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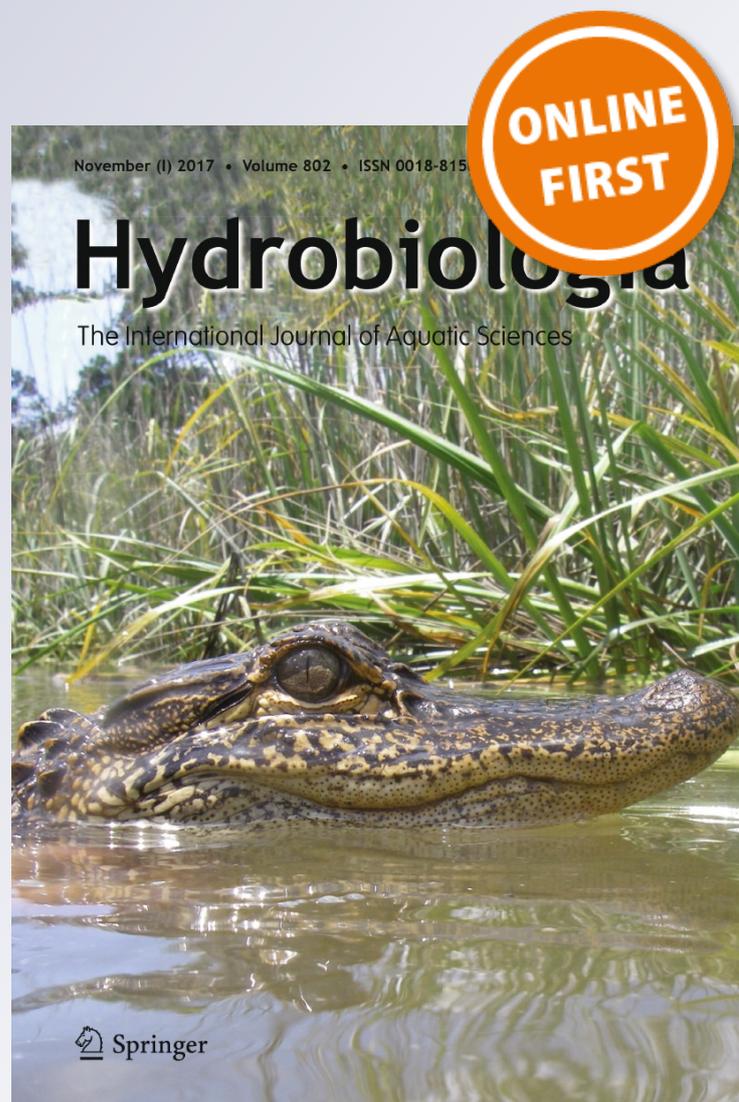
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Macrophyte-induced thermal stratification in a shallow urban lake promotes conditions suitable for nitrogen-fixing cyanobacteria

M. P. Vilas  · C. L. Marti · C. E. Oldham · M. R. Hipsey

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Abstract Densely vegetated shallow lakes often experience low water column nutrient levels and reduced phytoplankton growth, but in some cases a high phytoplankton biomass can co-exist with submerged macrophytes. The conditions that favour phytoplankton blooms within areas colonized by submerged macrophytes remain largely unexplored. We investigated changes in water quality variables and phytoplankton community composition data in relation to macrophyte-induced thermal stratification in a shallow urban lake. The results indicate that submerged macrophytes may favour internal loadings of phosphorus and low DIN:TP ratios, in periods when macrophyte-induced thermal stratification of the water column is common. Blooms of nitrogen-fixing cyanobacteria occurred under the strongly stratified

conditions triggered by the high macrophyte biomass and elevated canopy, even though nitrogen limitation was apparent during the whole growing season. These findings suggest that submerged macrophytes can promote blooms of nitrogen-fixing cyanobacteria once they are sufficiently tall and dense to induce stable water column conditions.

Keywords Shallow lakes · Submerged macrophytes · Thermal stratification · Phytoplankton · Cyanobacteria · Blooms

Introduction

The notion that shallow lakes tend to experience frequent vertical mixing (Padisák & Reynolds, 2003) is challenged by the fact that these lakes are often colonized by submerged macrophytes that can strongly stratify the water column during the daytime by dissipating turbulent kinetic energy and absorbing heat (Herb & Stefan, 2005; Andersen et al., 2017). Submerged macrophytes act as a barrier to wind-induced mixing, thereby inducing thermal stratification and impeding vertical transport of gases, and dissolved and particulate materials. When vertical transport of gases is impeded, oxygen consumed at the lake bottom cannot be replaced, potentially allowing for bottom anoxia (Branco et al., 2005). In such circumstances, reducing conditions can occur along with redox-driven phosphorus release from the bottom

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sediments (Søndergaard et al., 2003). Increased phosphorus concentrations in the water column may promote phytoplankton production that in turn shades the submerged macrophytes (Jeppesen et al., 2007). Moreover, bottom-water anoxia causes submerged macrophytes to experience stress, exposes them to phytotoxins, and triggers poor root growth that may diminish their growth or cause a sudden die-off (Borum et al., 2005; Lamers et al., 2013; Sand-Jensen et al., 2015). Upon death, macrophytes release the nutrients stored in their biomass into the water column (Rogers & Breen, 1982) and the sediments (Carpenter, 1981), creating the potential for large phytoplankton blooms (Watts, 2000).

While several studies have documented anoxic conditions (Caraco et al., 2006; Goodwin et al., 2008) and increased nutrient loadings within macrophyte beds (Barko & James, 1998; Mi et al., 2007; Boros et al., 2011), the widely accepted view is that vegetated shallow lakes have moderate to low water column nutrient levels (Phillips et al., 2016). Submerged macrophytes can reduce nutrient load directly by absorbing nutrients from both water column and sediments (Chambers et al., 1989) and indirectly by stabilizing the sediments, decreasing sediment resuspension (Horppila & Nurminen, 2003) and oxygenating areas around under-ground tissues (Lemoine et al., 2012). Given that nutrient load is associated with phytoplankton biomass (Muylaert et al., 2010), it is not surprising that most studies report negative effects of submerged macrophytes on phytoplankton biomass (Søndergaard & Moss, 1998; van Donk & van de Bund, 2002). In addition, submerged macrophytes contribute to a reduction in phytoplankton biomass through several other mechanisms: reducing the turbulent kinetic energy and thus favouring sedimentation of large non-buoyant cells (Van den Berg et al., 1998); reducing nitrogen concentrations by inducing denitrification (Holmroos et al., 2014) and direct uptake (Rogers & Breen, 1980); producing allelopathic substances that hinder phytoplankton development (Pakdel et al., 2013); providing refuge to zooplankton and thereby increasing the grazing pressure on phytoplankton (Jeppesen et al., 1997); and attenuating underwater light (Søndergaard & Moss, 1998). As a result, the phytoplankton community composition can be dominated by small phytoflagellates with low biomass that are mixotrophic and use dissolved organic carbon as food source (Fonseca &

Bicudo, 2010) and cyanobacteria, which can employ strategies such as buoyancy regulation and nitrogen fixation to give them a competitive advantage where they may be limited by light and nitrogen (Søndergaard & Moss, 1998; Takamura et al., 2003).

