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RESEARCH ARTICLE

Impacts of elevated atmospheric CO_2 concentration on terrestrial-aquatic carbon transfer and a downstream aquatic microbial community

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Abstract

Under higher atmospheric CO_2 concentrations, increases in soil moisture and, hence in terrestrial-aquatic carbon transfer are probable. In a coupled terrestrial-aquatic experiment we examined the direct (e.g. through changes in the CO_2 water concentration) and indirect (e.g. through changes in the quality and quantity of soil leachates) effects of elevated CO_2 on a lake microbial community. The incubation of soils under elevated CO_2 resulted in an increase in the volume of leachates and in both chromophoric dissolved organic matter (CDOM) absorption and fluorescence in leachate. When this leachate was added to lake water during a 3-day aquatic incubation, we observed negative direct effects of elevated CO_2 on photosynthetic microorganism abundance and a positive, indirect effect on heterotrophic microbial community cell abundances. We also observed a strong, indirect impact on the functional structure of the community with higher metabolic capacities under elevated CO_2 along with a significant direct effect on CDOM absorption. All of these changes point to a shift towards heterotrophic processes in the aquatic compartment under higher atmospheric CO_2 concentrations.

Keywords Direct and indirect CO_2 effects · Global change · Soil leachates · Allochthonous carbon · CDOM · Legacy

Introduction

Although the effect of surface water run-off from terrestrial ecosystems to adjacent aquatic systems have been relatively well studied (Cole and Caraco 2001; Le et al. 2016), at present little is known about how aquatic ecosystems will respond to inputs of terrestrial organic matter under high atmospheric CO_2. Several authors have hypothesized that under high atmosphere CO_2 concentrations plant transpiration will be lower, leading to higher soil water contents (Field et al. 1995; Leakey et al. 2009). This in turn means that, during rain events, soils will become saturated more rapidly leading to increased leachate production and run-off (Butcher et al. 2014). Given that leachates and run-off are, in many ecosystems, the main hydrological processes via which organic matter, particles and microorganisms are transported into adjacent surface waters (Hillel 2003; Dimitriou 2011; Orchard et al. 2013; Causse et al. 2015), increases in either of these pathways could have important consequences for aquatic systems.

The quantity and quality of dissolved organic carbon (DOC) in soil leachate and run-off are strongly influenced

Emma Rochelle-Newall and Audrey Niboyet contributed equally to this work (co-first authors).

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by soil organic carbon content, soil erosion, and vegetation cover, and by amounts and intensities of rainfall (Jacinthe et al. 2004; Worrall and Burt 2005; Mailapalli et al. 2012; Janeau et al. 2014; Trinh et al. 2016). Moreover, under elevated CO_2 it is anticipated that along with an increase in soil moisture content, there also will be modifications in vegetation cover and in soil organic carbon content (Drigo et al. 2010). Considering that the latest projections (IPCC 2013) point towards an increase in the volume and/or intensity of precipitation episodes that will lead to increases in leachate and run-off, it is probable that the export of organic matter from terrestrial systems to downstream aquatic ecosystems will increase. However, although this framework exists, the ecological effects of the transfer of organic matter on aquatic microbial processes under high atmospheric CO_2 concentrations have yet to be fully investigated.

In freshwater lakes, up to 60% of the microbial carbon demand can be supported by inputs of organic carbon from the surrounding catchment (Kritzberg et al. 2004; Cole et al. 2006). Therefore, any change in the supply of organic matter from the catchment can be expected to alter the ecological functioning of the system. Moreover, the strong links between microbial processes and dissolved organic matter (DOM) concentrations indicate that changes in microbial community composition and metabolic capacity are tightly linked to shifts in the quantity and quality of DOM (Gómez-Consarnau et al. 2012; Pommier et al. 2014; Le et al. 2016). While the influence of changing DOM quantity and quality on bacterial genetic and functional diversity is starting to be revealed, a comprehensive understanding of how aquatic microbial communities respond to the interactive and indirect effects of altered DOC bioavailability in overland flow from soils and high atmospheric CO_2 is lacking.

Chromophoric (or coloured) dissolved organic matter (CDOM) is the fraction of the bulk dissolved organic carbon (DOC) pool that absorbs light in the ultra violet and visible regions. Thus, samples with high CDOM absorption values are browner than those with low CDOM absorption values. Similar to the bulk DOC pool, CDOM can derive from both allochthonous (i.e. terrestrial sources) and autochthonous sources with different general chemical structures and optical characteristics (Vodacek et al. 1997; Rochelle-Newall and Fisher 2002b; Steinberg et al. 2004). Changes in the optical characteristics of CDOM (or FDOM, the fluorescent fraction of the bulk organic matter pool) can be considered to indicate changes within the CDOM pool (Yamashita et al. 2011; Ishii and Boyer 2012). CDOM, by extension, can represent an interesting proxy for measuring changes in the bulk DOM pool.

The amount of CDOM in a sample has generally been measured as the absorption of a filtered sample at 355 nm (Green and Blough 1994; Del Vecchio and Blough 2004; Rochelle-Newall et al. 2004). However, other work has pointed out the value of using other indices e.g. absorption at 254 nm, spectral slopes between specific wavelengths or the ratio between spectral slopes (Helms et al. 2008; Fasching et al. 2016) each of which provides different information on the organic matter present. Spectral slopes (determined over the range 275–295 nm, 355–450 nm) provide an insight into the source and diagenetic state of CDOM while the spectral slope ratio ($Sr[S275-295 \div S355-450\ \text{nm}]$) provides an indication of molecular weight with higher numbers indicating lower molecular weights (Helms et al. 2008). The fluorescence of CDOM has also been widely used as an indicator of the presence of CDOM as it provides a rapid and sensitive measure of CDOM in aquatic samples (Hoge et al. 1993; Rochelle-Newall et al. 2014).

In both soils and aquatic systems, microbial processes play an important role in the production of CDOM (Rochelle-Newall and Fisher 2002b; Nelson et al. 2004). It can therefore be hypothesized that the changes within the system that affect microbial processes will also influence CDOM characteristics. Moreover, the presence of CDOM in an aquatic system can have important implications for food-web structure as well as on the biogeochemical cycling of elements as increasing CDOM is generally associated with lower bioavailability for microbial processes (Karlsson et al. 2009).

While the impact of CO_2 on CDOM production in a coastal system has already been investigated, at present less is known about the impact of terrestrial run-off under high atmospheric CO_2 concentrations on CDOM dynamics in lakes. This is particularly interesting as given the strong links between microbial carbon cycling and CDOM production, any shift in soil microbial processes that is also linked to a shift in carbon source, such as a change in root exudation under high CO_2 (Torbert et al. 2000; Hill et al. 2015) may well have strong implication for aquatic carbon cycling and storage.

