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Leachates and elemental ratios of macrophytes and benthic algae of an Andean high altitude wetland

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ABSTRACT

*In wetlands, macrophytes and filamentous algae constitute an important carbon source for the total content of Dissolved Organic Matter (DOM) of the environment. Mallín wetland meadows are highly diverse and rare habitats in Patagonia, that can be characterized as wet meadows with a dense cover mainly dominated by herbaceous plants. We carried out a field study comparing elemental composition (C:N:P) of benthic algae (*Spirogyra *sp. and* Zygnema *sp.) and the submerged macrophyte (*Myriophyllum quitense*) from a high latitude wetland (local name: mallín). Besides we performed laboratory experiments in order to study the effect of ultraviolet radiation (UVR) on the optical properties and nutrient release of DOM from leachates of these benthic algae and submerged macrophyte. The obtained results indicated that macrophyte leachates could contribute significantly to changes in the optical characteristics of the wetlands while benthic algae contribute with leachates with low photoreactivity. Finally, nutrient release differs among plant species and season: benthic algae leachates release more P in spring, while* M. quitense *releases more of this nutrient in autumn. These results suggested that the different colonization may contribute differentially to the chemical environment of the wetland.*

Key words: high altitude wetlands, submerged macrophytes, dissolved organic matter, carbon nitrogen phosphorus, ultraviolet radiation

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1. INTRODUCTION

Patagonian wetlands occupy approximately a 5% of the total Patagonia area (Iriondo 1989). Among these there is a particular kind of wetland called by the Mapuche name mallin. These wetlands, which are similar to bogs, can be characterized as wet meadows with a dense cover mainly dominated by herbaceous plants belonging to families Juncaceae, Cyperaceae and Poaceae with a high net primary production (Raffaele 1999). Mallín wetland meadows are highly diverse habitats in western Patagonia (Raffaele 2004) and they are located either in steppe or forest environments in the Andes Cordillera. The steppe mallín wetlands are important resources for native and introduced species and in particular very significant for local economies based on cattle and sheep (Leon *et al*. 1998; Raffaele 1999; Hauenstein *et al.* 2002). On the contrary, mallín wetlands located in the mountains constituted flooded mountain meadows that are included in protected areas such as National Parks (Raffaele 2004), and protected from cattle grazing. However, due to their high altitude location, they are exposed to harsh environmental factors like ice and snow (provoking a shortage in plant growth) and high ultraviolet radiation (UVR). These wetlands are hydrologically complex environments (Iriondo *et al*. 1974), with the bottom covered by the submerged macrophyte *Myriophyllum quitense* (Ezcurra & Brion 2005), and filamentous benthic algae (*Zygnema* and *Spirogyra*). Although, there are studies regarding hydrology, seed banks, flora and fauna including microbial communities (Markgraf 1984; Raffaele 1996; Del Valle 1998; Cardoso *et al*. 2010), little is known about the elemental composition of plants, algae, and their leachates.

Macrophytes and filamentous algae constitute an important carbon source for the total content of dissolved organic matter (DOM) of wetlands (Wetzel 2001). These benthic producers release synthesized DOM during photosynthesis or through leaching of aged and dead tissues and filaments (Bertilsson & Jones 2003). The DOM entering the aquatic environment can remain in the wetland water (Naiman & Melillo 1984), and then be incorporated into the aquatic community through bacteria activity (Triska *et al*. 1989; Bastidas Navarro *et al*. 2009). On the other hand, DOM represents a significant component absorbing solar radiation in aquatic ecosystems, particularly in the ultraviolet region of the light spectrum (290 to 400 nm) (Morris *et al*. 1995; Schindler & Curtis 1997; Osburn *et al*. 2001). This energy absorption may lead to a variety of photochemical processes, including the photodegradation of DOM that could be related to changes in spectral and molecular properties (Bertilsson & Tranvik 2000; Farjalla *et al*. 2001; Helms *et al*. 2008). In addition, UVR increases macrophyte degradation and leachate production (Mans *et al*. 1998; Anesio *et al*. 1999), and the photochemical processes may result in an important source and sink of essential nutrients such as nitrogen and phosphorus (Lewis 2002; Qualls & Richardson

