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Artificial light at night decreases biomass and alters community composition of benthic primary producers in a sub-alpine stream

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Abstract

Artificial light at night (ALAN) is recognized as a contributor to environmental change and a biodiversity threat on a global scale. Despite its widespread use and numerous potential ecological effects, few studies have investigated the impacts on aquatic ecosystems and primary producers. Light is a source of energy and information for benthic autotrophs that form the basis of food webs in clear, shallow waters. Artificial night-time illumination may thus affect biomass and community composition of primary producers. We experimentally mimicked the light conditions of a light-polluted area (approximately 20 lux, white LED) in streamside flumes on a sub-alpine stream. We compared the biomass and community composition of periphyton grown under ALAN with periphyton grown under a natural light regime in two seasons using communities in early (up to 3 weeks) and later (4–6 weeks) developmental stages. In early periphyton, ALAN decreased the biomass of autotrophs in both spring (57% at 3 weeks) and autumn (43% at 2 weeks), decreased the proportion of cyanobacteria in spring (54%), and altered the proportion of diatoms in autumn (11% decrease at 2 weeks and 5% increase at 3 weeks). No effects of ALAN were observed for later periphyton. Further work is needed to test whether streams with frequent physical disturbances that reset the successional development of periphyton are more affected by ALAN than streams with more stable conditions. As periphyton is a fundamental component of stream ecosystems, the impact of ALAN might propagate to higher trophic levels and/or affect critical ecosystem functions.

Light pollution that results from the extensive use of artificial light at night (ALAN) is a global phenomenon and one of the fastest-spreading environmental alterations induced by humans (Hölker et al. 2010*a*; Falchi et al. 2016). ALAN can have several effects on the natural environment (Longcore and Rich 2004; Hölker et al. 2010*b*). So far, ecological effects of ALAN have been commonly examined at the level of single species (Gaston et al. 2015) while fewer studies address higher ecological levels such as communities or ecosystems functions (e.g., Davies et al. 2012; Becker et al. 2013; Meyer and Sullivan 2013). Moreover, studies of ecological effects of ALAN have largely focused on terrestrial habitats, while the interest in aquatic systems is relatively recent (Perkin et al. 2014*a*; Brüning et al. 2015; Hölker et al. 2015; Honnen et al. 2016) despite the fact that freshwaters are often exposed to ALAN from adjacent urban and sub-urban areas (Ceola et al. 2015).

Light serves as a source of both energy and environmental information for primary producers (Hegemann et al. 2001). The intensity, spectral quality, timing and duration of light all affect photosynthesis and growth of aquatic primary producers as well as their biochemistry and community composition (Richardson et al. 1983; Falkowski and Laroche 1991; Khoeyi et al. 2012). As a result of human population growth and increased urbanization, previously ALAN-naïve freshwater environments, e.g., streams, rivers, and littoral habitats of lakes are increasingly exposed to artificial illumination at night. In such shallow, clear waters, periphyton often forms the base of the food web (Stevenson 1996). Periphyton is a complex benthic community of algae, bacteria, and fungi embedded in a polysaccharide matrix (Wetzel 2001). These benthic communities are predominantly composed of

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autotrophs and dominate primary production of small and mid-sized streams (Dodds et al. 1999). Periphyton is therefore an important food resource for primary consumers and plays a key role in nutrient and carbon cycling in streams and rivers (Stevenson 1996; Law 2011). Due to its sensitivity to alterations of physical, chemical, and biological environmental conditions, periphyton is commonly used in biological monitoring (Lowe and Pan 1996).

Nocturnal artificial light can stimulate photosynthesis (Aube et al. 2013); however, it is unclear whether the light levels typically found in ALAN-illuminated aquatic environments, which are of low intensity in comparison to sunlight and of an unnatural spectral composition, produce measurable and relevant effects on their biomass and community composition. Poulin et al. (2014) found that ALAN (by high-pressure sodium lamps, emitting predominantly yellow light) at a light level of 0.08 μmol m^{-2} s^{-1} (approximately 6.6 lux, as low as 0.004–0.08% of natural mid-day irradiance) affected the physiology in unicellular cyanobacteria in laboratory cultures, although no effects on growth were observed. Hölker et al. (2015) found an increase in the abundance of photoautotrophs (diatoms, cyanobacteria) in sediments after 5 months of exposure to ALAN (by high-pressure sodium lamps) of approximately 0.09 μmol m^{-2} s^{-1} (6.8–8.5 lux). Periphyton is composed of several groups of autotrophs that all differ in light optima and minimum light requirements for growth and photosynthesis. Cyanobacteria and diatoms are generally considered to be better adapted to grow under low light intensities compared to green algae (Richardson et al. 1983; Langdon 1988), and therefore might benefit from low-light typically supplied by ALAN. Different light regime may thus cause differential responses among taxa, resulting in shifts of competitive equilibria and changes in periphyton community composition (Litchman 1998).

Natural light/dark cycles detected by photoreceptors provide information for the regulation of several physiological processes (Kianianmomeni and Hallmann 2014). As one of the most regular and predictable environmental fluctuations, light/dark cycles drive rhythmic changes in biological processes such as synthesis of various cellular components, DNA repair, growth and development in many organisms, often through a circadian clock (Brand and Guillard 1981; Fortunato et al. 2015 and references therein). In the majority of photosynthetic organisms, the regulation of a circadian clock is mediated by cryptochromes and other flavin blue-light receptors (Fortunato et al. 2015). Algae can detect light as low as moonlight (approximately 0.1 lux) (Bünning and Moser 1969) and cyanobacteria are also documented to detect and respond to changes in light intensity and spectral quality (Mullineaux 2001). Therefore, the disruption of natural light/dark cycles by ALAN may cause dysfunction in circadian rhythms and thereby light-driven physiological processes. If sensitivities differ among taxa, also an altered light/dark regime could represent a selection pressure potentially altering community composition.

