

Photosynthesis, growth, and distribution of plants in lowland streams—A synthesis and new data analyses of 40 years research

Kaj Sand-Jensen¹ | Tenna Riis² | Kenneth Thorø Martinsen¹ 

¹Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Copenhagen, Denmark

²Department of Biology, Aarhus University, Aarhus C, Denmark

Correspondence

Kenneth Thorø Martinsen, Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Universitetsparken 4, 3rd floor, 2100 Copenhagen, Denmark.
Email: kenneth.martinsen@bio.ku.dk

Funding information

Independent Research Fund Denmark; Danmarks Frie Forskningsfond, Grant/Award Number: 0217-00112B; VELUX Foundation

Abstract

1. This paper synthesises insights and offers new quantitative analyses of data gathered during 40 years of stream research by Danish researchers and international associates. Lowland Danish streams mostly drain fertile cropland and contain high nutrient concentrations saturating maximum yield and growth rate of plants. Concentrations of carbon dioxide (CO₂) are variable, although usually high, supporting a range of wetland and permanently submerged species.
2. All terrestrial and most amphibious species are obligate CO₂ users, while the majority of permanently submerged species can supplement their inorganic carbon demand with bicarbonate. In lake outlets, the average CO₂ concentration was close to air saturation during summer, whereas sites with no lake influence were 9-fold supersaturated. The 20% increase of atmospheric CO₂ concentrations over the past 40 years has marginal influence on CO₂ concentrations in the streams.
3. In lake outlets with low CO₂ concentrations, calculations on 33 stream species showed essentially no underwater photosynthesis by temporarily submerged terrestrial species, low rates by amphibious species, and high rates by permanently submerged bicarbonate users. Underwater photosynthetic rates increased at sites with high CO₂ concentrations (no lake influence): they were lowest for terrestrial wetland species (mean 1.8 mg O₂ g dry weight⁻¹ hr⁻¹), followed by homophyllous (3.0) and heterophyllous amphibious species (5.6), and highest among permanently submerged species (15.0). Terrestrial and amphibious species grew very slowly when CO₂ levels were low, but rapidly in CO₂ rich water, or when in contact with air above the water's surface. Decreasing CO₂ concentrations from upstream to downstream caused lower photosynthesis rates of amphibious species, while photosynthesis by bicarbonate users was consistently high.
4. The relative abundance of terrestrial and amphibious species decreased significantly as CO₂ resources decline from upstream to downstream, while the abundance of permanently submerged species increased as streams progressed.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Freshwater Biology* published by John Wiley & Sons Ltd.

However, plant abundance as a function of CO₂ concentrations did not differ markedly among the plant groups.

5. We conclude that photosynthesis and growth of species of different plant types under controlled experimental conditions resembling high in situ nutrient availability are closely related to inorganic carbon supplies, while their representation in plant assemblages is influenced by the spatially and temporally highly diverse ecological conditions from upstream to downstream in the mostly CO₂-rich lowland streams.
6. Submerged terrestrial and amphibious species restricted to CO₂ use for photosynthesis can partly escape the slow gas diffusion under water by growing in very CO₂-rich streams and having apical leaves in contact with air. Thus, strong restrictions on their growth and existence should be identified at sites where CO₂ concentrations are consistently low and no air contact is possible, while co-limitation by nutrients is likely to be marginal.
7. Generally, rising atmospheric CO₂ is irrelevant to photosynthesis of permanently submerged plants because of their ability to use bicarbonate and the high CO₂ supersaturation in most stream sites. In contrast, photosynthesis and growth of amphibious and terrestrial plants under water are highly dependent on CO₂ concentrations varying from close to air saturation in lake outlets to supersaturation in headwaters.

KEYWORDS

amphibious plants, bicarbonate users, CO₂ limitation, obligate CO₂ users, stream macrophytes

1 | INTRODUCTION

Streams are open windows for carbon dioxide (CO₂) emissions to the atmosphere due to the commonly observed 5–15 times greater CO₂ supersaturation in the water than in air (Cole et al., 2007; Rebsdorf et al., 1991). Extensive research has shown the significance of stream emissions in stream networks and in regional and global carbon budgets (Lauerwald et al., 2015; Sand-Jensen & Staehr, 2012; Wallin et al., 2013). It has become apparent that the CO₂ concentrations driving emissions are highly variable among sites and over time due to changing input and output along the stream network determined by a combination of physical, chemical, and biological processes (Neal et al., 1998; Stets et al., 2009; Wallin et al., 2011). In-stream CO₂ concentrations are often highest at upstream sites fed by CO₂-rich groundwater with declining concentrations downstream due to an increase in water residence time accompanied by ongoing emissions to the atmosphere mediated by water turbulence and consumption in photosynthesis (Finlay, 2003; Maberly et al., 2015; Neal et al., 1998). The CO₂ decline is even larger during passage through eutrophic lakes located in the stream network. While CO₂ is highly supersaturated upstream of lakes, phytoplankton photosynthesis in lakes reduces outlet CO₂ concentrations to below or near the air equilibrium; also known as air saturation (Sand-Jensen & Staehr, 2012). The observed spatial variability of CO₂ concentrations may have a strong influence on photosynthesis, growth, and

biodiversity of plants growing under water, as many stream plants are restricted to CO₂ use. This contrasts with plants capable of using bicarbonate (HCO₃⁻) to supplement their inorganic carbon demand by different biochemical investment in enzymes and membrane transporters, ensuring higher internal CO₂ concentrations and more efficient carboxylation (Maberly & Madsen, 2002). Despite CO₂ supersaturation, which is common in streams, obligate CO₂ users may have difficulty taking up much underwater CO₂ because gas diffusivity is low (Madsen & Sand-Jensen, 1987). Thus, coupled to the CO₂ variability, we expect a decrease in underwater photosynthesis of obligate CO₂ users along the streams, while HCO₃⁻ users can maintain photosynthesis at about 30%–40% of maximum photosynthesis in alkaline water at very low CO₂ concentrations (Madsen & Maberly, 1991; Madsen & Sand-Jensen, 1987; Sand-Jensen, 1983).

The present synthesis and new quantitative analysis draw on data and insights from Danish researchers and international associates who have studied inorganic carbon sources in streams and their use in photosynthesis and growth of stream plants for almost 40 years (e.g. Manolaki et al., 2020; Sand-Jensen, 1983). Throughout the 40-year period, the streams under investigation had high average CO₂ concentrations (c. 140–170 μM): 7.9–9.2-fold supersaturated relative to air-saturated water (Rebsdorf et al., 1991). Compared to these levels, the increase in dissolved CO₂ concentrations at air saturation by 20% (c. 15–18 μM) between 1983 and 2020 due to the rise of CO₂ in the atmosphere (c. 343–412 μatm) is minor. Thus, the rise of

atmospheric concentrations did not influence the CO₂ gradient from stream water to air, and observed changes of stream CO₂ concentrations in certain regions were caused by changes in land use (e.g. acidification of the groundwater due to nitrification of ammonium applied as fertiliser; Rebsdorf et al., 1991).

Concerning the possible influence of nutrient sources, Denmark is a lowland country with fertile soils and intensively cultivated cropland occupying the majority (60%) of the land area, while natural areas and forests occupy much less (<20%, Sand-Jensen & Lindegaard, 2004). The streams draining natural areas are relatively rich in dissolved inorganic nutrients (overall mean: 24 µg ortho-P/L and 840 µg ammonium + nitrate-N/L), while farmland streams are very nutrient rich (80 µg P/L and 5700 µg N/L; Sand-Jensen & Lindegaard, 2004). Tissue concentrations of nitrogen and phosphorus in vascular stream plants are high; Kern-Hansen and Dawson (1978) found no indication of biomass yield limitation by nutrients in 19 streams. Cavalli et al. (2015) evaluated potential growth rate limitation of 13 species collected from many Danish streams. Applying the experimentally derived tissue thresholds of 2% N and 0.2% P of dry weight (DW) for 95% maximum growth rate (Colman et al., 1987; Demars & Edwards, 2007; Gerloff & Kromholz, 1966), they found tissue concentrations markedly above the thresholds for all submerged species, while only a few samples of terrestrial and amphibious species were below the thresholds. Experiments on submerged plants in a groundwater-fed stream by nutrient amendment or root removal consistently found high tissue concentrations (i.e., 2.5%–3.4% N and 0.53%–0.70% P of DW) and no influence on growth rates (Madsen & Cedergren, 2002). Thus, nutrients do not appear to influence the growth rate and spatial distribution of submerged plants in the majority of Danish streams, while in few instances amphibious and terrestrial species may be nutrient limited. In contrast, extensive experiments over the years have shown the influence of dissolved inorganic carbon on photosynthesis and growth of stream plants (e.g., Maberly & Madsen, 2002; Madsen & Maberly, 1991; Manolaki et al., 2020; Riis et al., 2009; Sand-Jensen, 1983; Sand-Jensen & Frost-Christensen, 1998, 1999). Measurements of CO₂ and HCO₃⁻ concentrations in 50 Danish stream networks offer an opportunity to evaluate, under field conditions, the experimentally tested performance of plant species that differ in their modes of carbon acquisition.

About 170 vascular plant species exist under water in Danish streams, corresponding to about 10% of the native national species pool (Riis et al., 2001). About 75 of these species are primarily terrestrial (i.e., secondary water plants) that grow in streams for some period (Sand-Jensen et al., 1992). Stream environments also include 45 species of amphibious plants with either the same (homophyllous) or different (heterophyllous) leaf form in air and under water. Differences of the cuticle and the epidermis wall result in higher CO₂ conductance in aquatic photosynthesis for leaves acclimated to water as opposed to air (Frost-Christensen & Floto, 2007; Frost-Christensen et al., 2003). Heterophyllous species with strap-formed, or filamentous aquatic leaves may have higher photosynthesis rates than homophyllous species with broader leaves

(Mommer et al., 2005). Danish streams host 50 species that are permanently submerged, and most of these can use HCO₃⁻ (Sand-Jensen et al., 1992). We examined the responses of species from these four plant groups (terrestrial, amphibious-homophyllous, and amphibious-heterophyllous, and permanently submerged HCO₃⁻ users) to variable CO₂ concentrations among stream sites to evaluate their photosynthesis and growth. Species representing the four plant groups are shown (Figure 1).

The ability of terrestrial and amphibious plants to persist under water enables them to occupy the spaces where stream's water level fluctuates widely, leaving plants to alternate between submergence and air exposure (Riis et al., 2001; Winkel et al., 2016). Moreover, in the small shallow streams, plants grow from small individuals under water in winter and spring to tall individuals exposed to air later during summer and autumn (Voeselek et al., 2004). Both plant types acclimate to shifts between air and water by forming new leaves that use CO₂ to photosynthesise more efficiently in the environment to which they are exposed (Frost-Christensen et al., 2003; van Veen & Sasidharan, 2021).

The distribution of plant species is influenced by interrelated geomorphological and environmental conditions and interacting biotic processes (Baatrup-Pedersen & Riis, 2004). This complexity gives rise to extensive variation in the composition of plant assemblages among stream sites (Demars & Harper, 2005). Increase in width and water depth probably reduces the proportions of terrestrial and amphibious species relative to permanently submerged species from shallow headwaters with closer contact with land and air compared to deeper downstream sites with weaker contact. Moreover, changes in temperature, substratum, and water clarity among sites influence the plant assemblages (Baatrup-Pedersen & Riis, 2004). Within this complexity of interacting factors, we examined whether the form of inorganic carbon used for photosynthesis influenced plant assemblages.