2003). Since Patagonia is under the effect of the Antarctic ozone hole (Villafañe *et al*. 2001) a particular interest in these reactions rises from the increment of UVR caused by the reduction in the stratospheric ozone layer (Osburn *et al*. 2001; Zepp *et al*. 2003; Vernet 2006). Although there is lack of information about how UVR affects mallín wetlands dynamics, a strong effect of UVR on high altitude lakes of Patagonia was pointed out by previous studies (Zagarese & Williamson 2000). Accordingly, an increase in DOM photobleaching due to increasing levels of UVR radiation was predicted (Osburn *et al*. 2001), and in turn the interaction of UVR with DOM can result in a nutrient source for aquatic microcommunities (Bastidas Navarro & Modenutti 2010).

The importance of macrophyte and filamentous algae in wetland dynamics was recognized (Wigand *et al*. 2000; Egertson *et al*. 2004). However, the elemental constitution of these producers and how their leachates influence the optical properties of natural water by the input of DOM with different chemical features is still poorly known. This is of particular importance because it will affect the environmental nutrient cycling. In addition, as these environments are exposed to high UVR, a high interaction of short wavelength with this DOM coming from macrophytes and benthic algae is expected, resulting in important photochemical processes. Thus, our main hypothesis is that DOM will interact with UVR differentially according to the different sources. Because of its tissue complexity we expect that vegetal elemental composition and leachates of *M. quitense* will be richer in nutrients than those of *Zygnema* and *Spirogyra*. In order to test this hypothesis, we performed a field study to analyze the elemental composition of macrophytes and algae, and laboratory experiments analyzing the effect of UVR on DOM optical properties and nutrient release of their leachates.

2. METHODS

2.1. Study Area

The study was carried out in the mallín Los Patos located at an elevation of 1500 m above sea level, on the western slope of Cerro Chall-Huaco (41°16'S and 71°20'W). The area is included in the Nahuel Huapi National Park, Patagonia, Argentina. At the centre of the meadow there are two streams, and on the western side there is a small pond (Area = 1.5 ha) (Fig. 1). The surrounding vegetation is a subalpine forest of the deciduous *Nothofagus pumilio* (Poepp. et Endl.) Krasser. The flora of this wetland includes 52 native vascular species and 3 species of bryophytes (Raffaele 1993; Raffaele 1996). In particular, the pond is colonized by two aquatic plants: the emergent *Carex subantarctica* and the submerged *Myriophyllum quitense*, however the bottom is also covered by green filamentous algae of the genera *Spirogyra* and *Zygnema*.

