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RESEARCH ARTICLE

Temperature and substrate chemistry as major drivers of interregional variability of leaf microbial decomposition and cellulolytic activity in headwater streams

Encarnación Fenoy[1], J. Jesús Casas[1,2,*], Manuel Díaz-López[1], Juan Rubio[1], J. Luís Guil-Guerrero[3] and Francisco J. Moyano-López[1]

[1]Department of Biology and Geology, ceiMar, University of Almería, Ctra. Sacramento s/n, 04120 Almería, Spain, [2]Andalusian Centre for the Evaluation and Monitoring of Global Change, CAESCG, 04120 Almería, Spain and [3]Department of Agronomy, University of Almería, Ctra. Sacramento s/n, 04120 Almería, Spain

*Corresponding author: Department of Biology and Geology, University of Almería, Ctra. Sacramento s/n, CITE II-B, 04120 Almería, Spain. Tel: +34950015501; Fax: +34950015476; E-mail: jjcasas@ual.es

One sentence summary: Water temperature and leaf litter quality are majors factors affecting microbial decomposition and cellulolitic activity in headwater streams.

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ABSTRACT

Abiotic factors, substrate chemistry and decomposers community composition are primary drivers of leaf litter decomposition. In soil, much of the variation in litter decomposition is explained by climate and substrate chemistry, but with a significant contribution of the specialisation of decomposer communities to degrade specific substrates (home-field advantage, HFA). In streams, however, HFA effects on litter decomposition have not been explicitly tested. We evaluated responses of microbial decomposition and β-glucosidase activity to abiotic factors, substrate and decomposer assemblages, using a reciprocal litter transplant experiment: 'ecosystem type' (mountain vs lowland streams) $\times$ 'litter chemistry' (alder vs reed). Temperature, pH and ionic concentration were higher in lowland streams. Decomposition for both species was faster in lowland streams. Decomposition of reed was more accelerated in lowland compared with mountain streams than that of alder, suggesting higher temperature sensitivity of decomposition in reed. Q_{10} (5°C–15°C) values of β-glucosidase activity were over 2. The alkaline pH and high ionic concentration of lowland streams depleted enzyme activity. We found similar relationships of decomposition or enzyme activity with abiotic factors for both species, suggesting limited support to the HFA hypothesis. Overall, our results suggest a prime role of temperature interacting with substrate chemistry on litter decomposition.

Keywords: β-glucosidase activity; pH; ionic strength; HFA hypothesis; aquatic hyphomycetes; substrate recalcitrance

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INTRODUCTION

Organic matter decomposition is a key component of the global carbon cycle that is being altered by human activity (i.e. global warming and land-use changes) (Chapin *et al.* 2009). Low-order streams are ecosystems fundamentally subsidised by organic detritus (i.e. leaves) from the surrounding terrestrial habitats, notably in forested regions (e.g. Wallace *et al.* 2015). Thus, natural or human-induced changes affecting leaf litter decomposition have the potential to alter stream food webs (Graça *et al.* 2015). In terrestrial ecosystems, climate, substrate chemistry and decomposers community composition are the fundamental drivers of leaf litter decomposition (Gholz *et al.* 2000; Keiser *et al.* 2011). Temperature, moisture and substrate chemistry might together explain about 70% of variation of leaf litter decomposition in forest soils (Gholz *et al.* 2000; Parton *et al.* 2007), but factors contributing to the remaining variance and their relative contribution are yet uncertain (e.g. Freschet, Aerts and Cornelissen 2012). Among these factors, the specialisation of decomposer communities on the litter type characteristic of its native ecosystem, commonly referred to as the 'home-field advantage' hypothesis (HFA; Hunt *et al.* 1988; Gholz *et al.* 2000), might contribute significantly to decomposition. For instance, in forest soils litter mass loss can be 4.2% faster on average at the home environment (Wang, Zhong and He 2013), notably in undisturbed forest ecosystems (Austin *et al.* 2014).

In low-order streams, microbial decomposition of leaf litter is largely driven by temperature, through increased microbial biomass and/or enzymatic activity at elevated temperatures (Friberg *et al.* 2009; Boyero *et al.* 2011; Ferreira and Chauvet 2011; Ferreira and Canhoto 2015; Ferreira, Chauvet and Canhoto 2015). A global experiment in low-order streams (Boyero *et al.* 2011) reported that temperature explained up to 40% of the variation of microbial decomposition, but the authors concluded that factors other than temperature likely influenced litter breakdown rates, accounting for the considerable residual variability detected. This is to be expected given that low-order streams capture, as a whole, much of the catchment's environmental heterogeneity due to their intimate connection with the surrounding landscape (Lowe and Likens 2005). Such remarkable variability in many abiotic (i.e. water chemistry) and biotic (i.e. riparian and aquatic communities) factors, operating at regional and local scales, may significantly affect leaf litter decomposition in streams (Graça *et al.* 2015), interacting synergistically or antagonistically with temperature, potentially masking its effect on decomposition. This is particularly true for streams in the Mediterranean basin, where landscape heterogeneity is elevated at multiple scales (e.g. Casas *et al.* 2006, 2011), besides the consideration of this region as a global 'hot-spot' in terms of high rates of climate change and land transformation (see reviews in García-Ruíz *et al.* 2011; Cooper *et al.* 2013).

