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Effects of brownification and warming on algal blooms, metabolism and higher trophic levels in productive shallow lake mesocosms

Running head: Brownification and warming effects on lakes

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Abstract

An increase of dissolved organic carbon (DOC) in inland waters has been reported across the northern temperate region but the effects of this on whole lake ecosystems, often combined with other anthropogenic stressors like nutrient inputs and warming, are poorly known. The effects of these changes on different component of the ecosystem were assessed in an experiment using twenty-four large (3,000 L) outdoor mesocosms simulating shallow lakes. Two different temperature regimes (ambient and ambient +4ºC) combined with three levels of organic matter (OM, added as filtered peaty water), simulating the DOC increase that is predicted to take place over the next 4 to 21 years were used. Neither temperature nor OM had significant effects on net ecosystem production, respiration or gross primary production. Phytoplankton chlorophyll *a* concentration was not significantly affected by warming, however in summer, autumn and winter it was significantly higher in mesocosms receiving intermediate OM levels (July – Feb DOC concentrations 2 - 6 mg L^{-1}). Summer cyanobacterial blooms were highest in intermediate, and lowest in the highest OM treatments. OM concentration also influenced total macroinvertebrate abundance which was greater in spring and summer in mesocosms with intermediate and high OM. Fish abundance was not significantly affected by OM concentration, but abundance was greater in ambient (55 fish subsample^{-1}) compared to heated mesocosms (17 fish subsample^{-1}) and maximum abundance occurred two weeks later compared to heated mesocosms. The results suggest that changes in OM may have a greater effect on shallow lakes than temperature and that phytoplankton, especially cyanobacteria, benefit from intermediate OM concentrations, therefore, nuisance algal blooms might increase in relatively clear shallow eutrophic lakes where DOC concentrations increase.

1. Introduction

Widespread increases in dissolved organic carbon (DOC) concentrations, so-called 'brownification' (Graneli, 2012), have been reported from inland waters across northern Europe and North America (Driscoll *et al.*, 2003, Roulet & Moore, 2006, Skjelkvale *et al.*, 2005, Vuorenmaa *et al.*, 2006). In the UK, Evans *et al.* (2005) showed a mean DOC increase of 91% from 1988 to 2003 in 22 upland streams and lakes. The mechanisms responsible for this increase are subject to intense debate. Possible causal factors include: increased precipitation resulting in higher soil runoff and changing catchment flow paths (Hongve *et al.*, 2004, Weyhenmeyer *et al.*, 2016); changes in atmospheric nitrogen deposition (Pregitzer *et al.*, 2004) and land management (Yallop *et al.*, 2010); a decline in acid deposition (Monteith *et al.*, 2007, Sawicka *et al.*, 2017); rising temperatures resulting in increased microbial enzyme activity and consequently greater levels of OM decomposition within the catchment (Evans *et al.*, 2005, Freeman *et al.*, 2001). Indeed a combination of environmental factors, such as dry summers or droughts followed by a wet autumn or high precipitation, can lead to short-term increases in dissolved OM (Lepisto *et al.*, 2008). However, for the long-term DOC increase, evidence from recent years show that for UK sites, acid deposition is the main factor driving the DOC increase (Evans *et al.*, 2006), while for southern Scandinavia it is increased rainfall (Erlandsson *et al.*, 2008). Whatever the cause or causes, it is clear that climate change and other anthropogenic impacts are playing a major and critical role in raising DOC concentrations in surface waters at many places .

1.1 *Effects of DOC increase in aquatic systems*

The consequences of DOC increases in aquatic systems are also complex and can have far-reaching effects on the physics, chemistry and biology of a lake (Fig. 1). Humic substances

strongly attenuate light in the photosynthetically active radiation (PAR) region of the electromagnetic spectrum, especially in the shorter waveband including ultra violet radiation, and by reducing the underwater light climate can influence plankton-based food chains (Jones, 1992). This can affect lake thermal properties by trapping more solar energy at the surface (Köhler *et al.*, 2013) and reducing the depth of the epilimnion (Strock *et al.*, 2017). It can also affect productivity: Carpenter *et al.* (1998) showed that when DOC increased from 5 to 17 mg C L^{-1} attenuation increased and suppressed chlorophyll concentration and primary production. The productivity of nutrient-poor 'brown' lakes has been shown to be limited by light rather than nutrients and so terrestrial OM can control benthic primary production (Karlsson *et al.*, 2009). As a consequence, increased DOC concentrations might result in a decrease of zooplankton and higher trophic level biomass as a response to reduced food availability.

Bacteria utilise organic carbon as an energy source in respiration and their biomass increases with an increase in water colour and allochthonous carbon (Hessen, 1985, Tranvik, 1988). With increased bacterial respiration, aquatic systems are expected to become more heterotrophic as a result of elevated DOC (Hessen, 1992, von Einem & Graneli, 2010). However in a study of twenty-three lakes, Solomon *et al.* (2013) found no correlation between respiration (R) or gross primary production (GPP) and DOC concentration. In humic lakes with large inputs of dissolved organic carbon, the pelagic food chain can be driven by organic carbon fixed in the catchment (Jansson *et al.*, 2000) since mixotrophic phytoplankton can consume bacteria and are also able to utilise DOC directly (e.g. Graneli *et al.*, 1999). Zooplankton can benefit from mixotrophic phytoplankton and/or bacterial carbon via the microbial loop, but direct ingestion of particulate OM has also been described (Daniel *et al.*, 2005, Jones, 1992). While around 50% of allochthonous DOM is comprised of carbon,

a further proportion is nitrogen, i.e. dissolved organic nitrogen (DON; Willett *et al.* (2004)), and phosphorus, i.e. dissolved organic phosphorus (DOP; Jones *et al.* (1988)). With growing evidence of bioavailability (Rofner *et al.*, 2016), DON and DOP form a large potential source of nutrients for aquatic organisms. Even though these nutrients often enter a lake in relatively recalcitrant organic molecules, they can become available to organisms following mineralisation into inorganic nutrients by photolysis (Southwell *et al.*, 2011), bacterial action (Vähätalo *et al.*, 2003), or in the case of algae, via the production of extracellular phosphatases (Nedoma *et al.*, 2003). Thus, increasing DOM concentrations in aquatic systems could result in greater availability of N and P to primary producers and could be incorporated into higher trophic levels (Brett *et al.*, 2017).

For higher trophic levels, the role of increasing DOM is unclear. A survey of 10 American lakes suggest that browning has a negative effect on cladoceran as well as copepod production (Kelly *et al.*, 2014). However, experiments with increased DOC concentrations of 8 to 11 mg L^{-1} increased phyto- and zooplankton productivity (Kelly *et al.*, 2016). Fish populations can be affected by increased supply of allochthonous carbon in two ways: 1) changes in the availability of food, and 2) reduction in light penetration, affecting prey capture by predators reliant on visual cues (Karlsson *et al.*, 2009, Wissel *et al.*, 2003). Craig *et al.* (2017) found a strong negative impact of DOC concentration on bluegill populations, especially on size and lifetime fecundity, and Karlsson *et al.* (2009) showed decreasing fish production with decreasing light climate in nutrient–poor lakes. However, currently, limited data are available, especially for nutrient-rich systems where effects of elevated DOC concentrations on higher trophic levels are likely to be complex and, therefore, difficult to predict. Further, conflicting results highlight the need for more experimental studies.

1.2 *Effects of multiple stressors (DOC and climate change) on aquatic systems*

Aquatic and terrestrial ecosystems have already warmed in recent decades and will continue to do so as global temperatures continue to rise. Air temperature increases of 1.5 to 5ºC during the remainder of this century are forecast, but dependent on what mitigation measures are taken and how rapidly they are implemented, temperatures may rise by as much as 6.4ºC in this period (IPCC, 2007, IPCC, 2014). Maximum daily precipitation and the duration and frequency of heavy precipitation events are also forecast to increase (IPCC, 2014, Kundzewicz *et al.*, 2006). These factors, together with the ongoing recovery of surface waters from acidification, mean that an increase of OM and nutrient transport into lakes is to be expected in the future (Monteith *et al.*, 2015). Together with a multitude of direct factors, including elevated temperature and increased runoff and inflow rates, the indirect effects of DOC increase (increased microbial enzyme activity (Evans *et al.*, 2005, Freeman *et al.*, 2001) and changes in land management (Yallop *et al.*, 2010)) can cause a suite of changes in lakes. Among the affected water bodies, shallow lakes and wetlands, which tend to be numerically predominant among inland waters (Verpoorter *et al.*, 2014), are particularly susceptible to the effects of environmental change, because their small volumes and reduced tendency to stratify in summer makes them more directly responsive to weather conditions (Mooij *et al.*, 2005).

Regarding inorganic N and P, eutrophication remains a major anthropogenic pressure for an increasing number of lakes. With current climate predictions of warming and increases in precipitation in some regions, a further rise in aquatic dissolved and particulate organic material and inorganic nutrient concentrations is likely. Such changes could have major implications for aquatic biodiversity and the biogeochemical functioning of lakes, and exacerbate the cost of water treatment for drinking water supply (Köhler *et al.*, 2013, Ritson *et al.*, 2017).

Given the wide range of interacting physical, chemical and biological mechanisms that influence lake functions, the response of freshwater food webs to DOC increases, especially when in combination with other stressors are difficult to predict. Further, conflicting results highlight the need for experimental large-scale studies. To date, most research has focused on DOM and DOC increase in nutrient-poor systems (e.g. Karlsson *et al.*, 2009, von Einem & Graneli, 2010). However, there is a need to understand how brownification will impact productive systems affected by eutrophication in concert with a changing climate. We therefore investigated the impacts of warming and variation in allochthonous inputs of DOM on the productivity of shallow lake systems dominated by pelagic production, using a mesocosm experiment. Here, we focus on responses to two temperature regimes and three OM additions on these simulated eutrophic shallow lakes over one year. Our hypotheses were that: (i) shading, resulting from increasing DOM, would reduce phytoplankton biomass (chlorophyll-*a*); (ii) higher trophic level biomass (macroinvertebrates and fish) would consequently decline; and (iii) respiration would increase, while gross primary production would decrease, with increasing OM content, leading to more heterotrophic systems. We also hypothesised that (iv) higher temperatures, particularly in more eutrophic conditions, would increase primary production, and thus phytoplankton biomass; and (v) that higher trophic level biomass (macroinvertebrates and fish) would increase as a result of higher food availability and higher chances of offspring survival with higher temperatures under mixed water column conditions.