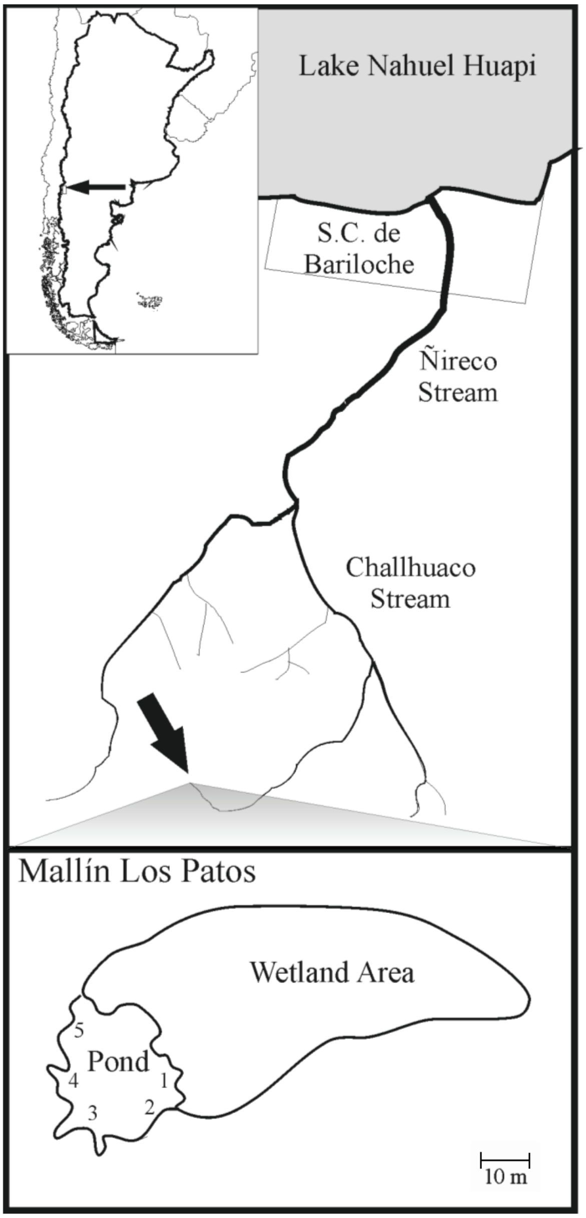

Fig. 1. Geographical location of study area. Numbers indicate the sampling points.

2.2. Field study

The pond was sampled on two different occasions: in late spring (December 2009) and autumn (April 2010) at 5 different sampling points (Fig. 1). Water temperature and dissolved oxygen were measured using an oxymeter (YSI Pro ODO Yellow Spring Instruments, USA), and pH was measured with a pH-meter (HI 8424 Hanna Instruments, USA). Redox potential was measured in the water, and in the sediments with a combined Pt electrode with Ag/AgCl internal reference (HI-3131B Hanna Instruments). Water samples were obtained with a limnological bottle (2 L). Macrophytes and algae were sampled at each sampling point on each sampling occasion. The plants and algae were immediately carried to the laboratory in polyethylene bags. In addition, the vegetation cover was estimated by measuring directly the different areas covered by the different species.

2.3. Laboratory procedures

Total phosphorus (TP), total dissolved phosphorus (TDP) and total nitrogen (TN) in the pond water were determined according to APHA, (2005) and Valderrama (1981), and chlorophyll-*a* concentration was determined according to Nusch (1980).

The elemental analysis of vegetal tissue and algae was carried out after carefully washing the vegetal material with distilled water. In *Myriophyllum quitense* the shoot and root parts were separated and dried at 60 ºC for 48 hours up to a constant weight. Afterwards, dry plants and algae were crushed to powder with a clean mortar and approximately 2 mg of dry weight (DW) was destined for elemental analysis. Carbon (C) and nitrogen (N) concentration in the tissues were measured with a CN analyzer (Thermo Finnigan Flash EA 1112). Phosphorus was measured after combustion of 2 mg DW of tissue at 450 ºC during 1 hour followed by the ascorbate-reduced molybdenum method (Apha 2005).

Macrophyte and algae leachates were prepared with algae and plants collected in the field, which were carefully rinsed in distilled water and dried at room temperature. Finally, 1 g of algae or macrophyte was placed in 100 mL of MilliQ water and left there at 4 °C for 48 h following Anesio (2000). In order to eliminate any potential presence of bacteria in the samples all the glassware was sterilized, and the obtained water was filtered through precombusted GF/F filter and then through sterile membrane filters of 0.2 μm pore size. All glassware was previously cleaned with diluted clorhidric acid (0.5 N), rinsed with MilliQ water, and then combusted at 450 °C for 1 hour to make sure that it was carbon free. Similarly, the GF/F filters (Osmonics) were precombusted at 450 °C for 1 hour.

2.4. Experimental design

The optical features of algae and macrophyte leachates were studied in two experiments: Experiments 1 (late spring: December 2009) and 2 (autumn: April 2010). Both experiments consisted in the exposure of leachates of green filamentous algae and *Myriophyllum quitense* to different light treatments for four hours: (1) exposed to UVR+PAR, (2) exposed to PAR, and (3) DARK (wrapped in aluminium foil). Experiments were carried out in an incubator at 15 ± 1 °C with a PAR intensity of 92 μmol m^{-2} s^{-1} (daylight fluorescent tubes). The UVR was provided by two UVA-340 fluorescent tubes (Q-Panel Lab Products, Cleveland, OH, U.S.A.). The UVR spectrum of these light tubes closely resembles the solar spectrum between 280 and 350 nm (Shick *et al.* 1999). During the incubation, water received 35 μW cm^{-2} nm^{-1} of the 340 nm band, and this irradiance level is equivalent to surface noon summer sunlight in Andean lakes. In the PAR treatment, UVR cutoff was reached using a polyethylene filter. The optical features of this polyethylene, with cutoff at 380 nm and 85% transmittance above 380 nm, were checked before the experiment using a double-beam spectrophotometer Shimadzu UV 2450. Light exposure was carried out in 20 mL quartz tubes. Quartz tubes were distributed in a frame at 30 cm distance from the light source following Bertilsson & Tranvik (2000). In both experiments, exposure was carried out in three replicates.