The influence of direct effects of water chemistry on litter decomposition in streams has been widely tested: i.e. the concentration of dissolved nutrients (e.g. Woodward *et al.* 2012), ionic concentration (e.g. Suberkropp and Chauvet 1995) and pH (e.g. Dangles *et al.* 2004). However, indirect effects of abiotic factors are less profusely studied in these ecosystems. Water chemistry and other abiotic factors may also exert indirect, historical effects on decomposition by acting as ecological filters for community composition (e.g. Casas *et al.* 2011) and functional traits of species, likely affecting both the chemistry of terrestrial/riparian leaf litter inputs (substratum) and decomposers capabilities.

As in terrestrial ecosystems (Aerts 1997; Makkonen *et al.* 2012), leaf litter chemistry is also a major determinant of decomposition rates in streams (Lecerf and Chauvet 2008; Schindler and Gessner 2009), depending to a great extent on its concentration in structural carbohydrates. Cellulose represents almost half of primary production and its decomposition is a key process in the C cycle (Sinsabaugh, Antibus and Kinkins 1991). Thus, the evaluation of its hydrolysis into glucose is considered a good tool to test possible functional differences in microbial assemblages (Gessner *et al.* 2007; Schneider *et al.* 2012), since they are much affected both by the aforementioned abiotic factors (Suberkropp and Chauvet 1995; Gulis and Suberkropp 2003) as well as by the chemistry of leaf litter inputs (Woodward 2009; Bärlocher *et al.* 2013). However, as temperature sensitivity of decomposition appears to increase with increasing molecular complexity of the substrate ('temperature–quality' hypothesis *sensu* Bosatta and Ågren (1999); Davidson and Janssens (2006)), determining how much decomposition rates respond to the interaction between substrate recalcitrance and temperature, in interplay with other abiotic and biotic factors, is, therefore, a critical issue to understand ecosystem responses to global changes (Conant *et al.* 2011; Gonçalves, Graça and Canhoto 2013).

Regarding microbial communities, most studies, both in soil and streams, have concentrated on the effects of their diversity on decomposition (see reviews in Hättenschwiler, Fromin and Barantal 2011; Graça *et al.* 2015), but with controversial findings. A different approach to consider in this issue is the fact that microbial communities may adapt to exploit the prevalent litter inputs in the physic-chemical setting of their ecosystem, which will accelerate litter decomposition at home (HFA; Hunt *et al.* 1988; Gholz *et al.* 2000; Ayres *et al.* 2009; Strickland *et al.* 2009; Wang, Zhong and He 2013; but see Makkonen *et al.* 2012). If true, taxonomically and functionally dissimilar microbial communities are expected in ecosystems widely differing in physic-chemical features and prevalent chemical composition of litter inputs, which could lead to 'HFA' effects, theoretically determining differential responses of decomposition to abiotic factors and substrate chemistry. However, while in some terrestrial ecosystems significant HFA effects on litter decomposition have been demonstrated (see above), to our knowledge this hypothesis has not been explicitly tested in stream ecosystems.

The present work was designed to evaluate the relative importance of abiotic factors (temperature, pH and ionic content), interacting with a microbial community putatively adapted to exploit a basal resource of a given chemical composition, on litter decomposition and cellulolytic activity. For this purpose, we performed a reciprocal litter transplant in a combined 'ecosystem type' and 'litter chemistry' experiment. The enzyme selected as indicator of functional adaptations (resource/environment) of the microbiota was β-glucosidase, since it plays a pivotal role in the final step of cellulose degradation (e.g. Seidle and Huber 2005) and has been widely used in ecological studies (Kourtev, Ehrenfeld and Huang 2002; Artigas *et al.* 2011; Sinsabaugh and Shah 2011; Ylla, Romaní and Sabater 2012). In addition, the potential functional diversity of endoglucanases in the cellulase complex present in the leaf-degrading microbiota was evaluated using zymograms.

We hypothesised that decomposition rate and cellulolytic activity for each leaf species would be higher in its native ecosystem due to historical resource and abiotic environmental conditions to which microbial community has adapted. Differences between regions are expected to be more pronounced on the more recalcitrant leaf substrate (*Phragmites*) due to its putative higher intrinsic temperature sensitivity.

MATERIALS AND METHODS

Study sites and experimental design

Six permanent streams from two adjacent regions—three streams per region—with sharp biogeoclimatic contrast were selected in southern Spain. Streams from the Sierra Nevada mountains (hereafter mountain streams) are located at elevations ranging between 1300 and 1400 m a.s.l, drain forested watersheds (i.e. pine afforestation) with siliceous lithology and have a flow regime influenced by snowmelt. Their riparian vegetation is dominated by alder (*Alnus glutinosa* (L.) Gaertner) and willow (*Salix* spp.). Streams of the semiarid lowland of Almería (hereafter lowland streams) are located at elevations ranging between 50 and 300 m a.s.l and drain watersheds dominated by scrublands over calcareous-gypsum soils. The riparian vegetation of lowland streams is dominated by common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) and oleander (*Nerium oleander* L.). Additional environmental information on these regions is given in Casas *et al.* (2011). We performed a reciprocal litter transplant in a combined 'ecosystem type' and 'litter chemistry' experiment, by using two species of leaf litter—*A. glutinosa* (hereafter *Alnus*) and *P. australis* (hereafter *Phragmites*)—as representatives of mountain and lowland streams, respectively. A key trait differentiating litter chemistry of both species is that polymerised silicic acid strongly binds with cellulose in reed leaf litter (Ma and Yamaji 2006), which might confer higher recalcitrance and, thus, temperature sensitivity of decomposition to this substrate.

