

Key Points:

- Extensive diel and depth variation in temperature, oxygen and pH occurred in dense littoral macrophyte stands in a small mesotrophic lake
- Metabolism of oxygen and dissolved inorganic carbon were closely coupled and photosynthesis and calcification peaked before noon
- Sensor measurements reveal the extensive variability of physical mixing, chemical gradients, and metabolism in dense macrophyte stands

Supporting Information:

Supporting Information may be found in the online version of this article.

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Ecosystem Metabolism and Gradients of Temperature, Oxygen and Dissolved Inorganic Carbon in the Littoral Zone of a Macrophyte-Dominated Lake

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Abstract Dense submerged macrophyte stands in lakes may promote alternating daytime stratification and nighttime convective mixing, driving extensive spatiotemporal variations in water temperature, oxygen, pH, and inorganic carbon (DIC). We set out to investigate environmental conditions and ecosystem metabolism in the macrophyte-dominated littoral zone (0.6 m depth) of a shallow, mesotrophic lake and compared this with the pelagic zone (3.0 m deep). We found that, during summer, vertical water column gradients only occasionally occurred and were weak in the pelagic zone, while dense littoral macrophyte stands of charophytes exhibited strong diel changes and steep daytime temperature and oxygen depth gradients. Oxygen showed daytime surface supersaturation and bottom anoxia, alternating with nighttime mixing. In spring, before charophytes appeared, the vertical gradients were largely absent. Ecosystem metabolism was primarily positive in spring, but areal rates of daily gross primary production (GPP) and closely related respiration increased 6-fold in summer. Ecosystem metabolism calculated based on oxygen or DIC was very similar, with a 1:1 M basis. Daytime DIC loss by CaCO₃ precipitation on charophyte surfaces averaged 6.3% of total DIC loss, and this loss was restored during nighttime. The contribution of shallow littoral macrophyte communities to whole-lake summer GPP exceeded that estimated for phytoplankton. Environmental conditions in the macrophyte-covered littoral zone differed markedly from pelagic waters with steep vertical gradients in temperature and chemistry during daytime including anoxic micro-habitats in stark contrast to deep pelagic waters. The study highlights the significant influence of the littoral zone and macrophytes on whole-lake ecosystem processes.

Plain Language Summary In shallow water in clear-water lakes where light reaches the bottom, dense stands of submerged plants may develop. Dense plant stands promote stratification of the water column resulting in large differences in water temperature, oxygen, pH, and dissolved inorganic carbon between surface and bottom waters. We set out to investigate how dense plant stands in the shallow water of a nutrient-poor lake influence environmental conditions and compare this to open waters. During summer, we found small differences between the surface and bottom water at the open water site, while pronounced differences developed in dense plant stands in the shallow water site. During daytime, oxygen was high in surface water with high photosynthesis, while respiration consumed virtually all oxygen in dim light below the plant canopy close to the bottom. During nighttime, differences vanished due to surface cooling, which promotes mixing of the entire water column. Two independent methods showed a close 1:1 balance between oxygen production and consumption and complementary carbon consumption and production in photosynthesis and respiration, respectively. Our study highlights the differences between shallow and open waters and the prominent role that plants may play in productivity and environmental conditions in the whole lake.

1. Introduction

Submerged charophytes and angiosperms attached to the sediment can develop a dense cover in shallow lakes with clear water (Blindow et al., 2014; Sand-Jensen et al., 2019). Many charophyte and angiosperm species have experienced a profound decline during the last 100 years due to drainage reclamation of land for agriculture (Baastrup-Spohr et al., 2013; Sand-Jensen et al., 2000). Moreover, many lakes have experienced excessive eutrophication and phytoplankton blooms, and consequently a loss of submerged macrophytes due to shading (Hilt et al., 2013; Sand-Jensen et al., 2017). Recently, macrophytes have colonized many new habitats that have

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been established in sand, gravel and chalk pits, as well as in rainwater basins within towns and along highways, to improve nature quality and biodiversity (Søndergaard et al., 2018).

In small, shallow lakes, high macrophyte density and dominance can have important consequences for environmental variability, carbon dynamics, and ecosystem metabolism (Ribaud et al., 2018; Sand-Jensen et al., 2019). Dense macrophyte stands have strong vertical light attenuation and thus concentrated warming of surface waters (Dale & Gillespie, 1977). A high density of macrophytes also leads to strong dissipation of wind-driven turbulence (Herb & Stefan, 2005). In ponds with a dense charophyte cover, high shoot density promotes the formation of vertical temperature and chemical gradients (Andersen, Kragh, & Sand-Jensen., 2017; Andersen, Sand-Jensen et al., 2017). Thus, oxygen often becomes highly supersaturated at the surface of charophyte canopies during daytime, while bottom waters with dim light simultaneously develop hypoxia or anoxia (Andersen, Kragh, & Sand-Jensen., 2017; Andersen, Sand-Jensen et al., 2017). The lack of oxygen influences chemical processes and challenges animals living in the sediment or firmly attached to basal macrophyte tissue which must tolerate anoxia, while mobile animals might migrate to the pelagic zone or move above the canopy to more suitable environmental conditions. Surface cooling during nighttime usually induces vertical mixing of oxygen-rich surface water with oxygen-depleted bottom water, removing the vertical oxygen and other physicochemical gradients (Holgersen et al., 2016).

In ponds with dense charophyte cover, dissolved inorganic carbon (DIC) follows an inverse diel course to oxygen with gradual depletion in surface waters that may lead to afternoon depressions of photosynthesis at low CO₂ and elevated pH (Kragh et al., 2017). Charophytes and many angiosperms continue to photosynthesize, though at lower rates, by using bicarbonate, assimilating CO₂ and precipitating CaCO₃ on their surfaces (i.e., Ca²⁺ + 2 HCO₃⁻ + H₂O → CO₂ + CaCO₃ + O₂; McConnaughey (1991)). At high pH and CO₃²⁻ concentrations in the water column, calcification can also occur in the water column when the solubility of Ca²⁺ and CO₃²⁻ is greatly exceeded (Müller et al., 2016). Thus, calcification increases the DIC decline and reduces the photosynthetic quotient between O₂ and DIC (Sand-Jensen et al., 2018). At the same time, alkalinity is reduced by two equivalents for every CO₂ molecule assimilated, while alkalinity remains constant and pH increases when no carbonate precipitation takes place (i.e., HCO₃⁻ + H₂O → CO₂ + OH⁻ + O₂; Sand-Jensen et al. (2018)). DIC and calcification rates can be calculated from continuous recordings of alkalinity, pH, and temperature (Andersen et al., 2019), and lake metabolism from diel changes in DIC, when accounting for precipitation-dissolution of CaCO₃ and the chemically enhanced CO₂ exchange with the atmosphere (Peeters et al., 2016). However, gross primary production (GPP), ecosystem respiration (R), and net ecosystem production (NEP) are often estimated solely using oxygen, while using DIC in addition to oxygen could provide improved insights on ecosystem metabolism (Torgersen & Branco, 2007).

The earlier, traditional perception that ponds and shallow lakes are vertically well-mixed is not always valid (Holgersen et al., 2022). Consequently, the spatiotemporal variability of environmental conditions in the littoral zone has not received proper attention. Because ponds have recurring stratification-mixing dynamics and associated chemical gradients, we propose that similar conditions may develop in dense macrophyte stands in the littoral zone of larger, shallow lakes, where standard measurements at deeper stations in the pelagic zone show permanent mixing. The development of vertical gradients of temperature and oxygen through dense, extensive stands of *Potamogeton crispus* in a shallow Australian lake supports this proposition (Vilas et al., 2017, 2018). We used small, continuous data-logging sensors to measure light, temperature, oxygen, pH, and conductivity in the littoral zone of a north temperate, small, shallow, mesotrophic lake during spring before macrophyte development and during summer with dense macrophyte canopies. We test the overall hypothesis that physicochemical conditions are markedly different between the littoral vegetated zone and the open pelagic waters where measurements traditionally have been conducted. Specifically, our hypotheses were: (1) the littoral macrophyte community at high biomass density during summer experiences recurring daytime temperature stratification and nocturnal mixing; (2) spatiotemporal variations in dissolved oxygen and DIC are closely coupled to metabolism and mixing regime; and (3) when CO₂ exchange with the atmosphere and precipitation-dissolution of CaCO₃ are accounted for, ecosystem metabolism can be determined using both oxygen and DIC to reveal the potential contribution of CO₂-generating anaerobic processes.