The effect of submerged macrophytes on the environmental conditions and phytoplankton community composition in shallow lake ecosystems has been mostly inferred from comparative studies of vegetated and non-vegetated lakes (Søndergaard and Moss, 1998; Takamura et al., 2003); less attention has been given to the variability that arises during seasonal growth periods in submerged macrophytes. Furthermore, almost no attention has been given to the response of the phytoplankton community structure to changes in the chemical variables that might emerge during periods when macrophyte-induced thermal stratification is common. Therefore, our study aims to explore the variability in the chemical characteristics and the phytoplankton community composition in a shallow lake during seasonal growth of the submerged macrophyte *Potamogeton crispus* L. (curly pondweed). For this purpose, we measured pH, nitrogen oxides (NO_x), ammonium (NH_4), total nitrogen (TN), total phosphorus (TP), filterable reactive phosphorus (FRP), chlorophyll-a (Chl-a), water column temperature, phytoplankton composition and abundance, and *P. crispus* biomass during periods when macrophyte-induced thermal stratification was absent and periods when macrophyte-induced thermal stratification was common. Our rationale was that the anoxic conditions that may develop during thermal stratification would enhance internal nutrient loadings, resulting in higher water column phosphorus levels. Moreover, macrophyte growth may reduce water column dissolved inorganic nitrogen (DIN) levels, leading to low DIN:TP ratios. This line of reasoning led us to our first hypothesis: (H1) the chemical characteristics are significantly different between periods when macrophyte-induced diurnal stratification is present and periods when it is absent. The different environmental conditions are expected to cause cyanobacteria to thrive during the stratified period because they benefit from environments rich in phosphorus (Downing et al., 2001) and the greater water column stability may shift the competitive balance towards buoyant species and away from sinking phytoplankton species (Huisman et al., 2004). Specifically, we predict that nitrogen-fixing

species will have a competitive advantage under nitrogen limited conditions (Schindler, 1977; Wood et al., 2010). This reasoning led us to our second hypothesis: (H2) the phytoplankton community composition is significantly different between periods when macrophyte-induced thermal stratification is present and periods when it is absent.

Materials and methods

Study site

Lake Monger is a eutrophic shallow lake located in a heavily urbanised suburb of Perth (Western Australia, Fig. 1). It has a total surface area of 68.2 ha and the bottom sediments are on average 11.9 m above Australian Height Datum (AHD). Twenty-three storm water drains feed into the lake and it overflows to the Swan-Canning River Estuary when mean water depth exceeds 1.1 m. The lake receives few stormwater inputs during the summer (Lund & Davis, 2000). As a result, the mean lake water level varies seasonally

ranging from 0.3 m in autumn to 1.2 m in spring. Water temperature varies from a maximum of 34°C in summer to a minimum of 11°C in winter. In the presence of submerged macrophytes, the water column is stratified during the day and fully mixed during the night (Leoni et al., 2016).

Lake Monger is characterized by high primary productivity and has a sedimentation rate of 1–1.5 cm year⁻¹ (Pierce, 1997). The lake sediments are characterized by high levels of organic matter (OM > 40%, Qiu & McComb, 2000). Dense stands of the submerged macrophyte *P. crispus* colonize the lake (Leoni et al., 2016). *P. crispus* has been described as a perennial or winter annual plant (Bolduan et al., 1994). In Lake Monger, it grows during the spring and most of the summer and declines towards the end of summer releasing the nutrients stored in its biomass into the water column (Leoni et al., 2016).

Field measurements

Data used in this investigation were collected during two growing seasons of the submerged macrophyte *P.*

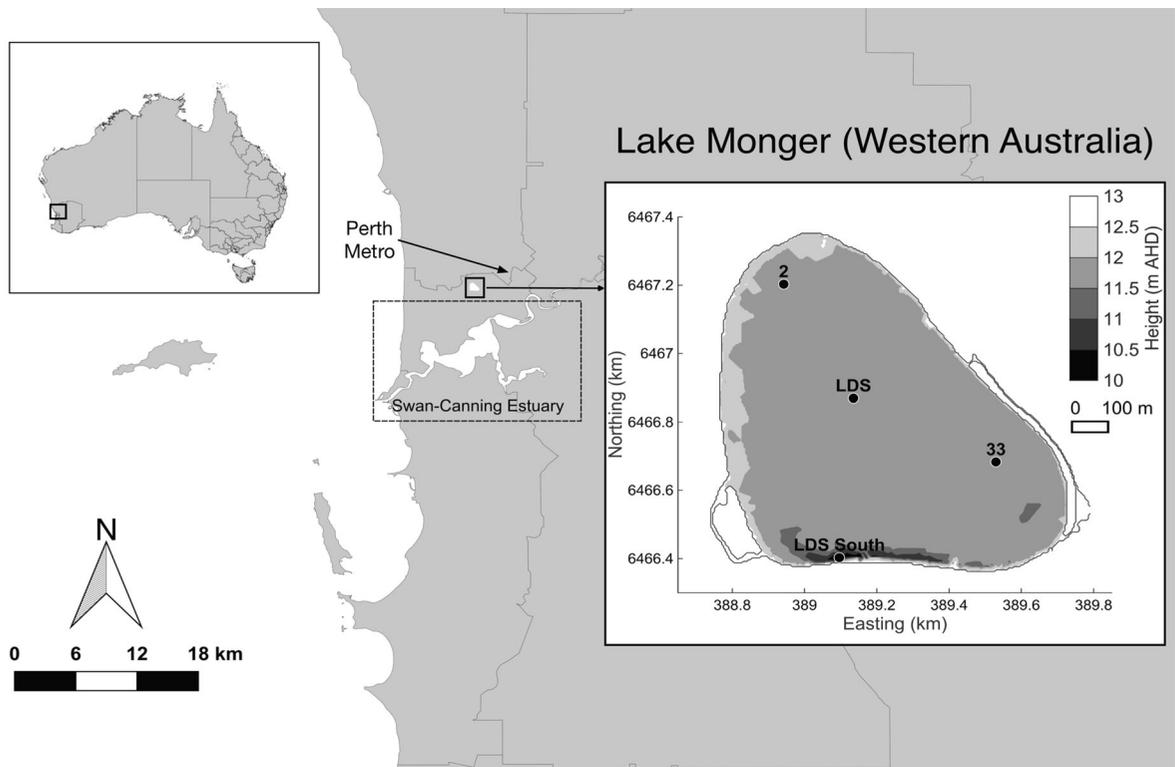


Fig. 1 Location of the study site and sampling stations (2, 33, LDS and LDS South)

crispus: (1) November 2014–January 2015 and (2) November 2015–January 2016.

Temperature, wind speed and water level

Air temperature data were sourced from the Australian Bureau of Meteorology (station: Perth Metro, located approximately 4 km east of the lake, Fig. 1) and were available at 15-min intervals (<http://www.bom.gov.au>). The thermal structure near the lake centre (Fig. 1) was continuously monitored, commencing in December 2010 with a Lake Diagnostic System (LDS) (Imberger, 2004). It was equipped with a fast response thermistor chain with 5 underwater sensors measuring water temperature at 0.2, 0.4, 0.6, 0.8 and 1 m below the surface and wind speed and direction sensors located at 2 m above the water surface. The sampling interval was set to 30 s. Weekly water level data were obtained from a water level scale located at the lake outlet.

