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Impact of nutrients and water level changes on submerged macrophytes along a temperature gradient: a Pan-European mesocosm experiment

Running head: Climate change effects on lake macrophytes

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Abstract

Submerged macrophytes are of key importance for the structure and functioning of shallow lakes and can be decisive for maintaining them in a clear water state. The ongoing climate change affects the macrophytes through changes in temperature and precipitation, causing variations in nutrient load, water level and light availability. To investigate how these factors jointly determine macrophyte dominance and growth, we conducted a highly standardised pan-European experiment involving the installation of mesocosms in lakes. The experimental design consisted of mesotrophic and eutrophic nutrient conditions at 1 m (shallow) and 2 m (deep) depth along a latitudinal temperature gradient with average water temperatures ranging from 14.9 to 23.9 °C (Sweden to Greece) and a natural drop in water levels in the warmest countries (Greece and Turkey). We determined Percent Plant Volume Inhabited (PVI) of submerged macrophytes on a monthly basis for five months and dry weight at the end of the experiment. Over the temperature gradient, PVI was highest in the shallow mesotrophic mesocosms followed by intermediate levels in the shallow eutrophic and deep mesotrophic mesocosms, and lowest levels in the deep eutrophic mesocosms. We identified three pathways along which water temperature likely affected PVI, exhibiting: (1) a direct positive effect if light was not limiting, (2) an indirect positive effect due to an evaporation-driven water level reduction, causing a non-linear increase in mean available light, (3) an indirect negative effect through algal growth and, thus, high light attenuation under eutrophic conditions. We conclude that high temperatures combined with a temperature-mediated water level decrease can counterbalance the negative effects of eutrophic conditions on macrophytes by enhancing the light availability. While a water level reduction can promote macrophyte dominance, an extreme

reduction will likely decrease macrophyte biomass and, consequently, their capacity to function as a carbon store and food source.

Keywords: climate change, mesocosm, latitudinal gradient, nutrients, water temperature, water level, macrophytes, shallow lakes

Introduction

Submerged macrophytes are of key importance for the structure and functioning of shallow lakes (Scheffer, Carpenter, Foley, Folke, & Walker, 2001; Burks et al., 2006; Hilt, Brothers, Jeppesen, Veraart, & Kosten, 2017). Among other services, they provide habitat and shelter for zooplankton, macroinvertebrates and small fish (Burks, Jeppesen & Lodge, 2001; Blindow, Hargeby, & Hilt, 2014), stabilise the sediment (Madsen, Chambers, James, Koch, & Westlake, 2001) and serve as a carbon store (Brothers et al., 2013) and food source (Schmieder, Werner & Bauer, 2006; Paice, Chambers, & Robson, 2017). At the same time, macrophytes compete with phytoplankton and periphyton for light and nutrients and have the potential to maintain shallow lakes in a clear water state (Scheffer & Jeppesen, 2007; Van Donk & Van de Bund, 2002; Hilt et al., 2006; Phillips, Willby, & Moss, 2016). However, submerged macrophytes are highly sensitive to environmental changes, including alterations in light availability, nutrients and temperature (Lacoul & Freedman, 2006; Bornette & Puijalon, 2011). All three parameters are expected to be simultaneously influenced by climate change. To anticipate how they will affect shallow lake macrophytes, a good understanding of their isolated and interacting effects is required.

Regional climate models predict higher summer temperatures for all of Europe, accompanied by higher precipitation in Northern but lower precipitation in Central and Southern Europe (IPCC, 2014). Changes in precipitation will likely translate to changes in lake water levels. This effect might be particularly strong in the Mediterranean region where the prolonged dry periods (Milly, Dunne & Vecchia, 2005; Trenberth et al., 2014), together with temperature-driven evaporation and growing demand for water use, are expected to dramatically alter lake hydrology and cause a decline of water levels (Coops, Beklioğlu, & Crisman, 2003; Beklioğlu, Altınayar, & Tan, 2006; Papastergiadou, Kagalou, Stefanidis, & Retalis, 2010; Beklioğlu et al., 2017). However, water column depth is an important factor affecting light availability, which in turn is a crucial component for the competitive success of submerged macrophytes against other primary producers (Sand-Jensen & Borum, 1991; Roberts, Kroker, Körner, & Nicklisch , 2003). A reduction of water column depth increases the light

availability at the sediment and the mean available light (MAL) over the water column non-linearly. These two aspects of light availability are particularly critical at the beginning of the growing season for short-growing plants, but also for tall-growing ones because MAL is likely decisive for whether the entire water volume can be used for efficient photosynthesis and the formation of dense macrophyte stands (Chen et al., 2016; Lauridsen, Mønster, Raundrup, Nymand, & Olesen, 2020). Empirical evidence from various climate regions including subtropical (Mazzeo et al., 2003; Havens, East, & Beaver, 2007), northern temperate (Nõges & Nõges, 1999; Rip, Ouboter, & Los, 2007; Liira, Feldmann, Mäemets, & Peterson, 2010) and Mediterranean climates (Beklioğlu et al., 2006; Coppens et al., 2016a; Özkan et al., 2010; Bucak et al., 2012; Beklioğlu et al., 2017) confirms a positive effect of a water level decline on submerged macrophyte growth due to improved light conditions in the water column and expansion of the littoral zone (Coops et al., 2003; Beklioğlu et al., 2006; Beklioğlu & Tan, 2008; Kosten et al., 2009; Stefanidis & Papastergiadou, 2013).

On the other hand, an extreme water level decline and drought conditions could hamper macrophyte growth through increased air exposure, causing dry out of above-ground macrophytes (Thomaz, Pagioro, Bini, & Murphy, 2006; Loverde-Oliveira, Huszar, Mazzeo, & Scheffeer, 2009) or a direct disruption of the littoral zone (Blindow, 1992; Blindow, Andersson, Hargeby, & Johansson, 1993; Beklioğlu et al., 2006). Moreover, macrophyte growth may also be restricted by overgrowth of filamentous algae and cyanobacteria that are favoured by the improved light conditions at lower depth and the higher temperatures, as well as by increased resuspension (Bresciani, Bolpagni, Braga, Oggioni, & Giardino, 2012; Jeppesen et al., 2015; Hilt et al., 2017).

Changes in precipitation are also expected to affect nutrient availability. Increased precipitation may possibly enhance surface runoff and in this way increase the nutrient loading of lakes and trigger eutrophication (Jeppesen et al., 2011). In contrast, more intense and prolonged droughts and a substantial reduction of surface runoff are often accompanied by decreased external nutrient loading (Jeppesen et al., 2009, 2011; Özen, Karapınar, Kucuk, Jeppesen, & Beklioğlu, 2010; Coppens et al., 2016a). The reduced nutrient loading may, though, be counterbalanced by a higher internal nutrient loading, which combined with a reduced lake volume and diminished nutrient retention

capacity may lead to rising nutrient concentrations (Özen et al., 2010; Jeppesen et al., 2015; Coppens et al., 2016a). Although plant growth can benefit from nutrient-rich sediments (Barko, Gunnison, & Carpenter, 1991; Carr & Chambers, 1998; Angelstein et al., 2009; Martin & Coetzee, 2014), excessive nutrients can indirectly and negatively influence the abundance and richness of submerged macrophytes by stimulating phytoplankton growth, thereby increasing the light attenuation (Sand-Jensen, Riis, Vestergaard, & Larsen, 2000; Jeppesen et al., 2014; Olsen et al., 2015). Moreover, high nutrient concentrations can stimulate periphyton growth, resulting in an additional reduction of the light reaching the plants (Jeppesen et al., 2014; Cao et al., 2015; Olsen et al., 2015). Eventually, the submerged macrophytes may even disappear, which in turn enhances turbidity (Moss, 1990; Scheffer et al., 2001).

Temperature might not only indirectly affect macrophytes due to its influence on nutrient loading and evaporation, also direct effects are conceivable. Knowledge of this is scarce, but photosynthetic rates will expectedly increase with rising temperatures (Farquhar, von Caemmerer, & Berry, 1980; Brown, Gillooly, Allen, Savage, & West, 2004), and this will likely translate into higher growth rates. Accordingly, several studies report a positive effect of temperature on the photosynthetic rate, growth rate, biomass and distribution of macrophytes, albeit considerable differences occur between species (Madsen & Brix, 1997; Rooney & Kalff, 2000; Riis et al., 2012; Li, Lan, Chen, & Song, 2018). Submerged macrophytes may benefit from higher temperatures, potentially augmenting their resistance to higher nutrient levels as they can grow faster to the surface (McKee et al., 2002; Romo et al., 2004; Kosten et al., 2009). Yet, due to the higher sensitivity of respiration than of photosynthesis to a temperature rise, growth rates might only increase up to an optimum temperature beyond which they will decline (Körner, 1991; Riis et al., 2012). At high temperatures and high nutrient conditions, phytoplankton and periphyton might outcompete submerged macrophytes, since they also have an advantageous position in terms of light availability (Köhler, Hachol, & Hilt, 2010; Olsen et al., 2015). In addition, increased sediment respiration with warming (Liboriussen et al., 2011) can reduce the oxygen supply for the roots of plants and subsequently their

biomass (Sand-Jensen, Møller, & Borum, 2015). Thus, the effect of temperature on submerged macrophytes is likely highly contingent on other environmental factors.

We conducted a highly standardised Pan-European mesocosm experiment to better understand the complex effect of the ongoing and future climate change on submerged macrophyte abundance and dominance in shallow lakes caused by the simultaneous changes in temperature, nutrient availability and water level – three of the most important determining factors for macrophytes. The unique experimental set-up combined a replicated factorial design consisting of two nutrient conditions (mesotrophic: low nutrient (L) and eutrophic: high nutrient (H)) and two water levels (1 m: shallow (S) and 2 m: deep (D)) with a gradient design along a latitudinal temperature gradient ranging from Sweden to Greece. This made it possible to set up the mesocosms within lakes to ensure natural water temperature conditions and water level fluctuations due to precipitation or evaporation. An inoculation with local fauna and flora ensured natural adaptation of the communities to the tested climate conditions.

We hypothesised that (Figure 1): 1) Mesotrophic conditions in combination with low water levels (SL mesocosms) lead to favourable light conditions that enable high macrophyte growth (measured as Percent Plant Volume Inhabited (PVI %)). In this situation, higher temperatures benefit macrophyte growth directly; 2) high water levels with mesotrophic conditions (DL mesocosms) or low water levels with eutrophic conditions (SH mesocosms) reduce the MAL, which in turn reduces macrophyte growth. In this situation, higher temperatures only have a dampened direct positive effect on macrophyte growth; 3) high water levels with eutrophic conditions (DH mesocosms) reduce the MAL to a critical level for macrophyte growth. In this situation, higher temperatures do not directly benefit macrophyte growth; 4) higher temperatures indirectly stimulate macrophyte growth due to evaporation-driven reduction of water levels with an accompanying increase in MAL. We expected these indirect effects of temperature to be particularly conspicuous at low water level conditions (SL and SH mesocosms) where MAL is exceptionally sensitive to changes in water column depth (Appendix S2, Figure S1).