Environmental characterisation of streams

During the course of the field experiment we took triplicate measurements (2, 5 and 11 weeks) of electrical conductivity (EC), pH and dissolved oxygen in each stream with a multiparametric probe (HACH® model HQ-30d, Loveland, CO, USA). With the same periodicity, stream discharge was measured as described in Casas *et al.* (2011), and we collected water samples: an aliquot of which was used to measure alkalinity by acid titration to an end point of pH 4.5, and the rest of the water sample was filtered through glass fibre filters (APFC, Millipore®, Darmstadt, Germany) and frozen (–20°C) until analysed to determine, by standard methods (APHA 2005), the concentration of the following chemical parameters: Cl^-, argentometric titration method; SO_4^{2-}, turbidimetric method; NO_3^--N, ion chromatography; soluble reactive phosphorus (SRP), colourimetric determinations involving the formation of the phosphomolybdic acid blue complex. Water temperature was recorded hourly with HOBO Pendant (Onset Computer Corporation, Bourne, MA, USA) loggers during the full incubation period.

Initial leaf litter characteristics

Senescent leaves were collected just after abscission using litterfall traps in the case of *Alnus* and directly from standing-dead shoots in *Phragmites*. The leaves were collected from the riparian zone of a single stream per region, transported to the laboratory and dried at room temperature for 2 weeks. The toughness of 25 senescent leaves per plant species was measured on moistened material using a calibrated texturometer (TA.XT2 Plus, Stable Micro Systems, London, UK). A constant needle tip surface area (0.38 mm^2) was used for all measurements, thus measures of toughness were expressed in units of mass (g).

Thereafter, leaf litter was oven-dried (70°C, 72 h) and grounded to <1 mm particle size to be used for triplicate chemical analyses. Nitrogen (N) and C were determined using a Perkin Elmer series II CHNS/O elemental analyser (Perkin-Elmer, Shelton, CT, USA), with results expressed as %N and %C of leaf dry mass. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined using an ANKOM 200/220 fibre analyzer (ANKOM Technologies, Macedon, NY, USA). Percentages of hemicellulose, cellulose and lignin were calculated using the following equations: hemicellulose = (%NDF – %ADF) – %ash; cellulose = (%ADF – %ADL) – %ash; and lignin = %ADL – %ash. Ash concentration (% ash) was determined by incineration at 550°C for 5 h. The concentration of silica (Si) in leaf litter was determined using inductively coupled plasma atomic emission spectroscopy (Thermo ICAP 6500 duo, Thermo Fisher Scientific, Cambridge, UK), after microwave sample digestion in nitric acid (65%) and hydrogen peroxide (30%).

Leaf litter bags and decomposition

Portions (5.0 ± 0.2 g dry mass) of leaf litter were spray moistened and introduced in bags (15 × 15 cm) of 1-mm mesh size, which allowed water circulation inside the bag but avoided the action of macroinvertebrates. At the end of December 2012, leaf bags—20 bags per species and stream—were incubated tied to iron stakes anchored to the stream bed in riffles along a 50-m stream reach. Four bags per stream and species were retrieved after 48 h—to estimate leaching loss—and after 2, 5 and 11 weeks. Leaf bags were transported to the laboratory in an icebox kept at 4°C. The leaves were carefully removed from the bags, rinsed with filtered stream water to eliminate fine particles, oven-dried (70°C, 72 h) and weighed to determine % remaining dry mass to calculate decomposition rates. The four remaining leaf bags per species and stream were retrieved after 5 weeks of incubation, processed as above but, instead of being oven-dried, the material of the four bags was pooled, ground with a blender and used to measure ergosterol and prepare enzyme extracts (see below). Five weeks of incubation was considered enough for maximum colonisation and partial degradation of the plant material by fungi in these streams (Casas *et al.* 2011).

Fungal biomass

Ergosterol was determined as a measure of fungal biomass in samples. Lipids were extracted from litter with alkaline methanol, the crude extract cleaned and concentrated by solid-phase extraction, and ergosterol finally purified and quantified by high-performance liquid chromatography (Gessner and Schmitt 1996). Ergosterol was detected at 282 nm and quantified against ergosterol standards (Fluka, Buchs, Switzerland). The lipid concentration was converted into fungal biomass using a factor of 5.5 μg ergosterol mg^{-1} fungal dry mass (Gessner and Chauvet 1993) and the results were expressed as milligram fungal biomass per gram leaf dry mass.

Determination of activity and functional parameters of β-glucosidase

After incubation for 5 weeks, samples were grounded and homogenised in distilled water (1:3 w/v) with a blender. The extracts were centrifuged (12 000× *g* 15 min; 4°C) and the supernatant obtained was used as crude enzyme extract. The dry mass of samples was estimated by differential gravimetry of 1 g of each plant material after oven drying (70°C, 72 h; $n = 3$). The activity of β-glucosidase was evaluated by determining the hydrolysis of p-nitrophenol-β-D-glucopyranoside 5 mM with readings at 405 nm. All assays were performed in triplicate.

We evaluated the effect of the main factors of divergence between streams from both regions—temperature, pH and ionic strength (IS)—on β-glucosidase activity. The effect of temperature was evaluated incubating extracts at temperatures ranging between 5°C and 70°C. The enzyme efficiency under natural thermal conditions was calculated as the activity—measured at the average temperature registered in the stream of origin—expressed as a % with respect to the maximum activity measured at optimal conditions. Values of activity were used to calculate enzyme activation energy (E_a) and temperature coefficients (Q_{10}). The E_a was calculated from the Arrhenius plots constructed with values of activity measured within the above-mentioned temperature range. Temperature coefficients (Q_{10}), for the approximate temperature range (5°C–15°C) of divergence between the two regions, were obtained using the equation:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)},$$

where R_2 and R_1 are reaction rates observed at temperatures T_2 and T_1, respectively.