At the initial and final time of each experiment, spectrophotometric scans were carried out from 250 nm to 790 nm in 10 cm quartz cuvettes using a double-beam spectrophotometer Shimadzu UV2450. Dissolved Organic Carbon (DOC) was measured with a Shimadzu TOC VCSH carbon analyzer. For the nutrient release analysis, at initial and final time, Soluble Reactive Phosphorus (SRP) concentrations were obtained through the ascorbate-reduced molybdenum method.

2.5. Data Analysis

Absorbance units were converted to absorption coefficients (a_d). The ratio a_{250}:a_{365} was used as a measure of the relative size of DOM molecules (Pérez & Sommaruga 2006), and water color was determined as the absorbance coefficient at 440 nm (a_{440}) according to Pace & Cole (2002).

Differences in limnological characteristics between spring and autumn were analyzed using a *t*-test. A Two Way ANOVA ("Plant" and "season" as factors) was applied in order to determine significant differences in optical characteristics and nutrient content between both leachates. Differences in elemental ratios, a_{250}:a_{365} ratio, and SRP concentration were analyzed through a One Way ANOVA. Post hoc comparisons were made using the Tukey Test with an overall significance level of p = 0.05. All statistical analyses were performed using SigmaStat 3.1 and Statistica 6.0.

3. RESULTS

In Mallín Los Patos limnological features changed in the two sampling occasions (Tab. 1). Higher mean temperature values were recorded in spring (*t*-test, p = 0.009) in coincidence with a lower dissolved oxygen concentration (*t*-test, p = 0.202). Nevertheless, dissolved oxygen concentration was always near saturation (85% in spring and 94% in autumn). Water redox potential exhibited high values in both water and sediments. Redox values in the water were significantly higher in spring (*t*-test, p = 0.027), while in the sediments no significant differences between seasons were observed (*t*-test, p = 0.189). In spring, there were higher dissolved organic carbon concentrations as well as higher water color values (high a_{440}), but lower mean molecular size (high a_{250}:a_{365}) (*t*-test, p = 0.023 for DOC, $p \leq 0.001$ for a_{440} and a_{250}:a_{365}). In spring also higher concentrations of TP, and TDP were observed (*t*-

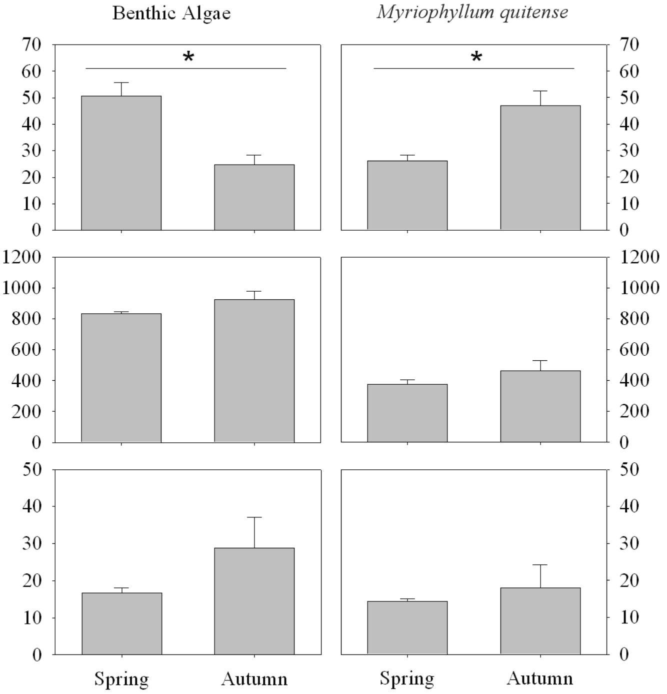

Fig. 2. Elemental relationships (C:N, C:P and N:P ratios) in the tissues of studied species in spring and autumn. (*) indicates significant differences (p <0.05) between seasons.

test, p <0.001 for both cases). Finally, chlorophyll-*a* concentrations were low in both seasons (≈2.5 µg L^{-1}), though significantly higher in autumn (*t*-test, p <0.001) (Tab. 1).

