Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night

Franz Höller1, Christian Wurzbacher1,2, Carsten Weißenborn1, Michael T. Monaghan1,2, Stephanie I. J. Holzhauer1 and Katrin Premke1,3

1Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 301/310, Berlin 12587, Germany
2Berlin Center for Genomics in Biodiversity Research, Königin-Luise-Strasse 6–8, Berlin 14195, Germany
3Leibniz Centre for Agricultural Landscape Research (ZALF), Eberswalderstrasse 84, Müncheberg 15374, Germany

An increasing proportion of the Earth’s surface is illuminated at night. In aquatic ecosystems, artificial light at night (ALAN) may influence microbial communities living in the sediments. These communities are highly diverse and play an important role in the global carbon cycle. We combined field and laboratory experiments using sediments from an agricultural drainage system to examine how ALAN affects communities and alters carbon mineralization. Two identical light infrastructures were installed parallel to a drainage ditch before the start of the experiment. DNA metabarcoding indicated that both sediment communities were similar. After one was lit for five months (July–December 2012) we observed an increase in photoautotroph abundance (diatoms, Cyanobacteria) in ALAN-exposed sediments. In laboratory incubations mimicking summer and winter (six weeks each), communities in sediments that were exposed to ALAN for 1 year (July 2012–June 2013) showed less overall seasonal change compared with ALAN-naïve sediments. Nocturnal community respiration was reduced in ALAN-exposed sediments. In long-term exposed summer-sediments, we observed a shift from negative to positive net ecosystem production. Our results indicate ALAN may alter sediment microbial communities over time, with implications for ecosystem-level functions. It may thus have the potential to transform inland waters to nocturnal carbon sinks.

1. Introduction

Artificial light at night (ALAN) is increasingly recognized as a major component of global environmental change [1,2]. Through its effects on the behaviour and physiology of organisms, ALAN is a potentially important driver of ecosystem dynamics [3–6]. There is increasing evidence that ALAN can trigger physiological and behavioural responses in organisms even at very low light levels (below 1 lux) [5,7], making it an ecologically significant light source despite being only a small fraction of daytime sunlight.

Light can impact biological systems in different ways. It serves as an information source to organisms that, along with temperature, drives daily and seasonal cycles of behaviour, phenology and physiological change [5,8]. Light is also the main source of energy for most primary producers and thus a key factor influencing the community structure, biomass and productivity of phototrophic communities [9].

Freshwater ecosystems may be particularly susceptible to changing light regimes at night. ALAN is found where humans are found, and half of the human population lives within 3 km of a surface freshwater body [10]. Experiments on the effects of ALAN in aquatic systems to date have demonstrated physiological and behavioural responses of individual species [3], and community effects over one [11,12] or several [13] generations. ALAN may have consequences for aquatic primary producers because photosynthesis can occur at very low light levels. Minimum light requirements for growth among microalgae are around...
1 μmol photons m$^{-2}$ s$^{-1}$ (approx. 50 lux) [14]; however, marine photolithotroph algae are thought to grow at under 0.01 μmol photons m$^{-2}$ s$^{-1}$ (approx. 0.5 lux), which is slightly above the light of a full moon on a clear night (0.005 μmol m$^{-2}$ s$^{-1}$, approx. 0.3 lux) [15]. Poulin et al. [16] found that night-time irradiance levels comparable with near shore street lighting (0.08 μmol m$^{-2}$ s$^{-1}$, approx. 6.6 lux) influenced photophysiological processes in Microcystis aeruginosa, a common freshwater cyanobacterium.

An important next step is to determine whether ALAN can influence aquatic microbial community composition (MCC) over time (i.e. species composition and relative abundance) and whether the impact extends to emergent functional traits such as community metabolism and associated carbon turnover. Microbial communities represent an excellent system for studying temporal changes in community structure because of their short generation times relative to plant and animal communities [17]. One possible effect could be a community shift in favour of species or genotypes that are better suited to survive and reproduce in nocturnal low-light environments. This may then affect ecosystem-level functions such as carbon and nutrient turnover.

Community respiration (CR) and net ecosystem production (NEP) are two of the most pivotal processes in aquatic ecosystem metabolism. They incorporate biochemical pathways that make organic carbon molecules and energy available to microorganisms [18] and exhibit a strongly positive relationship with temperature [19]. As ecosystem metabolism is the primary control on carbon cycling in the biosphere, there is substantial interest in understanding the controls on aquatic ecosystem metabolism [20]. Freshwater systems are hot spots for carbon cycling on the landscape scale and play a significant role in global and regional carbon cycles [21,22]. Consequently, understanding the controls on ecosystem metabolism and factors influencing aquatic microbial communities is essential for predicting carbon cycle response to ongoing environmental change such as the dramatic increase in ALAN.

