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PRIMARY RESEARCH PAPER

Microbial carbon production and transfer across trophic levels is affected by solar UVA and phosphorus

Manuel Villar-Argaiz · Juan M. Medina-Sánchez · Presentación Carrillo

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Abstract Processes and environmental factors that alter carbon fixation and fluxes are key to understanding ecosystem function and impacts of global change stressors. We tested the effects of ultraviolet A radiation (UVA) and nutrients on the ^{14}C production of particulate (bacteria, algae and zooplankton size fractions) and dissolved organic carbon. Experiments were carried out in situ on two occasions during the ice-free period of a high mountain lake (Sierra Nevada, Spain). The production of organic carbon was strongly modulated by nutrients and moderately by UVA. While UVA generally reduced primary production, this effect was alleviated by nutrient enrichment. Nutrients had opposite effects to UVA on basal trophic levels by inhibiting primary production and stimulating the bacterial incorporation of algal carbon exudates in the midsummer experiment, and vice versa in the early fall experiment. Also, nutrients had a pronounced effect stimulating particulate carbon in zooplankton only in midsummer when organisms were actively growing. These results suggest that, while UVA effects are restricted to the basal trophic levels of primary producers and bacteria, effects of nutrients transfer up the food web. However, higher carbon accrual in zooplankton during midsummer may not result into higher growth due to inefficient carbon absorption.

Keywords UVA radiation · Nutrients · Zooplankton · Algae · Bacteria · Carbon

Introduction

Ecosystem function includes the processes that facilitate energy transfer along food webs, and is indelibly associated with the processes that allow and regulate carbon fixation and cycling. These are in turn influenced by the numerous interactions that occur among species (Fischer et al., 2006), as well as between species and environmental factors (Traill et al., 2010). Among these factors, the relevance of solar radiation and nutrients in sustaining ecosystems was recognized in one of the earliest papers in trophic ecology (Lindeman, 1942). Because solar radiation and nutrients are required resources for plant photosynthesis, their impact extends into food webs due to nutritional constraints at the herbivore-plant interphase (Sterner & Elser, 2002).

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M. Villar-Argaiz (✉) · J. M. Medina-Sánchez · P. Carrillo
Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain
e-mail: mvillar@ugr.es

P. Carrillo
Instituto del Agua, Universidad de Granada, 18071 Granada, Spain

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Ultraviolet radiation (UVR) has also been identified as a factor that plays an important role in aquatic biota and ecosystems (Helbling & Zagarese, 2003). However, while a great deal of work has examined the effects of the most energetic wavelengths of UVB (280–320 nm), there is comparatively less information on the effects of UVA (320–400 nm) (Rautio & Tartarotti, 2010). The specific reasons why attention should also centre on the impact of UVA include (1) the greater irradiance of UVA versus UVB reaching the Earth's surface (Rautio & Tartarotti, 2010); (2) the low coefficient of extinction of UVA compared to UVB causing high irradiance penetration into the water column (Williamson et al., 1996) and, therefore, major biological damage despite the lower energy per photon compared to shorter UV wavelengths (Bothwell et al., 1995) and (3) the profuse evidence that UVA influences a wide variety of ecosystem functions, including bacteria growth (Sieracki & Sieburth, 1986; Ruiz-González et al., 2013), phytoplankton production (Carrillo et al., 2002) and zooplankton survival and reproduction (Zellmer, 1998).

The sparse information on the effects of UVA on ecosystem structure and function is still controversial as it can affect microbes, plants and animals in multiple ways (Paul & Gwynn-Jones, 2003). Thus, several studies have shown great variability in UVA tolerance among phytoplankton species (Sommaruga, 2001; Helbling & Zagarese, 2003). Such differences have been associated with the capacity to migrate downward in the water column, but also with the biochemical defences and the bioaccumulation of photoprotective compounds (Sommaruga et al., 2005), or the photorepair mechanisms through photolyases (Kim & Sancar, 1993). In addition, numerous investigations have reported the divergent role of UVA on heterotrophic bacteria. In their recent review, Ruiz-González et al. (2013) showed a great variability in the response of bacteria to UVA, from total inhibition to strong stimulation. Likewise, numerous laboratory and field studies have shown that zooplankton are negatively affected by UVB (Helbling & Zagarese, 2003), although UVA also induces mortality in cladocerans (Zellmer, 1998) and copepods (Tartarotti et al., 2000). While a disproportionate amount of research has focussed on the direct effects of UVR on zooplankton, more work needs to be done to assess the effects that UVA exerts on the basal trophic levels of phytoplankton and bacteria by altering the nutritional behaviour of zooplankton. For example, one would expect that UVA could affect zooplankton grazing and recycling rates, and in turn the production and relationship between primary producers and heterotrophic microbes (Reche et al., 1997) and, thereby, influence the efficiency at which energy is transferred in microbial and grazing food chains. More work is therefore needed to determine how UVA regulates food web attributes such as carbon production and transfer when the full food web complexity is taken into account.

Because ozone does not shield ecosystems from the long-wave UVA radiation, one might think that ambient solar UVA should be unaffected by regional or global change. However, many multiple anthropogenic stressors (Christensen et al., 2006) may influence plankton exposure and sensitivity to UVA. For example, it has been shown that acid precipitation and temperature increase can reduce the amount of chromophoric dissolved organic matter (Schindler et al., 1996), although this effect could be counterbalanced by higher DOC release from soil at northern latitudes (Pumpanen et al., 2014) where higher autumn and winter precipitations are expected to increase (Intergovernmental Panel on Climate Change, 2013). Also, deposition of atmospheric pollutants or mineral dust transported by aeolian processes may affect the penetration of natural radiation in the water column (Mladenov et al., 2011). While allocthonous aerosols may well influence the optical properties of the water column, they also contribute to total nutrient budget of aquatic and terrestrial ecosystems (Mahowald, 2011). It has been shown that the amount of allocthonous nutrients into ecosystems is associated with changes in land use and regional aerosol transport and deposition patterns (Intergovernmental Panel on Climate Change, 2013), and their contribution becomes increasingly important in sites where nutrients are scarce, such as many oligotrophic remote lakes (Psenner, 1999; Camarero & Catalan, 2012). One crucial question is how nutrients representing an appropriate simulation of natural aerosol intrusions alter the effects of UVA on ecosystems. Given the many-fold importance of carbon production at basal trophic levels and the efficiency at which this is transferred along the food web (Dickman et al., 2008), it is imperative to elucidate the role of UVA and nutrients on these processes.