Tab. 1. Limnological characteristics of Mallín Los Patos. Values are given as average ± standard deviation.

	Spring	Autumn
Temperature (ºC)	13.33 ± 1.91	7.55 ± 1.88
Dissolved Oxygen (mg L^{-1})	9.2 ± 1.24	11.3 ± 2.27
pH	6.51 ± 0.83	6.24 ± 0.71
Redox Potential (mV)(water)	197 ± 15.51	114 ± 43.61
Redox Potential (mV)(sediments)	275 ± 80.10	202 ± 58.94
DOC (mg L^{-1})	1.1 ± 0.02	0.4 ± 0.08
a_{250}:a_{365}	3.50 ± 0.10	2.61 ± 0.06
a_{440}	1.68 ± 0.04	1.08 ± 0.03
TP (µg L^{-1})	20 ± 0.81	8 ± 0.06
TDP (µg L^{-1})	8± 0.34	5 ± 0.06
TN (µg L^{-1})	n.d.	65 ± 5.07
Chlorophyll-*a* (µg L^{-1})	2 ± 0.01	3 ± 0.05

The vegetation cover exhibited different patterns of spatial distribution between spring and autumn. During spring, the bottom coverage of *M. quitense* was about 80%, while the benthic algae remained lower than 15%. On the other hand, in autumn, benthic algae bottom coverage increased up to 60%, whereas *M. quitense* constituted less than 30%.

We observed differences in the elemental ratios between the studied species and seasons (Fig. 2). The C:N ratio showed significant differences between seasons for both plants, while benthic algae (*Spirogyra* and *Zygnema*) exhibited higher values in spring (*t*-test, p = 0.007) *M. quitense* showed higher ratios in autumn (*t*-test, p = 0.025). On the other hand, the C:P elemental ratios showed no significant differences during both seasons for both plants (*t*-test, p = 0.184 for benthic algae, and p = 0.258 for *M. quitense*). Benthic algae exhibited N:P ratio higher than 20, whereas the submerged macrophyte was always below 20 (Fig. 2) and this relationship remained similar during both seasons (*t*-test, p = 0.280 for benthic algae, and p = 0.613 for *M. quitense*).

Leachates of both plants also presented differences in their optical and chemical features (Tab. 2). Benthic algae leachates exhibited lower values in the a_{250}:a_{365} ratio indicating higher molecular size than in *M. quitense* (Two Way ANOVA, p <0.001 for "Species" factor, a posteriori Tukey test, p <0.001), and these values did not differ between seasons (Two Way ANOVA, p = 0.363 for "Season" factor). We also observed differences in C (DOC) and P (SRP) in the two leachates, *M. quitense* being richer in C and P than benthic algae in both seasons (Two Way ANOVA, p <0.001, *a posteriori* Tukey test p <0.001) (Tab. 2).

Tab. 2. Optical features and nutrient content of leachates of the macrophyte and the benthic algae.

	Benthic Algae		*M. quitense*	
	Spring	Autumn	Spring	Autumn
a_{250}:a_{365}	3.00 ± 0.05	2.91 ± 0.09	3.51 ± 0.10	3.52 ± 0.03
a_{440}	2.19 ± 0.01	2.29 ± 0.10	2.63 ± 0.12	3.68 ± 0.01
DOC (mg g^{-1})	58.4 ± 0.05	54.8 ± 0.40	70.7 ± 0.02	90.4 ± 0.68
SRP (mg g^{-1})	3.4± 0.29	4.1 ± 0.96	7.4 ± 0.73	7.8 ± 0.21