2. Methods

2.1 *Mesocosm facility and set-up*

The CEH Aquatic Mesocosm Facility (CAMF, https://www.ceh.ac.uk/our-science/research-facility/aquatic-mesocosm-facility) is located at the Hazelrigg Experimental Field Site (Lancaster University) in the north west of England (54.014°N, -2.777°E) and comprises thirty-two fibreglass mesocosms, each 1.0 m deep and 2.0 m in diameter (3000 L) arranged in four rows with eight mesocosms in each (Fig. 2a). Water temperature within each mesocosm was measured every minute by two platinum resistance thermometer (PRT) sensors shaded by a plastic cover, sited at a depth of 45 cm, radially offset by 30 cm from the mesocosm side wall and logged by a Campbell® Scientific Data Logger (model CR1000, Campbell Scientific Ltd., Loughborough, U.K.). To prevent thermal stratification, purpose-built mixing units, comprising two plastic flower pots (15 cm diameter) suspended in the middle of the mesocosm, were moved slowly up and down through 0.8 m of the water column by a motorised pulley. The mixers were operated on a one minute on/one minute off cycle in all mesocosms between 7 a.m. and 7 p.m. from May 2012 until the end of the experiment (February 2013) to break up stratification resulting from incoming solar radiation. Preliminary experiments showed that this prevented stratification from forming. Dissolved oxygen was measured automatically every 5 minutes by a Waterra In-Situ RDO PRO dissolved oxygen probe (Waterra Ltd., Solihull, U.K.) calibrated before deployment in each mesocosm at a depth of 40 cm and 40 cm from the wall of the mesocosm. Photosynthetically active radiation (PAR) was measured every minute by an Apogee Instruments Quantum sensor (Apogee Instruments Inc., Logan, U.S.A.) mounted above the PRT sensors described above at a depth of 43 cm, on the south facing side of each mesocosm, radially offset by 30 cm from the mesocosm side wall. A weather station within the mesocosm compound, (Vaisala weather transmitter WXT520, Vaisala Oyj, Helsinki, Finland) measured wind speed, wind direction, precipitation, air temperature, barometric pressure and relative humidity,

logged at five minute intervals on the same Campbell® Scientific Data Logger as used for the temperature regulation.

In November 2011, four months prior to the start of the experiment, a mixture of sediment, from a nearby lake (Windermere, Cumbria, 54.388°N, -2.945°E, ~70% by volume), and washed silver sand (~30% by volume) was added to each mesocosm to a depth of approximately 10 cm together with 1500 L of lake water (Windermere) and 1500 L of rainwater. The CEH experimental facility is located in North West England and consequently experiences a relatively high annual rainfall (500 to 600 mm during winter), also during the period in which the experiment was established. The sediment and water was cross-mixed among all mesocosms to maximise homogeneity. The heating commenced two months before the start of the experiment.

2.2 *Experimental treatments*

Twenty-four mesocosms were used in a randomised block design, comprising six treatments and four replicates (eight mesocosms were unused). Three different OM treatments (no organic matter addition, NOM, low organic matter, LOM, and high organic matter, HOM) were factorially combined with the two temperature treatments (ambient, A and heated: ambient + 4 °C, H). Each treatment was randomly assigned to each of the four rows (Fig. 2c).

An electric heating element was placed in each mesocosm approximately 10 cm above the sediment surface (Fig. 2b). In heated mesocosms, the heating element was connected to a 110 V transformer with the power input controlled by the data logger. In unheated mesocosms, the elements were not connected to a power source. One data logger controlled and recorded data from a group of eight mesocosms. A custom program was used to control the heating system and maintain a continuous 4°C temperature difference between the heated and

ambient, non-heated mesocosms to ensure the heated mesocosms follow the daily and seasonal temperature cycles of the ambient temperature tanks. The reference ambient temperature was derived from the mean ambient temperature for each group of eight mesocosms. The 4°C temperature increase is within the range expected in the next 80 years (IPCC, 2007, IPCC, 2014).

Peat originating from south-west Scotland was stored in empty tanks (3000 L) and mixed with rain water. The pH of the peat/water mix was increased to pH 7.6 by one addition of sodium hydroxide to ensure a similar pH to the experimental mesocosms. A wooden lid was placed on top of the tanks to prevent photobleaching of the dissolved OM (Reche *et al.*, 1999). Water from these peat holding tanks was filtered through 50 μm gauze before adding to the mesocosms. We aimed to simulate a short-term and long-term increase of median OM concentrations in natural lakes and based it on current literature values: Global DOC concentrations from more than 7,500 lakes were in the range of $0.1 - 332$ mg L^{-1} with a median of 5.71 mg L^{-1} (Sobek *et al.*, 2007). In the UK, DOC concentrations in 11 lakes of the UK Acid Waters Monitoring Network were in the range 1-7 mg L^{-1} (Evans *et al.*, 2006), but can be higher for small and shallow lakes which dominate inland waters numerically and are particularly important for carbon storage (Downing *et al.*, 2008, Verpoorter *et al.*, 2014). During a 15 year period, the concentration of DOC across a range of UK upland water lakes and streams increased by around 6% per site per year (Evans *et al.*, 2005). Prior to the start of the experiment, an initial dose of filtered peat water was added: HOM mesocosms received 8 mg C L^{-1} and LOM mesocosms received 2 mg C L^{-1}. No peat water was added to the NOM mesocosms. During the experiment, 5 and 1 mg C L^{-1} were added every four weeks to the HOM and LOM mesocosms respectively, consisting of DOC and particulate organic carbon <50 μm . The DOC concentration in the peat holding tanks was measured according to

Tipping et al. (2009) and the particulate organic carbon content of the <50 μm fraction was determined by loss on ignition, one to three days before addition to treatments.

At two-week intervals all mesocosms received low Redfield ratio based nutrient additions of nitrogen (300 μg N L^{-1}) and phosphorus (42 μg P L^{-1}) as sodium nitrate (NaNO$_3$) and potassium dihydrogen orthophosphate (KH$_2$PO$_4$), respectively. Evaporative losses from the mesocosms in summer were compensated for by adding deionised water to maintain nutrient and ionic concentration.

2.3 *Inoculation of mesocosms*

Phytoplankton was inoculated into the mesocosms via the Windermere sediment and lake water (1500 L) added during the experimental set-up. Zooplankton were collected with a 180 μm net from Windermere and were added to the mesocosms three months prior to the start of the experiment. Macroinvertebrates were collected by kick netting along the shores of Windermere and were stored in a tank before adding to the mesocosms two months prior to the start of the experiment. Two weeks after the macroinvertebrate inoculation, invertebrates were cross-mixed among mesocosms via standardised sweep-net samples to ensure similar starting conditions. During December 2011, 144 adult three-spined sticklebacks (*Gasterosteus aculeatus*) were collected by hand-net from a local river (R. Darwen, Lancashire, ~30 km from CAMF) and transferred to the CEH Lancaster aquarium where they were placed in 30 L glass aquaria (approx. 30 fish/aquarium), each supplied with a constant flow (800 mL/min) of untreated lake water (Blea Tarn Reservoir) at 8.0 – 11.0°C. The fish were maintained under a short daylength (7.5 h : 16.5 h: L:D) and were fed bloodworm three times weekly. Three randomly selected males and three females were added to each mesocosm one month prior to the start of the experiment. At the time of transfer to the

mesocosms in late January 2012 the mean body mass and length ($\pm$ SEM) of the female fish was 1.58 ± 0.5 g and 55.2 ± 0.5 mm (n = 72) respectively and that of the males 1.49 ± 0.5 g and 53.4 ± 0.5 mm (n = 72).

The fish and invertebrate organisms used in this study were captured, transported and maintained in accordance with commonly employed ethical guidelines for the use of animals in research (e.g. the APA Guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research).

2.4 *Monitoring programme*

Conductivity, pH and salinity were measured every 2 weeks with a Hydrolab DS5X multiparameter datasonde (OTT Hydromet GmbH, Kempten, Germany), suspended mid-depth in each mesocosm for a minimum of 3 minutes. The *in situ* dissolved oxygen and PAR sensors were manually cleaned at least every two weeks to prevent biofilm growth. Respiration (R), gross primary production (GPP) and net ecosystem production (NEP) were calculated as μmol $O_2 \, L^{-1} \, day^{-1}$ according to Staehr *et al.* (2010). Oxygen saturation was calculated as a function of temperature and salinity and corrected for barometric pressure. The Schmidt coefficient was calculated from water temperature, and piston velocity was calculated from wind speed at 10 m height, in turn calculated from wind speed at 2 m height measured by the weather station. For these calculations, high-frequency data were summarised as 5-minute means and whenever one or two water temperature measurements were missing, data were linearly interpolated. The mixed depth was determined as 0.8 m, the maximum depth of the mixer. Attenuation coefficients (k) for PAR were calculated from PAR measured at the surface and a depth of 0.45 m in the mesocosm using the Beer-Lambert law (surface losses were not included). No surface PAR measurements were measured during

this study. However, a comparison of time-series from a surface PAR sensor installed in 2015 at 2 m height at CAMF with PAR (LP02 pyranometer, Hukseflux Thermal Sensors B.V., Delft, NL) measured at an adjacent weather station (Climatological Station Number 7236) resulted in a correlation with an R^2 of 0.98. The LP02 sensor was installed in May 2012 at the weather station, allowing us to calculate values from May 2012 until February 2013 based on the regression.

Water chemistry was measured every two weeks on a depth-integrated sample. Total nitrogen (TN) and total phosphorus (TP) were measured following Johnes & Heathwaite (1992), soluble reactive phosphorus (SRP) and total oxidised nitrogen (TON) (nitrate, NO_3-N and nitrite, NO_2-N) were measured following Mackereth et al. (1978). Whenever TON values were lower than the detection limit (0.01 mg L^{-1}), the detection limit was used for calculations. The concentration of DOC was measured following Tipping *et al.* (2009) every two weeks until the 17th of April, then every four weeks until the end of the experiment. TN and TP in the peat water of the peat holding tanks was measured once, in mid-January 2013. Phytoplankton biomass was determined as chlorophyll-*a* by boiling methanol extraction of Whatman GF/C filters and spectrophotometric analyses (Talling, 1974) every 2 weeks. Phytoplankton blooms were defined as mesocosm samples with a chlorophyll-*a* concentration above 60 μg L^{-1}, a threshold selected between the moderate probability of adverse health effects threshold of 50 μg chlorophyll-*a* L^{-1} set by the WHO (2003), and the OECD maximum chlorophyll-*a* boundary for eutrophic lakes of 75 μg chlorophyll-*a* L^{-1} (Organisation for Economic Co-operation and Development, 1982). Whenever a phytoplankton bloom was detected, species were identified with an inverted microscope. Zooplankton samples were taken after sunset when the animals were more equally distributed within the water column. Ten-litre integrated water samples were taken with a 1-m long tube,

filtered onto 50 μm gauze and transferred into small vials before preservation with 10% ethanol. Sub-samples of at least 100 individuals were counted under a microscope from samples collected every 6 to 8 weeks. Copepod numbers include copepodid stages. Nauplii were not included in the copepod counts nor were rotifers or *Daphnia* neonates containing egg yolk reserves.