In an earlier study, we examined the abundance of plant groups along 208 reaches in 40 Danish streams (Riis et al., 2001). Permanently submerged and amphibious species had high and almost similar plant cover (45% and 38%, respectively), but primarily terrestrial species were present as well (17%). Among the 10 most frequent species, seven species were amphibious and *Sparganium emersum*, *Callitriche platycarpa*, and *Callitriche cophocarpa* were the most common overall (Riis et al., 2000). *Callitriche* species form floating leaf rosettes in shallow water during summer, gaining access to atmospheric CO₂ (Madsen & Breinholt, 1995). *Sparganium emersum*, which resembles the anatomy and morphology of *Vallisneria spiralis*, may offer a transport pathway from roots buried in CO₂-rich organic sediment, through air lacunae, to leaves (Winkel & Borum, 2009). Thus, supplementary CO₂ supplies from air or sediment may influence the distribution of *Callitriche* and *Sparganium*.

The overall goal of this synthesis is to combine earlier extensive experimental data on photosynthesis and growth of many stream species of four plant types with new findings related to their measured abundance and calculated performance at stream sites with

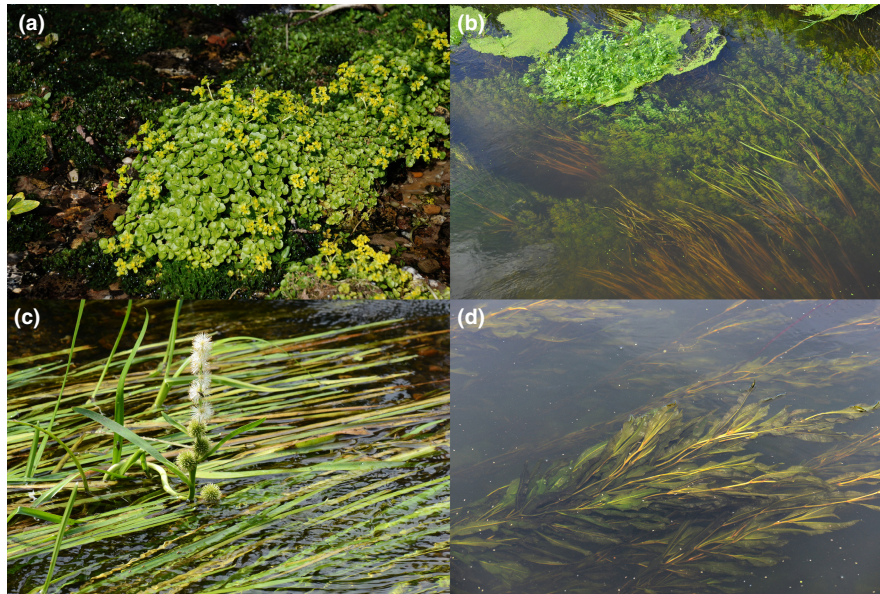


FIGURE 1 Species representing the four different plant groups in Danish streams. (a) Primarily terrestrial species (secondary water species), *Chrysosplenium oppositifolium* with basal leaves under water and apical leaves in air in a small, very shallow stream (about 0.1 m deep). (b) Homophyllous amphibious species, *Berula erecta* with basal leaves under water and some apical leaves in air in a small stream (about 0.9 m deep). (c) Heterophyllous amphibious species, *Sparganium emersum* with strap-formed submerged leaves, floating leaves, aerial leaves, and an inflorescence in a deeper stream (about 1.1 m deep). (d) Permanently submerged species, *Potamogeton lucens* in a very deep stream (about 3 m deep). Photos by Jens Christian Schou, shown with permission

widely divergent CO_2 and HCO_3^- concentrations. Specifically, our first hypothesis was that underwater photosynthesis increased from terrestrial, over amphibious to permanently submerged species. We made these determinations by combining CO_2 and HCO_3^- concentrations at many sites with photosynthesis of 33 species as measured in the laboratory at ambient field concentrations of inorganic carbon as well as very low and very high CO_2 concentrations. The second hypothesis was that photosynthesis of 11 intensively studied amphibious or permanently submerged species would gradually decrease in water simulating inorganic carbon conditions in headwaters, upstream and downstream of lakes. The third hypothesis was that the daily metabolic balance would match the directly measured growth rates, under water and in air, of eight rooted species of CO_2 users and 12 species of HCO_3^- users. Finally, the fourth hypothesis was that the abundance of plant groups was related to site dimensions and CO_2 concentrations along the stream network. The possibility of co-limitation by nutrients is also discussed.

2 | MATERIAL AND METHODS

2.1 | Study design

Our synthesis and quantitative analyses use numerous data from almost 40 years of field measurements and experimental studies in Danish lowland streams. The analyses can be grouped as: (1) inorganic carbon concentrations at 236 sites in 50 lowland streams; (2) photosynthetic response to inorganic carbon of 33 plant species

tested at low, ambient, and high CO_2 concentrations (Sand-Jensen et al., 1992) and 11 species tested at multiple CO_2 and HCO_3^- concentrations (many papers); (3) relative growth rates (RGRs) of eight amphibious species in air and under water and 12 species of permanently submerged species measured at multiple CO_2 and HCO_3^- concentrations (many papers); (4) relative abundance of stream plants at 159 of the sites in (1) (Baattrup-Pedersen & Riis, 2004; Riis et al., 2000).

2.2 | Inorganic carbon availability in the field (1)

Bicarbonate and CO_2 concentrations were measured in samples collected in the morning (8:00–11:00) during mid-summer in 50 Danish lowland, high-alkalinity streams. The majority of sites (203) had no or distant influence of lakes, while 33 sites were located downstream of lakes. Along 13 streams, HCO_3^- and CO_2 concentrations were measured at the upper reaches of headwaters as well as immediately upstream and downstream lakes in the stream network in the morning and the afternoon (14:00–17:00) on the same day. Water temperature, pH, specific conductivity, and total alkalinity (Gran titration; Sand-Jensen & Staehr, 2012) were used to calculate dissolved inorganic carbon (DIC, sum of $[\text{CO}_2]$, $[\text{HCO}_3^-]$, and $[\text{CO}_3^{2-}]$), HCO_3^- , and CO_2 according to Mackereth (1978). Total alkalinity averaged 2.80 meq/L and no streams had high humic content, ensuring reliable calculations of DIC, HCO_3^- , and CO_2 (Abril et al., 2015); 94% of all CO_2 measurements were below 50 μM in 33 lake outlets and below 300 μM at 203 sites with no lake influence. These CO_2 thresholds correspond to about

3- and 18-times supersaturation of the stream water relative to air saturation (c. 17 μM).

2.3 | Photosynthetic response to inorganic carbon: Laboratory analysis (2)

Several papers have examined the photosynthetic response to variable inorganic carbon supply in laboratory experiments with plant species collected from Danish streams during summer (e.g., Baattrup-Pedersen et al., 2013; Frost-Christensen et al., 2003; Madsen, 1984; Madsen & Breinholt, 1995; Madsen & Maberly, 1991; Madsen & Sand-Jensen, 1987; Sand-Jensen, 1983; Sand-Jensen & Frost-Christensen, 1998, 1999; Sand-Jensen et al., 1992; Vadstrup & Madsen, 1995). All experiments were performed under well-stirred conditions at relatively high photosynthetic irradiance ($>400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 400–700 nm). Incubation temperature was mostly 15°C, close to the mean summer temperature for Danish streams. In a few cases, incubation temperature was 12 or 20°C; one experiment on *Callitriche cophocarpa* from a spring-fed brook used the ambient temperature of 8°C (Madsen & Maberly, 1991). Laboratory conditions thus intended to replicate natural conditions, but no further attempt was made to correct for the influence of variable water temperature on the rate of photosynthesis.

2.3.1 | Thirty-three species from four plant groups (2)

The most comprehensive experimental study measured photosynthesis of 33 species collected from eight streams (Sand-Jensen et al., 1992). Most collections and incubations (c. 80%) were with species from high alkalinity (4.16–4.74 meq/L) and CO_2 -rich waters (215–260 μM). Experiments were conducted at 15°C in ambient water at unaltered pH and reduced pH by HCl addition to elevate the CO_2 concentration (800 μM , Sand-Jensen et al. (1992)). The CO_2 compensation point, where photosynthesis compensates for respiration and oxygen exchange is zero, was calculated from end-pH and DIC in drift experiments (Maberly & Madsen, 1998). End pH was also measured for HCO_3^- users, which seem to offer a CO_2 compensation point that reflects the combined influence of low CO_2 ($<0.5 \mu\text{M}$), high pH (>10.2), and reduced HCO_3^- concentration relative to CO_3^{2-} . In addition to the 33 species, photosynthesis was measured at many CO_2 concentrations on one species from each of the four plant types of interest in the current study (figure 6 in Sand-Jensen et al. (1992)).

All measurements show that photosynthesis by CO_2 users increased in linear proportion to CO_2 at low concentrations (Figure 2). The slopes of photosynthesis versus CO_2 concentration for the two intervals between the CO_2 compensation point and either

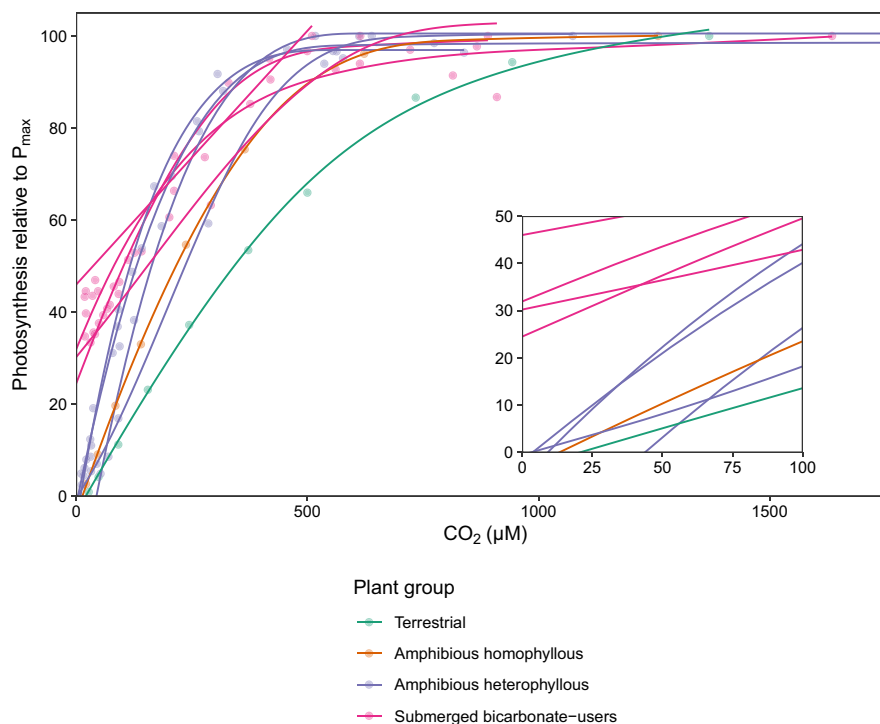


FIGURE 2 Photosynthesis of different plant species belonging to four groups as a function of CO_2 concentrations in the water. The terrestrial (secondary water) plant is *Epilobium hirsutum*. Amphibious plants are represented by Homophyllous amphibious, *Berula erecta*, and Heterophyllous amphibious (three blue lines), *Callitriche cophocarpa*, *Sparganium emersum*, and *Sparganium erectum*. Data for permanently submerged bicarbonate users (four red lines) are taken from studies of *Elodea canadensis* (HCO_3^- 1 and 2 mM), *Potamogeton crispus* (HCO_3^- 5.0 mM), *Potamogeton pectinatus* (HCO_3^- 5.0 mM), and *Ranunculus peltatus* (HCO_3^- 2.3 mM) Inset: photosynthesis $<100 \mu\text{M CO}_2$. Photosynthetic rates are shown relative to maximum rates at high CO_2 concentrations. Data from: Sand-Jensen (1983), Madsen and Sand-Jensen (1987), Madsen and Maberly (1991), and Sand-Jensen et al. (1992)

the ambient or the highly elevated CO_2 concentration (800 μM) were quite similar for all 23 non-permanent underwater species (Figure S1). However, slopes became slightly steeper and the difference between the slopes for the lower and higher CO_2 concentration interval, respectively, decreased from the photosynthetically less active terrestrial species to the more active heterophyllous amphibious species. The relationship for the lower concentration interval was used in all calculations of mean photosynthesis across the 236 field sites with available CO_2 concentrations. This lower CO_2 interval covers 90% of concentrations observed in the field.

