Seasonal patterns of organic matter stoichiometry along a mountain catchment

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Seasonal patterns of organic matter stoichiometry along a mountain catchment

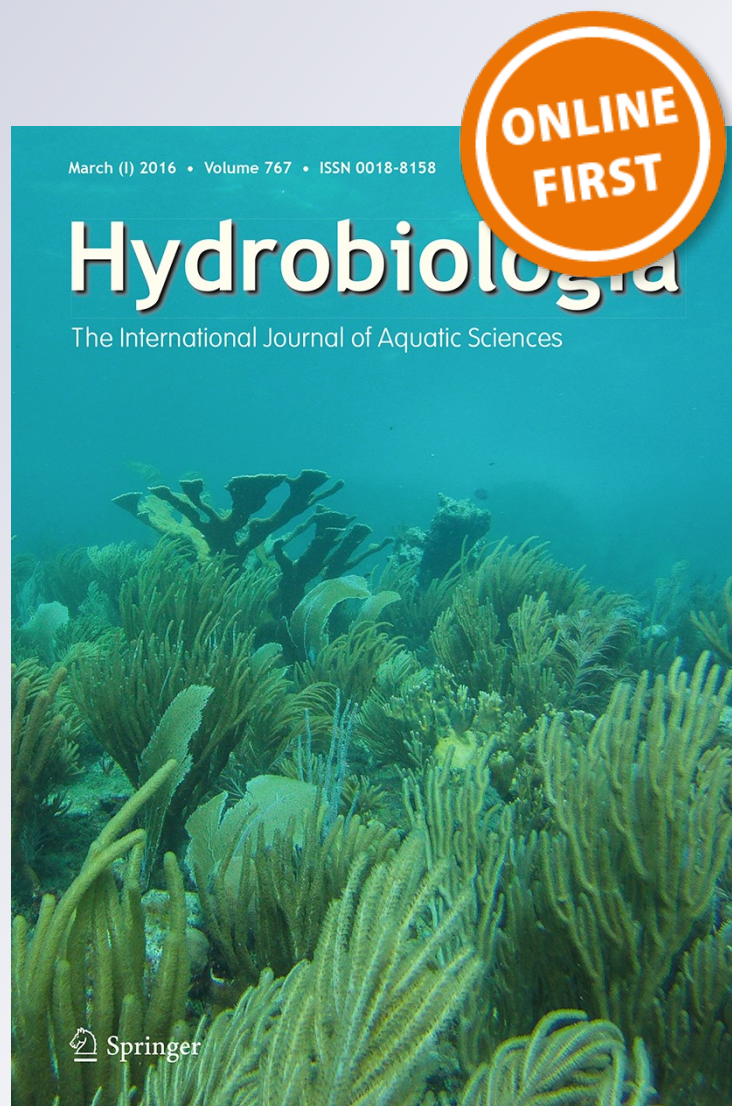**Verónica Díaz Villanueva · Marcela Bastidas Navarro · Ricardo Albariño**

Abstract Organic matter (OM) nutrient content is expected to change as OM is transported from upstream forested reaches downstream to open land, and these changes could depend on seasonal patterns. The aim of this study was to analyze OM carbon nitrogen and phosphorus content, from headwater reaches to open reaches downstream. We sampled four sites along a mountain catchment over the course of one year and quantified fine particulate organic carbon (FPOC) in transport and dissolved organic carbon (DOC) concentration and elemental content. Both FPOC and DOC concentration decreased downstream, and changes in OM stoichiometry depended on the season. Stoichiometry of FPOM and DOM along the catchment was similar in autumn, with higher C:P and C:N ratios upstream, which could be associated with the relative importance of allochthonous versus autochthonous OM origin. Conversely in spring, C:N was very low in the forest sites. Our results highlight that headwaters running through pristine forests are a source of POM and DOM for downstream ecosystems, and that the quality of such resources depends on season.

Introduction

Most headwater catchments connect forested mountain areas with open valleys transporting allochthonous organic matter (OM) that enters the stream from the forest. However, on its way downstream, part of this OM is physically transformed and biologically processed. A conspicuous body of literature explores OM inputs, transformation and transport (Webster & Meyer, 1997; Pusch et al., 1998; Tank et al., 2010). However, only recently have OM biochemical/optical and elemental quality been analyzed in the longitudinal stream axis (Frost et al., 2009; Green & Finlay, 2010; Akamatsu et al., 2011; Finlay et al., 2011; Sakamaki & Richardson, 2013). Changes in fine particulate OM (FPOM) stoichiometry downstream are expected as a consequence of two contrasting mechanisms that would lead to opposite outcomes. On one hand, downstream reaches that are wider and with more light, usually have higher primary production, so

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fragments of periphyton dislodge, entering the bulk of FPOM in transport. This process may contribute to a decrease in the C:N ratio downstream (Akamatsu et al., 2011; Sakamaki & Richardson, 2013). At the same time, OM that arrives from upstream reaches may be partially processed by heterotrophic organisms that consume C, N, and P according to their internal needs. For example, detritivorous insects retain nutrients and return feces to the environment, usually with higher C:P and C:N ratios than those of the litter they feed on (Cross et al., 2005; Yoshimura et al., 2008), so a decline in the nutritional quality of FPOM might be expected.

As regards dissolved OM (DOM), it appears to exist some controversy among authors, as some of them have stated that DOM increases downstream (Hood et al., 2006; Finlay et al., 2011), while others found the opposite (Frost et al., 2006; Stephens & Minor, 2010; Laudon et al., 2011). The result depends on the scale of the comparison and the landscape through which the catchment runs, for example, the proportion of wetlands and forested or cultivated areas (Frost et al., 2006; Wilson & Xenopoulos, 2008; Laudon et al., 2011) or the presence of upstream lakes (Larson et al., 2007). However, DOM quality along catchments generally references optical characteristics and suggests higher bioavailability downstream because of a higher primary production (Halbedel et al., 2013). Few studies have focused on DOM nutrient content, mostly on dissolved organic nitrogen (DON) (Hood et al., 2005; Balcarczyk et al., 2009), and they found a correlation between C:N and aromaticity, suggesting that highly aromatic DOM is N-depleted.

