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RESEARCH ARTICLE

Combined effects of water temperature and nutrients concentration on periphyton respiration – implications of global change

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With the increase in global mean surface temperature predicted for the near future, stream water temperature will also increase. Simultaneously, water quality is likely to decrease (e.g., due to increases in nutrient and pollutant concentrations). The objective of this study was to evaluate the individual and combined effects of increases in water temperature and nutrients concentration on periphyton respiration, as a surrogate for stream metabolism. Stones naturally colonized with periphyton in an unpolluted mountain stream in Central Portugal were sampled seasonally over a year, and incubated in the laboratory under two water temperatures (ambient and 4°C elevated) and two nutrients concentration levels (ambient and $\sim$6$\times$ higher inorganic dissolved nitrogen, $\sim$2$\times$ higher soluble reactive phosphorous concentrations). Overall, increases in water temperature stimulated periphyton respiration to a larger extent than did increases in nutrients concentration. In spring, the simultaneous increase in water temperature and nutrients concentration stimulated periphyton respiration beyond expected from the individual effect of each factor. These results indicate that synergistic interactions between factors might occur under certain environmental conditions, suggesting that care should be taken when predicting the combined effect of changes in multiple factors from their individual effects. The observed stimulation of periphyton respiration promoted by increased temperature and nutrients concentration can lead to changes in streams carbon budgets, with a positive feedback for global warming, as more CO_2 might be released to the atmosphere.

Keywords:
Global changes / Metabolism / Stream / Synergistic effects

1 Introduction

Models considering a doubling in atmospheric CO_2 concentration predict that global mean air temperature will increase by 1.1–6.4°C over this century [1]. Water temperature of streams and rivers is expected to follow this increase by raising 0.3–0.9°C for each degree increase in air temperature [2, 3]; additional increases in water temperature of up to 6°C can be achieved if shading by the riparian vegetation is lost [4]. This raise in temperature is likely to affect the metabolism of individuals and communities [5], which may lead to changes in community structure, species distribution, interspecific relations, biodiversity [6–8], and ecosystem processes such as carbon mineralization, primary production, and denitrification [9, 10]. The effects of increases in temperature on ecosystem processes are anticipated to be stronger in naturally colder environments (e.g., high latitude and altitude, cold months) since at low temperatures enzymatic activities are temperature limited [11].

Many water bodies worldwide are presently suffering from impoverished water quality and this scenario will be aggravated in the future. Global warming will lead to higher evaporation and evapotranspiration rates [12] which, associated with increased water abstraction for human

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activities, higher inputs of nutrients and pollutants due to intensification of agriculture and urbanization, and higher waste water production [13], will result in increased concentration of pollutants and nutrients in streams [14], unless mitigation measures are undertaken to restore and protect streams. Nutrient enrichment of freshwaters generally stimulates the activity of microbial communities and leads to a eutrophic state [15], stimulating carbon transformation [16, 17], and decreasing water quality [18, 19].

Stream metabolism is a universal aquatic process, which includes primary production and respiration [15, 20]. It can be highly sensitive to environmental changes since it depends on complex interactions among trophic levels, carbon and nutrient cycling and energy conversions (reviewed by Young et al. [21]). Most studies on the effects of temperature on stream metabolism report significant increases in respiration rates and in gross primary production with temperature [10, 22], since enzymatic activity is temperature dependent [5]. Nutrient enrichment usually also stimulates respiration and primary production [23, 24]. Nevertheless, the effect of simultaneous changes of water temperature and water quality on stream metabolism has rarely been addressed [10], despite evidence from other systems suggesting that the effects of factors associated with global changes acting per se might not be sufficient to predict the effects of factors acting in combination [25–27].

The goal of this study was to assess the individual and combined effects of changes in water temperature and nutrients concentration on periphyton respiration, seasonally over 1 year. Stones colonized with periphyton were collected from an unpolluted mountain stream in Central Portugal and incubated in the laboratory under varying temperature and nutrient conditions. We predicted that periphyton respiration, and consequently stream metabolism, would increase both with temperature and with nutrients concentration. A stronger effect on respiration rates promoted by the interaction of both factors combined was expected. Season (i.e., ambient water temperature) was expected to determine the extent to which increases in temperature and decreases in water quality affect respiration rates.