Typically habitats dominated by periphyton experience disturbance at a sub-annual timescale, and the development of periphyton communities in these systems follow characteristic patterns of colonization and succession. Biomass accrual over time leads to the establishment of three-dimensional, spatially complex biomass matrices (Biggs 1996), and a shift in growth forms from the dominance of small, adnate diatoms toward higher abundance of erect, stalked forms, and finally to filamentous forms of green algae and cyanobacteria (Hudon and Bourget 1983; Biggs 1996). Physical disturbances such as fluctuations in flow associated with discharge peaks or increased wave action, and sediment transport caused by floods and storms, can erode periphyton biomass and thus alter or reset its successional state. Successional patterns may thus be associated with a notable vertical sub-structuring and differentiation of a periphyton matrix. Such development of a microscale architecture is strongly influenced by light conditions and modifies the environmental conditions within the matrix itself, modulating a community response to light (Boston and Hill 1991). Moreover, seasonal variation in environmental conditions causes strong seasonal differences in periphyton community composition (Biggs 1996). In temperate latitudes, variation in light regime is one of the major drivers of seasonal patterns in species composition and autotrophs are in general better adapted to lower light conditions in winter and spring, and higher light conditions in summer and autumn (Kirk 1994; Laviale et al. 2009). Thus, it is likely that the sensitivity of periphyton to ALAN will vary across seasons as well.

We used streamside artificial flumes fed by a sub-alpine stream to investigate the effects of night-time illumination on periphyton. We mimicked the light conditions of light-polluted areas of urban and sub-urban streams and measured its effects on biomass and community composition of periphyton in early ("developing") and late ("pre-established") developmental stages. We conducted the experiment in two seasons (spring and autumn) to account for seasonal differences in community composition and (non-ALAN associated) environmental conditions. We hypothesized that ALAN would stimulate photosynthesis, resulting in higher biomass of periphyton. Furthermore, we expected ALAN to differentially affect the major autotrophic groups in periphyton, thereby altering periphyton community composition. We also expected the effects of ALAN to depend on the periphyton developmental stage, with later stages of spatially complex communities being less sensitive to ALAN.

Materials and methods

Study site and experimental design

Experiments were conducted in a set of five metal flumes situated in the riparian zone of the Fersina stream in Trentino Province, Northeastern Italy (46° 04′ 32″ N, 11° 16′ 24″

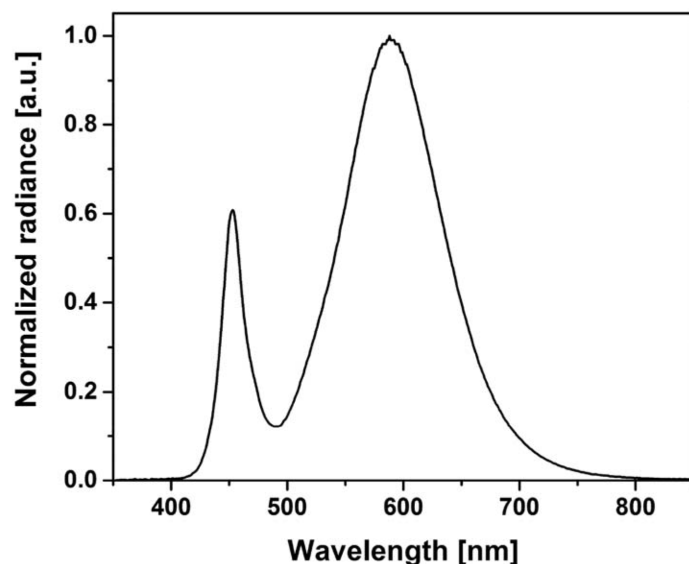

Fig. 1. Spectral composition of LED lights used in the study (12 V, 3000 K, Barthelme, Nürnberg, Germany).

E) at 577 m asl in spring and autumn 2014. The Fersina is a 2nd order snowmelt-fed gravel-bed stream originating at an altitude of 2005 m. It is approximately 14 km long, with a 171 km^2 watershed receiving the contribution of numerous small streams that descend from lateral valleys. The streamside flumes on the Fersina have been used for ecohydrological studies on periphyton (Cashman et al. 2016) and benthic macroinvertebrates (Carolli et al. 2012; Bruno et al. 2013, 2016). The flume system is located on the right bank, with no history of direct exposure to ALAN in the entire upstream section. It consists of five metal, U-shaped flumes that are 20 m long and 30 cm wide with either 30 cm (flumes A–C) or 50 cm (flumes D, E) high side walls. Flumes are directly fed by water that is diverted from the stream through a loading tank equipped with a sluice gate for discharge regulation. A metal mesh (3 × 5 cm opening) prevents large material and fish from entering the flumes while allowing the colonization by periphyton and macroinvertebrate fauna. A baseflow of 0.05 m^3 s^{-1} and velocity of 0.4 m s^{-1} were established by manipulating a sluice gate in all flumes 6 months before starting the experiment and kept constant throughout the experimental period. The flume bottom was covered with a 20 cm thick layer of cobbles of approximately 10 cm diameter and a layer of gravel and sand deposited by the water flow.

On 04 March (for spring sampling) and 01 September (for autumn sampling), we evenly distributed 16 white unglazed ceramic tiles (9.8 cm × 19.6 cm) into each flume along its entire length. The tiles were used as substrate for the development of periphyton. Each was placed on top of the cobble layer, centrally in the flumes at a maximum water depth of 5 cm. We left the tiles for 26 d in spring and 22 d in autumn in order to facilitate the natural development of a "pre-established" community prior to the beginning of the experimental treatment (Oemke and Burton 1986 and references therein). The growth time in September was shorter due to faster periphyton growth, likely a consequence of higher water temperature.

On 31 March and 24 September, artificial light was installed by mounting battery-powered warm-white LED strips (12 V, Barthelme, Nürnberg, Germany; 3000 K color temperature measured with spectroradiometer specbos 1211UV, JETI, Jena, Germany; Fig. 1) on wires above either the upstream or the downstream section of each flume (chosen randomly). This experimental setup resulted in a design with a total of five lit sections and five control sections in 10 flume sections of 10 m length. Lightproof plastic foil curtains were hung on steel wires between half-flume sections and longitudinally between the flumes, to prevent the LED light from spreading into the control sections, which were exposed to the natural light/dark regime. Curtains were removed during the day to allow direct sunlight to reach all flume sections. The light levels were measured below the water surface with an ILT1700 underwater photometer (International Light Technologies, Peabody, Massachusetts, U.S.A.) after astronomical twilight on the nights of the new moon, on 30 March and 23 September (Table 1). Mean illumination in the lit sections amounted to 20.3 ± 1.8 lux (mean and SD, $n = 20$; approximately 0.31 μmol m^{-2} s^{-1}), a light level comparable to those found in urban environments (Hale et al. 2013). A timer was used to automatically turn the lights on and off at civil twilight and dawn over a period of 3 weeks. The length of the illumination period was chosen to cover the full range of natural nocturnal light levels, i.e., from new moon to full moon illumination. A longer illumination period was avoided in order to avoid periphyton reaching senescence phase in succession, which could drive community changes independently from ALAN. We measured flow velocity using a hand-held current meter (Global Water Flow Probe, Global Water Instrumentation, College Station, Texas, U.S.A.), and physico-chemical parameters using a WTW handheld meters for oxygen, pH, conductivity, and turbidity (WTW GmbH, Weilheim, Germany) (Table 1; Supporting Information Table S1).