Besides longitudinal changes, concentration and quality of OM in transport may depend on seasonal patterns. Among the numerous factors that affect both POM and DOM, snowmelt runoff in spring was found to increase organic nitrogen content (Hood et al., 2005; Petrone et al., 2007), and autumn storm events increase inputs from the terrestrial environment (Wilson & Xenopoulos, 2008; Singh et al., 2014; Garcia et al., 2015), supplying soil particles and decomposing leaf litter. Besides, forest phenology is a strong factor in determining both OM inputs, with important differences between deciduous and perennial forests (Singh et al., 2014) which differ in the timing of leaf litter input, and also light penetration and hence primary production, which generally increase in spring (Akamatsu et al., 2011; Halbedel et al., 2013).

Here, our aim was to study patterns of FPOM and DOM related to stoichiometric ratios (C:N:P) along the longitudinal axis of a headwater catchment and to test the hypothesis that longitudinal changes in OM stoichiometry depend on the season. With this purpose, we quantified FPOM in transport and DOM at four sites located within the catchment, from the high mountain forest to the open valley, and we analyzed their C:N, C:P and N:P ratios.

Materials and methods

Study site

The study was carried out in the Chall-Huaco catchment (41°13′S; 71°20′W), a tributary of Lake Nahuel Huapi, in the Nahuel Huapi National Park, North Patagonia, Argentina. The area is not inhabited, and there are only a few trails for hiking activities. The climate is cold-temperate, with a mean annual temperature of 8°C and rainfall around 1700 mm year^{-1} mainly in autumn, with snow accumulations during winter and early spring. Soils are Andisols, weakly developed, of sandy loam texture, characterized by a high capacity to stabilize OM (organic C > 8%), store water, buffer pH and retain P, (bioavailable $P < 15$ mg kg^{-1}) (Mazzarino et al., 1998; Satti et al., 2003). Steep slopes characterize the landscape. Channel configuration alternates interspersed step-pool reaches with a pool-riffle structure. Glacial and fluvial forces shaped the lower catchment, with smooth slopes, and morphology determined by run-riffle pool habitats.

The upper reach (1500–1200 m a.s.l.) drains an old growth forest of *Nothofagus pumilio* (Poep. and Endl.) Krasser (lenga beech). In the Patagonian Andes, this deciduous beech constitutes the highest mountain belt of the temperate forest, up to the timberline (Villalba et al., 1997). *Nothofagus pumilio* leaves constitute almost 100% of the material that enters the stream, which reaches 200 g m^{-2} in May, and in the stream it accumulates in packs of up to 1 m^2 and 30 cm deep (Albariño et al., 2009). In the downstream reach (1050 m a.s.l.) the vegetation is open, mostly dominated by shrubs like *Diostea juncea* (Gillies and Hook.) Miers., *N. antarctica* (Forest.) Oerst., and *Schinus patagonica* (Phil.) I.M. Johnst. The hydrological regime presents peak discharges in autumn (rain)

and spring (snowmelt) and the lowest values in early autumn. The streambed is dominated by cobbles and boulders. The presence of wood logs is more frequent at the upper, forested reaches.

According to previous studies of the same river sites (Buria et al., 2010; Díaz Villanueva et al., 2010) benthic leaf litter is higher in autumn (24 ± 8 g dry mass (DM) m^{-2} in S1, 34 ± 3 g DM m^{-2} in S2 and 23 ± 10 g DM m^{-2} in S4). The amount of benthic leaf litter was only 30% higher in S1 and S2 than in S4, revealing the importance of OM transport and retention downstream. Periphyton chlorophyll a concentration (Chl a) is higher in spring and downstream (0.56 ± 0.15 μg Chl a cm^{-2} in S1, and 3.83 μg Chl a cm^{-2} in S4). In S1, periphyton is composed entirely of diatoms, while other groups appear downstream,

including the chrysophyte *Hydrurus foetidus*, the dominant alga in spring.

We established four sampling sites along the Chall-Huaco catchment (Fig. 1). The two upper sites, S1 and S2, run through the *N. pumilio* forest while the two downstream sites, S3 and S4, run through the open valley (stream order and catchment area in Table 1). The distance between S1 and S4 was 4.3 km. We considered four periods: Autumn was split in two: early autumn (sampled three times in April,) and late autumn (three times in May and once in June), because differences in discharge are high: heavy rains begin in May coincidently with the highest leaf litter input. In winter (July and August), it was not possible to have access to the sampling sites because of the snow. The other sampling dates were in spring (three times, once
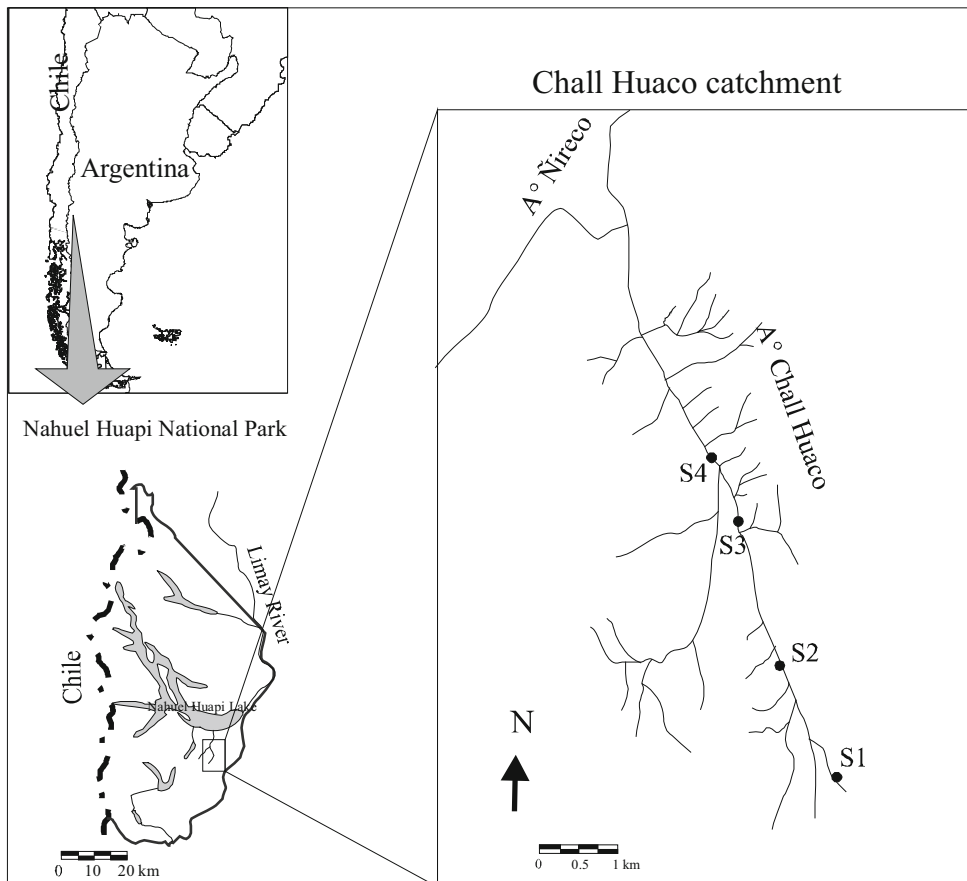
Fig. 1 Map of the Chall-Huaco catchment, located in the Nahuel Huapi National Park, Argentina. Sampling sites (S1–S4) are indicated on the map