Photosynthesis of HCO_3^- users does not stop as CO_2 approaches very low concentrations at high pH (about 1–2 μM at pH 9.2–9.5), but typically continues by use of HCO_3^- up to pH 10.2–10.8 (Maberly & Madsen, 1998). In detailed measurements on six HCO_3^- users, the photosynthetic rate extrapolated to zero CO_2 averaged 35.8% ($SD \pm 5.7$) of maximum photosynthesis (P_{max} ; Sand-Jensen, 1983; Madsen & Sand-Jensen, 1987; Madsen & Maberly, 1991; Sand-Jensen et al., 1992). For the 10 HCO_3^- users tested only at two concentrations, i.e., ambient and high CO_2 of 800 μM that saturated photosynthesis (Sand-Jensen et al., 1992), the extrapolation to zero CO_2 yielded a similar mean value (35.3% of P_{max}). Thus, we used the equation for photosynthesis for the interval between CO_2 in ambient water and 800 μM to calculate the range of photosynthesis for all species at the 236 sites. Subsequently, we calculated mean and median values of photosynthesis for species and plant types among the 33 lake outlets and the 203 sites with no lake influence.

2.3.2 | Eleven species studied in detail (2)

The rate of photosynthesis has been measured at a wide variety of CO_2 and HCO_3^- concentrations for 11 representatives of the four plant types that are found in Danish streams (Madsen & Maberly, 1991; Madsen & Sand-Jensen, 1987; Sand-Jensen & Frost-Christensen, 1998, 1999; Sand-Jensen et al., 1992). We calculated their photosynthetic rate in the morning and afternoon in reaches in headwaters where CO_2 concentrations are particularly high, as well as upstream and downstream of lakes in 13 stream networks.

2.4 | Daily metabolic balance and growth rates (3)

Both photosynthesis and respiration by three amphibious CO_2 users were measured in carbon units for above-ground shoots collected from three streams (Sand-Jensen & Frost-Christensen, 1999). Laboratory conditions replicated the assumed natural conditions of 12 hr of photosynthesis at high illumination at the field sites and 12 hr of dark respiration, and the data gathered were used to determine the daily metabolic balance between formation of organic carbon by photosynthesis and loss by respiration. This daily metabolic balance was converted to the RGR by, firstly, assuming that organic carbon comprised 40% of the plant DW and, secondly, calculating the daily biomass increments (i.e., the daily metabolic balance, ΔB)

relative to the biomass the day before (B_0). In this case, the period (T , days) for calculation in the general formula is 1 day:

$$\text{RGR} (d^{-1}) = \ln((B_0 + \Delta B)/B_0)/T$$

The calculated daily metabolic balance and its conversion to RGR do not subtract the costs of producing and sustaining the root system. However, these measures provide an indication of the ability of the species to grow and survive in a variety of field conditions. The growth estimates were compared with direct measurements of RGR of entire plants among the same amphibious species and five additional amphibious species. Moreover, RGRs of 12 permanently submerged species were measured under different levels of inorganic carbon supply in water and atmospheric air over several days (T , e.g., Madsen, 1993; Madsen & Breinholt, 1995; Madsen & Brix, 1997; Madsen & Cedergreen, 2002; Madsen & Sand-Jensen, 1987; Manolaki et al., 2020; Pedersen & Sand-Jensen, 1997; Riis et al., 2009; Sand-Jensen & Frost-Christensen, 1999).

2.5 | Observed plant abundance among stream sites (4)

Abundance of species and plant groups were measured at 159 sites in 30 Danish lowland streams based on quantitative analyses of species cover in more than 40,000 quadrats (0.2 \times 0.2 m); approximately 250 quadrats across the stream along a 100-m reach (Baattrup-Pedersen & Riis, 2004; Riis et al., 2000). We coupled these data to stream dimensions and CO_2 concentrations available for the same sites collected at the same time as the vegetation analysis. At each site, we created 10 transects, 10 m apart along a 100-m reach. At each transect, we recorded water depth and species cover for every 0.2-m interval from shore to shore. These measurements were used to calculate the species cover and the average wetted cross-sectional areas (A , m^2) for each reach. Dissolved nutrients are high and saturating to plant growth in most Danish streams (Manolaki et al., 2020). This implies that nutrient variability does not influence the distribution of plant species among stream sites in these nutrient-rich Danish streams and in other nutrient-rich lowland streams in countries with intensive agriculture and dense human populations.

The percentage plant cover of species was analysed along the stream network (Baattrup-Pedersen & Riis, 2004; Riis et al., 2000). Individual species were combined into three categories: terrestrial wetland species, amphibious species, and permanently submerged species. The two types of amphibious species (homophyllous and heterophyllous) were combined into a single category to ensure a more robust analysis as homophyllous species had a relatively low abundance. In addition, the abundances of *Callitriche* spp. (mainly *C. platycarpa* and *C. cophocarpa*) and *Sparganium* spp. (primarily *Sparganium emersum* and secondarily *Sparganium erectum*) were calculated separately as they may exhibit a contrasting preference for air contact that may influence their distribution. *Callitriche* spp. form floating leaf rosettes in shallow water, whereas submerged

populations of *Sparganium* spp. in deeper water form long, strap-shaped leaves. *Sparganium* spp. were also combined or kept separate from permanently submerged species. The distributions of plant groups and species were analysed in relation to both wetted area and CO₂ concentration along all sites.

2.6 | Statistics

We tested the importance of stream site dimensions (wetted area) and CO₂ concentrations on relative occupancy (response variable, proportion) of the three plant groups using beta-regression modelling. Beta-regression is suitable for regression problems when the response is continuous and bounded between 0 and 1, and avoids the potential drawbacks associated with alternative analysis (i.e. transformation of the response variables followed by linear regression; Cribari-Neto & Zeileis, 2010). Wetted area and CO₂ concentrations (predictor variables) were log₁₀-transformed to improve normality. To avoid values of exactly 0 or 1, proportions were transformed as described in Douma and Weedon (2019). Candidate models were compared using the Akaike information criterion with correction for small sample sizes. Beta-regression models were fitted using the *betareg* R-package (Cribari-Neto & Zeileis, 2010; R Core Team, 2021).

3 | RESULTS

3.1 | Stream water CO₂ and HCO₃⁻ (1)

Carbon dioxide and HCO₃⁻ concentrations varied markedly between stream sites located in lake outlets and sites with no lake influence. In 33 lake outlets, the CO₂ concentration averaged 18.4 μM (median 12.8 μM) and the water was below air-saturation in 60% of measurements (Figure 3). Measurements at 203 sites with no lake influence showed a mean CO₂ concentration of 147 μM (median 121 μM), and the water was supersaturated in all cases. The HCO₃⁻ concentration was lower in lake outlets (mean 1.44 mM) compared with sites with no lake influence (mean 2.41 mM), hence the molar concentration of CO₂ relative to HCO₃⁻ was much lower in lake outlets (mean 1.3%)

compared with sites with no lake influence (9.2%). Low concentrations of CO₂ and low proportions relative to HCO₃⁻ in lake outlets suggest that the ability of submerged species to use HCO₃⁻ may be particularly important here.

3.2 | Photosynthesis across all species and stream sites (2)

Mean photosynthesis measured in ambient water from the collection sites increased gradually from the terrestrial, over the amphibious, and to the permanently submerged species reaching a 7-fold difference (Figure 4). The CO₂ compensation point dropped in the same order, from mean concentrations close to air saturation for terrestrial wetland species (16.8 μM), over slightly under-saturated concentrations for the two types of amphibious species (11.8 and 8.4 μM), to concentrations close to zero for permanently submerged HCO₃⁻ users (0.3 μM) reaching pH of 10.2–10.8 in pH-drift experiments (Maberly & Madsen, 1998). These patterns accord with the first hypothesis.

Photosynthesis averaged across 203 sites with no lake influence and among species belonging to the four plant types also increased gradually from terrestrial species (1.8 mg O₂ g DW⁻¹ h⁻¹), over homophyllous (3.0) and heterophyllous amphibious species (5.6), to permanently submerged species (15.0, Figure 5). Overall, terrestrial species performed poorly under water at stream sites with low CO₂ concentrations due to strong CO₂-related constraints on their photosynthesis, while amphibious species attained appreciable photosynthesis and permanently submerged species did even better, due to their use of HCO₃⁻. The performance also varied considerably among species within each plant group. *Cardamine amara*, a terrestrial wetland species, performed as well as many amphibious species, and some homophyllous amphibious species did as well as some heterophyllous amphibious species.

The difference in aquatic photosynthesis among plant groups was even more pronounced when photosynthesis was compared across 33 lake outlets with low CO₂ concentrations (Figure 5). Mean photosynthetic rates of the three groups of obligate CO₂ users were close to zero (0.08 for terrestrial, 0.19 for homophyllous amphibious,

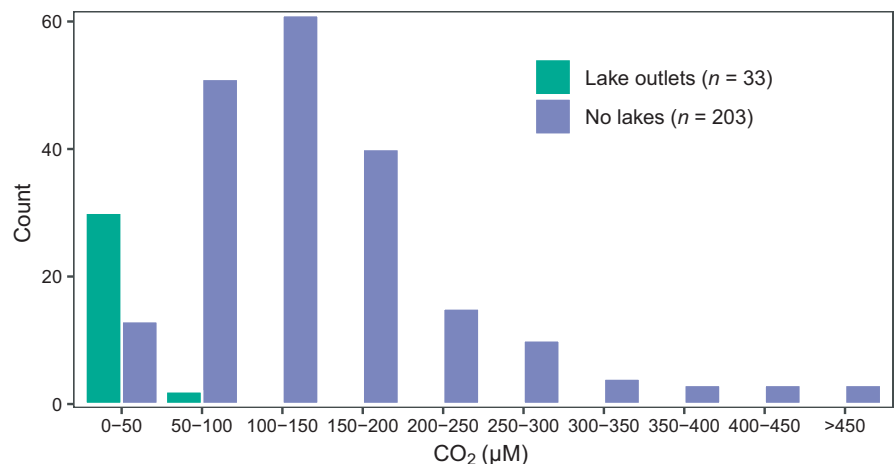


FIGURE 3 Frequency distribution of CO₂ concentrations in streams at 33 sites downstream of lakes and 203 sites with no lakes