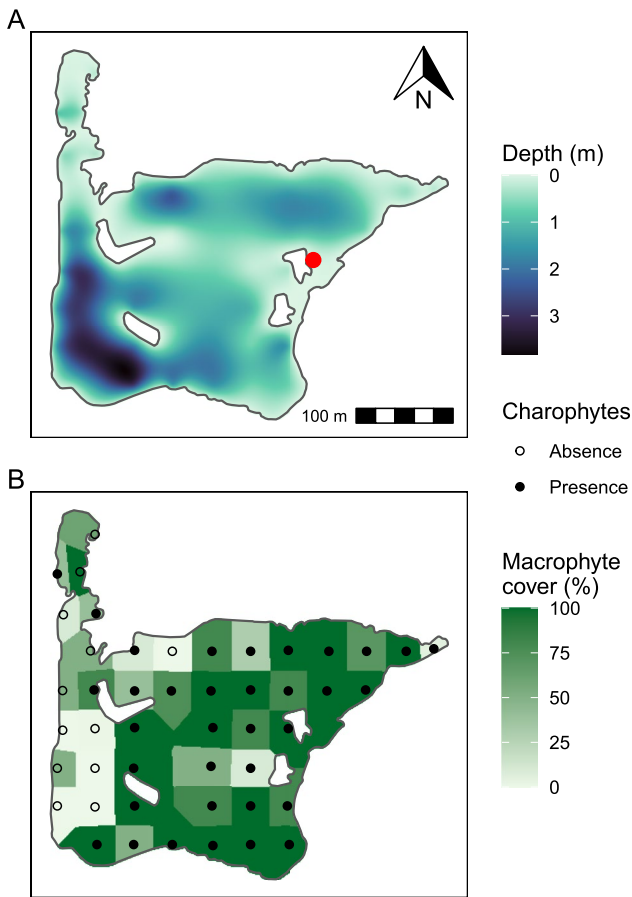


Figure 1. (a) Bathymetric map of Lake Nymølle. The red point shows the location of the littoral zone site. (b) Summer submerged macrophyte cover (0%–100%) in Lake Nymølle at 47 locations (points). Solid points mark the presence of charophytes.

2. Methods

2.1. Study Lake

Lake Nymølle (55.6277°N, 12.1551°E), Denmark, is a small (7.1 ha) and shallow (0.9 and 3.5 m, mean and maximum depth) groundwater-fed lake (Figure 1a). It is located in a former sand and gravel pit abandoned around 2003 and transformed into a nature and recreational park. The lake has transparent, alkaline water and calcareous mineral sediment. The lake is dominated by submerged macrophytes with a negligible cover of floating-leaved or emergent macrophytes. We collected high-frequency measurements of vertical physicochemical dynamics in a charophyte stand (littoral zone) during summer 2019, when the stand was dominated by *Chara globularis* and *Chara virgata*, and in spring 2020 before charophyte development. Furthermore, we collected water samples and vertical profiles at the deepest part of the lake (pelagic zone) and performed a lake-wide inventory of submerged macrophytes.

2.2. Measurements in Pelagic Zone

Water samples and measurements of Secchi depth and vertical profiles were collected at midday every month from May through September 2019, November 2019, and February 2020. Depth profiles of temperature, dissolved oxygen, and pH were measured for every 0.5-m depth increment (YSI Multiprobe, Yellow Springs, Ohio, USA). As water column stratification was negligible, water samples retrieved from the surface, mid-depth, and above the bottom were mixed. Measurements of alkalinity, total nitrogen, total phosphorus, and chlorophyll *a* were conducted according to standard methods (Egemoose et al., 2020). Dissolved calcium was measured in September 2019 by EDTA titration (FBL Methods, 1992).

2.3. Submerged Macrophyte Survey

In late June 2019, we conducted a lake-wide inventory of submerged macrophytes and their coverage. In 47 regularly distributed sample points, we measured depth, identified species, measured canopy height and visually estimated the cover of each species and the total cover in an area of approximately two by two meters. Sampling was conducted from a boat using either a bathyscope or, at greater depths, a rake. The applied method is similar to that used in the national monitoring program (Johansson & Lauridsen, 2014). We created a bathymetric map using thin-plate spline interpolation and a map of total macrophyte cover, canopy height and water depth above the canopy using nearest-neighbor interpolation (Douglas et al., 2021; Hijmans, 2022).

2.4. Measurements in the Littoral Zone Site

In the middle of a 20 m wide littoral macrophyte belt and about 15 m away from deeper water (>2 m; Figure 1a), we deployed high-frequency sensors to measure water temperature, light, oxygen, pH, and specific conductivity every 10 min. Measurements were performed during summer 2019 (water depth = 0.66 m) and spring 2020 (water depth ranging from 0.57 to 0.67 m). Sensors were mounted at different depths on thin steel pegs pushed into the lake bottom. Water temperature and light (lux) were measured using 3–5 sensors (HOBO UA-002-64, Onset Computers, Bourne, Ma, USA) distributed vertically through the water column. Dissolved oxygen was measured in two or three depths (MiniDOT, PME Vista, CA, USA) and calibrated at 100% air saturation. pH was measured in one depth (pHTemp2000 MadgeTech data logger with an Omega pH electrode) and calibrated at pH 4.0 and 7.0. Specific conductivity (HOBO U24-001) was measured at two or three depths. Above (0.5 m) the water surface, we measured wind speed (HOBO S-WSET-A). Unfortunately, wind data were only logged during a short period (27 April–4 May 2020) due to instrument failure. Instead, this data was obtained from a weather

station (DMI, 2022) located 18.4 km away. We found a significant linear relationship between wind speed at the weather station ($wind_{DMI}$) and the lake site ($wind_{lake}$) specified as a linear model with a squared term and without an intercept (reported as the estimate (\pm S.E.)); $wind_{lake} = 0.38(\pm 0.02) \cdot wind_{DMI} - 0.02(\pm 0.003) \cdot wind_{DMI}^2$; $R^2 = 0.68$, p -value < 0.001) which was used to predict wind speed at the littoral site throughout the period. During summer 2019, we estimated the vertical light attenuation coefficient (K_d) each day in the charophyte stand as the slope of \log_e (lux) versus depth as described in Martinsen, Kragh, et al. (2022). In summer 2019, macrophyte biomass (as dry weight at 105°C) was determined from three replicate samples (30 × 30 cm), while stand height was measured at seven points.

2.4.1. Dissolved Inorganic Carbon

To obtain a continuous time series of DIC, we used the relationship between alkalinity and specific conductivity in Lake Nymølle to calculate alkalinity from continuous measurements of specific conductivity, which, in combination with measurements of pH, enabled the determination of DIC at high temporal resolution. The relationship between alkalinity and specific conductivity was obtained through dilution series with water from Lake Nymølle and demineralized water in 2019 and 2020 (Table S1 in Supporting Information S1). The alkalinity of the water was determined by acidimetric titration (Gran, 1952). Finally, DIC was calculated from pH, alkalinity, and water temperature using the *AquaEnv* R-package (Hofmann et al., 2010). Bicarbonate and calcium are by far the dominant anions and cations, respectively, and the main contributors to the specific conductivity. Both ions are simultaneously removed or re-dissolved if $CaCO_3$ precipitation and dissolution take place ($2 HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3$). Thus, we regard our procedure as satisfactory because the contribution of other dissolved ions to changes in the specific conductivity due to metabolism and $CaCO_3$ precipitation and dissolution is minor.