As the direct effects of elevated atmospheric CO_2 concentrations on terrestrial and aquatic ecosystems have already been investigated (Leakey et al. 2009; Low-Décarie et al. 2014), the research presented here mainly aimed to study the indirect effects (due to changes in allochthonous inputs) of increased atmospheric CO_2 concentration on terrestrial and then aquatic ecosystems. Here, we assessed the direct, i.e. through its effect on the water CO_2 concentration, and indirect, i.e. through changes in the quantity and quality of soil leachates, effects of a higher CO_2 atmospheric concentration and the interaction of these two factors on a lake microbial community. To do this, we examined the short-term impact of leachates from soil monoliths maintained under ambient or elevated CO_2 during 8 months on a lake microbial community, itself maintained under ambient or elevated CO_2 during 72 h. The objectives of this work were to investigate the impact of elevated CO_2 on (1) the quantity and quality

of organic carbon of leachates from soils, (2) the response of a lake microbial community to an addition of that organic carbon, (3) the potential interactions between the addition of organic carbon and the direct effect of elevated CO_2.

Materials and methods

The experiment was conducted in two steps (Fig. 1). First, 12 soil monoliths were cultivated during 8 months under ambient or elevated CO_2 concentrations (6 replicates for each treatment) with regular simulated moderate rains. An important rain event was simulated at the end of the 8-month growth period. The leachate from each of the 12 soil monoliths was collected separately. The leachate can therefore be considered as providing an integrative signal of the impact of elevated CO_2 on the soil monoliths as it takes into account the complex effects of elevated CO_2 on the terrestrial ecosystems over the 8-month treatment period.

Each leachate was divided into two parts, which were added to replicated lake microbial communities, one under ambient CO_2 concentration and one under elevated CO_2 concentration, and compared, in each CO_2 condition, to lake water controls without leachate. The term leachate is used to describe the water collected at the bottom of the soil monolith to simplify the text. However, it is probable that the sample collected contains a mix of leachate (water that flows through the soil and dissolves solutes during its passage) and of run-off (which occurs when the soil infiltrability is exceeded and water pools at the soil surface (Hillel 2003)). This is due to the nature of the soil monoliths and although care was taken during preparation of the soil monoliths, the production of run-off cannot be entirely ruled out.

Soil monoliths

Twelve grassland monoliths ($50 \times 50 \times 40$ cm) were left to grow under ambient or elevated CO_2 concentrations in growth chambers in a glasshouse at the University of Paris-Sud (Orsay, France) (Fig. 1). The soil used in the experiment was collected at the CEREEP-Ecoton IDF in Saint Pierre-lès-Nemours (France) at two depths 0–20 cm (pH_{H2O} of 6.58, 0.09% N, 0.11% C) and 20–40 cm (pH_{H2O} of 7.56, 0.05% N, 0.06% C) and the two layers were reconstituted in the monoliths. The soil monoliths were planted with *Dactylis glomerata* (common name Cocksfoot, or orchard grass, very common in temperate pastures) at a density of 2000 seeds m^{-2}, and fertilized with NPK 14-7-14, Multicote 12 slow-release granules (20 g m^{-2}, equivalent to 30 kg N ha^{-1}) at the time of sowing. The soil monoliths were watered with tap water at regular intervals throughout the experiment (from 25 October 2012 to 25 June 2013). The grass was cut three times during the experiment (in February 2013, April 2013 and June 2013) to promote vegetation establishment, each time, above-ground biomass was clipped to 5 cm above the soil surface, and plants were left to re-grow. The soil monoliths were grown in twelve individual chambers under a CO_2 controlled atmosphere (Niboyet et al. 2017). Six chambers were ventilated with ambient air from outside the greenhouse (Ambient CO_2; 463 ± 2 ppm) (Fig. 1). Six other chambers (High CO_2; 702 ± 3.5 ppm) were ventilated with ambient air enriched with CO_2. The elevated atmospheric CO_2 concentration chosen in the terrestrial experiment is close to the middle of the range of the IPCC scenarios for 2100 (IPCC 2013), and comparable to the CO_2 concentration used in many other global change studies on grassland ecosystems (see discussion in Niboyet et al. 2011). CO_2 concentrations were monitored daily throughout the experiment using a portable carbon dioxide analyser (M170 Measurement Indicator, Vaisala, Helsinki, Finland). On June 25th 2013, each of the twelve monoliths was watered (+ 15 L over 36 h, corresponding to a rather heavy, but realistic rain event of 60 mm over 36 h), and the resulting leachates were collected by drainage from the bottom of the monoliths 24 h later in acid washed glass bottles for the coupled aquatic experiment. Leachates derived from soil monoliths placed under ambient CO_2 are hereafter called Leach-A leachates (A for ambient) and leachates derived from soil monoliths placed under elevated CO_2 are hereafter called "Leach-H leachates" (H for high) (Fig. 1). Sub-samples of leachate were collected for the determination of DOC and CDOM and the remaining samples were stored at 4 °C until they were used for the aquatic experiment (within 24 h).

Aquatic microcosms

In order to assess the impact of soil leachates on an aquatic microbial community, the soil leachates were incubated in lake water under ambient (381 ± 25 ppm) or elevated atmospheric CO_2 (750 ± 31 ppm) in two climatic chambers (ECOLAB) of the ECOTRON IDF (Saint Pierre-lès Nemours, France) for 72 h. This experimental design allowed crossing different factors (type of leachate $\times$ pCO_2 level; Fig. 1). A detailed technical description of the ECOLAB is available in Verdier et al. (2014). Light intensity (450 µmol m^2 s^{-1}) was supplied by warm white fluorescent lamps (JBL SOLAR TROPIC, 54W, 4000K) with a light: dark cycle of 12:12 h. Temperature (°C) and pH (total scale) were continuously measured by a probe (SPT21T pH + ATC, Consort, Turnhout, Belgium) connected to a data-logger console (D291, Consort, Turnhout, Belgium) after a two-point calibration using Metrohm buffer solutions (pH4 and pH7). Temperature was 19.83 (± 0.26) °C and 19.80 (± 0.33) °C (t-test, NS) and pH 8.33 (± 0.20) and 8.07 (± 0.22) (t-test, $p < 0.001$) in the ambient and high CO_2 climatic chambers, respectively.