Materials and methods

Experimental set-up and sampling

The mesocosm experiments were carried out along a latitudinal gradient in six countries: Sweden, Estonia, Germany, Czech Republic, Turkey and Greece (Table A1), thus realising a gradient design for the temperature parameter. To ensure natural water temperature conditions, the mesocosms were installed within the lakes using floating pontoon bridges and arranged in two rows with 8 mesocosms along each. Within each country, a 2×2 factorial design was employed with two water levels (shallow: 1 m, deep: 2 m) and two nutrient levels (high: 200 µg L^{-1} total phosphorus (TP) and 2.0 mg L^{-1} total nitrogen (TN), low: 25 µg L^{-1} TP and 0.5 mg L^{-1} TN), representing mesotrophic (low) and eutrophic (high) conditions. The resulting four treatments – shallow with low nutrient level (SL), shallow with high nutrient level (SH), deep with low nutrient level (DL) and deep with high nutrient level (DH) – were replicated randomly four times.

The experiment lasted six months from May to November 2011. The lakes selected for the experiment fulfilled strict criteria and the synchronised set-up, samplings and experimental manipulations were performed according to a common protocol that was strictly followed by all countries. A detailed description of the experimental set-up and the common protocol is given in Landkildehus et al. (2014).

Fibreglass mesocosms from the same manufacturer with a diameter of 1.2 m and heights of 1.2 m (shallow) and 2.2 m (deep) were used in all countries. First, a 10 cm sediment layer consisting of 90% washed sand (diameter < 1 mm) and 10% lake sediment taken from a nearby oligo-mesotrophic lake was added to the mesocosms. Before the addition, the lake sediment was equilibrated to the desired initial nutrient levels (25 µg L^{-1} TP in the low and 200 µg L^{-1} TP in the high nutrient

mesocosms) in the laboratory for two months to eliminate the risk of the sediment acting as an uncontrolled source or sink of nutrients during the experiment. After sediment addition, filtered water from the oligo-mesotrophic lakes where the mesocosms were installed was added to the mesocosms except in Germany and the Czech Republic where tap water was used as the TP concentrations of their lake water were higher than 25 µg L^{-1} TP. However, in these countries, we ensured that the water was dechlorinated and not harmful to most organisms. To ensure adaptation of natural biota to experimental conditions in all countries, mixed plankton and sediment inocula collected from five local lakes (with a TP gradient of 25 to 200 µg L^{-1}) were added to the mesocosms four days after set-up.

Four days after the sediment and plankton inoculation, macrophytes and fish were added to the mesocosms. The submerged macrophyte Eurasian watermilfoil (*Myriophyllum spicatum*) was used because it is a common and dominant plant in all countries. The plants were collected just before initiation of the experiment and kept cool until transplantation in all countries except Germany. There, the plants were collected during the autumn of the previous year and overwintered in a greenhouse to guarantee the availability of watermilfoil at the beginning of the experiment. Before planting, the macrophytes were left in carbonated water for 15 minutes to eliminate invertebrates and snails. Five to ten cm long apical shoots of eight plants were planted evenly in the central zone (diameter 0.5 m) of each mesocosm. Stones, weighing around 5 g, were attached to the plants to allow them to reach the sediment. Six three-spined stickleback individuals, *Gasterosteus aculeatus*, were added to the mesocosms in a 1:1 sex ratio, except in Sweden and Greece where two individuals of roach (*Rutilus rutilus*) and six western mosquitofish (*Gambusia affinis*), respectively, had to be used instead for different local reasons (see Landkildehus et al., 2014). These two fish species feed on similar food sources as three-spined stickleback (Hynes, 1950; Offill & Walton, 1999), however.

After the completion of the set-up, dissolved mixtures of calcium nitrate ($Ca(NO_3)_2$) and disodium phosphate (Na_2HPO_4) were added monthly to the mesocosms as N and P sources to achieve the

aimed nutrient levels. Detailed information about loading rates can be found in Landkildehus et al. (2014) and Coppens et al. (2016b).

Water temperature, water depth and chemical and biological variables were measured monthly. The water samples for determination of chemical and biological variables were kept frozen until analysis for TP, TN and chlorophyll-*a* (Chl-*a*), which were determined using standard methods in each country. Water temperature was determined *in situ* using a multi-probe tool.

Light attenuation coefficient (K_d) and mean available light (MAL) estimation

Photosynthetically active radiation (PAR) profiles were measured in each mesocosm monthly with a light meter. The measurements were conducted at midday (between 11 a.m. and 1 p.m.) every 10 cm throughout the water column until the light had decreased up to 10% of the incoming light. The diffuse attenuation coefficient of light (K_d) was calculated according to the Beer-Lambert law, computing and averaging a light attenuation coefficient for each layer:

$$K_d = \frac{1}{N-1}\sum_{i=1}^{N-1}\frac{ln\frac{E_{zi}}{E_{zi+1}}}{Z_{i+1}-Z_i} \quad (2)$$

where E_{zi} is PAR at $z_i th$ depth, and $E_{zi+1}$is PAR at $z_{i+1} th$depth.

Subsequently, mean K_d values for July to November were calculated. Light profiles were missing for August and September in Estonia (due to errors in data loggers) and the missing attenuation coefficients were therefore linearly interpolated since none of the attenuation coefficients from the other countries indicated strong seasonality. Moreover, due to the extreme water level decrease in the Greek shallow mesocosms, it was not possible to measure light from September onwards. We used the same high attenuation coefficient from the last available measurements for the missing

months because visual observation indicated similar high light attenuation throughout the experiment (Scharfenberger et al., 2019).

Mean available light (MAL) over the water column was estimated based on the Beer-Lambert law as follows:

$$MAL = \frac{PAR_0}{K_d * d}(1 - \beta)(1 - exp(-K_d * d)) \quad (3)$$

where d is water depth, PAR_0 incident PAR at the surface and K_d the attenuation coefficient, and $\beta = 0.1$ is a correction factor for reflection and backscatter (Staehr, Sand-Jensen, Raun, Nilsson, & Kidmose, 2010). MAL has the same unit as PAR_0 and increases linearly with this; in contrast, it decreases exponentially with increasing water depth d and increasing attenuation coefficient K_d.

Macrophyte growth

Macrophyte coverage and water depth in each mesocosm were determined monthly from July to November. Presence of macrophyte species other than *M. spicatum* was also recorded at each sampling event. Macrophyte coverage was estimated by visual inspection. The following scale was used: 0: no plants, 1: 0-5%, 2: 5-25%, 3: 25-50%, 4: 50-75%, 5: 75-95% and 6: 95-100% for coverage. The estimated scale values were converted to mid-range percentages, i.e.: 0 ≙0%, 1 ≙2.5%, 2 ≙15%, 3 ≙37.5%, 4 ≙62.5%, 5 ≙85% and 6≙ 97.5%. Furthermore, the average height of the plants and water depth were recorded during each sampling event. PVI was calculated using the following formula by Canfield et al. (1984):

$$PVI(\%) = \frac{coverage(\%) \times average\ plant\ height}{water\ depth} \quad (1)$$

PVI calculations were based on the total coverage of all macrophytes and filamentous algae.

At the end of the experiment, the aquatic vegetation in all mesocosms was harvested, determined to species level and dried at 60 °C for 24 hours to calculate the dry weight of macrophytes and, if

present, filamentous algae. Biomass per area (g m^{-2}) was calculated by dividing the measured weight by the surface area of the mesocosms. Dry weight data for each macrophyte species were available in all countries except Greece. Four mesocosms (one DL, two DH and one SH) in Germany and three mesocosms (two SL and one SH) in Czech Republic were excluded from the analyses since they were flooded or sank during the experiment.

Data analyses

Although the experimental period was from May to November, we used the data collected between July and November 2011 for data analyses since it took some months for the macrophytes to stabilise. Mesocosm-wise averages of all physicochemical variables and PVI were calculated for the period July-November 2011. In Greece, where an extreme water level decrease from October onwards prevented sampling, we calculated mean TP, TN and Chl-*a* for the period July-October only. All statistical analyses were conducted in R version 3.6.0 (R Core Team, 2019).

Regression analysis

Linear mixed-effects regression was used to test the effects of temperature, nutrient and depth levels on mean PVI, macrophyte biomass (DW), Chl-*a*, K_d and MAL using the "*nlme"* package (Pinheiro et al., 2019). Data from all the mesocosms were analysed together in a single model using nutrient, depth and temperature as fixed factors and country as random factor. The intercept was changed to the average of the investigated temperature range at 18 °C. Model selection for the random effect structure was based on a model with the full set of potentially fixed effects using REML (restricted maximum likelihood) estimation. Model validation was based on visual inspection of the residual plots, including their relation to all predictor variables. In cases of violation of the normality assumption, the dependent variables were ln or ln+1 (natural logarithm) transformed. However, for better readability, we adopted the simplified notation (without ln or ln+1 abbreviations) throughout the text. In case of violation of the heterogeneity assumption, we

modelled the residual variance structure. The decision for the best residual variance structure was based on Akaike information criteria (AIC). Model selection for the fixed effect structure was based on the lowest AICc (AIC with correction for small sample sizes) from all potential models ("MuMIn" package, Bartón, 2015) using ML (maximum likelihood) estimation. Final models with optimal fixed and random effect structures were again estimated using REML estimation and reported. The model fit was assessed by conditional (variance explained by fixed effects) and marginal (variance explained by fixed and random effects) coefficients of determination ("MuMIn" package, Bartón, 2015) and by the squared correlation between predicted and original values. In cases of significant factor and covariate interactions, we conducted a pairwise comparison of trends and a pairwise comparison between treatments in 0.1 °C steps over the temperature gradient using two-tailed t-tests for pairwise comparisons of least-square-means ("emmeans" package; Lenth, 2020). Effect sizes were calculated using standardised predictors following Gelman (2008). The same basic approach was used when alternative covariates were tested instead of temperature or when testing temperature and treatment effects for other variables.

Correlation-based data analyses

The linear and the monotonic relationships between and among all predictor and response variables were investigated using principal component analysis (PCA, "prcomp" function in the stats package, R Core Team, 2019) and Spearman correlation, respectively. All variables were scaled and centered before PCA.

To disentangle the effects of highly correlated variables, semi-partial Spearman correlation was used ("spcor" function in "ppcor" package; Kim, 2012). Semi-partial Spearman correlation coefficient r and percentile 95% confidence intervals were bootstrapped over mesocosms with 1000 repetitions ("boot" package, Canty & Ripley, 2015; "boot" and "boot.ci" function).

Regression tree analysis for PVI was used to determine threshold values for the most important predictor variables as identified by the regression tree ("partykit" package, Horthorn & Zeileis, 2015 Hothorn, Hornik, & Zeileis, 2006).

Estimation of water level change effects on MAL and PVI

To assess the effect of water level change on MAL, theoretical MAL values were calculated based on equation (3) assuming constant water levels of 0.9 m and 1.9 m for the shallow and deep mesocosms, respectively (i.e. d = 0.9 (S) or d = 1.9 (D)). Potential negative effects of water column depth on K_d, as indicated by the semi-partial Spearman correlation, were thereby not included in the calculation. Expected PVI values for constant water levels of 0.9 m and 1.9 m for the shallow and deep mesocosms, respectively, were predicted based on the mixed-effects regression model with MAL as a covariate.

Results

Water temperature gradient

During the experiment, the mean water temperature (July-November) was highest in Greece, followed by Turkey, Germany, Estonia, Czech Republic and Sweden. Overall, a temperature gradient from 14.9 °C to 23.9 °C was covered (Figure 2a; Appendix S1, Table S1).