Field surveys

During the *P. crispus* growing season in 2014–2015, water samples were collected at four different locations (Fig. 1) to identify spatial variability in chemical and phytoplankton composition data. Given that no significant spatial differences were identified (ANO-SIM, $P > 0.01$), in the second growing season (2015–2016), only sampling at the LDS station was undertaken. Water samples were collected at regular intervals (~ 2 – 3 weeks) at ~ 0.2 m from the surface at the locations previously described (Table 1). Bottom water samples (~ 0.8 m from the surface) were also collected at the LDS station during stratification of the water column (January) using a battery-powered

pump. Five-litre plastic bottles were filled at each sampling station and within 4 h of collection aliquots were subsampled for: identification and enumeration of phytoplankton (aliquot = 100 ml, preserved with Lugol's iodine solution), chlorophyll-a (Chl-a, aliquot = 2 l, filtered through glass microfiber Whatman filters of 0.7 μm pore size and 45 mm diameter), total nutrients (aliquot = 50 ml), and dissolved inorganic nutrients (aliquot = 50 ml, filtered through 0.45 μm cellulose acetate membrane syringe filters). Nutrients and Chl-a samples were refrigerated on ice for transport and frozen at -20°C for later analysis. At each sampling station pH (accuracy: 0.05 pH units) and temperature (accuracy: 0.001 $^{\circ}\text{C}$) profiles were measured using a fine-scale profiler (F-probe, Imberger & Head, 1994).

Macrophyte biomass was sampled at the LDS station during both growing seasons using a quadrat (0.1–0.2 m^2 —depending on the plant density). Plants within the quadrat were uprooted and removed with a rake. Duplicate or triplicate samples were collected, placed into zip lock bags and transported to the laboratory. In the laboratory, macrophyte samples were washed and dried at 60 $^{\circ}\text{C}$ to a constant mass, and weighed. Canopy height was estimated by randomly choosing two/three points around the LDS station.

During the *P. crispus* growing season 2015–2016, two field experiments were performed to account for the diurnal variability in the physical and chemical characteristics. The first experiment was carried out on 4 November 2015 and corresponded to low above-ground plant biomass and short canopies. The second experiment was carried out on 7 January 2016 and corresponded to high above-ground biomass and tall plant canopies. In both field experiments, water samples were collected from 04:00 to 22:00 h at

Table 1 Samples collected during *P. crispus* seasonal growth. Sampling stations are shown in Fig. 1

<i>P. crispus</i> growing season	Month/year	Number of samples	Sampling stations	Thermal stratification at the LDS
2014–2015	November 2014	4	LDS, 2, LDS South, 33	No
	December 2014	10	LDS, 2, LDS South, 33	No
	January 2015	12	LDS, 2, LDS South, 33	Yes
2015–2016	November 2015	3	LDS	No
	December 2015	3	LDS	No
	January 2016	4	LDS	Yes

regular intervals of 2–2.5 h. A multi-parameter water quality meter (AAQ-RINKO, JFE Advantech Inc., Japan) was used to compare diurnal patterns in dissolved oxygen (DO), redox potential and photosynthetic active radiation (PAR) between these two experiments. The AAQ-RINKO was deployed at the LDS station at ~ 0.3–0.4 m from the bottom, measuring DO (digital sensor, accuracy: 1%), redox potential (accuracy: 0.1 mV), PAR (accuracy: 5% of reading), and water temperature (accuracy: 0.01°C) every 1 s for the duration of the diurnal experiment.

Laboratory analysis

Determinations of Chl-a, FRP, NO_x, NH₄, TN and TP were done following standard procedures (Rice et al., 2012). DIN was calculated as the sum of ammonium and nitrogen oxides. Phytoplankton enumeration was done using a 1 mL Sedgewick-Rafter counting chamber at ×400 magnification under light microscopy. Phytoplankton was identified to the lowest taxonomic level possible (mainly genus level) and abundances were expressed as cells ml⁻¹.

Data analysis

Statistical analysis

To test our hypotheses that chemical (H1) and phytoplankton (H2) data differed between periods when stratification was absent and periods when stratification was present, non-parametric multivariate analyses were used (Clarke, 1993). Non-metric multidimensional scaling (NMDS) ordination analysis was used to visualize differences between both groups (stratification common and stratification absent). NMDS was applied to Bray–Curtis dissimilarity matrices of square-root transformed chemical data (pH, NO_x, NH₄, TN, TP, FRP, DIN:TP) and log transformed phytoplankton abundance (genus level) data to reduce the effect of dominant taxa (Anderson et al., 2006). In both cases, the solution with the lowest stress value was selected (a stable two-dimensional solution was identified for all ordinations). Significant differences in chemical and biological variables (H1) and phytoplankton community composition (H2) between the two groups (stratification common and stratification absent) were evaluated by an analysis of similarities (ANOSIM, 999 permutations) given that

heterogeneity of dispersion between groups was not critical in our data (Anderson & Walsh, 2013). Multivariate analyses were conducted in R package *vegan* (Oksanen et al., 2016). The chemical variables and Chl-a were related to the ordination axes using the *envfit* function (Oksanen et al., 2016). This function fits environmental vectors onto the ordination space defined by the species data and generates a measure of fit as well as a significance value based on a permutation test (999 permutations). The *envfit* output was then used to determine the strength of the correlation between each chemical variable and the phytoplankton community structure. The non-parametric Wilcoxon rank-sum test (Wilcoxon, 1945) was used to assess differences across months in nutrient concentrations given its robustness to compare small groups of data (Blair & Higgins, 1980). This test was performed in MATLAB using the statistics toolbox.

Results

Strength of the thermal stratification increases with macrophyte biomass

Water column temperature difference of at least 1°C over 1 m was consistently recorded during the daytime after 21 December in the *P. crispus* growing season in 2014–2015 and after 19 December in the *P. crispus* growing season in 2015–2016 (Fig. 2a, b respectively). The development of diurnal thermal stratification coincided with an increase in submerged macrophyte biomass and canopy height. The strength of the stratification differed across growing seasons. During the *P. crispus* growing season in 2014–2015, a maximum temperature difference between surface and bottom waters of 11.7°C m⁻¹ was recorded (20 January 2015) (Fig. 2a). No such strong temperature differences were recorded during the second growing season in 2015–2016, and the maximum temperature difference during this period was only 6.4°C m⁻¹ (6 January 2016) (Fig. 2b). In the second growing season, surface and bottom waters fully mixed after the plants declined on 25 January 2016 (Fig. 2b), suggesting that the plants were responsible for the observed thermal stratification. Logistic growth functions fitted to changes in biomass data over time showed similar maximum growth rates for *P. crispus* in both growing seasons (mean ± SD = 0.073 ± 0.042 and 0.068 ± 0.028