2.4.2. Ecosystem Metabolism

We estimated ecosystem metabolism comprising GPP, respiration (R), and net ecosystem production (NEP = GPP-R) each day using diel changes in oxygen (GPP_{oxygen} and R_{oxygen}) and DIC (GPP_{DIC} and R_{DIC}) from the sensor just above the charophyte canopy. We determined these components using an inverse modeling approach based on maximum likelihood estimation (Solomon et al., 2013). The calculation is described in detail in Martinsen et al. (2017). In short, diel changes in O_2 and DIC are modeled as:

$$O_{2-t1} = O_{2-t0} + GPP_{oxygen} - R_{oxygen} + F + \epsilon \quad (1)$$

$$DIC_{t1} = DIC_{t0} - GPP_{DIC} + R_{DIC} + F + C + \epsilon \quad (2)$$

where O_{2-t1} , O_{2-t0} , DIC_{t1} , and DIC_{t0} are oxygen and DIC at consecutive time steps (10 min), F is the gas flux of O_2 and CO_2 across the air–water interface, and ϵ is an error term. GPP was modeled as a function of light (Jassby & Platt, 1976):

$$GPP = P_{max} * \tanh\left(\frac{P_{alpha} * lux}{P_{max}}\right) \quad (3)$$

where P_{max} is the maximum photosynthesis at light saturation and P_{alpha} is the initial slope. We modeled GPP as a non-linear function of light, which improved model fits (data not shown) compared to a linear GPP-light relationship. Christensen et al. (2013) found the same improvement when modeling ecosystem metabolism in a small charophyte-dominated lake and also that this was in agreement with shoot incubations at different light levels. R was modeled as a temperature-dependent process (Jørgensen & Bendoricchio, 2001):

$$R = R_{20} * 1.073^{(temp-20)} \quad (4)$$

where R_{20} is the respiration rate at 20°C, temp is the water temperature, and 1.073 is a standard value of temperature dependence equivalent to a Q_{10} of 2. The O_2 or CO_2 flux across the air–water interface was calculated as:

$$F = d * \alpha * k * (G_{sat} - G_{obs}) / z_{mix} \quad (5)$$

where G_{sat} and G_{obs} are the gas concentration at atmospheric saturation and actual concentration, respectively, d is a dummy variable indicating whether the sensor is above (1) or below (0) the thermocline (z_{mix}), k is the gas transfer velocity adjusted from k_{600} to in situ water temperature using Schmidt numbers (Jähne et al., 1987; Raymond et al., 2012). k_{600} was determined from wind speed using the mean of three empirical relationships

(Cole & Caraco, 1998; Crusius & Wanninkhof, 2003; Schilder et al., 2016). The chemical enhancement factor, α , was calculated using the theoretical model of Hoover and Berkshire (1969) as described in Wanninkhof and Knox (1996). Chemical enhancement is only relevant for CO₂ exchange and not O₂. The depth of the mixed layer Z_{mix} was determined from the temperature profiles using the *rLakeAnalyzer* R-package (Read et al., 2011).

A model of k is needed to quantify air–water gas exchange at each time step for accurate estimates of ecosystem metabolism (Equation 5). Applying existing empirical models developed in other systems can introduce error due to the influence of lake size and shape on k (Vachon & Prairie, 2013), for example, markedly different influence of wind on k between open water and near-shore sites (Schilder et al., 2016). Overestimation of k can thus bias estimates of net ecosystem metabolism, and for air–water exchange of CO₂, underestimating k may result in artificially estimates of high chemical enhancement factors as k determines the boundary layer thickness (Wanninkhof & Knox, 1996). We used the average of three empirical models developed in small lakes to reduce the potential error due to lake-specific characteristics and choice of methods. Furthermore, the generally high metabolic rates (Hoellein et al., 2013) in our study reduce the relative influence of these potential sources of error as the profound diel changes ensure a strong signal for modeling.

In addition to chemical enhancement, the DIC metabolism model also differs from that of O₂ model by including a term for calcification/dissolution processes (C) calculated from changes in alkalinity:

$$C = (\text{alkalinity}_{t1} - \text{alkalinity}_{t0}) * 0.5 \quad (6)$$

This stoichiometric relationship has been verified by coupled measurements of changes in alkalinity and calcium concentrations (Sand-Jensen et al., 2018, 2021). The model parameters P_{max} , P_{alpha} , and R_{20} were estimated for each day by minimizing the negative log-likelihood. Model fits were evaluated using Spearman rank correlation between observed and predicted values. Days with poor fits (2 days in 2020 spring data) or unrealistic parameter estimates (3 days in 2020 spring data) were discarded.

2.5. Statistics

GPP and R estimated using both oxygen and DIC were compared using standardized major axis regression (model II regression) in the *lmodel2* R-package (Legendre, 2018) because errors are present in both the response and predictor variables. The same approach was used to describe the relationship between R_{20} and GPP_{20} , which are the metabolic rates normalized to 20°C using Equation 4 and diel mean water temperature. Diel patterns in pH, DIC components, and the calcification rate were visualized using generalized additive models with a cyclic cubic spline smoother using the *mgcv* R-package (Wood, 2021). All data analysis and statistics were carried out using R (R Core Team, 2022).

3. Results

3.1. Physicochemical Dynamics in Pelagic Waters

In the deepest part of Lake Nymølle, the temperature gradient between the surface (0.25 m) and bottom (3 m) was small in monthly measurements from May to September (mean = 0.26, range = 0.07–0.5°C m⁻¹; Figure 2). Dissolved oxygen in surface waters was supersaturated at noon (mean = 128% and range = 110%–159%), while pH averaged 8.2 (range = 7.9–8.7) and CO₂ concentrations were close to air saturation (mean = 16.6 and range = 2.7–38 μmol L⁻¹). Depth gradients were negligible at all measurements except in June, when moderately lower temperature (surface = 22.3°C and bottom = 20.8°C) and markedly lower oxygen concentration (surface = 139% and bottom = 8%), and pH (surface = 7.9 and bottom = 7.2) occurred in the bottom waters compared to surface waters. Mean alkalinity in the water column was 1.7 (range = 0.92–2.9) meq L⁻¹ (Table S2 in Supporting Information S1). In September, the calcium concentration was 1.57 meq L⁻¹. Concentrations of total P, total N, and chlorophyll *a* were relatively low, corresponding to a mesotrophic, clear lake with the Secchi-disc often being visible at maximum depth (Table S2 in Supporting Information S1). Mean summer (June–September) chlorophyll *a* was 5.0 mg m⁻³ (±3.1 SD).