2 Methods

2.1 Experimental design

The individual and combined effects of an increase in water temperature and nutrients concentration on periphyton respiration were assessed by using stones naturally colonized with periphyton in an unpolluted mountain stream in Central Portugal. Stones were incubated at ambient water temperature (i.e., the temperature registered in the stream water at the time of stones collection, hereafter called "ambient temperature") or at 4°C above ambient temperature to simulate water temperature under a future global warming scenario (hereafter called "elevated temperature"; Table 1). This was obtained by controlling the temperature in the laboratory rooms. Water temperatures are predicted to increase up to 4.7°C for streams in the United States under a warming scenario [4]. In some areas of the world, air temperature is predicted to increase up to 7°C (e.g., Portugal; [28]). Therefore an increase in water temperature by 4°C is realistic for temperate streams in a global warming scenario.

Stones were incubated in water collected from an unpolluted mountain stream with low nutrients concentration (hereafter called "low NP") or water collected from a lowland stream surrounded by agricultural fields, with approximately three- to eightfold higher nutrient concentrations and ~5–7 higher conductivity to simulate water quality under a future global change scenario (hereafter called "high NP"; Table 1). Therefore, using water from a presently nutrient enriched stream seemed a realistic option to simulate future decline in water quality of presently oligotrophic streams. Incubation of stones was

Table 1. Temperature (ambient and elevated) and nutrients concentration (low NP and high NP) of the water used for metabolism measurements in the four seasons

	Temperature (°C)		DIN (μg L^{-1})		SRP (μg L^{-1})		Conductivity (μS cm^{-1})	
Seasons	Ambient	Elevated	Low NP	High NP	Low NP	High NP	Low NP	High NP
Spring	12	16	130	739	6.1	15.4	39	258
Summer	17	21	249	733	3.6	29.9	12	70
Autumn	12	16	172	1357	20.0	10.7	39	268
Winter	7	11	414	2491	3.3	6.3	29	144

The stones were collected from the stream at ambient temperature and low nutrients concentration.
DIN, dissolved inorganic nitrogen (NO_3-N + NO_2-N + NH_4-N); SRP, soluble reactive phosphorus (~PO_4^{-}-P).

performed four times over the year, one in each season, to cover natural changes associated with seasonal variations in the periphyton and in nutrients concentration, since these change together with hydrology and agricultural activities (Table 1).

The two factors, temperature and nutrients concentration, each one with two levels, were crossed in a complete factorial design, which resulted in a total of four treatments per season: ambient temperature–low NP (AL), ambient temperature–high NP (AH), elevated temperature–low NP (EL), and elevated temperature–high NP (EH). Each treatment was composed of three replicates, each containing two flat schist stones (occasionally only one, depending on stone size), naturally colonized with periphyton. Stones were incubated in the dark, and oxygen consumption (respiration) was the target variable.

2.2 Periphyton and water

Flat schist stones naturally colonized with periphyton were collected from a mountain stream in Central Portugal (Ribeira de São João, Lousã mountain, Central Portugal; 40°05′59″N, 8°14′02″W), where dissolved nutrient concentrations are usually low and average temperature is 17° C in summer and 7°C in winter (Table 1; see also [29]). Stones were transported to the laboratory in plastic boxes filled with stream water; care was taken during collection and transport to avoid stones contact with air to prevent desiccation of the periphyton. In the laboratory, the stones with periphyton were kept overnight in acclimatized rooms at the target temperatures, in the dark, with aerated stream water [9].

To test the effect of changes in nutrients concentration on periphyton respiration, water was collected from two streams: the stream where the stones were collected (low NP; Table 1) and a lowland nutrient enriched stream (high NP; Ribeira de São Paulo de Frades, Coimbra, Central Portugal; 40°14′87″N, 8°24′656″W; Table 1). The water was transported in 5 L plastic bottles, promptly filtered (filter paper) in the laboratory in order to remove particulate organic matter in suspension, transferred into plastic boxes (62 cm $\times$ 50 cm $\times$ 40 cm, water volume $\sim$95 L), and stored in acclimatized rooms at the target temperatures (both nutrient levels per temperature) for $<$2 days until being used for incubations. The water was aerated with aquarium pumps until the incubation began to ensure 100% saturation of dissolved oxygen (DO). Just before the incubations started, water samples were collected and frozen at $-$18°C until determination of nutrients concentration. Nitrate, nitrite, and ammonium concentrations were determined by ion chromatography (Dionex DX-120, Sunnyvale, CA, USA); soluble reactive phosphorus was determined by the ascorbic acid method [30].