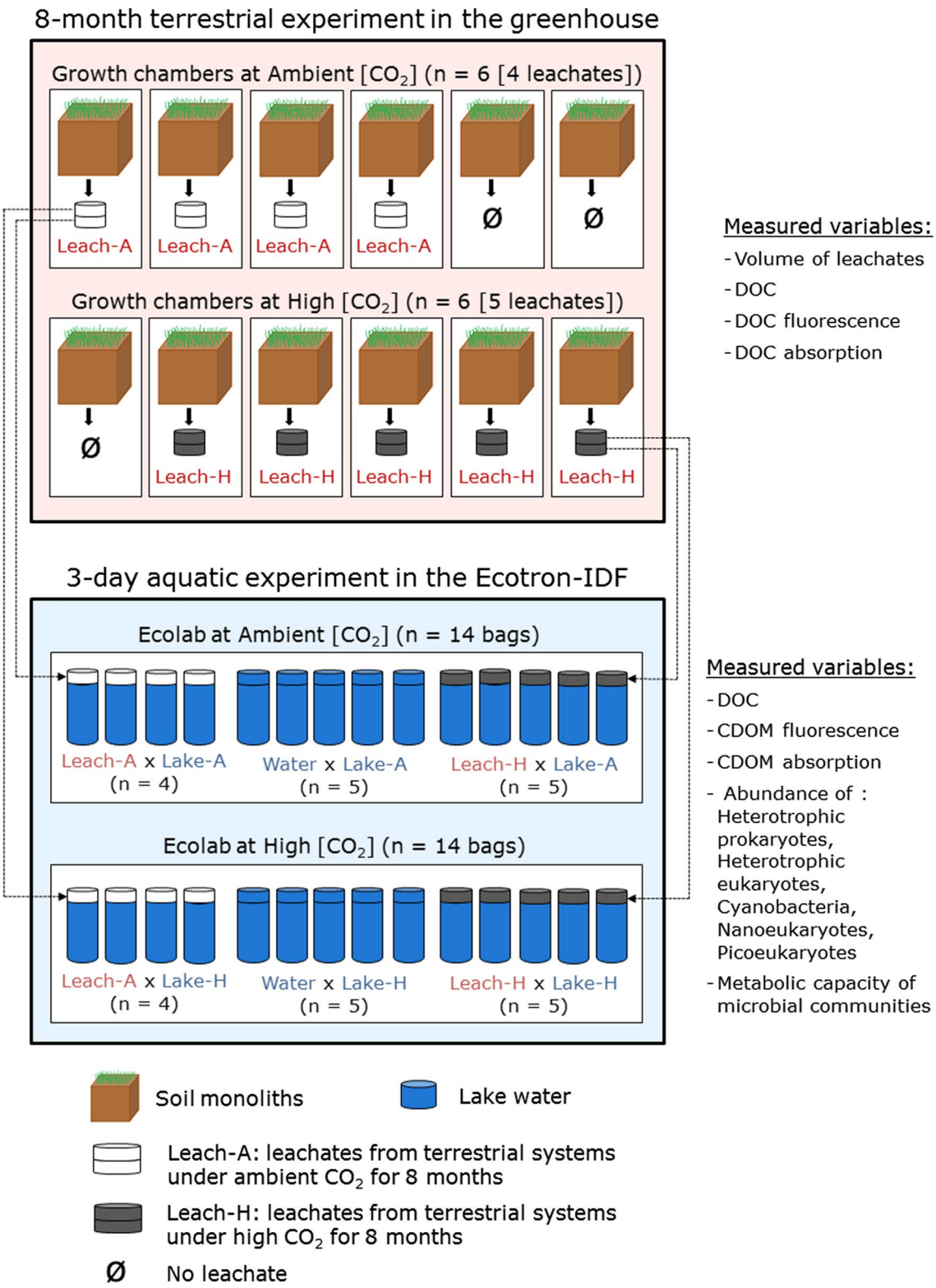

Fig. 1 Experimental setup. The coupled experimental set-up shown with both the long term (8 month) terrestrial and the short term (72 h) aquatic incubations detailed. As shown by the arrows, the leachates derived from the soil monoliths placed in each CO_2 treatment (Leach-A and Leach-H), were not pooled and each leachate was split and added to only two bags with lake water, one of which was incubated under ambient CO_2 (Lake-A) and the other under high CO_2 (Lake-H). Five lake water controls (Water×Lake A or Water×Lake B) with no leachate addition were also incubated at each CO_2 level. For clarity, arrows are presented for only one leachate collected under ambient CO_2 and for only one leachate collected under high CO_2. As also shown in the figure (Ø), for three soil monoliths, the quantities of leachate collected were insufficient to be used

For the incubations, 200 L of water were collected in the shallow Lake Créteil near Paris (48°46′N, 2°28′E) on June 27th, 2013 and immediately transported at the CEREEP ECOTRON IDF (80 km, south of Creteil) to start the experiment. In each climatic chamber 23 sterile transparent bags (5.4 L of volume, Whirlpak sample bag, model n° B01447) were half filled (2.7 L) with the water from the lake, prefiltered on a 30-µm sieve in order to remove zooplankton. Nine bags in each climatic chamber were used for the measurement of temperature and pH. Of these nine, three were covered with a black bag to block out light, however due to problems with gas exchange the data from these three bags was not used. A further three were left as whole lake water and a final three were filled with filtered lake water (0.2 µm). This last option was chosen as it provides an estimate of the impact of CO_2 changes on water with minimal biological activity. A clear effect of CO_2 treatment was observed on water pH during the whole experiment ($p < 0.0001$), confirming that the increase in atmospheric CO_2 in the high-CO_2 climatic chamber had spread to the aquatic environments (Supplementary materials, Fig.S1).

The remaining 14 bags were used to test the effects of soil leachates on the microbial community at ambient CO_2 concentrations, hereafter denoted as "Lake-A", and high CO_2 concentrations, hereafter denoted as "Lake-H" in the climatic chambers (Fig. 1). For 3 soil monoliths (two under ambient CO_2 and belonging to Leach-A treatment and one under high CO_2 and belonging to Leach-H treatment), the quantities of leachate were insufficient, thus, they were not added to lake microbial communities. This means that $n = 5$ for the control and the Leach-H treatment and $n = 4$ for the Leach-A treatment for each CO_2 level (Fig. 1). Thus, in each climatic chamber, the five bags chosen as a control (Water) did not receive soil leachate, a further four received the addition of Leach-A leachates and the last five received an addition of Leach-H leachates. The leachates for each terrestrial treatment were not pooled and each leachate was split and added to only two bags, one of which was incubated under ambient CO_2 (Lake-A) and the other under high CO_2 (Lake-H) (Fig. 1). The bags were randomly selected for each treatment to avoid systematic edge effects. The amount of leachate water was added to give an increase of 100 µM C in DOC concentration which reflects what may happen in a large lake located in a catchment of mainly agricultural and pastoral land-use based on the values of Caverly et al (2013) and Dhillon and Inmadar (2013). Caverly et al (2013), in the two-week period following the passage of a tropical storm, observed an export of 22 kg DOC ha^{-1} from a 21 ha catchment and Dhillon and Inmadar (2013) observed DOC concentrations of up to 1500 µM C in a stream during storm flow in a catchment where export was calculated to be 18 kg DOC ha^{-1}. Taking these values into account and assuming a lake of volume 300 000 m^3, located in a catchment of mainly agricultural and pastoral land-use of about 21 ha and considering an export of 18 kg C ha^{-1} exported to the lake, this would result in an increase in DOC concentration of about 100 µM C.