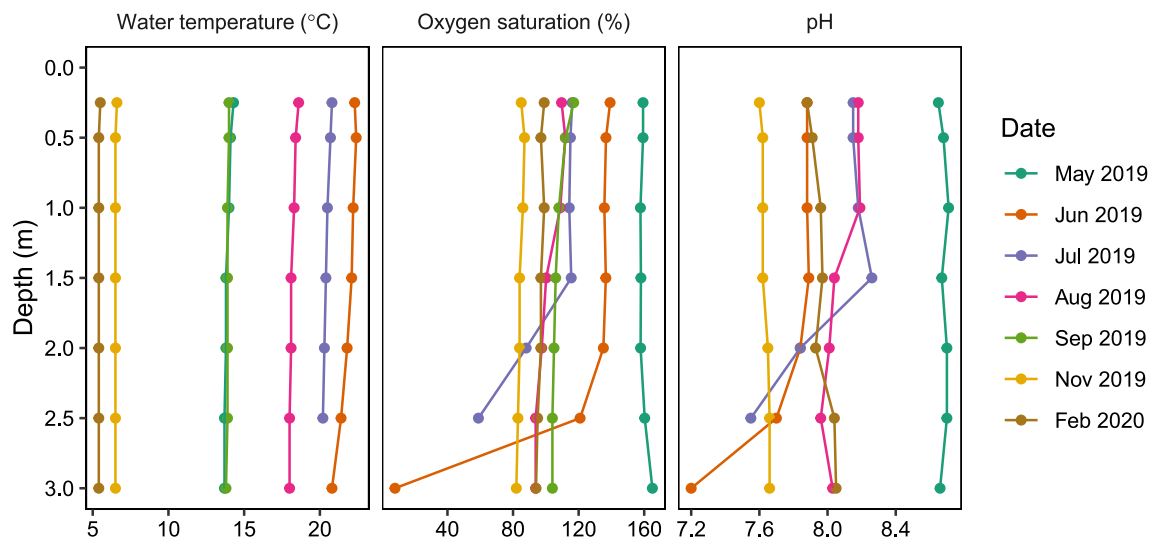


Figure 2. Pelagic zone vertical profiles (y-axis, m) of water temperature, oxygen, and pH (x-axis) on seven occasions in the pelagic zone in the deepest part of Lake Nymølle.

3.2. Submerged Macrophyte Inventory

The submerged macrophyte cover was well-developed throughout the lake, reaching the maximum depth of 3.0 m with a thin, scattered cover. Seven charophyte species and 10 angiosperm species or taxa were present (Table S3 in Supporting Information S1). Total cover of all macrophyte species and for charophytes alone were 69% and 61% respectively. The two most widespread species, *Chara vulgaris*, and *Chara virgata* were found at 24 and 16 of the 47 sampling sites across the lake (Figure 1b; Table S3 in Supporting Information S1). The same two species also dominated at the littoral site, where the dense stands (Figure 1c) had mean summer biomass of 1,231 g DW m⁻² (± 171 SD) and a mean height of 36 cm (± 4 SD).

3.3. Physicochemical Dynamics in the Littoral Zone

3.3.1. Light Conditions

During summer, light decreased steeply through the canopy, with a mean attenuation coefficient of 11 m⁻¹ (range = 7–12 m⁻¹). In the pelagic waters, the light attenuation coefficient was less than 0.7 m⁻¹ (estimated from Secchi-depth observations assuming that 10% of subsurface light reaches the Secchi-disc; Kirk (1994)).

3.3.2. Water Temperature

The water column in the shallow littoral zone warmed up during the daytime and cooled down during the nighttime. In spring, the mean daily water temperature increased from 5°C in early March to 15.2°C in early May, while it decreased from 24.5°C in late August to 15.2°C in mid-September (Figure 3). In spring, the water column was fully mixed with no or negligible vertical temperature gradients (Figure 3c). In summer, a recurring diel pattern with daytime stratification and nocturnal mixing of the water column developed on most days. On 27 August, the temperature at 0.08 m below the surface reached a daytime maximum of 26.2°C, while simultaneously, the temperature at 0.49 m below the surface within the macrophyte canopy was 20.7°C, corresponding to a vertical gradient of 13.4°C m⁻¹. The daytime vertical gradient usually disappeared by nighttime due to surface water cooling, which drove the dynamic pattern of daytime stratification and nocturnal mixing because surface water cooling induces convective mixing (Figure 3b). Mean and maximum predicted wind speeds were low in both summer (1.4 and 2.7 m s⁻¹) and spring (1.7 and 2.7 m s⁻¹) highlighting the influence of surface cooling. On 3 days in late August, the water column stayed permanently stratified day and night, while nocturnal mixing occurred on all other days, showing higher daytime and lower nighttime surface temperatures compared to the temperature course deeper into the canopy.

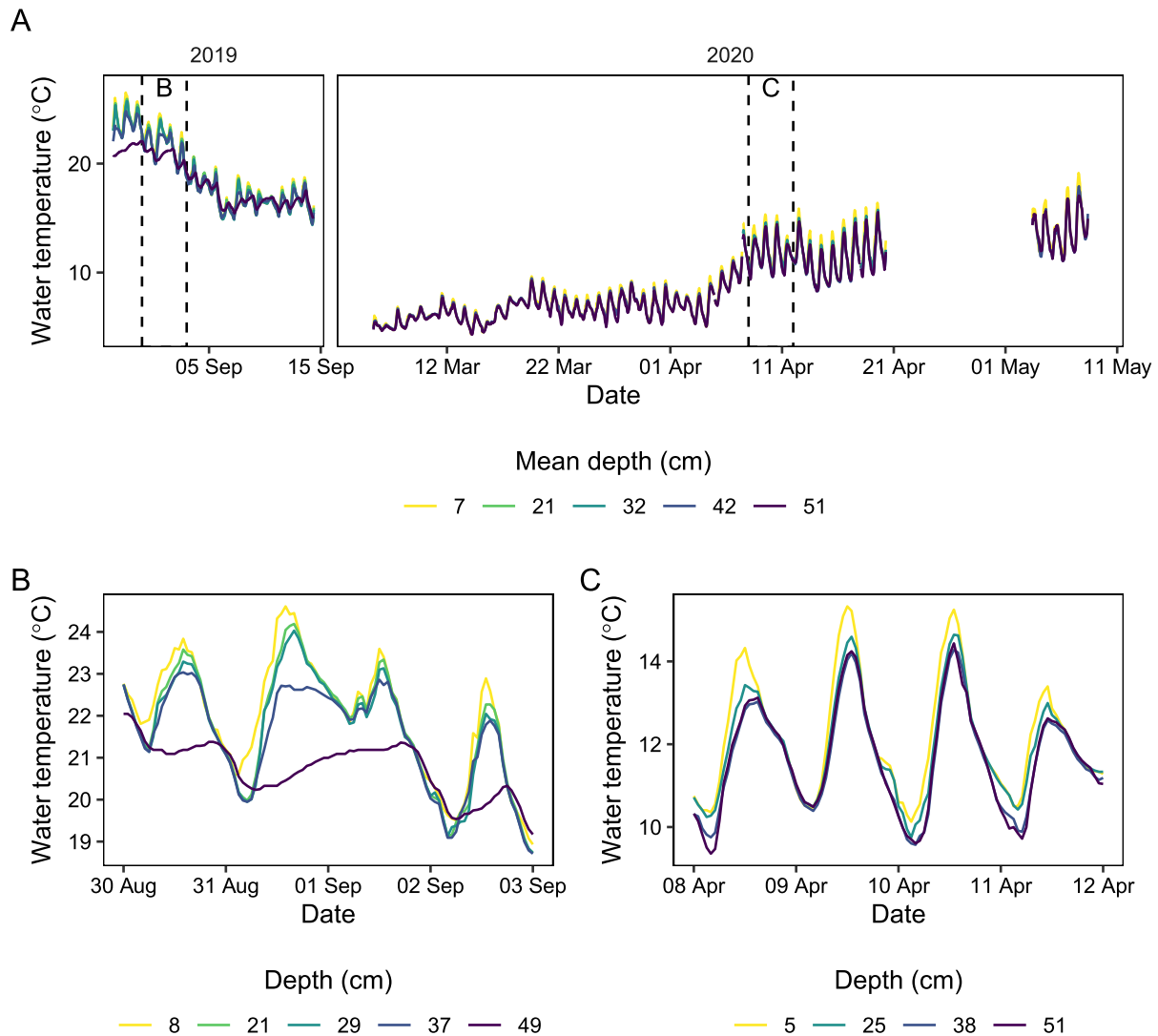


Figure 3. Water temperature dynamics in Lake Nymølle at four to five depths in the shallow littoral zone during summer 2019 and spring 2020 (a). Insets show the diel water temperature dynamics on 4 days in 2019 (b) and 2020 (c) in the periods denoted by the stippled lines. The macrophyte canopy was dense in August–September 2019 and very sparse in March–early May 2020.

