The MILAN Campaign

Studying Diel Light Effects on the Air–Sea Interface

In the following, the methods of data acquisition, sampling, and analytical procedures are listed. At the end, Table ES1 summarizes measurement precision of all methods used to analyze discrete water and aerosol samples presented in the main text of the manuscript.

**Meteorological data processing**

Temperature and wind speed analysis at the Institute for Chemistry and Biology of the Marine Environment (ICBM)-Terramare was based on data collected at 10-min intervals using a Davis Vantage weather station. Meteorological data at the catamaran S$^3$ (Ribas-Ribas et al. 2017) were collected at a higher frequency (1-min interval), which was averaged using a 10-min window. All wind speed measurements were corrected for wind speed at 10 m height ($U_{10}$; Kleemann and Meliss 1993). Large-scale meteorology analysis was based on ERA-Interim (Dee et al. 2011) data for mean sea level pressure and $U_{10}$ wind, while cloudiness was evaluated from low level cloud fraction. Air surface photosynthetically active radiation (PAR) was measured with a LI-190R sensor and global solar radiation was measured in the surface using a LI-200R pyranometer in the 400–1,100 nm range.

**Hydrology measurements**

Conductivity–temperature–depth (CTD) data were measured by a “SBE19plusV2” from Sea-Bird Electronics. The temperature data were calculated by using the ITS90 and the salinity data were calculated by PSS78 (Seabird toolbox). The acoustic Doppler current profiler (ADCP) 1,200 kHz from Teledyne RD Instruments was used to measure the current speed and current direction within the water column. On board R/V Otzum and R/V Senckenberg, a downward-looking ADCP was mounted. The following configuration was used: vertical resolution 0.25 m, mode 12, and as reference bottom track. The surface velocity of the raw data was smoothed by a median filter.

**Water sampling**

The catamaran S$^3$ was used to sample the sea surface microlayer (SML) by rotating glass disks. The underlying water (ULW) was sampled in parallel from 1 m water depth. Details about S$^3$ can be found in Ribas-Ribas et al. (2017). Large-volume (20 L) samples from SML and ULW were collected approximately every 3 h, yielding nine pairs of SML–ULW samples during a full 25-h diel cycle. Water samples were brought to the R/Vs for further processing in the ship- or land-based laboratories.

**Pigment analysis**

Samples for pigment analysis were filtered onto precombusted, acid washed glass fiber filters (Whatman, GF/C) and stored at 80°C until analysis. Pigment filters were extracted for 24 h in 90% ethanol after 30-min sonication on ice (GT SONIC). The extract was measured using a microplate reader (Synergy H1, BioTek) and pigment concentrations were calculated using the spectral deconvolution method (Thrane et al. 2015). Marker pigments were used according to Schlüter et al. (2016) to categorize main phytoplankton groups.

**Microbial cell counts**

Prokaryotic cell numbers were counted via flow cytometry after water samples were fixed, flash frozen in liquid nitrogen, and stored at −20°C. Prior to measurements, all samples were stained with SYBR Green solution. Counting was performed after addition of latex beads serving as an internal standard. Further details can be found in Stolle et al. (2010).

**Bacterial community composition**

Bacterial community composition from the solar simulator incubations was analyzed according to Stolle et al. (2011). Briefly, water samples were filtered onto 0.2 μm pore-size
polycarbonate filtered, frozen in liquid nitrogen, and stored at −80°C. Deoxyribonucleic acid (DNA) was extracted using a phenol-/chloroform protocol, after which the target sequence of the bacterial 16S rRNA gene was amplified using polymerase chain reaction (PCR). PCR products were analyzed using the fingerprinting technique single-strand conformation polymorphism (SSCP).

**Chromophoric and fluorescent DOM**

Samples for chromophoric dissolved organic matter (CDOM) were filtered through 0.2 μm polyethersulfone syringe filters and stored chilled (0°–4°C) in precombusted amber glass vials until analysis, which occurred within a few hours from the sample collection. CDOM absorbance was measured in a 1 cm pathlength quartz cuvette with a CAMSPEC M550 ultraviolet (UV)-visible spectrophotometer from 190 to 800 nm at medium speed, at 1 nm wavelength resolution, at room temperature (20°C) and corrected for Milli-Q water each day of analysis. Spectra were corrected for scattering by subtracting the average of absorbance values between 700 and 800 nm. Absorption coefficients $a(\lambda)$ were calculated from absorbance ($A_\lambda$) values as