Here, we examine the microbial communities in the uppermost layer of freshwater sediments of an agricultural drainage system in northern Germany. We employed a field experiment approach to compare ALAN-naive and ALAN-exposed sediments, and a common garden incubation to examine interactions between ALAN and temperature. We used DNA metabarcoding to examine bacterial and algal communities and we measured CR in incubated sediment cores at two different temperatures. Our experimental set-up allowed us to test three hypotheses, namely (i) that the presence of ALAN would lead to altered benthic MCC by favouring phototrophic organisms able to carry out photosynthesis at low light levels; (ii) that changes in MCC would lead to changes in ecosystem function, e.g. CR and NEP; and (iii) that seasonal changes in community structure and function would be less pronounced because of the potential loss of an important seasonal cue.

2. Material and methods

(a) Experimental field site

Sediment cores for both field and laboratory studies were taken from an agricultural drainage ditch at an experimental site in Westhavelland Nature Park, Brandenburg, Germany (52°59′ N, 12°46′ E). Located approximately 70 km northwest of Berlin, it is one of the darkest areas of Germany at night and is an official Dark Sky Reserve (www.darksky.org). The experimental site consists of two grassland fields that were under the same management regimen by the same agricultural business (mown twice per year, no fertilizer). Identical light infrastructures were installed in two sites along the drainage ditch in early spring 2012. Each site contains 12 street lights (20 m apart) arranged in three rows parallel to the ditch. The ditch itself is approximately 5 m wide and 50 ± 26 cm deep. The first row of lights is located 3 m from the water’s edge. There is little or no water flow in the ditch except during strong precipitation events. The main flow directions, as indicated in the topographic map for this area, is from the control site to the treatment site over a distance of around 800 m (Blatt 3340-NO Ferchesar, 1: 10.000). The experimental sites are separated by 600 m (Euclidian distance) and a row of trees ensures there is no influence of the experimental lights on the control site. Luminaires (Schreder Saphire 1) were mounted at 4.75 m height. Lights (70 W high-pressure sodium lamps, 2000 K, 96 lm W$^{-1}$; Osram Vialox NAV-T Super 4Y, Munich, Germany) were turned on in the light treatment site on 25 July 2012. Nocturnal light levels ranged from 13.3 to 16.5 lux at the water surface (approx. 0.18 μmol m$^{-2}$ s$^{-1}$) and 6.8–8.5 lux (approx. 0.09 μmol m$^{-2}$ s$^{-1}$) at the sediment surface (50 cm depth) when samples were collected. Light measurements in the field and laboratory were taken with an ILT1700 lux meter (range: 0.00167–1 670 000 lux, International Light Technologies, Peabody, MA, USA).

(b) Field sampling

Benthic microbial communities in the two sites were sampled in July 2012 (summer) before the start of the artificial light treatment, and in December 2012 (winter), five months after artificial illumination at the light site. A sediment core (Uwitec, Austria; inner diameter: 60 cm) was used to obtain surface sediment (0–2 cm depth) and individual samples (three replicates per site) were placed directly into 50 ml Falcon tubes and stored in the dark at −20°C prior to DNA extraction. For the laboratory incubation experiment, 28 sediment cores from each site were taken in each field at the end of June 2013. The first 2 cm of all 28 cores were pooled in one sterile container per site (totalling 1.6 l sediment each). Water samples were taken on the same day, filtered (GF/C-filter, Whatman Ltd, USA), and stored in a climate chamber until the start of the experiment (compare insets of figure 1a,c,d).

Dissolved oxygen, pH, conductivity, temperature, chlorophyll (YSI 6600 V2 data probe, YSI, Yellow Springs, USA) and precipitation (Vaisala Weather Transmitter WXT520, Vantaa, Finland) were measured at both sites over a three-week period before each of the field samplings in July and December 2012, and again in June 2013. In addition, water samples were taken in 2013 and 2014 to measure carbon and nutrients (dissolved organic carbon (DOC), total phosphorus (TP), soluble reactive phosphorus (SRP) and dissolved inorganic carbon (DIC)) according to standard chemical procedures [23,24]. DOC concentrations were determined with an organic carbon analyser (Shimadzu, TOC-V CPH, Duisburg, Germany) as non-purgeable carbon after acidification. DIC was measured using a nitrogen/carbon analyser (multi N/C 3100, Jenncalystics). Concentrations of TP and SRP were determined photometrically by the molybdenum blue method [25] using segmented flow analysis (Skalar Scan$^{++}$, Skalar Analytical B.V.). The two sites were similar in terms of morphology, catchment characteristics and abiotic parameters (electronic supplementary material, Table S1) of the ditch. There were small but significant differences in some chemical parameters between the two sites before the samples were taken (electronic supplementary material, table S1).