3.3.3. Dissolved Oxygen

In spring, vertical differences in oxygen saturation were very small (mean = 0.3%, range = -10.3%–15.4%) and very similar at all water depths (0.09, 0.27, and 0.46 m) in the littoral zone throughout the measuring period (Figure 4). The lack of vertical oxygen gradients is expected, as vertical differences in water temperature were negligible, indicating full vertical mixing in spring. The oxygen saturation increased during the day and declined during the night, with a minimum–maximum range of 90%–173% for the entire period (Figure 4c). Mean daily oxygen concentrations were mostly supersaturated (114% for the entire spring), indicating higher GPP relative to ecosystem respiration.

In summer, oxygen was measured just above the canopy and sediment surface. The diel oxygen course varied greatly between the two depths (Figure 4). During the daytime, dissolved oxygen increased rapidly (maximum 177%) in the upper canopy due to high photosynthesis. In contrast, the oxygen concentration was low, often zero, in dim light (approx. 1.5% of surface light) close to the sediment due to low photosynthesis relative to respiration in the stratified water column (Figure 4b). During most nights, oxygen concentrations increased in bottom waters and decreased in the upper canopy, attaining the same level due to efficient convective mixing. On some nights,

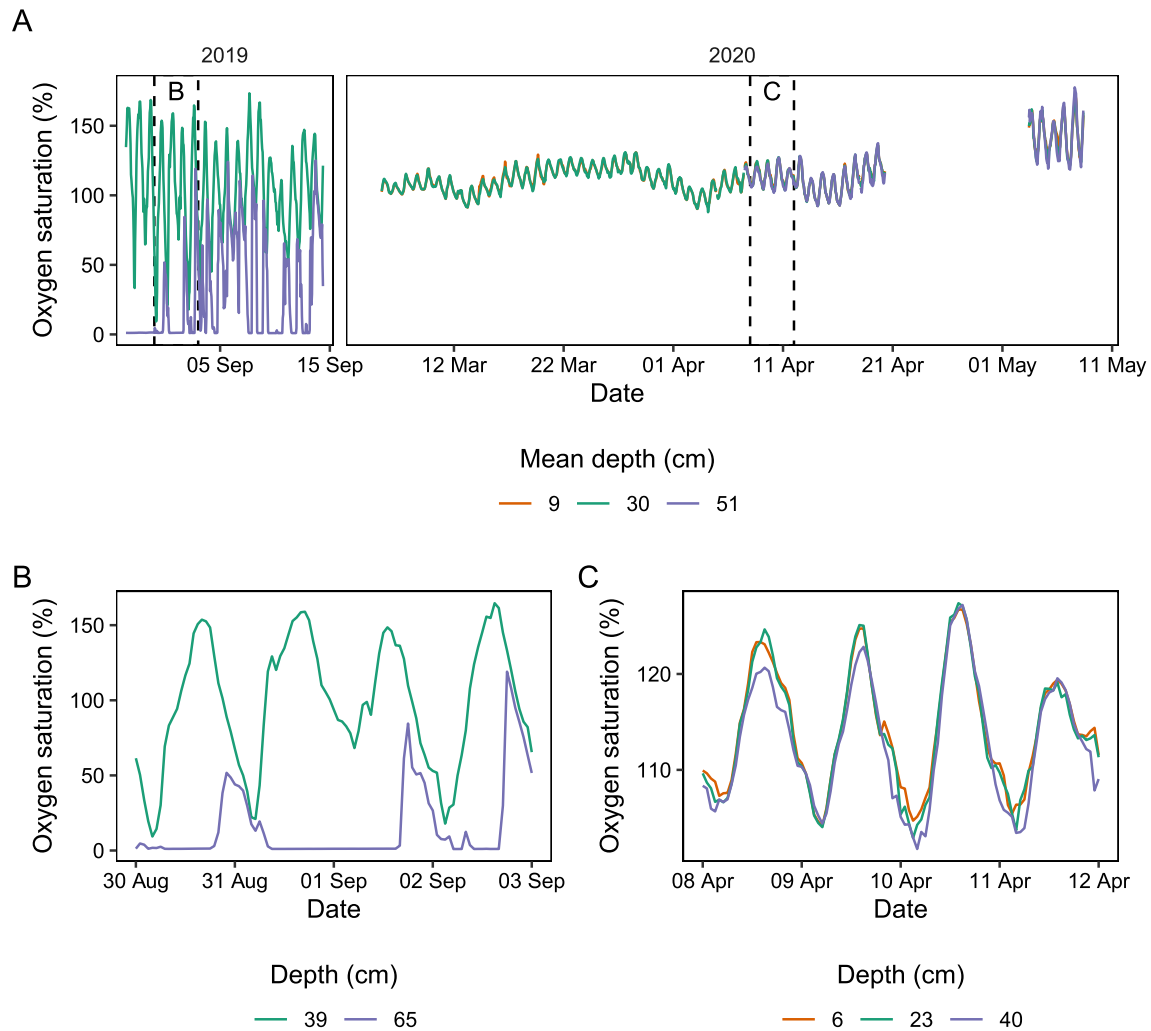


Figure 4. Dissolved oxygen dynamics in Lake Nymølle at two to three depths in the shallow littoral zone during summer 2019 and spring 2020 (a). Insets show the diel dissolved oxygen dynamics on 4 days in 2019 (b) and 2020 (c) in the periods denoted by the stippled lines. The macrophyte canopy was dense in August–September 2019 and very sparse in March–early May 2020.

however, the water column remained stratified and induced anoxia near the sediment that could last for several days. This was the situation during warm nights with insufficient cooling of surface waters and weak convective mixing. Mean oxygen concentration in the upper canopy for the entire period was 105%.

3.3.4. pH and DIC

pH and DIC followed regular diel patterns just above the charophyte canopy in summer (Figure 5). Diel variations of pH, averaged over the entire period, showed a steep increase from morning to noon, a plateau in the afternoon (around 15 o'clock), followed by decreasing pH during nighttime (Figure 6). Concentrations of CO_2 , HCO_3^- , and DIC followed a corresponding pattern, with concentrations declining steeply before noon with the fastest rate already at 9–10 o'clock, reaching a plateau at 15–16 o'clock and then increased during nighttime. The CO_2 decline was particularly steep in the early morning, and after 9 o'clock the CO_2 concentration was below $3 \mu\text{M}$ for the rest of the day. The calculated calcification rate peaked at 9–10 o'clock, when DIC declined most rapidly, and was zero at 15–16 o'clock, when carbonate concentrations were highest. This implies that calcification on the charophyte surfaces is directly related to photosynthesis and not to carbonate precipitation in the water column. Mean integrated calcification (daytime period) was 0.021 mM (range = -0.006 – 0.067) or 6.3% of the mean DIC decline at 0.33 mM (range = 0.15 – 0.48).

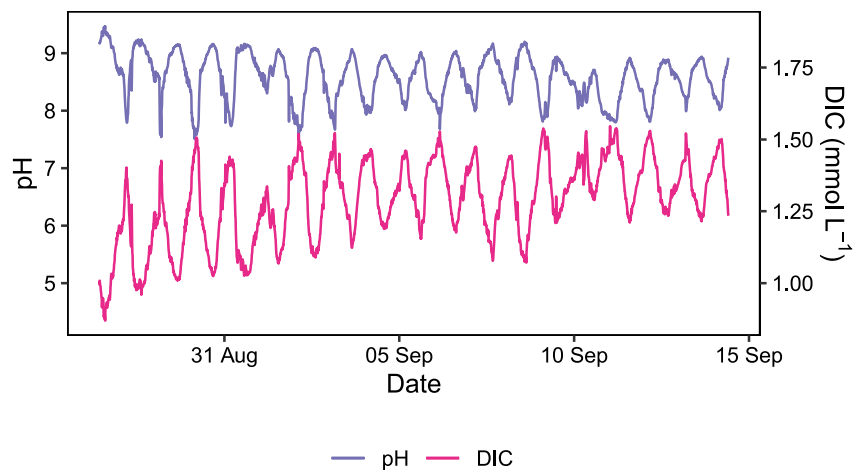


Figure 5. DIC and pH dynamics immediately above a dense charophyte canopy in the shallow littoral zone in Lake Nymølle in summer 2019. pH was measured at a high frequency (10 min), and DIC (secondary y-axis) was estimated from pH, alkalinity, and water temperature. Alkalinity was determined from continuous high frequency (10 min) measurements of specific conductivity.