All of the bags were attached to a grid installed above a water bath. The vertical movement of the grid (40 cm up and down and back, every 10 min) allowed the water in the incubation bags to be gently mixed by the movement of the bags and limited the formation of biofilms on the bag walls.

Samples for microbial abundance and characterization, DOC and CDOM were taken at the beginning (t_0) and at the end (t_f) of the experiment. No intermediate time points were sampled to avoid disturbing the climatic conditions in each chamber. Metabolic capacity (Biolog Ecoplates®) of the communities present was determined at the end of the incubation.

Dissolved organic carbon (DOC) concentration, absorption and fluorescence

DOC concentration and DOC absorption and fluorescence (or chromophoric dissolved organic matter or CDOM) in both the leachates and the aquatic incubations were determined on filtered (Whatman GF/F) samples collected in precombusted (450 °C, overnight) glass tubes, or amber bottles for CDOM, sealed with a Teflon lined cap. 30 ml were collected in duplicate for DOC concentration, preserved with 36 µl 85% phosphoric acid (H_3PO_4) and stored at ambient temperature and in the dark until measurement on a Shimadzu TOC VCSH analyzer. DOC absorption (m^{-1}) and fluorescence were measured on 125 ml samples following the method detailed in Rochelle-Newall et al. (2014) within 24 h of sampling, during which time they were stored at 4 °C in the dark. Shortly before measurement samples were left to warm to room temperature, mixed and re-filtered at 0.2 µm (Sartorius Minisart NML Syringe filters). Absorption was measured using a spectrophotometer (Specord 200, Analytic Jena) from 200 to 750 nm with a 10 cm or 1 cm quartz cuvette to avoid internal quenching at high concentrations of CDOM. Data are reported as absorption at 254 or 355 nm and are expressed as m^{-1}. The spectral slopes ($S_{275–295}$, $S_{280–450}$, $S_{355–400}$) were calculated using linear regression of the log-transformed absorption spectra over the selected ranges (275–295, 280–450, 355–450 nm). The spectral ratio (Sr) was determined as the ratio between $S_{275–295}$:$S_{355–400}$. Fluorescence was estimated with a Turner Trilogy® Fluorometer using the UV module insert. This module is used for CDOM measurements (excitation = 350 nm and emission wavelengths = 410–450 nm). Values are given as Turner Fluorescence Units (TFlU).

Abundances within aquatic microbial communities

Samples were fixed with paraformaldehyde (1% final conc.) at 4 °C in the dark for 1 h and then flash frozen in liquid nitrogen before being stored at -80 °C until analysis. Prokaryote counts (heterotrophic prokaryotes and cyanobacteria) from the aquatic incubations were assessed by flow cytometry using a Becton Dickinson FACScan (BD Biosciences, Oxford, UK) instrument equipped with a 15 mW 488 nm laser. Pigmented microorganisms were identified based on the autofluorescence of the chlorophyll *a* via orange fluorescence (FL2, 585/42 bandpass filter) *vs* red fluorescence (FL3, 650 nm longpass filter). Cells containing chlorophyll *a* were distinguished using side scatter (SSC) *vs* FL3 and distinct size classes were clustered to discriminate cyanobacteria from small and large picoeukaryotes. A subsample of each sample was stained with the nucleic acid stain SYBr Green I (Marie et al. 1997) in order to visualize populations of heterotrophic organisms using SSC green fluorescence (530/30 bandpass filter). Chlorophyll *a*-containing organisms were gated off plots of SSC vs FL1, having been identified in plots of FL3 *vs* FL1. Populations were enumerated using a syringe-pump calibrated to 0.5 and 1 µm microspheres (Polysciences, Eppelheim, Germany) according to Zubkov and Burkill (2006).

Metabolic capacity of aquatic microbial communities

The catabolic capacity of the microbial communities in the incubations was determined using Biolog Ecoplate® 96-well microplates. Each well was inoculated with 150 µl of sample. The plates were incubated in the dark (at 20 °C) for 96 h. Colour development (OD at 590 nm) was measured using a Bio-Rad Laboratories, Model 680 Microplate Reader every 24 h. The data from 96 h were used to determine average colour development for each group of substrates. The relative proportion of substrate utilization for each biochemical group of substrates (e.g. amine, polymers) within each triplicate plate was then determined following the method of Salles et al. (2009). The data were presented as radial charts to help visualization of the metabolic potential of the community as in Pommier et al. (2014).

Statistical analyses

Significant differences between CO_2 treatments and the significance of the interaction between the direct and indirect effects of elevated CO_2 were determined using ANOVA and a post-hoc Tukey test was used to determine the direction of the effects. Leachates from each terrestrial monolith were split in two and added to two different aquatic microcosms, one under ambient CO_2 and one under elevated CO_2. Thus, for the results of the aquatic microcosm experiment a linear-mixed model was used to include the terrestrial monolith as a random effect. In these cases the marginal R^2 was computed instead of the standard R^2 (Nakagawa et al. 2013). When needed variables were transformed for the residuals to attain normality and homoscedasticity. All statistical tests were performed using the program R (2013). The raw data and statistical results are presented in tabular form in the text and in the Supplementary Materials.