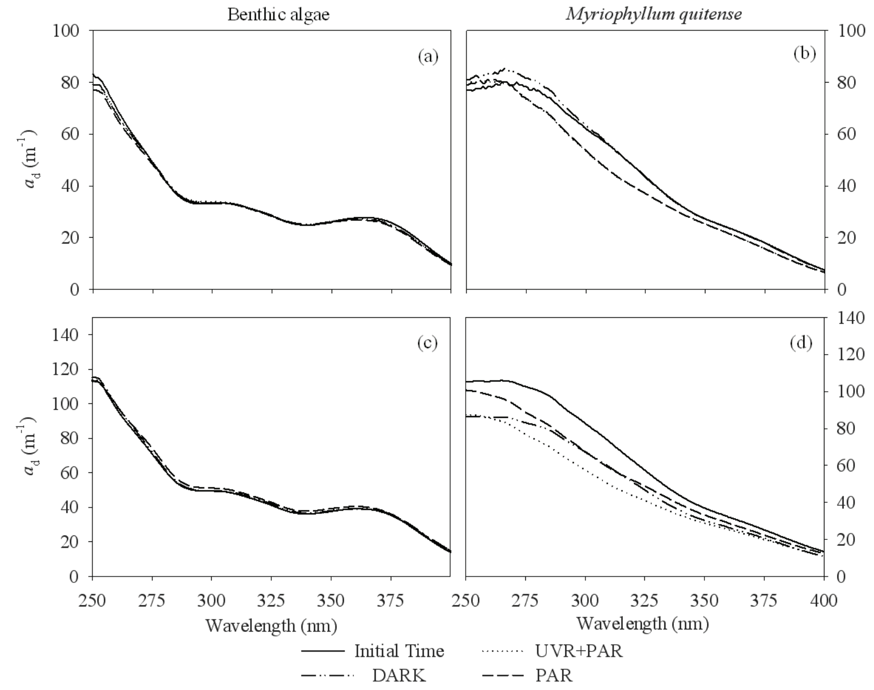

Fig. 3. Spectrophotometric absorbance curves in the leachates from benthic algae (left column) and *Myiriophyllum quitense* (right column) in spring (**a** and **b**) and in autumn (**c** and **d**) experiments.

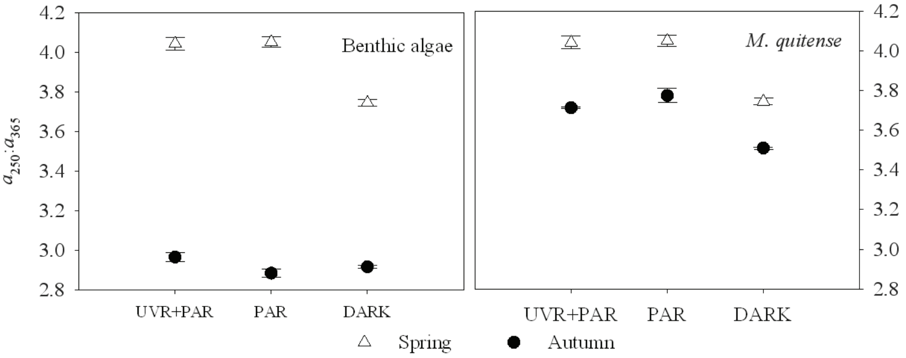

Fig. 4. Mean molecular weight (a_{250}:a_{365} ratio) in the leachates from benthic algae and *Myiriophyllum quitense* in both spring experiments.

In each species, in the two experiments the effect of light treatments on the optical features of the leachates showed similar trends (Fig. 3). Benthic algae leachates showed a bimodal pattern at the initial time, with maxima at 307 nm and at 370 nm (Fig. 3a and c); while *M. quitense* exhibited a unimodal spectrum with a maximum at 265 nm (Fig. 3b and d). However, the leachates from benthic algae did not exhibit changes after UVR+PAR or PAR exposure neither in the spectrophotometric scans nor in the a_{250}:a_{365} ratio (One Way ANOVA, p = 0.918, Fig. 4), indicating a low photoreaction of this DOM to light exposure regardless of light quality. On the contrary, *M. quitense* leachates showed significant changes in the spectrophotometric scans and in the a_{250}:a_{365} ratio between light treatments (One Way ANOVA, p <0.001 in both seasons, Fig. 4), with higher values in the UVR+PAR and PAR treatments (*a posteriori* Tukey test, p <0.001 for UVR+PAR and PAR *vs* DARK for both seasons).

