compared to extended drying-wetting cycles. The impacts of macrophyte beds due to WLFs during the colder season (such as winter)
might be smaller than in summer months. This is because macrophyte beds would be less desiccated and decompose slowly during the colder season. In addition, phytoplankton blooms are less likely to occur during winters compared to summers. As for macrophyte management, the above-ground biomass of desiccated macrophytes could be harvested to reduce the nutrient release after rewetting. Only above-ground biomass was recommended to harvest is because 1) the below-ground biomass (roots, bulbs, or even seeds) have the potential to regrow or re-germinate after rewetting, and 2) this harvesting method would reduce the disturbance to sediment thus minimizing nutrient release after rewetting. The biomanipulation of fish, such as reducing the planktivorous and benthivorous fish abundance, or increasing the piscivore fish abundance, can also be considered during WLFs to reduce the risk of phytoplankton blooms by decreasing the fish grazing pressure on zooplankton.

5.3 Future research

This thesis investigated the impacts of macrophyte decomposition and its interaction with sediments as a nutrient source supplying phytoplankton blooms after drying then rewetting. However, phytoplankton blooms during WLFs could also be driven by other factors worth considering in future studies, including the morphology of lakes and reservoirs (e.g. depth, or basin morphology), the physico-chemical parameters of inlet water and its retention time, structure of trophic levels, and various climatic zones (Zohary and Ostrovsky 2011; Bakker and Hilt 2016). Further work is also needed on the role of macrophyte seed banks during WLFs. The availability of macrophyte seed banks and the longevity of propagules of submerged macrophytes during water level drawdown are unknown for most species (Bakker et al. 2013). In addition, the quantity and quality of soil organic matter and the changes in sediment redox conditions could also be important factors determining nutrient dynamics in the littoral zone (Watts
Macrophyte loss and regrowth can alter the habitats or food resources available for the aquatic community. Therefore, there is also value in quantifying the impact of changes in macrophyte community on other trophic levels or food-webs during WLFs, such as the role of fish on water quality during WLFs. These proposed work would provide the comprehensive information required to build models to better understand nutrient cycling, energy flows (food-web changes), and phytoplankton blooms during WLFs. Model outputs might provide practical instructions for managers to develop detailed water level management plans which are tailored to specific reservoirs or macrophyte species.
Table S4-1. The mean value (± SD) of nutrient concentrations in the water column of each treatment after rewetting (on days 0, 3, 7, 14, 28). Codes for treatment abbreviations explained in Table 3-1. CK_D and CK_W refer to the control treatment without litter and living macrophytes in previously dried and constantly wet sediment treatments.

<table>
<thead>
<tr>
<th>Codes</th>
<th>Days after rewetting</th>
<th>TDN (mg N L⁻¹)</th>
<th>TDP (mg P L⁻¹)</th>
<th>DON (mg N L⁻¹)</th>
<th>NH₄⁺ (µg N L⁻¹)</th>
<th>NOₓ (µg N L⁻¹)</th>
<th>DOP (µg P L⁻¹)</th>
<th>PO₄³⁻ (µg P L⁻¹)</th>
</tr>
</thead>
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<tr>
<td>CK_D</td>
<td>0 d</td>
<td>0.12 ± 0.01</td>
<td>0.07 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>0.01 ± 0.00</td>
<td>38.57 ± 1.05</td>
<td>4.83 ± 1.58</td>
<td>2.08 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>3 d</td>
<td>0.82 ± 0.14</td>
<td>0.57 ± 0.01</td>
<td>0.35 ± 0.05</td>
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<td>40.23 ± 0.38</td>
<td>53.81 ± 16.63</td>
<td>1.95 ± 1.31</td>
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<tr>
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<td>TDP (mg P L⁻¹)</td>
<td>DON (mg N L⁻¹)</td>
<td>NH₄⁺ (µg N L⁻¹)</td>
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<td>PO₄³⁻ (µg P L⁻¹)</td>
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### Supplementary Tables

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