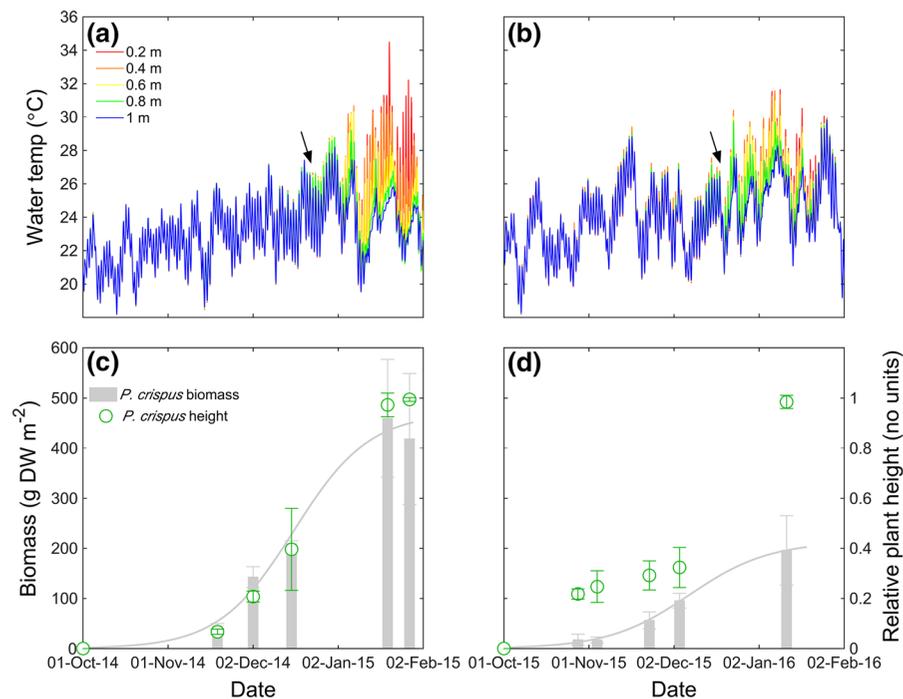


Fig. 2 Time series of water temperature (°C) recorded by the LDS station at 0.2 m (red), 0.4 m (orange), 0.6 m (yellow), 0.8 m (green) and 1 m (blue) depth for *P. crispus* growing seasons in 2014–2015 (a) and 2015–2016 (b). *P. crispus* mean biomass (grey bars), standard deviation (grey error bars), relative height (green circles), relative height standard deviation

(green error bars) and adjusted logistic growth for biomass data in the growing seasons 2014–2015 (c) and 2015–2016 (d). The black arrow in (a) and (b) indicates the date after which thermal stratification consistently developed during the day. The relative height was obtained by dividing plant height by total water depth obtained from a water level scale

day⁻¹ for 2014–2015 and 2015–2016 respectively, Fig. 2c, d). However, the estimated maximum biomass was significantly higher in the *P. crispus* growing season in 2014–2015 compared with 2015–2016 (470.63 ± 79.25 and 213.43 ± 36.22 g DW m⁻² respectively), suggesting that the higher biomass in January 2015 may have strengthened the diurnal stratification.

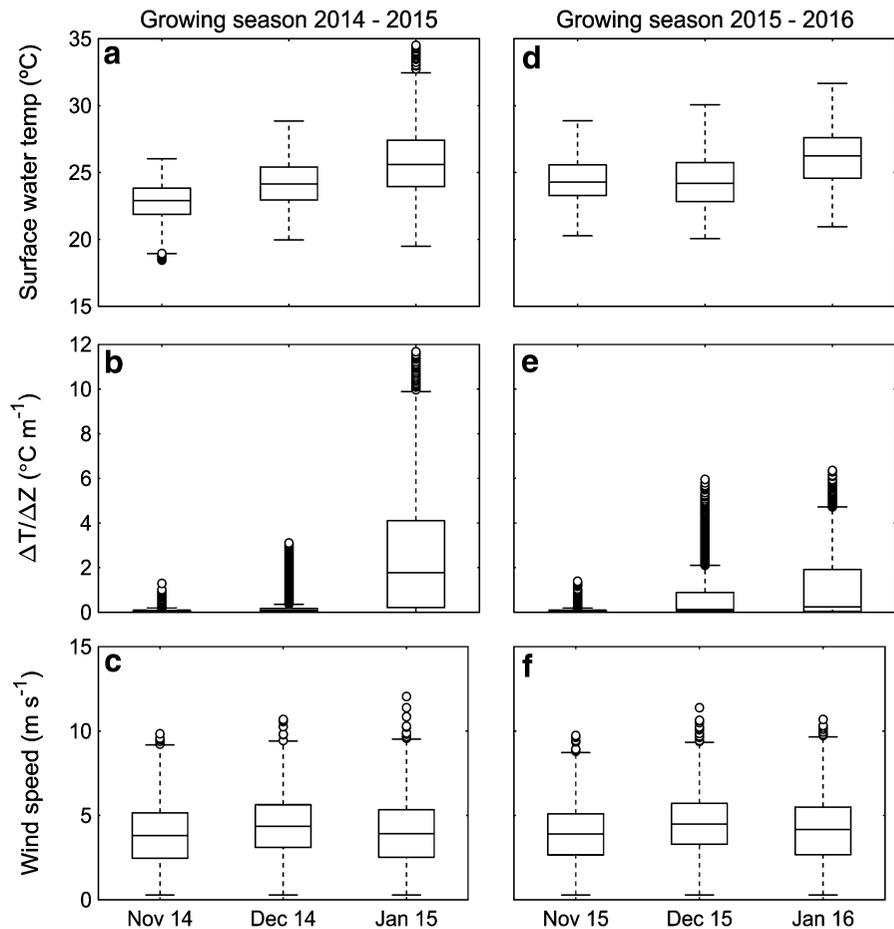
A slight increase in the median surface water temperature (0.2 m depth) at the LDS station was observed from November 2014 to January 2015 (Fig. 3a). The median surface water temperature also increased from December 2015 to January 2016 (Fig. 3d). As previously described, the temperature difference between surface and bottom waters was more pronounced in January 2015 than in January 2016 (Fig. 3b, e), while the median wind speed was almost identical (Fig. 3c, f). Moreover, there were no differences in midday air temperature (mean \pm SD = 30.3 ± 3.9 and 28.2 ± 5.3 °C for January 2015 and January 2016, respectively; data not shown)

or water depth at the LDS station (mean \pm SD = 1.03 ± 0.07 and 1.20 ± 0.05 m for January 2015 and January 2016, respectively; data not shown) across the two periods.

Chemical characteristics before and during thermal stratification of the water column

TN, DIN, TP and FRP exhibited distinct trends that were maintained across seasons (Fig. 4). TN increased from November to January, and concentrations in January 2015 were significantly higher than in January 2016 ($P < 0.05$) (Fig. 4a, f). DIN followed a different pattern from that of TN as it decreased from November to December and increased from December to January as diurnal stratification became common. DIN was higher in January 2015 than January 2016 (median of 16.9 and 10.0 $\mu\text{g l}^{-1}$ respectively) (Fig. 4b, g). The two orders of magnitude difference between TN and DIN in January 2015 indicates that much of the TN

Fig. 3 Comparison of monthly surface water column temperature at the LDS ($^{\circ}\text{C}$) (a), $\Delta T/\Delta Z$ ($^{\circ}\text{C m}^{-1}$) (b), and wind speed (m s^{-1}) (c) in the *P. crispus* growing season in 2014–2015. Comparison of monthly surface water column temperature at the LDS ($^{\circ}\text{C}$) (d), $\Delta T/\Delta Z$ ($^{\circ}\text{C m}^{-1}$) (e), and wind speed (m s^{-1}) (f) in the *P. crispus* growing season in 2015–2016. $\Delta T/\Delta Z$ was calculated from the maximum and minimum depths in which water temperature was measured