Macroinvertebrates and fish were sampled once in February and March 2012, then every two weeks until mid-July 2012. A standardised 3 minute net sweep, using a 500 μm net (24 x 24 cm) and conducted by the same individual on each occasion, was used to collect pelagic samples. For sediment, a sample with an area of 0.0225 m^2 was collected with an Ekman grab and sieved through a 1 mm sieve. Abundances of fish and major groups of macroinvertebrates were determined in white trays and the sampled organisms together with the sediment were then returned to their respective mesocosm. Handling times were minimised to prevent stress and mortality. The total abundance of fish and macroinvertebrates in the pelagic and sediment sample were analysed together for each treatment as they were present in the net sweep as well as Ekman grab samples. No macrophytes were added to the mesocosms, but some germinated from the sediment. Filamentous algae growth on the mesocosm rim was detected from May 2012 onwards and measured monthly in a depth of ~5 cm as thickness in cm.

2.5 *Statistical method*

All data were analysed using a mixed effects, nested, randomised-block ANOVA with a temporal autocorrelation structure. Fixed effects of temperature, OM and their interaction and random effects of mesocosm nested within the factor block were used; all data were checked for normality and any non-normal variables were log-transformed before analysis. All

statistical analyses were carried out in the nlme package of R (Pinheiro *et al.*, 2011, R Development Core Team, 2007). For bloom chlorophyll-*a* concentrations > 60 μg L^{-1} fixed factors consisted of temperature, OM and the type of algae dominating the bloom (cyanobacteria or not cyanobacteria).

3. Results

The warming treatment only had a significant effect on increasing conductivity and decreasing dissolved oxygen concentration and fish abundance (Table 1). There were no significant interactions between temperature and the level of OM for any variable (Table 1). Consequently, the main focus of this manuscript is on the dissolved OM treatments. For clarity reasons, data for the two temperature regimes within each DOC treatment were combined and presented as means for Figs 3, 4, 5, 6 and S1. Likewise, in figures depicting the few warming treatment outcomes, data for the three OM treatments were combined and presented as means.

3.1 *Physical conditions*

The water temperatures in heated and unheated mesocosms followed an expected seasonal pattern and the heated treatment was on average 3.996 ºC warmer than ambient (F (1,15) = 1335.7, *p*<0.0001; Fig. 3a). The standard deviation of differences within a treatment over the experimental period was 0.14 ºC for heated and 0.18 ºC for unheated mesocosms. The OM treatments (Fig. 3b) had a mean DOC concentration averaged across the entire year of the experiment (n = 136) of 3.7 ± 0.2 mg L^{-1} in the NOM, 4.6 ± 0.2 mg L^{-1} in the LOM and 8.5 ± 0.6 mg L^{-1} in the HOM mesocosms and these concentrations were significantly different from each other, (F (2,15) = 240.7, *p*<0.0001; Fig. 3b). At the start of the experiment, DOC concentration was relatively low in the HOM mesocosms but increased steadily during

spring, reaching 9.5 mg L^{-1} in May. The difference between DOC in our control and treated mesocosms (NOM and HOM) equated to the amount of change that might be expected over the next 4 years and 21 years, respectively. Underwater PAR and the PAR attenuation was significantly affected by the DOC treatment (Table 1; Fig. 3c, d). Accordingly, in the HOM treatments the mean daily attenuation coefficient was over 7.4 m^{-1} which equates to a 1% PAR depth of about 0.62 m (Table 1, Fig. 3d). The mean daily attenuation coefficient in LOM and NOM was 4.3 m^{-1} and 2.8 m^{-1} respectively, and for both treatments the 1% PAR depth exceeded the depth of the water column (90 cm). The OM additions were accompanied by synchronous increases in attenuation in LOM and HOM treatments with subsequent declines most likely resulting from uptake by organisms and photodegradation.

3.2 *Chemical conditions*

Although levels varied over time, annual mean concentrations of TN and TP were higher in the LOM and HOM than in the NOM treatments (Table 1. Fig. 4a, b). In contrast, mean TP and TN concentrations in heated and unheated mesocosms were not significantly different (Table 1). Mean TON and SRP concentrations were significantly affected by OM treatments (Table 1, Fig. 4c, d). In both cases, mean concentrations increased in the HOM treatments towards the end of the experiment. In all treatment groups, pH and conductivity was high (Fig. 4e, g) and significantly different between OM treatments for pH and the warming treatment for conductivity (Table 1); mean pH values were lower in HOM treatments than in LOM and NOM treatments, reflecting the influence of organic acidity.

3.3 *Species abundance, biomass and ecosystem-level effects*

Despite significant effects of temperature and OM treatments on oxygen concentrations in the mesocosms (Table 1, Fig. 4f, h), no significant effect on R, GPP or NEP (Table 1) was detected over the course of the experiment. Dissolved oxygen was oversaturated most of the

time and net ecosystem production was positive in all treatments (Table 1, Fig. S1) indicating that the systems were autotrophic throughout the year even though there were occasional heterotrophic episodes in the HOM treatments (Fig. S1). Accordingly, the concentration of phytoplankton chlorophyll *a* was very high from summer onwards (Fig. 5a), peaking in early autumn, and high levels persisted until the experiment was terminated in February. In HOM treatments, phytoplankton chlorophyll *a* increased in spring as TN and TP increased, reaching 32 μg L^{-1} at the beginning of April (Fig. 5a). The highest chlorophyll *a* concentration in the NOM and HOM mesocosms occurred in October. The highest annual mean phytoplankton chlorophyll *a* concentrations occurred in the LOM treatments, reaching a mean of 130 μg L^{-1} in September (Fig. 5a) and this was the treatment with the highest net ecosystem production (Table 1). During blooms (chlorophyll *a* concentration above 60 μg L^{-1}) species were determined and differentiated between cyanobacteria species (*Aphanizomenon* sp., *Dolichospermum* spp., *Microcystis* sp., *Woronichinia* sp. and unidentified pico cyanobacteria) and blooms dominated by other phytoplankton species (*Carteria* sp., *Monoraphidium* sp., *Scenedesmus* sp., *Peridinium* sp., *Cryptomonas* sp., *Eudorina* sp. and *Plagioselmis* sp.), occurring in various mesocosms from around June onwards. According to the bloom species identified, the phytoplankton biomass value was classified as a cyanobacterial bloom or a non-cyanobacterial bloom (Table 2, Fig. 6). Cyanobacterial blooms were particularly high and long-lived in the LOM treatments (Figs. 5a, 6, Table 2).

Zooplankton mainly consisted of calanoid and cyclopoid copepods at the beginning of the experiment, however in April and May, *Bosmina* sp. was the dominant zooplankter, while from July onwards the total zooplankton abundance was low with a mean of 1.45 ± 4.77 individuals L^{-1} (see Fig. S1). Over the course of the experiment, zooplankton abundance was not significantly affected by any treatment, but on average the density was over 8-fold lower

in heated compared to unheated mesocosms due to the *Bosmina* sp. peak (Table 1, Fig. S1). The low macroinvertebrate abundances (Fig. 5b) observed until May might have been caused by low spring temperatures in 2012 (Fig. 3a). Macroinvertebrates were more abundant in treatments receiving OM compared to the NOM group in summer (Fig. 5b, Table 1), but not affected by warming (Fig. S1, Table 1). The macroinvertebrates in the pelagic and benthic samples consisted of Ephemeroptera, Amphipoda, Arachnida, Hemiptera, Diptera, Trichoptera, Gastropoda, Bivalvia, Platyhelminthes, Coleoptera, Chaoborus, Isopoda and Annelida. Chironomidae, Corixidae, Oligochaeta and Mollusca accounted for 86% of all macroinvertebrates and were chosen for detailed analysis (see Table1), however only Chironomidae showed a significant response to warming, with reduced abundances in heated treatments (Table 1, Fig. 5c). Juvenile sticklebacks were not detected before the end of April, possibly because the lower than normal spring temperatures caused a delay in the onset of spawning. Afterwards, fish numbers increased in the heated treatments, before a sharp increase in the ambient treatments in June (Fig. 5d), although no significant difference in abundance between OM treatments was found (Table 1, Fig. S1). Macrophyte growth was only visible in NOM mesocosms due to water colour and/or high algae growth in LOM and HOM mesocosms. *Elodea nutallii* was the dominant species with some *Potamogeton berchtoldii*. Filamentous algae growth on the mesocosm rim was generally low, with a mean length from the mesocosm rim of 1.2 ± 0.3 cm, 1.3 ± 0.4 cm and 1.4 ± 0.4 cm for NOM, LOM and HOM respectively (F $(2,15) = 0.3$, $p=0.76$) and a mean of 1.3 ± 0.5 cm and 1.3 ± 0.4 cm for ambient and heated treatments, respectively (F $(1,15) = 0.1$, $p=0.79$).

4. Discussion

4.1 *Overview and major findings*

In order to understand how our freshwater ecosystems and resources may be impacted by the combined pressures of 'brownification' and climate warming, it is important to constrain experimental conditions within realistic bounds. The present study focusses on brownification in combination with climate warming in eutrophic systems which has not been tackled so far. The results of the present study show that neither warming nor increased concentrations of OM had an impact upon R, GPP or NEP, however increased levels of OM significantly altered water chemistry. While fish and chironomids reacted strongly to warming, OM addition under eutrophic conditions increased total macroinvertebrate abundance. Phytoplankton biomass decreased with DOC concentrations > 10 mg L^{-1}, but intermediate DOC concentrations < 7 mg L^{-1} enhanced phytoplankton biomass in eutrophic conditions, and in particular promoted cyanobacteria blooms.