2.3 Metabolism chambers and oxygen consumption

Periphyton respiration was measured in closed chambers (metabolism chambers; $\sim$19 cm $\times$ 14 cm $\times$ 12 cm, volume 3.76–4.71 L), made of acrylic glass. A pump (camper water pump, Eco-plus 12 V, Comet, Florida, USA), connected to a battery (12 V, 60 Ah, 510 A, DiaMec, China), circulated the water inside the chambers (200 mL s^{-1}), allowing a homogeneous distribution of nutrients and oxygen within each chamber. Chambers were always incubated completely submerged in the 95 L plastic boxes filled with stream water. Each chamber was submerged in the corresponding treatment box and received 1–2 stones. Care was taken when placing the stones inside the chambers to avoid air bubbles being trapped inside the chamber before measurements. The incubations were made in the dark and DO (% and mg L^{-1}) was determined using an oxymeter (Oxi 3210 SET 1, WTW, Weilheim, Germany) at the beginning of incubations and hourly for at least 4 h; the difference in oxygen concentration for each time interval corresponded to oxygen consumption by the periphyton.

2.4 Stone area, periphyton biomass, and chlorophyll-*a*

At the end of all incubations, the upper surface of the stone was scraped with a toothbrush and rinsed with distilled water to remove the periphyton into a tray. We assumed that the colonization of the below surface of the stones was negligible because the stones were embedded in the stream sediment that was very compact; therefore, considering the whole area of the stone would underestimate the respiration. The suspended material was filtered through ignited, pre-weighed fiberglass filters (Millipore APFF04700, Millipore, MA, USA; filter pore 0.7 μm). Two filters were obtained per chamber, enclosed individually in Petri dishes and frozen at $-$18°C until used for biomass and chlorophyll-*a* (Chl-*a*) determination; the dish with the filter for Chl-*a* extraction was wrapped with aluminum foil for protection against light exposure. Filters were freeze-dried overnight (LY3TTE, Snijders Scientific, Tilburg, Netherlands) and weighed ($\pm$0.01 mg). One filter was used for Chl-*a* extraction using a standard procedure [30], whereas the other filter was ignited (550°C, 4 h) and reweighed for ash free dry mass (AFDM) determination.

After periphyton removal, the active surface area of the stone was covered with aluminum foil, and the foil was dried (105°C, 30 min) and weighed ($\pm$0.01 mg). A linear regression between aluminum foil area (m^2) and foil dry mass (g) was applied to calculate the active surface area of the stone: area $=$ (foil dry mass $+$ 0.0002)/0.0027. Stone

volume was determined by the volumetric method; the dislocated water volume was subtracted from the total chamber volume in order to calculate the actual volume of water in each chamber.

2.5 Data analysis

Differences in periphyton biomass and Chl-*a* concentration among seasons were assessed by one-way ANOVA (Statistica 7 software, StaSoft, OK, USA). Differences in periphyton biomass, Chl-*a* and respiration among treatments within each season were assessed by two-way ANOVAs, with temperature and nutrients concentrations as categorical variables (Statistica 7 sofware). A Fisher LSD multicomparison test ($\alpha = 0.05$) was used when necessary. The individual effect of increased temperature and nutrients concentration on periphyton respiration rates across seasons were assessed using a paired *t*-test (1-tail; Statistica 7 software).

Respiration rates were determined as oxygen variation over the incubation time, corrected for water volume and periphyton area or AFDM [9]:

$$R = \frac{\Delta O_2}{\Delta t} \times \frac{V}{S \text{ or AFDM}}$$

where R is the respiration rate (mg O_2 m^{-2} h^{-1} or mg O_2 mg^{-1} AFDM h^{-1}), ΔO_2 is the change in oxygen concentrations during incubation (mg O_2 L^{-1}), Δt is the time interval between measurements (h), V is the water volume in the chamber (L), S is the periphyton area (m^2), and AFDM is the periphyton biomass (mg; adapted from [9]).