$$a(\lambda), \text{m}^{-1} = 2.303A_\lambda,$$

where $L$ is the pathlength of the cuvette (0.01 m). As CDOM absorption is exponential, the absorption spectral slope $S$ (nm$^{-1}$) was determined by a standard equation by linear regression of log-transformed absorption spectra against the wavelength:

$$a(\lambda) = a_\lambda e^{-S(\lambda-\lambda_0)},$$

with $a(\lambda_0)$ being the absorption coefficient at a reference wavelength $\lambda_0$. We used multiple 20-nm wavelength intervals in a stepwise (1 nm) linear regression analysis according to Loiselle et al. (2009). Spectral slope $S$ computed between 275 and 295 nm defines the spectral dependence of CDOM $a(\lambda)$, providing information on dissolved organic matter (DOM) chromophores. Slope $S$ varies with DOM source and reflects DOM biological and chemical modification. For example, $S$ increases with salinity, tracing mixing, and coastal inputs processes. Slope $S$ also increases with progressive CDOM photobleaching during irradiations and is inversely related with DOM molecular weight (Helms et al. 2008).

Fluorescent dissolved organic matter (fDOM) was continuously measured during deployment of the catamaran S$^2$, as reported in Ribas-Ribas et al. (2017).

**Dissolved organic carbon: Bulk concentration and composition.** Samples for DOM fractions were filtered through 0.45 μm polyethersulfone (PES) syringe filters and stored chilled (0°–4°C) in precombusted glass “TOC” vials until analysis, which occurred within 4 days of sample collection. Samples were analyzed on the next-generation Model 9 liquid chromatography, organic-carbon detection, organic nitrogen detection system (DOC Labor, Karlsruhe, Germany; Huber et al. 2011). Briefly, a 1 mL of whole water is injected onto a size exclusion column (2 mL min$^{-1}$; HW50S, Tosoh, Japan) with a phosphate buffer (potassium dihydrogen phosphate 1.2 g L$^{-1}$ plus 2 g L$^{-1}$ disodium hydrogen phosphate × 2 H$_2$O, pH 6.58) and separated into five “compound-group specific” DOM fractions: (i) biopolymers (likely hydrophobic, high molecular weight, largely non-UV absorbing extracellular polymers); (ii) “humic substances” (higher molecular weight, UV absorbing); (iii) “building blocks” (lower molecular weight, UV absorbing humics); (iv) low molecular weight “neutrals” (hydrophilic or amphiphilic, non-UV absorbing); and (v) low molecular weight acids. All peaks were identified and quantified with bespoke software (LabVIEW 2013) provided by DOC Labor normalized to IHSS HA and FA standards.
**Surfactants.** Phase sensitive alternating current (ac) voltammetry (*out-of-phase* signal, frequency 77 Hz, amplitude 10 mV) was used for determination of SAS concentration (surfactant activity) in marine samples. Quantification of SAS is based on measuring the adsorption effect on the Hg electrode and is determined from the change in capacity current (Aic) at the potential (E) −0.6 V (Čosović and Vojvodić 1982), after 30 s of accumulation. Both the SML and the ULW samples have been studied as original samples after filtration through 0.7 µm Whatman GF/F filters (preburned at 450°C for 5 h) and salinity correction to 35 [corresponding to 0.55 M NaCl (99.99% Suprapure, Sigma-Aldrich)]. Surfactant activity is expressed as the equivalent amount of the selected standard of non-ionic surfactant tetra-octylphenolethoxylate (eqT; in mg dm⁻³) as described in detail previously (Čosović and Vojvodić 1998).

Electrochemical analyser Autolab-type II (Eco Chemie B. V., The Netherlands) equipped with a GPES 4.6 software (Eco Chemie B. V., The Netherlands) was used for AC voltammetry measurements in a standard 50 cm³ polarographic Metrohm cell equipped with a three-electrode system: a hanging mercury drop electrode (HMDE, Metrohm, Switzerland), a reference Ag/AgCl/3 mol L⁻¹ KCl electrode, and a platinum coil as an auxiliary electrode. The SAS analyses were performed in triplicate, and the precision was typically below 5%.