In the present study, we have explored the general hypothesis that production of dissolved and particulate

organic carbon at the basal trophic levels of algae and bacteria and the efficiency at which carbon is conveyed to zooplankton consumers is altered by UVA, nutrients and their interaction. On the basis of previous studies and data from the same study system, we used controlled enclosure experiments conducted in the field to test the following specific hypotheses: (1) UVA decreases primary production and stimulates bacterial production, resulting in lower herbivore incorporation of particulate production; (2) nutrient enrichment attenuates the negative effects of UVA on primary production, enhancing zooplankton incorporation and assimilation of particulate organic production and (3) production of all trophic levels is stimulated after enrichment with nutrients.

Methods

Study site

Experiments were conducted in lake La Caldera, located at 3050 m.a.s.l. in the Mediterranean mountains of Sierra Nevada, Spain (36°55′–37°15′N, 2°31′–3°40′W). The lake water is highly transparent (>10% photosynthetic active radiation [PAR, 400–700 nm] penetrates to the maximum depth of 14 m), rarely stratifies, and the ice-free season normally extends from late June to late October. Measurements of UVR and PAR in 2003 revealed greater incidence of daily doses in midsummer compared to early fall due to the higher solar angle, although penetration into the water column increased in early fall due to higher lake transparency (Carrillo et al., 2002). Thus, the lake surface received 2.1 and 1.8 W m^{-2} of UVB in summer relative to early fall, out of which 14% reached 5 m in summer but 25% in early fall. Similarly, while UVA irradiance at lake surface slightly decreased from 68 in summer to 59 W m^{-2} in early fall, the percentage of surface irradiance that reached 5 m increased from 19% in summer to 28% (original data in Carrillo et al., 2008a). Fish are absent and the pelagic community is relatively simple. *Daphnia* are scarce and the calanoid copepod, *Mixodiaptomus laciniatus* (Lilljeborg), is the dominant crustacean zooplankton in the lake comprising ~90% in abundance. Phytoplankton is the principal carbon food compartment for zooplankton, as bacteria do not contribute more than 5% to the total particulate carbon (Villar-Argaiz et al., 2002a). Additional characteristics of this site are described elsewhere (Villar-Argaiz et al., 2002a).

Field sampling

Water samples for bacteria, phytoplankton and zooplankton were collected weekly during the ice-free period of the lake at four depths (0.5, 5, 8 and 10.5 m) at a maximum depth station (Z_{max} = 14 m). Detailed collection protocols and original data are published elsewhere (Villar-Argaiz et al., 2002a).

Experimental procedures

The experiments were conducted in cloudless days at two different occasions (August 27 and October 10 1997) representing high and low PAR and UVA conditions in the midsummer and in the early fall experiments, respectively. Figure 1 depicts the general experimental design employed for each of the experiments. To test the effects of UVA on food web response variables (see below), we manipulated light (UVA+PAR vs. PAR treatments). To test the effects of nutrients and whether the effect of UVA varied with nutrient conditions, we manipulated phosphorus (nutrients added to give a final N:P molar ratios of 16 and 5; hereafter $N:P_{16}$ and $N:P_5$ treatments, respectively) under each of the light treatments described above (Fig. 1). For each light treatment, an additional treatment received no nutrients and served as a control (hereafter $N:P_c$) to give a 2 (light) × 3 (nutrient) factorial design with three replicates (Fig. 1).

For the experiments, a composite lake water sample constructed by mixing equal volumes of lake water was collected with a 6-l horizontal Van Dorn sampler from four depths (0.5, 5, 8 and 12 m). Water was prescreened through 40-μm mesh to remove zooplankton, mixed in a 50 l dark-covered barrel, and transferred to three smaller barrels. Two of these barrels received phosphate to give final N:P molar ratios of 16 and 5 according to the concentrations of inorganic nutrients (NO_2^-, NO_3^-, NH_4^+ and SRP) in the lake water one day before the experiments. Specifically the barrels were spiked with 19.5 and 63.1 $\mu g l^{-1}$ of $P\text{-}PO_4^{3-}$ to a final ratio of 16 and 5, respectively, in August and with 12.7 and 40.9 $\mu g l^{-1}$ of $P\text{-}PO_4^{3-}$ to a final ratio of 16 and 5, respectively, in October. A third barrel with no nutrients added served as a control. The initial molar

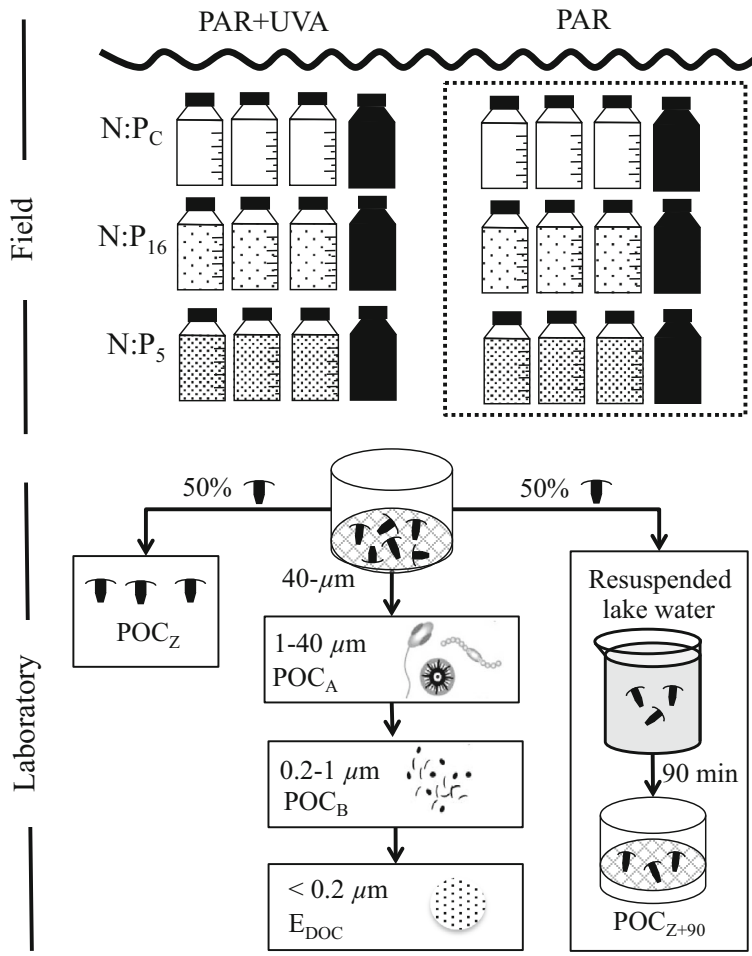

Fig. 1 Scheme illustrating the experimental field and laboratory experiments. Field enclosures are indicated by the experimental flasks and the density of dots define the enrichment with nutrients from no dots (no nutrients added, N:P_c), to intermediate (nutrients added at a N:P of 16, N:P_{16}) and high dot density (nutrients added at a N:P of 5, N:P_5). Incubations at 5 m depth for the three nutrient treatments were performed under two light regimes: UVA+PAR using Pyrex flasks and PAR using Pyrex flasks covered by Plexiglas filters (shown as *dotted lines*). For the laboratory procedures, water from each experimental flask was 40 µm filtered and zooplankton suspended in a petri dish. Half of the individuals were used to estimate zooplankton incorporation of particulate organic carbon (POC_Z, *left arrow*), and the other half was used to estimate particulate carbon absorbed in zooplankton after 90 min (POC_{Z+90}, *right arrow*). The serial filtration of the filtrate allowed to estimate the production of particulate organic carbon in the algal (POC_A), bacterial (POC_B) or dissolved (E_{DOC}) size fractions (*middle arrow*)