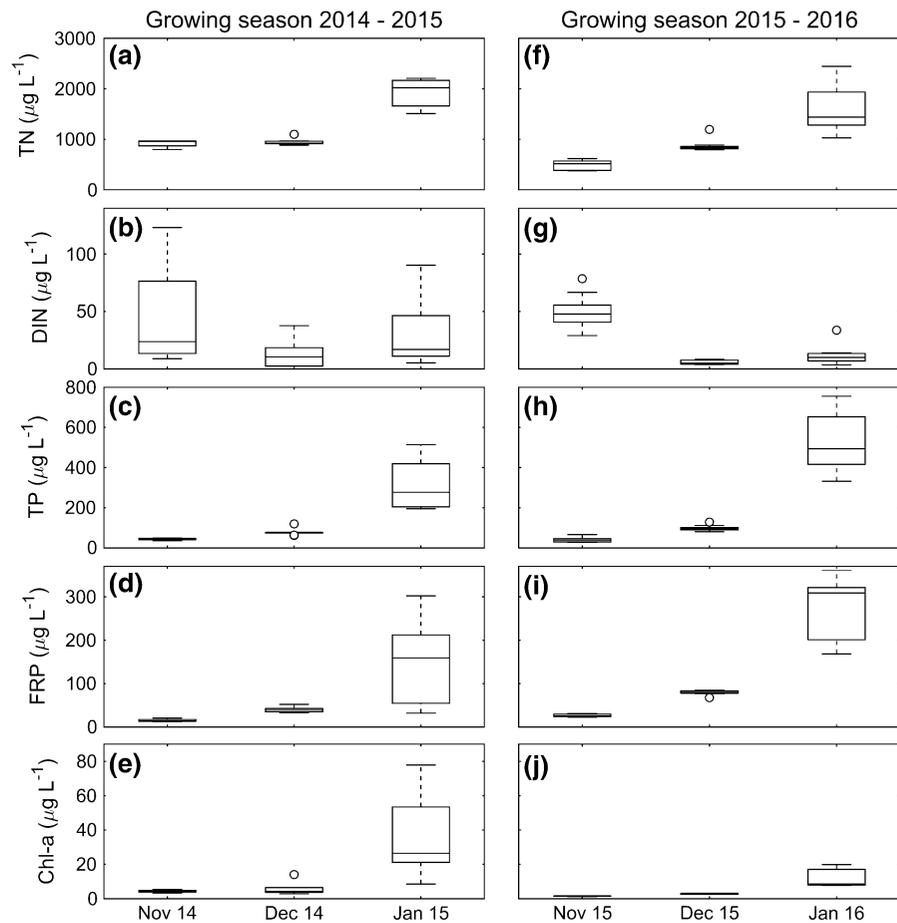


was detrital or dissolved organic material and therefore not readily available for phytoplankton. TP and FRP increased over November–January, with a rapid increase from December to January (Fig. 4c, d). Significantly higher FRP levels were recorded in January 2016 compared with January 2015 (median of 308 and 159 $\mu\text{g l}^{-1}$, respectively). Chl-a was generally at moderate to low concentrations ($< 20 \mu\text{g l}^{-1}$), with the exception of January 2015 when values were as high as 80 $\mu\text{g l}^{-1}$ (Fig. 4e). Despite high levels of FRP were recorded in periods when diurnal thermal stratification was common in both growing seasons (January 2015 and January 2016), the high Chl-a levels were only measured in January 2015.

During the diurnal field experiment performed on 4 November 2015 (Fig. 5a–e) the following occurred: no thermal stratification developed (Fig. 5a); near bottom DO increased during daytime and decreased during nighttime, but never dropped below 50% saturation

(Fig. 5b); near bottom redox potential exceeded 200 mV (Fig. 5b); bottom PAR measurements exceeded 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at midday (Fig. 5c); NH_4 declined during the daytime; NO_x decreased after dawn and then remained reasonably constant (Fig. 5d); and surface FRP remained low ($< 30 \mu\text{g l}^{-1}$) (Fig. 5e). In contrast, for the field experiment performed on 7 January 2016 (Fig. 5f–j), near bottom waters were hypoxic at 04:00 h (Fig. 5g), corresponding to the end of the stratified period of the previous day. At this time, near bottom redox potential remained < -50 mV (Fig. 5g), there was no light (Fig. 5h), and NH_4 /FRP were maximal (Fig. 5i, j). These observations suggest that the antecedent stratified conditions resulted in insufficient downward transport of DO to balance consumption, and thus promoted anoxia and phosphorus release from the sediments. As surface and bottom waters mixed at 04:30 h, near-bottom DO levels increased to 100% saturation and remained fairly

Fig. 4 Comparison of monthly total nitrogen (TN) ($\mu\text{g l}^{-1}$) (a), dissolved inorganic nitrogen (DIN) ($\mu\text{g l}^{-1}$) (b), total phosphorus (TP) ($\mu\text{g l}^{-1}$) (c), filterable reactive phosphorus (FRP) ($\mu\text{g l}^{-1}$) (d) and chlorophyll-a (Chl-a) ($\mu\text{g l}^{-1}$) (e) from all sampling stations in the *P. crispus* growing season 2014–2015; and monthly total nitrogen ($\mu\text{g l}^{-1}$) (f), dissolved inorganic nitrogen ($\mu\text{g l}^{-1}$) (g), total phosphorus ($\mu\text{g l}^{-1}$) (h), filterable reactive phosphorus ($\mu\text{g l}^{-1}$) (i) and chlorophyll-a ($\mu\text{g l}^{-1}$) (j) at the LDS station in the *P. crispus* growing season 2015–2016



constant during the day. Bottom PAR measurements remained above $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ after 9:00 h and attained a maximum of $270 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 16:00 h (Fig. 5j). During the daytime, surface and bottom NH_4 and surface FRP concentrations dropped while no changes in NO_x and bottom FRP were observed. Near-bottom FRP levels matched the surface concentrations when bottom and surface waters mixed at 21:00 h (Fig. 5e, f).

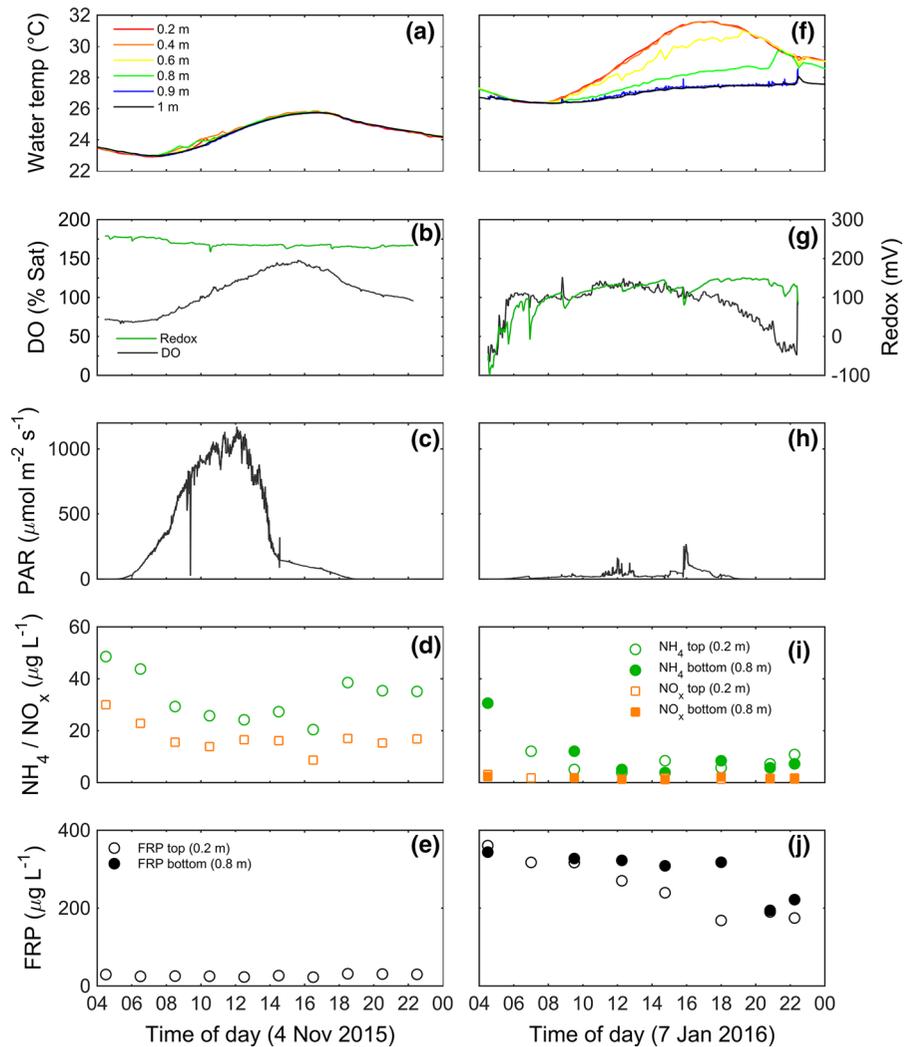
A non-metric multidimensional scaling ordination using Bray–Curtis dissimilarities was used to visualize variation that occurred in the chemical variables (pH, NO_x , NH_4 , TN, TP, FRP and DIN:TP) between periods when thermal stratification was absent (November–December) and periods when thermal stratification was common (January) in both growing seasons (Fig. 6). A clear separation among water samples was visualized on the NMDS ordination with no overlap between periods when diurnal stratification was common (grey) and absent (black). The