Additional information on the SAS chemical characteristics may be gained from examining surfactant activity values normalized to DOC concentrations (Čosović and Vojvodić 1998; Frka et al. 2009; Gašparović et al. 2007). Higher normalized surfactant activities indicate a higher contribution of hydrophobic substances to the bulk organic matter. As determined from the behavior of representative model substances, the highest normalized values can be ascribed to highly hydrophobic substances such as fatty acids (2.39 for linoleic acid), while humic acids and proteins (0.19–0.34) and some polysaccharides like xanthan (0.04) have a low surfactant activity contribution to DOC.

**Lipid analysis.** Total lipid and lipid class quantitation was performed by Iatroscan thin layer chromatography–flame ionization detection (TLC–FID; Iatroscan MK-VI, Iatron, Japan). Lipids were separated on Chromarods-SIII thin layer rods and quantified by an external calibration with a standard lipid mixture. Quantified lipid classes include hydrocarbons (HC), lipid degradation indices (DI) [fatty acid methyl esters (ME), free fatty acids (FFA), alcohols (ALC), 1,3-diacylglycerols (1,3DG), 1,2-diacylglycerols (1,2DG) and monoacylglycerols (MG)], waxes (WE), phytoplankton energy reserves [triacylglycerols (TG)], membrane lipids including three phospholipids (PL) [phosphatidylglycerols (PG), phosphatidylethanolamines (PE), and phosphatidylcholines (PC)], glycolipids (GL) [sulfoquinovosyldiacylglycerols (SQDG), monogalactosyldiacylglycerols (MGDG), and digalactosyldiacylglycerols (DGDG)], sterols (ST), and pigments (PIG). The standard deviation accounted for 3%–11% of the signal magnitude of lipid classes. A detailed description of the procedure is described in Gašparović et al. (2015).

**Dissolved amino acids.** For total dissolved hydrolysable amino acids (DHAA), 5 mL of sample were prefiltred through 0.45 µm pore size filters (Acrodisk filters) and filled into precombusted glass vials (8 h, 500°C) and stored at −20°C until analysis. Analysis was performed according to Lindroth and Mopper (1979) and Dittmar et al. (2009) with some modifications, as described in more detail in Engel and Galgani (2016).

**Gel-like particles.** The abundance and area of gel particles was determined microscopically (Engel 2009). Depending on the concentration of gel particles in the water, 5–10 mL of the sample was filtered onto a 0.4 µm Nucleopore membrane (Whatman). Transparent exopolymer particles (TEP) were stained with 1 mL of an Alcian Blue solution for 3 s. Coomassie stainable particles (CSP) were stained accordingly with 1 mL Coomassie Brilliant Blue G (CBBG) for 30 s. Filters for both types of gel particles were mounted on Cytoclear slides and stored at −20°C.
until analysis. Microscopic images (1,388 × 1,040 pixels) of two filters per sample were taken at 200× magnification with a microscope (Axio Scope.A1, Zeiss) equipped with a camera (AxioCam MRC, Zeiss) using the Axio Vision LE64 Rel. 4.8 software (Zeiss). All particles bigger than 0.2 µm² were analyzed. ImageJ was used for image analysis.

**Iodide.** Iodide was measured in filtered samples (GF/F) using cathodic stripping square wave voltammetry (Campos 1997).

**Solar simulator.** The unfiltered water sample was subsampled into 12 aliquots in a series of ~430 ml quartz irradiation flasks. Of these, four were designated as fully irradiated (Ir: flasks uncovered), four as dark controls (DC: double foil wrapped), and four as temperature controls (TC: double foil wrapped). One further aliquot was measured at the beginning of the experiment (0 h). Ir and DC samples were irradiated under controlled laboratory conditions in a “solar simulator” (Uher et al. 2017), and TC samples were stored at 4°C in a refrigerator. The solar simulator holds a 300 W Xenon-arc (LOT Oriel) lamp of 300 nm transmission cut off, with spectral irradiance of 124.4 W m⁻² nm⁻¹ (300–800 nm). At intervals of 3, 6, 9, and 24 h one subsample of each designation was removed for SAS analysis (see section above).