N:P ratio for the control was 960 in the midsummer experiment and 1579 in the early fall experiment. Water from each barrel was immediately used to fill two sets of four 300-ml Pyrex flasks (three clear and one dark) assigned to each of the light treatments (UVA+PAR and PAR). Each experiment yielded 12 experimental flasks for each light treatment and a total of 24 (Fig. 1).

Each flask received between 180 and 200 live individuals of the copepod *M. laciniatus* that were gently collected by vertically integrated tows (80-µm mesh size) at lake maximum depth and individually pipetted into 0.2-µm filtered lake water on the same day of the experiment. The stocking density of zooplankters was calculated in order to avoid food depletion based on the abundance of phytoplankton cells the week before experiments and the highest feeding rate of 500 cells copepod^{-1} h^{-1} reported for *M. laciniatus* copepodites in the studied lake (Carrillo et al., 1995). Thus, 200 copepodites feeding at this rate

may have filtered a maximum of 8 and 9% of the cells in the incubation flasks during the midsummer and early fall experiments, respectively. An estimation of zooplankton filtration rates using Peters & Downing's equation (Peters & Downing, 1984) relating filtering rate to body dry weight in calanoid copepods indicated that copepods would have filtered less than 10% of the total cells in midsummer and early fall experiments, providing support for estimations using Carrillo et al. (1995) ingestion rate of 500 cells copepod^{-1} h^{-1}. Copepods added to the flasks belonged to similar stages (copepodite stages CII on August 27 and CIII-CIV on October 10) because the life cycle of this calanoid includes a mass hatching of eggs as thaw approaches followed by the development of a single cohort during the ice-free season (Villar-Argaiz et al., 2002b).

Immediately before incubations, each flask was added with 1 MBq of $NaH^{14}CO_3$ (specific activity: 310.8 MBq mmol^{-1}, NEN Dupont). All flask sets were horizontally held during in situ incubations at 5 m under surface (UVA+PAR samples) or at 5 m under Plexiglas UF3-sheets (PAR samples) for 4 h symmetrically distributed around noon. The incubation at 5 m resembled the mean lake depth during a wet hydrological year (Carrillo et al., 2002), and therefore reproduced mean ambient UV radiation conditions for the lake. According to the latter study, the inhibitory effect of UVR on primary production is particularly important at the intermediate depth of 5 m, and is mainly due to UVA. The optical properties of the cut-off filters and Pyrex glass used in the light treatments were tested prior to the experiments with a double-beam spectrophotometer (Perkin-Elmer Lambda 40). Plexiglas transmitted 90% PAR and completely blocked UVA and UVB, whereas Pyrex glass transmitted ~92% PAR, between 82 and 96% UVA, and 0% in the UVB spectrum. After incubation, flasks were transported cold and dark to the laboratory within 1 h.

In the laboratory, animals were removed by filtration through 40-μm and suspended in a Petri disc with 0.2-μm filtered composite lake water following the scheme depicted in Fig. 1. Half of the animals were then retained in a 40-μm mesh, rinsed several times with deionized water and individually pipetted under a dissecting microscope into scintillation vials to estimate zooplankton net incorporation of organic carbon (POC_Z), following a procedure similar to DeMott et al. (1998). The rest of the alive animals were placed in 50-ml vials containing 40-μm filtered composite lake water to allow for gut clearance. Vials were kept in the dark on a lab bench at lake temperature (11°C on August 27 and 8°C on October 10) for 90 min, after which animals were retained in a 40-μm mesh, rinsed with deionized water and collected in scintillation vials as described above. This set of animals was used to calculate particulate organic carbon absorbed in zooplankton (POC_{Z+90}) after C-loss processes via egestion (Lampert, 1984; Thor & Wendt, 2010). The time of 90 min allowed for gut evacuation was estimated from reported faecal pellet production rates of ~1 faecal pellet ind^{-1} h^{-1} (Panfferhöfer & Knowles, 1979; Butler & Dam, 1994) and rates of ca. 1.2 faecal pellet ind^{-1} h^{-1} observed for *M. laciniatus* in La Caldera Lake (personal observation). Zooplankton absorption efficiency (AE_Z), i.e. the fraction of incorporated carbon that was taken up by the gut, was calculated as $AE_Z = POC_{Z+90}$ per individual/POC_Z per individual × 100.

Filtrates after removing zooplankton from the flasks were used to calculate organic carbon production in particulate and dissolved organic fractions below zooplankton. Laboratory procedures have been extensively described elsewhere (Carrillo et al., 2002). Briefly, organic carbon production was determined by serial filtration of an aliquot of 60 ml through 1 and 0.2-μm Nucleopore filters to segregate the particulate organic ^{14}C retained in algal (between 1 and 40 μm, hereafter POC_A), bacterial incorporation of algal carbon exudates (between 0.2 and 1.0-μm, hereafter POC_B) and dissolved (excreted organic carbon <0.2 μm, hereafter E_{DOC}) fractions. Total organic particulate carbon (TOPC) was calculated as the sum of all particulate organic fractions, i.e. $TOPC = POC_A + POC_B + POC_Z$. Percentage of total organic particulate carbon incorporated by zooplankton (%POC_Z) was calculated as the fraction of total organic particulate carbon production incorporated by zooplankton, i.e. $\%POC_Z = POC_Z/TOPC \times 100$.

All filters (POC_Z, POC_{Z+90}, POC_A and POC_B) and a 4 ml subsample of the dissolved fraction (E_{DOC}) were placed in scintillation vials, acidified with 100 μL of 1 N HCl to remove DI^{14}C and allowed to stand open in a hood for 24 h as recommended by Lignell (1992). Vials were counted with 16 ml of liquid scintillation cocktail (Beckman Ready Safe) in a scintillation counter equipped with autocalibration (Beckman LS 6000 TA). The total CO_2 in the lake water was calculated from measurements of the alkalinity and pH

(APHA, 1992). In all calculations for organic carbon production below zooplankton, dark values were subtracted from corresponding light values.