Table 1 Catchment area, stream order, altitude and mean values of oxygen saturation levels (%), oxygen concentration, conductance, temperature, inorganic nutrients (SRP: phosphorus, Nitrate, and ammonium) and chlorophyll *a* concentration in the water column at the four sampling sites (mean $\pm$ standard error)

	S1	S2	S3	S4
Catchment area (km^2)	0.496	3.529	6.403	20.618
Stream order	2	3	3	4
Altitude (m a.s.l.)	1530	1215	1083	1048
Discharge ($l\ s^{-1}$)				
Early autumn	5	11	18	210
Late autumn	34	92	167	625
Spring	69	244	365	1155
Summer	12	44	69	565
$[O_2]$ ($mg\ l^{-1}$)	10.7 ± 0.4	10.3 ± 0.4	10.4 ± 0.3	10.6 ± 0.3
Conductance ($\mu S\ cm^{-1}$)	71.8 ± 2.2	73.8 ± 3.7	82.6 ± 2.8	71.5 ± 3.4
Temperature (°C)				
Mean	4.7 ± 1.0	5.8 ± 0.9	7.9 ± 1.1	6.9 ± 1.0
Max.	11.7	11.5	14.1	13.5
Min.	0.2	2.3	3.2	2.9
SRP ($\mu g\ l^{-1}$) ($N = 11$)				
Early autumn	2.8 ± 0.3	5.2 ± 0.8	2.9 ± 0.6	2.3 ± 0.5
Late autumn	3.0 ± 0.5	3.1 ± 0.5	3.1 ± 0.4	3.0 ± 0.6
Spring	<2	2.9 ± 0.6	<2	<2
Summer	4.0 ± 0.1	4.1 ± 0.1	<2	<2
Nitrate ($\mu g\ N\text{-}NO_3\ l^{-1}$) (Feb.–June)	4.5–9.5	23.42–1.3	Not measured	Only June: 23.00
Ammonium ($\mu g\ N\text{-}NH_4\ l^{-1}$)	4.8–15.6	7.7–5.8	Not measured	Only June: 2.7
Chlorophyll *a* ($\mu g\ l^{-1}$)				
May	0.19 ± 0.02	0.32 ± 0.02	0.46 ± 0.08	0.44 ± 0.12
Dec.	0.60 ± 0.07	0.76 ± 0.09	1.91 ± 0.10	1.31 ± 0.14

in October, once in November and once in December) and summer (twice, once in February and once in March). Sampling was carried out always between 10 and 12 AM, to minimize diurnal changes.

Physical variables

At each sampling site and date, water conductance (chemical conductivity corrected by temperature), water temperature, and dissolved oxygen were measured with a multiprobe instrument (YSI85). Stream width and depth were recorded, and water velocity was measured with a digital flowmeter (Global Water, California, USA) to calculate stream discharge. Light intensity (Photosynthetically Active Radiation, PAR) was averaged from the instantaneous measures collected with a QSL-2100 Irradiance Sensor (Biospherical Instruments, Inc., San Diego, California) along a longitudinal 30-m transect by wading the stream channel.

Particulate and dissolved organic matter

Fine particulate and dissolved OM was sampled at four sites of the Chall-Huaco catchment. Fine particulate OM in transport (0.7 μm < FPOM < 1 mm) was obtained by pre-filtering water through an acid-washed 1-mm-sieve followed by filtration onto a pre-weighed and combusted GF/F filters (pore size 0.7 μm, Whatman). The filters were weighed after drying at 60°C for 48 h. Carbon content in FPOM was analyzed by filtering stream water and measuring the dry sample in a CN analyzer (Thermo Finnigan Flash EA 1112). Particulate organic P and N were calculated from the difference between total P (TP) and dissolved P (TDP) or total N

(TN) and dissolved (TDN), respectively. Total phosphorus (TP) and total nitrogen (TN) were determined from non-filtered samples, and the dissolved fraction from filtered (GF/F) samples. Both non-filtered and filtered samples were digested with persulphate potassium at 125°C at 1.5 atm for 1 h. Afterwards, P concentration was measured as soluble reactive phosphorus (SRP), through the ascorbate-reduced molybdenum method (APHA, 2005) and N was measured after acidification with H_2SO_4 (Valderrama, 1981).