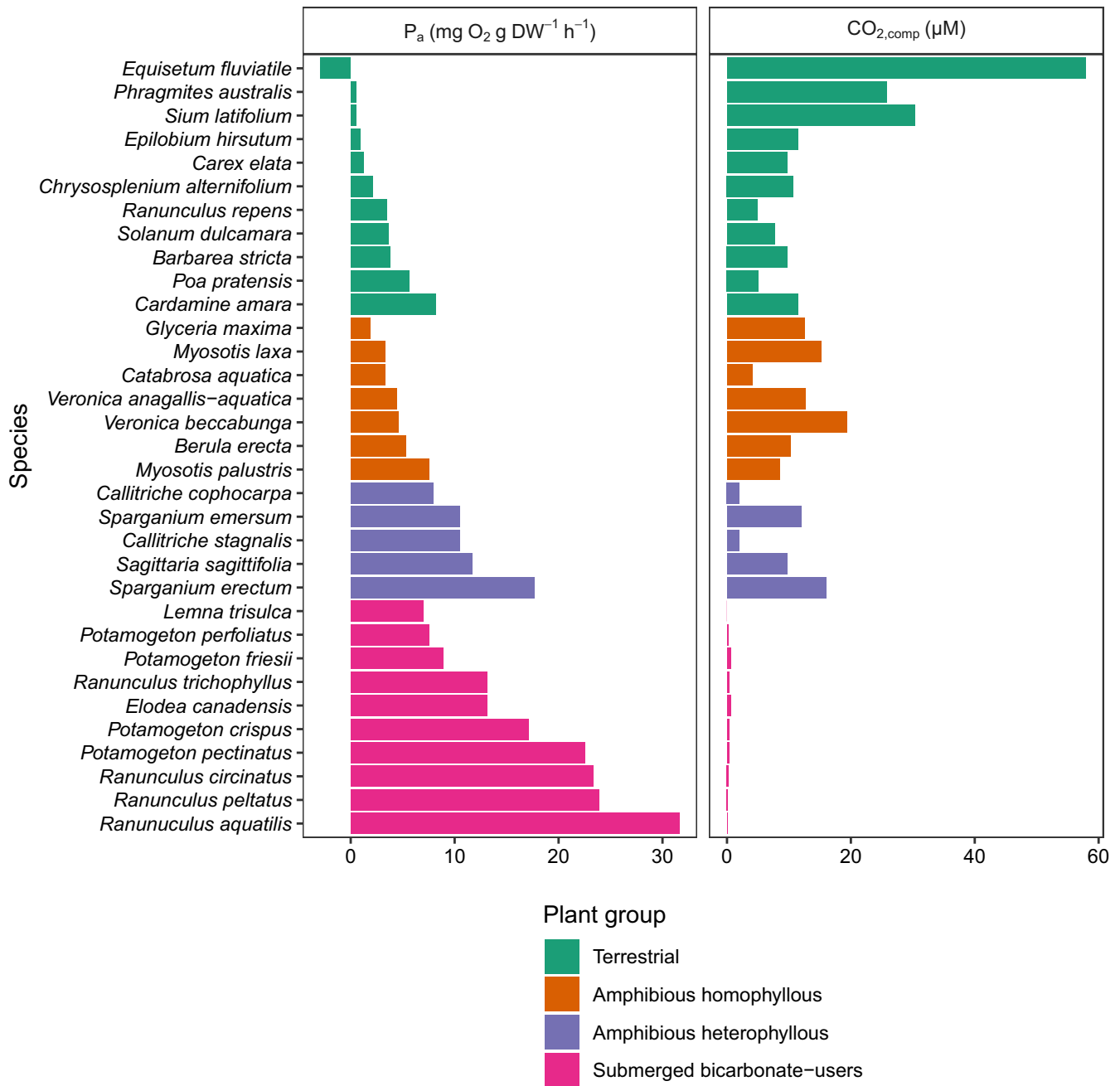


FIGURE 4 Photosynthesis in ambient water (left panel) and CO₂ compensation point (right panel) of species belonging to four plant groups. Calculated from data in Sand-Jensen et al. (1992)

and 0.44 mg O₂ g DW⁻¹ h⁻¹ for heterophyllous amphibious species), while permanently submerged HCO₃⁻ users performed well (11.4 mg O₂ g DW⁻¹ h⁻¹). The mean percentage of photosynthetic rates in lake outlets, relative to sites without lake influence, was 0 for terrestrial species, 6% for homophyllous amphibious species and 8% for heterophyllous amphibious species, while the percentage was high (72%) for permanently submerged HCO₃⁻ users. It is evident that CO₂ users can barely photosynthesise when submerged at the low CO₂ concentrations immediately downstream of lakes, even when exposed to summer sunshine, while HCO₃⁻ users do well. These patterns accord with the second hypothesis.

3.3 | Eleven species in stream-lake networks (2)

We calculated the CO₂ and HCO₃⁻ concentrations as well as the performance of 11 intensively studied species in the morning and in the afternoon along 13 stream networks. In lake outlets, median CO₂ concentrations were markedly below air saturation in the morning (8.6 µM) and more so in the afternoon (5.5 µM), although a few lake outlets were supersaturated (Figure 6). Immediately upstream of the lakes, median CO₂ concentrations in both morning and afternoon were about 6-fold supersaturated (97 and 87 µM) and headwaters were about 12-fold supersaturated (188 and 195 µM). At high CO₂

FIGURE 5 Mean photosynthesis of species within four plant groups for 33 lake outlets (green) and 203 stream sites with no lake influence (blue). The median (diamond) is shown for all species within plant groups. Calculated from data in Sand-Jensen et al. (1992)

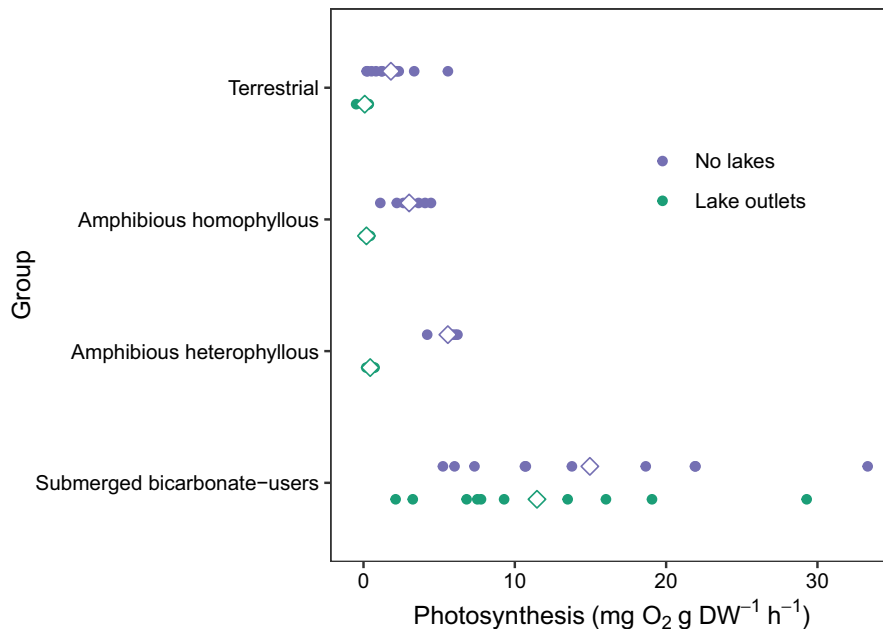
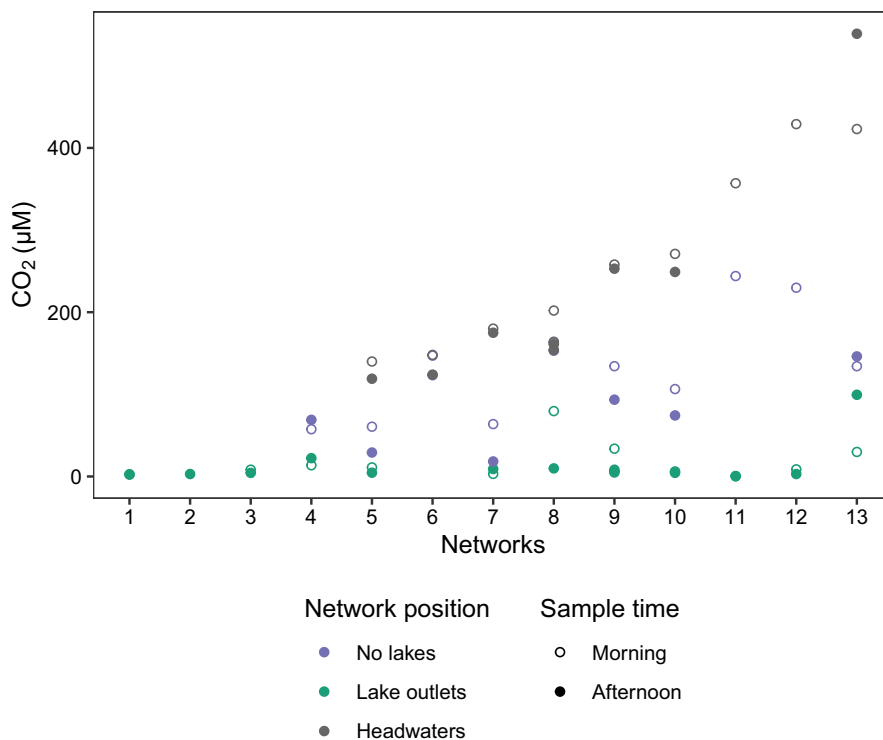


FIGURE 6 Carbon dioxide concentrations in the morning (open point) and in the afternoon (closed point) in 13 stream networks in headwaters (grey), lake outlets (green), and sites with no lake influence (blue)



concentrations in upstream sites and headwaters, differences in morning and afternoon CO₂ were small.

In lake outlets, medians of calculated net photosynthetic rates of CO₂ users were close to zero; they were mostly positive in morning hours due to higher CO₂ concentrations and negative in the afternoon, when CO₂ concentrations were lower and respiration exceeded gross photosynthesis (Figure 7). In certain lake outlets with higher CO₂ concentrations, all CO₂ users attained positive photosynthesis. The HCO₃⁻ users did well in the lake outlets both in the morning and in the afternoon. Their photosynthetic rates were

between 9.9 and 15.2 mg O₂ g DW⁻¹ h⁻¹ and resembled the rates calculated for the 10 HCO₃⁻ users in 33 lake outlets (Figure 5). These patterns supported the second hypothesis.

At sites immediately upstream of lakes, calculated photosynthetic rates were positive for all CO₂ users and about 1.5-fold higher in the morning (medians between 3.5 and 13.6) than in the afternoon (2.0–9.6 mg O₂ g DW⁻¹ h⁻¹; Figure 7). Bicarbonate users had higher rates (medians 10–21.3 mg O₂ g DW⁻¹ h⁻¹) that declined by only 10% from the morning to the afternoon. In the headwaters, CO₂ concentrations and photosynthesis were consistently high; some CO₂ users