Sampling procedure

On the first sampling day of each growth period (31 March and 24 September), we sampled four tiles from each flume section ("pre-established periphyton") and deployed 12 new, clean tiles evenly along each flume section for the later collection of "developing" periphyton, so that each flume contained a total of 32 tiles. From this point onward, we sampled four replicate tiles with periphyton of identical developmental stage from each flume section on a weekly basis for 3 weeks (Table 2). This allowed us to analyze pre-established periphyton using a replicated before-after-control-impact (BACI) design, while

developing periphyton was analyzed as a time series because all tiles were uncolonized at the start of the experiments.

Tiles were carefully removed from the flumes to minimize biomass loss due to sloughing. Any non-periphytic material (e.g., Simuliidae larvae) attached to the sides and the bottom was removed with forceps. Each tile was placed into a plastic box (23 × 14 × 6.5 cm) and carefully covered with pre-filtered (Whatman GF/F glass-fiber filter, 0.7 μm nominal pore size) water from the flumes. We measured periphyton biomass in the field using an in situ deployable fluorometer (BenthoTorch, bbe Moldaenke GmbH, Schwentinental, Germany). This instrument is designed for rapid quantification of biomass of benthic autotrophs based on in vivo chlorophyll *a* fluorescence at 690 nm, and for assessment of community composition by discrimination of diatoms, green algae, and cyanobacteria based on the fluorescence of marker pigments with fluorescent signatures at 470 nm, 525 nm, and 610 nm (bbe Moldaenke 2013; Harris and Graham 2015). Studies that examined the accuracy and sensitivity of the BenthoTorch (BT) suggested it as a useful tool for examining patterns over sites and time (Harris and Graham 2015). The accuracy of BT measurements was found to decline with BT Chl *a* concentrations >4 μg cm^{-2} (Harris and Graham 2015; Echenique-Subiabre et al. 2016), when discrepancies were found in the relative percentages of different groups obtained by the BT and the results obtained by standard laboratory procedures such as spectrophotometric determination of Chl *a* and analysis of biovolume with a microscope (Kahlert and Mckie 2014; Harris and Graham 2015). We took eight 1-cm^2 BT measurements of undisturbed periphyton for each tile, distributed across the tile surface. All measurements were performed in the morning (08:00 h to 12:00 h). The periphyton was then scraped from each sampled tile with a razor and a tooth brush and the tile was rinsed with pre-filtered flume water. The resulting periphyton suspension was collected into a 250 mL plastic bottle, labelled and stored on ice pending analysis in the laboratory within 24 h.

Table 1. Environmental parameters averaged over the experimental period for the two investigated seasons ($n = 20$).

	Spring		Autumn	
	Mean	**SD**	**Mean**	**SD**
Conductivity (μS cm^{-1})	95.67	12.80	142.70	1.88
Temperature (°C)	6.6	1.3	13.4	0.1
Oxygen (mg L^{-1})	11.59	0.96	8.83	0.11
Oxygen (%)	101.2	6.0	90.1	1.3
pH	7.7	0.8	8.1	0.1
Turbidity (NTU)	1.53	0.34	0.39	0.17
Velocity (m s^{-1})	0.37	0.18	0.34	0.11
Light at night in D sections (lux)	0.0027	0.0008	0.0012	0.0006

Laboratory procedures

The total volume of the periphyton suspension was determined with a measuring cylinder. After vigorous shaking, aliquots for determination of dry mass (DM) were concentrated on pre-combusted, pre-weighed 25 mm Whatman GF/F glass-fiber filters by vacuum filtration, dried at 65°C until constant weight and re-weighed. Additional aliquots for pigment analysis were concentrated on filters and stored in 2 mL safety reaction vessels. These filters were transferred to −80°C for a minimum of 48 h to stimulate cell lysis and subsequently freeze-dried and stored at −20°C pending analysis by high-performance liquid chromatography (HPLC) (Waters, Millford, Massachusetts, U.S.A.). Pigments were analyzed only for pre-established periphyton, following the procedure described in Woitke et al. (1994) and Shatwell et al. (2012). Pigments were identified and quantified by their retention time and absorption spectra from standards and the literature (Jeffrey et al. 1997). Chl *a* was calculated as the sum of the true Chl *a* and chlorophyllids *a*, and determined as a mean of the absorption readings at 440 nm and 410 nm wavelength. Chlorophyll *b*, chlorophyll *c*, and fucoxanthin were determined from the absorption readings at 440 nm.

Data analysis

We used Pearson's correlation analyses to compare the measurements obtained by the BT with those based on HPLC-derived data and the measurements of DM. Spring and autumn sets of samples were analyzed separately, as there are seasonal differences in the periphyton community composition. To test for effects of ALAN on total biomass (log-transformed), absolute (log-transformed) and relative biomass of the major groups (diatoms, green algae, and cyanobacteria as

Table 2. Overview of tile manipulations and sampling dates in two experimental seasons.