**Ambient aerosols.** For ambient aerosol particle sampling, a high volume Digital sampler DHA-80 (Walter Riemer Messtechnik, Germany) was installed on the roof of the ICBM-Terramare at a height of ~3 m. Submicron aerosol particles (PM₁) were collected on preheated 150 mm quartz fiber filters (Munktell, MK 360) at a flow rate of 500 L min⁻¹. Furthermore, size-resolved particle sampling was done using a five stage Berner impactor (Hauke, Austria) with 50% cutoffs at 0.05, 0.14, 0.42, 1.2, 3.5, and 10 µm aerodynamic particle diameter and a flow rate of 75 L min⁻¹. Day and night samples (with a respective sampling time of 12 h from 0600 to 1800 UTC) were collected.

**Sea spray aerosol chamber.** The sea spray aerosol chamber was operated such that three discreet seawater samples (~100 L volume; cycles 01, 02, and 03) were used to generate nascent sea spray aerosol. Each experiment was run for approximately 24 h during which the seawater was recirculated within the sea spray chamber by the plunging jet system at a series of constant flow rates. Each experiment was conducted at a constant temperature that matched the ambient conditions at which the seawater sample was collected. Particle-free dry air flowed through the headspace of the chamber at a constant rate depending upon which instruments were connected. The flow of particle-free dry air into the chamber was always higher than the volume of air sampled such that excess air flowed out of a one-way flutter valve.

Each experiment consisted of two measurements phases. First, aerosol number size distributions were determined at three different plunging jet flow rates over a period of several hours. Aerosol number size distributions of particles with diameters between 0.015 nm and 10 µm were measured continuously using a custom built differential mobility particle sizer (DMPS) and an optical particle size spectrometer (OPSS; FIDAS Palas GmbH, Germany) using an ~8 min integration time. Following this period of measurements, the chemical composition of the aerosol was determined over nominal 24 h periods using a low pressure impactor (Dekati Inc., Finland,) operating at 30 L min⁻¹. During this period the plunging jet was operated at a single constant flow rate. The low pressure impactor was configured using quartz fiber substrates and the exposed substrates were transferred to precleaned glass vials where they were extracted immediately in 50 mL ultrapure water for 1 h in an ultrasonic bath. Following extraction the samples were split into fractions for total organic carbon analysis, surfactant analysis, inorganic ion analysis, sugars analysis, and microbial analysis.
Measurements of the CO\textsubscript{2} transfer velocity (kw). An autonomous drifting buoy (Ribas-Ribas et al. 2018) was deployed to measure partial pressure of CO\textsubscript{2} (pCO\textsubscript{2}) in the air, at a water depth of 1.2 m, and inside a floating chamber. pCO\textsubscript{2} was determined using an infrared gas analyzer (OceanPack LI-COR LI-840x, SubCtech GmbH, Germany; range: 0 to 3,000 µatm ± 1.5%).

Aqueous pCO\textsubscript{2} was measured for 40 min, followed by two measurements taken in the floating chamber for 15 min. Air in the floating chamber was completely replaced with ambient air before each measurement. Air measurements were taken for 2 min following every floating chamber cycle. The CO\textsubscript{2} fluxes were calculated using the following equation:

\[
F_{CO_2} = \frac{dpCO_2}{dt} \cdot \frac{V}{STR},
\]

where \(dpCO_2/dt\) is the slope of the pCO\textsubscript{2} change in the floating chamber, \(V\) is the volume of the floating chamber, \(S\) represents the surface area of the floating chamber, \(T\) represents the water temperature at a depth of one m from \(S\), and \(R\) is the gas constant (Ribas-Ribas et al. 2018).

Measurements were excluded when the regression for the slope was \(R^2 < 0.90\). The equation of the gas transfer velocity \(k_w\) is

\[
k_w = \frac{F_{CO_2}}{K(pCO_2\text{water} - pCO_2\text{air})}.
\]

The solubility coefficient \(K\) depends on the temperature and the salinity of the seawater and was calculated according to Weiss (1974). Finally, \(k_w\) was standardized to \(k_{660}\) with the following formula:

\[
k_{660} = k_w \left( \frac{660}{Sc_{CO_2}} \right)^{-n_{Sc}}.
\]

where \(Sc_{CO_2}\) is the temperature-dependent Schmidt number (Wanninkhof 2014). The Schmidt number exponent \((n_{Sc})\) depends on the wind speed. For low wind speeds of less than 3.7 m s\textsuperscript{-1}, we used \(n_{Sc} = 2/3\) and for higher wind speeds we adjusted \(n_{Sc} = 1/2\) (Guérin et al. 2007). Details of \(k_w\) calculation were published in Ribas-Ribas et al. (2018).