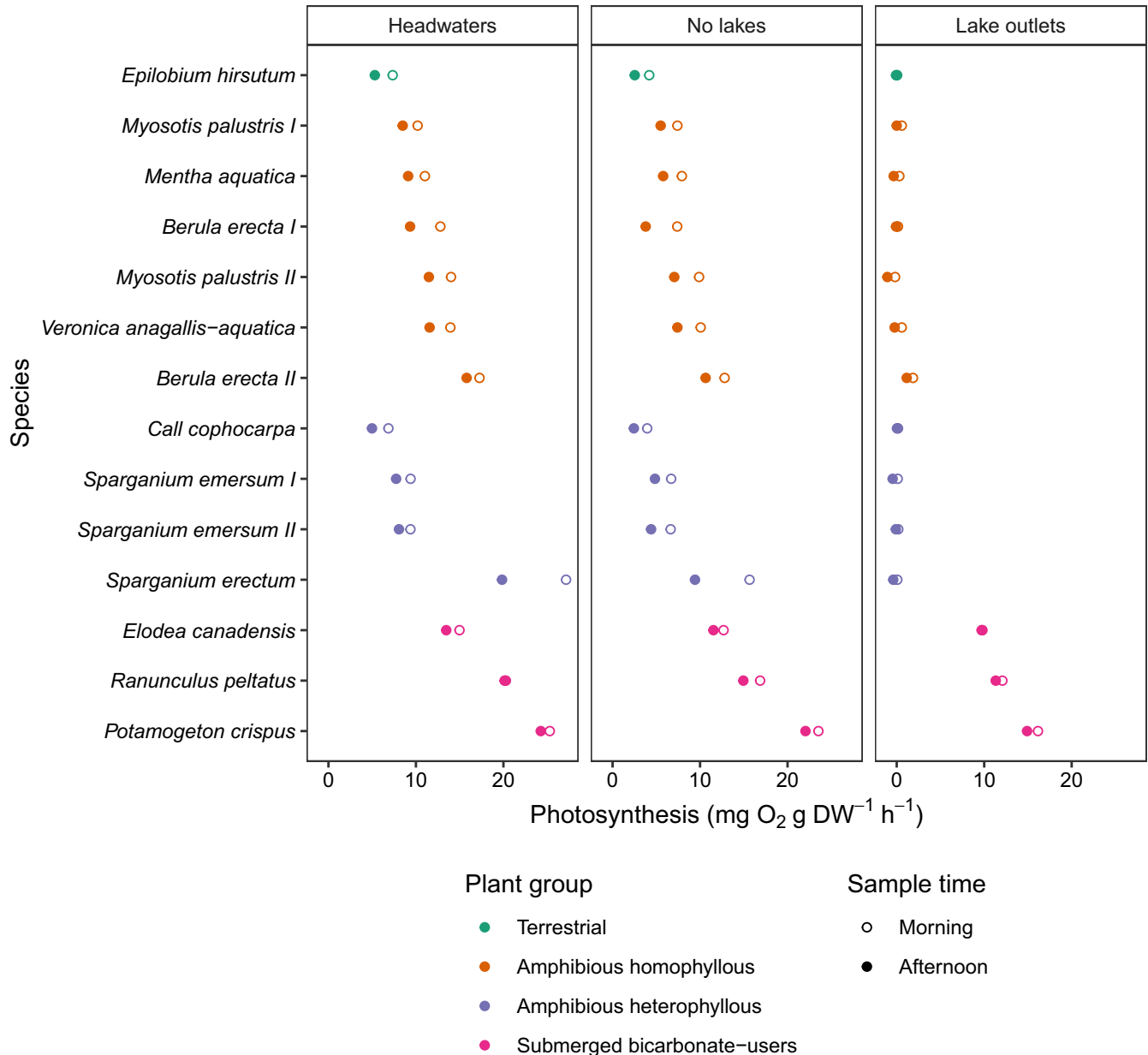


FIGURE 7 Mean calculated photosynthesis of 11 species among four plant types in the morning (open point) and the afternoon (closed point) in headwaters, distant from lakes, and upstream and downstream lakes in 13 stream networks. Data calculated from field data on inorganic carbon and experimental photosynthesis data from Sand-Jensen (1983), Madsen and Sand-Jensen (1987), Madsen (1991), Madsen and Maberly (1991); Sand-Jensen et al. (1992); Madsen and Breinholt (1995), and Sand-Jensen and Frost-Christensen (1999)

attained the same maximum photosynthetic rates here as HCO_3^- users (approximately $25 \text{ mg O}_2 \text{ g DW}^{-1} \text{ h}^{-1}$).

3.4 | Daily metabolic balance and relative growth rate under water and in air (3)

For three common amphibious CO_2 users, *Mentha aquatica*, *Myosotis palustris*, and *Veronica anagallis-aquatica*, data on the photosynthesis- CO_2 relationship and dark respiration enabled calculation of the daily metabolic balance for a 12-h light and 12-h dark cycle in 13 stream networks (Table 1). The mean daily metabolic balance for

the green shoots was negative in most lake outlets (-11 to $-20 \text{ mg O}_2 \text{ g DW}^{-1} \text{ day}^{-1}$): few individuals from these amphibious species can survive if they are permanently submerged in these conditions. However, in two of 13 lake outlets, CO_2 concentrations were higher and the daily metabolic balance was positive (data not shown). At the generally higher CO_2 concentrations upstream of the lakes, the mean daily balance was positive (41 – $53 \text{ mg O}_2 \text{ g DW}^{-1} \text{ day}^{-1}$) for the three species and even more so in the headwaters (107 – $143 \text{ mg O}_2 \text{ g DW}^{-1} \text{ day}^{-1}$). Those positive metabolic balances for the green shoots, without accounting for the respiratory cost of root systems, corresponded to daily RGR of 38 – 49×10^{-3} upstream of lakes and 95 – 125×10^{-3} in headwaters supporting the third hypothesis (Table 1).

TABLE 1 Metabolic balances of three amphibious stream plants

Location	<i>Mentha aquatica</i>	<i>Myosotis palustris</i>	<i>Veronica anagallis-aquatica</i>
Headwaters	111 (± 13)	107 (± 11)	143 (± 16)
No lakes	53 (± 11)	47 (± 10)	41 (± 14)
Lake outlets	-20 (± 6)	-11 (± 5)	-20 (± 7)

Note: Daily metabolic balance of three amphibious species in 13 stream networks in headwaters and at sites immediately upstream and downstream of lakes. Calculated from CO_2 concentrations in the morning and in the afternoon at the sites and measurements of photosynthesis- CO_2 relations and dark respiration in experiments (Sand-Jensen & Frost-Christensen, 1999). A 12-h light and 12-h dark period was used for calculations. Mean values ($\pm \text{SEM}$) as $\text{mg O}_2 \text{ g DW}^{-1} \text{ day}^{-1}$.

Daily RGRs of rooted amphibious species measured in field and laboratory experiments were negative or close to zero in water, where CO_2 concentration is close to air saturation (20–25 μM), while daily RGR increased to about 100×10^{-3} in highly supersaturated water (Figure 8). Daily RGRs resembled those calculated from the metabolic balance (Table 1). Amphibious species' RGR did not change with HCO_3^- concentration. Thus, the faster conversion to CO_2 at higher HCO_3^- concentrations through carbonate buffer reactions was not reflected by RGR. The HCO_3^- users' RGR increased from low to higher concentrations of CO_2 and HCO_3^- . Bicarbonate users grew when CO_2 concentration was at the air saturation level, and they grew faster as HCO_3^- concentrations increased. For example, *Ranunculus aquatilis* in air-saturated water and 0.55 mM HCO_3^- attained half of the maximum daily RGR of 63×10^{-3} obtained when HCO_3^- exceeded 1.5 mM (Madsen, 1993). Increasing the CO_2 concentration 20-fold above air saturation (350 μM) raised the daily RGR further (96×10^{-3}), indeed to the same level as achieved by amphibious species at the same high CO_2 concentration (Figure 8). This growth stimulation of HCO_3^- users by elevated CO_2 at all HCO_3^- concentrations is consistent with the CO_2 stimulation of their photosynthesis (Figure 2; Maberly & Madsen, 2002).

The strong limitation of photosynthesis and growth that terrestrial wetland and amphibious species experience in air-saturated water vanishes when plants extend above the water's surface or have a floating leaf rosette (Frost-Christensen et al., 2003; Madsen & Breinholt, 1995; Sand-Jensen & Frost-Christensen, 1999). Thus, the amphibious species attained daily RGR between 25 and 125×10^{-3} with air contact, while values were very low (-8 – 25×10^{-3}) when completely submerged in water with low CO_2 close to air saturation (Figure 8). In water supersaturated 25–30 times with CO_2 (i.e., 500–600 μM), the species grew much better, although generally slower than with air contact.

3.5 | Plant abundance along streams (4)

Combining all streams and sites, we analysed the influence of site dimension (i.e., wetted area) and CO_2 concentration on plant

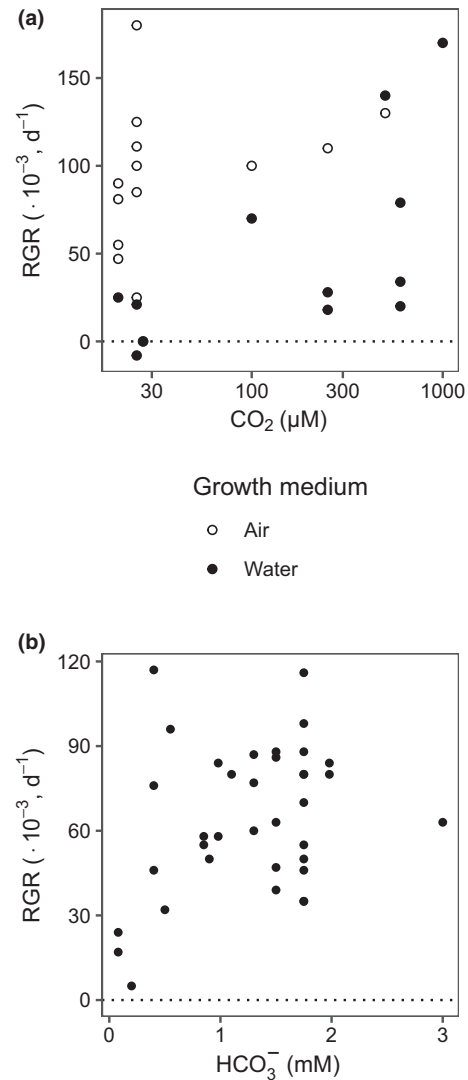


FIGURE 8 Daily relative growth rates (RGRs) of different species as a function of CO_2 and bicarbonate concentrations in the water. (a) Amphibious CO_2 users with (open point) and without (closed point) air contact. (b) Permanently submerged bicarbonate users. Data compiled from Madsen (1993), Madsen and Breinholt (1995), Vadstrup and Madsen (1995), Madsen and Brix (1997), Pedersen and Sand-Jensen (1997); Sand-Jensen and Frost-Christensen (1999), Madsen and Cedergreen (2002); Riis et al. (2009), and Manolaki et al. (2020)

abundance. The best beta-regression model included wetted area, plant group, CO_2 concentration, and an interaction between wetted area and plant group, showing that plant groups differed in their response to site dimension (Table S1). The proportion of *Callitriche* spp., all amphibious, and terrestrial species in the plant assemblage decreased significantly with increasing wetted area downstream (Figure 9a). In contrast, the populations of *Sparganium* spp. and submerged HCO_3^- users increased significantly downstream in wider and deeper reaches. The scatter of data points in the relationship of relative abundance of plant types to the wetted area was substantial (data not shown) due to extensive variations in location,

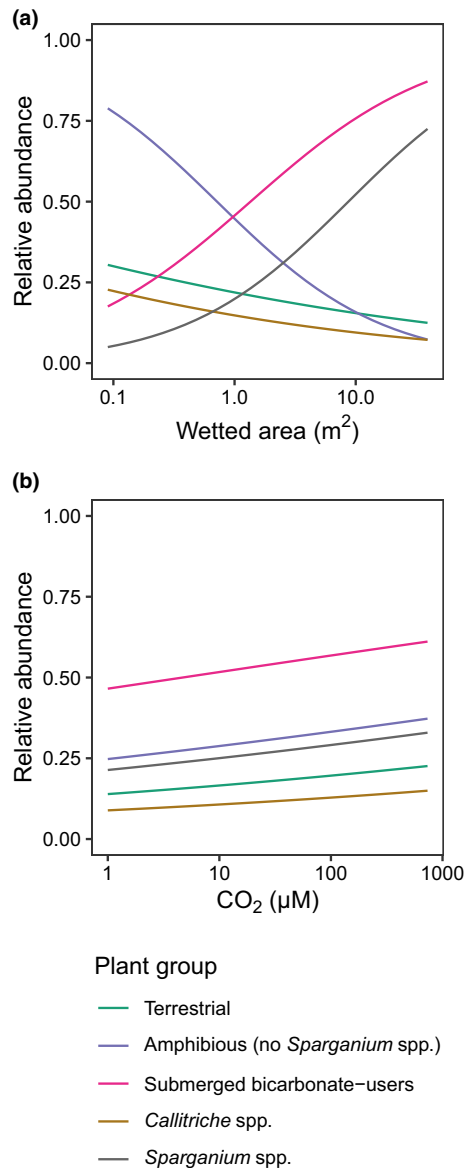


FIGURE 9 Relative abundance of species and plant types in the plant assemblages as a function of wetted area (a) and CO₂ concentrations (b) along the stream network. Relative abundance and wetted area at the sites are on logarithmic axes and data are modelled using beta-regression. Based on field data on inorganic carbon and wetted area and modified and re-calculated data on plant abundance from Riis et al. (2001)

morphometry, and management, all of which are likely to co-vary with the wetted area.