(c) DNA metabarcoding of sediment microbial communities

DNA was extracted from all sediment samples (field samples from July and December 2012 and incubated cores) using a
phenol–chloroform/CTAB method [26]. Approximately 350 µl of sediment was used for each extraction. A mechanical bead-beating step was performed using MMX400 (Retsch GmbH, Germany) for two rounds of 3 min at a frequency of 28.5 s⁻¹. DNA was quantified using a NanoDrop spectrometer (PEQLAB Biotechnologie GmbH, Germany) and uniformly diluted to a concentration of 52 ng µl⁻¹. One microtitre of this was used to amplify the V8 region of the ribosomal small subunit marker gene with universal primers (926F and 1392R) from [27].

Two amplicon libraries were constructed, one for the field study, which was sequenced with 454 pyrosequencing (GS Junior Titanium bench top sequencer, Roche, 454 Life Science), and one for the incubation experiment, which was sequenced with a MiSeq (Illumina). For the 454 sequencing, barcoded fusion primers were used with conditions as described previously [27]. The protocol was modified by using the Herculase II Fusion DNA Polymerase system (Agilent Technologies) with 0.25% DMSO with 30 cycles of amplification, and by increasing annealing and denaturation times to 45 s. The master mix and samples were prepared for amplification on a sterile bench to prevent foreign DNA contamination. Amplicons were gel-checked, purified, pooled and subjected to emulsion PCR following the steps of the GS Junior Titanium Series manual for Lib-L amplicon libraries (Roche, 454 Life Science). Illumina sequencing used the same native primers (926F and 1392R) without barcodes, no DMSO in the PCR reaction and an annealing temperature of 50 °C. PCR products were purified with magnetic beads (MagBio HighPrep, GC biotech) and the library preparation was carried out with Nextera XT DNA Sample Prep Kit (Illumina) in combination with the 96 Nextera XT Index Kit (Illumina) following the manufacturer’s instructions. During the preparation, the amplicons are shortened by a transposase. The resulting sequencing library was subjected to sequencing with the two-times 300 bp reads (V3 chemistry, Illumina).

(d) Laboratory incubations

Transparent incubation cores (acrylic, inner diameter 53 mm; height 300 mm) were filled with 100 g of sediment sampled in June 2013 from either the lit or control site (after around 1 year of ALAN at the experimental site). Incubation cores with the sediment were gently filled up with the filtered ditch water that was collected on the same day that sediment was collected. It is likely that oxygenation, which occurred while sediments were being prepared for the experiment, resulted in a loss of Archaea from these samples (see §3). For the incubations, 10 cores from each site were placed into a temperature-controlled plant-growth climate chamber (KBW-720; Binder, Germany). The upper section of the chamber was programmed for dark (control) conditions at night and the lower section was programmed for ALAN (treatment) conditions (four treatments × five replicates). The sections were separated by a dark opaque plastic to prevent light exchange. In each level, light-cassettes (five compact fluorescent lamps, LT–T8 30 W COLOURLUX plus, 6500 K, NARVA GmbH, Germany) were placed 50 cm above the incubation cores and these were used for daytime illumination (3220 ± 284 lux 50 cm below at the incubation cores, approx. 45 µmol m⁻² s⁻¹). In the treatment section, four LED strips (6300 K, Barthelme GmbH, Nürnberg, Germany) were installed on the rear panel and were used for night-time illumination of the treatment incubation cores (71 ± 4 lux, approx. 1.3 µmol m⁻² s⁻¹). LED intensity was regulated over a pulse-width modulator (FG-Elektronik, Germany) with a potentiometer. LED cycles were controlled by a clock timer (type SB-1, Unitec, Germany). An aeration system prevented the generation of oxygen gradients. Temperature was monitored with data loggers (Gemini-Data-Loggers, UK). A minimal temperature difference of 0.2 °C between upper and lower chambers was noted over the whole experiment.