The effect of pH was evaluated within the range 4–9, using the following buffers: citrate (4 and 5), phosphate (6) and Tris-HCl (7, 8 and 9), 50 mM in all cases. Also, we measured enzyme activity at the average pH of the stream, which was expressed as a percentage of the maximum activity measured at optimal pH in each case. We evaluated the stability under different pH values by pre-incubating extracts in the above-mentioned buffers for different time periods (1, 2 and 4 h) and measuring residual activity in relation to the maximum.

We also calculated an adaptation of Q_{10} temperature coefficient to estimate the change in activity as pH increased one unit, for the pH range (7–8) in which both regions differ, approximately. For this purpose, we used reaction rates obtained from the pH stability test. The formula of 'Q_1' pH coefficient would be as follows:

$$Q_1 = \left(\frac{R_2}{R_1}\right)^{\left(\frac{1}{pH_2 - pH_1}\right)},$$

where R_2 and R_1 are reaction rates observed at pH_2 and pH_1, respectively.

The effect of IS on the activity of β-glucosidase was evaluated on extracts obtained from both plant species sampled incubated in the stream with higher EC (Río de Aguas, lowland). We used buffers with the same pH and total molar concentration, but differing in IS:

pH 6.0: IS 66 and 252 mM (citrate and phosphate, respectively)

pH 7.0: IS 46 and 108 mM (Tris-HCl and phosphate, respectively)

pH 8.0: IS 28 and 143 mM (Tris-HCl and phosphate, respectively).

Zymograms for endocellulase activity

We used aliquots of extracts previously concentrated using Amicon® Ultra-15 (10.000 MWCO) (Millipore, Billerica, MA, USA) for SDS-PAGE zymograms. These were prepared using 11% polyacrylamide and 0.4% carboxymethyl cellulose as substrate for the activity of endocellulases. The gel was rinsed in sodium citrate buffer (50 mM, pH 5, containing 25% isopropanol) for 30 min, and, thereafter, was incubated for 1 h in sodium citrate buffer (50 mM, pH 5) at 50°C to allow enzyme hydrolysis of the substrate. After rising pH with NaOH 0.1 N, gels were stained with Congo red (0.1%) for 30 min and destained in 1 M NaCl to reveal protein bands with cellulase activity. The soluble protein concentration in extracts was determined by the Bradford method (1976) using bovine serum albumin as standard.

Statistical analyses

We used mixed-model nested ANOVAs to test for differences in environmental variables among regions and streams ('region' fixed factor; 'stream' random factor nested within region), and two-sample *t*-tests to compare the physic-chemical characteristics of leaf litter between species.

Decomposition rates were calculated by fitting the % dry mass remaining over time (2, 5 and 11 weeks) to linear models ($M_t = M_0 - bt$) and exponential models ($M_t = M_0\, e^{-kt}$), where M_t is the remaining dry mass at time t, M_0 is the initial dry mass (corrected for leaching), and b and k are the linear and exponential decomposition rates, respectively. Streams differed in temperature, thus coefficients were also calculated with degree-days as the independent variable, which were computed from records of daily mean water temperature. We used three-way mixed-model ANCOVAs to compare decomposition rates—'region' and 'species' as fixed factors and 'stream' as random factor—with time (days) or thermal sum (degree-days) as the covariate.

Two-way ANOVA was used to test the effects of region (stream as experimental unit) and species on fungal biomass, decomposition efficiency (ratio of decay rate to fungal biomass; Gonçalves, Graça and Canhoto 2013), cellulolytic activity and its operational parameters as a function of temperature (efficiency and energy of activation) and pH, as well as on the number of isoenzymes. Three-way ANOVA was used to test for differences of enzymatic stability at different pH ('region', 'species' and 'pH' as factors; stream treated as experimental unit). We tested the effect of IS, at different pH values, on the residual enzymatic activity, in leaf litter extracts from the stream with the highest IS (Aguas), using repeated-measured ANOVA (three measures over time; 'IS', 'pH' and 'species' as factors).

To assess the relative effect of temperature and pH on enzyme activity we compared paired (by stream) Q_{10} and Q_1 mean values for each species within a given region using paired *t*-test. Furthermore, to evaluate the effect of region and species on differences in activity caused by temperature and pH (Q_{10}–Q_1), we used two-way ANOVA.

We used partial least squares (PLS) regression (Abdi 2003) to evaluate the relative importance of environmental variables as predictors of decomposition rates and cellulolytic activity: both activities, at the temperature and at the pH of the stream. Separate PLS models were developed for each dependent variable and leaf litter species to test the HFA hypothesis directly, by assuming that, given the large differences in environmental variables between regions, results would support the hypothesis if inverse relationship—among dependent variable and predictors—would be found between the two leaf litter species. All models were constructed with the autofit function in order to obtain the highest predictive (Q^2) value. A PLS model was considered significant when Q^2 exceeds a critical value of 0.097, and models with $Q^2 > 0.4$ were considered good (Eriksson *et al.* 2006). The relative influence of each predictor in a PLS model was expressed as the variable importance on projection (VIP). Predictors with a VIP >1 were considered as the most influential for the model (Eriksson *et al.* 2006).