For the dissolved fraction of organic C, samples were filtered through pre-combusted GF/F filters. A Shimadzu analyzer (TOC-VCSH) was used to measure dissolved organic carbon (DOC) concentration. Inorganic P concentration was obtained from filtered samples as SRP. Inorganic nitrogen (NH_4^+ and NO_3^-) was measured only in February and June (low and high discharge periods, respectively). Ammonium determination was measured with the indophenol blue method (APHA, 2005), and NO_3^- was measured as NO_2^- after reduction through a cadmium-copper column (Wood et al., 1967) and quantified spectrophotometrically. Dissolved organic P (DOP) was calculated from the difference between TDP and SRP. As inorganic N was measured only once, organic N calculation was not possible; however, as inorganic N values were very low, and are generally low in Patagonian lakes, (between 0.6 and 18% of the TN, (Diaz et al., 2007)) we assumed that TDN would be a good reflection of dissolved organic N (DON). Perakis & Hedin (2002) reported that, contrary to the relatively polluted areas of the Northern Hemisphere, in Patagonian forest DON may account for 61–97% of total *N* in running waters.

Specific ultra-violet absorbance at 254 nm ($SUVA_{254}$) was used as indicative of the amount of humification or aromaticity within the samples (Weishaar et al., 2003). Filtered stream water was analyzed by spectrophotometric measures using a double beam spectrophotometer (Shimadzu UV2450) in 10-cm quartz cuvettes at 250 nm. The absorption coefficient (a_{254}) was obtained following Kirk (1994):

$$a = 2.303 A l^{-1},$$

where *a* is the absorption coefficient (m^{-1}), A is the absorbance, and l is the cuvette path length (m). $SUVA_{254}$ was calculated by dividing a_{254} by the DOC concentration in mg l^{-1}.

Chlorophyll *a*

Chlorophyll *a* concentration in seston was measured in May and November, by filtering 500 ml stream water ($N = 3$) through GF/F filters. The pigment extraction was done with 90% hot ethanol and then frozen for 24 h (Nusch, 1980). Measurements were carried out using a fluorometer (Turner designs 10-AU, Sunnyvale, USA).

Benthic leaf litter

Seasonal changes in the amount of benthic coarse particulate OM in some points of this catchment have been reported in Buria et al. (2010) and Díaz Villanueva et al. (2010). Stoichiometric relations of lenga beech leaf litter were obtained from stream bottom samples collected in May at each site. They were dried at 60°C for 72 h and a subsample was ground to powder with a mortar and pestle, then carbon and nitrogen were quantified in a CN analyzer (Thermo Finnigan Flash EA 1112). To measure P concentration, another subsample was weighed and combusted at 500°C for 4 h, and the ashes were dissolved in water to measure SRP as explained above.

As lenga beech leaves constitute 95% of the leaf litter input (Albariño et al., 2009), their leaching might be responsible for much of the DOC that enters the streams. So, we quantified carbon and nutrients derived from lenga beech leachates in the laboratory. Freshly fallen leaves were collected from the ground and air dried for 72 hs (fresh mass, FM) at room temperature. Afterwards, a correlation factor was obtained to estimate dry mass (DM) from FM. One gram of FM was submerged into 150 mL MilliQ water in sterile Erlenmeyers and left in the dark at 4°C for 48 h. Afterwards, DOC concentration was quantified with a TOC (as explained earlier) and expressed as mg C per g leaf DM. $SUVA_{254}$ of leachates was obtained as described above. Also SRP and TDP were measured in leachates, and organic P was calculated as the difference between TDP and SRP.

Statistical analyses

Statistical differences in DOM and POM quantity and stoichiometric ratios among sites in the four seasons were tested with RM-ANOVA, considering that each

date is a repeated measure and the site is the factor (one factor, four levels) using SPSS. Where differences were found, a posteriori tests were conducted with the Holm-Sidak method, with an overall significance level of $P < 0.05$. Normality and homoscedasticity were confirmed prior to ANOVA. Differences in Chl *a* concentration and benthic lenga beech litter stoichiometric ratios among sites were analyzed with one-way ANOVA.

To analyze the magnitude of the individual effects, Eta squared (h^2) in the effect size in ANOVA was calculated as follows:

$$h^2 = SS_{effect} / SS_{total},$$

where SS_{effect} is the Sum of Square for the factor (site, date, and the interaction) and SS_{total} is the total sum of squares for all effects, interactions, and errors in the ANOVA.

Results

Field characteristics

According to the bimodal hydrological regime dominating streams in Northern Patagonia, the highest discharge was registered in mid-autumn (May), caused by rains, Followed by spring (November) due to snowmelt with the lowest occurring in early autumn (April) (Table 1). Inorganic nutrients (SRP, NO_3^- and NH_4^+) were always low at all sampling sites; in particular, SRP was below the detection level during spring and summer. Oxygen concentration and conductance remained almost constant, but temperature was higher at the two lowest sites (Table 1). Light intensity varied from 214 (summer) to 319 $\mu E\ m^{-2}\ s^{-1}$ (late autumn) in the forest and from 612 $\mu E\ m^{-2}\ s^{-1}$ (late autumn) to 2000 (summer) in the open sites.