The incubation experiment consisted of two different phases, designed to mimic summer and winter conditions of the ditch sediment ecosystem. Starting with the summer conditions, the temperature was maintained at 20.0 ± 0.03 °C with a light: dark cycle of 16.5 L: 7.5 D (daylight from 05.00 to 21.30). Throughout the night (21.30 to 05.00), control incubation cores were in complete darkness and treatment incubation cores were lit with LED lights. The summer experiment lasted six weeks, after which temperature and photoperiod were linearly reduced over a period of 12 days, to acclimatize the cores to winter conditions. Winter temperature was maintained at 6.0 ± 0.09 °C with 7.5 L: 16.5 D (daylight from 08.30 to 16.00). In the treatment chamber, cores were again lit with LED lights throughout the night (from 16.00 to 08.30). Water samples were taken at the start of the experiment and at the end of each incubation period (20 °C and 6 °C) to measure DOC, DIC, TP and SRP.

CR in the sediment cores was measured using a needle-type oxygen microsensor (Optode, PreSens, MicroX T3, Regensburg, Germany) after mixing the water column [28,29]. This non-invasive method was used to measure oxygen concentration through a septum directly in the water column 1 cm above the sediment of the experimental cores. This resulted in seven measurements per core at 20 °C and 16 measurements per core at 6 °C. To obtain values, oxygen concentrations were measured three to six times starting 30 min after hermetically sealing the sediment core. The amount of consumed oxygen was calculated over time and averaged over all repeated measurements. Single CRday measurements differed significantly with the progression of the incubations (ANOVA, at 20 °C and at 6 °C, p < 0.001), while CRnight measurements were independent from the measuring date (ANOVA).

(e) Data analysis

Chemical parameters were compared using a two-tailed t-test. Data were checked for homogeneity of variance (Levene test) and normal distribution (Shapiro–Wilk test) and transformed (In or x 1.1 to x 2) if needed. Alternatively, a Welch test or a Mann–Whitney U-test was applied. Tests for each data subset were Bonferroni-corrected for multiple comparisons (n = 6). In the subsets, we tested for (i) the differences between the two sediment types, (ii) the differences between the two subsequent temperatures, and (iii) the differences between the light treatments and the controls. The significance level for all tests in this study was set to 0.05.

The number of DNA sequence reads varied across all samples; thus a random subsample of 3650 reads (the size of our smallest sample) for the field samples and 4254 reads for the laboratory incubation samples was used to make data comparable. Laboratory incubation sequences consisted of paired reads and thus first had to be overlapped using MOTHUR [30] by defining a minimum length of 190 and a minimum overlap of 25 with a maximum of five mismatches. All sequences (field and laboratory sequences) were then submitted to the SILVA NGS pipeline [31] using default settings (www.arb-silva.de/ngs/). This resulted in a taxon-abundance table. The SILVA NGS table (number of reads for each taxonomic path, corresponding to an organism group with the maximum taxonomic depth of 20) was used as input for R. Krons&321;&321; charts [32] were created using the group means. Subsequently, MCC was analysed by multivariate statistics using the vegan package [33]. Non-classified species (‘no relatives’) were excluded from all datasets prior to statistical analysis, which resulted in 1141 taxonomic paths for the field sites and 2195 taxonomic paths for the laboratory experiment. Taxonomic turnover (βsorensen) was calculated with the Sorensen index (binary) and the Shannon index was calculated using a subsampled matrix with the sequencing read counts. For the MCC, we analysed the field samples using non-metric multidimensional scaling (NMDS). The matrix was transformed...
into a probability presence/absence matrix (function ‘drarefy’ with the smallest number of sequences) and served as the basis for calculating the NMDS ordination by applying Euclidean distances (figure 1a). The ordination was rotated so that the first axis corresponds to season. The separation of the sampling dates and sites was visualized by drawing standard-error ellipses around group centroids with a confidence level of 0.95. This was supported by a permutational multivariate analysis of variance (PERMANOVA). Seasonal differences in major taxonomic groups between the ALAN-naive and ALAN-exposed areas (figure 2a) were examined by subtracting each December value from each July value. We removed all minor microbial groups. Significance was tested with a Mann–Whitney U-test and was not corrected for multiple comparisons.

We analysed the samples from the laboratory set-up in a similar way. We started the analysis of the MCC by testing the possible influence of temperature and CR night on the MCC. This was done by calculating a partial constrained redundancy analysis (RDA) based on the original quantitative matrix (figure 1b). The effect of the two sediment types was thereby removed. Afterwards, the CR night was fitted into the ordination by R (envfit, p < 0.05). (Online version in colour.)