Hypothesis 1: Favourable light conditions in the SL mesocosms allow high macrophyte growth and a direct positive temperature effect

Over the entire temperature gradient (14.7 °C-24.2 °C), the SL mesocosms had a significantly higher PVI compared with the deep mesocosms (DL and DH) (Figure 3a; Table 1, 2b) and also compared with the SH mesocosms over the warmer temperature range (Table 2b). PVI increased significantly under mesotrophic (L) conditions (21.9% [PVI $°C^{-1}$] (L)) along the temperature gradient and was more than twice higher than the values observed in the eutrophic mesocosms (Table 1, 2a).

Concomitantly, the mean available light (MAL) in the SL mesocosms was significantly higher than in all the other treatments (geometric mean: 156.0 [µmol photons m^{-2} s^{-1} $°C^{-1}$]) (Figure 3b; Table 2a, 2b) and increased significantly over the entire temperature gradient (SL: 9.8% [µmol photons m^{-2} s^{-1} $°C^{-1}$]) (Table 1, 2a). The increase was significantly higher in the SL mesocosms than in all the other treatments (Table 2b).

The high MAL in the SL mesocosms (geometric mean: SL: 8.6 [µmol g Chl-*a* l^{-1}]) was accompanied by significantly lower Chl-*a* compared with the eutrophic mesocosms (SH and DH) and comparable with those in the DL mesocosms (Figure 3c, Table 1, 2a, 2b). In all treatments, Chl-*a* increased with temperature at the same rate (10.2% [µg Chl-*a* l^{-1} $°C^{-1}$]; however, this trend was only significant at the 94% level (Table 1, 2a).

The attenuation coefficient (K_d) was significantly lower in the SL mesocosms than in the SH mesocosms for the entire temperature gradient and significantly lower compared with the DH mesocosms for the warmer temperature range (Figure 3d; Table 1, 2b). However, K_d was significantly higher in the SL mesocosms than in the DL mesocosms along the colder temperature range (Table 2b). The increase in K_d with increasing temperatures was significant (6.1% [m^{-1} °C^{-1}] and comparable with that in the DL mesocosms (Table 1, 2a, 2b). However, the increase was significantly lower than in the eutrophic mesocosms (Table 2b).

Hypothesis 2: Reduced light availability in the DL or SH mesocosms allows only reduced macrophyte growth and a dampened direct positive temperature effect

PVI in the SH mesocosms did not differ significantly from the PVI in the DL mesocosms but was significantly higher than in the DH mesocosms (Figure 3a; Table 1, 2b). The DL mesocosms had a significantly higher PVI compared with the DH mesocosms in the higher temperature range (Table 2b). The PVI increase with temperature in the DL mesocosms was the same as in the SL mesocosms (21.9% [PVI °C^{-1}] (L)). Similarly, PVI increased with temperature at the same rate in the SH and the DH mesocosms and was not significant (8.4% [PVI °C^{-1}] (H)) (Table 1, 2a).

MAL was significantly higher in the SH mesocosms than in the DL mesocosms over the entire temperature gradient (geometric mean: SH: 129.7 [µmol photons m^{-2} s^{-1} °C^{-1}], DL: 79.3 [µmol photons m^{-2} s^{-1} °C^{-1}]) (Figure 3b; Table 2b). The MAL trend over the entire water temperature gradient in the SH mesocosms (SH: 8.9% [µmol photons m^{-2} s^{-1} °C^{-1}])) was significantly higher than in the DL mesocosms and not significantly different from the SL mesocosms. The trend in MAL for the DL mesocosms (DL: 2.3% [µmol photons m^{-2} s^{-1} °C^{-1}]) was significantly higher than in the DH mesocosms. However, for both the DL and SH mesocosms the confidence intervals include zero (Table 1, 2a, 2b).

Chl-*a* in the SH mesocosms was significantly higher compared with the DL mesocosms (geometric mean: SH: 17.7 [µg Chl-*a* l^{-1}], DL: 6.6 [µg Chl-*a* l^{-1}]) (Figure 3c; Table 2a, 2b).

The differences between the DL and SH mesocosms in regard to Chl-*a* were accompanied by a significantly higher K_d in the SH mesocosms compared with the DL mesocosms (geometric mean: SH: 2.9 [m^{-1}], DL: 1.4 [m^{-1}]) (Figure 3d; Table 2a, 2b). In both treatments, K_d significantly increased with water temperature with approximately equal rates (SH: 6.1% [m^{-1} °C^{-1}], DL: 9.6% [m^{-1} °C^{-1}]) (Table 1, 2a, 2b).

Hypothesis 3: Unfavourable light conditions in the DH mesocosms reduce macrophyte growth to critical levels, preventing them from benefiting from higher temperatures.

The DH mesocosms had the lowest PVI (geometric mean: DH: 1.1 [PVI %]), which was significantly lower compared with the SH mesocosms for the entire water temperature gradient and the DL mesocosms in the warmer temperature range (Figure 3a; Table 1, 2a,2b). The PVI increase with temperature in the DH mesocosms was similar to that in the SH mesocosms, and thus significantly lower than in the mesotrophic mesocosms (Table 2a, 2b).

MAL levels in the DH mesocosms were significantly lower than in all other treatments (geometric mean DH: 56.7 [µmol photons m^{-2} s^{-1} °C^{-1}]) (Figure 3b; Table 2a, 2b), and the change in MAL with water temperature was significantly lower in the DH than in all other treatments and not significant (DH: - 1.9% [µmol photons m^{-2} s^{-1} °C^{-1}]), (Table 1, 2a, 2b).

Chl-*a* was significantly higher in the DH mesocosms than in all the other treatments (geometric mean at the reference of 18 °C: DH: 37.6 [µg Chl-*a* l^{-1}]) (Figure 3c; Table 2a, 2b).

K_d in the DH mesocosms was significantly lower than in the SH mesocosms only for the colder temperature range and significantly higher than in the SL mesocosms only for the warmer temperature range (Figure 3d, Table 2b). However, for the entire temperature range, K_d was significantly higher in the DH mesocosms than in the DL mesocosms (Table 2b). The increase in K_d with water temperature was significant (Table 2a) and significantly higher compared with the eutrophic mesocosms but not the SH mesocosms (DH: 13.3% [m^{-1} °C^{-1}]) (Table 1, 2b).

Differentiating between water temperature and MAL as drivers of PVI

PCA and the Spearman correlation coefficient identified MAL as the strongest co-variate with PVI (Figure 4a, 4b, respectively). Mixed-effects regression confirmed a significant increase of PVI with increasing MAL, estimated to be slightly higher (not significant, though) in the eutrophic compared with the mesotrophic mesocosms (H: 1.3% PVI % µmol photons m^{-2} s^{-1}, L: 0.9% PVI % µmol photons m^{-2} s^{-1}) (Figure 3e; Table 1, 2a). In the range from 38 to 189 µmol photons m^{-2} s^{-1}, PVI was significantly higher under mesotrophic (L) than eutrophic (H) conditions for the same MAL value (Table 2b). The variance explained by the mixed-effect regression model with MAL as covariate was (marginal R^2 = 0.47 and conditional R^2 = 0.47) higher than for the model with water temperature as covariate (R^2 = 0.31 and conditional R^2 = 0.39) (Table 1).

The importance of MAL after accounting for all other drivers was further confirmed by the almost twice as large semi-partial Spearman correlation coefficient compared with the Spearman correlation coefficient for water temperature (r = 0.46, r = 0.20, respectively; Table 3). This is in line with the results of the regression tree analysis, which identified MAL as the single-most-important variable in explaining the variability in PVI. The regression tree identified MAL levels of 76 µmol photons m^{-2} s^{-1} and 199 µmol photons m^{-2} s^{-1} as thresholds for differentiating between low, medium and high PVI probability (Appendix S3, Figure S2).

Hypothesis 4: An indirect temperature effect on macrophyte growth due to evaporation-driven water level reduction and consequent MAL improvement

The highest water level decline was recorded in Greece (approximately 82 cm (S), 85 (D)), followed by Turkey (approximately 46 cm (S), 51 cm (D)), while only small changes (from -2 to +15 cm)

occurred in the other countries (Figure 2b). The highest water level decline was recorded in Greece from July to August and in Turkey from August to September.

Although the increase in MAL was only significant for the SL mesocosms over the entire water temperature gradient (Table 2a), we found a considerable potential for increases in MAL in Turkey and Greece when comparing the theoretical MAL values, i.e. those expected if the water level would not have changed due to evaporation, with those actually measured (Figure 5). In this case, by concomitantly increasing K_d values as measured (Table 2a), approximate MAL improvements were DH: 18% (TR) and 44 % (GR); DL: 15% (TR) and 38% (GR); SH: 29% (TR) and 158% (GR); SL: 26 % (TR) and 100% (GR). This means that water level-driven increases in MAL had the potential to improve light conditions in Turkey from expected medium PVI levels to high PVI levels (SH) and in Greece from expected low PVI levels to medium PVI levels (SH > DH) and from medium PVI levels to high PVI levels (SL) (Figure 5).

This is in line with our results from the PCA, Spearman and semi-partial correlation, which indicates that MAL was most strongly driven by changes in water column depth followed by K_d and PAR (Figure 4a, 4b, respectively; Table 3).

The effect of algae abundance on light attenuation

We found a significant increase of K_d over the tested Chl-*a* gradient (2-186 µg Chl-*a* l^{-1}) (Figure 3f; Table 1). This increase was significantly higher in the shallow (0.7% [m^{-1} µg Chl-*a* l^{-1}]) than in the deep (0.3% [m^{-1} µg Chl-*a* l^{-1}]) mesocosms (Table 1, 2a, 2b). Accordingly, K_d differed between treatments for the same amount of Chl-*a*. The SH mesocosms had a significantly higher K_d over the entire Chl-*a* gradient compared with all the other treatments (for the DH mesocosms only for Chl-*a* >14 µg l^{-1}). The DL mesocosms had a significantly lower K_d over the Chl-*a* gradient compared with all the other treatments (for the SL mesocosms for Chl-*a* >50 µg l^{-1}). K_d in the DH and SL mesocosms

differed significantly within a range from 2 to 9 µg Chl-*a* l^{-1} and from 115 to 186 µg Chl-*a* l^{-1} (Table 2b).

The semi-partial correlation further confirmed the strong positive correlation between Chl-*a* and K_d but revealed a significant amount of shared variance by K_d with water temperature and water column depth independent of Chl-*a* (Table 3).

Evidence by the linear mixed-effects regression for strong relations between K_d, Chl-*a* and nutrient availability (Table 1) was supported by the results from the correlation analysis. PCA and Spearman correlation indicated a strong positive association between Chl-*a*, TP, TN and K_d (Figure 4a, 4b, respectively). Semi-partial Spearman correlation for Chl-*a* further confirmed a strong positive influence of TP on Chl-*a* after removal of the influence of other potential drivers (WT, MAL, WD) (Table 3). It also indicated that water temperature independent of TP was a key driver for Chl-*a* (Table 3).