4.2 *Response of phytoplankton and primary productivity to organic carbon addition*

Since nitrate and phosphate were added equally to all mesocosms and the sediment was the same in all mesocosms, the higher TN and TP concentrations in the LOM and HOM treatments most likely arise from the release of nutrients from the peat (the mean TP and TN concentration of the peat water in the three peat holding tanks in January 2013 was 0.41 mg L^{-1} and 8.13 mg L^{-1}, respectively) or, additionally in the case of N, by cyanobacteria N-fixation. OM in freshwaters acts as a source of carbon, nitrogen and phosphorus for the phytoplankton community but also attenuates light (Köhler *et al.*, 2013). Contrary to our hypothesis that, as a consequence of the shading effect of OM, phytoplankton biomass would decrease with increasing OM concentration, phytoplankton growth in fact benefited from brownification in these shallow systems, confirming the results from Kelly et al. (2016). During early spring, DOC concentrations were likely not high enough (below 8 mg L^{-1} in the HOM treatments) to have a shading effect, and phytoplankton growth appeared to benefit

from increased SRP and TON concentrations. From late spring until the end of autumn, SRP and TON concentrations were drawn down by primary production indicating nutrient limitation. During this time, the chlorophyll *a* concentrations in the HOM treatments were lower than the other two treatments, indicating light limitation with DOC rising above 10 mg L^{-1} and a 1% PAR depth of 0.7 m. Similar findings were reported for a 50 day long Canadian enclosure experiment, during which DOC addition caused an increase in chlorophyll *a* with DOC concentrations below 10 mg L^{-1} (Graham & Vinebrooke, 2009). In our eutrophic experiment, the addition of OM caused a decrease in phytoplankton growth only at very high DOC concentrations, above 10 mg L^{-1}; globally, of the lakes reported by Sobek *et al.* (2007), an estimated 35% had a DOC concentration above 10 mg L^{-1}. In contrast, a Swedish mesocosm experiment (Nicolle *et al.*, 2012) with similar depth demonstrated that brown water additions led to the predicted decrease in chlorophyll *a* concentrations, although in that study nutrient concentrations were lower in brown water mesocosms than in the present study. Despite the higher phytoplankton growth in spring, DO was lowest in HOM mesocosms and NEP indicated a heterotrophic system possibly caused by mixotrophic phytoplankton as well as bacterial activity. However, over the whole year, the mesocosms were autotrophic for most of the time. Indeed, R and GPP showed a strong linear correlation ($r^2 = 0.95$, data not shown), thus R was mostly driven by phytoplankton biomass. In contrast, Hanson *et al.* (2003) found negative NEP at DOC >10 mg L^{-1} in 25 lakes in northern Wisconsin and Michigan.

In the LOM treatments, simulating the DOC concentrations predicted to be typical for UK lakes in the immediate future, we observed high phytoplankton biomass during summer, autumn and winter. Similar findings were reported for a microcosm experiment conducted in an alpine lake in the US, in which low additions of DOC (1.1 mg L^{-1}) also stimulated

phytoplankton density and growth (Kissman *et al.*, 2013). Highest phytoplankton biomass was recorded in August and September, and was also visible in the NEP data, however the increase of NEP during May and June is surprising given the increase of macroinvertebrates at the same time. However, as we did not find significant between-treatment differences for R, GPP or NEP, we cannot conclude that altering the OM content of the mesocosms (with a depth of 1 m) had any effect on NEP.

4.3 *Response of phytoplankton species composition to organic carbon addition*

In the present study, the high phytoplankton chlorophyll *a* concentration in the LOM treatments was caused by the occurrence of blooms, consisting mostly of *Aphanizomenon* sp., in several mesocosms. The high phytoplankton abundance and long bloom duration in the LOM mesocosms was reflected in the TN concentrations, probably as a result of some cyanobacteria being able to fix nitrogen from the atmosphere (heterocysts were observed in the samples) in addition to release from organic matter addition. Hansson *et al.* (2013) and Ekvall *et al.* (2013) found cyanobacteria (*Microcystis;* although unable to fix nitrogen) to benefit from both elevated temperatures and brownification, however total cyanobacterial biomass remained unaffected in their mesocosm experiment. Typically, cyanobacteria blooms can be associated with high nutrient loading, water column stratification and high surface water temperatures ($> 20\,°C$) (Paerl, 1996). In our experiment, mechanical mixers were used to prevent stratification within the mesocosms, but increased nutrient concentrations within the range for eutrophic lakes and increased water surface temperatures in July might well have played a role in bloom formation. The low bloom formation in the HOM treatment is likely to have been caused by low light availability in the water column and the prevention, by mixing, of buoyant cyanobacteria from accumulating substantially at the surface in order to exploit high light levels (Paerl & Huisman, 2008).

Apart from the potential toxicity, cyanobacterial dominance is undesirable for natural systems, as it enhances surface light absorption, has negative effects on biodiversity as well as ecosystem services (Brookes & Carey, 2011). Shallow lakes can switch between stable states: a clear, plant dominated state with high biodiversity, and a turbid state dominated by phytoplankton or suspended sediment (Scheffer *et al.*, 1993). Such switches between states are influenced by increased nutrient loading. However, recent evidence suggests that nutrients alone cannot cause such a shift and an external driver is necessary (Janssen *et al.*, 2014, Scheffer & van Nes, 2007). The evidence from the present study suggests that increased allochthonous OM in combination with an increase in nutrient concentration stimulates phytoplankton bloom formation and thus favours the establishment of a turbid state. Macrophytes in shallow lakes might suffer by brownification via reduced light availability limiting plant growth in the sediment, and by phytoplankton domination before plants have a chance to become established. However, in a natural system, with plants already established, brownification might lead to a different response.

4.4 *Response of higher trophic levels to organic carbon addition*

With increasing phytoplankton biomass in spring, total macroinvertebrate abundance increased in mesocosms with added OM. A direct trophic level effect, via elevated allochthonous carbon stimulating macroinvertebrate abundance, might be the obvious mechanism underlying this observation. However, indirect uptake of allochthonous OM particles by macroinvertebrates or stimulation of higher trophic levels through the microbial loop (Azam *et al.*, 1983) fuelled by DOC increase, is also possible. Even though fish biomass showed no statistically significant effect overall as a result of increased OM, the peak in numbers of juvenile fish sampled in June was greatest in the HOM treatments, followed by

LOM and NOM, as was also the case for macroinvertebrate abundance. Fish predation pressure during summer was generally high and was likely to be the cause for the decline in zooplankton and macroinvertebrates. Surprisingly, fish predation pressure in the HOM treatments was as high as in the other treatments, indicating the ability of sticklebacks to feed under low light conditions. This is consistent with previous observations that prey consumption by planktivorous fish is not markedly affected by elevated turbidity (De Robertis *et al.*, 2003) and that turbidity affects prey selectivity in sticklebacks, rather than overall consumption rates (Sohel *et al.*, 2017). Thus, our second hypothesis, that higher trophic level biomass would decline with increasing OM content was not supported over the first half of the experiment. Cyanobacteria blooms can alter the trophic structure in lakes considerably, especially if toxins are produced which can be harmful to aquatic organisms (Codd *et al.*, 2005). Even if no toxins are produced, cyanobacteria constitute a low quality food source to higher trophic levels (Paerl, 1988), such as zooplankton and macroinvertebrates in the present study. However, overall mean macroinvertebrate abundance was highest in the LOM mesocosms in the present study, but decreased strongly in July, when phytoplankton biomass increased. The duration of the study might have been too short to see effects of brownification on the top trophic level, as Kelly *et al.* (2016) did find larger differences two years after manipulating DOC concentrations within a lake manipulation, compared to one year after manipulation.

4.5 *Warming effects on biological components*

Surprisingly, phytoplankton biomass showed very similar abundances and phenology in ambient and warmed mesocosms, contradicting our fourth hypothesis. The lack of a temperature effect on phytoplankton biomass could result from abnormal weather during the experimental period, however air temperatures were between the mean daily maximum and

minimum temperature (1981-2010) (Met Office regional climates: North West England:climate https://www.metoffice.gov.uk/climate/uk/regional-climates/nw#temperature). However, our results are in accordance with a mesocosm experiment in Denmark, where nutrients were found to have an overriding effect over temperature: chlorophyll *a* was elevated at high nutrient levels while the effect of temperature was insignificant (Zhou *et al.*, 2018). Cyanobacteria are known to flourish under warm conditions and often gain competitive advantage compared to other phytoplankton taxa at higher temperatures (Elliott *et al.*, 2006, Jöhnk *et al.*, 2008). The reasons underlying the lack of higher cyanobacteria bloom formation in warmed mesocosms are not clear, however the low amount of available nitrate and phosphate during the summer months might be a contributory factor.

As food availability in the heated mesocosms was not different to that in the mesocosms maintained at ambient temperature, higher trophic level biomass was not expected to differ between treatments. However, fish abundance showed a clear phenological response with juveniles being detected earlier in the heated mesocosms, as reported from various sites (Thackeray *et al.*, 2013, Thackeray *et al.*, 2010), likely due to acceleration of processes (earlier spawning, shorter incubation time, earlier hatching, more rapid growth of juveniles) in the warmer environment. A peak of *Bosmina* spp. during May and June 2012 and the higher abundance of chironomidae in the unheated mesocosms might have caused the high abundance of fish in comparison to the warmed mesocosms if food was limiting in the warmed mesocosms. However, not knowing how many adults spawned in each treatment makes it difficult to interpret fish abundance further. The lower chironomid abundances in warmed mesocosms from May onwards might indicate a higher emergence in warmed treatments. Conductivity was higher in heated mesocosms, in common with previous

mesocosm experiments (Feuchtmayr *et al.*, 2009, McKee *et al.*, 2003), and possibly resulting from increased mineral weathering in the sediment. However, conductivity decreased from September 2012 as a consequence of rainwater dilution.

5. Conclusion and strategies to address the effects of large scale environmental change in lakes

Climate change and anthropogenic impacts have already caused, and most likely will continue to do so in the future, an increase of OM in aquatic systems, warming and increased eutrophication. In the present study, small amounts of allochthonous carbon from a peat source, coupled with increases of nutrients, had a marked effect on processes within experimental shallow lake mesocosms. Although the extrapolation of experimental results to natural shallow lakes has its limitations, the outcomes of pelagic algal nutrient limitation mesocosm experiments have been shown to be applicable to natural systems (Spivak *et al.*, 2011). For shallow lakes, turbid states are not desirable, and high cyanobacteria biomass in particular should be prevented along with the often accompanying elevated toxin concentrations. Both the data presented here, and from mesocosm experiments reported by Ekvall *et al.* (2013), show either an increase in cyanobacteria biomass or toxin concentration when future scenarios of brownification, or brownification and temperature, are simulated. Identifying strategies to counteract these negative effects of climate change is challenging, particularly since the transfer of OM from catchments to lakes, as well as the effects of climate change on soil carbon stores, is still poorly understood. Thus, more research and monitoring, as well as the use of similar metrics (Sepp *et al.*, 2018), is needed to identify the factors responsible for increases in OM within aquatic ecosystems and to evaluate possible land use changes in catchments with high OM discharge. Improved management is

particularly important for sites where peat is removed as well as sites undergoing land use changes and afforestation which can lead to DOM export into lakes (Haddaway *et al.*, 2018, Kritzberg, 2017). In addition to a change in catchment management practices, riparian vegetation could provide an important buffer in order to reduce the transfer of both OM and nutrients from catchments into streams. Increasing the retention of dissolved and particulate matter within rivers and streams via stream restoration and in-stream structures along with less intensive agriculture could also reduce concentrations downstream. Particular emphasis should be given to forested, peatland- and wetland-rich catchments as well as regions suffering from permafrost degradation; their management will play a vital role in the overall control of OM discharge.