3.3.5. Ecosystem Metabolism

The daily GPP and R calculated from diel changes of oxygen concentrations were low in the shallow littoral zone in spring and about 6-fold and significantly higher in summer (*t*-test, $p < 0.001$; Table S4 in Supporting Information S1), reflecting higher summer temperature and development of the dense charophyte biomass. The charophytes were the dominating primary producers, while microbenthic and filamentous algae developed quickly and contributed periodically in late spring before charophytes took over. Metabolic rates decreased from late August to early September and onwards, along with lower incoming light and temperature (Figure 7). Daytime NEP was positive on 74% of the days in spring and 53% of the days in summer.

Daily GPP, R, and NEP in summer calculated from diel DIC changes, CO_2 exchange with the atmosphere, and CaCO_3 precipitation and dissolution were of the same molar magnitude and closely followed the same course from day-to-day as metabolism based on diel oxygen changes. Thus, regression analysis (model II regression reported as estimate (95% CI); slope = 0.99 (0.94–1.05), intercept = -24.8 (-27.2 – -22.6)) showed a close 1:1 relationship between GPP, R, and NEP based on diel changes in oxygen and DIC with no significant differences for GPP (paired *t*-test, $p = 0.08$) and R ($p = 0.4$) while NEP was slightly higher ($p < 0.001$) when derived from DIC compared to oxygen (Figure 8a). The mean photosynthetic ($\text{PQ} = \text{O}_2$ produced/ CO_2 consumed) and respiratory ($\text{RQ} = \text{CO}_2$ produced/ O_2 consumed) quotients were 0.94 (0.86–1.01 95% CI) and 0.97 (0.84–1.09 95% CI) respectively. Daily R and GPP normalized to 20°C also showed a linear relationship close to 1:1 for both oxygen and DIC during summer (Figure 8b; Table S5 in Supporting Information S1). However, later in September, R exceeded GPP, probably reflecting the beginning senescence of the charophytes.

4. Discussion

4.1. Stratification and Mixing Patterns

Daytime stratification and nocturnal mixing occurred regularly in Lake Nymølle's littoral zone during summer. Daytime warming of the surface water established a vertical temperature gradient (1.5 – $13.8^\circ\text{C m}^{-1}$), being steepest in late August and early September, when incoming light and temperatures were highest. In comparison, the vertical temperature gradient at noon in the pelagic zone was weak from May to September (0.07 – 0.5°C m^{-1}). Earlier studies have shown that a dense cover of submerged macrophytes in ponds and small lakes (e.g., surface area < 1 ha) and the littoral zone of larger lakes can induce strong temperature gradients by concentrating light absorption and warming in the upper canopy, and simultaneously dissipating turbulent energy and mixing (Dale & Gillespie, 1977; Lövested & Bengtsson, 2008). During summer in Lake Nymølle, the charophyte stand was 0.36 m tall, and the high biomass density ($1,230 \text{ g DW m}^{-2}$) is known to promote thermal stratification through high light attenuation and dampened turbulence (Martinsen et al., 2017). The results confirm that daytime

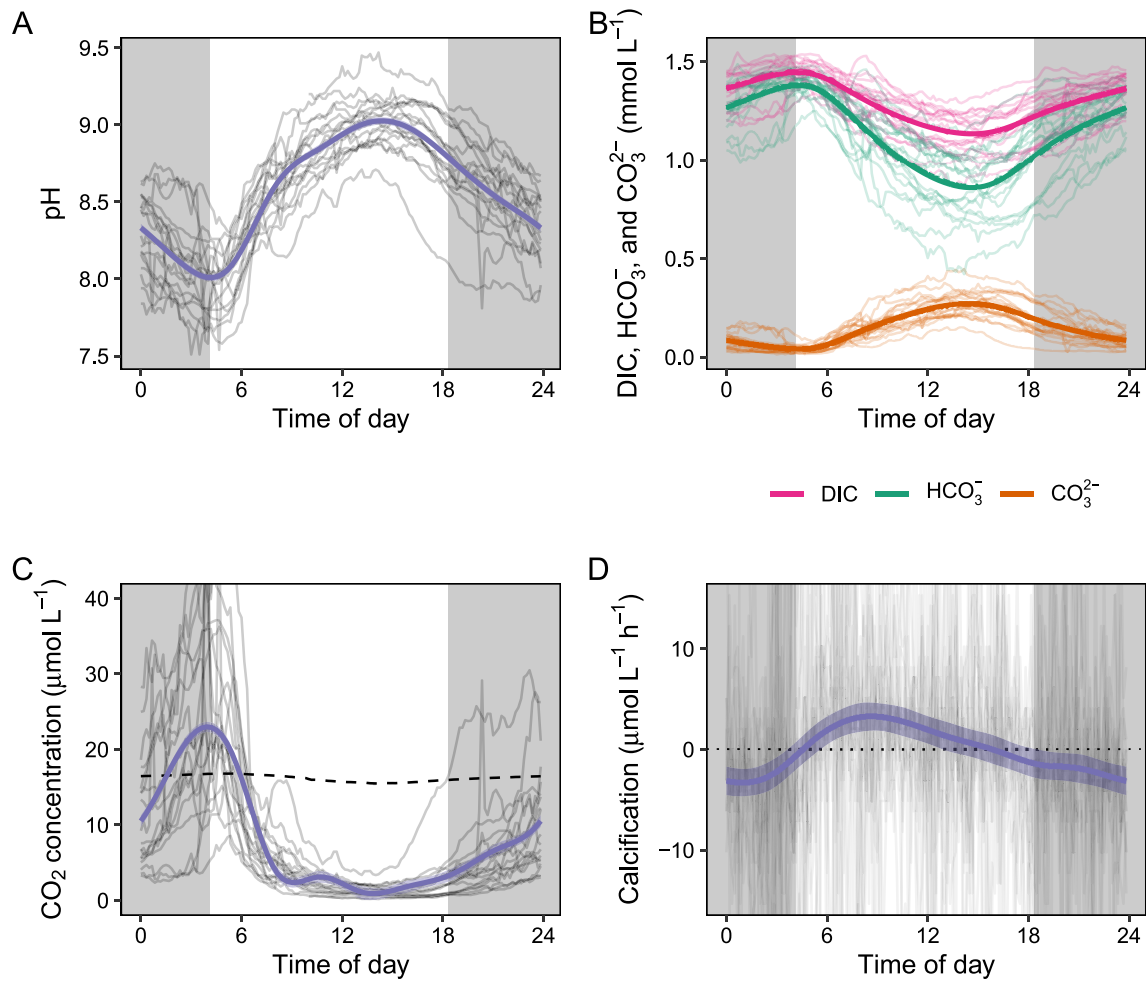


Figure 6. Average diel patterns during summer in pH (a), DIC, HCO₃⁻, and CO₃²⁻ concentration (b), CO₂ concentration (c), and calcification rate (d) in the littoral zone above the charophyte canopy. In C, the stippled line shows the CO₂ concentration at air saturation. High-frequency data (10 min) are shown as thin solid lines. Diel patterns are modeled using generalized additive models with a cyclic cubic spline smoother, and shaded regions are 95% CI. Gray boxes show nighttime periods.