Water temperature, nutrients and depth effects on dry weight (DW)

Dry weight (DW) decreased with water temperature. The estimated average decrease was significantly greater in the shallow than in the deep mesocosms (-0.6% [mg DW m^{-2} °C^{-1}] (D), -14.8% [mg DW m^{-2} °C^{-1}] (S)) (Figure 6a; Table 1, 2a, 2b). However, although the differences between these trends were significant, the trends themselves were not. Overall, due to the differential decrease in DW in the deep and the shallow mesocosms, DW became more similar between depth treatments (D vs S mesocosms) with increasing temperatures. Over the entire water temperature gradient, DW was significantly higher in the SL than in the SH mesocosms and in the DL than in the DH mesocosms, otherwise, the pairwise differences were only significant in the colder temperature ranges (Table 2b). However, the explained variance by the water temperature-nutrient-depth model was overall low (marginal $R^2 = 0.16$ and a conditional $R^2 = 0.17$; Table 1).

Relationships between macrophyte dry weight (DW) and PVI

Dry weight (DW) increased with PVI (Figure 6b; Table 1). The estimated average increase was greatest in the DL mesocosms, followed by DH, SH and SL (DL: 15.9% [mg DW m^{-2} PVI $\%^{-1}$], DH: 10.4% [mg DW m^{-2} PVI $\%^{-1}$], SH: 8.5% [mg DW m^{-2} PVI $\%^{-1}$], SL: 5.2% [mg DW m^{-2} PVI $\%^{-1}$]) (Table 1 & 2a). While these trends were significant for all treatments, only the trends between the DL and the SL mesocosms were significantly different in the post-hoc pairwise comparison; accordingly, DW differed significantly between these two treatments over the 15.4-53% PVI range (Table 2B). The explained variance by the PVI-nutrient-depth model was overall high (marginal R^2 = 0.46 and conditional R^2 = 0.66; Table 1).

Even though water temperature explained considerably less than PVI of the variability in DW, it has explanatory power for DW in its own right, as indicated by a significant semi-partial Spearman correlation coefficient of r = -0.33 after accounting for the variability shared with PVI (Table 3). PCA and Spearman correlation indicated a strong negative correlation between DW and nutrients, Chl-*a* and high attenuation; however, this is likely due to the strong relation between DW and PVI as indicated by the semi-partial correlation (Figure 4a, 4b, respectively).

Discussion

Understanding how submerged macrophytes will respond to land use and global climate change is essential for maintaining their functional role in shallow lakes. However, this requires insight into the complex interactions between the biotic and environmental factors that influence macrophyte growth and dominance. In our pan-European mesocosm experiment mimicking shallow lake ecosystems along a temperature gradient, we investigated the interactive effect of temperature, nutrients and water level on macrophytes. These three variables are among the most important drivers of macrophyte growth and are anticipated to be affected by climate change. Our results suggest that water temperature can have a direct positive effect on Percent Plant Volume Inhabited by submerged plants (PVI) if light availability in the water column is not reduced by nutrient-driven excessive algal growth or a water depth. However, water temperature can have an even larger indirect effect on PVI due to an evaporation-driven reduction in the water level, causing a non-linear increase in mean available light (MAL). Thus, a temperature-driven water column depth reduction has the potential to mitigate the adverse effects on MAL by temperature- and nutrient-driven algal growth, particularly in very shallow systems (< 1 m).

In line with our hypotheses 1 - 3, results from regression and correlation-based analyses identified mean available light (MAL) in the water column to be the most proximate and important driver for PVI (Table 1, 3). This is supported by several studies showing the correspondence between better light conditions and macrophyte growth (Chen et al., 2016; Li et al., 2018). However, semi-partial correlation indicates that temperature, despite its high correlation with MAL (Figure 4a, 4b), also had a direct positive effect on PVI. Importantly, we identified several significant pathways over which the complex interactions between temperature, nutrients and depth are likely able to control MAL and thus PVI (Figure 7).

In the first pathway, we have a direct positive temperature effect. In line with hypothesis 1, this pathway was likely important for the strong PVI increase in our shallow mesocosms with low

nutrient level (SL) and medium (> 76 µmol photons m^{-2} s^{-1}) or good light conditions (> 199 µmol photons m^{-2} s^{-1}) over the entire temperature gradient (Figure 7; Appendix S4, Figure S4). Accordingly, earlier studies have shown that macrophytes at warmer temperatures can grow taller and thereby get access to more light than at lower temperatures (Barko & Smart, 1981; Rooney & Kalff, 2000). Moreover, a longer growing season in the south and higher metabolic rates and growth rates could contribute as well (Patrick et al., 2012; Velthius et al., 2017; Zhang et al., 2019; 2020; Hansson et al., in press). On the other hand, under the overall low light conditions (<= 76 µmol photons m^{-2} s^{-1}) of the deep mesocosms with high nutrient level (DH), we did not observe an increase in PVI with temperature (Hypothesis 3). These differences in responses between our shallow and deep mesocosms concur with the metabolic theory predicting an Arrhenius type increase in growth rates with rising temperatures under non-limiting conditions (Brown et al., 2004).

However, contrary to our expectations (Hypothesis 2), PVI in the deep mesocosms with low nutrient level (DL) increased with temperature comparable with the rates in the SL mesocosms, while the increase in the shallow mesocosms with high nutrient level (SH) was as low as in the DH mesocosms. This was the case despite considerably higher MAL levels in the SH than in the DL mesocosms, which additionally increased more strongly with temperature in the former compared with the latter. Furthermore, while MAL explains the PVI in the SH mesocosms well, especially in the higher temperature ranges, the PVI in the DL mesocosms, particularly in Greece, was higher than expected by the MAL values (Figure 3e). This indicates that while PVI in the DL mesocosms might still have directly benefited from temperature under light conditions over a critical MAL, this was not the case in the SH mesocosms. We can only speculate that this may be due to high growth of periphyton, which is known to have the potential to outcompete macrophytes through its efficient use of resources (light and nutrients) and through shading (Zhang et al., 2019; 2020). Such an explanation would be in line with the strong relation of PVI with MAL but not with temperature in the SH mesocosms. Moreover, periphyton acting as an unconsidered link would be consistent with findings also from this mesocosm experiment showing that periphyton increased significantly with temperature, and was significantly higher at eutrophic conditions in Greece (Mahdy et al., 2015,

Table 3 therein). An important role of periphyton could also reconcile the direct adverse effect of nutrients as indicated by semi-partial correlation analysis, which seems contradictory to many studies reporting positive effects of nutrients on macrophytes including *M. spicatum* (Anderson & Kalff, 1986; Cao, Wang, & Zhu, 2012).

In the second pathway, a change in MAL over the water temperature gradient was strongly mediated by temperature-driven increases in Chl-*a* (≈ 10.2% µg Chl-*a* l^{-1} per °C). The temperature-driven increase in Chl-*a* in combination with the overall higher Chl-*a* levels in the eutrophic mesocosms (Figure 3c) led to high light attenuation, particularly in the warmer temperature ranges (Figure 3d). The strong role of Chl-*a* in controlling attenuation was supported by the high effect size of Chl-*a* in the mixed-effects regression relating the attenuation coefficient K_d to Chl-*a* (Table 1) and by the high semi-partial correlation coefficient (Table 3). However, mixed-effects regression also indicated that for the same Chl-*a* concentration, the K_d levels tended to be higher in the shallow than in the deep mesocosms (for SL only at higher Chl-*a* levels) and that they increased more strongly with Chl-*a* in the shallow compared with the deep mesocosms (Figure 3f). A negative influence of depth on K_d, which was also confirmed by the semi-partial correlation, could be explained by the fact that lower water levels have a lower capacity to attenuate wind-induced turbulence and thus favour resuspension (Evens, 1994; Håkanson, 2005; Skinner, 2012). For the shallow mesocosms with high nutrient level, this effect might have been further amplified by higher availability of sedimented organic material originating from the high Chl-*a* levels in those systems. Although semi-partial correlation indicated a positive relation between water temperature and K_d, it is unclear how water temperature could directly affect higher K_d (Table 3).

In the third pathway, MAL was strongly influenced by water level and thus by temperature-driven water level changes, with potential improvements in MAL of up to 158% (SH Greece) (Figure 5). Most importantly, evaporation-driven water level reduction had the potential to shift light conditions from expected low PVI levels to medium ones (Greece DL, SH), and from expected medium PVI levels to high ones (Turkey SH and Greece SL). As we expected in hypothesis 4, a highly positive impact of evaporation-driven water level reductions on PVI is further supported when

comparing measured PVI with predicted PVI levels based on theoretical MAL and measured MAL. These predictions were based on the mixed-effects regression model between PVI and MAL (Appendix S6, Figure S11; Table 1). While the estimated PVI levels based on measured MAL predict well the measured ones, expected PVI levels based on theoretical MAL would have been considerably lower than the measured and predicted PVI. These results demonstrate the potentially important impact of water level change on submerged macrophytes that has been observed in several other warm lakes (Beklioğlu et al., 2006; Beklioğlu & Tan, 2008; Bucak et al., 2012, Jeppesen et al., 2015). However, in our estimation of theoretical MAL values, we did not consider the potential of higher water levels reducing K_d (as discussed above). Thus, we might have overestimated the beneficial effect of the water level reduction on MAL.

PVI measures the relative dominance of macrophytes in the water column. High PVI indicates a stronger potential of macrophytes for providing shelter for other organisms, which influences the water flow and hydraulics, stabilises the sediment and thus contributes to maintaining the clear-water state. However, dry weight (DW) is a measure of the plant biomass. Thus, these two metrics differ in predicting macrophyte growth. For example, 5 cm and 200 cm deep water columns could have the same high PVI but may differ considerably in DW, indicating great differences in the role of macrophytes in terms of, for instance, food provisioning and carbon storage. In our experiment, DW was measured only at the end of the experiment but had an overall strong relation to average PVI (Figure 6b; Table 1, 3). Moreover, as expected, the same PVI level was associated with higher DW in the DL compared with the SL mesocosms. This difference increased with increasing PVI levels, reflecting the smaller water volume in the shallow mesocosms compared with the deep mesocosms. Likely, this is also the reason why DW tended to decrease with increasing water temperature particularly in the shallow mesocosms as this reflects the increasingly declining water volume due to the evaporation-driven reduction in water levels. A decrease in macrophyte biomass due to extreme lowering of the water table was observed in Lake Stymfalia, Greece (Papastergiadou, Retalis, Kalliris, & Georgiadis, 2007). Likewise, in a shallow tropical lake in Brazil (Loverde-Oliveira et al., 2009) and a shallow Mediterranean lake in Sicily (Barone & Naselli-Flores, 2010), submerged macrophytes

disappeared after a strong water level decline that shifted the lake ecosystems to a turbid state. Apart from its strong relation to PVI and the negative influence of water temperature likely mediated by the changes in water volume, we were not able to conclusively explain the overall high variability in DW with the tested drivers.