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Table 1 Analysis of mean annual conditions in the mesocosm treatments. Results derived from a mixed model nested randomised-block ANOVA with repeated measures. Fixed factors were temperature and organic matter and random factor mesocosm was nested within the factor block. Overall mean values during the experimental period for each treatment (ambient- A, heating – H, no organic matter addition – NOM, low organic matter addition- LOM, high organic matter addition – HOM) ± standard deviation are given together with p-values. Significant p-values (95% confidence interval) are indicated in bold. F values and degrees of freedom are given in Table S1.

	Warming (W)			Organic matter (OM)		
	Mean A ± SD	Mean H ± SD	p	Mean NOM ± SD	Mean LOM ± SD	Mean SD
Photosynthetically active radiation (μmol m^{-2} s^{-1})	46.6 ± 13.6	48.1 ± 32.4	0.6892	74.5 ± 18.8	52.5 ± 43.5	1
Light attenuation coefficient (k) (m^{-1})	5.1 ± 4.3	5.0 ± 4.0	0.7578	3.1 ± 2.8	4.5 ± 4.0	
Total phosphorus (mg L^{-1})	0.08 ± 0.04	0.07 ± 0.03	0.2038	0.06 ± 0.03	0.08 ± 0.05	0.09
Total nitrogen (mg L^{-1})	0.80 ± 0.36	0.82 ± 0.32	0.7164	0.63 ± 0.29	0.92 ± 0.56	0.8
Soluble reactive phosphate (μg L^{-1})	11.2 ± 5.2	13.1 ± 5.3	0.2289	4.9 ± 2.7	5.7 ± 3.6	2
Total oxydised nitrogen (mg L^{-1})	0.14 ± 0.08	0.18 ± 0.08	0.1548	0.07 ± 0.06	0.10 ± 0.07	0.3
pH	7.9 ± 0.4	7.8 ± 0.4	0.4064	8.0 ± 0.5	7.9 ± 0.4	7.7
Conductivity (μS cm^{-1})	124.8 ± 12.2	158.5 ± 9.7	**<.0001**	139.8 ± 9.9	142.1 ± 13.2	14
Dissolved oxygen (mg L^{-1})	12.2 ± 2.0	11.5 ± 1.9	**0.0005**	12.4 ± 2.1	12.1 ± 2.0	1
Respiration (μmol O$_2$ L^{-1} day^{-1})	-5.0 ± 49.5	-1.0 ± 54.1	0.3525	-4.7 ± 62.2	1.7 ± 55.5	-5
Gross primary production (μmol O$_2$ L^{-1} day^{-1})	0.2 ± 57.8	7.0 ± 63.6	0.1812	0.8 ± 72.2	10.4 ± 65.2	-0
Net ecosystem production (μmol O$_2$ L^{-1} day^{-1})	5.1 ± 15.0	8.0 ± 15.6	0.1104	5.5 ± 17.2	8.7 ± 16.6	5
Chlorophyll a (μg L^{-1})	45.0 ± 29.7	43.4 ± 29.7	0.4880	36.1 ± 25.8	57.3 ± 45.6	39
Zooplankton (number L^{-1})	365.1 ± 6.0	44.1 ± 4.6	0.0743	289.2 ± 5.2	89.0 ± 3.6	23
Total macroinvertebrates (number subsample^{-1})	28.2 ± 19.2	22.1 ± 14.8	0.1347	18.0 ± 10.7	31.8 ± 23.7	25
Chironomidae (number subsample^{-1})	18.0 ± 13.5	11.7 ± 8.0	**0.0225**	13.0 ± 9.2	18.5 ± 12.8	13
Corixidae (number subsample^{-1})	5.0 ± 6.9	6.3 ± 9.0	0.7119	0.5 ± 0.8	8.3 ± 12.2	8
Oligochaeta (number subsample^{-1})	1.8 ± 1.8	1.5 ± 1.5	0.4408	1.3 ± 1.4	2.1 ± 2.1	
Mollusca (number subsample^{-1})	6.8 ± 9.5	4.3 ± 5.8	0.5600	3.1 ± 2.7	8.3 ± 12.6	
Fish (number subsample^{-1})	13.1 ± 12.5	5.8 ± 5.5	**0.0104**	7.3 ± 6.6	10.0 ± 10.0	11

Table 2 Analysis of mean bloom conditions in the mesocosm treatments. Results derived from a mixed model nested randomised-block ANOVA with repeated measures. Fixed factors were temperature and organic matter and random factor mesocosm was nested within the factor block. Overall mean values during the experimental period for each treatment (ambient- A, heating – H, no organic matter addition – NOM, low organic matter addition- LOM, high organic matter addition – HOM, cyanobacteria – CY, other phytoplankton – OTH) ± standard deviation are given together with p-values. Significant p-values (95% confidence interval) are indicated in bold. F values and degrees of freedom are given in Table S1.

		Bloom chlorophyll a (μg L^{-1})
Warming (W)	Mean A ± SD	103.9 ± 57.5
	Mean H ± SD	107.4 ± 49.1
	p	0.280
Organic matter (OM)	Mean NOM ± SD	104.5 ± 49.1
	Mean LOM ± SD	129.8 ± 64.2
	Mean HOM ± SD	78.2 ± 16.8
	p	**0.035**
Dominating phytoplankton (DP)	Mean CY ± SD	127.4 ± 63.6
	Mean OTH ± SD	82.0 ± 22.2
	p	**0.027**
W x OM	p	0.322
W x DP	p	0.697
OM x DP	p	**0.028**
W x OM x DP	p	0.371