Rationality of the methodology used

The assignation of the size fractions in this study is well justified by the lack of major size overlap among trophic levels (Medina-Sánchez et al., 2004) and the inappreciable abundance of protozoa at the experimental dates in lake La Caldera. Because of the absence of autotrophic picoplankton (<2 μm) and the non-significant retention of bacteria in 1-μm filters (Medina-Sánchez et al., 2006), the organic ^{14}C retained on the 0.2-μm filters corresponded to the algal or zooplankton exudates incorporated by heterotrophic bacteria. Likewise, E_{DOC} corresponded to the total excretion of organic ^{14}C (< 0.2-μm fraction) not incorporated by heterotrophic bacteria (Medina-Sánchez et al., 2004).

We experimentally assessed zooplankton net incorporation rate as the particulate organic carbon actively incorporated by the zooplankton by means of a single ^{14}C incorporation technique. This procedure is based on the incapability of copepods to uptake dissolved ^{14}C. Consequently, ^{14}C actively incorporated by zooplankton corresponds with primary production consumption, although bacteria should not be disregarded due to their capacity to incorporate bicarbonate (Alonso-Sáez et al., 2010). Previous feeding trials indicated that reliable ^{14}C counts required at least 100 copepods, what explained the higher than ambient densities of zooplankton used in the incubation flasks. Also the short incubation time of 4 h precluded phytoplankton depletion in the flasks due to the relatively low filtration rate of copepods (see arguments above). The advantage of using this technique in contrast to others (dilution method, microspheres, labelled algae, etc.) lies in that it offers detection sensitivity (Steeman-Nielsen, 1952), causes minimal disturbance to the biotic assemblages, and measurements were carried out in as near to environmental conditions as possible. Our goal was not to measure instantaneous ingestion rates, but rather short-term estimations of zooplankton net incorporation of organic carbon. Measurements therefore provided with an evaluation of zooplankton capability to control lower trophic levels on which they rely for food. A similar method, using ^{3}H-thymidine as a radiotracer, has been successfully applied to estimate bacterivory by protist and mixotrophic flagellates (Caron, 2000; Medina-Sánchez et al., 2004). In this paper, the term absorption was used for gut uptake as defined by Thor & Wendt (2010). Briefly, absorption equals ingestion minus egestion, but does not take into account other expenses associated with transport across membranes or transformation of biomolecules (Thor & Wendt, 2010).

Statistical analysis

The effects of UVA, nutrients and their interaction on POC_A, POC_B, POC_Z, E_{DOC}, TOPC, $\%POC_Z$ and AE_Z were tested by two-way ANOVA. The effect size of UVA or nutrients for each response variable was quantified as the difference between mean values for the treatment and the control divided by mean values for the control (PAR and unenriched treatments, respectively). Differences among treatments were tested by post hoc Newman–Keuls' test. Normality (by Shapiro–Wilks' W-test), homoscedasticity (by Cochran's and Levene's tests) and correlation between means and standard deviations were checked for each data group in order to verify the assumptions required by ANOVA. The data were log transformed when these conditions were not met. The statistical analyses were performed using Statistica 7.1 for Windows software (Statsoft, 1997).

Results

Lake biological dynamics

The phytoplankton community was rather simple and was dominated by the chrysophyceae *Chromulina nevadensis* (Sánchez-Castillo) from thaw to late August when it was replaced by the chlorophyceae *Chlorella* sp. (Fig. 2A). Seasonal changes in zooplankton abundance (mean values for the water column) ranged from 6 to 34 individuals l^{-1}, and maximum density was reached towards late summer (Fig. 2B). Calanoids represented by the copepod *M. laciniatus* comprised 90% of the mean zooplankton abundance during the ice-free period. Other less abundant species included the cladocerans *Daphnia pulicaria* (Hrbacék) and *Chydorus sphaericus* (Müller), the rotifer *Hexarthra bulgarica* (Wiszniewsky),

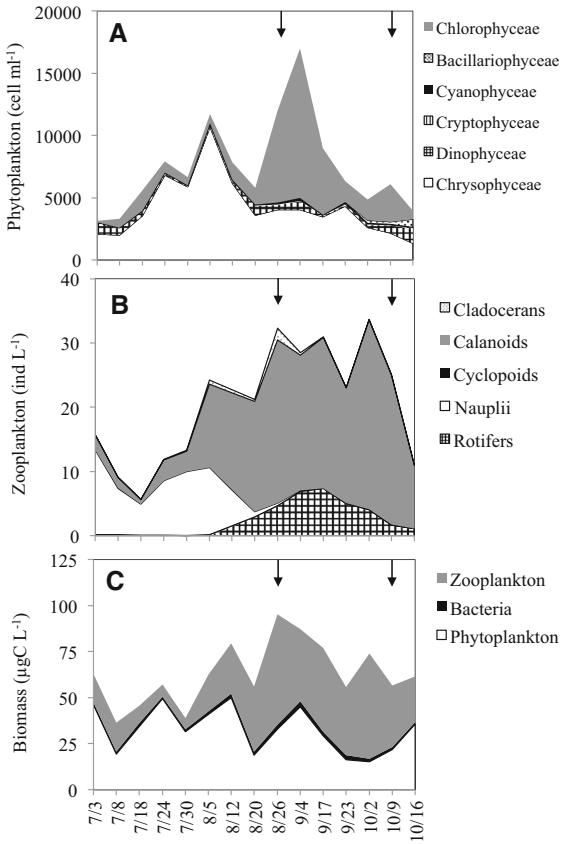

Fig. 2 Temporal dynamics of the relative abundance of phytoplankton (**A**), zooplankton (**B**) and biomass contribution of various planktonic groups (**C**) in lake La Caldera during the ice-free period of 1997. Organism abundance and biomass are means of four depths for the water column. *Arrows* indicate dates for the experiments

the cyclopoid *Acanthocyclops vernalis* (Fisher) and the calanoid *Diaptomus cyaneus* (Gurney). Figure 2C shows the temporal contribution of bacteria, phytoplankton and zooplankton fractions to the total pool of organic matter in lake La Caldera. The contribution of bacteria remained relatively stable and never contributed more than 5% to the total particulate matter. Phytoplankton was the principal storage compartment and zooplankton became more important as the ice-free season progressed (Fig. 2C), coinciding with the prevalence of copepodite and adult stages of the dominant zooplankter *M. laciniatus* towards the end of the season. By the time zooplankton experiments were initiated, phytoplankton mean abundance and carbon concentration in the lake water were 11 997 cells ml^{-1} and 32.7 µgC l^{-1} on August 27, and 6069 cells ml^{-1} and 21.7 µgC l^{-1} on October 10 (Fig. 2A, C).