Results

Effects of CO_2 on soil leachates

Soil monoliths incubated under high CO_2 (Leach-H) produced significantly greater (107%) leachate volumes (Table 1). DOC concentrations were significantly lower in Leach-H, but this difference disappeared when adjusted for volume. Significant differences between treatments were observed for the absorptions at 254 nm (a_{254nm}) and at 355 nm (a_{355nm}), with lower absorptions observed in Leach-H as compared to Leach-A. Similar to absorption, the fluorescence also differed significantly between treatments, with lower fluorescence observed in Leach-H as compared to Leach-A. Interestingly, and in contrast to DOC, when fluorescence, a_{254nm} or a_{355nm} were normalized

Table 1 Volumes, total carbon lost and optical parameters for the leachates

Treatment	Volume (ml)	DOC (mg l^{-1})	Tot C lost (mg)	Fluorescence (TFlU)	a_{254} (m^{-1})	a_{355} (m^{-1})	Flu* (TFlU per mgC l^{-1})	a_{254}* (m^{-1} per mgC l^{-1})	a_{355}* (m^{-1} per mgC l^{-1})
Leach-A (se)	694 (39)	36.45 (1.13)	25.25 (1.18)	29,033 (889)	168.35 (5.34)	29.27 (0.72)	796.95 (11.86)	4.62 (0.09)	0.80 (0.02)
Leach-H (se)	1436** (209)	19.47*** (1.51)	28.54 NS (5.14)	22,719** (1028)	123.22*** (6.27)	22.64*** (0.97)	1180.36*** (45.95)	6.39 NS (0.20)	1.18 NS (0.05)

Flu* is carbon normalized CDOM fluorescence, a_{254}* and a_{355}* are the carbon normalized CDOM absorption at 254 and 355 nm. Leach-A: ambient pCO_2 and Leach-H: high pCO_2 soil incubations. Statistics indicate the difference between Leach-A and Leach-H

***P < 0.001; **P < 0.01; *P < 0.05; NS: not significant

to volume, the significant effect remained, with the volume normalized values being significantly lower in Leach-H. In contrast, when fluorescence, a_{254nm} and a_{355nm} were normalized to C, no significant treatment effect was found.

Aquatic incubations

Microbial communities and metabolic capacities

The Lake Créteil water added to the aquatic microcosms was dominated by prokaryotes (Supp.mats. Table S1). After 72 h, significant direct and indirect effects on the abundances of the autotrophic and heterotrophic eukaryotes and prokaryotes were found (Fig. 2; Table 2). For example, while there was no significant direct effect of CO_2 (Lake A as compared to Lake H) on heterotrophic prokaryotes, heterotrophic eukaryotes, or the ratio of autotrophic to autotrophic + heterotrophic functional groups, there was a significant indirect effect of leachate CO_2 treatment (Leach A as compared to Leach H; Table 2).

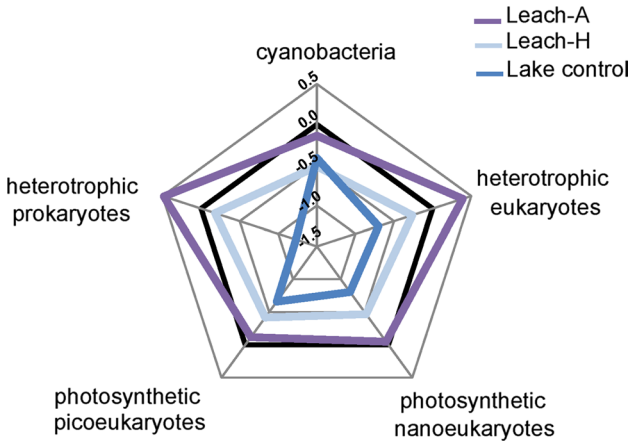

Fig. 2 Impact of CO_2 concentration and leachate additions on relative abundances of microbial community components. Lines indicate the direct effect of high CO_2 for each leachate addition and lake water controls. Values are calculated by subtracting the abundances of a specific component in the Lake-A incubations (with Leach-A, Leach-H or Water) from those in the corresponding Lake-H incubation. Thus, negative values indicate a decrease in abundance in high CO_2 relative to ambient CO_2 (Lake-H has reduced abundances relative to Lake-A) and positive values indicate an increase in abundance (Lake-H has increased abundances relative to Lake-A). The statistical test results are provided in Table 2

The direct and indirect effect of increased CO_2 concentration on the metabolic capacity of the lake communities is shown in Fig. 3. Relative to the Water/Lake-A incubation, the Water/Lake-H incubation resulted in a significant decrease of the metabolic capacity of communities for the amine and carboxylic acids (Fig. 3, Water polygon). The utilization of the other substrate groups did not change significantly. Moreover, the Leach-H/Lake-H community had a significantly lower metabolic capacity than the Leach-H/Lake-A community (Leach-H polygon), particularly for phenolic acids. In contrast, the metabolic capacity of the Leach-A/Lake-H community was significantly higher for all substrates relative to that of Leach-A/Lake-A (Fig. 3, Leach-A polygon), particularly for amines and phenolic and carboxylic acids.

DOC and CDOM

DOC concentration increased significantly over the 72 h incubation (Fig. 4a), particularly for the Water controls. Fluorescence increased significantly (Fig. 4b) less for the Lake-H than Lake-A incubations (Table 3), indicating a negative direct effect of higher CO_2 on CDOM production. On the other hand, the indirect effect of CO_2 (leachate CO_2 history) on fluorescence was not significant. There was, however, a consistent significant positive direct effect of higher CO_2 on changes in both a_{254nm} and a_{355nm} (Fig. 4c,

Table 2 Direct (CO_2 concentration in the climatic chamber, "CO_2") and indirect (legacy of the CO_2 effect in leachates, "Leachate") CO_2 effects on lake microbial community abundances

	Cyano	Pico	Nano	Het prok	Het Euk	Auto /(auto + hetero)
Leachate	NS	NS	**	*	NS	*
CO_2	**	**	**	NS	NS	NS
Direction of effect	Lake-A > Lake-H	Lake-A > Lake-H	Lake-A > Lake-H Water > Leach-H	Water > Leach-A	–	Water < Leach-A
Marginal R^2	0.30	0.35	0.45	0.33	0.21	0.28

The interaction between the leachate (indirect effect) and CO_2 treatment (direct effect) has been removed from the model because it was never significant. The linear model included a random effect corresponding to the terrestrial monolith. The direction of significant effects was determined using post-hoc Tukey tests. When necessary, i.e. for Pico, Nano, Het Euk and Auto/(Auto + Hetero), variables have been log transformed for the residuals to attain normality and homoscedasticity. Cyano: Cyanobacteria; Pico: picoplankton; Nano: nanoplankton; Het Prok: Heterotrophic prokaryotes; Het Euk: Heterotrophic eukaryotes; Auto /(Auto + Hetero) : Autotrophic abundance/(Autotrophic + Heterotrophic abundance)