The distribution of CO₂ concentrations was strongly skewed to high levels and only 14% of measurements were below 2.5-fold air saturation (c. 45 µM) in the combined dataset for plant abundance and CO₂ at field sites. Beta-regression modelling showed that the influence of CO₂ concentration on plant abundance was similar for the different plant groups (i.e., the interaction between the two was not important; Figure 9b; Table S1). The relationship of plant group abundance to site dimension supported the fourth hypothesis, while the lack of significant spatial relations to underwater CO₂ did not.

4 | DISCUSSION

4.1 | Photosynthesis and growth of stream plants

All terrestrial and amphibious species were restricted to CO₂ use. The CO₂ compensation point offers an immediate measure of the ability to photosynthesise when the CO₂ concentration is close to air saturation. When completely submerged in air-saturated water, low photosynthesis prevents species from growing and surviving in most cases (Madsen et al., 1996). Thus, the mean CO₂ compensation point of submerged terrestrial species (16.8 µM) was close to the CO₂ concentration at air saturation leaving no photosynthetic surplus. Moreover, the mean CO₂ compensation points of the two amphibious plant types (11.8 and 8.4 µM) left little room for photosynthesis in air-saturated water to support the nocturnal respiratory costs and enable growth. In contrast, the median CO₂ compensation points of leaves in the air were much lower for many of the same terrestrial and amphibious species (2.6–3.6 µM) and the CO₂ conductance (i.e. photosynthesis relative to CO₂ concentration) was several-fold higher than under water, which ensured high photosynthesis and growth with air contact (Nielsen, 1993).

In CO₂-supersaturated water at stream sites with no lake influence, terrestrial wetland species obtained sufficient underwater photosynthesis to survive, but not enough to grow well. However, some terrestrial species and all amphibious species acclimate to submergence by increasing their CO₂ conductance (3–70-fold) through the formation of thinner filamentous or narrower leaves and the reduction of cuticle resistance (Frost-Christensen & Floto, 2007; Mommer et al., 2005, 2006; Nakayama et al., 2017; Nielsen, 1993). A few terrestrial species even attained higher underwater photosynthesis than amphibious species. The underwater photosynthetic performance of terrestrial plants was generally lower than that of amphibious plants, which was generally lower than permanently submerged plants in accordance with our first hypothesis. However, when observed from one species to the next, the change was gradual and with some overlap between plant groups.

The heterophyllous amphibious, non-European species, *Hygrophila difformis* have submerged filamentous leaves that apparently are able to use HCO₃⁻ to some extent (Horiguchi et al., 2019). Several species of heterophyllous European *Ranunculus* (e.g., *R. aquatilis*, *Ranunculus peltatus*, *Ranunculus trichophyllus*) have submerged filamentous leaves as their main leaf form and are capable of very efficient HCO₃⁻ use. Although we classify the *Ranunculus* species as submerged, when flowering, they form floating leaves with stomata that take up atmospheric CO₂ and have a well-developed cuticle to reduce transpiration (Nielsen & Sand-Jensen, 1993). Thus, boundaries between plant groups are fuzzy, due to the gradual intraspecific and interspecific changes in leaf anatomy, morphology, and physiology induced by phytohormones (e.g., abscisic acid and ethylene) in response to either air or water exposure (van Veen & Sasidharan, 2021).

In lake outlets with very low CO₂ concentrations, the submerged terrestrial and amphibious species photosynthesised at only 0%–8%

of the rates calculated at stream sites with no lake influence. In contrast, permanently submerged species capable of using HCO_3^- attained high photosynthetic rates at all stream sites, and rates for lake outlets were only modestly lower (c. 70%) relative to those at stream sites with higher CO_2 concentrations. These patterns correspond to our second hypothesis. Thus, regarding underwater photosynthesis, the HCO_3^- users are superior to amphibious species at all streams sites and particularly at low CO_2 concentrations in lake outlets.

The differences in aquatic photosynthesis and daily metabolic balances of green shoots between CO_2 users and HCO_3^- users concur with the daily RGR of rooted individuals in accordance with our third hypothesis. Thus, the amphibious CO_2 users died or barely survived in water close to air saturation irrespective of the HCO_3^- concentration, whereas the HCO_3^- users grew well when the HCO_3^- concentration surpassed the 1 mM level that is typical of alkaline waters (Madsen, 1993; Madsen et al., 1996). In water highly supersaturated with CO_2 (approximately 500–600 μM) both plant types may attain high RGR (c. 0.1 day^{-1}) corresponding to a doubling of the biomass within only one week. Amphibious species with the entire shoot exposed to air or with air contact of a floating leaf rosette (e.g., *Callitriche cophocarpa*) attained RGR of the same magnitude as at very high CO_2 concentrations under water, implying that the high diffusivity of CO_2 in air compensates for the low atmospheric CO_2 concentrations and stimulates growth (Madsen & Breinholt, 1995).

The improved growth in air was confirmed for the amphibious species, *Berula erecta*, *Mentha aquatica*, and *Myosotis palustris* in a comparison of 62 populations growing exposed to air and in neighbouring stream populations under water (Sand-Jensen & Jacobsen, 2002). Biomass and leaf production were about 4-fold higher with air exposure compared with submergence. The reduced performance under water can be explained mainly by lower CO_2 diffusivity, but the lower light availability and higher pressure drag of flowing water on plants and sediment, which causes shoot flapping, shoot dislodgement, and sediment erosion, may reduce the biomass as well (Madsen et al., 1993; Sand-Jensen & Frost-Christensen, 1998). Flowering, pollination, and seed production are also negligible for most submerged amphibious species (Schou et al., 2017). The advantages of submerged growth to amphibious species include expansion of their niche into the water, escaping intense competition on land, and surviving winter frost (Haslam, 2014). Flowing water also offers easy downstream dispersal of vegetative propagules followed by colonisation (Andersson et al., 2000; Boedeltje et al., 2003; Riis, 2008; Sand-Jensen & Frost-Christensen, 1999). The disadvantages of underwater growth are balanced by advantages to the extent that amphibious plants attain the same overall abundance as the permanently submerged plants in the small, shallow streams in Denmark and other European lowlands (Haslam, 2014; Riis et al., 2000).

4.2 | Influence of other environmental factors

Dissolved inorganic and total nutrient concentrations (inorganic + organic) were high in Danish streams in the 1990s and have remained

high ever since, although slightly decreasing in the farmland streams (Sand-Jensen & Lindegaard, 2004). Contemporary median concentrations of total nutrients in farmland streams (110 μg TP/L; 4.1 mg TN/L) and in natural streams at sites with no lake influence (60 μg TP/L; 1.0 mg TN/L; Thodsen et al., 2016) have remained above concentrations that are likely to induce limiting tissue concentrations for maximum growth rate of submerged plants and the vast majority of amphibious and terrestrial stream plants (Cavalli et al., 2015; Madsen & Cedergren, 2002; Madsen et al., 1998). The same conditions apply to nutrient-rich farmland streams throughout Europe (European Environmental Agency, 2009). Among amphibious and terrestrial species, Cavalli et al. (2015) reported a low proportion of tissue N and P slightly below the critical threshold for maximum growth rate suggesting the possibility of co-limitation by CO_2 and nutrients under submerged conditions. However, photosynthesis under water for these plant types virtually stopped by CO_2 limitation, which we therefore regard as much more important than nutrient limitation.

A special situation exists in outlets from lakes where phytoplankton growth influences CO_2 , nutrients and water clarity. In 92 alkaline Danish lakes, divided into deep and shallow, and located in calcareous catchments, the annual medians of nutrient (56 and 86 μg TP/L; 0.77 and 1.09 mg TN/L) and chlorophyll *a* concentrations (18 and 42 μg /L) were high and Secchi-depths were moderately low (0.9 and 2.0 m; Johansson et al., 2016). Although light limitation may influence plant growth in lake outlets with deep water, most lake outlets had shallow waters along the examined reaches (median 0.42 m). Moreover, concentrations of total nutrients were high, but phytoplankton growth in the lakes during summer will reduce dissolved nutrients in outlets to low levels (Johansson et al., 2016). At the same time CO_2 concentrations are particularly low in the lake outlets and co-limitation of growth by light and nutrients is possible. Nonetheless, the low CO_2 concentration remains particularly critical for CO_2 users under water because it strongly decreases photosynthesis and may prevent growth.

In nutrient-poor streams in uncultivated upland and mountain regions, co-limitation of plant growth by inorganic carbon and nutrients is more likely to occur. At many stations in the Scottish River Spey catchment, median concentrations of dissolved inorganic nutrients were extremely low (i.e., 1 μg ortho-P L^{-1} , 13 μg $\text{NO}_3^- + \text{NH}_4^+ \text{-N/L}$). However, only 7% and 19% of the foliar samples from submerged vascular plants had concentrations below the standard critical threshold for growth rate limitation by N and P, respectively (Demars & Edwards, 2007). Thus, there is a possibility of co-limitation by inorganic carbon and nitrogen under such nutrient-poor conditions. It is not possible to evaluate the issue further due to lack of field measurements of inorganic carbon and direct growth experiments.

4.3 | Distribution of stream plant groups

The occurrence of terrestrial and amphibious plants (excluding *Sparganium* spp.) decreased downstream with increasing stream

dimension as the streams became broader and deeper. The opposite pattern, with occurrences significantly increasing downstream, was exhibited by permanently submerged species (with or without *Sparganium* species) and selected groups of HCO_3^- users (e.g., *Potamogeton* spp., data not shown). The representation of species varied considerably among individual streams, probably because plant distribution is strongly related to longitudinal connectivity and associated means of dispersing and regenerating the populations (Demars & Harper, 2005; Riis, 2008). The management regime (e.g., channelisation and weed cutting) that also varies among streams markedly influences species distribution (Baatrup-Pedersen et al., 2002; Baatrup-Pedersen & Riis, 1999, 2004) and contribute to high scatter when data are combined from many sites and streams.

The observed positive relation of the abundance of terrestrial wetland and amphibious species to narrow, shallow streams was anticipated because these plants can easily expand into the water from land. Moreover, their shoots can elongate and obtain air contact in shallow water and thereby induce faster growth, compared to the conditions in deep water further downstream. Also, deep downstream sites usually have faster stream velocities than the bushy amphibious species can tolerate and they cannot reach into well-illuminated surface water as easily as permanently submerged species can (Baatrup-Pedersen & Riis, 1999; Sand-Jensen, 2008).