Experimental production of organic carbon

Figure 3A shows the relative contributions of particulate and dissolved fractions to the total production of organic carbon in the experiments. In general, algae contributed more than 50% to the total carbon production of any given treatment (80–90% in the control with no nutrients added) in the midsummer experiment, and a substantial fraction of the production was incorporated into zooplankton. As opposed to first period, POC_A was highly reduced and E_{DOC} comprised a major fraction of the newly produced carbon in early fall (Fig. 3A).

Effects of UVA on organic carbon production

The results of the ANOVA showed that UVA explained a notable percentage of the variance in POC_A (20–29%) and POC_B (9–21%) (Table 1). However, the post hoc comparisons indicated that the individual effects exerted by UVA on particulate production generally differed between fractions and experiments (Table 2). Thus, while UVA decreased POC_A in both experiments, it strongly stimulated POC_B only in the early fall experiment (Table 2; Fig. 3B, C). In contrast, particulate carbon incorporated by zooplankton (POC_Z) and dissolved (E_{DOC}) and were unaffected by UVA (Table 2; Fig. 3D, E).

Interactive UVA and nutrient effects on organic carbon production

Interactive UVA × nutrient effects were significant for POC_A (only in the midsummer experiment), POC_B and E_{DOC}, but not for POC_Z (Table 1). Thus, the addition of nutrients led to a suppression of the inhibitory effects that UVA exerted on POC_A (Table 2; Fig. 3B). This was reflected in antagonistic UVA × nutrient interactive effects that explained a notable percentage of the variance in POC_A in the midsummer experiment (Table 1). Also, an inhibitory effect of UVA on POC_B emerged when nutrients were added at a N:P ratio of 5 in the midsummer experiment, while nutrients suppressed the stimulatory effect of UVA on POC_B in the early fall experiment (Table 2; Fig. 3C).

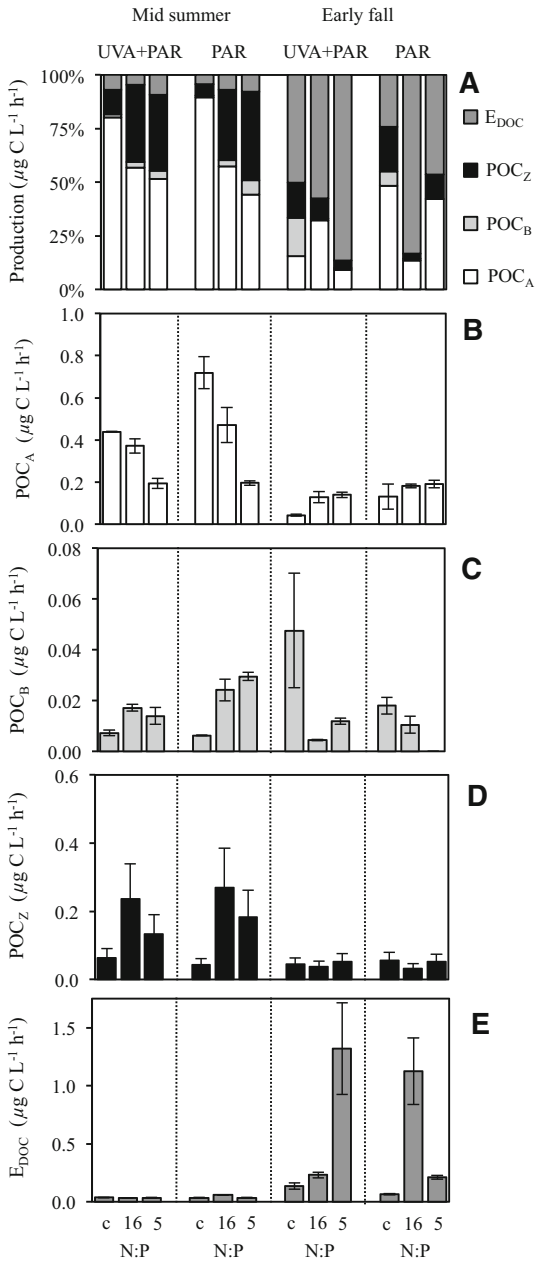

Fig. 3 Relative contribution of POC_A, POC_B, POC_Z and E_{DOC} (**A**), and mean production of POC_A (**B**), POC_B (**C**), POC_Z (**D**) and E_{DOC} (**E**) measured in the light and nutrient-enriched treatments during the midsummer and early fall experiments. Values in (**A**) are means for three replicates and *error bars* in (**B**, **C**, **D** and **E**) are means ± SD for the three replicates

After the enrichment with nutrients at the N:P of 16, E_{DOC} decreased in response to UVA in the midsummer experiment (Table 2; Fig. 3E). In the early fall experiment, the effects of UVA on E_{DOC} after nutrient enrichment were more complex, as it inhibited the production of E_{DOC} when nutrients were added at the N:P ratio of 16, but strongly increased E_{DOC} at the N:P ratio of 5 (Table 2; Fig. 3E).

Effects of nutrients on organic carbon production

The results of the ANOVA showed that nutrient enrichment explained most of the variance in POC_A (43–74%) and POC_B (52–58%) in the two experimental periods (Table 1). However, while nutrient enrichment decreased POC_A in midsummer (only at $N:P_5$), it increased POC_A in early fall under UVA (Table 3; Fig. 3B). In contrast, the enrichment with nutrients had opposed effects on bacteria by stimulating POC_B under all light conditions in midsummer but decreasing POC_B in early fall, although only significantly under UVA (Table 3; Fig. 3C). While nutrient enrichment increased E_{DOC} only in the $N:P_{16}$ treatment under PAR in midsummer, this effect was particularly marked in early fall when caused the higher E_{DOC} values in the $N:P_5$ treatment under UVA and in the $N:P_{16}$ treatment under PAR (Table 3; Fig. 3E).

POC_Z was also strongly enhanced by nutrient enrichment, but only in the midsummer experiment (Table 3; Fig. 3D). The stimulatory nutrient effect on POC_Z during this time was most marked for the $N:P_{16}$ treatment under PAR (Table 3; Fig. 3D). A similar effect was observed for carbon incorporated by zooplankton expressed as percentage of ingested particulate organic production. For example, under PAR treatment $\%POC_Z$ increased from 6 to 35% or 45% when nutrients were added at N:P ratios of 16 and 5, respectively (Fig. 4A). A slight decrease in $\%POC_Z$ for the $N:P_{16}$ treatment was observed in early fall (only significant under PAR; Table 3), a decline attributed to the decrease in POC_Z rather than the increase in POC_A and POC_B fractions (Figs. 3, 4A).