Fig. 1 Conceptual diagram of external stressors (bottom of figure) and lake internal interactions and effects (top of figure) of organic matter increase.
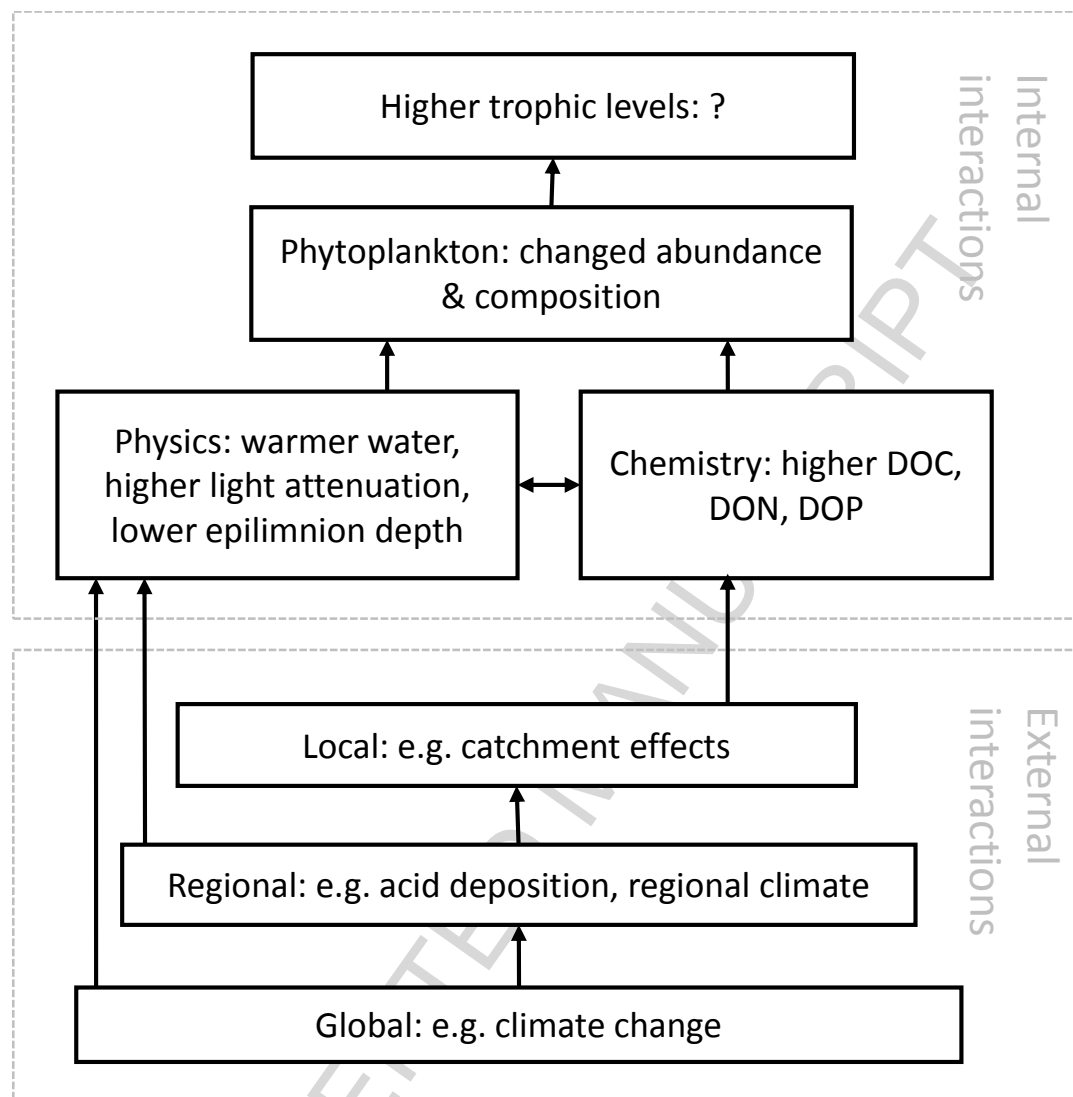
Fig. 2 The CEH Aquatic Mesocosm Facility (CAMF). (a) Photo, (b) heating elements in a mesocosm before the addition of sediment, and (c) layout of the 24 experimental treatments (two temperature treatments: heated (H) and ambient (A) and three organic matter treatments: no organic matter (NOM), low organic matter (LOM) and high organic matter addition (HOM)) within the 32 mesocosms at CAMF (the eight unused mesocosms are unlabelled).
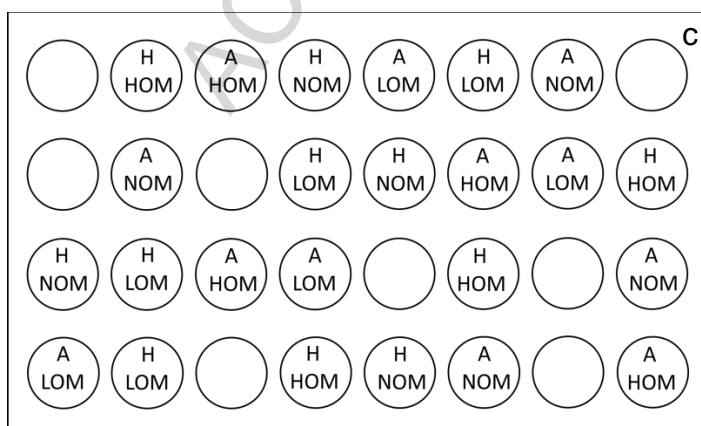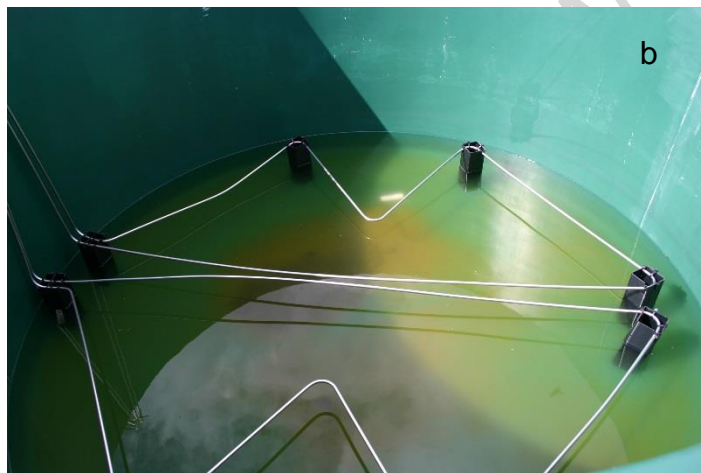
Fig. 3 Establishment of treatments and significantly different light conditions in the mesocosms. (a) Mean daily water temperature (ºC) for all heated (H) and ambient (A) mesocosms, and (b-d) no organic matter (NOM), low organic matter (LOM) and high organic matter (HOM) treatments for (b) mean ± SD dissolved organic carbon concentrations, (c) daily (24 hours) mean of photosynthetically active radiation (PAR) at 0.43 m depth shown as a 9 day rolling mean per treatment for clarity, and (d) PAR attenuation coefficient. Vertical grey bars in (b), (c) and (d) indicate addition of organic matter to LOM and HOM mesocosms. All values are means of eight mesocosms per treatment for the organic matter treatments and twelve mesocosms per treatment for the temperature treatments.
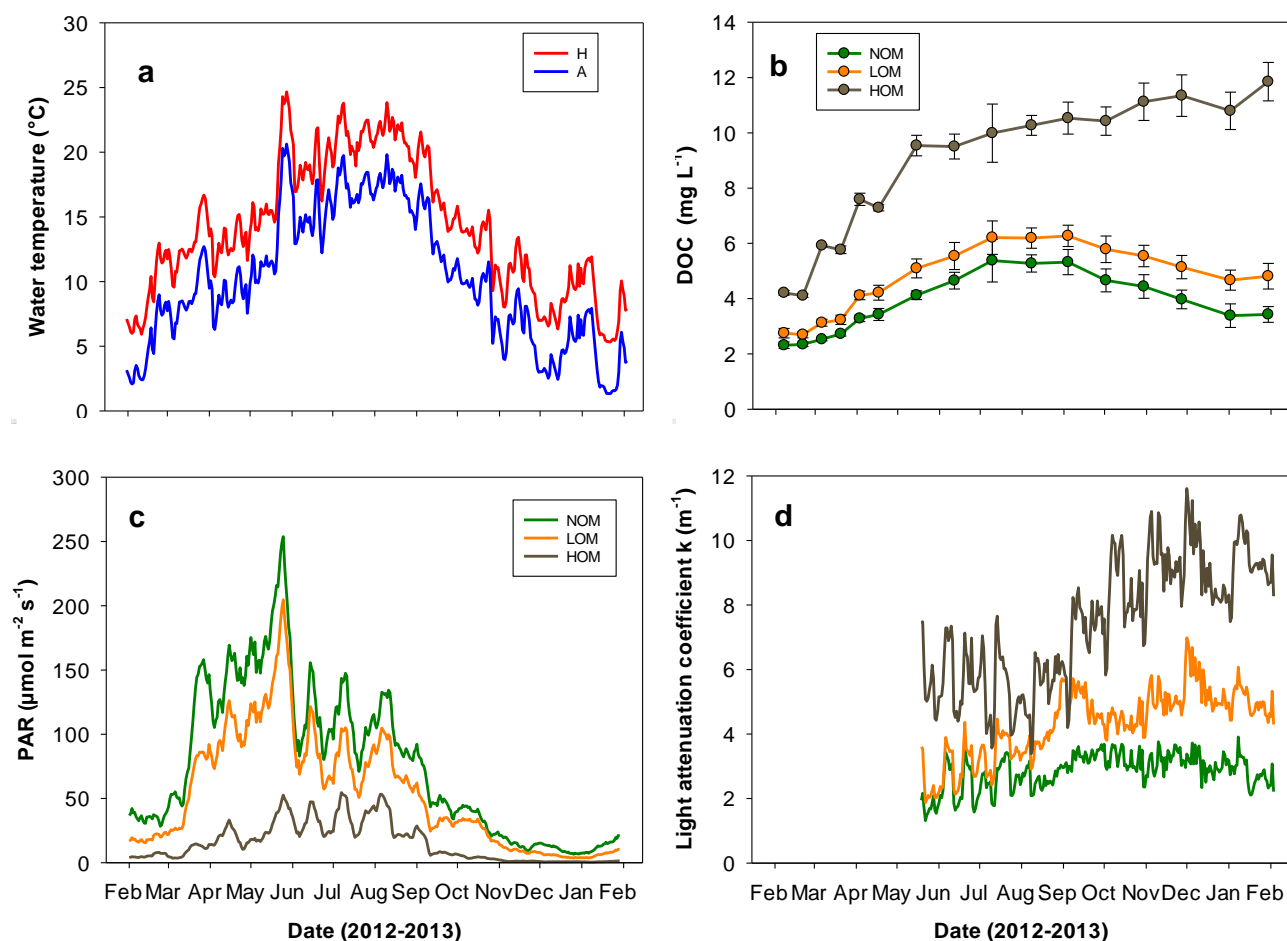
Fig. 4 Chemical and physical conditions in the different treatments for variables with statistically significant variation: (a) total phosphorus, (b) total nitrogen, (c) soluble reactive phosphate, (d) total oxydised nitrogen, (e) pH and (f) dissolved oxygen for the no organic matter (NOM), low organic matter (LOM) and high organic matter (HOM) treatments. (g) Conductivity and (h) dissolved oxygen for