Organic matter dynamics

Fine POC in transport decreased along the catchment (Fig. 2a; Table 2), being 42% lower at S4 than at S1. Although the decrease depended on the season, being more gradual in spring and summer, site explained 68% of the variation (Table 2). Chlorophyll *a* concentration in the FPOM fraction was higher in the open sites (S3 and S4) than in the forest sites (S1 and S2)
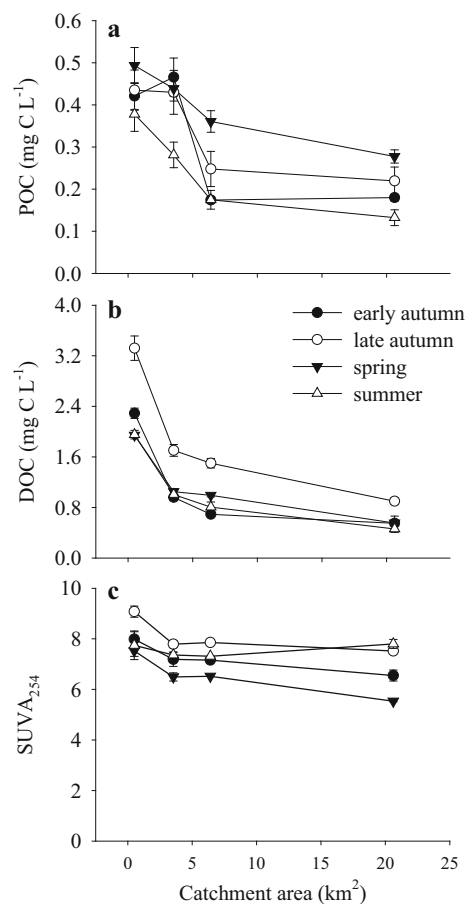
Fig. 2 a Fine particulate organic carbon (FPOC), **b** dissolved organic carbon concentration (DOC), and **c** specific ultra-violet absorbance at 254 nm ($SUVA_{254}$) at the different sampling sites (*error bars*: *standard error*)

both in December and May, and it was higher in all sites in December (Table 1).

Dissolved OM concentration ranged from 3.90 mg C l^{-1} at S1 in late autumn to 0.32 mg C l^{-1} at S4 in summer, showing a significant decrease from S1 to S4 (Fig. 2b; Table 2). Temporal dynamics were similar in all sampling sites, with the highest values in late autumn, which coincided with the end of leaf abscission time. DOM aromaticity significantly decreased downstream, but it was most strongly affected by the season, which explained 52% of the variation (Table 2; Fig. 2c). DOM aromaticity was much more variable during the year in S4, and in the summer it was as high as in S1.

Table 2 Results of the one-way repeated measure ANOVA. Eta squared (h^2) is the effect size in ANOVA

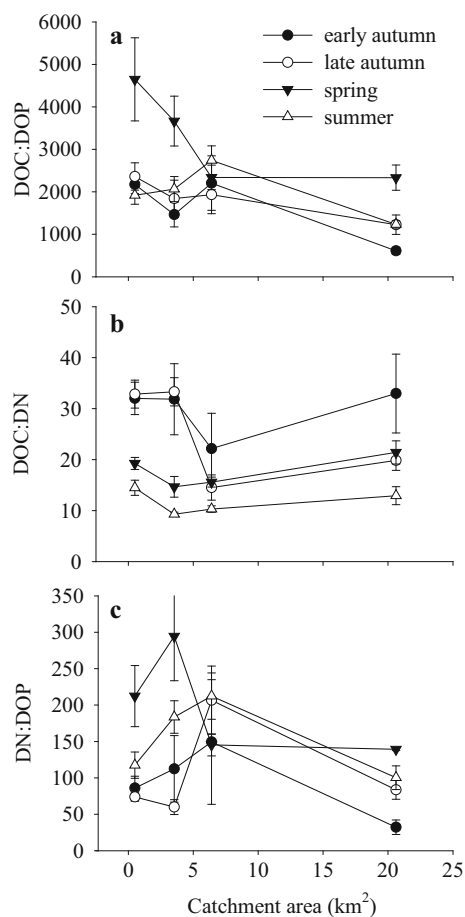|	Site			Season			Site × season			A posteriori
	F	P	h^2	F	P	h^2	F	P	h^2	
FPOC	114.74	<0.001	68.3	41.605	<0.001	19.97	4.97	0.001	6.5	Early and late autumn: S1 = S2 > S3 = S4 Spring: S1 = S2 > S3 > S4 Summer: S1 > S2 > S3 = S4
DOC	1871.3	<0.001	76.2	460.449	<0.001	18.75	35.27	<0.001	4.6	S1 > S2 > S3 > S4
SUVA	517.40	<0.001	33.0	291.067	<0.001	51.85	7.24	0.002	13.2	Early, late autumn and Spring: S1 > S2 = S3 > S4 Summer: S1 = S4 > S2 = S3
FPOM-C:P	9.90	0.005	10.18	48.715	<0.001	48.61	10.317	0.002	30.60	Early autumn: S4 > S3 = S2 > S1 Late autumn: S1 = S2 > S3 = S4
FPOM-C:N	3.21	0.083	4.30	18.673	<0.001	40.61	4.421	0.009	31.90	Late autumn and spring: S1 = S2 > S3 = S4
FPOM-N:P	5.29	0.032	16.22	9.94	<0.001	21.07	10.70	0.001	52.17	Early autumn: S4 > S3 > S1 = S4 Spring: S1 = S2 > S3 = S4 Summer: S4 > S3 = S2 = S1
DOC:DOP	12.49	0.002	22.14	12.565	<0.001	33.54	1.78	0.220	15.80	S1 = S2 = S3 > S4
DOC:DN	6.50	0.015	12.78	34.534	<0.001	54.82	3.53	0.028	15.40	Early autumn: S4 > S3 Late autumn: S1 = S2 > S3 = S4
DN:DOP	12.93	0.002	19.75	10.544	<0.001	27.00	2.97	0.062	25.94	Early autumn: S3 > S4 Late autumn: S3 > S2 Spring: S1 = S2 > S3 = S4
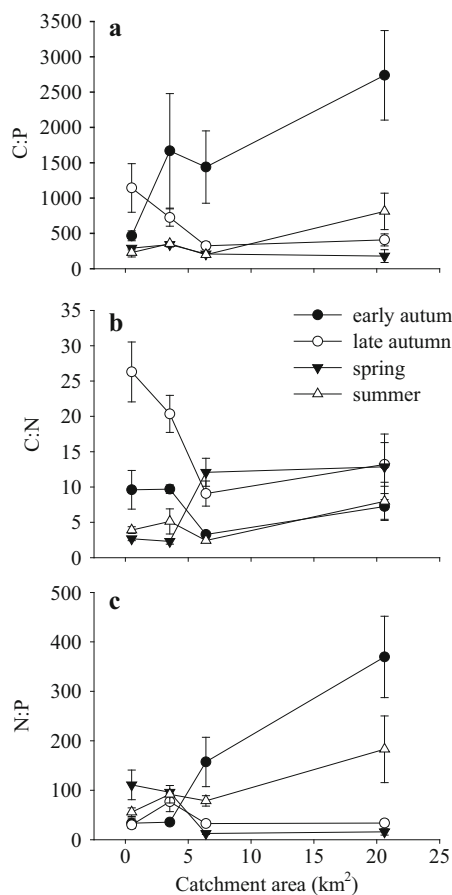
Fig. 3 Elemental atomic ratios in fine particulate organic matter **a** carbon:phosphorus; **b** carbon:nitrogen; and **c** nitrogen:phosphorus, at the different sampling sites (*error bars*: *standard error*)