Zooplankton absorption of particulate production

AE_Z was unaffected by UVA and copepods feeding at ambient nutrient conditions exhibited $AE_Z \sim 100\%$ regardless of the light treatment (Table 1; Fig. 4B). In contrast, AE_Z declined by 10–29% after nutrient enrichment in midsummer, although effects were only significant for the PAR treatment (Table 3; Fig. 4B). Similar declining trends were observed in the presence

Table 1 Effects of UVA and nutrient enrichment on production of particulate (POC_A, POC_B, POC_Z, TOPC) and dissolved (E_{DOC}) carbon fractions, and percentage of total organic particulate carbon incorporation (%POC_Z) and absorption efficiency (AE_Z) by zooplankton tested by two-way ANOVA

Variable	Factor	df1	df2	Midsummer experiment			Early fall experiment		
				F	*P*	PV	*F*	*P*	PV
POC_A	UVA	1	12	28.94	***	11.91	20.36	***	34.27
	Nutrient	2	12	89.44	***	73.63	12.74	***	42.90
	UVA × nutrient	2	12	11.55	***	9.51	0.79	ns	2.64
	Error					4.94			20.19
POC_B	UVA	1	12	21.14	***	16.66	9.38	**	12.16
	Nutrient	2	12	36.61	***	57.67	19.96	***	51.79
	UVA × nutrient	2	12	10.32	**	16.24	7.90	**	20.49
	Error					9.43			15.56
POC_Z	UVA	1	12	0.30	ns	0.89	0.12	ns	0.78
	Nutrient	2	12	10.03	**	60.65	1.11	ns	14.47
	UVA × nutrient	2	12	0.36	ns	2.20	0.47	ns	6.20
	Error					36.25			78.56
E_{DOC}	UVA	1	12	19.98	***	23.25	1.01	ns	0.80
	Nutrient	2	12	7.01	**	16.31	19.49	***	30.64
	UVA × nutrient	2	12	19.98	***	46.50	37.64	***	59.14
	Error					13.94			9.43
TOPC	UVA	1	12	7.04	*	17.08	4.79	*	0.14
	Nutrient	2	12	11.85	**	53.28	1.61	ns	32.63
	UVA × nutrient	2	12	0.84	ns	3.55	0.16	ns	55.98
	Error					26.09			11.24
%POC_Z	UVA	1	12	0.15	ns	0.17	5.97	*	15.66
	Nutrient	2	12	34.78	***	82.76	9.89	**	51.95
	UVA × nutrient	2	12	1.17	ns	2.78	0.17	ns	0.88
	Error					14.28			31.50
AE_Z	UVA	1	12	0.05	ns	1.44	8.66	ns	23.02
	Nutrient	2	12	4.54	*	55.88	2.43	ns	12.91
	UVA x nutrient	2	12	0.75	ns	3.27	4.06	*	21.55
	Error					39.42			42.52

df1, *df2* are degrees of freedom, *PV* percentage of explained variance calculated as sum of squares of treatment/total sum of squares, *ns* not significant

P, the significance level (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

of UVA in early fall, although effects were not significant (Table 3).

Discussion

This study responds to the current demand for integrative research on the effect of ambient stressors on multiple trophic levels. A schematic model represents the seasonal significance of UVA, nutrients and their interaction on this food web dominated by three trophic levels (Fig. 5). Consistently with our first hypothesis, we found that UVA played a role, although moderate, in determining the production of carbon in algal and bacterial fractions. The decrease in primary production due to UVA in both experimental periods is consistent with the photoinhibitory effect found for this and other systems (Helbling et al., 2001; Carrillo et al., 2002). However, by stimulating the production in the bacterial fraction at early fall, UVA steered the food webs towards the preponderance of microbial components. Several mechanisms may account for the positive effect of UVA on bacteria. Our results do not show an effect of UVA increasing E_{DOC}. One likely explanation is that UVA may activate the photolyase enzymes responsible for bacterial capacity to repair DNA damage (Davidson & van der Heijden, 2000; Medina-Sánchez et al.,

Table 2 Individual effect size of UVA tested by two-way ANOVA (see Table 1) and post hoc Newman–Keuls' test on production of particulate (POC_A, POC_B, POC_Z, TOPC) and dissolved (E_{DOC}) carbon fractions, and percentage of total organic particulate carbon incorporation ($\%POC_Z$) and absorption efficiency (AE_Z) by zooplankton

Experiment	Factor	N:P treatment	POC_A		POC_B		POC_Z		E_{DOC}		TOPC		$\%POC_Z$		AE_Z	
			Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*
Midsummer	UVA	$N:P_c$	−38.6	***	16.7	ns	58.3	ns	0.02	ns	−33.1	ns	125.9	ns	−9.5	ns
		$N:P_{16}$	−21.1	ns	−27.1	ns	−12.3	ns	−48.3	**	−18.2	ns	7.5	ns	6.5	ns
		$N:P_5$	−1.70	ns	−55.6	***	−27.8	ns	0.01	ns	−17.2	ns	−12.7	ns	12.8	ns
Early fall	UVA	$N:P_c$	−66.7	*	199.9	**	−29.4	ns	105.6	ns	−34.4	ns	17.1	ns	−0.32	ns
		$N:P_{16}$	−27.8	ns	−58.1	ns	10.1	ns	−79.5	**	−23.6	ns	51.1	ns	−1.33	ns
		$N:P_5$	−26.3	ns	0.01	ns	6.7	ns	530.9	***	−15.3	ns	21.0	ns	−5.14	ns

Δ expresses the magnitude (as percentage) and sign (–, inhibitory; no sign, stimulatory) of each individual effect; *ns* not significant

P, the significance level (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Table 3 Individual effect size of nutrient enrichment tested by two-way ANOVA (see Table 1) and post hoc Newman–Keuls' test on production of particulate (POC_A, POC_B, POC_Z, TOPC) and dissolved (E_{DOC}) carbon fractions, and percentage of total organic particulate carbon incorporation ($\%POC_Z$) and absorption efficiency (AE_Z) by zooplankton