The expected respiration rates in a scenario of global changes (elevated temperature–high NP, EH) were calculated from the stimulation of respiration rates of periphyton in ambient temperature and low nutrients concentration (present scenario, AL) by an increase in temperature alone (EL) or an increase in nutrients concentration alone (AH), assuming no interaction between factors: Expected $R_{EH} = R_{AL} + (R_{EL} - R_{AL}) + (R_{AH} - R_{AL})$. Expected R_{EH} rates were plotted as black bars on the graphs for comparison with the observed R_{EH}. If observed R_{EH} rates $<$ expected R_{EH} rates, the interaction between factors is antagonistic; if observed R_{EH} rates $=$ expected R_{EH} rates, the interaction between factors is additive, i.e., predictable from their individual effects; if observed R_{EH} rates$>$expected R_{EH} rates, the interaction between factors is synergistic. A single sample *t*-test (Statistica 7 software) was used to determine if the observed periphyton respiration rate was significantly different from the expected rate in each season.

The percentage increase in respiration rates with simultaneous increase in temperature and nutrients concentration (future scenario, EH) over ambient levels (present scenario, AL) was calculated as: Increase in respiration rate (%) $= (R_{EH} - R_{AL}) \times 100)/R_{AL}$.

3 Results

3.1 Periphyton chlorophyll-*a* and biomass

Chl-*a* and periphyton AFDM were determined to quantify the periphyton on the stones used in the incubations. Chl-*a* concentrations were higher in summer (0.34 mg Chl-*a* m^{-2}), intermediate in spring (0.10 mg Chl-*a* m^{-2}) and low in autumn and winter (0.03 and 0.04 mg Chl-*a* m^{-2}, respectively; one-way ANOVA, $p<0.001$; Fig. 1). Chl-*a* concentration was not significantly different among treatments, except in autumn when it was higher in the ambient temperature–low NP treatment (0.075 mg Chl-*a* m^{-2}) than at any other treatment (0.014–0.028 mg Chl-*a* m^{-2}; Fisher LSD, $p < 0.009$). Periphyton biomass concentrations were higher in autumn and in winter (75.91 and 52.55 g AFDM m^{-2}, respectively), and lower in spring and summer (2.53 and 2.32 g AFDM m^{-2}, respectively; one-way ANOVA, $p < 0.001$; Fig. 1). No significant differences in periphyton biomass were also found among treatments within each season (two-way ANOVA, $p > 0.149$). Therefore, the amount of periphyton colonizing the stones within each incubation trial was similar across treatments. The active surface area of the stones varied slightly between 200 and 300 cm^2.

3.2 Periphyton respiration

O_2 consumption by the periphyton, on a per area basis (Fig. 2) and per biomass basis (Fig. 3), were higher in spring across treatments.

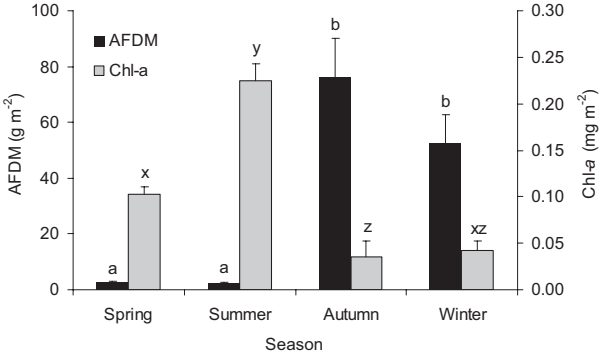

Figure 1. Seasonal periphyton biomass (g AFDM m^{-2}) and chlorophyll-*a* concentration (mg Chl-*a* m^{-2}; mean $\pm$ 1 SE). Different letters indicate significant differences among treatments (one-way ANOVA followed by Fisher LSD, $p < 0.050$).