differences in the chemical characteristics between unstratified and stratified periods were found to be significant by ANOSIM test ($R = 0.82$, $P = 0.001$).

Cyanobacteria blooms occurred only during stratified conditions

Phytoplankton data collected in both growing seasons were visualized using a NMDS ordination (Fig. 7a). The NMDS stress value was of 0.14 suggesting a good representation of our data. Phytoflagellates such as *Cryptomonas*, *Teleaulax*, *Peridinium* and *Trachelomonas* were positioned within the unstratified period (black). Cyanobacteria taxa (*Anabaena*, *Microcystis*, *Aphanocapsa*, *Aphanothece*) were located within the stratified period (grey). The stratified period was also comprised of epiphytes such as *Miriactinium* and *Scenedesmus*. The overall NMDS ordination did not show a strong separation between periods when thermal stratification was common and absent. This result was

Fig. 5 Diurnal variation in water temperature at the LDS (°C) (a), bottom dissolved oxygen (% saturation) at 0.9 m depth (b), bottom redox potential (mV) at 0.9 m depth (b), PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at 0.9 m depth (c), NH_4 and NO_x ($\mu\text{g l}^{-1}$) (d), and FRP ($\mu\text{g l}^{-1}$) (e) on 4 November 2015, before *P. crispus* commonly stratified the water column. Diurnal variation in water temperature at the LDS (°C) (f), bottom dissolved oxygen (% saturation) at 0.9 m depth (g), bottom redox potential (mV) at 0.9 m depth (g), PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at 0.9 m depth (h), NH_4 and NO_x ($\mu\text{g l}^{-1}$) (i), and filterable reactive phosphorus ($\mu\text{g l}^{-1}$) (j) on 7 January 2016, while *P. crispus* commonly stratified the water column



supported by ANOSIM test, which showed that despite being significant ($P = 0.001$), the difference in phytoplankton composition between both groups was weak ($R = 0.35$). Chemical variables significantly related to the ordination of the phytoplankton community structure were: TN, pH, TP, DIN:TP, FRP, and NO_x ($P < 0.05$) (Fig. 7b). TN provided the best fit ($R^2 = 0.53$, $P < 0.001$), followed by FRP ($R^2 = 0.47$, $P < 0.001$), and pH ($R^2 = 0.45$, $P < 0.001$). Total phosphorus ($R^2 = 0.39$, $P < 0.001$), DIN:TP ($R^2 = 0.36$, $P < 0.001$), and NO_x ($R^2 = 0.28$, $P < 0.005$) explained less of the variation.

The phytoplankton community composition was different between the two growing seasons. The *P. crispus* growing season in 2014–2015 was characterized by a shift in dominance from phytoflagellates in

November 2014 to cyanobacteria (> 90% of total cell counts) in January 2015. High Chl-a levels were recorded in January 2015 (Fig. 4e), with *Anabaena* the dominant genus (57% of total cyanobacteria cell counts). In contrast, no such high Chl-a levels were measured in the *P. crispus* growing season in 2015–2016 (Fig. 4j), when cyanobacteria was the dominant genus throughout the season.

Discussion

This study highlights the importance of macrophyte-induced stratification in driving temporal variations in water quality. Thermal stratification has been previously shown to strongly influence hypolimnetic

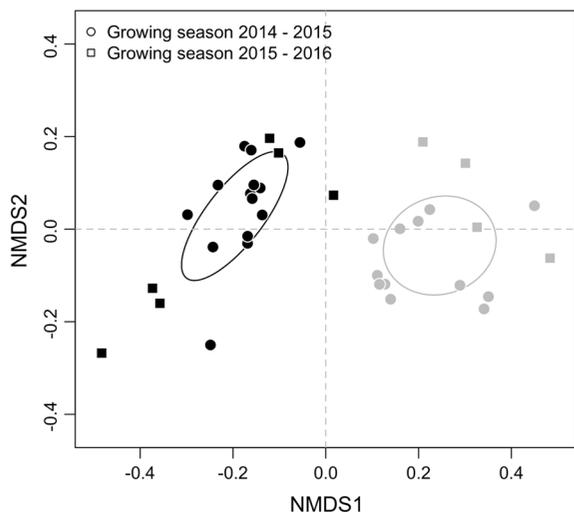


Fig. 6 NMDS based on Bray–Curtis dissimilarities of square-root transformed chemical data displaying groups in which stratified conditions were absent (black) and in which stratified conditions were common (grey) during both *P. crispus* growing seasons. Ellipses show the standard deviation of the group centroid

oxygen levels within submerged macrophyte beds (Chimney et al., 2006). Here, we demonstrate that low hypolimnetic oxygen levels can occur during the night, when thermal stratification results in insufficient vertical transport to balance DO consumption within the macrophyte bed. This observation is supported by a separate analysis in which we showed that bottom-water anoxia only occurs during stratified conditions and that nighttime convective mixing re-oxygenates bottom waters (Vilas et al., in review). In the present study, oxic conditions were sustained during the day, even at low light intensities (January 2016), supporting previous observations that *P. crispus* is able to photosynthesize and elongate at low light intensities (Chambers, 1982; Tobiessen & Snow, 1984).