Experiment	Factor	Light treatment	POC_A		POC_B		POC_Z		E_{DOC}		TOPC		$\%POC_Z$		AE_Z	
			Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*	Δ	*P*
Midsummer	$N:P_{16}$	UVA+PAR	−15.2	ns	143	*	273.7	*	−18.2	ns	22.9	ns	199.5	*	−16.3	ns
		PAR	−34.0	***	289	***	575.0	*	58.2	***	0.5	ns	529.6	**	−28.9	*
	$N:P_5$	UVA+PAR	−56.1	***	90.4	*	105.3	*	−4.6	ns	−34.0	ns	210.1	**	−10.2	ns
		PAR	−72.6	***	400	***	350.0	*	−4.6	ns	−46.7	*	702.5	***	−28.0	*
Early fall	$N:P_{16}$	UVA+PAR	200	*	−91.3	***	−8.3	ns	70.4	ns	28.3	ns	−33.5	ns	−1.8	ns
		PAR	38.5	ns	−38.0	ns	−41.2	ns	1612	***	10.0	ns	−48.5	*	−0.8	ns
	$N:P_5$	UVA+PAR	223	*	−80.0	**	33.3	ns	877	***	52.5	ns	−22.1	ns	−4.3	ns
		PAR	46.2	ns	−40.0	ns	−11.8	ns	218	ns	18.0	ns	−24.6	ns	0.6	ns

Δ expresses the magnitude (as percentage) and sign (–, inhibitory; no sign, stimulatory) of each individual effect; *ns* not significant

P, the significance level (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

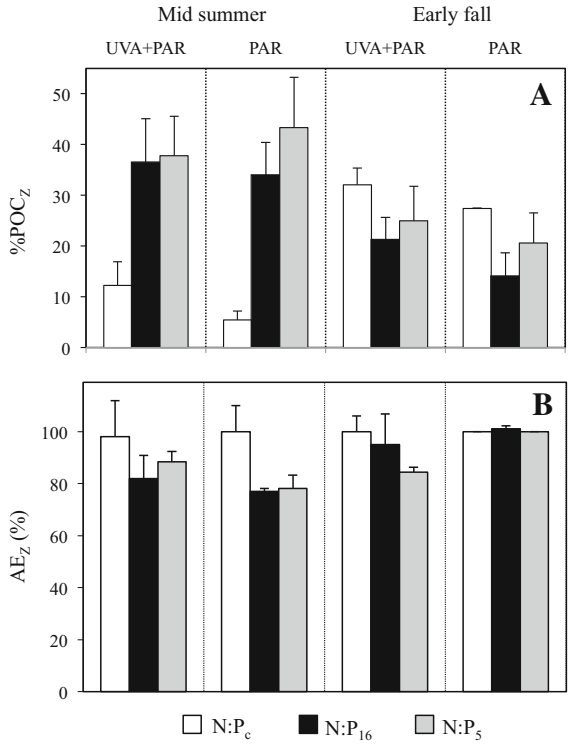

Fig. 4 Percentage of particulate organic carbon incorporated (%POC$_Z$) (**A**) and assimilated (%AE$_Z$) (**B**) by zooplankton measured in the light and nutrient-enriched treatments during the summer and early fall experiments. *Error bars* represent the mean ± 1 SD

2002). However, our first hypothesis was only partially supported because zooplankton incorporation and absorption of particulate carbon were basically unaffected by UVA. These findings suggest that UVA-mediated effects on trophic interactions at the base of food webs may not transfer to the higher trophic level of consumers, at least for the 4 h incubation time in this study. Unlike reports of hampered zooplankton ability to assimilate food (Zellmer et al., 2006), AEs varied little due to UVA and remained close to the maximal efficiency of 100%. Although values for AEs were higher that those reported in the literature for moderate to high resource prey concentrations (DeMott et al., 1998; Thor et al., 2007), absorption efficiency is known to vary according to prey abundance (Montagnes & Fenton, 2012). Thus, recent models in marine copepods indicate AE values that converge towards maximal absorption efficiencies at low prey concentration (Thor & Wendt, 2010), conditions that prevailed during the experiments and are usual for the studied lake. Although it is important to highlight that the observed responses are possibly attributable to the short-term incubations of 4 h, previous studies have established that zooplankton have evolved numerous adaptive mechanisms to high UV exposure including the synthesis of UV absorbing compounds such as carotenoids or mycosporine-like amino acids (Hairston, 1976; Hansson & Hylander, 2009). We suggest that the dominance of calanoid species such as *M. laciniatus* in these highly oligotrophic fish-less lakes is likely a combination of their capability to cope with high UVR doses, their high assimilation efficiencies and their lower food threshold compared to other zooplankton species such as *Daphnia* (Lampert & Muck, 1985).

In support of our second hypothesis, UVA × nutrient interactive effects on algal and bacterial incorporation of algal carbon exudates were generally significant, and antagonistic in the case of algae in midsummer. This indicates that the detrimental role of UVA inhibiting algal production was alleviated in the presence of nutrients. These findings are consistent with Medina-Sánchez et al.'s (2006) observations for this lake, and could be explained by the metabolic adjustments of P-limited algae that decrease carbon fixation in response to a sudden increase in phosphorus. However, and contrary to predictions, effects did not lead to higher carbon consumption by zooplankton. This finding reinforces the absence of a UVA effect on carbon trophic pathway through zooplankton irrespective of nutrients.

Our third hypothesis that nutrients enhance particulate production in all trophic levels was not fully supported, because the enrichment with nutrients had diametrically opposed impact on the production of carbon in algal and bacterial fractions (Fig. 5). By strongly inhibiting algal production and stimulating bacterial fraction in midsummer, and vice versa in early fall, nutrients might not only foster the coexistence between algae and bacteria, but also revealed as key ecological control influencing carbon production at basal trophic levels in food webs of oligotrophic lakes. The simultaneous reduction in bacterial carbon production after enrichment with nutrients is consistent with the idea that algae, released from a severe top–down control, are able to efficiently compete with bacteria for the available nutrients. This hypothesis is supported by the increase in algal production and dissolved organic carbon after nutrient enrichment in