All tests were performed on transformed variables, except pH, to make the variances homoscedastic, using log(x+1), or

Table 1. Environmental variables (mean ± SE; $n = 3$) measured in each stream, and the average for each region. Different superscript letters indicate significant ($P < 0.05$) differences among streams determined by post-hoc Tukey HSD test, following mixed-model nested ANOVA. Also shown are *F* values for the overall comparison between regions and among streams.

Location or comparison	O_2 (mg L^{-1})	O_2 (%)	Temperature (°C)	pH	EC (μS cm^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Cl^- (mg L^{-1})	SO_4^{2-} (mg L^{-1})	NO_3^--N (mg L^{-1})	SRP (μg L^{-1})	Discharge (L s^{-1})
Sierra Nevada											
Aldeire	10.8 ± 0.2^a	95.1 ± 0.7^{b,c}	3.8 ± 1.0^b	6.8 ± 0.1^d	41 ± 1^c	18 ± 2^c	12 ± 2^c	4 ± <1^c	0.38 ± 0.03^b	5 ± 2	48 ± 1^a
Jérez	10.7 ± 0.4^{a,b}	93.9 ± 1.1^{b,c}	3.8 ± 1.3^b	6.7 ± 0.1^d	48 ± 4^c	21 ± 1^c	10 ± 2^c	6 ± 1^c	0.17 ± 0.04^c	3 ± 2	52 ± 1^a
Lanteira	11.2 ± 0.3^a	94.9 ± 0.6^{b,c}	3.1 ± 1.1^b	6.9 ± 0.1^d	42 ± 2^c	17 ± 1^c	16 ± 2^c	5 ± 1^c	0.44 ± 0.05^b	3 ± 1	43 ± 1^a
Region average	**11.0 ± 0.2**	**95.0 ± 0.4**	**3.5 ± 0.7**	**6.8 ± 0.1**	**43 ± 1**	**19 ± 1**	**13 ± 1**	**5 ± <1**	**0.33 ± 0.02**	**4 ± 1**	**48 ± 1**
Semiarid lowland											
Aguas	9.5 ± 0.6^b	97.6 ± 5.3^b	15.5 ± <0.1^a	8.1 ± 0.1^c	3150 ± 17^a	159 ± 7^b	240 ± 26^a	1629 ± 52^a	0.98 ± 0.08^a	4 ± 2	39 ± 1^a
Negras	11.3 ± 0.1^a	107.2 ± 0.8^a	13.1 ± 0.4^a	8.5 ± <0.1^{a,b}	2645 ± 9^a	263 ± 10^a	177 ± 33^{a,b}	416 ± 54^b	0.14 ± 0.04^c	6 ± <1	3 ± 1^c
Vícar	8.7 ± 0.1^{c,b}	87.5 ± 0.7^c	13.9 ± <0.1^a	8.3 ± <0.1^{b,c}	996 ± 10^b	253 ± 14^a	121 ± 7^b	196 ± 12^b	0.36 ± 0.01^b	4 ± 1	9 ± 1^b
Region average	**9.1 ± 0.1**	**92.6 ± 1.3**	**14.2 ± 0.1**	**8.3 ± 0.0**	**2073 ± 7**	**225 ± 6**	**172 ± 24**	**747 ± 223**	**0.73 ± 0.03**	**4 ± 1**	**18 ± 5**
Comparison											
Region	2.1^{ns}	0.6 ^{ns}	62.6***	90.1***	109.7***	185.1***	74.6***	50.5**	0.5^{ns}	1.7^{ns}	6.1^{ns}
Stream	11.9***	11.9***	14.5***	6.9**	198.0***	19.9***	5.5**	34.2***	127.0***	0.5^{ns}	95.3***

$^{***}P < 0.001$; $^{**}P < 0.01$; ns, not significant ($P > 0.05$), Boldface = average value for each region.

arcsine $\sqrt{x}$ for percentages. We used Tukey HSD tests for pair-wise post-hoc comparisons when factors had more than two levels. All tests were carried out using Statistica software (Statsoft, Tulsa, OK, USA), except PLS regressions that were performed with XLSTAT (Addinsoft, New York, NY, USA).

RESULTS

Environmental setting

The sharp contrast in biogeoclimatic setting between regions was mirrored in stream's environmental conditions. Mountain streams had significantly lower temperature, pH, EC, alkalinity, Cl^- and SO_4^{2-} compared to lowland streams (Table 1). However, lowland streams formed a group more heterogeneous compared to mountain streams, primarily due to greater among-streams variability in EC, SO_4^{2-}, NO_3^--N and discharge in the lowland (Table 1).

Initial leaf litter characteristics

Nitrogen concentration did not significantly differ between species, while C concentration was significantly higher in *Alnus* leaf litter (Table 2). Significantly higher concentrations in cellulose, hemicellulose, Si and ash, but lower in lignin, were measured in leaves of *Phragmites* when compared to *Alnus*. The higher concentration of fibre and Si in leaf blades of *Phragmites* likely determined its significantly higher toughness compared to *Alnus* (Table 2). From these data we also estimated the amount of non-structural carbohydrates that was higher in *Alnus* (42%) than in *Phragmites* (25%).