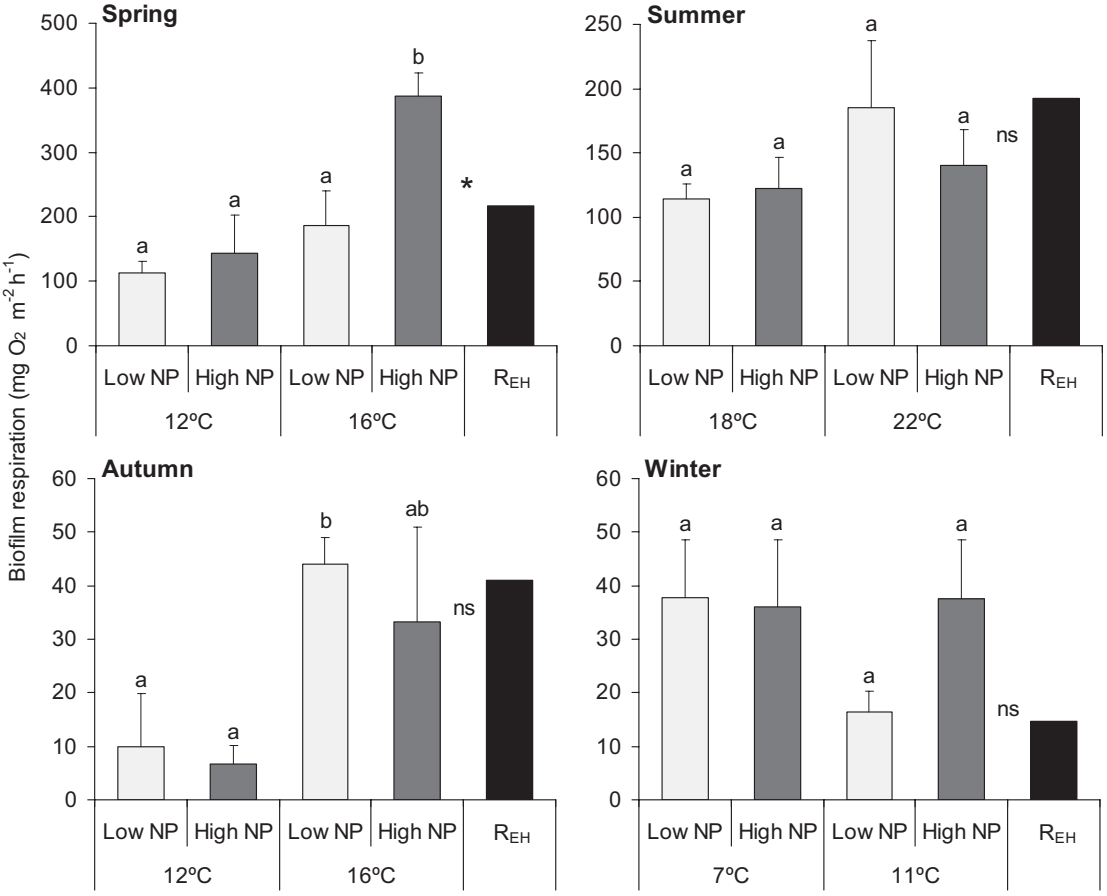

Figure 2. Periphyton respiration on aerial basis (mg O_2 m^{-2} h^{-1}; mean ± 1 SE) at two temperatures and two nutrient concentrations (low NP or high NP) for each season. Different letters indicate significant differences among treatments (two-way ANOVA followed by Fisher LSD, $p < 0.050$). The black bars indicate the respiration rate expected under elevated temperature and nutrients concentration, assuming no interaction between factors (R_{EH}). *Significant differences between observed and expected respiration rates under elevated temperature and high nutrients concentration; ns, indicates non-significant differences (single sample *t*-test, $p < 0.050$).

Temperature significantly stimulated periphyton respiration per area in autumn for the low NP treatment (from 9.83 to 43.97 mg O_2 m^{-2} h^{-1}; Fisher LSD, $p = 0.016$). In spring, respiration rates per area were significantly higher under elevated temperature and nutrients concentration (EH) than at any other treatment (Fisher LSD, $p < 0.013$). No significant differences were observed among treatments in winter and summer (two-way ANOVA, $p > 0.140$). However, the respiration rates per area in elevated temperature and low NP treatment tended to be lower than in all other treatments in winter, while in summer there was a tendency for the opposite (Fig. 2). The absence of significant differences was probably a consequence of the reduced number of replicates used ($n = 3$) that translated into higher variability within treatments. Overall, the increase in temperature had a more significant impact on periphyton respiration per area (paired *t*-test, $p = 0.048$) than did the increase in nutrients concentration ($p = 0.188$).