	Season	Tiles deployed	No. of weeks prior to the treatment	Pre-treatment sampling	Lights turned on	During–treatment sampling			End of treatment sampling	No. of weeks of exp. treatment for each collected tile
Pre-established periphyton	Spring	04 Mar	4	31 Mar	31 Mar	-	-	-	23 Apr	3
	Autumn	01 Sep	3	24 Sep	24 Sep	-	-	-	16 Oct	3
Developing periphyton	Spring	31 Mar	-	-	31 Mar	07 Apr	14 Apr	21-Apr	-	1, 2, 3
	Autumn	24 Sep	-	-	24 Sep	01 Oct	08 Oct	14-Oct	-	1, 2, 3

distinguished by the BT) we used linear mixed-effects models (LMM) (Zuur et al. 2009) as available in the nlme package (Pinheiro et al. 2015) for R (Version 3.1.3, R Core Team 2015). We included treatment ("lit" and "control") and time ("before" and "after" for pre-established periphyton, and "2 weeks" and "3 weeks" for developing periphyton) as fixed factors in the model, while flume and tile were defined as nested random factors to avoid pseudoreplication and account for spatial dependency between replicate tiles and sections within the individual flumes. When the observed variance differed between the levels of fixed factors (treatment or time), these were used as variance covariates (Zuur et al. 2009). The same model was used to test if ALAN affected the ratios of photosynthetic pigments, and the ratio of Chl *a* : DM. Chl *a* : DM is a commonly used indicator for the proportion of autotrophic biomass in the periphyton community (Stevenson 1996) and is related to physiological acclimation of periphyton to light conditions, as intracellular concentrations of photosynthetic pigments increase in adaptation to low light intensities (Falkowski and Laroche 1991). Changes in pigment ratios may indicate changes in intracellular pigment concentrations or reflect alterations in the community composition (Jeffrey et al. 1997).

For pre-established periphyton, the experimental design followed a replicated BACI approach. Therefore, any effect of ALAN is represented by the interaction term treatment x time. For developing periphyton, the starting phase was the same for all treatments (no periphyton) and the effect of ALAN is considered to be directly represented by the treatment main effect. Pairwise comparisons of significant interactions were performed using the glht function from the multcomp package for R (Hothorn et al. 2008) with Benjamini-Hochberg *p* value adjustments.

Results

Comparison of BT- and HPLC-based measurements

Chl *a* is a commonly used proxy of autotroph biomass, as it is present in all algae and cyanobacteria. The BT uses in vivo fluorescence of Chl *a* to estimate the total biomass of autotrophs in the periphyton, and of marker pigments to differentiate between the three groups, i.e., diatoms, green algae, and cyanobacteria (bbe Moldaenke 2013). The BT measurements for total biomass of autotrophs in pre-established periphyton were correlated with the concentrations of Chl *a* determined by HPLC ($r = 0.93$, $p < 0.01$, Supporting Information Fig. S1a). The BT-measured biomass of diatoms was also correlated with the concentrations of their marker pigments (i.e., Chl *c*: $r = 0.95$, $p < 0.01$, Supporting Information Fig. S1b; fucoxanthin $r = 0.94$, $p < 0.01$, Supporting Information Fig. S1c). In contrast, the BT-measured biomass of green algae was only weakly correlated with Chl *b* ($r = 0.34$, $p < 0.01$, Supporting Information Fig. S1d). Since phycocyanins cannot be identified by our used HPLC protocol, it was not possible to compare the BT-measured biomass of cyanobacteria with HPLC measurements. Both proxies for autotroph biomass, BT-based total biomass of autotrophs and HPLC-based concentration of Chl *a*, correlated strongly with the directly measured DM of the periphyton that includes autotrophs, non-autotrophs, and non-living material such as detritus in periphyton (BT total: $r = 0.93$, $p < 0.01$, Supporting Information Fig. S1e; Chl *a*: $r = 0.94$, $p < 0.01$; Supporting Information Fig. S1f). In total, only 0.7% of periphyton biomass measurements in our experiments were above 4 μg cm^{-2}, the reported upper threshold for unbiased and accurate BT performance (Harris and Graham 2015; Echenique-Subiabre et al. 2016). Because of the strong correlations observed and the low number of measurements potentially affected by the instrument accuracy, we concluded that the BT provided accurate estimates of autotroph biomass and present only BT-based data hereafter. Because the biomass of green algae measured with the BT was only weakly correlated with their marker pigment Chl *b* identified by HPLC, green algae were not further analyzed.

Biomass

The biomass of newly developing periphyton was below the detection limit of the BT (0.01 μg cm^{-2}) at 1 week of growth, but was detectable and measurable at 2 weeks and 3 weeks. The total biomass of autotrophs (μg cm^{-2}), increased over time in both lit and control periphyton in both seasons (Fig. 2a,b; LMM, spring: time $F_{1,70} = 99.38$, $p < 0.0001$, autumn: time $F_{1,72} = 18.79$, $p < 0.0001$). In spring, significant interaction was found between treatment and time (LMM: treatment $\times$ time $F_{1,70} = 8.56$, $p = 0.005$). Pairwise comparisons indicated that the biomass of autotrophs did not differ between lit and control periphyton at 2 weeks of treatment (Fig. 2a, $p = 0.13$), but that at 3 weeks the autotroph biomass in the lit periphyton was significantly lower (57%, based on median values) than in the control (Fig. 2a, $p = 0.008$). In autumn, there was a significant effect of treatment (LMM: treatment $F_{1,72} = 4.20$, $p = 0.04$), and no significant interaction (LMM: treatment $\times$ time $F_{1,70} = 1.99$, $p = 0.16$). At 2 weeks of treatment, the biomass of autotrophs in the lit periphyton was 43% lower (median, Fig. 2b) than of the control periphyton ($p = 0.01$), while at 3 weeks there was no difference between the two treatments ($p = 0.65$). In pre-established periphyton, the total biomass of autotrophs increased over time in both lit and control periphyton in both seasons (Fig. 2c,d; LMM, spring: time $F_{1,72} = 36.9$, $p < 0.001$, autumn: time $F_{1,72} = 191.7$, $p < 0.001$). ALAN had no effect on the biomass of autotrophs in pre-established periphyton in either season (LMM, spring: treatment $\times$ time: $F_{1,72} = 0.10$, $p = 0.76$, autumn: treatment $\times$ time $F_{1,72} = 0.64$, $p = 0.43$).