Fig. 4 Elemental atomic ratios in dissolved organic matter **a** dissolved organic carbon:dissolved organic phosphorus; **b** dissolved organic carbon:dissolved nitrogen; and **c** dissolved nitrogen:dissolved organic phosphorus, at the different sampling sites (*error bars*: *standard error*)

Organic matter stoichiometry

All the changes in OM stoichiometry along the catchment, both dissolved and particulate, depended on season (significant interaction term), except for DOM C:P and N:P ratios (Table 2). In fact, the effect of the season in the stoichiometry of FPOM was higher than the effect of site and of the interaction (Table 2). In general, FPOM-C:P was higher in late autumn (except in S1) (Fig. 3a). FPOM-C:N was higher in late autumn in the forest, but in the open sites it was higher both in late autumn and spring (Fig. 3b). In contrast, N:P was more affected by the interaction between both factors than by any of the two separately, being higher downstream in summer and early autumn (low discharge seasons) (Fig. 3c; Table 2).

The lowest DOC:DOP values were found downstream; however, the effect of season was stronger

than the effect of site (Table 2), and the higher values were found in spring (Fig. 4a). Differences in DOC:DN between forest and open sites were significant in late autumn (Table 2), and there was a seasonal pattern of being lower in spring–summer than in autumn (Fig. 4b). The same occurred with N:P ratio (DN:DOP) (Table 2), with a tendency of being higher in the forest in spring (Fig. 4c). So, DOM in spring had less P but more N and this occurred both in forest and open sites.

Lenga beech leaf litter and leachate stoichiometry

Benthic lenga beech leaf litter collected in May did not vary significantly in nutrient stoichiometry along the

Table 3 Benthic lenga leaf litter stoichiometric ratios in the four sampling sites (May samples) and p value for the one-way ANOVA among sites

	C:P	C:N	N:P
S1	2213 ± 192	78.6 ± 7.8	29.2 ± 5.5
S2	1479 ± 16	84.0 ± 7.6	16.5 ± 1.0
S3	1418 ± 206	97.0 ± 10.5	15.4 ± 3.7
S4	1725 ± 35	96.2 ± 11.5	19.4 ± 2.8
P	0.052	0.47	0.17
Senescent fresh leaves	1729b	91a	19b

Bottom row: stoichiometric ratios of senescent lenga leaves, according to (a) Satti et al. (2003) and (b) Diehl et al. (2007)

longitudinal gradient (Table 3). Elemental ratios were also similar to the ratios of freshly fallen leaves reported by other authors (Table 3).

Lenga beech leachate had 23.16 ± 1.89 mg C g^{-1} and 54.98 ± 0.21 µg organic P g^{-1}, resulting in a DOM-C:P ratio of 1088.6 ± 89.1. Much of the P in leaves leached as SRP (284.42 ± 1.11 µg P g^{-1}). If we consider both organic and inorganic P, C:P was 418. As C:P in leaves (mean value: 1708) was higher than C:P in leachate, lenga beech leaves lose proportionally more P than C after entering the water. The SUVA$_{254}$ of the leachate was 26.78.

Discussion

The hypothesis that longitudinal changes in OM stoichiometry depend on the season was confirmed by the significant interaction between site and date. However, while FPOM and DOM concentrations were more affected by site, their stoichiometry was more affected by season. Concentrations of FPOM and DOM were higher upstream as we expected since forests are major sources of OM in streams (Meyer et al., 1998; Pusch et al., 1998) and it was in accordance to previous studies of the same river sites (Buria et al., 2010; Díaz Villanueva et al., 2010), where benthic leaf litter was 30% higher in S1 and S2 than in S4. On the other hand, changes in OM stoichiometry along the catchment strongly depended on the season, but the factors affected differently FPOM and DOM. While site and season interacted to alter FPOM nutrient composition, interactions in the DOM C:P and N:P (but not C:N) were not significant, meaning that these differences were an additive function of site and season. These contrasting results might be due to the different processes that undergo

DOM and FPOM. It is probable that FPOM in transport was more transformed than DOM, while differences along the catchment in DOM nutrient content were more affected by the relative contribution of leaching from the dominant source of OM in these streams at different times of year.

Longitudinal decrease in FPOM concentration was more abrupt in autumn, while in spring the increase of FPOM downstream reduces the difference with upstream sites. Different seasonal patterns between the forest and the open sites may illustrate different origins of FPOM, and/or differences in FPOM motility, due to discharge pulses. Downstream increases in spring may respond to FPOM mobilization caused by higher discharges due to snowmelt and runoff (Table 1) and also may reflect the increment in periphyton production, which may enter the water column by dislodgement. The latter was reflected in our study with a higher chlorophyll *a* concentration in spring and downstream.

Longitudinal depletion of DOC concentration was even more abrupt (it was 24–28% lower downstream than upstream), with similar seasonal dynamics in all sampling sites, showing a clear coincidence with leaf abscission (higher values in autumn). Biofilms can rapidly immobilize DOM, reducing export to downstream reaches (Romaní et al., 2004). However, some dilution effect is also possible. DOM derived from lenga beech leaf leachates was highly aromatic and might be responsible for the higher aromaticity of DOM upstream. Although SUVA$_{254}$ was lower downstream, we would have expected a higher decrease because algae-derived DOM is less aromatic. This result would suggest that a high proportion of DOM in lower reaches might derive from leaf litter leachates produced in the forest.