***P < 0.001; **P < 0.01; *P < 0.05; NS: not significant

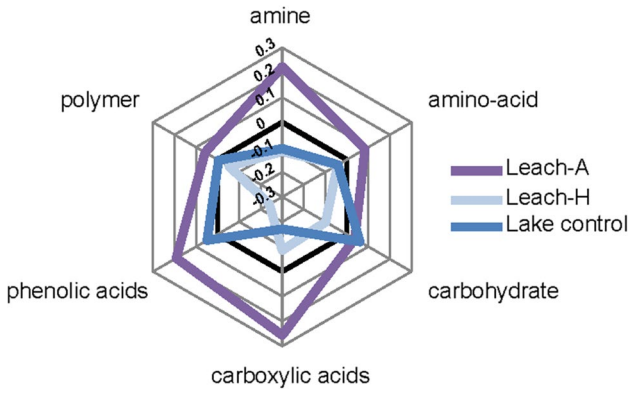

Fig. 3 Impact of CO_2 and leachate additions on a) the metabolic potential (as measured by Biolog EcoPlates®) of the incubated lake water communities. Lines indicate the direct effect of high CO_2 for each leachate addition and lake water controls. The position on each of the six axes indicates the mean potential degradation rate of that group of substrates after a 96-h incubation. Values are calculated by subtracting the mean potential degradation rate in the Lake-A incubations (with Leach-A, Leach-H or Water) from those in the corresponding Lake-H incubation. Thus, negative values indicate a decrease in mean degradation rate in high CO_2 relative to ambient CO_2 (Lake-H has reduced degradation rates for that group of substrates relative to Lake-A) and positive values indicate an increase in mean degradation rate (Lake-H has increased degradation rates for that group of substrates relative to Lake-A)

d; Table 3) indicating the CDOM absorption increased under higher CO_2. Interestingly, while we found a significant indirect effect of leachate addition on a_{355nm}, with higher absorption in the Leach-H addition, this was not the case for a_{254nm}. There was also a significant interaction effect of CO_2 and leachate history at both of these wavelengths (Table 3).

Comparison of the spectral slopes also showed some differences between treatments (Supp. mats. Fig. S2, Table 3). A significant direct effect of CO_2 was found for all the spectral slopes ($S_{275–295}$ nm, $S_{280–450}$ nm and $S_{355–400}$ nm) with the decrease in the spectral slopes being higher in the Lake-H incubations. However, a significant indirect effect was only observed for $S_{280–450}$ nm and $S_{355–400}$ nm, with Leach-A and Water being significantly lower than the Leach-H addition. We also observed a significant interaction effect for $S_{355–400}$ nm. When the spectral slope ratio, Sr, a proxy for apparent molecular weight, was tested both indirect and direct effects were significant, as was the interaction effect (Table 3).

Discussion

Impacts of elevated CO_2 on leachate production

Here we present the results from an experiment that aimed at determining the short-term impact of leachate from planted soil monoliths that were grown under ambient or high CO_2 concentration during 8 months (indirect effect) on a natural microbial lake water community that was itself incubated under ambient or high CO_2 (direct effect). In this two-phase experiment that couples a long term, integrative, terrestrial incubation with a short time aquatic incubation, we show that there were both significant direct and indirect effects of elevated CO_2, and interactions between the two, on the optical properties of the organic matter and, presumably as a consequence, on both the abundance and metabolic capacities of the microbial communities.

Leachates can be considered as providing an integrated signal of the impact of elevated CO_2 on the terrestrial monoliths as they take into account the complex effects of elevated CO_2 on the soil/plant system and this over the 8-month period of the experiment. Thus, any addition of leachate to an aquatic ecosystem takes into account not only the immediate impact of addition of organic matter but also takes into account the legacy of the terrestrial ecosystem from which that organic matter originates, in this case, its exposition to ambient or elevated CO_2.

We observed an increase in the volume of leachate released from the soil monoliths as well as an alteration of the quality of the leachate under elevated CO_2. It has already been shown that elevated CO_2 may decrease plant stomatal conductance, which often results in decreased plant transpiration when not offset by increases in leaf area, and in decreased plant water loss through increased water use efficiency (Hungate 1999; Adair et al. 2011). Declines in plant transpiration and in plant water use under elevated CO_2 might thus result in increased soil moisture leading to more rapid soil saturation during precipitation events and higher runoff under high CO_2 (Field et al. 1995). This mechanism likely explains the higher leachate volumes observed in our study under elevated CO_2. Indeed, elevated CO_2 significantly increased soil moisture in our terrestrial experiment, and the volume of leachate released from the soil monoliths was positively correlated to soil moisture (Supp. mats. Fig. S3). Increased leachate volumes should also lead, everything else being equal, to a dilution of the dissolved solutes in the soil and hence the concentration of carbon and nutrients in leachate. However, our results show that elevated CO_2 resulted in a change not only in the quantity of carbon in the leachate due to dilution but also in a change in the optical quality of the organic carbon present. We also observed an increase in the carbon normalized fluorescence (TFlU*) under high CO_2 (Table 1). These changes in the carbon normalized optical quality suggest that the chemical composition of the dissolved organic matter was different between the two CO_2 levels (Rochelle-Newall et al. 2014). In other words, this suggests that the legacy of elevated CO_2 on