Breakdown rates

Leaf mass loss, corrected for leaching, fitted to negative exponential models slightly better than to linear models (average $R^2 = 0.90$ and $R^2 = 0.88$, respectively). Thus, exponential models instead of linear ones were used to compare decomposition rates (Fig. 1). Decomposition rates calculated on a time basis were significantly affected by leaf litter species ($F = 83.6$, $P < 0.001$)—higher in *Alnus* than in *Phragmites*—region ($F = 14.8$, $P < 0.05$)—higher in lowland than in mountain streams—and stream ($F = 9.2$, $P < 0.05$). The significant interaction found between region and species ($F = 31.3$, $P < 0.01$) revealed that, while *Alnus* litter decomposed significantly faster than *Phragmites* in mountain streams, no significant differences between species were detected in lowland streams. On average, decomposition rate of *Alnus* was 1.5 times higher in lowland than in native mountain streams, while that of *Phragmites* was 3.4 times greater in its native region compared to the mountain one. Decomposition rates computed on a thermal sum (dd^{-1}) basis did not differ significantly between regions ($F = 2.4$, $P > 0.05$) or among streams ($F = 5.1$, $P > 0.05$), although differences between species ($F = 94.1$, $P < 0.001$) and the interaction region x species ($F = 32.7$, $P < 0.01$) still remained significant (Fig. 1).

Fungal biomass and decomposition efficiency

Fungal biomass in leaf litter did not differ significantly between species or regions ($F = 1.43$, $P = 0.26$ and $F = 0.05$, $P = 0.83$; respectively). Noteworthy is, however, the tendency for both species of higher average fungal biomass in its native region (Fig. 2). Decomposition efficiency was significantly higher in the lowland compared to the mountain region ($F = 37.48$, $P < 0.001$). No significant effect of species was detected ($F = 0.02$, $P = 0.88$) (Fig. 2).

Table 2. Variables (mean ± SE; $n = 3$) used to characterise leaf litter (on leached material) of the two species assayed. Also shown are *t*-values and significance level of the comparisons.

Species or comparison	Toughness (g)	%C	%N	%Hemicellulose	%Cellulose	%Lignin	%Ash	%Si
Alnus glutinosa	69.8 ± 1.7	48.45 ± 0.40	1.64 ± 0.06	18.2 ± 1.9	10.9 ± 0.4	11.5 ± 2.4	6.30 ± 0.01	0.03 ± <0.01
Phragmites australis	238.1 ± 23.3	39.75 ± 0.87	1.50 ± 0.03	22.5 ± 1.7	27.6 ± 1.7	3.4 ± 0.9	12.45 ± 0.13	2.21 ± 0.06
Comparison	−13.7***	9.0***	2.0^{ns}	−6.4**	−12.6***	6.3**	−53.46***	− 23.87**

***$P < 0.001$; **$P < 0.01$; ns, not significant ($P > 0.05$).

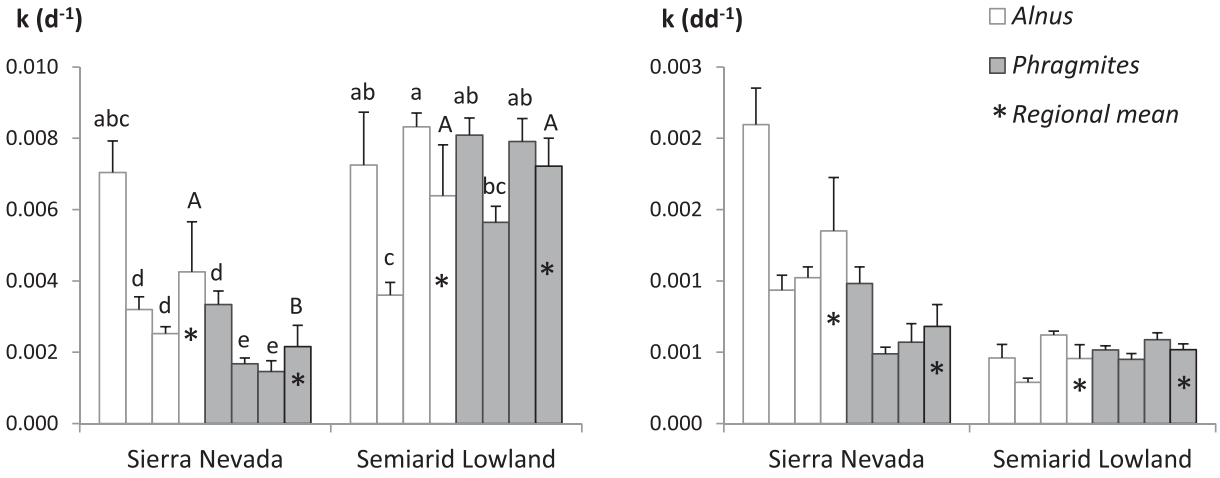

Figure 1. Decomposition rates ($k \pm$ SE) results for each of the three streams per region adjusted to negative exponential decay models for litter of *A. glutinosa* and *P. australis*. The bars with an asterisk represent the mean value for each data series. Different letters indicate significant differences ($P \leq 0.05$) between streams (lowercase letters) or regions (capital letters).