the heated (H) and ambient (A) treatments. Dissolved oxygen is shown as 9 day rolling means per treatment and standard deviations are not shown to aid clarity. Vertical grey bars in (a) and (b) indicate addition of organic matter to LOM and HOM mesocosms. Immediately after each fortnightly sampling, nutrients (N and P) were added to all mesocosms. All values are means of eight mesocosms per treatment for the organic matter treatments and twelve mesocosms per treatment for the temperature treatments.
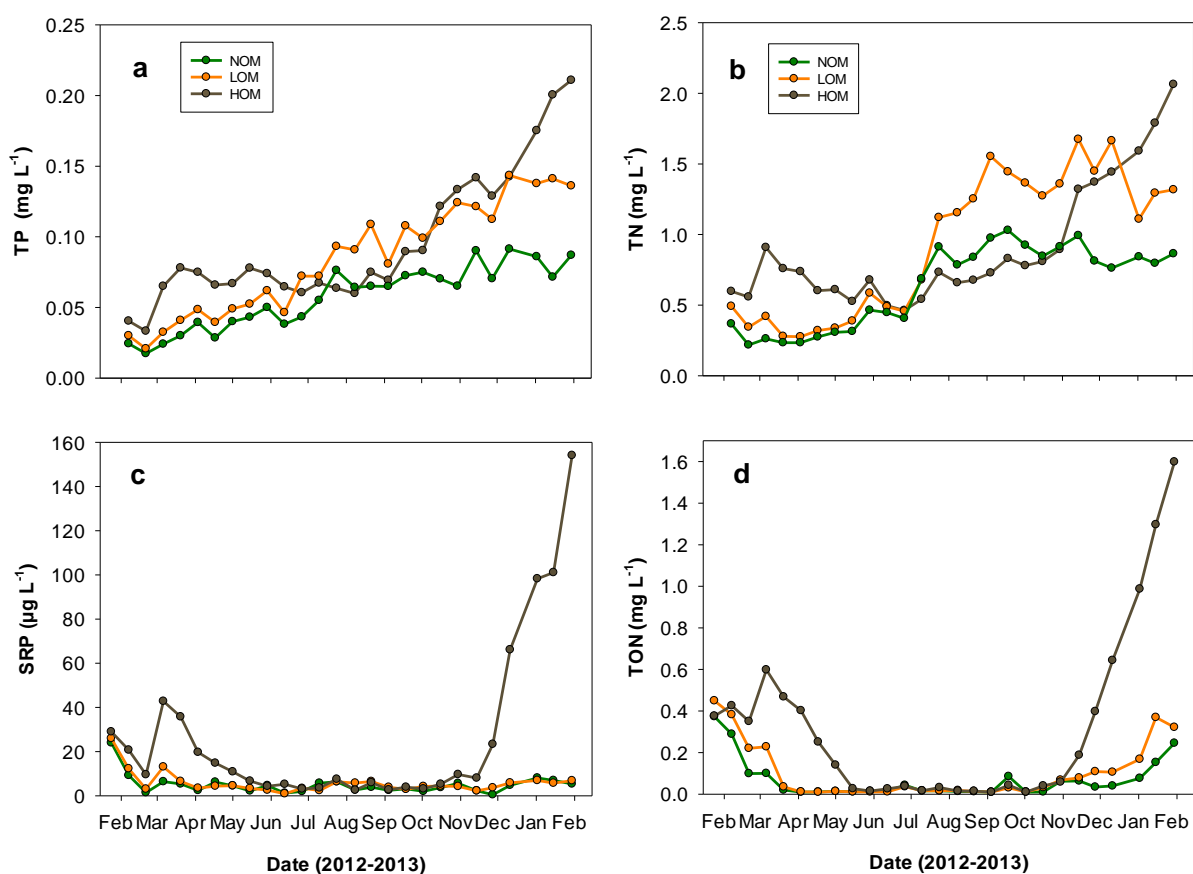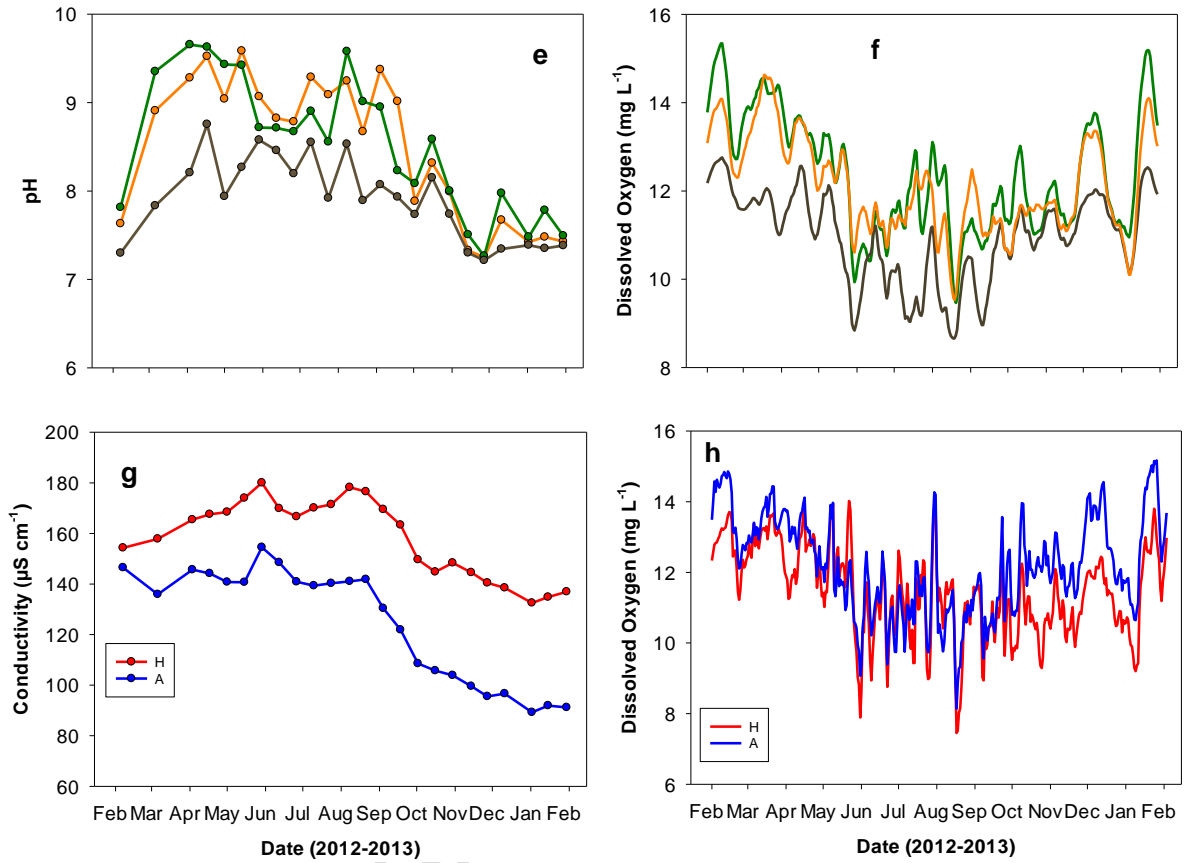
Fig. 5 Biological parameters in the different treatments for variables with statistically significant variation. (a) Chlorophyll *a*, and (b) total macroinvertebrate abundances for the no organic matter (NOM), low organic matter (LOM) and high organic matter (HOM) treatments during the experimental period. Chironomidae (c) and fish abundances (d) for the heated (H) and ambient (A) treatments. Macroinvertebrate and fish abundances are given as number per subsample, each subsample consisting of a standardised pelagic sweep net sample pooled with a benthic sediment sample. Macroinvertebrate and fish abundances were not determined after mid-July 2012. All values are means of eight mesocosms per treatment for the organic matter treatments and twelve mesocosms per treatment for the temperature treatments.
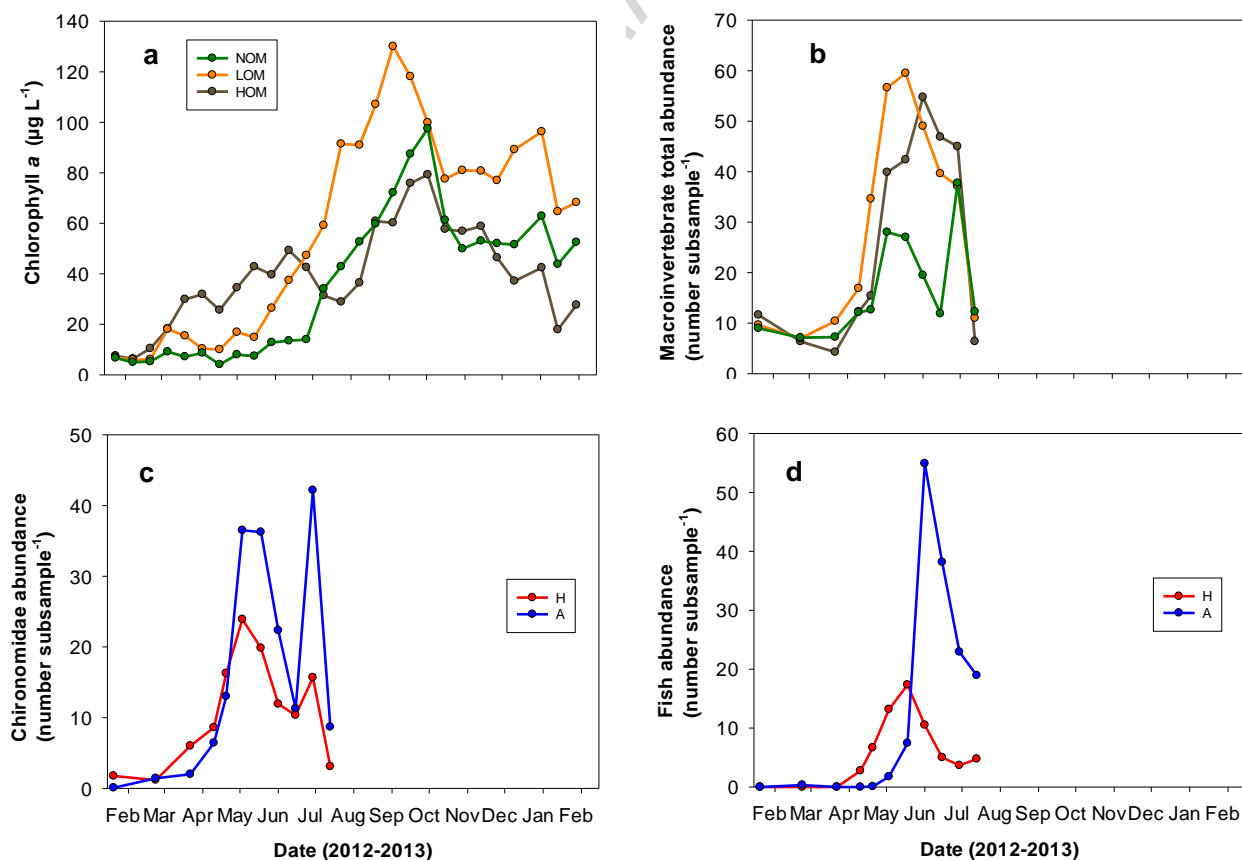
Fig. 6 Mean chlorophyll *a* in different treaments when blooms dominated by cyanobacteria or other phytopankton. No organic matter (NOM), low organic matter (LOM) and high organic matter (HOM) treatments shown as boxplots for all data during the experiment for blooms dominated by cyanobacteria (CY) and blooms dominated by other phytoplankton (OTH). See Table 2 for results of statistical analysis.
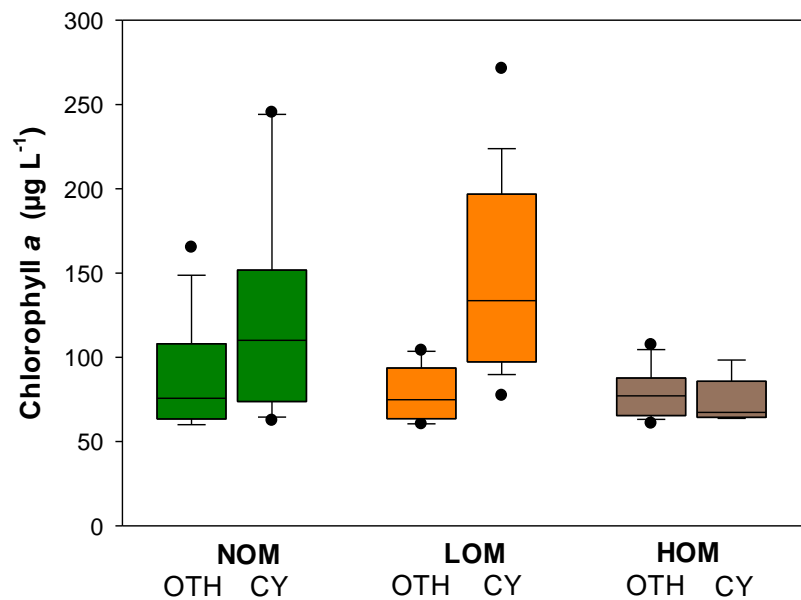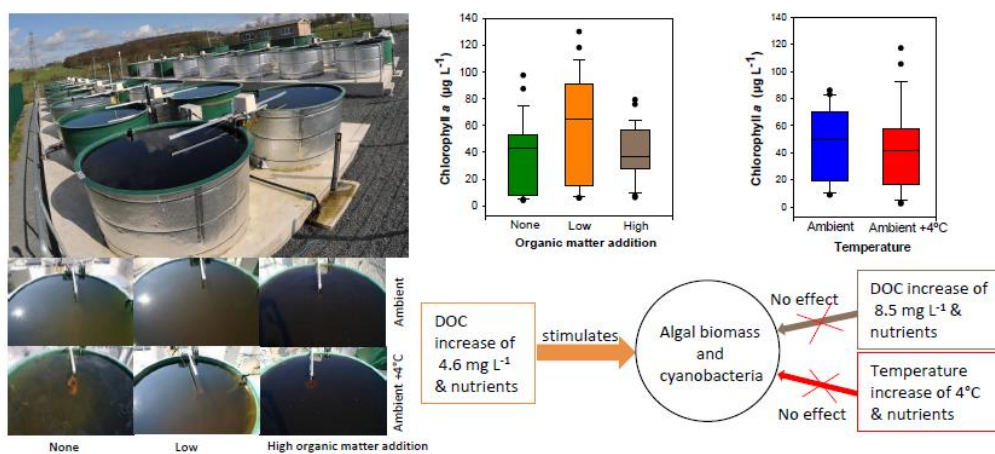
Graphical abstract