The expected respiration rates per area under elevated temperature and nutrients concentration for each season were calculated assuming no interaction between factors (Fig. 2). In spring the observed respiration rate per area was significantly higher than the expected value (*t*-test, $p = 0.044$), indicating synergistic effects between factors; in winter, although no significant difference was detected ($p = 0.124$), the tendency was the same. In autumn and summer the observed respiration rate was similar to that expected (*t*-test, $p = 0.742$ and 0.150, respectively) possibly indicating additive effects between factors (Fig. 2).

The percentage of increase in respiration rates per area when changes from present (ambient temperature and low NP) to future scenario (elevated temperature and high NP) were simulated varied by one order of magnitude across seasons. The highest increase in respiration rate per area occurred in spring and autumn, when an increase in temperature and nutrients concentration resulted in a

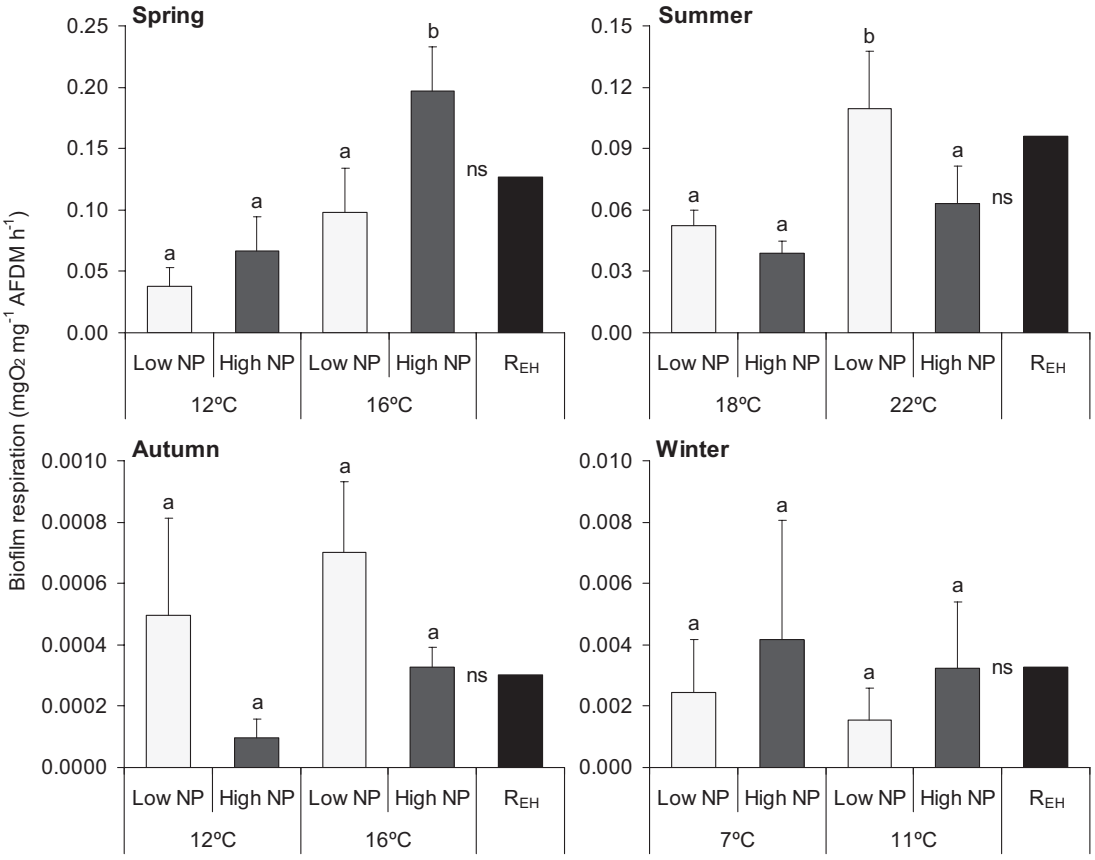

Figure 3. Periphyton respiration on mass basis (mg O_2 mg^{-1} AFDM h^{-1}; mean $\pm$ 1 SE) at two temperatures and two nutrient concentrations (low NP or high NP) for each season. Different letters indicate significant differences among treatments (two-way ANOVA followed by Fisher LSD, $p < 0.050$). The black bars indicate the respiration rate expected under elevated temperature and nutrients concentration, assuming no interaction between factors (R_{EH}). *Significant differences between observed and expected respiration rates under elevated temperature and high nutrients concentration; ns, indicates non-significant differences (single sample *t*-test, $p < 0.050$).

respiration rate increase of ~240%. In summer, the increase in respiration rate was of ~20%, while in winter respiration rate was not stimulated by the simultaneous increase in temperature and nutrients concentration.

The patterns of respiration per biomass were very similar to those of respiration per area. Respiration rates per mass ranged from 0.0001 to 0.1969 mg O_2 mg^{-1} AFDM h^{-1} across treatments and seasons. In spring, respiration rate per mass was highest at the elevated temperature and high NP treatment (Fisher LSD, $p < 0.048$). In summer, elevated temperatures significantly stimulated respiration rates only in the low NP treatment (Fisher LSD, $p = 0.026$). In autumn and winter neither temperature nor nutrients concentration affected respiration rates (two-way ANOVA, $p = 0.126$ and 0.494, respectively), which might partially result from limited replication. In general, temperature also had a more significant impact (paired *t*-test, $p = 0.040$) than did nutrients concentration (paired *t*-test, $p = 0.283$).