Variations on stoichiometric ratios of FPOM along the catchment depended on the season (significant interaction, Table 2), with a strong season effect (48%). FPOM is a pool of matter that derives from different origins, composed by leaf litter and other plant fragments, feces, periphyton sloughs, and soil particles (Akamatsu et al., 2011; Sakamaki & Richardson, 2013). Each of these components has a particular stoichiometric ratio that influences FPOM stoichiometry. Changes along the catchment depended on the season, perhaps as a consequence of the interacting effects of FPOM mobilization dependent on discharge and the variation in proportion of FPOM from different origins according to the season. Through our data, it was not possible to determine the origin of FPOM for each season. However, we can compare FPOM stoichiometry with that of leaf litter, soil and periphyton. Except in early autumn, FPOM-C:P was lower than that of leaf litter (Table 3) and similar to that of periphyton (C:P = 144–235 May samples, unpublished data). Similarly, FPOM-C:N was always much lower than that of leaf litter but only slightly lower than C:N of soil (C:N = 21, reported for *N. pumilio* forest soil (Satti et al., 2003)); and, except in late autumn in the forest, it was very similar to periphytic C:N (near 7). We also acknowledge that FPOM stoichiometry would not only depend on its origin (which will always be a mix), but it also can undergo transformations. One transformation is due to heterotrophic consumption and another is microorganism colonization, that would result in an enrichment with P and N (Cross et al., 2005; Cleveland & Liptzin, 2007). Such numerous mechanisms would need further studies to determine how season modifies FPOM stoichiometry in these systems.

DOM stoichiometry also depended on the site and the season but the interaction between these factors was significant only for DOM-C:N. Like FPOM, DOM bulk also derives from different sources (leaf litter and soil leachates, algal exudates), but season and site did not interact. Seasonal dynamics of DOM inputs influenced DOM stoichiometry independently on site. Seasonal patterns showed that C:P increased in spring, indicating that DOM (both allochthonous and/or autochthonous) entering the stream, at this time is P-depleted. On the contrary, season and site interacted in DOM-C:N, which was higher in the forest in autumn, but decreased in spring and summer, reaching similar values as those in the downstream sites. We did not find a correlation of C:N with aromaticity as previously reported for other streams (Hood et al., 2005; Balcarczyk et al., 2009), suggesting that in our study sites, N might not derive only from aromatic molecules. These opposed patterns of C:P and C:N changes in the DOM fraction led to very high N:P ratios upstream in spring. In particular, N:P was always higher than 30 and mostly higher than 100, while biofilms usually have N:P ratios of 10–30 (Gibson & O'Reilly, 2012). Andean Patagonian catchments are generally very poor in inorganic P (Diaz et al., 2007), so it is probable that heterotrophic use of organic P was increased by the limiting inorganic P in the water.

Understanding the contribution of OM from forested headwaters to downstream reaches has long been of interest, but still some mechanisms governing nutrient cycling along the catchment remain unexplored. Natural patterns of OM export from headwaters are predicted to change due to human activities at catchment or at global scales driven by climate change (Kominoski & Rosemond, 2011). Andean Patagonian freshwaters are still among the most nutrient-poor and unpolluted in the world and comprise numerous headwater streams which feed large glacial-origin oligotrophic lakes, important freshwater reservoirs. This study provides a first insight into the different seasonal patterns in OM stoichiometry and raises new questions that could be the basis for further studies related to headwaters of the Patagonian Andes.

References

Akamatsu, F., S. Kobayashi, K. Amano, S. Nakanishi & Y. Oshima, 2011. Longitudinal and seasonal changes in the origin and quality of transported particulate organic matter along a gravel-bed river. Hydrobiologia 669: 183–197.

Albariño, R., V. Díaz Villanueva & L. Buria, 2009. Leaf Litter Dynamics in a Forested Small Andean catchment, Northern Patagonia, Argentina. Ecological advances on Chilean temperate rainforests Academia Press, Gent[Links] 183–211.

Apha, A. P. H. A., 2005. Standard methods for the examination of water, sewage, and wastewater. American Public Health Association, Washington DC.

Balcarczyk, K. L., J. B. Jones, R. Jaffé & N. Maie, 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry 94: 255–270.

Buria, L., R. Albariño, V. Díaz Villanueva, B. Modenutti & E. Balseiro, 2010. Does predation by the introduced rainbow trout cascade down to detritus and algae in a forested small stream in Patagonia? Hydrobiologia 651: 161–172.

Cleveland, C. C. & D. Liptzin, 2007. C:N: P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85: 235–252.

Cross, W. F., J. P. Benstead, P. C. Frost & S. A. Thomas, 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50: 1895–1912.

Diaz, M., F. Pedrozo, C. Reynolds & P. Temporetti, 2007. Chemical composition and the nitrogen-regulated trophic state of Patagonian lakes. Limnologica–Ecology and Management of Inland Waters 37: 17–27.

Díaz Villanueva, V., L. Buria & R. Albariño, 2010. Primary consumers and resources: annual variation in two contrasting reaches of a Patagonian mountain stream. Annales de Limnologie–International Journal of Limnology 46: 21–28.

Finlay, J. C., J. M. Hood, M. P. Limm, M. E. Power, J. D. Schade & J. R. Welter, 2011. Light-mediated thresholds in stream-water nutrient composition in a river network. Ecology 92: 140–150.

Frost, P. C., J. H. Larson, C. A. Johnston, K. C. Young, P. A. Maurice, G. A. Lamberti & S. D. Bridgham, 2006. Landscape predictors of stream dissolved organic matter concentration and physicochemistry in a Lake Superior river watershed. Aquatic Sciences 68: 40–51.

Frost, P. C., L. E. Kinsman, C. A. Johnston & J. H. Larson, 2009. Watershed discharge modulates relationships between landscape components and nutrient ratios in stream seston. Ecology 90: 1631–1640.