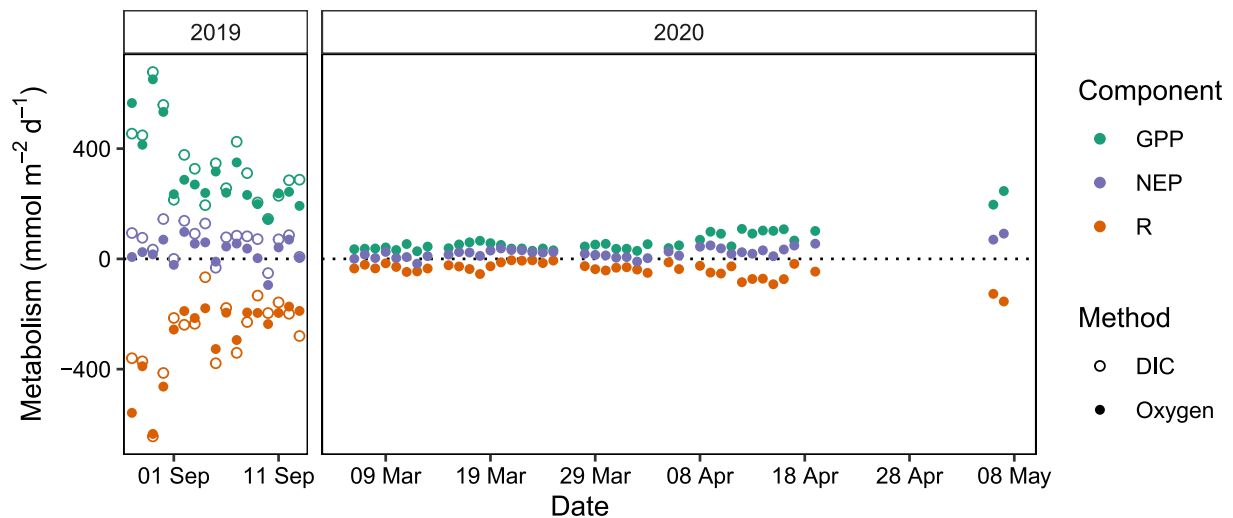


Figure 7. Areal rates of ecosystem metabolism (GPP, R, and NEP) in the littoral zone in Lake Nymølle estimated from diel changes in oxygen (closed point) and DIC (open point). The charophyte canopy was dense in August–September 2019 and very sparse in March–early May 2020.

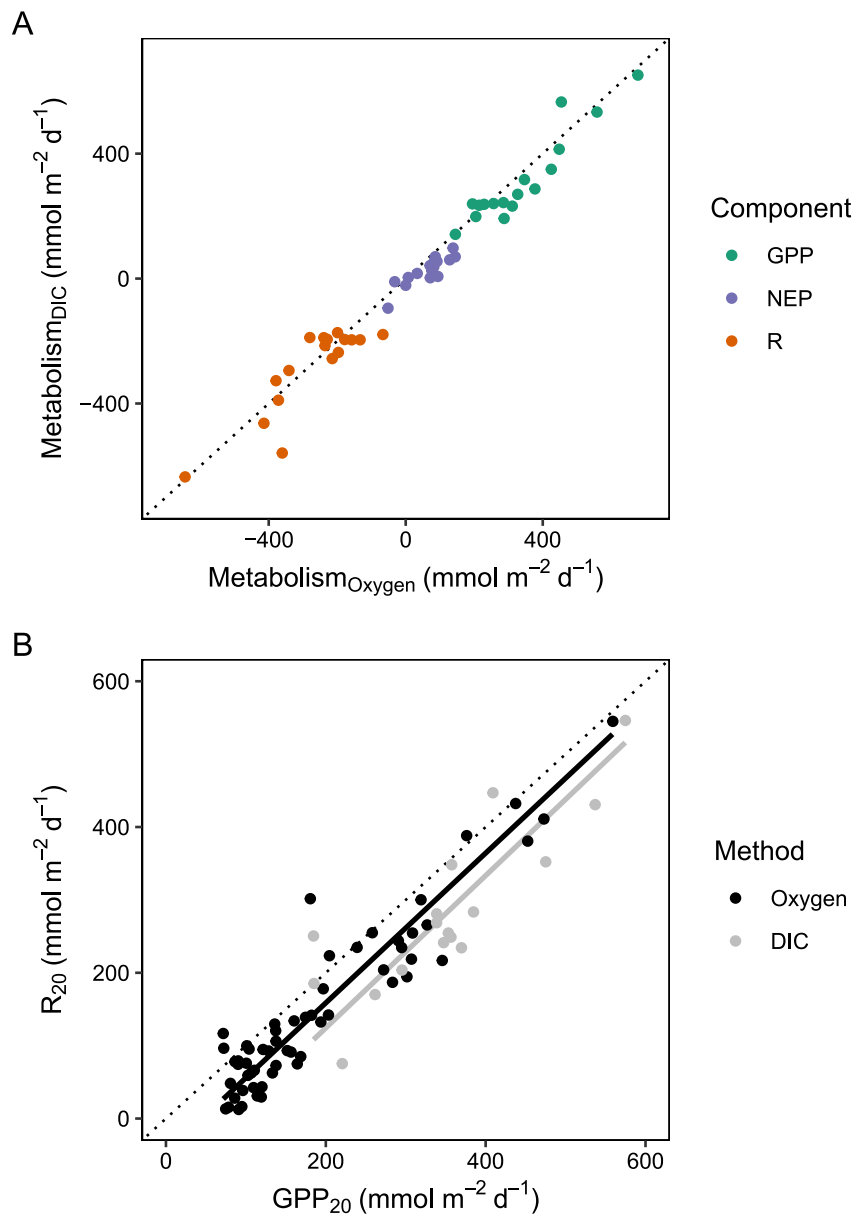


Figure 8. (a) Relationship between ecosystem metabolism in charophyte canopy in the littoral zone in summer estimated from diel changes in oxygen (x-axis) and DIC (y-axis). The dotted line is the 1:1 line. (b) Relationship between R and GPP normalized to 20°C. Lines are standardized major axis regression fits (model II regression).

stratification and nocturnal convective mixing take place in dense littoral stands of macrophytes in Lake Nymølle, supporting hypothesis 1 and suggesting that this may be a common feature in the littoral zones of larger lakes (e.g., surface area >10 ha) even at lesser macrophyte densities (Vilas et al., 2017, 2018). The role of surface cooling in water column mixing is very apparent due to the sheltered location of the littoral site and low wind speeds. Littoral zones make up the majority of global lake area (Seekell et al., 2021), further highlighting the potential influence of macrophyte-mediated stratification-mixing patterns in lakes.

Thermal stratification affects the vertical distribution of gases and solutes, since it prevents or reduces vertical exchange between surface and bottom waters (Boehrer & Schultze, 2008). During summer in Lake Nymølle's littoral macrophyte stand, daytime photosynthesis caused surface waters to become oxygen-supersaturated, while, simultaneously, respiration dominated in bottom waters, resulting in hypoxia or anoxia. The environmental conditions in the littoral zone, with pronounced differences in oxygen saturation across short vertical distances, was in

stark contrast to the conditions measured in spring before macrophytes appeared and at the pelagic site with no macrophytes, where oxygen was distributed more evenly. During summer in the littoral zone, vertical gradients of temperature and oxygen disappeared on most nights, due to convective mixing. In the same period, however, the water column remained stratified for consecutive days with warm nights, inducing long-term anoxia in bottom waters. The extensive anoxia was probably a consequence of stronger daytime stratification and warmer nights with lower temperature differences between air and water, resulting in insufficient convective mixing. The same summer patterns appear in dense charophyte stands in ponds (Andersen, Kragh, & Sand-Jensen, 2017; Andersen, Sand-Jensen et al., 2017), supporting hypothesis 2. Thus, the basal green parts of charophytes in bottom waters, as well as their rhizoids in surface sediment, must be able to endure anoxia for consecutive days when vertical mixing fails. Furthermore, mobile animals likely have to seek more suitable micro-habitats. Mass movements of large snails out of anoxic charophyte canopies have been observed in ponds (Sand-Jensen et al., 2019) and other macroinvertebrates may perform similar diel migrations (Marklund et al., 2001). Fleeing behavior in response to anoxia or other steep chemical gradients are likely to occur in dense littoral macrophyte stands on warm, calm nights when vertical convection fails. Thus, littoral macrophyte stands are attractive to attached and mobile animals due to the surfaces, food and shelter they offer (Burks et al., 2002), while occasionally posing a severe risk due to oxygen depletion during warm periods (Vilas et al., 2017).