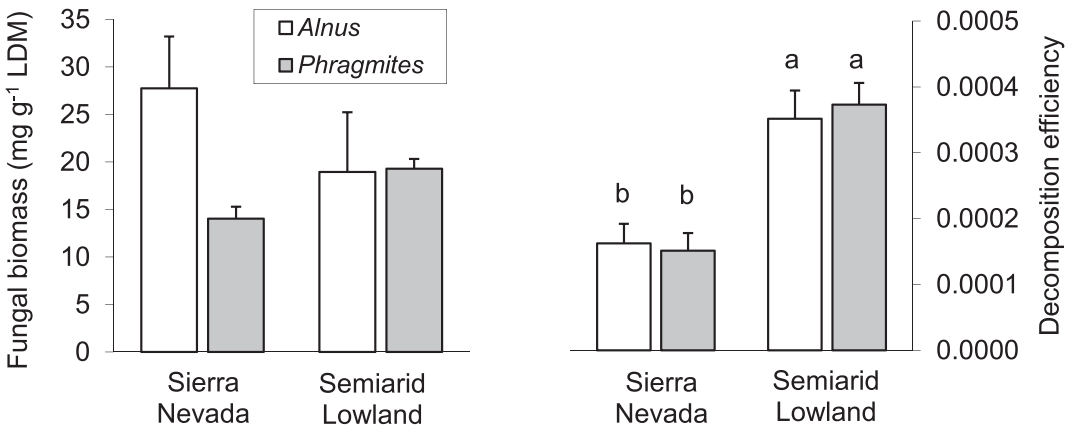

Figure 2. Fungal biomass and decomposition efficiency (mean + SE) observed in the two leaf litters in both regions.

Activity and functional parameters of β-glucosidase

Maximum activity was measured at temperatures between 40°C and 70°C, without significant differences between regions or species; however, activity at the stream temperature and enzymatic efficiency (% of the maximum activity measured at the average temperature in each stream) significantly differed between regions, but not between species (Table 3). The energy of activation (Ea) for β-glucosidase activity was not significantly affected by region (mountain: 37.70 ± 2.47 kJ mol^{-1}; lowland: 31.13 ± 3.92 kJ mol^{-1}; $F = 1.55$, $P = 0.25$) or species (*Alnus*: 37.94 ± 1.12 kJ mol^{-1}; *Phragmites*: 30.89 ± 4.41 kJ mol^{-1}; $F = 2.02$, $P = 0.20$).

Maximum activity of β-glucosidase was recorded at pH between 5 and 6 in all cases, being not significantly affected by region or species, as occurred with the enzymatic efficiency (Table 4). In contrast, activity measured at the average pH of the stream was significantly higher in the lowland, and for *Alnus*, compared to the mountain region and *Phragmites*, respectively; also, a significant interaction region × species was detected, that was driven by significantly higher values in lowland vs mountain streams for *Alnus*, but a lack of significant differences between regions for *Phragmites* (Table 4).

Maximum enzyme stability against pH was obtained at pH = 7, except for *Alnus* extracts from lowland streams (pH = 9) (Fig. 3). Stability was significantly influenced by region ($F = 43.5$, $P < 0.001$; mountain streams > lowland streams), species ($F = 38.8$, $P < 0.001$; *Phragmites* > *Alnus*) and pH ($F = 11.8$, $P < 0.001$) (Fig. 3). It is worth to highlight the significant effect

Table 3. β-glucosidase activity (mean ± SE, $n = 3$ measures per stream and species) as a function of temperature in decomposing litter of *Alnus* and *Phragmites* incubated for 5 weeks in streams from Sierra Nevada and the semiarid lowland of south-eastern Spain. All measurements were carried out at pH 7. Also shown are *F* values and significance level, following two-way ANOVA, to test the effect of region, species and the interaction region x species.

Factor or comparison	Maximum activity (μmol g^{-1} DM h^{-1})	Temperature of maximum activity (°C)	Activity at temperature of the stream (μmol g^{-1} DM h^{-1})	Efficiency (%)
A. glutinosa				
Sierra Nevada	**79.7 ± 14.5**	**60.0 ± 10.0**	**4.4 ± 1.2**	**6.1 ± 1.9**
Aldeire	51.5 ± 1.3	40.0 ± <0.1	4.5 ± 0.1	8.7 ± 0.1
Jérez	87.8 ± 8.2	70.0 ± <0.1	6.5 ± 1.1	7.4 ± 1.2
Lanteira	99.9 ± <0.1	70.0 ± <0.1	2.3 ± 0.1	2.3 ± 0.1
Semiarid lowland	**152.1 ± 69.4**	**50.0 ± 5.8**	**100.0 ± 86.0**	**42.8 ± 25.6**
Aguas	92.8 ± <0.1	50.0 ± <0.1	13.5 ± 0.1	14.5 ± 0.2
Negras	290.3 ± 9.9	40.0 ± <0.1	272.1 ± 1.2	93.9 ± 3.5
Vícar	73.1 ± <0.1	60.0 ± <0.1	14.5 ± 0.2	19.9 ± 0.3
P. australis				
Sierra Nevada	**92.1 ± 49.4**	**43.3 ± 3.3**	**8.4 ± 2.3**	**11.9 ± 2.6**
Aldeire	49.1 ± 0.2	40.0 ± <0.1	6.7 ± 0.1	13.6 ± 0.1
Jérez	190.7 ± 6.2	50.0 ± <0.1	12.9 ± 0.1	6.8 ± 0.2
Lanteira	36.5 ± 0.25	40.0 ± <0.1	5.6 ± 0.1	15.3 ± 0.5
Semiarid lowland	**63.0 ± 16.5**	**43.3 ± 3.3**	**18.0 ± 8.5**	**27.2 ± 7.1**
Aguas	64.5 ± <0.1	50.0 ± <0.1	9.1 ± 0.1	14.1 ± 0.1
Negras	90.8 ± 1.1	40.0 ± <0.1	34.9 ± 0.2	38.4 ± 0.3
Vícar	33.8 ± 0.9	40.0 ± <0.1	9.9 ± 0.4	29.2 ± 1.9
Comparison				
Region	0.2^{ns}	0.5^{ns}	5.3^{*}	5.6^{*}
Species	1.0^{ns}	3.2^{ns}	0.7^{ns}	0.1^{ns}
Region x species	1.0^{ns}	0.5^{ns}	2.2^{ns}	0.8^{ns}