Microsensor studies. The microsensors were deployed in an automated microsensor setup (Unisense A/S, Denmark) which could be programmed to log the sensor signals and control the sensor position in a profiling sequence. Prior to measurements, the O\textsubscript{2} and pH sensors were calibrated in solutions of known oxygen concentration and pH value, respectively. To avoid the deforming effect on the uppermost water layer of capillary action with the sensors, the sensors were deployed under water with the tip pointing up (Fig. SB1 in the “Milan Objectives” sidebar). Typically, profiles were measured in 10–50 µm steps from a few millimeters below the surface, up through the surface to the overlying air, allowing a detailed resolution of the parameters across the air–water interface.
Table ES1. Measurement uncertainties for methods analyzing discrete water and aerosol samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Figure in main text</th>
<th>No. of replicates</th>
<th>Relative standard deviation/accuracy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial abundance</td>
<td>Fig. 6</td>
<td>1</td>
<td>&lt;5%</td>
<td>Rahiff et al. (2017)</td>
</tr>
<tr>
<td>CDOM</td>
<td>Fig. 7</td>
<td>3</td>
<td>0.5%</td>
<td><a href="https://camspec.eu/">https://camspec.eu/</a></td>
</tr>
<tr>
<td>fDOM</td>
<td>Fig. 7</td>
<td>1</td>
<td>0.2 mg L$^{-1}$</td>
<td>Ribas-Ribas et al. (2017)</td>
</tr>
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<td>DOC</td>
<td>Fig. 8</td>
<td>1</td>
<td>2.6%</td>
<td>DOC Labor, Karlsruhe, Germany</td>
</tr>
<tr>
<td>Biopolymers</td>
<td>Fig. 8</td>
<td>1</td>
<td>4.1%</td>
<td>DOC Labor, Karlsruhe, Germany</td>
</tr>
<tr>
<td>Humic substances</td>
<td>Fig. 8</td>
<td>1</td>
<td>3.9%</td>
<td>DOC Labor, Karlsruhe, Germany</td>
</tr>
<tr>
<td>Building blocks</td>
<td>Fig. 8</td>
<td>1</td>
<td>6.9%</td>
<td>DOC Labor, Karlsruhe, Germany</td>
</tr>
<tr>
<td>LMW neutrals</td>
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<td>1</td>
<td>3.5%</td>
<td>DOC Labor, Karlsruhe, Germany</td>
</tr>
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<td>LMW acids</td>
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<td>1</td>
<td>2.0%</td>
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<td>SAS</td>
<td>Fig. 9</td>
<td>3–5</td>
<td>&lt;5%</td>
<td>Rickard et al. (2019)</td>
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<td>Lipids</td>
<td>Fig. 10</td>
<td>2</td>
<td>&lt;21%</td>
<td>Penezić et al. (2010)</td>
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<td>TEP</td>
<td>Fig. 11</td>
<td>Average: 14%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSP</td>
<td>Fig. 11</td>
<td>Average: 13%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodide</td>
<td>Fig. 12</td>
<td>2–5</td>
<td>See individual error bars in Fig. 12</td>
<td>Campos (1997)</td>
</tr>
<tr>
<td>pH microsensor</td>
<td>Fig. 14</td>
<td>1</td>
<td>0.01 units</td>
<td><a href="http://www.unisense.com/pH">www.unisense.com/pH</a></td>
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<tr>
<td>O$_2$ microsensor</td>
<td>Fig. 14</td>
<td>1</td>
<td>&lt;2%</td>
<td>Revsbech (1989)</td>
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<td>WSOC</td>
<td>Fig. 15</td>
<td>1</td>
<td>2.0%</td>
<td>Cavalli et al. (2010)</td>
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<td>0.3%</td>
<td>van Pinxteren et al. (2009, 2012)</td>
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<td>Fluoride</td>
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<td>van Pinxteren et al. (2009, 2012)</td>
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<td>van Pinxteren et al. (2009, 2012)</td>
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<tr>
<td>Phosphate</td>
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<td>1.6%</td>
<td>van Pinxteren et al. (2009, 2012)</td>
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<tr>
<td>Sodium</td>
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<td>0.7%</td>
<td>van Pinxteren et al. (2009, 2012)</td>
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<td>Ammonium</td>
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<td>1.8%</td>
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<td>3.8%</td>
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REFERENCES