The observed respiration rates did not differ significantly from those expected at any season (*t*-test, $p > 0.160$) indicating possible additive effects between both factors (Fig. 3).

When the scenario changed from present to future the highest increase in respiration rates per mass occurred in spring (~430%). In summer and winter the respiration rates also increased (21 and 32%, respectively), in opposition to autumn (−34%).

4 Discussion

Increases in water temperature and nutrients concentration, acting per se, are known to stimulate stream metabolism [9, 10, 15, 21]. However, simultaneous changes in water temperature and quality are anticipated in a climate change scenario [14]. Despite this, we are aware of only one study addressing the combined effect of these two factors on

stream metabolism [10]. Recently, the effects of increases in both temperature and nutrients concentration on lake sediment respiration [31] and on stream biofilm formation and activity [32] were also addressed under controlled conditions. Here, we determined the combined effect of an increase in water temperature and in nutrients concentration, as those expected in a global change scenario, on periphyton respiration, as a surrogate of stream metabolism, seasonally over 1 year.

Periphyton respiration rates varied across seasons. Rates were higher in spring than at any other season, which can be attributed to the combination of high temperature and light availability in spring. These environmental conditions are known to stimulate periphyton activity [33–35], including respiration [36, 37]. On the contrary, the lowest periphyton respiration rates were found in autumn and winter. The still relatively closed canopy in autumn and low water temperatures in winter in headwater streams most likely limited periphyton growth [35, 38, 39] and consequently the rates of periphyton respiration. Such patterns have already been reported in other studies [40–43], with respiration rates usually higher during warm seasons and lower during cold seasons. The higher values for periphyton biomass recorded during autumn and winter were attributed to anamorphic organic matter (e.g., fine particulate organic matter derived from litter decomposition) trapped in the polysaccharide matrix, since the high AFDM values in the cold seasons were not companied by high Chl-*a* concentrations or high respiration rates. If this is true, the Chl-*a* concentration and respiration rates in autumn and winter were underestimated since not all AFDM was living mass.