4.2. Dissolved Inorganic Carbon Dynamics

During summer, diel changes in pH and DIC followed relative stable patterns in the littoral macrophyte canopy with little variation in the diel fluctuations from day-to-day. When averaged over the summer period, the CO₂ concentration dropped rapidly from above air saturation (22 μM) at sunrise to sixfold undersaturation (2.5 μM) already at 9 o'clock and the rest of the day (Figure 6). DIC concentrations also decreased rapidly before noon and became stable at 15–16 o'clock, showing an afternoon depression of photosynthesis (Kragh et al., 2017). Afternoon values of high pH and low CO₂ in Lake Nymølle also suggest inorganic carbon limitation of photosynthesis in the littoral stand, as also observed in experiments with *Chara virgata* (Christensen et al., 2013). High oxygen concentrations in the afternoon may contribute to the reduction of photosynthesis by photorespiration (Van et al., 1976). In contrast, afternoon depression of photosynthesis is unlikely to occur in the pelagic zone where the sparse phytoplankton community cannot induce marked depletion of DIC and CO₂.

The calculated calcification took place before noon and occurred simultaneously with the DIC decrease. The precipitation most likely occurred at the charophyte surfaces linked to the formation of pH polarity with acid zones (pH down to 6) having HCO₃⁻ uptake and alkaline zones (pH up to 10) having CaCO₃ precipitation (McConnaughey, 1991; McConnaughey & Whelan, 1997) and not in the water column in the afternoon despite slightly higher pH and CO₃²⁻ concentrations. The mean calcification rate during summer was modest relative to the decrease of DIC (6.3%). It is known that charophytes prefer to use CO₂ and gradually increase the proportion of calcification, approaching 50%, at high photosynthetic rates, high HCO₃⁻ and low CO₂ concentrations in the water (Sand-Jensen et al., 2018). During summer, the daytime decline of DIC caused by photosynthesis in Lake Nymølle is fully restored during nighttime respiration in the charophyte canopy and the underlying sediment. However, during spring, the build-up of the charophyte biomass with dense carbonate crusts on their surfaces causes a net uptake from the water (Andersen et al., 2019; Herbst et al., 2018). The encrusted carbonate is exported to the calcareous sediment upon charophyte senescence (Sand-Jensen et al., 2021), while DIC and calcium can be replenished by groundwater input and sediment respiration (McConnaughey et al., 1994).

4.3. Ecosystem Metabolism in the Littoral Zone

During summer, when the plant biomass, temperatures, and incoming light are at their highest, high rates of GPP and R drive extensive diel variations in oxygen and DIC, which are much greater than those in the pelagic zone. The contribution of the littoral zone to whole-lake GPP can be estimated using our measurements of GPP in the littoral zone during summer and the lake macrophyte survey. Shallow areas (<1 m) with dense cover of charophytes (75%–100%) similar to our littoral zone site represents 33% of the lake area. Pelagic GPP of phytoplankton is estimated by multiplying: (a) the mean of chlorophyll measurements in summer (June–September), (b) the mean water depth with phytoplankton measured above the macrophyte canopy across the lake, (c) the median light-saturated GPP of phytoplankton (15 mg O₂ mg⁻¹ chl. h⁻¹; Krause-Jensen and Sand-Jensen (1998)), and (d) the measured mean summer day length having high irradiances exceeding 50% of the daily mean expected to

saturate photosynthesis in surface waters. Averaged across the lake, this yields daily GPP of littoral charophyte stands of 3.32 and 0.95 g O₂ m⁻² for phytoplankton (29% of GPP of the littoral charophyte stands). The profound contribution of submerged macrophytes and benthic microalgae to whole-lake processes has also been highlighted by other studies of small shallow oligotrophic-mesotrophic lakes (Brothers & Vadeboncoeur, 2021; Van de Bogert et al., 2012).

Ecosystem metabolism estimates calculated from DIC and oxygen yield two independent measures of lake metabolism and is, as expected, of similar molar magnitude (Hanson et al., 2003; Peeters et al., 2016), supporting hypothesis 3. Obtaining metabolism estimates from both methods simultaneously may have several advantages, nevertheless, only the oxygen method is commonly used, although ecosystem processes are usually calculated in carbon units. Simultaneous estimates of metabolism enables determination of PQ and RQ which otherwise has to be assumed. The quotients depends on the dominating metabolic pathways of the system and provides information on ecosystem functioning. A RQ above 1, that is, biological CO₂ production exceeding O₂ consumption, suggest a contribution from anaerobic processes or the predominant organic substrates (Berggren et al., 2012; Vachon et al., 2020). Similarly, PQ is influenced by calcification-precipitation dynamics which might be particularly important in systems dominated by submerged macrophytes (Sand-Jensen et al., 2018).

One advantage of the DIC approach is the ability to include the anaerobic release of CO₂ without any subsequent oxygen consumption, for example, denitrification, sulfate and ferric-iron reduction accompanied by permanent sedimentation of iron sulfides (Torgersen & Branco, 2007). Seepage of anoxic water rich in sulfide, ferrous-iron and ammonium into littoral plant stands could result in low PQ and high RQ due to oxygen consumption in oxidation of sulfide, ferrous-iron and ammonium. Apparently, these processes had negligible influence in our study, where surface waters above the plant stand remained oxic at all times, ensuring oxidation of reduced iron and sulfur if formed and seepage of anoxic water being minimal. The PQ and RQ generally were close to 1.0 in the littoral zone during the studied summer period suggesting that metabolism primarily involved carbohydrate formation in photosynthesis followed by respiration of the same magnitude of carbohydrates and, thus, negligible net growth in biomass. If extensive net growth takes place, we may anticipate PQ markedly above 1.0 (e.g., 1.4) when nitrate is the main N-source to net growth of plant biomass and PQ close to 1.0 when ammonium is the main source (Laws, 1991). However, it is also more cumbersome to collect the required data for DIC metabolism as pH and conductivity sensors are needed, as well as alkalinity measurements, or, preferably, DIC is measured by advanced semi-automatic collection and acidification of water samples, and infrared gas analyzer measurements of DIC converted to CO₂ (Bass et al., 2012).

4.4. Conclusions

Water temperature, oxygen, inorganic carbon, and ecosystem metabolism in charophyte stands in the littoral zone of Lake Nymølle show pronounced spatiotemporal variation in environmental conditions within the same day and over short distances (cm scale). The high biomass of charophytes drives large diel fluctuations in temperature and oxygen resulting in diel stratification-mixing patterns and high metabolic rates. The littoral zone may make up a large proportion of shallow lakes, and if the cover of submerged macrophytes is high, it may have a disproportionate influence on whole-lake carbon cycling as shown by comparison of GPP of littoral macrophytes and pelagic phytoplankton in Lake Nymølle. Littoral habitats with dense vegetation may thus create environmental conditions that drastically differ from those of pelagic habitats traditionally studied. Further research should aim to reconcile the importance of littoral habitats in whole-lake metabolism.

Data Availability Statement

Data and scripts used for the analysis and figures are available from an online repository (<https://doi.org/10.17894/ucph.63ac767d-3ee4-493c-a387-2f7280f81252>; Martinsen, Zak, et al., 2022).

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