Our experimental design was special in that we combined a factorial design with a gradient design, by which we realised the tested temperature gradient by setting up the experiment along a latitudinal gradient. While a gradient design is preferable for the temperature parameter, where nonlinear dynamics can be expected (Brown et al., 2004, Kreyling et al. 2018), the latitudinal approach allowed us to set up the mesocosms in lakes and thus obtain near natural water temperature climates, including precipitation- and evaporation-driven water level changes. Moreover, for the inoculation, we were able to use local flora and fauna adapted to the respective climates. These aspects, rarely considered in an experimental design so far, come, however, with the disadvantage that not only temperature but also other climate aspects change along the latitudinal gradient, including the water level. Therefore, although the gradient design provides us with a controlled temperature gradient, we need to consider co-correlation in the experimental analysis. Furthermore, our study mainly considered one dominant tall-growing submerged macrophyte species (Eurasian watermilfoil), while other species, particularly low-growing ones, played only a minor role (Appendix G). However, different macrophyte species (submerged, emergent or floating) have various preferences regarding nutrients and light conditions. While emergent macrophytes are mainly impacted by hydrological changes, submerged species are mostly influenced by temperature, light availability in the water column and other indirect effects on water quality (Short, Kosten, Morgan, Malone, & Moore, 2016; Hansson et al., in press). Likewise, the unplanned need of using two other fish species than three-spined stickleback in Greece and Sweden may potentially have had unexpected cascading effects despite their similar diets. Moreover, the need of using of tap water in Germany and Czech Republic might have had unpredictable consequences. We somehow considered these effects by using country as a random effect in the statistical analyses but cannot exclude the possibility of their potential influence on the results. Controlled mesocosm experiments offer a

mechanistic understanding of community-level responses to individual and multiple stressors under consideration of a complex ecosystem context. They offer a middle world between laboratory experiments (high control and replications but low complexity) and field experiments (low control and replication but high complexity) (Fordham, 2015; Stewart et al., 2013). However, they also inherit some problems from both approaches. For example, unlike natural systems, they are not impacted by species turnover, migration events, and – maybe the most important factor for our experiment – natural wave and wind disturbance, which may potentially counteract the positive effects of water level reduction on plant growth in natural ecosystems. On the other hand, the higher degree of complexity in mesocosm experiments comes with a certain loss of control, meaning that some aspects can only be interpreted in a correlation sense. Therefore, cautious interpretation is needed when upscaling our results to natural shallow lakes. For instance, our experimental results indicate that periphyton may be a potential neglected competitor in the complex setting of multiple changing drivers, an aspect that needs to be investigated in future experiments.

In conclusion, our study provides evidence that climate change-driven changes in water temperature, nutrients and water level lead to complex interactions that directly and indirectly control macrophyte growth, partly in opposing directions. Particularly, we showed that an evaporation-driven water level decrease has the potential to mitigate the adverse effects of increased light attenuation driven by high algal growth in consequence of temperature-nutrient interactions. Therefore, with the current climate change predictions, the expected water level decrease might override the negative effect of diminished light availability on macrophytes caused by nutrients, as observed in some other studies (Bécares et al., 2008, Özkan et al., 2010; Kosten et al., 2011). This may result in larger sediment stabilisation and a potentially greater role of macrophytes as a refuge for various organisms. However, while the relative dominance of macrophytes benefits from water level reductions as long as these are not too extreme, a water level decrease is likely accompanied by a reduction in macrophyte biomass, leading to a diminished role of macrophytes as an important food source and carbon store. In summary, we expect that

while short-term drought episodes with moderate water level reductions can stabilise macrophyte dominance, longer and intense drought periods, accompanied by extreme water level reductions, may adversely affect the development of macrophytes and their potential to stabilise lakes in the clear-water state.

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Data Availability Statement

The data of this study are available from the corresponding authors upon reasonable request.

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Tables

Table 1. Results of best AIC linear mixed-effects regression models with depth (D) and nutrient (N) levels as fixed factors, water temperature (WT), Chl-*a* or mean available light (MAL) as covariates and country as random factor. Treatment baseline for the depth treatment was the factor "shallow" (S) and for the nutrient treatment the factor "low" (L). PVI (%) and DW (dry weight) were (ln+1) transformed; all other variables were ln transformed. Significant p-values are highlighted in bold. Effect size is regression estimates based on standardised variables. The three reported R^2 values refer to marginal (variance explained by fixed factors), conditional (variance explained by fixed and random factors) and pseudo (squared correlation coefficient between estimated and true value) R^2, respectively.

Response	Predictor	Effect size	Estimate	Std. error	T-value	p-value	R^2
ln(PVI +1)	Int (18 °C)	1.85	0.73	0.31	2.37	**0.02**	0.31, 0.39, 0.43
	WT	0.85	0.08	0.08	1.06	0.29	
	D (S)	1.07	1.37	0.33	4.13	**0.00**	
	N (L)	0.86	1.14	0.28	4.04	**0.00**	
	D:N	-0.58	-0.58	0.37	-1.57	0.12	
	WT:N	0.72	0.12	0.05	2.21	**0.03**	
ln(PVI +1)	Int (115 µmol photons m^{-2} s^{-1}))	1.85	1.57	0.15	10.71	**0.00**	0.47, 0.47, 0.44
	MAL	1.47	0.01	0.00(2)	8.49	**0.00**	
	N (L)	0.56	0.56	0.21	2.71	**0.01**	
	MAL:N	-0.44	(-)0.00(3)	0.00(2)	-1.71	0.09	
ln(DW + 1)	Int (18 °C)	1.36	0.71	0.33	2.15	**0.03**	0.16, 0.17, 0.37
	WT	-0.51	-0.01	0.10	-0.06	0.95	
	D (S)	0.55	0.56	0.25	2.22	**0.03**	

	N (L)	0.75	0.75	0.21	3.52	**0.00**	
	WT:D	-0.95	-0.15	0.06	-2.50	**0.01**	
ln(DW + 1)	Int (11%)	1.41	1.26	0.36	3.48	**0.00**	0.46, 0.66, 0.42
	PVI	2.00	0.10	0.02	4.54	**0.00**	
	D (S)	0.05	0.26	0.31	0.84	0.40	
	N (L)	0.21	0.61	0.31	1.98	0.05	
	D:N	-0.41	-0.72	0.42	-1.70	0.09	
	PVI:D	-1.26	-0.02	0.03	-0.68	0.50	
	PVI:N	-0.13	0.05	0.04	1.30	0.20	
	PVI:D:N	-1.54	-0.08	0.04	-1.94	0.06	
ln(Chl-*a*)	Int (18 °C)	2.73	3.65	0.23	16.14	**0.00**	0.42, 0.52, 0.41
	WT	0.45	0.10	0.05	1.91	0.06	
	D (S)	-0.19	-0.73	0.22	-3.31	**0.00**	
	N (L)	-1.18	-1.62	0.24	-6.88	**0.00**	
	D:N	0.85	0.96	0.29	3.31	**0.00**	
ln(K_d)	Int (18 °C)	1.12	1.21	0.07	16.7	**0.00**	0.71, 0.86, 0.59
	WT	0.56	0.12	0.02	5.23	**0.00**	
	D (S)	0.14	0.14	0.05	2.70	**0.01**	
	N (L)	-0.33	-0.33	0.04	-9.06	**0.00**	
	WT:D	-0.20	-0.03	0.02	-1.84	0.07	
	WT:N	-0.20	-0.03	0.01	-2.76	**0.01**	
ln(K_d)	Int (30 µg Chl-*a* l^{-1})	1.12	1.14	0.13	8.83	**0.00**	0.37, 0.73, 0.51
	Chl-*a*	0.39	0.00(3)	0.00	4.43	**0.00**	
	D (S)	0.15	0.23	0.05	4.25	**0.00**	
	N (L)	-0.28	-0.20	0.05	-3.60	**0.00**	

	D:N	-0.17	-0.14	0.08	-1.66	0.10	
	Chl-*a*:D	0.33	0.00(4)	0.00	4.82	**0.00**	
ln(MAL)	Int (18 °C)	4.60	4.06	0.14	28.86	**0.00**	0.63, 1, 0.62
	WT	0.31	-0.02	0.04	-0.37	0.71	
	D (S)	0.75	0.82	0.04	20.57	**0.00**	
	N (L)	0.26	0.33	0.04	8.73	**0.00**	
	D:N	-0.15	-0.15	0.05	-2.70	**0.01**	
	WT:D	0.53	0.10	0.01	9.21	**0.00**	
	WT:N	0.14	0.04	0.01	3.37	**0.00**	
	WT:N:D	-0.19	-0.03	0.02	-1.87	0.07	

Table 2. a) Back-transformed intercept and slope for all dependent variables. Due to the back transformation, the intercept has the same unit as the dependent variables and depicts the geometric mean. The values in brackets show the lower and upper 95% confidence interval of the geometric mean. The back-transformed slope can be interpreted as the percentage change of the response variable for one-unit change in the respective covariate. The values in brackets are the lower and upper back-transformed 95% confidence intervals given also as a percentage change. Due to the ln+1 (natural logarithm) transformation, the percentage interpretation is approximate. Further, for y = 0, percentage changes are not defined. **b**) Value range of significant differences between treatments and significance of differences between slopes for all dependent variables. The treatment range of significant differences gives the range of the covariate in which the treatments are significantly different (<= 0.05 level) according to a two-tailed t-test for pairwise comparisons of least-square-means ("emmeans" package; Lenth, 2020). The contrast slope column indicates which of the slopes are significantly different.

a)

Dependent variable	Covariate (value at intercept)	Intercept (back-transformed; value of dependent variable at the intercept)				Slope (back-transformed; approx. percentage change of dependent variable per unit change of the covariate)			
		DH	DL	SH	SL	DH	DL	SH	SL
ln(PVI + 1)	WT (18 °C)	1.1 (-0.1, 3.5)	5.5 (2.7, 10.4)	7.1 (2.7, 17.0)	13.2 (7.1, 23.9)	8.4 (-6.9, 26.1)	21.9 (7.2, 38.6)	same as DH	same as DL
ln(PVI + 1)	MAL (115 µmol photons m^{-2} s^{-1})	3.8 (2.3, 6.0)	7.4 (4.8, 11.2)	same as DH	same as DL	1.3 (1, 1.6)	0.9 (0.7, 1.2)	same as DH	same as DL
ln(DW + 1)	WT (18 °C)	1.0 (-0.1,	3.3 (0.9,	2.6 (0.5,	6.6 (2.3,	-0.6 (-17.9, 20.4)		-14.8 (-30.0, 3.7)	

		3.8)	9.1)	7.4)	16.6)				
ln(DW + 1)	PVI (11 %)	2.5 (0.4, 7.9)	5.5 (1.7, 14.3)	3.6 (0.9, 9.8)	3.1 (0.7, 9.0)	10.4 (5.7, 15.3)	15.9 (9.0, 23.1)	8.5 (4.9, 12.1)	5.2 (2.2, 8.2)
ln(Chl-*a*)	WT (18 °C)	37.6 (20.6, 68.1)	6.6 (3.5, 12.0)	17.7 (10.5, 29.2)	8.6 (4.9, 14.6)	10.2 (-0.4, 22.0)			
ln(K_d)	WT (18 °C)	2.3 (1.8, 3.0)	1.4 (1.0, 1.9)	2.9 (2.1, 3.8)	1.8 (1.2, 2.4)	13.3 (8.0, 18.7)	9.6 (4.8, 14.7)	9.6 (3.5, 16.1)	6.1 (0.4, 12.2)
ln(K_d)	Chl-*a* (30 µg Chl-*a* l^{-1})	2.1 (1.2, 3.4)	1.6 (0.9, 2.5)	2.9 (1.8, 4.5)	1.8 (1.0, 3.0)	0.3 (0.2, 0.4)		0.7 (0.6, 0.8)	
ln(MAL)	WT (18 °C)	56.7 (39.2, 81.8)	79.3 (54.9, 114.3)	129.7 (90.0, 186.6)	156.0 (108.4, 224.3)	-1.6 (-9.7, 7.2)	2.3 (-6.1, 11.4)	8.9 (-0.2, 18.9)	9.8 (0.5, 20.0)

b)