Overall, periphyton respiration was more stimulated by a short-term increase in temperature than by a short term increase in nutrients concentration. Long-term exposure may result in increased primary production leading to increased biomass of producers and therefore higher respiration rates. However, here we were only interested in the nutrient effect without the confounding factor of increased biomass. The effect of nutrient enrichment on periphyton respiration might have gone largely undetected due to differences in N:P ratio among seasons; the difference in nutrient concentrations between low and high NP treatments (4–8$\times$ for N and 1–3$\times$ for P) might have resulted in P limitation, which is a common scenario in many streams [44]. Besides this, the high thickness of the periphyton (e.g., in autumn and winter) might have precluded the nutrients and oxygen from reaching the entire mat, therefore decreasing nutrient availability within the periphyton [45–47] and turning the lower layers "inactive" (reviewed by [48]). Nevertheless, Liboriussen et al. [31] also reported stronger effects of warming than of nutrient enrichment on lake sediment respiration.

Our results are contrary to the reports indicating that periphyton respiration rates are usually stimulated by an increase in nutrient availability [15, 23, 24, 32]. This could be partially explained by (i) P limitation as mentioned above, (ii) by the low number of replicates ($n = 3$), which could not encompass the high variability within treatments, and (iii) by short-term exposure, which may differ from a continuous nutrient exposure of natural periphytic assemblages. However, our results were consistent with those of Flury and Gessner [49] that reported that microbial variables such as respiration might be little affected by nutrient enrichment and temperature increase.

The stimulation of periphyton respiration with increasing temperature at both nutrient levels in autumn suggests that the future increase in water temperature might enhance stream metabolism in both oligotrophic and eutrophic streams. In spring, oligotrophic streams might be protected from the effects of warming given that increased temperature only stimulated periphyton respiration under high nutrients concentration. Díaz-Villanueva et al. [32] also found stronger stimulation of biofilm activity in the laboratory with warming under high than low nutrient conditions. Lower sensitivity of oligotrophic systems to warming, mediated by nutrient limitation, was also suggested considering microbial-induced litter decomposition [27]. Contrarily, however, lake sediment respiration was stimulated by warming (3.7–6.6°C above ambient) at low nutrients concentration (20–50 μg total P L^{-1}, 320–680 μg total N L^{-1}) but not in enriched conditions (addition: 2.7 mg P m^{-2} d^{-1}, 27.1 mg N m^{-2} d^{-1}) [31].

Moreover, in spring, the stimulation of periphyton respiration induced by nutrient enrichment (78%; the only season where it was observed) was equivalent to that induced by an increase of 4°C in temperature (66%). This indicates that in some cases the effects of warming might be similar to those of eutrophication, and that some currently eutrophic streams might serve as models to predict the effects of global warming on oligotrophic streams (also suggested by [27]). The stimulation of periphyton respiration beyond expected with simultaneous increase in temperature and nutrients concentration in spring indicates synergistic interactions between both factors, and suggests that it is not always possible to predict the combined effects of multiple factors acting in concert.

We anticipated stronger effects of warming in the colder season (winter) due to temperature limitation of enzymatic activity [5, 11]. This was, however, not the case here. Also, similar temperature effects would be expected in spring and autumn given that both seasons had the same ambient temperature (12°C). However, the temperature effect was stronger in spring than in autumn. These differences between expected and observed patterns might be due to differences in water quality between

seasons, since the prediction regarding temperature is made on the basis that all else remains equal. Also, community composition might play an important role mediating how ecosystem function responds to changes in environmental factors [32]. Since periphyton community structure changes seasonally [42], and distinct species might have distinct temperature and nutrient optima [50], distinct communities might respond differently to the environmental changes of similar magnitude.

An increase in respiration rates result in increased carbon (C) released into the atmosphere, which might further stimulate global warming (positive feedback; [10, 51]). However, the extent to which respiration will affect global warming depends on the response of primary production to the increase in temperature; if warming stimulates primary production more than respiration, then the storage of C in these systems would increase, which could act as a negative feedback on global warming, or at least counteract the positive feedback by respiration. However, respiration has been found to be stimulated by warming to a larger extent than primary production [10, 11], making the net ecosystem metabolism of forested headwaters, which is usually heterotrophic by definition [52], even more heterotrophic in a global change scenario. Besides this, modifications of C budgets under simultaneous increase of water temperature and nutrients concentration might have severe consequences on the functioning of small headwater streams and reaches downstream [27]. A stimulation of C respired from the system will remove higher amounts of C from streams, which might result in faster disappearance of carbon sources for higher trophic levels, impairing mass transfer along the food chain.

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The authors have declared no conflict of interest.

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