Figure 1. MCC presented as NMDS (axis 1 was always aligned with the seasonal shift/temperature) and as partial RDA in (a) field sediments and (b–d) incubated cores. Ellipses indicate the standard error around the centroid for each core type (origin and experimental treatment). Insets are Krona charts with two taxonomic levels of the microbial community displayed at the start of each experiment (red, Bacteria; brown, Eukarya; blue, Archaea; green, no relatives; C, ALAN-naive (control) sediment; T, ALAN-exposed (treatment) sediment). (a) Ditch sediment samples were analysed using a weighted presence/absence matrix and Euclidean distances, calculated and presented as NMDS. (b) Laboratory-incubated cores were examined using partial RDA of the sediment community. Sediment type explained 6.4% of the variation. The residuals were constrained with temperature and the mean night respiration. The RDA model was significant for both terms (ANOMA, temperature p < 0.001; CR night p < 0.05, 2000 permutations). (c,d) NMDS ordinations of laboratory-incubated cores (analogous to (a)) with the two sediment sources (c: ALAN-exposed; d: control). The arrows mark significant parameters which were fitted into the ordination by R (envfit, p < 0.05). (Online version in colour.)
comparisons). Last, we employed a permutation analysis on the laboratory incubation data for identifying single taxonomic groups at the deepest level that was resolved. This was written as a function in R, which randomly subsampled the community matrix 500 times and performed a Mann–Whitney U-test per taxonomic group for each subsampling in order to cope with the type I error caused by the subsampling. The $P_q$-values show the fraction of non-significant Mann–Whitney U-tests in 500 random subsamples. The significance level for the $P_q$-value was set to 0.05 without additional correction for multiple comparisons.

3. Results

(a) Field experiment

The microbial communities (Bacteria, Archaea and Eukarya) in the freshwater sediments of the two ditch sites (ALAN-naive and ALAN-exposed) showed a large degree of overlap in their overall structure at the start of the study in July 2012 (figure 1a, inset) and in taxonomic composition ($\beta_{\text{act}} = 0.339$ between ditches; $\beta_{\text{res}} = 0.321$ between replicates within each site; electronic supplementary material, figure S1). After five months of illumination, the communities at the two sites remained similar (PERMANOVA, $p > 0.05$) although the NMDS ordination on the two sampling dates detected a significant difference between the two sites in December 2012 (figure 1a). There was a significant seasonal trend (PERMANOVA, $p < 0.01$), with seasonal difference appearing to be smaller at the ALAN-treated site.

From the taxon list generated from DNA metabarcodes, a number of microbial groups were present that could potentially respond to artificial illumination (figure 2a). Of these, there was an increase in the relative abundance of photoautotrophic Stramenopiles (dominated by diatoms in our samples, see electronic supplementary material, figure S1) in the ALAN-exposed sediments five months after lights had been switched on. Mixotrophic organisms (Cryptophyceae and Dinophyceae) were either unaffected or were reduced after five months of illumination. Some of the major heterotrophic microbial groups (Bacteroidetes and Deltaproteobacteria) also were reduced after illumination.

(b) Laboratory incubation experiment

Starting conditions in the sediments from the two sites (ALAN-naive and ALAN-exposed) differed slightly but consistently in DOC concentrations (ALAN-exposed > ALAN-naive, mean $\Delta$DOC = 2.7 mg l$^{-1}$, mean DOC = 23.3 ± 3.1 mg l$^{-1}$). Concentrations of DIC, TP and SRP were similar. Mean SRP concentrations were 4.9 ± 2.8 µg l$^{-1}$. Following the experiment, cores exhibited significant differences between the two temperature regimes in all measured parameters (table 1). Microbial
communities at the start of the experiment were similar in the two sites ($\beta_{\text{str}} = 0.388$ between sites; $\beta_{\text{rep}} = 0.385$ between replicates within each site; see insets of figure 1c,d). Differences primarily were the result of differing abundance (i.e. relative number of sequencing reads) at the lowest taxonomic level (electronic supplementary material, figure S1 and table S2). The microbial communities from all incubations were structured significantly by temperature (6°C and 20°C, partial RDA, $p < 0.001$) and respiration (CRnight; partial RDA, $p < 0.05$; figure 1b). Qualitative MCC of each sediment type revealed that the ALAN-exposed sediment community showed no group separation at all (PERMANOVA, $p > 0.05$), i.e. no seasonal response to the temperature trigger or a light response.