Garcia, R. D., M. Reissig, C. P. Queimaliños, P. E. Garcia & M. C. Dieguez, 2015. Climate-driven terrestrial inputs in ultraoligotrophic mountain streams of Andean Patagonia revealed through chromophoric and fluorescent dissolved organic matter. Science of The Total Environment 521: 280–292.

Gibson, C. A. & C. M. O'Reilly, 2012. Organic matter stoichiometry influences nitrogen and phosphorus uptake in a headwater stream. Freshwater Science 31: 395–407.

Green, M. & J. C. Finlay, 2010. Patterns of hydrologic control over stream water total nitrogen to total phosphorus ratios. Biogeochemistry 99: 15–30.

Halbedel, S., O. Büttner & M. Weitere, 2013. Linkage between the temporal and spatial variability of dissolved organic matter and whole stream metabolism. Biogeosciences Discussions 9: 18253–18293.

Hood, E., M. W. Williams & D. M. McKnight, 2005. Sources of dissolved organic matter (DOM) in a Rocky Mountain stream using chemical fractionation and stable isotopes. Biogeochemistry 74: 231–255.

Hood, E., M. N. Gooseff & S. L. Johnson, 2006. Changes in the character of stream water dissolved organic carbon during flushing in three small watersheds, Oregon. Journal of Geophysical Research: Biogeosciences (2005–2012) 111: G1.

Kirk, J. T., 1994. Estimation of the absorption and the scattering coefficients of natural waters by use of underwater irradiance measurements. Applied optics 33: 3276–3278.

Kominoski, J. S. & A. D. Rosemond, 2011. Conservation from the bottom up: forecasting effects of global change on dynamics of organic matter and management needs for river networks. Freshwater Science 31: 51–68.

Larson, J. H., P. C. Frost, Z. Zheng, C. A. Johnston, S. D. Bridgham, D. M. Lodge & G. A. Lamberti, 2007. Effects of upstream lakes on dissolved organic matter in streams. Limnology and Oceanography 52: 60–69.

Laudon, H., M. Berggren, A. Ågren, I. Buffam, K. Bishop, T. Grabs, M. Jansson & S. Köhler, 2011. Patterns and dynamics of dissolved organic carbon (DOC) in boreal streams: the role of processes, connectivity, and scaling. Ecosystems 14: 880–893.

Mazzarino, M., M. Bertiller, T. Schlichter & M. Gobbi, 1998. Nutrient cycling in Patagonian ecosystems. Ecología Austral 8: 167–181.

Meyer, J. L., J. B. Wallace & S. L. Eggert, 1998. Leaf litter as a source of dissolved organic carbon in streams. Ecosystems 1: 240–249.

Nusch, E., 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Archive für Hydrobiologie Beih 14: 14–36.

Perakis, S. S. & L. O. Hedin, 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. Nature 415: 416–419.

Petrone, K., I. Buffam & H. Laudon, 2007. Hydrologic and biotic control of nitrogen export during snowmelt: a combined conservative and reactive tracer approach. Water Resources Research 43: n/a-n/a.

Pusch, M., D. Fiebig, I. Brettar, H. Eisenmann, B. K. Ellis, L. A. Kaplan, M. A. Lock, M. W. Naegeli & W. Traunspurger, 1998. The role of micro-organisms in the ecological connectivity of running waters. Freshwater Biology 40: 453–495.

Romaní, A., H. Guasch, I. Muñoz, J. Ruana, E. Vilalta, T. Schwartz, F. Emtiazi & S. Sabater, 2004. Biofilm structure and function and possible implications for riverine DOC dynamics. Microbial Ecology 47: 316–328.

Sakamaki, T. & J. S. Richardson, 2013. Nonlinear variation of stream-forest linkage along a stream-size gradient: an assessment using biogeochemical proxies of in-stream fine particulate organic matter. Journal of Applied Ecology 50: 1019–1027.

Satti, P., M. J. Mazzarino, M. Gobbi, F. Funes, L. Roselli & H. Fernandez, 2003. Soil N dynamics in relation to leaf litter quality and soil fertility in north-western Patagonian forests. Journal of Ecology 91: 173–181.

Singh, S., S. Inamdar, M. Mitchell & P. McHale, 2014. Seasonal pattern of dissolved organic matter (DOM) in watershed sources: influence of hydrologic flow paths and autumn leaf fall. Biogeochemistry 118: 321–337.

Stephens, B. M. & E. C. Minor, 2010. DOM characteristics along the continuum from river to receiving basin: a comparison of freshwater and saline transects. Aquatic Sciences 72: 403–417.

Tank, J. L., E. J. Rosi-Marshall, N. A. Griffiths, S. A. Entrekin & M. L. Stephen, 2010. A review of allochthonous organic matter dynamics and metabolism in streams. Journal of the North American Benthological Society 29: 118–146.

Valderrama, J. C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine chemistry 10: 109–122.

Villalba, R., J. A. Boninsegna, T. T. Veblen, A. Schmelter & S. Rubulis, 1997. Recent trends in tree-ring records from high elevation sites in the Andes of northern Patagonia. Climatic Change 36: 425–454.

Webster, J. R. & J. L. Meyer, 1997. Organic Matter Budgets for Streams: A Synthesis. Journal of the North American Benthological Society 16: 141–161.

Weishaar, J. L., George R. Aiken, Brian A. Bergamaschi, Miranda S. Fram, Roger Fujii & K. Mopper, 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science & Technology 37: 4702–4708.

Wilson, H. F. & M. A. Xenopoulos, 2008. Ecosystem and seasonal control of stream dissolved organic carbon along a gradient of land use. Ecosystems 11: 555–568.

Wood, E. D., F. Armstrong & F. A. Richards, 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. Journal of the Marine Biological Association of the United Kingdom 47: 23–31.

Yoshimura, C., M. O. Gessner, K. Tockner & H. Furumai, 2008. Chemical properties, microbial respiration, and decomposition of coarse and fine particulate organic matter. Journal of the North American Benthological Society 27: 664–673.