Carbon dioxide concentrations decreased significantly at sites located further downstream in stream networks in both our and other studies (Maberly et al., 2015; Neal et al., 1998). This may contribute to distribution patterns that indicate terrestrial and amphibious preferences for the most CO_2 -rich headwaters, in contrast to permanently submerged HCO_3^- users that benefit from the deeper, warmer downstream waters without being severely restricted by the lower CO_2 concentrations (Demars & Trémolières, 2009). CO_2 concentrations were predominantly high, but some sites had low concentrations. Thus, the differences between species groups in their abundance relative to CO_2 were not significant as we hypothesised, and perhaps veiled further by the co-variation of CO_2 with site dimensions. Although the influence of CO_2 on plant assemblage composition was not significant in the studies under review here, its influence is supported by two other studies. In a study of a complex of many factors in upper catchments of the Danube and Rhine, the CO_2 concentration was the best predictor of a plant community index (Demars & Trémolières, 2009). In a groundwater-fed stream, Maberly et al. (2015) found that underwater populations of amphibious CO_2 user, *Berula erecta* disappeared when CO_2 decreased downstream.

5 | CONCLUSIONS

This synthesis shows the pronounced influence of CO_2 and HCO_3^- concentrations on underwater photosynthesis and growth of stream plants in experimental studies, and the potential importance of air contact for amphibious plant growth. In contrast, the evaluations of field distributions and abundances of plants are much less distinct,

probably due to the influence of multiple interrelated geomorphological and environmental conditions and interacting biotic processes (Baatrup-Pedersen et al., 2013, 2016). Thus, the high occurrence of amphibious species in small lowland streams is promoted by their ability to expand from the transition zone between land and water and disperse with the streamflow. Moreover, amphibious plants may survive submerged during frosty winters, grow well at CO_2 supersaturation in spring, and attain air contact, faster growth, and seed production during summer. Once in the air, the importance of CO_2 supply from the water diminishes. Site-characteristic stream dimensions, steepness, and width of the land-water transition zone, as well as dissolved inorganic carbon concentrations, may all influence the occurrence of the different plant types and species in the stream assemblages. Thus, while photosynthesis and growth of species of different plant types under controlled experimental conditions are shown to be closely related to inorganic carbon supplies, this was not the case for their representation in plant assemblages under spatially and temporally highly diverse ecological conditions in the mostly CO_2 -rich lowland streams. A focused analysis of the influence of stream CO_2 on submerged plant assemblages awaits measurements encompassing the full CO_2 gradient under relatively similar conditions of other environmental variables, almost resembling a controlled experiment.

ACKNOWLEDGMENTS

We thank Independent Research Fund Denmark (0217-00112B) for a grant to K.S.J. and the project: "Supporting climate and biodiversity by rewetting low-lying areas", with which K.T.M. is associated. T.R. was supported by the VELUX Foundation. We thank David Stuligross for constructive comments and careful proofreading. Jens Christian Schou gave permission to use photos.

CONFLICT OF INTEREST

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

DATA AVAILABILITY STATEMENT

Data on photosynthesis and growth of stream plants are available in the previously published studies cited in the main text, and data on stream CO_2 concentrations and site dimensions are available from the authors upon request.

ORCID

Kenneth Thorø Martinsen  <https://orcid.org/0000-0001-8064-513X>

REFERENCES

- Abril, G., Bouillon, S., Darchambeau, F., Teodoru, C. R., Marwick, T. R., Tamoo, F., Ochieng Omengo, F., Geeraert, N., Deirmendjian, L., Polsenaere, P. & Baorges, A. V. (2015). Technical Note: Large overestimation of $p\text{CO}_2$ calculated from pH and alkalinity in acidic, organic-rich freshwaters. *Biogeosciences*, 12, 67–78. <https://doi.org/10.5194/bg-12-67-2015>

- Andersson, E., Nilsson, C., & Johansson, M. E. (2000). Plant dispersal in boreal rivers and its relation to the diversity of riparian flora. *Journal of Biogeography*, 27, 1095–1106. <https://doi.org/10.1046/j.1365-2699.2000.00481.x>
- Baatrup-Pedersen, A., Göthe, E., Riis, T., & O'Hare, M. T. (2016). Functional trait composition of aquatic plants can serve to disentangle multiple interacting stressors in lowland streams. *Science of the Total Environment*, 543, 230–238. <https://doi.org/10.1016/j.scitotenv.2015.11.027>
- Baatrup-Pedersen, A., Larsen, S. E., & Riis, T. (2002). Long-term effects of stream management on plant communities in two Danish lowland streams. *Hydrobiologia*, 481, 33–45. <https://doi.org/10.1023/A:1021296519187>
- Baatrup-Pedersen, A., Madsen, T. V., Riis, T., & Cavalli, G. (2013). Photosynthetic performance of submerged macrophytes from lowland stream and lake habitats with contrasting CO₂ availability. *New Phytologist*, 198, 1135–1142. <https://doi.org/10.1111/nph.12203>
- Baatrup-Pedersen, A., & Riis, T. (1999). Macrophyte diversity and composition in relation to substratum characteristics in regulated and unregulated Danish streams. *Freshwater Biology*, 42, 375–385. <https://doi.org/10.1046/j.1365-2427.1999.444487.x>
- Baatrup-Pedersen, A., & Riis, T. (2004). Impacts of different weed cutting practices on macrophyte species diversity and composition in a Danish stream. *River Research and Applications*, 20, 103–114. <https://doi.org/10.1002/rra.722>
- Boedeltje, G., Bakker, J. P., Bekker, R. M., Van Groenendael, J. M., & Soesbergen, M. (2003). Plant dispersal in a lowland stream in relation to occurrence and three specific life-history traits of the species in the species pool. *Journal of Ecology*, 91, 855–866. <https://doi.org/10.1046/j.1365-2745.2003.00820.x>
- Cavalli, G., Baatrup-Pedersen, A., & Riis, T. (2015). Nutrient availability and nutrient-use efficiency in plants growing in the transition zone between land and water. *Plant Biology*, 18, 301–305. <https://doi.org/10.1111/plb.12397>
- Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., & Melack, J. (2007). Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems*, 10, 172–185. <https://doi.org/10.1007/s10021-006-9013-8>
- Colman, J. A., Sorsa, K., Hoffmann, J. P., Smith, C. S., & Andrews, J. H. (1987). Yield and photosynthesis-derived critical concentrations of tissue phosphorus and their significance for growth of Eurasian water milfoil *Myriophyllum spicatum* L. *Aquatic Botany*, 29, 111–122. [https://doi.org/10.1016/0304-3770\(87\)90090-8](https://doi.org/10.1016/0304-3770(87)90090-8)
- Cribari-Neto, F., & Zeileis, A. (2010). Beta regression in R. *Journal of Statistical Software*, 34, 1–24. <https://doi.org/10.18637/jss.v034.i02>
- Demars, B. O. L., & Edwards, A. C. (2007). Tissue nutrient concentrations in freshwater aquatic macrophytes: High inter-taxon differences and low phenotypic response to nutrient supply. *Freshwater Biology*, 52, 2075–2086. <https://doi.org/10.1111/j.1365-2427.2007.01817.x>
- Demars, B. O. L., & Harper, D. M. (2005). Distribution of aquatic vascular plants in lowland rivers: Separating the effects of local environmental conditions, longitudinal connectivity and river basin isolation. *Freshwater Biology*, 50, 418–437. <https://doi.org/10.1111/j.1365-2427.2004.01329.x>
- Demars, B. O. L., & Trémoières, M. (2009). Aquatic macrophytes as bio-indicators of carbon dioxide in groundwater fed rivers. *Science of the Total Environment*, 407, 4752–4763. <https://doi.org/10.1016/j.scitotenv.2009.04.017>
- Douma, J. C., & Weedon, J. T. (2019). Analysing continuous proportions in ecology and evolution: A practical introduction to beta and Dirichlet regression. *Methods in Ecology and Evolution*, 10, 1412–1430. <https://doi.org/10.1111/2041-210X.13234>
- European Environmental Agency (2009). *Nitrate and Phosphorus Concentrations in European Freshwater Bodies between 1992/1993 and 2003*. https://www.eea.europa.eu/ds_resolveuid/EEA2FAD2-C0A5-49F9-84F2-F8222A089A8F
- Finlay, J. C. (2003). Controls of streamwater dissolved inorganic carbon dynamics in a forested watershed. *Biogeochemistry*, 62, 231–252. <https://doi.org/10.1023/A:1021183023963>
- Frost-Christensen, H., & Floto, F. (2007). Resistance to CO₂ diffusion in cuticular membranes of amphibious plants and the implication for CO₂ acquisition. *Plant, Cell & Environment*, 30, 12–18. <https://doi.org/10.1111/j.1365-3040.2006.01599.x>
- Frost-Christensen, H., Jørgensen, L. B., & Floto, F. (2003). Species specificity of resistance to oxygen diffusion in thin cuticular membranes from amphibious plants. *Plant, Cell & Environment*, 26, 561–569. <https://doi.org/10.1046/j.1365-3040.2003.00986.x>
- Gerloff, G. C., & Kromholz, P. H. (1966). Tissue analysis as a measure of nutrient availability for growth of angiosperm aquatic plants. *Limnology and Oceanography*, 11, 529–534. <https://doi.org/10.4319/lo.1966.11.4.0529>
- Haslam, S. M. (2014). *River Plants of Western Europe*. Cambridge University Press.
- Horiguchi, G., Nemoto, K., Yokoyama, T., & Hirotsu, N. (2019). Photosynthetic acclimation of terrestrial and submerged leaves in the amphibious plant *Hygrophila difformis*. *AoB PLANTS*, 11, plz009. <https://doi.org/10.1093/aobpla/plz009>
- Johansson, L. S., Søndergaard, M., Jeppesen, E., Landkildehus, F., Kjeldgaard, A., Sortkjær, L., Windolf, J., & Bøgestrand, J. (2016). Lake 2015 (in Danish with English summary). NOVANA. Aarhus University, DCE – National Center for Environment and Energy, number 207. <http://dce2.au.dk/pub/SR207.pdf>
- Kern-Hansen, U., & Dawson, F. H. (1978). The standing crops of aquatic plants of lowland streams in Denmark and the inter-relationships of nutrients in plants, sediment and water. *Proceedings of the European Weed Research Society, 5th International Symposium on Aquatic Weeds*, 143–150.
- Lauerwald, R., Laruelle, G. G., Hartmann, J., Ciais, P., & Regnier, P. A. G. (2015). Spatial patterns in CO₂ evasion from the global river network. *Global Biogeochemical Cycles*, 29, 534–554. <https://doi.org/10.1002/2014gb004941>
- Maberly, S. C., Berthelot, S. A., Stott, A. W., & Gontero, B. (2015). Adaptation by macrophytes to inorganic carbon down a river with naturally variable concentrations of CO₂. *Journal of Plant Physiology*, 172, 120–127. <https://doi.org/10.1016/j.jplph.2014.07.025>
- Maberly, S. C., & Madsen, T. V. (1998). Affinity for CO₂ in relation to the ability of freshwater macrophytes to use HCO₃⁻. *Functional Ecology*, 12, 99–106. <https://doi.org/10.1046/j.1365-2435.1998.00172.x>
- Maberly, S. C., & Madsen, T. V. (2002). Freshwater angiosperm carbon concentrating mechanisms: Processes and patterns. *Functional Plant Biology*, 29, 393–405. <https://doi.org/10.1071/PP01187>
- Mackereth, F. (1978). Water analysis: Some revised methods for limnologists. *Freshwater Biological Association Scientific Publication*, 36, 117.
- Madsen, T. V. (1984). Resistance to CO₂ fixation in the submerged aquatic macrophyte *Callitriche stagnalis* Scop. *Journal of Experimental Botany*, 35, 338–347. <https://doi.org/10.1093/jxb/35.3.338>
- Madsen, T. V. (1991). Inorganic carbon uptake kinetics of the stream macrophyte *Callitriche cophocarpa* Sendt. *Aquatic Botany*, 40, 321–332. [https://doi.org/10.1016/0304-3770\(91\)90078-j](https://doi.org/10.1016/0304-3770(91)90078-j)
- Madsen, T. V. (1993). Growth and photosynthetic acclimation by *Ranunculus aquatilis* L. in response to inorganic carbon availability. *New Phytologist*, 125, 707–715. <https://doi.org/10.1111/j.1469-8137.1993.tb03919.x>
- Madsen, T. V., & Breinholt, M. (1995). Effects of air contact on growth, inorganic carbon sources, and nitrogen uptake by an amphibious freshwater macrophyte. *Plant Physiology*, 107, 149–154. <https://doi.org/10.1104/pp.107.1.149>