Dependent variable	Covariate (value at intercept)	Treatment range of sig. difference [unit of covariate]	Contrast slope
ln(PVI + 1)	WT (18 °C)	D H - S H : 14.7 - 24.2 D H - D L : 16.0 - 24.2 D H - S L : 14.7 - 24.2 S H - D L : none S H - S L : 19.3 - 24.2 D L - S L : 14.7 - 24.2	H - L*

ln(PVI + 1)	MAL (115 µmol photons m^{-2} s^{-1})	H - L: 38 - 189	H - L
ln(DW + 1)	WT (18 °C)	D H - S H: 14.7 - 15.8 D H - D L : 14.7 - 24.2 D H - S L: 14.7 - 21.1 S H - D L: 22.6 - 24.2 S H - S L: 14.7 - 24.2 D L - S L: 14.7 - 15.8	D - S*
ln(DW + 1)	PVI (11 %)	D H - S H: none D H - D L : none D H - S L: none S H - D L: none S H - S L: none D L - S L: 15.4 - 53	D H - S H D H - D L D H - S L S H - D L S H - S L D L - S L*
ln(Chl-*a*)	WT (18 °C)	D H - S H: 14.7 - 24.2 D H - D L: 14.7 - 24.2 D H - S L: 14.7 - 24.2 S H - D L: 14.7 - 24.2 S H - S L: 14.7 - 24.2 D L - S L: none	No interactions with WT
ln(K_d)	WT (18 °C)	D H - S H : 14.7 - 18.1 D H - D L : 14.7 - 24.2 D H - S L : 17.9 - 24.2 S H - D L: 14.7 - 24.2 S H - S L: 14.7 - 24.2 D L - S L: 14.7 - 18.1	D H - S H D H - D L* D H - S L* S H - D L S H - S L* D L - S L
ln(K_d)	Chl-*a* (30 µg Chl-*a* l^{-1})	D H - S H : 14 - 186 D H - D L : 2 - 186 D H - S L : 2 - 9 and 115 - 186 S H - D L: 2 - 186 S H - S L: 2 - 186 D L - S L: 50 - 186	D - S*

ln(MAL)	WT (18 °C)	D H - S H : 14.7 - 24.2	D H - S H*
		D H - D L: 14.7 - 24.2	D H - D L*
		D H - S L: 14.7 - 24.2	D H - S L*
		S H - D L: 14.7 - 24.2	S H - D L*
		S H - S L: 14.7 - 24.2	S H - S L
		D L - S L: 14.7 - 24.2	D L - S L*

Table 3. Bootstrapped semi-partial Spearman correlation coefficients r with 95% percentile confidence intervals. Bold indicates correlation coefficients where the confidence interval does not include zero. "-" denotes cells of variables not used as predictors for the particular variable. WT: water temperature, PVI: Percent Plant Volume Inhabited, DW: dry weight, Chl-*a*: chlorophyll-a, K_d: attenuation coefficient, MAL: mean available light, TP: total phosphorus, WD: water column depth, PAR: photosynthetically active radiation.

	WT	**MAL**	**TP**	**Chl-*a***	**WD**	**PVI**	**PAR**	**K_d**
PVI	**0.20 (0.02, 0.36)**	**0.46 (0.29, 0.60**)	**-0.20 (-0.32, -0.05)**	0.10 (-0.05, 0.26)	-	-	-	-
DW	**-0.33 (-0.47, -0.18)**	0.01 (-0.13, 0.14)	-0.11 (-0.22, 0.01)	-	0.08 (-0.04, 0.22)	**0.58 (0.44, 0.71)**	-	-
Chl-*a*	**0.21 (0.06 ,0.36)**	**-0.25 (-0.37, -0.13)**	**0.45 (0.32, 0.59)**	**-**	**-0.15 (-0.29, -0.01)**	-	-	-
K_d	**0.25 (0.06, 0.42)**	-	-	**0.48 (0.33, 0.61)**	**-0.27 (-0.41, -0.12)**	-	-	-
MAL	-	-	-	-	**-0.74 (-0.82, -0.65)**	-	**0.44 (0.34, 0.55)**	**-0.51 (-0.63, -0.40)**

Figures

Figure 1. Schematic representation of the hypothesised temperature-nutrient-depth effect on macrophyte growth (PVI). The highlighted grey area indicates that at higher temperatures additional effects on PVI due to water level reduction are expected. SL: shallow mesocosm with low nutrient level, SH: shallow mesocosm with high nutrient level, DL: deep mesocosm with low nutrient level, DH: deep mesocosm with high nutrient level.

Figure 2. (a) Country-wise monthly water temperature (°C) and (b) water level change (cm) during the experiment (July-November) averaged over all mesocosms (Table A1). SE: Sweden, EE: Estonia, CZ: Czech Republic, GE: Germany, TR: Turkey, GR: Greece.

Figure 3. Scatterplot for (a) PVI, (b) mean available light (MAL), (c) chlorophyll-*a* (Chl-*a*), (d) attenuation coefficient (K_d) against water temperature, e) PVI against MAL and f) K_d against Chl-*a* at the original scale. Note that due to the back transformation, the predictions of the mixed effect model (lines) represent the geometric mean (Table 2). Mixed-effects regression models at the scale of model estimation (ln-transformation) with 95% confidence intervals are shown in Appendix S4, Figure S3 to S8. DH: deep mesocosm with high nutrient level, DL: deep mesocosm with low nutrient level, SH: shallow mesocosm with high nutrient level, SL: shallow mesocosm with low nutrient level, CZ: Czech Republic, EE: Estonia, GE: Germany, GR: Greece, SE: Sweden, TR: Turkey.

Figure 4. Linear (a) and monotonic (b) relationship between and among all predictor and response variables. a) Principal components analysis (PCA) of all predictor and response variables. The first axis (Dim1) explains 35.1% and the second axis (Dim2) 32.7% of the variation. Shaded and coloured areas indicate treatment-wise point concentration confidence ellipses with a confidence level of 0.99. Coloured and enlarged group symbols within the ellipses (dot, triangle, square, cross) indicate the barycentres of the respective groups. b) Spearman correlation network of all predictor and response

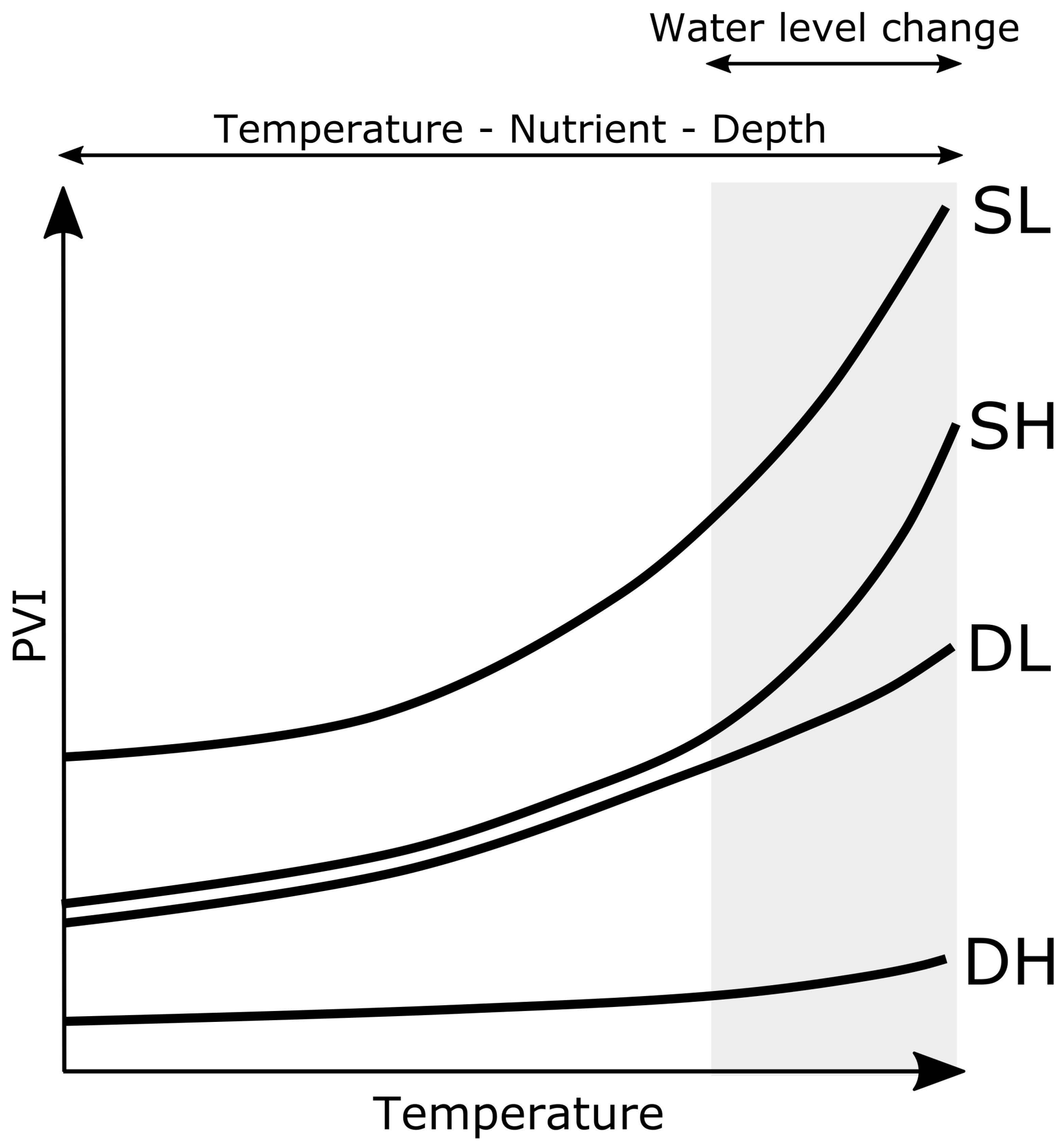
variables. Plotted are significant correlations with a Spearman correlation coefficient ρ >= 0.4. WT: water temperature, PAR: photosynthetically available radiation, PVI: Percent Plant Volume Inhabited, MAL: mean available light, DW; dry weight, TP; total phosphorus, TN: total nitrogen, Chl-*a:* chlorophyll-*a*, K_d: light attenuation coefficient, WC: water column depth. Values indicate the Spearman correlation coefficients.