Highlights:

- Studied the effects of increased DOC, eutrophication and temperature on freshwaters

- An experimental outdoor large-scale mesocosm facility was used over a year

- Algal biomass appeared unaffected by warming

- DOC at low concentration increased algal biomass and stimulated cyanobacteria

- DOC at high concentration did not affect algal biomass, possibly due to shading.
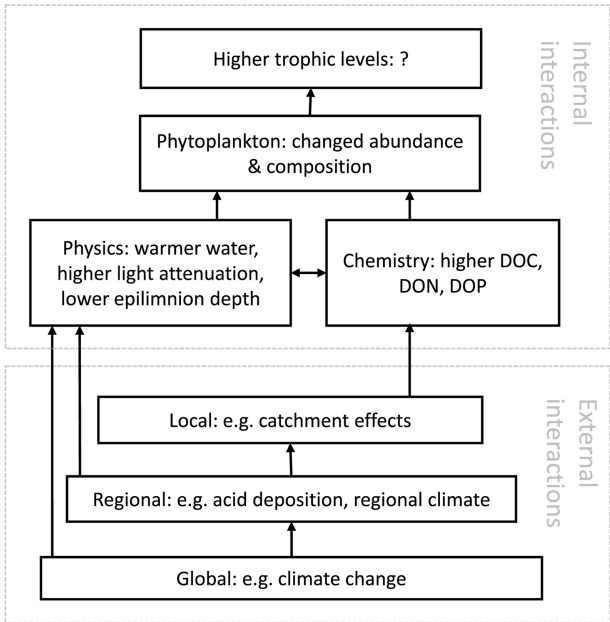
Figure 1
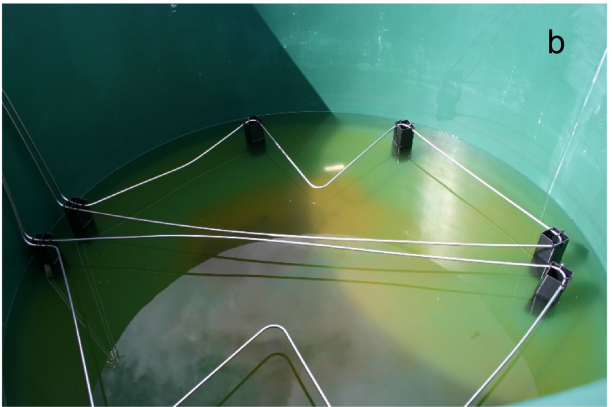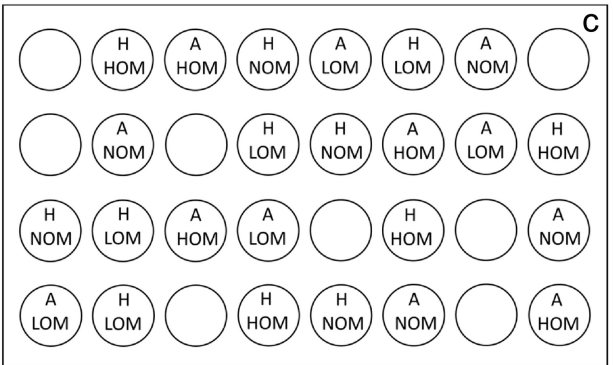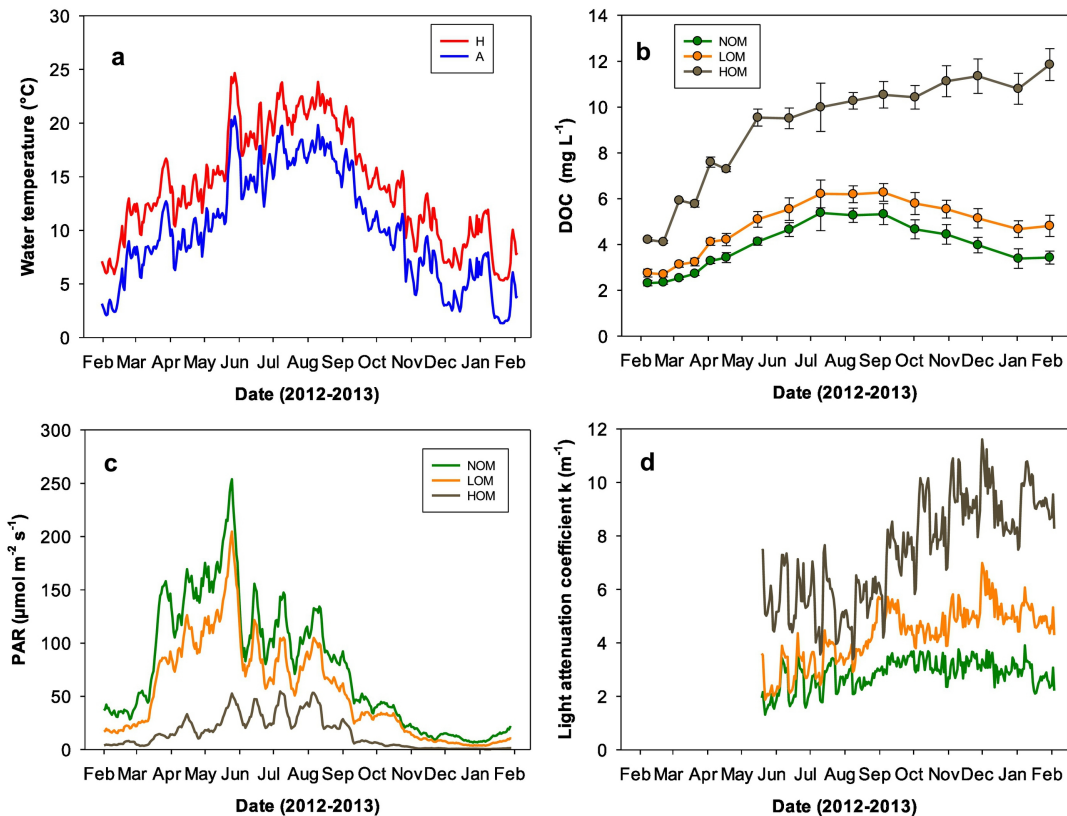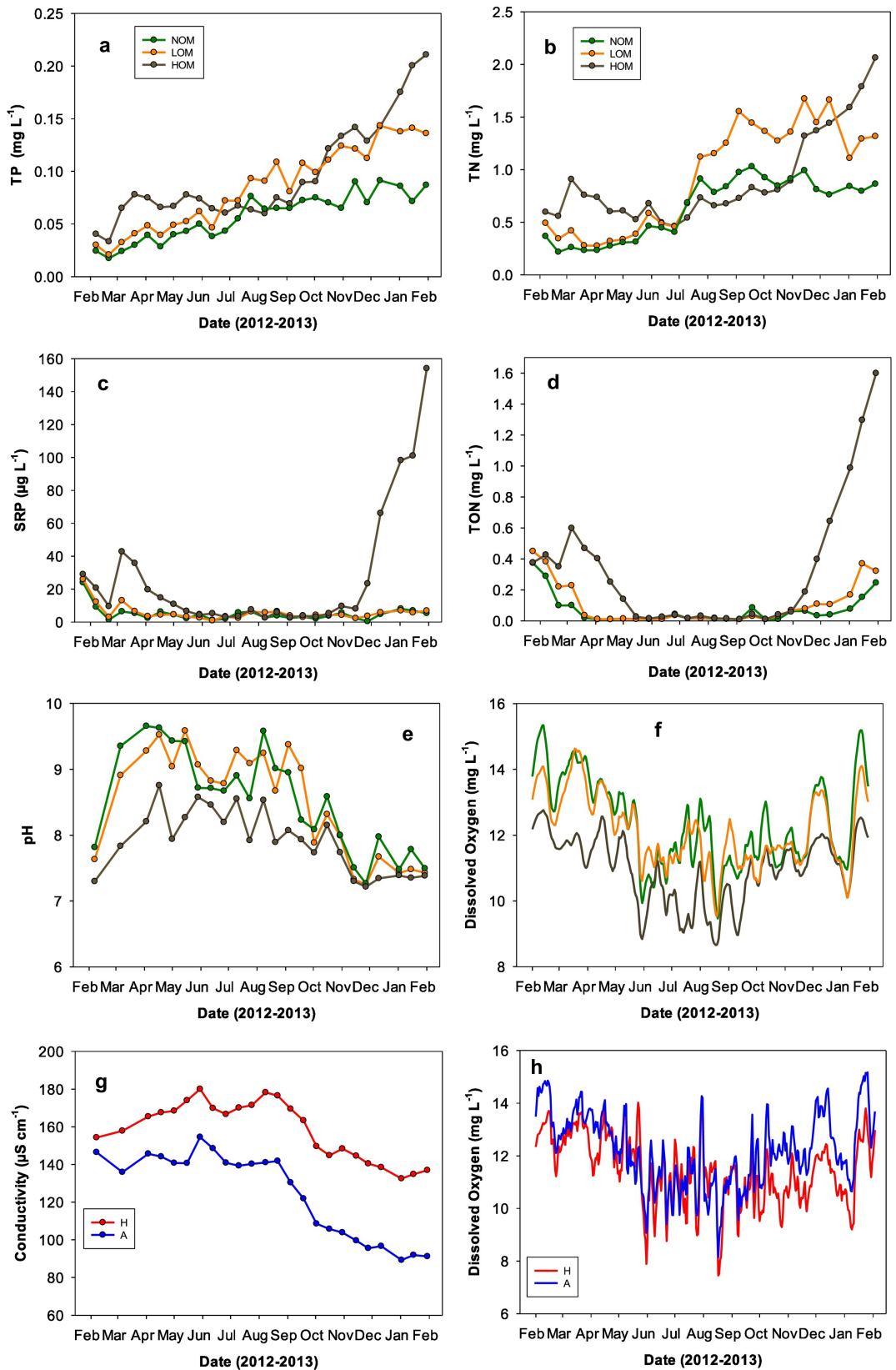
Figure 2

Figure 3

Figure 4

Figure 5

Figure 6