Macrophyte-induced diurnal stratification affects water column nutrients

The ordination analysis captured a strong separation between water samples collected in periods when macrophyte-induced thermal stratification of the water column was common and when it was absent. The ANOSIM R statistic of 0.82 indicated that most of the observed variation was explained by diurnal stratification (Fig. 6). As expected, an increase in phosphorus (TP and FRP) was observed in periods when

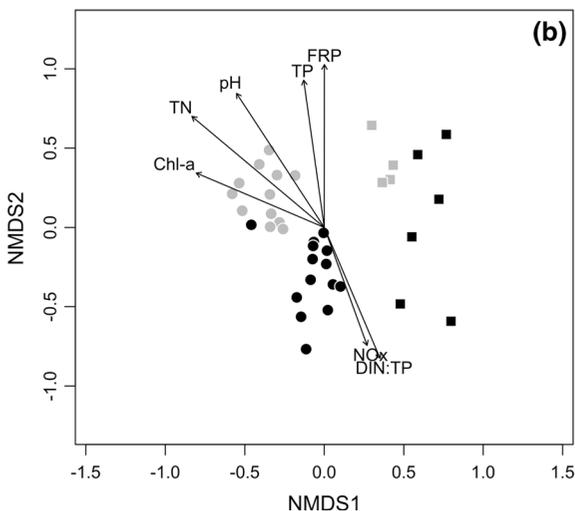
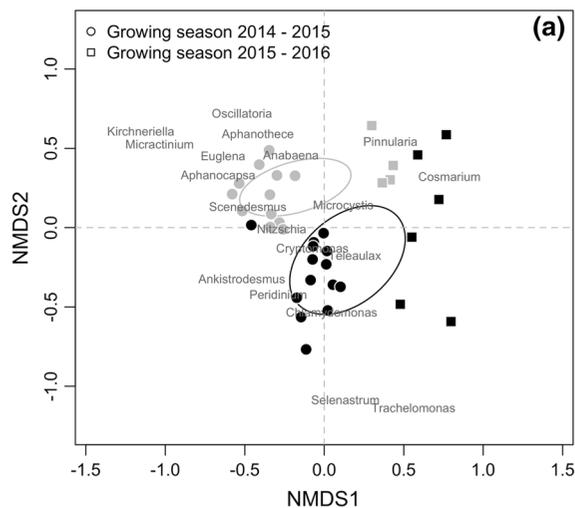


Fig. 7 NMDS based on Bray–Curtis dissimilarities of log-transformed phytoplankton abundance data displaying groups in which stratified conditions were absent (black) and in which stratified conditions were common (grey) during both *P. crispus* growing seasons (a). Ellipses show the standard deviation of the group centroid. Significant biological and chemical vectors fitted on the ordination plot are also shown (b). The value of each biological and chemical variable increases towards the direction of the arrow and significance is indicated by arrow length

macrophyte-induced diurnal stratification was common in both growing seasons (Fig. 3). Increased phosphorus concentrations within dense stands of *P. crispus* have been previously reported in both natural and experimental conditions (Rogers & Breen, 1980; Mi et al., 2007), and have been attributed to the anoxic conditions that occur when submerged macrophytes impede vertical mixing (Boros et al., 2011). By

restricting vertical mixing *P. crispus* obstructs the ventilation of bottom waters, which results in bottom hypoxia or anoxia during the night (Chimney et al., 2006; Vilas et al., in review). In such conditions, reduction of iron oxyhydroxides may occur, and thus the release of the absorbed phosphorus into solution (Søndergaard et al., 2003). At the beginning of the field experiment on 7 January 2016, when bottom hypoxia and highly reducing conditions were recorded, phosphorus levels were high. By the end of the experiment, when oxic conditions were measured, phosphorus concentrations had decreased substantially (Fig. 5g, j). Assuming that this same stratification pattern was maintained on the subsequent day, we calculated FRP fluxes from changes in bottom-water FRP concentrations to demonstrate that the doubling of FRP within a 6-h period is plausible. Since water samples were collected at ~ 0.2 m from the bottom of the lake, we used a distance from the bottom of 0.2 m and found a FRP flux of $97.5 \text{ mg m}^{-2} \text{ day}^{-1}$. Similar FRP fluxes were reported in other shallow lakes (Jensen & Andersen, 1992), supporting our conclusion of FRP release during the night. In addition, previous studies in Lake Monger have documented sediment iron levels ranging between 102 and $324 \text{ mg g}^{-1} \text{ DW}$ (Lund & Davis, 2000; Qiu & McComb, 2000). This supports our conclusion that nighttime anoxia caused the reduction of iron oxyhydroxides, and thus the release of phosphorus from the sediments.

Two alternative mechanisms for the internal FRP loadings are: (a) pH driven FRP release from the sediments (Boros et al., 2011); and (b) active translocation of FRP from the sediments to the water column mediated by *P. crispus* (Mi et al., 2007). Although an increase in pH was positively associated with the stratified samples, it is unlikely that this mechanism alone could have caused FRP to double in concentration over the night, particularly since bottom-water pH usually decreases over the night due to microbial respiration (results not shown). Mechanism (b) is unlikely since other laboratory studies indicate no phosphorus leakage during *P. crispus* growth (Welsh & Denny, 1979).

Overall, TN increased in January in both growing seasons. This was expected since an increase in macrophyte biomass causes accumulation of macrophyte detritus and enhances zooplankton production (Schriver et al., 1995). In both growing seasons DIN

levels decreased from November to December. There are three plausible mechanisms for this decline: (a) *P. crispus* decreased water column DIN levels through stem uptake (Rogers & Breen, 1980); (b) water column DIN was removed through denitrification (Holmroos et al., 2014); or (c) *P. crispus* growth reduced sediment resuspension and enhanced sedimentation, thus decreased the release of DIN from resuspended organic particles (Holmroos et al., 2014). In a separate analysis we showed that the macrophytes in Lake Monger can stratify the water column once they occupy 50% of the water depth (Vilas et al., in review). In addition, we observed that bottom-water anoxia only develops once the plants have caused the water column to stratify (Vilas et al., in review). Since denitrification is considered to be an anaerobic process inhibited by oxygen, we argue that the decline from November to December is either a result of macrophyte uptake or enhanced sedimentation.

Slightly higher DIN levels were recorded in January compared with December in both growing seasons. As the macrophytes grow in height they provide a suitable environment for particle sedimentation, including macrophyte litter which is readily available for rapid mineralization (Soana et al., 2012). The increase in NH_4 observed at 4:00 h on 7 January 2016 indicates OM mineralization (Holmroos et al., 2014). If we calculate NH_4 fluxes in a similar way to FRP we obtain a flux of $18.7 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$, which is at the lower end of previously reported values of $12.6\text{--}25 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$ in eutrophic shallow lakes (Reddy et al., 1996; Nowlin et al., 2005) and $56\text{--}532 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$ in *Vallisneria spiralis* L. beds (Soana et al., 2015). The decrease in NH_4 during the daytime suggests that either oxic conditions allowed for ammonia oxidation to occur or that NH_4 was rapidly lowered due to macrophyte or phytoplankton uptake. The relatively constant NO_3 concentrations may also indicate that either nitrification was balanced by denitrification or NO_3 uptake. Denitrification is often enhanced by bioturbation activities (Hölker et al., 2015). While we did not quantify benthic fauna, we observed high densities of Chironomid larvae that could have aerated bottom sediments and created localized zones suitable for denitrification. *P. crispus* and phytoplankton uptake could have also contributed to maintain low NO_3 and NH_4 concentrations during the daytime. DIN uptake rates in *V. spiralis* beds have been estimated to range between

1008 and 2800 mg DIN m⁻² day⁻¹ (Soana et al., 2015). At such rates DIN would have been rapidly depleted from the system, suggesting that either *P. crispus* possess lower uptake rates than *V. spiralis* or that other mechanisms were contributing to nitrogen cycling.