In pre-established periphyton, the ratio of Chl *a* : DM was not affected by artificial nocturnal illumination in either season (Supporting Information Table S3. LMM, spring: treatment $\times$ time $F_{1,70} = 0.03$, $p = 0.86$, autumn: treatment $\times$ time $F_{1,69} = 0.46$, $p = 0.50$), indicating that the proportion of

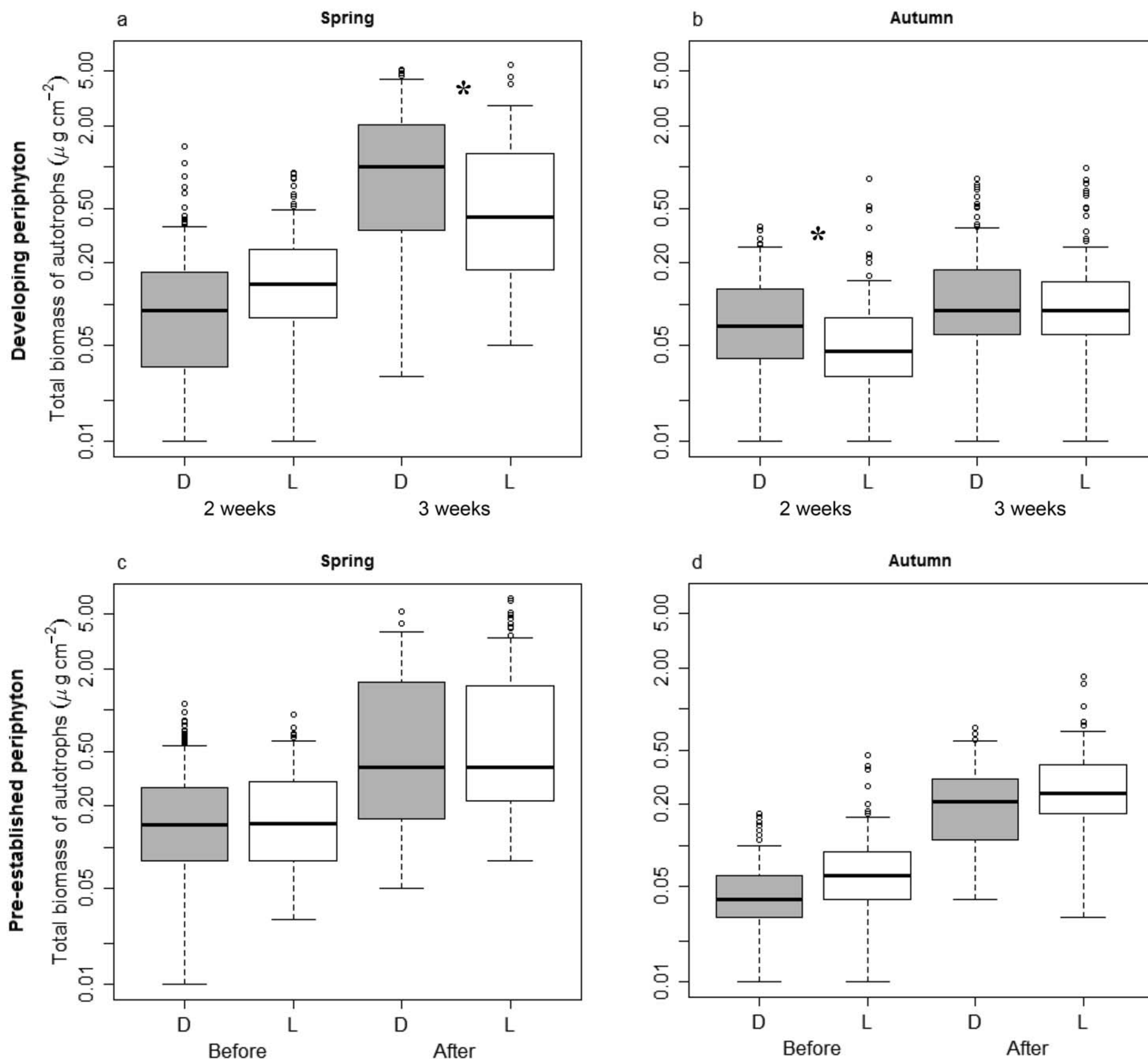

Fig. 2. Total biomass of autotrophs (μg cm^{-2}) measured in two experimental seasons with the BT in: (**a**, **b**) developing periphyton (single measurements $n = 1265$); (**c**, **d**) pre-established periphyton (single measurements $n = 1263$). Box: median, IQR; whisker: range (5–95% values). Data on Log-scale. Asterisk indicates significant difference between the two treatments ($p < 0.05$, linear mixed models and pairwise comparisons with Benjamini-Hochberg correction).

autotrophs in the periphyton community did not change due to different light environment induced by ALAN, nor that periphytic algae responded to ALAN with an increase in the intracellular concentration of photosynthetic pigments.

Community composition

Diatoms remained the dominant autotrophs in both lit and control periphyton in developing and pre-established communities at all times (Fig. 3). The proportion of diatoms increased with time in both seasons, and in both developing (Fig. 3a,b; LMM, spring: time $F_{1,71} = 12.25$, $p < 0.002$, autumn: time $F_{1,73} = 40.21$, $p < 0.001$) and in pre-established periphyton (Fig. 3c,d; LMM, spring: time $F_{1,72} = 248.18$, $p < 0.001$, autumn: time $F_{1,72} = 0.11$, $p < 0.001$). In developing periphyton, the proportion of diatoms did not differ between lit and control periphyton in spring (Fig. 3a; LMM: treatment