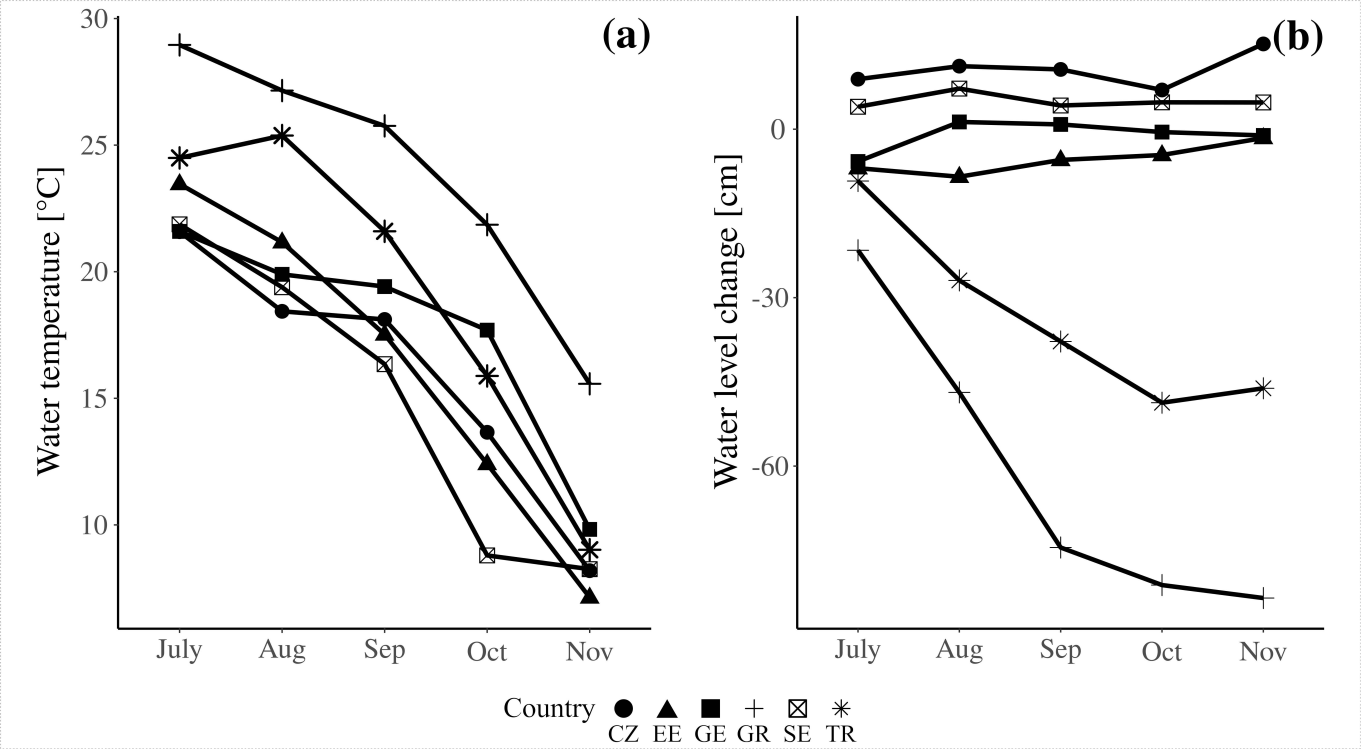
Figure 5. Boxplot of treatment- and country-wise measured and estimated mean available light (MAL). Estimated MAL levels were calculated based on measured PAR and attenuation coefficients (K_d) but assuming unchanged water levels of 0.9 m and 1.9 m for the shallow and deep mesocosms, respectively. Dashed lines indicate thresholds for expected low (MAL < 76 µmol photons m^{-2} s^{-1}), medium (76 µmol photons m^{-2} s^{-1} >= MAL < 200 µmol photons m^{-2} s^{-1}) and high (MAL >= 200 µmol photons m^{-2} s^{-1}) PVI levels as estimated by regression tree analysis (Appendix S3, Figure S2). DH: deep mesocosm with high nutrient level, DL: deep mesocosm with low nutrient level, SH: shallow mesocosm with high nutrient level, SL: shallow mesocosm with low nutrient level, CZ: Czech Republic, EE: Estonia, GE: Germany, GR: Greece, SE: Sweden, TR: Turkey.

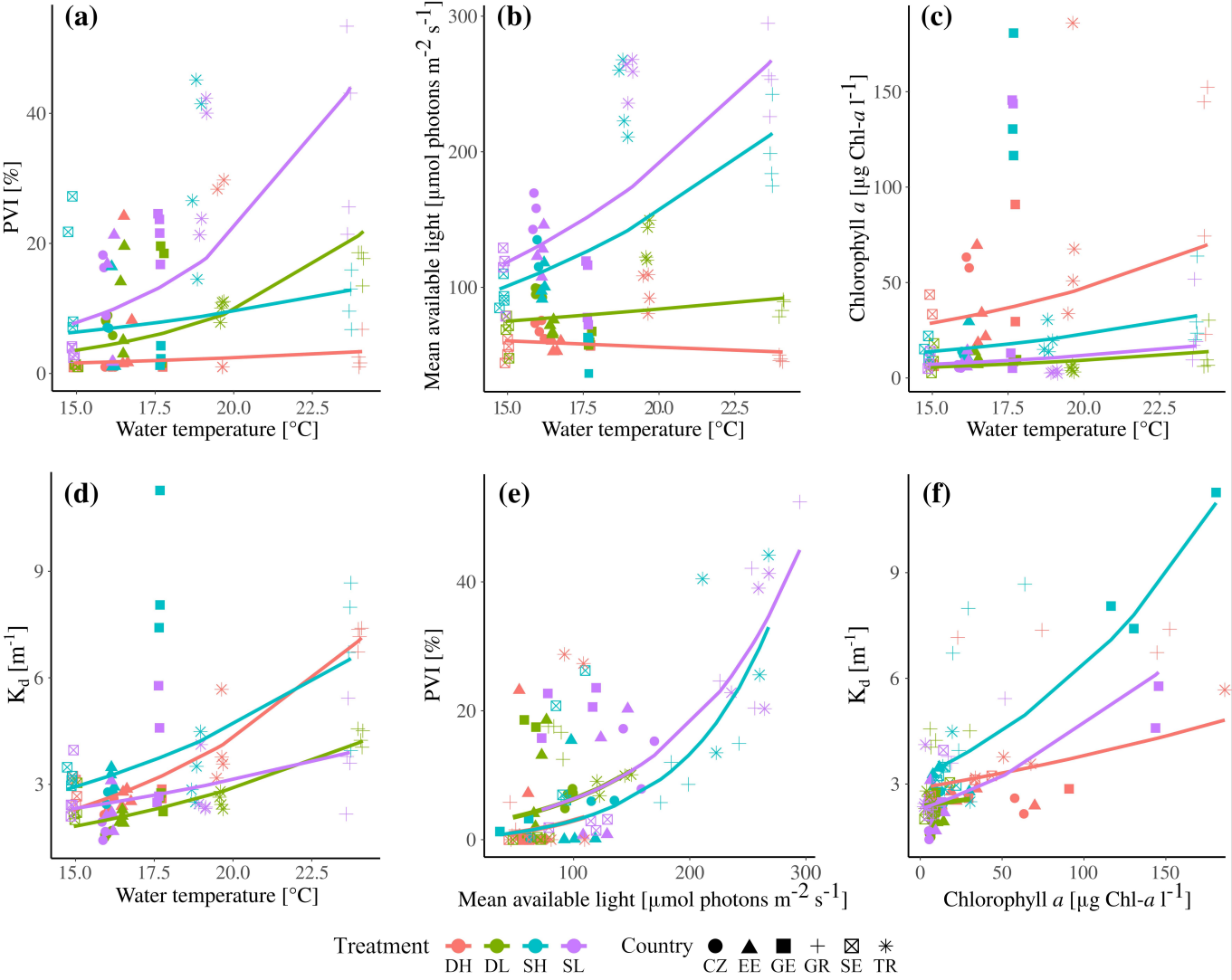
Figure 6. Scatterplot for dry weight (DW) against a) average water temperature gradient and b) average PVI at the original scale. Note that due to the back transformation, predictions of the mixed effect model (lines) represent the geometric mean (Table 2). Mixed-effects regression models at the scale of model estimation (ln-transformation) with 95% confidence intervals are shown in Appendix S5, Figure S9 and S10. DH: deep mesocosm with high nutrient level, DL: deep mesocosm with low nutrient level, SH: shallow mesocosm with high nutrient level, SL: shallow mesocosm with low nutrient level, CZ: Czech Republic, EE: Estonia, GE: Germany, GR: Greece, SE: Sweden, TR: Turkey.

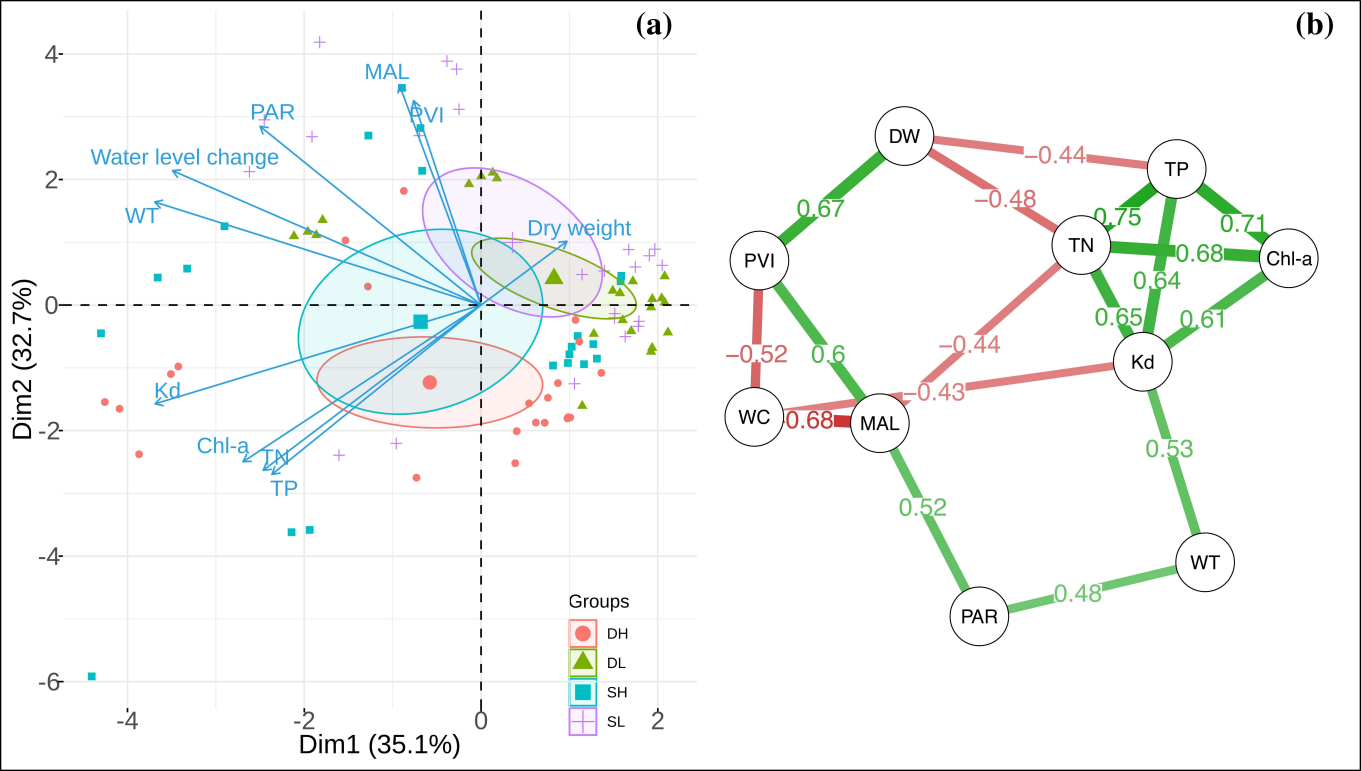
Figure 7. Model based on data analysis results. Evidence from our experiment suggests that water temperature has a direct positive effect on PVI and an even larger indirect effect due to its negative influence on water levels and thus on mean available light (MAL). However, water temperature also has a positive effect on chlorophyll-*a* (Chl-*a*), which in turn increases attenuation and thus reduces