Analyzing nutrient release by the leachates, we found that in the spring experiment benthic algae release more P in the DARK treatment (One Way ANOVA, p = 0.002, a posteriori Tukey test, p <0.003 for UVR+PAR and PAR *vs* DARK), while no significant differences were observed in the autumn experiment (One Way ANOVA, p = 0.078) (Fig. 5a, b). On the contrary, we found that *M. quitense* in the spring experiment did not exhibit differences in P release (One Way ANOVA, p = 0.176, Fig. 5), while in the autumn experiment significant differences were observed (One Way ANOVA, p <0.001) with more P released in the DARK treatment (Fig. 5c, d).

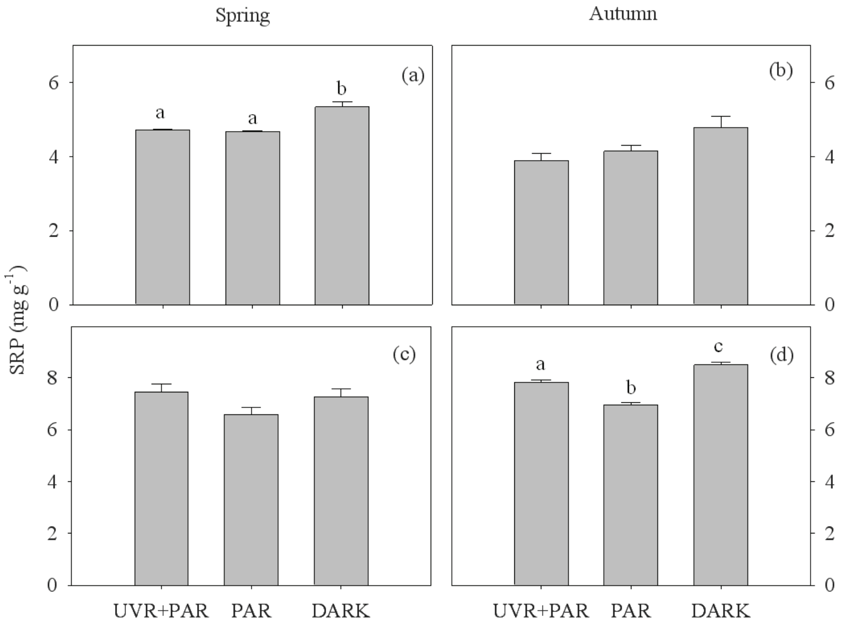

Fig. 5. SRP concentrations for benthic algae (**a** and **b**) and *Myriophyllum quitense* (**c** and **d**) in spring and autumn experiments. Letters on the bars indicate significant differences (p <0.05) between light treatments (UVR+PAR, PAR and DARK).

4. DISCUSSION

Most lakes and many streams and rivers have a large proportion of their basins potentially available to macrophytes and epiphytes. Given the extent of habitat colonization and the high production rates (Moss 1998) macrophytes and benthic algae are important sources of dissolved organic matter (DOM) (Bertilsson & Jones 2003). In Mallín Los Patos the whole bottom can be colonized by macrophytes and epiphytes; thus, these plants can be assumed to be very important for the ecosystem dynamics. In particular, our results showed that the submerged macrophyte *Myriophyllum quitense* and the green filamentous algae (*Spirogyra* and *Zygnema*) are important sources of dissolved organic carbon, providing about 55 and 80 mg g^{-1} DW of benthic algae and *M. quitense*, respectively. These species also constitute a source of phosphorus that would be directly released to the water (3.75 and 7.58 mg P g^{-1} DW for benthic algae and *M. quitense*, respectively).

Interestingly, we determined that the dissolved oxygen concentration in water was near saturation, and aerobic conditions were also observed in the sediments (high redox potential) suggesting aerobic decomposition of organic matter in this environment. In wetlands, aerobic conditions are assumed to be determinants for mycrorrhizal fungi colonization (Daleo *et al.* 2007). The observed redox potential values in sediments may favor the development of mycorrhizal fungi. Thus, possible symbiotic associations would also play an important role in nutrient allocation in wetland macrophytes.