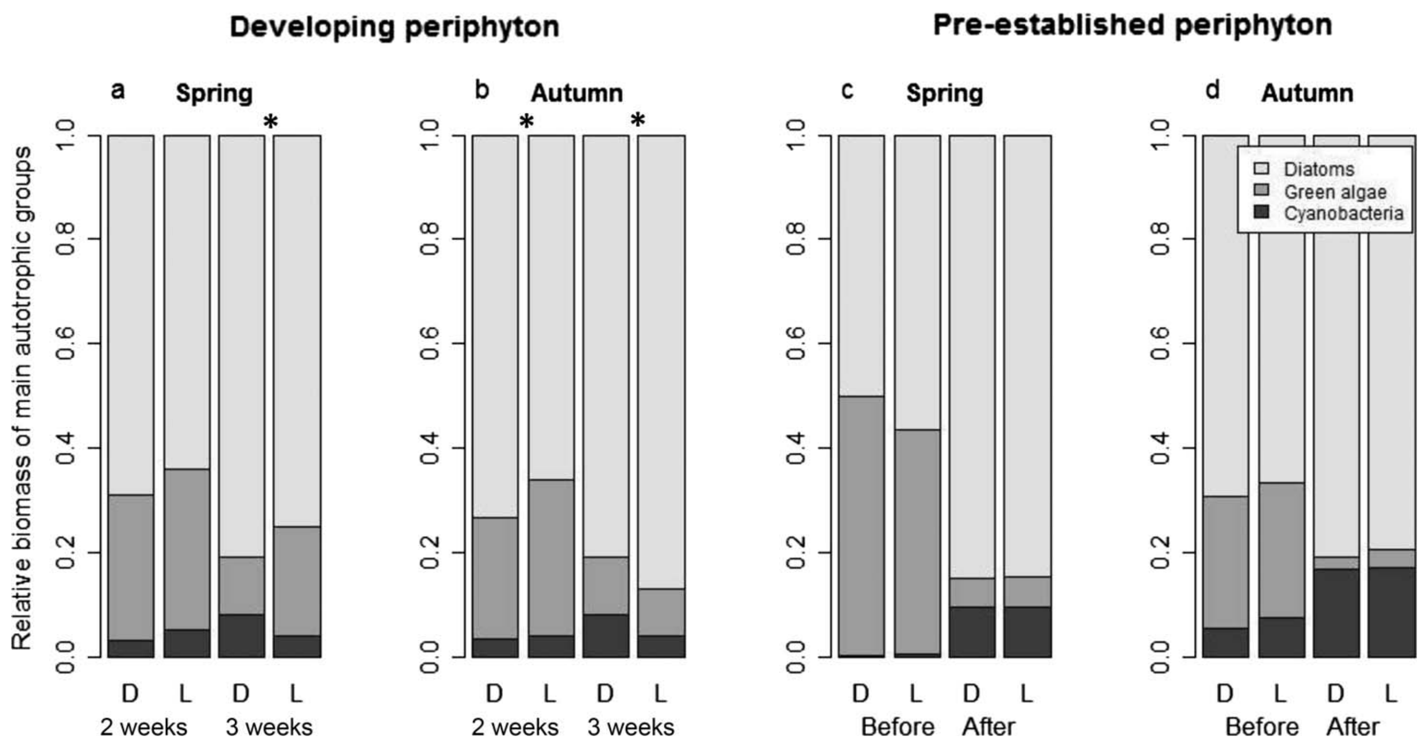

Fig. 3. Relative biomass of major autotrophic groups (diatoms, green algae, and cyanobacteria) measured in two experimental seasons with the BT in: (**a**, **b**) developing periphyton (single measurements $n = 1265$); (**c**, **d**) pre-established periphyton (single measurements $n = 1263$). Asterisk indicates significant difference in the proportion of diatoms or cyanobacteria between the two treatments ($p < 0.05$, linear mixed models and pairwise comparisons with Benjamini-Hochberg correction).

$F_{1,70} = 0.73$, $p = 0.40$, treatment × time $F_{1,70} = 0.96$, $p = 0.33$). The absolute biomass of diatoms did not differ between the treatments at 2 weeks, but it was 60% lower in the lit periphyton at 3 weeks of treatment compared to the control (Supporting Information Table S2; LMM: treatment × time $F_{1,70} = 8.93$, $p = 0.004$. Pairwise comparisons: lit to control at 2 weeks $p = 0.18$, at 3 weeks $p = 0.003$). In autumn, the proportion of diatoms in developing periphyton showed a significant interaction between treatment and time (Fig. 3b; LMM: treatment × time $F_{1,73} = 9.93$, $p = 0.002$). Pairwise comparisons indicated that at 2 weeks of treatment in autumn the lit periphyton had 11% lower (median) proportion of diatoms ($p = 0.01$), but at 3 weeks of treatment 5% higher proportion relative to the control ($p = 0.04$). A similar trend was observed for the absolute biomass of diatoms (Supporting Information Table S2), but there was no significant difference in absolute diatom biomass between lit and control developing periphyton (LMM: treatment $F_{1,72} = 3.25$, $p = 0.07$, treatment × time $F_{1,72} = 3.14$, $p = 0.08$). In pre-established periphyton, ALAN treatment had no effect on the proportion of diatoms in either season (Fig. 3c,d; LMM spring: treatment × time $F_{1,72} = 2.90$, $p = 0.09$, autumn: treatment × time $F_{1,72} = 0.23$, $p = 0.63$), nor on the absolute biomass of diatoms (Supporting Information Table S2; LMM, spring: treatment × time $F_{1,72} = 0.0001$, $p = 0.99$, autumn: treatment × time $F_{1,72} = 0.56$, $p = 0.46$).

The proportion of cyanobacteria increased with time in both seasons, and in both developing (Fig. 3a,b; LMM, spring: time $F_{1,72} = 42.99$, $p < 0.001$, autumn: time $F_{1,74} = 6.16$, $p = 0.01$) and in pre-established periphyton (Fig. 3c,d; LMM, spring: time $F_{1,74} = 110.74$, $p < 0.001$, autumn: time $F_{1,74} = 33.48$, $p < 0.001$). The biomass of cyanobacteria was generally low (< 1 μg cm^{-2}). In developing periphyton, there was a significant effect of treatment in spring (LMM: treatment $F_{1,70} = 5.59$, $p = 0.02$), and a significant interaction between treatment and time (LMM: treatment × time $F_{1,70} = 6.47$, $p = 0.01$). Pairwise comparisons indicated that at 2 weeks of treatment the proportion did not differ between lit and control periphyton ($p = 0.82$), but at 3 weeks the proportion of cyanobacteria in lit periphyton was 54% lower (median) than in the control ($p = 0.005$). A similar pattern was observed for the absolute biomass of cyanobacteria in spring (Supporting Information Table S2), where a significant interaction between treatment and time (LMM: treatment × time $F_{1,70} = 11.47$, $p = 0.001$) resulted from similar ($p = 0.83$) biomass between the treatments at 2 weeks, but 81% lower (median, $p < 0.001$) biomass in the lit periphyton at 3 weeks compared to the control. In autumn, there was no difference in the proportion of cyanobacteria between lit and control periphyton (LMM: treatment $F_{1,72} = 1.80$, $p = 0.18$, treatment × time $F_{1,72} = 2.00$, $p = 0.16$) and also no difference in their absolute biomass (LMM: treatment $F_{1,72} = 2.08$, $p = 0.15$, treatment × time

$F_{1,72} = 1.74$, $p = 0.19$). However, the absolute biomass of cyanobacteria was frequently below the detection limit (Supporting Information Table S2). In pre-established periphyton, the proportion of cyanobacteria was not affected by ALAN treatment (Fig. 3c,d; LMM spring: treatment × time $F_{1,72} = 0.003$, $p = 0.96$, autumn: treatment × time $F_{1,72} = 0.40$, $p = 0.53$), nor was the absolute biomass of cyanobacteria (Supporting Information Table S2; LMM, spring: treatment × time $F_{1,72} = 0.0009$, $p = 0.98$, autumn: treatment × time $F_{1,72} = 2.16$, $p = 0.14$).