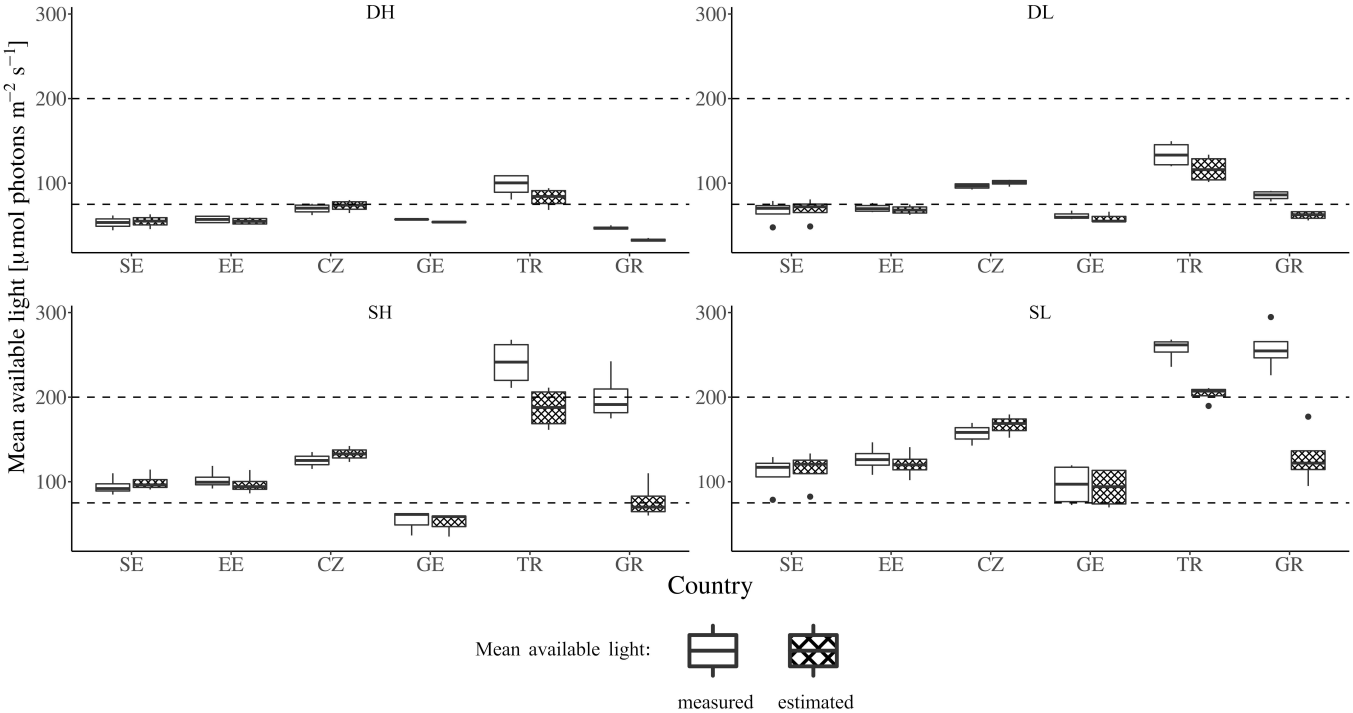
MAL again. Nutrients have a direct positive effect on Chl-*a* levels and an indirect negative effect on PVI, again mediated by reduced MAL levels.

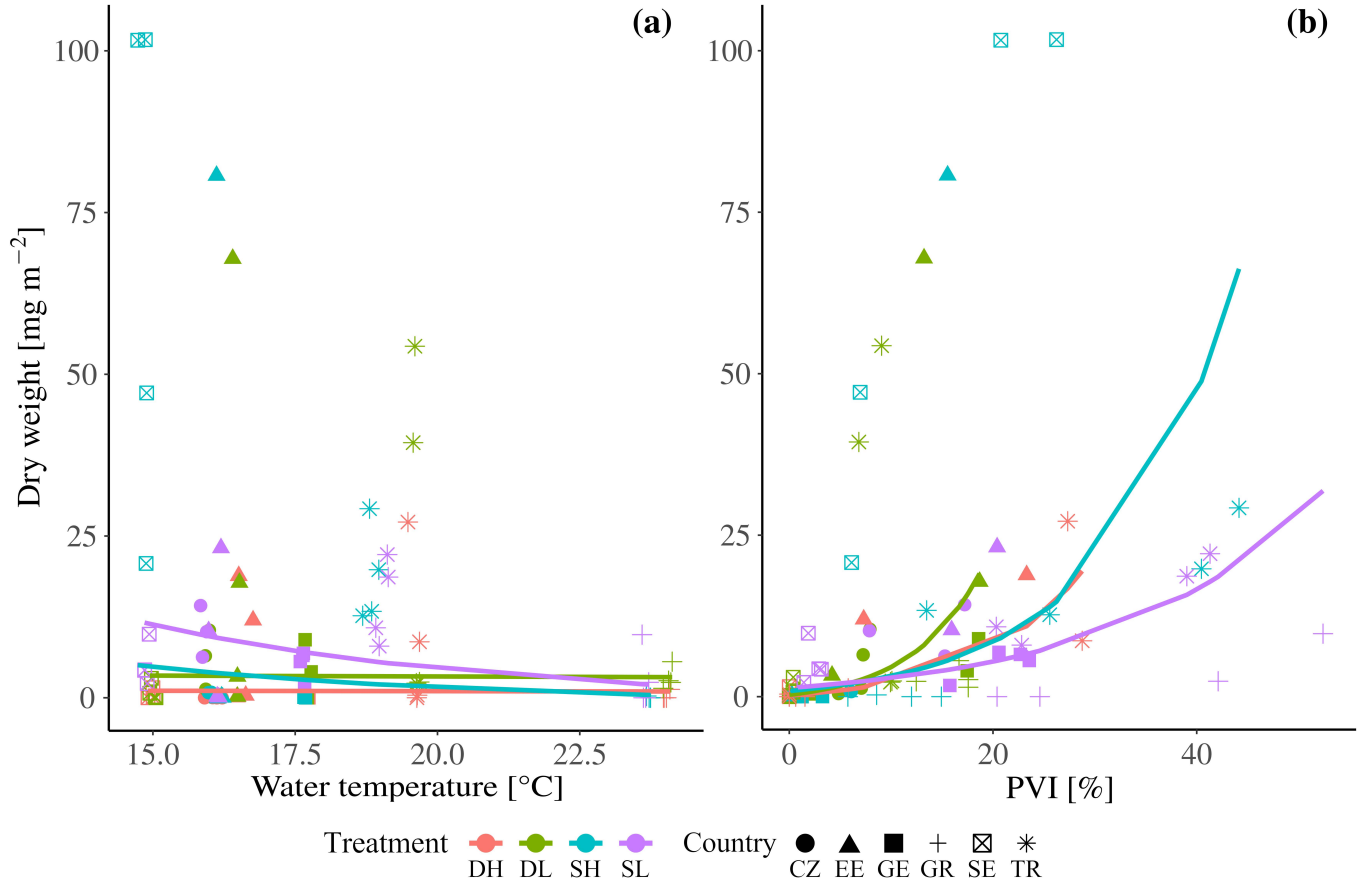

Water level change
Temperature - Nutrient - Depth
SL
SH
DL
DH
PVI
Temperature

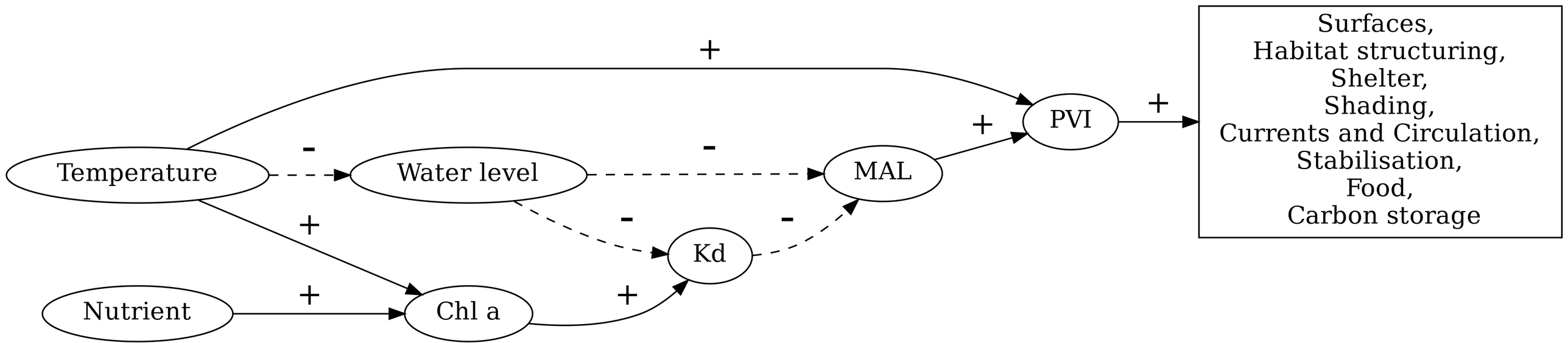

(a)
(b)
Water temperature [°C]
Water level change [cm]
30
25
20
15
10
0
-30
-60
July
Aug
Sep
Oct
Nov
Country
CZ
EE
GE
GR
SE
TR


(a)
PVI [%]
Water temperature [°C]
(b)
Mean available light [µmol photons m-2 s-1]
Water temperature [°C]
(c)
Chlorophyll a [µg Chl-a l-1]
Water temperature [°C]
(d)
Kd [m-1]
Water temperature [°C]
(e)
PVI [%]
Mean available light [µmol photons m-2 s-1]
(f)
Kd [m-1]
Chlorophyll a [µg Chl-a l-1]
Treatment
DH DL SH SL
Country
CZ EE GE GR SE TR


(a)
MAL
PAR
PVI
Water level change
WT
Dry weight
Kd
Chl-a
TN
TP
Dim2 (32.7%)
Dim1 (35.1%)
Groups
DH
DL
SH
SL
(b)
DW
TP
PVI
TN
Chl-a
Kd
WC
MAL
WT
PAR
−0.44
−0.48
0.67
0.75
0.71
0.68
0.64
0.65
0.61
−0.52
0.6
−0.44
−0.43
0.68
0.53
0.52
0.48


DH
DL
SH
SL
300
200
100
SE
EE
CZ
GE
TR
GR
Mean available light [µmol photons m^{-2} s^{-1}]
Country
Mean available light:
measured
estimated


(a)
(b)
Dry weight [mg m^{-2}]
100
75
50
25
0
15.0
17.5
20.0
22.5
Water temperature [°C]
0
20
40
PVI [%]
Treatment
DH
DL
SH
SL
Country
CZ
EE
GE
GR
SE
TR


Temperature
Water level
MAL
PVI
Surfaces,
Habitat structuring,
Shelter,
Shading,
Currents and Circulation,
Stabilisation,
Food,
Carbon storage
Nutrient
Chl a
Kd
+
-
+
-
+
-
+
-
+
+
+