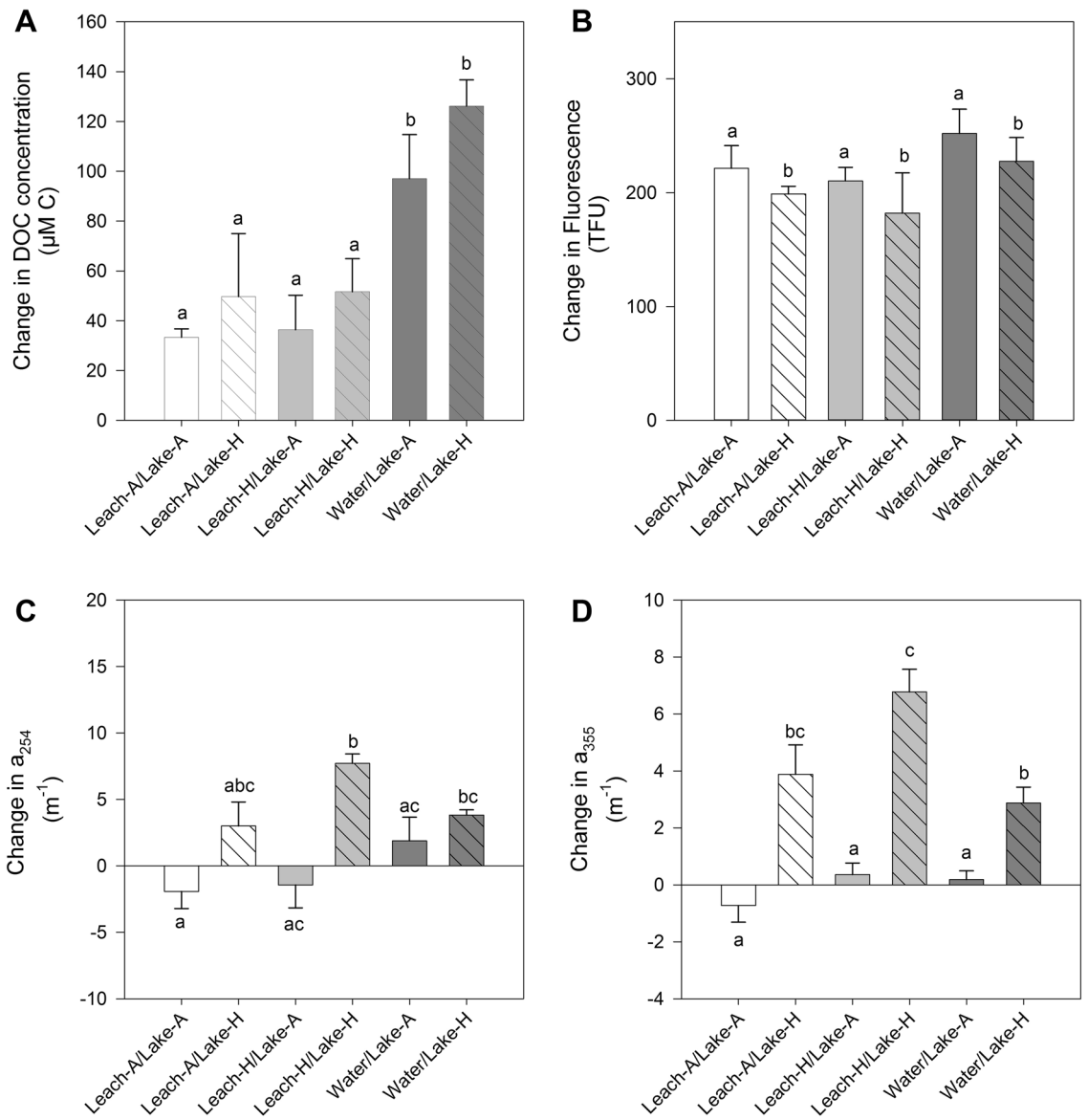

Fig. 4 Changes (T_f – T_0) in DOC (µM C), CDOM fluorescence (TFlU) and absorption (a_{254} nm and a_{355} nm, m^{-1}) in the lake water experiment. The prefix Leach-A corresponds to the incubations with leachates from the soil monoliths incubated under ambient CO_2 concentration (pCO_2 ~ 460 ppm), the prefix Leach-H corresponds to the leachates from soil monoliths incubated under high CO_2 concentration (pCO_2 ~ 700 ppm) and Water corresponds to the lake water controls to which no leachate was added. Lake-A (bars without hatching) correspond to the lake water incubations (with and without leachates) incubated under ambient CO_2 and Lake-H (with hatching) to those incubated under high CO_2 concentrations. Mean and SE (n = 4 or 5, see Fig. 1) are presented. Different letters indicate a statistically significant difference; the full statistical results are presented in Table 3

the terrestrial ecosystem can also be observed in the leachate and not just in the plant biomass as shown previously (Niboyet et al. 2017). It is not clear which changes led to the increase in fluorescence in the dissolved carbon pool. However, given the importance of microbial processes in the production of CDOM (Rochelle-Newall and Fisher 2002b; Nelson et al. 2004), it is probably related to a shift in soil microbial activities or to the composition of microbial communities that is also linked to a shift in carbon source, itself due to changes in root exudation under high CO_2 (Torbert et al. 2000; Hill et al. 2015). Nevertheless, this shift towards higher fluorescence per mg l^{-1} DOC suggests that the biodegradability of the Leach-H leachate may be lower than that of the Leach-A leachate, as increasing CDOM is generally associated with lower bioavailability for microbial processes (Blough and Del Vecchio 2002; Rochelle-Newall and Fisher 2002b) (but see (Romera-Castillo et al. 2011)).

Table 3 Results of the ANOVA test on the changes (Tf-T0) in optical characteristics of the aquatic incubations in Fluorescence, in Absorption at 254 nm or 355 nm (a_{254} and a_{355}), in spectral slope (S) between 275 and 290 nm ($S_{275-295}$), 280–450 nm ($S_{280-450}$) and 355–400 nm ($S_{355-400}$) and the change in the spectral ratio (Sr)

	Change in DOC	Change in fluorescence	Change in a_{355}	Change in a_{254}	Change in $S_{275-295}$	Change in $S_{280-450}$	Change in $S_{355-400}$	Change in Sr
Leachate	***	NS	**	NS	NS	**	***	***
CO_2	NS	*	***	***	*	***	***	***
Leachate x CO_2	–	–	*	*	–	–	**	***
Direction of effect	Water > Leach-H, Leach-A	Lake-H < Lake-A	Lake-H > Lake-A Leach-H > Water, Leach-A	Lake-H > Lake-A	Lake-A > Lake-H	Lake-A > Lake-H Water, Leach-A > Leach-H	Lake-A > Lake-H Water, Leach-A > Leach-H	Lake-H > Lake-A Leach-H, Leach-A > Water
R^2	0.51	0.32	0.81	0.59	0.22	0.87	0.91	0.86

The direction of significant simple effects was determined using post-hoc Tukey tests. The interaction was removed from the model when non-significant (-).When needed the variable was transformed for the residuals to attain normality and homoscedasticity (Sqrt(X + 2) transformation for change in a_{355}, and log(X + 1) transformation for change in Sr)

***P < 0.001; **P < 0.01; *P < 0.05; NS: not significant

Impacts of elevated CO_2 on aquatic functioning

During the short-term aquatic incubations, we observed a significant direct effect of increased CO_2 on absorption at 254 and 355 nm (Fig. 3) and increased Sr under higher CO_2. In other words, under high CO_2, the water was 'browner' and there was a significant decrease in the apparent molecular weight. Why these changes occurred under higher CO_2 concentration merits some reflection. It has been previously shown that while phytoplankton are probably not a direct source of aquatic CDOM, at least at the wavelengths examined, they do provide the precursor material for heterotrophic bacterial processes (Nelson et al. 1998; Rochelle-Newall and Fisher 2002a, b). We observed significantly fewer photosynthetic organisms in the Lake-H incubations than in the Lake-A incubations along with higher heterotrophic organism abundances, consistent with a recent mesocosm study of marine prokaryotes (Endres et al. 2014). This significant, negative direct effect of CO_2 on autotroph abundance is in contrast to what has previously been reported by Jansson et al. (2012) for boreal and sub-arctic lakes. Interestingly, Jansson et al (2012) also reported a strong relationship between CO_2 concentrations and DOC concentrations as found here.