The C:N:P elemental ratios of the studied species showed differences that could be explained by species-specific relationships (Sterner & Elser 2002; Demars & Edwards 2007; Osborne & Kovacic 2007). C:N:P stoichiometry of benthic microalgae has been indicated as largely variable in response to different levels and types of nutrient limitation (Hillebrand & Sommer 1999). In our study, we observed that benthic algae C:N exhibited lower values in autumn, that can be associated with a higher growth and reproductive rate (González *et al.* 2010). On the contrary C:P elemental ratio did not vary in the two seasons. According to Loladze & Elser (Loladze & Elser 2011) N:P ratio higher than 16 implies a P limitation. The observed N:P ratios were around or over 20 suggesting that benthic algae are P limited. Thus, if algae are limited by nutrients, as it is often the case in natural conditions (Elser *et al.* 1990), other algae and bacteria may compete for them. In benthic algae, under P limitation, the C:P ratios increased with decreasing growth rates and with higher growth when the ratio was around 130 (Hillebrand & Sommer 1999). A different situation was observed for *M. quitense*, in which N:P elemental ratio was below 20 though higher than 16, and C:N and C:P were higher in autumn, indicating a higher growth rate in spring. These results also support the differences observed in the cover of both plants, with dominance of benthic algae in autumn and of *M. quitense* in spring.

Different macrophyte species allocate nutrients differentially in their structural tissues, and therefore DOM coming from these species will have different nutrient content (Davis 1983). In that sense, we have hypothesized that the elemental composition and leachates of *M. quitense* would be richer in nutrients than those of benthic algae. Our results support this hypothesis, and in particular we observed a higher P release in autumn.

However, we determined that benthic algae leachates released more P in spring than in autumn, suggesting that nutrient release sources could vary according to the alternative dominance of benthic algae and macrophytes.

Leachates are chemically heterogeneous compounds with labile and refractory fractions (Peret & Bianchini 2004). The absorbance spectra obtained for benthic algae and *M. quitense* showed maximum absorbance at different wavelengths, suggesting that these plants have differences in the released DOM. Ultraviolet range (UV-A and UV-B), promotes the phototransformation of the DOM structure, molecular weight and optical properties (Reche *et al.* 2001; Brinkmann *et al.* 2003; Obernosterer & Benner 2004), including nutrient release (Maranger & Pullin 2003). Our laboratory experiments indicated that both leachates reacted differently to light. In autumn, benthic algae did not exhibit changes in spectrophotometric absorbance spectra or a_{250}:a_{365} ratio, indicating that this DOM is mostly refractory. These results were consistent with the undetectable SRP release after light (UVR+PAR or PAR) exposure. It has been mentioned that in algae of high altitude lakes UVR promotes the accumulation of phenolic compounds (Figueroa *et al.* 2009). On the other hand, light treatments induced changes in the absorbance spectra of M. quitense leachates and a decrease in the DOM mean molecular size after UVR and PAR exposure. This decrease of mean molecular size seemed to be related to a release of P after UVR exposure. Higher DOM photoreactivity has been observed in lakes with a high influence of autochthonous DOM (Reche *et al.* 2001). Similar results were obtained in field experiments in Lake El Trébol where natural lake water was exposed to solar radiation (Zagarese *et al.* 2001; Bastidas Navarro *et al.* 2009). However, other studies in shallow Andean lakes have indicated that leachates of *Potamogeton linguatus* and *Schoenoplectus californicus* macrophytes could contribute significantly to changes in the optical characteristics and nutrient release (Bastidas Navarro & Modenutti 2010). However, our results indicate that the photoreactivity of autochthonous DOM is species dependent, since leachates of *M. quitense* seemed to react to light very similarly to other macrophyte species but the studied benthic algae exhibited a very low photoreactivity.

5. CONCLUSIONS

We observed that the elemental composition and leachates of the submerged macrophyte *M. quitense* were richer in nutrients than those of benthic algae and exhibited a higher P release in autumn. Besides, we determined that the colonization of wetlands by benthic algae will contribute with a DOM with lower photoreactivity than that of macrophytes. Finally, benthic algae leachates release more P in spring than in autumn, suggesting that nutrient release sources could vary according to the alternative dominance of benthic algae and macrophytes.

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