- Madsen, T. V., & Brix, H. (1997). Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. *Oecologia*, 110, 320–327. <https://doi.org/10.1007/s004420050165>
- Madsen, T. V., & Cedergreen, N. (2002). Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology*, 47, 283–291. <https://doi.org/10.1046/j.1365-2427.2002.00802.x>
- Madsen, T., Enevoldsen, H., & Jørgensen, T. (1993). Effects of water velocity on photosynthesis and dark respiration in submerged stream macrophytes. *Plant, Cell & Environment*, 16, 317–322. <https://doi.org/10.1111/j.1365-3040.1993.tb00875.x>
- Madsen, T. V., Hahn, P., & Johansen, L. (1998). Effects of inorganic carbon on the nitrogen requirements of two submerged macrophytes, *Elodea canadensis* and *Callitriche cophocarpa*. *Aquatic Botany*, 62, 301–306. [https://doi.org/10.1016/S0304-3770\(98\)00087-4](https://doi.org/10.1016/S0304-3770(98)00087-4)
- Madsen, T. V., & Maberly, S. C. (1991). Diurnal variation in light and carbon limitation of photosynthesis by two species of submerged freshwater macrophyte with a differential ability to use bicarbonate. *Freshwater Biology*, 26, 175–187. <https://doi.org/10.1111/j.1365-2427.1991.tb01727.x>
- Madsen, T. V., Maberly, S. C., & Bowes, G. (1996). Photosynthetic acclimation of submersed angiosperms to CO₂ and HCO₃⁻¹. *Aquatic Botany*, 53, 15–30. [https://doi.org/10.1016/0304-3770\(95\)01009-2](https://doi.org/10.1016/0304-3770(95)01009-2)
- Madsen, T. V., Sand-Jensen, K., & Madsen, T. V. (1987). Photosynthetic capacity, bicarbonate affinity and growth of *Elodea canadensis* exposed to different concentrations of inorganic carbon. *Oikos*, 50(2), 176–182. <https://doi.org/10.2307/3565998>
- Manolaki, P., Mouridsen, M. B., Nielsen, E., Olesen, A., Jensen, S. M., Lauridsen, T. L., Baattrup-Pedersen, A., Sorrell, B. K., & Riis, T. (2020). A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms. *Journal of Environmental Management*, 274, 111181. <https://doi.org/10.1016/j.jenvman.2020.111181>
- Mommer, L., Pons, T. L., & Visser, E. J. (2006). Photosynthetic consequences of phenotypic plasticity in response to submergence: *Rumex palustris* as a case study. *Journal of Experimental Botany*, 57, 283–290. <https://doi.org/10.1093/jxb/erj015>
- Mommer, L., Pons, T. L., Wolters-Arts, M., Venema, J. H., & Visser, E. J. (2005). Submergence-induced morphological, anatomical, and biochemical responses in a terrestrial species affect gas diffusion resistance and photosynthetic performance. *Plant Physiology*, 139, 497–508. <https://doi.org/10.1104/pp.105.064725>
- Nakayama, H., Sinha, N. R., & Kimura, S. (2017). How do plants and phytohormones accomplish heterophylly, leaf phenotypic plasticity, in response to environmental cues. *Frontiers in Plant Science*, 8, 1717. <https://doi.org/10.3389/fpls.2017.01717>
- Neal, C., House, W. A., Jarvie, H. P., & Eatherall, A. (1998). The significance of dissolved carbon dioxide in major lowland rivers entering the North Sea. *Science of the Total Environment*, 210, 187–203. [https://doi.org/10.1016/S0048-9697\(98\)00012-6](https://doi.org/10.1016/S0048-9697(98)00012-6)
- Nielsen, S. L. (1993). A comparison of aerial and submerged photosynthesis in some Danish amphibious plants. *Aquatic Botany*, 45, 27–40. [https://doi.org/10.1016/0304-3770\(93\)90050-7](https://doi.org/10.1016/0304-3770(93)90050-7)
- Nielsen, S. L., & Sand-Jensen, K. (1993). Photosynthetic implications of heterophylly in *Batrachium peltatum* (Schränk) Presl. *Aquatic Botany*, 44, 361–371. [https://doi.org/10.1016/0304-3770\(93\)90077-A](https://doi.org/10.1016/0304-3770(93)90077-A)
- Pedersen, O., & Sand-Jensen, K. (1997). Transpiration does not control growth and nutrient supply in the amphibious plant *Mentha aquatica*. *Plant, Cell & Environment*, 20, 117–123. <https://doi.org/10.1046/j.1365-3040.1997.d01-12.x>
- R Core Team (2021). *R: A language and environment for statistical computing*.
- Rebsdorf, A., Thyssen, N., & Erlandsen, M. (1991). Regional and temporal variation in pH, alkalinity and carbon dioxide in Danish streams, related to soil type and land use. *Freshwater Biology*, 25, 419–435. <https://doi.org/10.1111/j.1365-2427.1991.tb01386.x>
- Riis, T. (2008). Dispersal and colonisation of plants in lowland streams: Success rates and bottlenecks. *Hydrobiologia*, 596, 341–351. <https://doi.org/10.1007/s10750-007-9107-0>
- Riis, T., Madsen, T. V., & Sennels, R. S. (2009). Regeneration, colonisation and growth rates of allofragments in four common stream plants. *Aquatic Botany*, 90, 209–212. <https://doi.org/10.1016/j.aquabot.2008.08.005>
- Riis, T., Sand-Jensen, K., & Larsen, S. E. (2001). Plant distribution and abundance in relation to physical conditions and location within Danish stream systems. *Hydrobiologia*, 448, 217–228. <https://doi.org/10.1023/A:1017580424029>
- Riis, T., Sand-Jensen, K., & Vestergaard, O. (2000). Plant communities in lowland Danish streams: Species composition and environmental factors. *Aquatic Botany*, 66, 255–272. [https://doi.org/10.1016/S0304-3770\(99\)00079-0](https://doi.org/10.1016/S0304-3770(99)00079-0)
- Sand-Jensen, K. (1983). Photosynthetic carbon sources of stream macrophytes. *Journal of Experimental Botany*, 34, 198–210. <https://doi.org/10.1093/jxb/34.2.198>
- Sand-Jensen, K. (2008). Drag forces on common plant species in temperate streams: Consequences of morphology, velocity and biomass. *Hydrobiologia*, 610, 307–319. <https://doi.org/10.1007/s10750-008-9446-5>
- Sand-Jensen, K., & Frost-Christensen, H. (1998). Photosynthesis of amphibious and obligately submerged plants in CO₂-rich lowland streams. *Oecologia*, 117, 31–39. <https://doi.org/10.1007/s004420050628>
- Sand-Jensen, K., & Frost-Christensen, H. (1999). Plant growth and photosynthesis in the transition zone between land and stream. *Aquatic Botany*, 63, 23–35. [https://doi.org/10.1016/s0304-3770\(98\)00107-7](https://doi.org/10.1016/s0304-3770(98)00107-7)
- Sand-Jensen, K., & Jacobsen, D. (2002). Herbivory and growth in terrestrial and aquatic populations of amphibious stream plants. *Freshwater Biology*, 47, 1475–1487. <https://doi.org/10.1046/j.1365-2427.2002.00890.x>
- Sand-Jensen, K., & Lindegaard, C. (2004). *Freshwater Ecology (in Danish)*. Gyldendal.
- Sand-Jensen, K., Pedersen, M. F., & Nielsen, S. L. (1992). Photosynthetic use of inorganic carbon among primary and secondary water plants in streams. *Freshwater Biology*, 27, 283–293. <https://doi.org/10.1111/j.1365-2427.1992.tb00540.x>
- Sand-Jensen, K., & Staehr, P. A. (2012). CO₂ dynamics along Danish lowland streams: Water-air gradients, piston velocities and evasion rates. *Biogeochemistry*, 111, 615–628. <https://doi.org/10.1007/s10533-011-9696-6>
- Schou, J. C., Moeslund, B., Baastrup-Spohr, L., & Sand-Jensen, K. (2017). *Danish water plants (in Danish)*. BFN-Press.
- Stets, E. G., Striegl, R. G., Aiken, G. R., Rosenberry, D. O., & Winter, T. C. (2009). Hydrologic support of carbon dioxide flux revealed by whole-lake carbon budgets. *Journal of Geophysical Research: Biogeosciences*, 114, G0100. <https://doi.org/10.1029/2008JG000783>
- Thodsen, H., Windolf, J., Rasmussen, J., Bøgestrand, J., Larsen, S. E., Tornbjerg, H., Tornbjerg, H., Ovesen, N. B., Kjeldgaard, A., & Wiberg-Larsen, P. (2016). Vandløb 2015 (in Danish, English summary). NOVANA. Aarhus University, DCE – National Center for Environment and Energy, number 206. URL: <http://dce2.au.dk/pub/SR206.pdf>
- Vadstrup, M., & Madsen, T. V. (1995). Growth limitation of submerged aquatic macrophytes by inorganic carbon. *Freshwater Biology*, 34, 411–419. <https://doi.org/10.1111/J.1365-2427.1995.TB00899.X>
- van Veen, H., & Sasidharan, R. (2021). Shape shifting by amphibious plants in dynamic hydrological niches. *New Phytologist*, 229, 79–84. <https://doi.org/10.1111/nph.16347>
- Voensenek, L., Rijnders, J., Peeters, A., Van de Steeg, H., & De Kroon, H. (2004). Plant hormones regulate fast shoot elongation under

- water: From genes to communities. *Ecology*, 85, 16–27. <https://doi.org/10.1890/02-740>
- Wallin, M. B., Grabs, T., Buffam, I., Laudon, H., Ågren, A., Öquist, M. G. & Bishop, K. H. (2013). Evasion of CO₂ from streams – The dominant component of the carbon export through the aquatic conduit in a boreal landscape. *Global Change Biology*, 19, 785–797. <https://doi.org/10.1111/gcb.12083>
- Wallin, M. B., Öquist, M. G., Buffam, I., Billett, M. F., Nisell, J., & Bishop, K. H. (2011). Spatiotemporal variability of the gas transfer coefficient (KCO₂) in boreal streams: Implications for large scale estimates of CO₂ evasion. *Global Biogeochemical Cycles*, 25, GB3025. <https://doi.org/10.1029/2010gb003975>
- Winkel, A., & Borum, J. (2009). Use of sediment CO₂ by submersed rooted plants. *Annals of Botany*, 103, 1015–1023. <https://doi.org/10.1093/aob/mcp036>
- Winkel, A., Visser, E. J. W., Colmer, T. D., Brodersen, K. P., Voeselek, L. A. C. J., Sand-Jensen, K., & Pedersen, O. (2016). Leaf gas films, underwater photosynthesis and plant species distributions in a

flood gradient. *Plant, Cell & Environment*, 39, 1537–1548. <https://doi.org/10.1111/pce.12717>

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Sand-Jensen, K., Riis, T., & Martinsen, K. T. (2022). Photosynthesis, growth, and distribution of plants in lowland streams—A synthesis and new data analyses of 40 years research. *Freshwater Biology*, 00, 1–17. <https://doi.org/10.1111/fwb.13915>