It is difficult to determine whether the significant direct effect of CO_2 is due to an acidification effect in the Lake-H incubations or to a fertilization effect in the Lake-A incubations as we do not have measurements of primary production or of respiration. The difference in pH between Lake-A and Lake-H (0.26 ± 0.062 in the filtered Lake-A and filtered Lake-H controls, Supp. Mats. Fig. S1) is small when compared to the large range of pH that can be observed in a natural lake system on diel and seasonal scales (Maberly 1996) though in our experiment this difference in pH was continuous for several days and not variable over time as in a lake. Moreover, we cannot exclude competitive interactions for limiting nutrients between heterotrophic prokaryotes and primary producers as demonstrated in previous laboratory experiments (Danger et al. 2007a, b) and suggested in theoretical models (Zou et al. 2016).

In a natural system and over a longer timescale, the shift towards increased CDOM absorption (or 'brownification') could create a feedback whereby increased CDOM would decrease light penetration, thereby decreasing primary production further (Karlsson et al. 2009). This should lead to a higher relative importance of heterotrophic processes, which would in turn lead to higher CDOM production, and perhaps nutrient limitation of eukaryotes due to higher competitive ability of prokaryotes (Danger et al. 2007b). Moreover, if we take into account that the majority of the phytoplankton are mixotrophs (Jones 2000), in situations of high nutrient competition and high prokaryote abundances, phytoplankton may switch from auto to heterotrophy by ingesting bacteria (Unrein et al. 2007; Hartmann et al. 2012). This would provide nutrients to the phytoplankton and reduce prokaryote densities and this effect could be even stronger as temperatures also rise (IPCC 2013). All of which would, in turn, lead to stronger and longer stratification of the water column and to the oligotrophisation of surface waters, further enhancing the competition between phytoplankton and prokaryotes.

The positive direct effect of higher CO_2 concentration on CDOM production is in contrast to a previous 20-day aquatic mesocosm experiment (Rochelle-Newall et al. 2004) where no effect was observed. However, in the present study,

soil leachate was added to lake water, likely providing more complex precursors for CDOM production. In contrast, Rochelle-Newall et al (2004) examined CDOM production in coastal water during a bloom of the coccolithophorid *Emiliania huxleyi*. This difference in carbon source (allochthonous *vs* autochthonous) likely resulted in a difference in chemical structure, as terrestrial organic matter is different from that of phytoplankton derived organic matter (Malcolm 1990; Hedges 1992; Yamashita et al. 2011; Rochelle-Newall et al. 2014).

Along with changes in the optical quality of the DOM, we also observed changes in the potential metabolic capacities of the microbial communities between treatments (Fig. 3). For example, potential metabolic capacity increased for communities incubated under high CO_2 (Lake-H) with ambient leachate. In other words, the size of the nutritional niche of this community increased, potentially indicating a reduction in the degree of specialization in carbon source utilization (Pommier et al. 2014). In contrast, little change or even a slight decrease in potential metabolic capacity was observed for Lake-H communities with Leach-H leachate or Lake water. This was particularly evident for the phenolic acid groups in the Leach-A/Lake-H incubation. Phenolic acids originate from terrestrial plant material and are therefore an indicator of an allochthonous organic carbon source (Malcolm 1990; Hedges 1992). The reduction in potential metabolic capacity may indicate a reduction in the size of the nutritional niche and therefore increased specialization of carbon utilization, potentially towards phenolic (e.g. terrestrial) carbon sources. This reduction in community metabolic capacity under high CO_2 may also have resulted from the decrease in photosynthetic microorganisms, especially cyanobacteria and nanophytoplankton. At present, it is difficult to determine conclusively the mechanisms behind this reduction in metabolic capacity in the Leach-A/Lake-A incubations; nevertheless, it does provide some interesting avenues for further investigation.

In summary, the direct effect of elevated CO_2 resulted in a significant reduction in photosynthetic organisms, an increase in CDOM absorption, a decrease in apparent molecular weight (Sr) and an increase in metabolic capacity. Indirect effects of increased CO_2 (leachate legacy) resulted in a significant increase in heterotrophic organism abundance and an increase in community metabolic activity. The ultimate result of this shift towards heterotrophic processes will be that future aquatic systems will rely more heavily on terrestrial carbon inputs, which may influence the capacity of these systems to act as a carbon sink (Maberly et al. 2013; Faithfull et al. 2015). If primary production is reduced due to light limitation caused by high CDOM concentrations (Karlsson et al. 2009), systems may become a source of CO_2 to the atmosphere, which could ultimately feedback on climatic change. While the influence of trans-biome fluxes of matter has already been emphasized (Loreau et al. 2003; Gravel et al. 2010; Harvey et al. 2017) and tested experimentally in a general context (Nakano and Murakami 2001; Harvey et al. 2016) and—less frequently—in a context of global warming (Fey et al. 2015), this is one of the first times that the influence of such fluxes is suggested when investigating the impact of elevated CO_2 concentration on aquatic ecosystems.

This was an exploratory study with a short timescale (72 h) and relatively small (2.5 l) aquatic microcosms. Our terrestrial and aquatic systems were also de-coupled as leachate was added once instead of via several simulated precipitation episodes. Nevertheless, by using a novel and original, albeit largely exploratory, experimental set-up that integrated and separated, through the use of a factorial set-up, the direct and indirect (or legacy) effects of increased CO_2 increases on a freshwater aquatic system we were able to show significant effects of both the terrestrial legacy of elevated CO_2 and the direct impacts of elevated CO_2 on a freshwater ecosystem. Although we do not provide definitive answers as to the impacts of elevated CO_2 on coupled terrestrial and aquatic ecosystems, the results help to fill a knowledge gap and in doing so open up some new potential directions for research. Many authors have highlighted the need to take into account both direct, i.e. changes in environmental conditions, and indirect, i.e. changes in cascading between organisms within food-webs, effects of anthropogenic impacts on ecosystems and even on meta-ecosystems and communities (Tylianakis et al. 2008; Hoekman 2010; O'Connor et al. 2013). It is clear that any future experiment will need to take into account the complexity of direct and indirect effects that are only hinted at in the results of this work. This will undoubtedly require the use of a novel combination of experimental and theoretical approaches in order to obtain more definitive answers to the question of how rising CO_2 concentrations are impacting the biosphere now and what will be that impact in the future.

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Author contributions GL, AN, ERN, LJ designed the experiments; GL, AN, LJ, ERN, SF, SC, ML, EDS, SB carried out the work; ERN, LJ, GL, SB, SF, AN interpreted the results and ERN, LJ, GL prepared the figures and wrote the manuscript that was revised and improved by all the coauthors.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

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