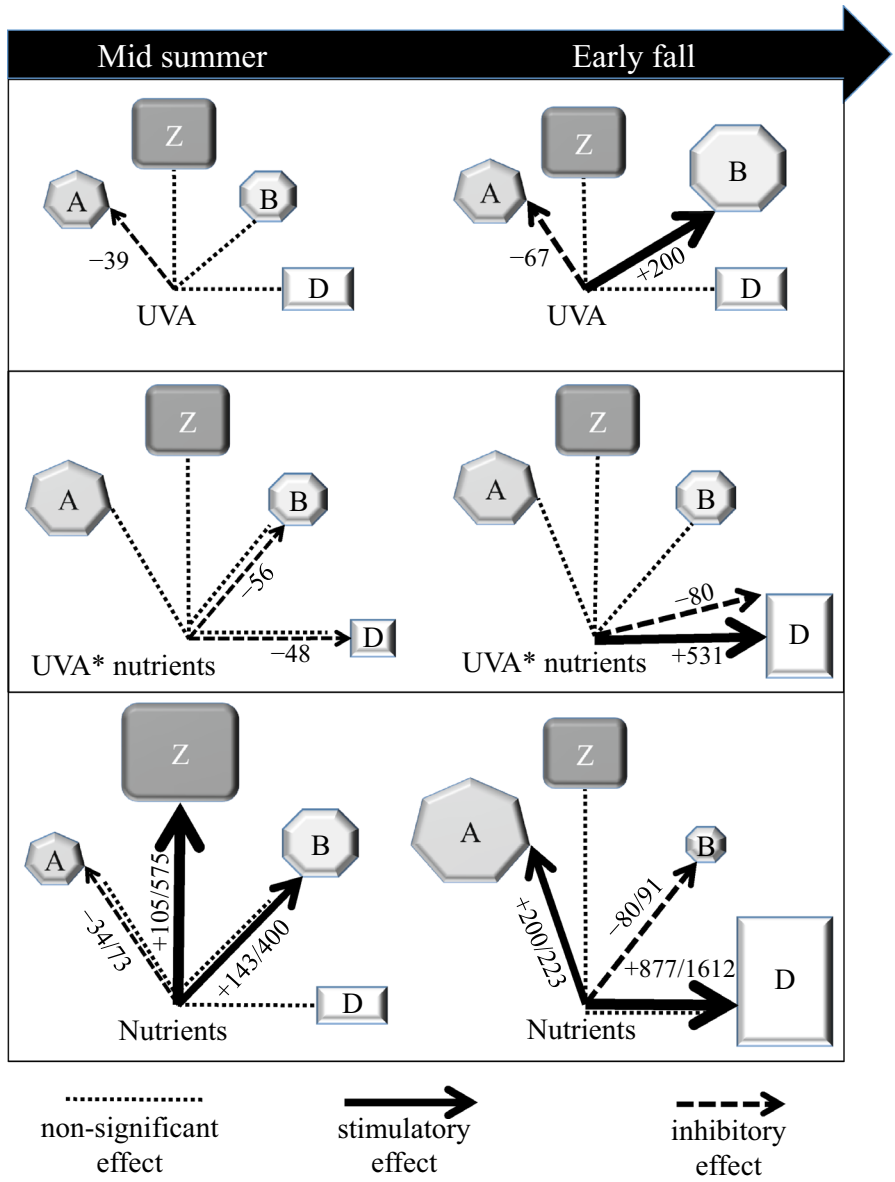

Fig. 5 Scheme illustrating the influence of UVA (*higher panel*), UVA in the presence of nutrients (*intermediate panel*) and nutrients (*lower panel*) on production of particulate (B, bacteria; A, algae; Z, zooplankton) and dissolved (D, dissolved) organic carbon fractions for the ice-free period. *Boxes* for B, A, Z and D are scaled to maximum sizes and arrow thickness is proportional to the effect. Numbers next to the *arrow* express the magnitude and sign (–, inhibitory; +, stimulatory) of the effects. For the lower panel, *slash-attached numbers* indicate the magnitude and sign for the effects of nutrients added at the N:P ratio of 16 (neither N-rich nor P-poor) and 5 (P-rich), respectively

early fall that would in turn alleviate bacterial dependence from the excretion of fresh carbon by algae, and with natural observations of a switch from commensalism to competition in the relationship between phytoplankton and bacteria towards the end of the season (Villar-Argaiz et al., 2002a; Carrillo et al., 2008b). In contrast to UVA, nutrients strongly enhanced zooplankton carbon accrual in midsummer, incorporating a higher share of the available particulate production. This finding could be explained by the dominance at this period of ambient immature copepodites with large nutrient and carbon demands for growth (Villar-Argaiz et al., 2002b). However, the decreased in absorption efficiency by almost 30% when nutrients were added supports the conclusion that not all incorporated carbon was used for growth, supporting the view that carbon absorption is stoichiometrically couple to the availability of phosphorus (Sterner & Elser, 2002). Conditions changed towards the end the ice-free period when a grown-zooplankton population, with lower carbon demands (primarily adult copepods), incorporated a lower share of the

available carbon and therefore exhibited a lower capability to regulate algal particulate production.

Ecological implications

The occurrence of natural inputs of allocthonous nutrients in alpine lakes of the Mediterranean region, similar to the ones simulated in this study, is a natural phenomenon associated with the atmospheric transport of dust aerosols from the Sahara desert (Bullejos et al., 2010). Previous studies in these ecosystems have demonstrated that, after these atmospheric depositions, phytoplankton blooms, and the recovery to previous oligotrophic conditions occurs shorty after (Villar-Argaiz et al., 2001). An important question is whether the incorporation of carbon by zooplankton after the enrichment with nutrients may turn into enhanced growth. Although this study did not report changes in growth of the animals, the decrease in the efficiency of carbon absorption by zooplankton in the enriched treatments in midsummer indicates that carbon was not efficiently invested in production. In fact, this idea is consistent with recent observations that moderate to high experimental nutrient inputs resulted in low herbivore growth (Villar-Argaiz et al., 2012), and with the long-term inter-annual observation of over 25 years reporting weakened coupling between phytoplankton and zooplankton despite phytoplankton steadily increase in the studied lake (Bullejos et al., 2010). Also, a study by Elser et al. (2010) showed that the effects of atmospheric nutrient depositions may result in decreased growth efficiency of zooplankton due to the existence of stoichiometric food quality constraints. A final implication of these findings is that inefficient trophic transfers from basal trophic production to zooplankton secondary production in higher than ambient nutrient conditions would reduce overall food chain performance, and possibly affect the trophic status of the ecosystem towards more eutrophic conditions (Villar-Argaiz et al., 2012).

These experiments support previous conclusions that response of ecosystems to relevant global factors cannot be made on single trophic assessments (Bothwell et al., 1994) and are in line with the extended view that the interactive nature of multiple environmental stressors is at least partially responsible for trophic interactions and ecosystem function (Carrillo et al., 2008a). In the Mediterranean region, where global change is expected to result in less rainfall and increased allocthonous aerosol inputs (Santese et al., 2007), alterations in nutrient regimes and optical UVA properties of the water column would have the potential to regulate carbon production in aquatic ecosystems. While these factors altered the balance in the production of carbon between the lower trophic levels of algae and bacteria, only nutrients seasonally affected the amount of carbon channelled to the higher trophic level of zooplankton. However, higher carbon accrual by zooplankton may not necessarily result in enhanced population growth due to the low absorption efficiency of carbon between basal and consumer trophic levels.

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