Pigments were only analyzed in pre-established periphyton, and the ratio of Chl *c* : Chl *a* was affected by ALAN treatment in spring (Supporting Information Table S3; LMM: treatment × time $F_{1,70} = 4.75$, $p = 0.03$). Pairwise comparisons indicated that before the treatment the ratio did not differ between lit and control periphyton ($p = 0.67$), but that after the treatment the lit periphyton had 14% lower (median) Chl *c* : Chl *a* ratio compared to the control ($p = 0.009$). A similar pattern was found for the ratio of fucoxanthin : Chl *a* in spring (Supporting Information Table S3), but there was no significant difference between lit and control periphyton (LMM, spring: treatment × time $F_{1,70} = 1.00$, $p = 0.32$). In autumn, neither the ratio of Chl *c* : Chl *a* (LMM: treatment × time $F_{1,70} = 0.23$, $p = 0.63$) nor the ratio of fucoxanthin : Chl *a* (LMM: treatment × time $F_{1,70} = 0.96$, $p = 0.33$) were affected by ALAN.

Discussion

We found reduced biomass of autotrophs in developing periphyton (up to 3 weeks) in the flume sections that experienced night-time illumination by white LED in both spring and autumn. The proportion of cyanobacteria decreased under ALAN in spring, while the proportion of diatoms was affected by ALAN in autumn, with an initial decrease in lit periphyton at 2 weeks of treatment, but a contrasting increase at 3 weeks relative to the control. The observed effects of ALAN on periphyton biomass and the proportion of cyanobacteria were stronger in spring than in autumn, while the opposite was measured for the proportion of diatoms. Seasonal variation in species composition driven by non-ALAN related environmental variables may therefore be an important modulator of periphyton response to ALAN. The sensitivity to ALAN depended on the periphyton developmental stage: significant effects were observed in early (up to 3 weeks) but not in later (4–6 weeks) developmental stages. Our results suggest that systems dominated by periphyton in early developmental stages may be more sensitive to ALAN. Therefore, ALAN might reduce resilience of periphyton communities in streams and shoreline habitats subjected to frequent physical perturbations that scour the periphyton biomass and reset periphyton development.

The presence of ALAN creates an environment with alternating phases of natural light during the day and low-level artificial light during the night. These light conditions have rarely been studied, however, there are several aspects that can be discussed and compared to the current literature. By replacing the dark phase in a natural light/dark cycle with low-light illumination, ALAN may provide conditions comparable to those of continuous illumination, with two alternating phases of light intensity. Continuous light can have both positive and negative effects on plants and microalgae for reasons that are still poorly understood (see reviews from Sysoeva et al. 2010; Velez-Ramirez et al. 2011). Many species of algae, plants, and lower plants display reduced growth, productivity, and photosynthetic efficiency, including reduced quantum yield and lower maximum rates of electron transport and Rubisco carboxylation (Brand and Guillard 1981; Velez-Ramirez et al. 2011 and references therein). The light intensities applied in these studies are of a constant level, usually several orders of magnitude higher than those applied in our experiment and in the range of daylight intensities (75–500 μmol m^{-2} s^{-1} compared to approximately 0.31 μmol m^{-2} s^{-1}); however, some of these effects have been demonstrated to occur at lower light levels as well (Poulin et al. 2014). Maintaining active photosynthesis under low-level ALAN might be energetically costly (Poulin et al. 2014; Hölker et al. 2015). Furthermore, Zevenboom and Mur (1984) reported that the cyanobacteria *Microcystis aeruginosa* required a dark period to obtain maximum growth rate. We observed that also in semi-natural conditions, replacing the dark phase of a natural light/dark cycle with low-light white LED illumination (approximately 20 lux) can reduce the biomass of periphyton.

Many cellular processes such as chloroplast differentiation, DNA repair, cell division, embryogenesis, and gametogenesis depend on light/dark cycles (Hegemann et al. 2001) and a dark period might be critical for stress recovery and repair (Gaston et al. 2013). Under continuous light, the clock genes in moss were shown to express arrhythmic profiles (Okada et al. 2009) and this may be also occurring in algae. The disruption of circadian clocks and dependent physiological and developmental processes might therefore explain the observed periphyton biomass decrease in early developmental stages under ALAN in our experiment.

The minimum light intensity that supports growth based on aerobic photosynthesis is considered to be between 0.01 μmol m^{-2} s^{-1} (approximately 0.5 lux) and 0.1 μmol m^{-2} s^{-1} (approximately 7.4 lux), slightly above the maximum light of a full moon on a clear night (0.005 μmol m^{-2} s^{-1}, approximately 0.3 lux) (see discussion in Raven and Cockell 2006 and references therein). These thresholds are far below the ALAN levels applied in our experiment (20 lux); therefore, it is likely that nocturnal photosynthesis did occur under ALAN. However, the ALAN treatment did not result in an increase of periphyton biomass. The potential positive effects of ALAN on biomass through nocturnal photosynthesis were likely offset by its negative effects, e.g., through disruption of the circadian clocks or energy costs

of maintaining an active photosynthesis, resulting in the neutral or negative effects on periphyton biomass that we observed.