In the ordinate (figure 1c), a weak effect of ALAN was visible, which corresponded to a decreased Shannon index (Mantel test, $p = 0.163$, $p < 0.01$). This was in strong contrast to the ALAN-naive sediment community at summer conditions previously exposed to ALAN (figure 3a, CLN (ALAN-naive sediment, lit at night, night measurements)). The exposure to ALAN had a clear effect on the CR at night (CRnight) (table 1). The CR during the day (CRday) was lower under summer conditions for the ALAN-naive sediment (figure 3b, CD (ALAN-naive sediment, dark at night, day measurements), and table 1). When compared directly, median CRnight was lower across all light-treated incubations except for the ALAN-naive sediment community at 20°C (figure 3b, CLN (ALAN-naive sediment, lit at night, night measurements)). This lower CR under ALAN conditions points to oxygen production by photosynthesis, especially in sediment communities at summer conditions previously exposed to ALAN (figure 3d, TLN (ALAN-exposed sediment, lit at night, night measurements)).

Changes in relative abundance of responsive taxa again included a potential increase in diatoms with light (Stramenopiles in figure 2b). Cyanobacteria also exhibited a pronounced response in cores from the ALAN-exposed site. The communities in the ALAN-naive sediment behaved similarly to the field data, i.e. the sediment communities showed clear seasonal differences (figure 2a). At the lowest available taxonomic level, several photautotrophic (Cyanobacteria, green algae) and heterotrophic organisms were affected by light in the ALAN-exposed sediment over the course of the experiment (table 2). In subsets of the data (i.e. at the 20°C incubations or the 6°C incubations), single photautotrophic groups increased in abundance (20°C, diatom Nitzschia; 6°C, Cyanobacteria; see detailed electronic supplementary material, table S2). We found no significant changes to taxonomic groups in the ALAN-naive sediment. Only in subsets of the ALAN-naive data (i.e. at the 20°C incubations or the 6°C incubations) could single non-phototrophic taxonomic groups be identified (see detailed table in electronic supplementary material, table S2).

4. Discussion

Artificial illumination near surface freshwaters has the potential to be a viable night-time energy source for microbial phototrophs, some of which can carry out photosynthesis at light levels that are near to those generated by ALAN. This could result in changes to the microbial community as conditions may favour taxa that benefit from nocturnal (low-level) light. These changes could, in turn, influence ecosystem-level processes such as carbon turnover. Such changes may also vary
temporally because day length and light levels (along with temperature) act as seasonal cues for many organisms in natural systems. We carried out field and laboratory experiments to examine changes to microbial community structure and carbon mineralization in sediments of an agricultural drainage system. The field experiment started when artificial lights were

Table 2. Significant changes in relative abundance of selected taxa in incubated cores following illumination at night in sediments pre-exposed to ALAN during the whole course of the experiment (both temperature regimes). Significance is based on a permuted Mann–Whitney U-test ($p < 0.05$). $Pq$-values represent the proportion of negative Mann–Whitney U-tests. P, photoautotroph; M, methylotroph; H, heterotroph.

<table>
<thead>
<tr>
<th>domain</th>
<th>ID</th>
<th>taxon</th>
<th>trend</th>
<th>$Pq$</th>
<th>function</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacteria</td>
<td>358</td>
<td>candidate division TM7</td>
<td>—</td>
<td>*</td>
<td>H</td>
<td>filamentous, stabilize biofilms</td>
</tr>
<tr>
<td></td>
<td>449</td>
<td>Cyanobacteria, Snowella</td>
<td>+</td>
<td>*</td>
<td>P</td>
<td>psychrophilic</td>
</tr>
<tr>
<td></td>
<td>455</td>
<td>Cyanobacteria, Subsection III, Family I</td>
<td>+</td>
<td>***</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>488</td>
<td>Cyanobacteria, MLE1–12</td>
<td>+</td>
<td>**</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>612</td>
<td>Firmicutes, Ruminococcaceae, Incertae sedis</td>
<td>—</td>
<td>*</td>
<td>H</td>
<td>isolated from rumen</td>
</tr>
<tr>
<td></td>
<td>802</td>
<td>Alphaproteobacteria, Hyphomicrobiurn</td>
<td>—</td>
<td>*</td>
<td>M</td>
<td>some photo-heterotrophy</td>
</tr>
<tr>
<td>eukaryota</td>
<td>1552</td>
<td>Chloroplastida, Charophyta, Mesotaenium</td>
<td>+</td>
<td>**</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1597</td>
<td>Chloroplastida, Chlorophyta, Scenedesmus</td>
<td>—</td>
<td>*</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1814</td>
<td>Opisthokonta, Nucleomycetes, LKM11</td>
<td>—</td>
<td>***</td>
<td>H</td>
<td>algal decomposer/parasite</td>
</tr>
<tr>
<td></td>
<td>1874</td>
<td>Alveolata, Ciliophora, Didinium</td>
<td>—</td>
<td>***</td>
<td>H</td>
<td>feeds on other ciliates</td>
</tr>
</tbody>
</table>