In summary, our findings support the first hypothesis that there would be significant differences in the lake chemical composition between periods when macrophyte-induced thermal stratification was common and when it was absent. Specifically, we showed that internal loadings of phosphorus can occur during periods when thermal stratification is common. This situation may hold for other densely vegetated shallow lakes. These results expand our understanding of the effect of submerged macrophytes on water quality since they indicate that nutrient uptake and deposition may be exceeded by nutrient release from the sediments during periods when macrophyte-induced stratification is common.

Changes in phytoplankton community composition with macrophyte-induced thermal stratification

Contrary to our expectations, the phytoplankton community composition could not be strongly differentiated between periods when thermal stratification was common and absent. Despite a statistically significant difference between both groups, the low ANOSIM R statistic ($R = 0.35$) indicates weak separation between groups. The differences in community structure observed between *P. crispus* growing seasons may have accounted for the low ANOSIM R statistic. While a weak separation was identified between stratified and unstratified conditions, some interesting patterns were evident in the ordination diagram. Taxa present across the unstratified period were characterized by the presence of flagella and by their ability to use macrophyte organic exudates (mixotrophy). This is consistent with previous observations correlating phytoflagellates presence to their ability to exploit environments rich in organic matter and lacking uniform light conditions, typical of submerged macrophyte beds (Søndergaard & Moss, 1998). On the other hand, the stratified period was characterized by the absence of phytoflagellates and the presence of cyanobacteria genera such as *Anabaena* and *Microcystis*. Fitting of biological and

chemical variables to the ordination plot revealed that pH, TP, FRP, TN, NO_x and DIN:TP were significantly correlated to the ordination of the phytoplankton community composition (Fig. 7). While TP, FRP, pH, TN were highest for the group in which stratified conditions were common, DIN:TP and NO_x were highest for the group in which stratified conditions were absent. This is in agreement with previous studies suggesting that cyanobacteria dominate in environments rich in phosphorus (Downing et al., 2001) and with low N:P ratios (Havens et al., 2003). Additionally, the positive correlation between pH and the group in which stratified conditions were common indicates that other adaptations such as the presence of carbon concentrating mechanisms (Reynolds, 2006; Unrein et al., 2010) could have contributed to the ability of cyanobacteria to dominate in periods when macrophyte-induced thermal stratification is common.

During the *P. crispus* growing season 2014–2015, cryptophytes dominated before thermal stratification (November 2014–December 2014) followed by high biomass of a nitrogen-fixing cyanobacteria in January 2015. In contrast, low biomass of cyanobacteria was consistently recorded between November 2015 and January 2016. Alternative state theory (sensu Phillips et al., 2016) assumes that when submerged macrophytes dominate, a clear water state low in phytoplankton biomass is usually favoured. It was therefore a surprise to observe a bloom of a nitrogen-fixing phytoplankton species, *Anabaena*, coexisting with the submerged macrophytes in January 2015. As previously discussed, low DIN:TP ratios, high phosphorus, low DIN, and high pH occurred when the water column stratified on a diurnal cycle. Although nitrogen-fixing cyanobacteria has been previously associated with low N:P ratios (de Tezanos Pinto & Litchman, 2010) and low DIN concentrations (Wood et al., 2010), there is ongoing debate about the use of inorganic nutrient pools and nutrients ratios to explain cyanobacteria dominance (Ferber et al., 2004; O'Neil et al., 2012). In this context, heterocyst abundance has been suggested as a better predictor of nitrogen-fixing cyanobacteria (Ferber et al., 2004; Wood et al., 2010). While we observed heterocysts in *Anabaena* during January 2015, the lack of quantitative data precludes us from concluding that nitrogen fixation provided *Anabaena* a competitive advantage under low DIN and N:P ratios.

Nitrogen fixation is an energetically costly process that requires adequate light (de Tezanos Pinto &

Litchman, 2010). In both growing seasons, the macrophytes reached the water surface and therefore *Anabaena* growth may also have been limited by light. However, as the macrophytes approached the water surface they caused stratified conditions during the daytime, which may have allowed *Anabaena* to accumulate near the water surface (Sherman et al., 1998). By concentrating at the water surface *Anabaena* could have gained access not only to light, but also to carbon dioxide (CO₂) diffusing into the lake from the atmosphere (Paerl et al., 2011), which may have helped it relieve the potential CO₂ limitation typical of *P. crispus* beds (Madsen & Sand-Jensen, 1991). The lack of strongly stratified conditions in January 2016 could have removed the competitive advantage of buoyancy control and may explain why *Anabaena* did not bloom in the second growing season. While it is plausible that the *Anabaena* bloom could have caused the stronger temperature difference between surface and bottom waters in the first growing season (Scheffer et al., 1997), our observation of thermal stratification prior to the algal bloom (Fig. 2) and a decline in Chl-a levels on 28 January 2015 ($\sim 17 \mu\text{g l}^{-1}$) coinciding with a strong thermal stratification indicates that the higher macrophyte biomass measured in the first season was responsible for the more stable water column. It is therefore hypothesised that the stronger stratification that developed in January 2015 may have allowed *Anabaena* to vertically migrate to obtain adequate light and CO₂, while possibly circumventing nitrogen limitation through fixation.

Implications for management and future climate conditions

P. crispus is commonly used in restoration programs in China (Wu et al., 2009), but is generally considered an invader and nuisance species elsewhere (Bolduan et al., 1994). Identifying the conditions under which *P. crispus* can enhance internal nutrient loadings and cyanobacteria blooms would be of value to lake managers. This study indicates that *P. crispus* can enhance internal loadings of phosphorus and low DIN concentrations when diurnal thermal stratification is common. This condition combined with the potential for nitrogen-fixing cyanobacteria to circumvent nitrogen limitation sets the scene for harmful algal blooms (HABs) to develop. HABs pose serious health threats

to humans and other organisms (Pettersson & Pozdnyakov, 2013). In our study, *Anabaena* bloomed in January 2015 but was absent in January 2016. It is suggested that the strong thermal stratification in January 2015 may have allowed *Anabaena* to overcome light limitation typical of conditions in submerged macrophyte beds, by enabling it to position at the water surface, thus allowing it to establish a surface bloom.

Promoting low sparse canopies would reduce or eliminate the effect of submerged macrophytes on thermal stratification, reducing the negative effects that submerged macrophytes may have on water quality. A reduction in canopy height could be achieved by cutting macrophytes to a specific height (Coops et al., 2002). In addition, a recirculation system to promote flushing of the macrophyte bed during stratified conditions may prevent bottom-water hypoxia, thus internal phosphorus loadings. Enhanced flushing is a cost effective management strategy to prevent internal loadings of phosphorus (Hickey & Gibbs, 2009). Although we suggest that dense/tall submerged macrophyte beds may have negative effects on ecosystem services, previous studies in Lake Monger indicate that large phytoplankton blooms (Chl-a $> 700 \mu\text{g l}^{-1}$) have occurred in the absence of the submerged macrophytes (Lund & Davis, 2000), suggesting that non-vegetated conditions can result in worse water quality due to intense phosphorus cycling (Nurminen & Horppila, 2009).

Global warming is expected to strengthen the column stability and to enhance internal loading of nutrients in lakes (Wilhelm & Adrian, 2008; Carey et al., 2012). In this study, macrophyte-induced thermal stratification emerged as a key factor that likely explains the bloom of *Anabaena* in a densely vegetated shallow lake. Therefore, one could speculate that global warming would favour the proliferation of nitrogen-fixing cyanobacteria within macrophyte beds. Further experimental work over periods of time spanning more than two seasonal cycles should be undertaken to further evaluate this hypothesis.

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