Periphyton growth and biomass are the result of an interaction between species traits available in the community, and external factors such as environmental conditions and grazing (Biggs et al. 1998). In our study, non-ALAN associated environmental conditions varied minimally across flumes (Supporting Information Table S1) due to the same inflowing water, short residence time, and the controlled flumes setting. Flumes were colonized by macroinvertebrate fauna; therefore, the indirect effects of ALAN on periphyton, due to potential changes in grazing activity by macroinvertebrates, cannot be excluded. However, the densities of macroinvertebrates in lit and control sections before the treatment were similar (number of individuals per m^2 of the substrate surface area, mean and SD averaged across five flume sections for each treatment: in spring 423 ± 129 for control and 417 ± 99 for lit sections; in autumn 1990 ± 1532 for control and 1818 ± 1291 for lit sections) (A. Manfrin, unpubl.). Immature stages of Baetidae and to a lesser extent Heptageniidae were predominant grazers/scrapers. Both Baetidae and Heptageniidae are common in mountain streams (Hieber et al. 2005) where they hide between the rocks and in crevices during the day and move to forage on epilithic periphyton, algae, and detritus at night (Bishop 1969). Both taxa are photophobic at night and the number of individuals that move to the upper surface of rocks was shown to decline by 85% under nocturnal illumination of 5 lux (Elliott 1968). Because the tiles were placed on top of the cobble layer, directly exposed to ALAN and without any cover, it is likely that periphyton on the lit tiles was grazed less, or at least not more than in the control sections. If grazers had any effect on the periphyton biomass, we would expect less grazing and thus higher periphyton biomass in lit sections relative to controls. So, while we cannot exclude indirect effects of ALAN on periphyton due to potentially ALAN-induced changes in grazing activity, the lower biomass we recorded in the lit sections suggests that grazers were not a strong determinant of the periphyton biomass.

The periphyton was largely composed of diatoms in our experiments. Diatoms are often the dominant group in streams and rivers worldwide (Biggs et al. 1998) and many species are adapted to light-limited conditions, such as heavy shade (Allan and Castillo 2007). The ability to grow under low light levels might provide them with a selective advantage over other groups in the periphyton community under ALAN conditions, as suggested by Hölker et al. (2015). The proportion of diatoms, as measured with the BT, only increased in periphyton in early developmental stages (up to 3 weeks) at 3 weeks of ALAN treatment in autumn, but a decreased proportion was observed at 2 weeks. The same pattern, although non-significant, was observed for the absolute biomass of diatoms. Since the periphyton communities significantly changed over time, the observed non-linear response of diatoms may be related to succession in species composition. In pre-established periphyton (4–6 weeks), ALAN did not affect the proportion of diatoms in either season, as measured with the BT. Diatoms are characterized by their marker pigments, Chl *c* and fucoxanthin (Jeffrey et al. 1997) and the ratio of Chl *c* : Chl *a* was found to be 14% lower in lit pre-established periphyton compared to the control in spring, as measured with HPLC. A similar pattern appeared for the ratio of fucoxanthin : Chl *a* in spring, but the difference was not statistically significant. That these patterns were found using HPLC but not BT indicates that pigment analysis by HPLC may be more sensitive than the BT in detecting changes in diatom proportions and thus community composition.

Similar to diatoms, cyanobacteria are also able to grow in low light environments (Richardson et al. 1983) where they can outcompete other species (Zevenboom and Mur 1984) but contrary to our expectations ALAN decreased their proportions in lit periphyton compared to the control. Cyanobacteria posess complex sensory systems that allow them to respond to changes in light intensity and spectral quality (Mullineaux 2001), and are also known to display circadian rhythms (Mullineaux 2001; Suzuki and Johnson 2001). A dark period was shown to be necessary for some species to obtainin a maximum growth rate (Zevenboom and Mur 1984). Their decreased biomass under ALAN suggests that cyanobacteria are sensitive to artificial nocturnal illumination that may have disrupted a circadian regulation or light/dark controlled physiological processes.

Primary producers are able to maximize their photosynthetic efficiency by changing intracellular concentrations of pigments in response to light conditions (Falkowski and Laroche 1991). This physiological acclimation is well documented in the laboratory, but rarely identified in the field (Descy et al. 2009). Photosynthetic pigments, e.g., chlorophylls and fucoxanthin, are known to increase in similar proportions under low light conditions (Descy et al. 2009), which might occur under low-level nocturnal artificial light as well. The ratios of Chl *a* : DM and fucoxanthin : DM were not affected by ALAN in the pre-established periphyton, indicating that periphyton did not acclimate to ALAN. However, ALAN decreased the ratio of Chl *c* : Chl *a* in spring. Since the periphyton communities significantly changed over time, this effect might be related to a different succession of species with different intracellular concentrations of Chl *c* in the two treatments.

The fact that effects of ALAN on both biomass and community composition were observed only for developing periphyton supports our hypothesis that periphyton sensitivity to ALAN is higher in early developmental stages compared to later ones. Thicker periphyton biofilms are resistant to high-light stress (Hill 1996) because of the light attenuation and self-shading that occur inside the complex periphyton matrix. This is likely also true for ALAN and might

explain the reduced sensitivity of periphyton in later developmental stages we observed. The observed seasonally different responses to ALAN may be due to the seasonal variation in community composition detected with the BT (Fig. 3) and with 18S-rRNA metabarcoding analysis targeting diatoms (M. Grubisic, unpubl.), as well as seasonal changes in environmental variables (Table 1).

Conclusions

The use of ALAN is increasing worldwide and therefore the ecological consequences of light pollution are increasing as well (Pawson and Bader 2014). It is known that ALAN affects aquatic microorganisms (Poulin et al. 2014; Hölker et al. 2015), insects (Perkin et al. 2014*b*; Honnen et al. 2016), and fish (Riley et al. 2012; Brüning et al. 2015). Our study shows that artificial nocturnal illumination, with white LED, can also influence biomass and community composition of aquatic primary producers, the basal food resource for consumers. A better mechanistic understanding of impacts of ALAN is necessary to predict long-term consequences and interactions with other factors such as trophic interactions or anthropogenic stressors such as eutrophication or climate change. Further research on underlying physiological responses, taxonomic sensitivity and the regulation of ecosystem metabolism may give an insight in the non-linear responses of ALAN observed in this experiment. Assessing effects of ALAN generated by different light sources, at different light levels and in different aquatic systems is urgently needed in order to identify and mitigate adverse ecological effects of light pollution.

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Conflict of Interest

None declared.

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