* $pq < 0.05$; ** $pq < 0.01$; *** $pq < 0.001$. 

Figure 3. CR (mg O$_2$ L$^{-1}$ h$^{-1}$) of the incubated sediment cores during the day (open box plots) and at night (shaded box plots) at 6°C (a,c) and 20°C (b,d). X-axis labels indicate sediment source (T, sediment from ALAN-exposed (treatment) ditch; C, sediment from ALAN-naive (control) ditch) and incubation treatment (L, lit at night; D, dark at night). N, night measurements. The horizontal line indicates the transition between gross ecosystem production (P) and respiration (R). Box plots (median, 25th percentile, 90th percentile, outliers) depict means of measurements made over the six-week period of each experiment. Asterisks denote significant difference between control and treatment conditions. (Online version in colour.)
systems that receive high levels of sunlight and low levels of light intensities might be beneficial in shallow aquatic high light environments [14]. Thus, the ability to use a large range of light intensities is crucial for the success of communities. Some dinoflagellates can photosynthesize and may thus be able to use ALAN. Others have higher maintenance energy demands and may be unable to use these low light levels below or close to our experimental ALAN level [14,36] and have light compensation points of growth and photosynthesis [37]. Finally, there is some evidence that intermittent near-dark periods influence photoacclimation to photoperiod in a species-specific way [38].

A long-term approach, including multiple generations of microbial communities [35], we further examined changes under controlled laboratory conditions. Phototrophs (diatoms, Cyanobacteria, green algae) again showed an increase in response to ALAN, but this was much more pronounced in the pre-exposed site. Collectively, these results suggest that an illumination period of over three months induced a weak response and that illumination for 1 year was sufficiently long to prompt a clear change in what had been an ALAN-naive freshwater microbial community. These data provide a starting point for future studies on rates of community change.

Several mechanisms might play a role in the response of Cyanobacteria, diatoms (e.g. Nitzschia) and green algae (e.g. Mesotaenium) to light at night. Some Cyanobacteria and diatoms have light compensation points of growth and photosynthesis below or close to our experimental ALAN level [14,36] and may thus be able to use ALAN. Others have higher maintenance energy demands and may be unable to use these low intensities. Some dinoflagellates can photosynthesize and grow at low photon flux densities but cannot tolerate relatively high light environments [14]. Thus, the ability to use a large range of light intensities might be beneficial in shallow aquatic systems that receive high levels of sunlight and low levels of ALAN. State transitions, which are a short-term acclimation mechanism allowing a cell to better balance the distribution of energy between photosystems I and II, might be beneficial for Cyanobacteria and diatoms to increase the efficiency of utilization of absorbed light energy under ALAN conditions close to our experimental ALAN level [37].

An important objective of our study was to test whether changes to the communities were accompanied by functional changes, namely to the process of carbon mineralization. In the experiment, we observed lower total CR in ALAN-exposed incubations, which supports our second hypothesis. It is not clear for all treatments whether this is indicative of greater primary production, but nonetheless indicates a significant link between the MCC and carbon turnover. Given the relatively low-light intensities of ALAN compared with sunlight, a shift to positive NEP might not be expected. Instead, indirect effects of ALAN, including changes in animal (e.g. herbivore) behaviour and plant phenology, could be a mechanism influencing primary production. However, in the treatment with ALAN-exposed sediment communities at 20°C, the majority of cores changed to overall autotrophy (negative respiration) at a level similar to that observed during the day. In this way, ALAN could increase NEP, which has implications for ecosystem function.

Night-time irradiance levels in the laboratory (71 lux, approx. 1.3 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)) were brighter than in our field study, but fell within the higher range of light levels reported for street lighting. Perkin et al. [12] measured surface light levels between 0.008 and 4 lux at selected urban stream locations. Meyer & Sullivan [13] reported light levels ranging from 8 to 12 lux at open or partly canopied streams adjacent to 'highly' lit areas. Hale et al. [39] estimated that 33% of the city of Birmingham (UK) is covered by natural areas of which 13% of the area contributed to the total city area with ALAN levels above 30 lux. The highest known surface light level, 150 lux (approx. 2.7 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \)), due to street light was reported for a small town close to the experimental field site [40]. This suggests that the experimental set-up mimicked a high but...
typical ALAN scenario close to a strong LED street light. However, future studies should also include lower light levels.

Research on inland water metabolism has shown that gross primary production, CR and NEP vary considerably over time [41,42]. Most standing inland waters have overall negative NEP and thus release CO₂ to the atmosphere, although NEP may be positive during the day and negative at night. However, circadian rhythms in ecosystem metabolism within a water body are primarily driven by changes in light and temperature [20]. Such day–night differences may be negated by the impact of ALAN as we have seen in our experiments. The resulting changes in microbial community composition may thus shift NEP from negative to positive at night (figure 4).

There have been few studies of seasonal changes in microbial community structure (e.g. [17,43]) and community function [44] in aquatic sediments. This is the first study of which we are aware that investigates how the overall community structure and function of sediment microbial communities may be influenced by ALAN. We found evidence that seasonal changes in community structure and function are less pronounced in sediments exposed to ALAN, supporting our third hypothesis. The field samples provide evidence because the seasonal effect was reduced in the ALAN-treated communities after five months of exposure. After 1 year of artificial illumination in the lit site, this trend continued with the pre-treated sediment no longer showing a clear seasonal response to temperature. In fact, results from the pre-treated sediment community suggest that ALAN may be the sole trigger and that it may override other seasonal triggers such as temperature. Consequently, this results in a loss of temporal structure and may lead to a loss of diversity.

ALAN represents a sustained perturbation that may have long-term cumulative effects. However, much of the available knowledge is based on short-term experiments within one generation, typically over days to weeks. These do not allow the consideration of response mechanisms, such as acclimation, adaptation, physiological, behavioural or even evolutionary compensatory mechanisms linked to environmental context and seasonal timing. Malicky [45] observed at a newly illuminated fuel station a high initial flight activity of insects during the first 2 years, which diminished rapidly in subsequent years.

Perkin et al. [12] suggested that artificial light might be an example of a typical ‘press’ driver [46] that elicits a ‘ramped’ or delayed and slowly increasing response from many organisms. It is thought that, over time scales of a few years, microbial composition usually differs from that of undisturbed communities [47]. To reliably predict the ecological consequences of ALAN in natural systems, it is critical to have a better understanding of longer term processes that moderate the susceptibility of communities to an illuminated environment [48].

5. Conclusion ALAN induced shifts in microbial community structure and ecosystem function and may lead to overall shifts in NEP. As ALAN increases in global extent [2], increased illumination of water bodies in rural areas could lead to diminished diurnal fluctuations and subsequently a shift from negative to positive NEP. In short, ALAN has the potential to transform inland waters to a carbon sink during the night. Additional studies are needed to verify whether this is the case in other aquatic systems. This information could greatly enhance our ability to predict system responses to global change.

Data accessibility. DNA sequences have been submitted to the European Nucleotide Archive (ENA) under the study accession number PRJEB3881.

Acknowledgments. This is publication 12 of the Berlin Center for Genomics in Biodiversity Research (http://www.begendiv.de). We are grateful to Susan Mbedi and Stefan Heller for their help in the field and laboratory, to Katrin Attermeyer for designing figure 4 and to Tom Chatwell for helpful comments.

Author contributions. F.H., M.T.M., C.W. and K.P. conceived the study. C.W. carried out the respiration measurements. C.W. and S.H. carried out the sediment sampling. C.W. and C.W. carried out the DNA metabarcoding. C.W. analysed the sequence data and carried out all community analyses. C.W., F.H. and K.P. analysed respiration data. All authors contributed to writing the manuscript.

Funding statement. Research was supported by the Verlust der Nacht project, funded by the Federal Ministry of Education and Research, Germany, BMBF-033L038A.

Conflict of interests. We have no competing interests.

References


14. Richardson K, Beadall J, Raven JA. 1983 Adaptation of unicellular algae to innundiance: an analysis of

Downloaded from http://rstb.royalsocietypublishing.org/ on May 22, 2017