$^{*}P < 0.05$; ns, not significant ($P > 0.05$), Boldface = average value for each region.

of the interaction region x pH ($F = 6.4$, $P < 0.001$). Enzyme activity in extracts from lowland streams showed higher sensitivity to acidic—with decrements of activity between 60% and 80% after 4 h incubation—or circumneutral conditions. In contrast, a slightly higher sensitivity to alkaline pH was measured in extracts from the mountain streams (Fig. 3).

For each species within both regions, Q_{10} coefficients were always significantly higher compared to Q_1 pH coefficients (all *t* values between –7 and –4, $P < 0.05$ in the four comparisons) (Fig. 4). Furthermore, while all mean Q_{10} temperature coefficients were clearly above 2, indicating that activity at least doubled with a 10°C temperature increase, all mean Q_1 pH coefficients resulted lower than 1, thus indicating a reduction in the activity with increasing pH (between 7 and 8). Neither species nor region exerted significant effects on differences between Q_{10} and Q_1 ($F = 3.69$, $P = 0.09$; $F = 1.19$, $P = 0.31$; respectively).

High IS produced a significant reduction in β-glucosidase activity in extracts from the stream with higher EC ($F = 665.3$, $P < 0.001$). This reduction was clearly detected in extracts of the two species for the three pH values tested, particularly after 2 h of incubation (Fig. 5).

Cellulase zymograms

A higher number of endoglucanase isoenzyme forms were detected in extracts from *Phragmites* compared to *Alnus* ($F = 22.84$, $P < 0.01$), regardless of the region ($F = 4.43$, $P = 0.07$) (Fig. 6). However, a significant effect of the interaction region x species was detected ($F = 7.14$, $P < 0.05$), which resulted from no differences between regions in the profiles measured in extracts from *Phragmites*, but a higher number of isoenzymes in *Alnus* from the lowland compared to mountain streams.

Effects of environmental factors on decomposition rates and enzyme activity

PLS regression models showed that decomposition rate and enzyme activity in *Phragmites* were significantly predicted by environmental factors, but that was not always the case in *Alnus* (Table 5). A high percentage of the variance of decomposition rates of *Phragmites* was explained by environmental factors, with temperature being the most important predictor (Table 5). Cellulolytic activity in *Phragmites*, both at the stream temperature (74% var. expl.) and the stream pH (66% var. expl.), were mainly predicted by water conductivity and temperature. For *Alnus*, only β-glucosidase activity measured at stream pH, was significantly predicted (81% var. expl.), mainly by water conductivity and temperature (Table 5). Overall, PLS regression models, when significant, revealed similar relationships between dependent variables and environmental factors for both species, which suggest limited support to the HFA hypothesis.

DISCUSSION

Our results strongly support the leading role of temperature, interacting with substrate chemistry, on decomposition and cellulolytic activity, compared to water pH and ionic concentration, in accordance with the 'temperature–quality' hypothesis (Bosatta and Ågren 1999). However, the part of our working hypothesis suggesting higher decomposition rates and cellulolytic activity in the native range of each leaf litter species—'HFA' hypothesis (Hunt *et al.* 1988; Gholz *et al.* 2000)—received much less support.

Acceleration of litter decomposition at higher water temperature has been frequently reported in correlative field studies (Irons *et al.* 1994; Fabre and Chauvet 1998; Friberg *et al.* 2009; Boyero *et al.* 2011) and laboratory experiments (Dang *et al.* 2009;

Table 4. β-glucosidase activity (mean ± SE, $n = 3$ measures per stream and species) as a function of pH in decomposing litter of *Alnus* and *Phragmites* incubated for 5 weeks in streams from Sierra Nevada and the semiarid lowland of south-eastern Spain. All measurements were carried out at 22°C. Also shown are F values and significance level, following two-way ANOVA, to test the effect of region, species and the interaction region x species.

Factor or comparison	Maximum activity (μmol g^{-1} DM h^{-1})	pH of maximum activity	Activity at pH of the stream (μmol g^{-1} DM h^{-1})	Efficiency (%)
A. glutinosa				
Sierra Nevada	**10.32 ± 2.13**	**6.0 ± <0.1**	**2.79 ± 0.50**	**27.39 ± 2.64**
Aldeire	7.22 ± 0.06	6.0 ± <0.1	1.82 ± 0.16	25.20 ± 1.42
Jérez	14.40 ± 0.22	6.0 ± <0.1	3.50 ± 0.06	24.32 ± 0.59
Lanteira	9.33 ± 0.04	6.0 ± <0.1	3.05 ± 0.07	32.65 ± 0.83
Semiarid lowland	**29.84 ± 11.61**	**5.5 ± 0.2**	**10.37 ± 2.54**	**38.46 ± 5.26**
Aguas	53.06 ± 1.22	5.7 ± 0.3	15.41 ± 0.27	29.04 ± 0.91
Negras	17.95 ± 0.45	5.7 ± 0.3	8.47 ± 0.21	47.21 ± 2.15
Vícar	18.50 ± 0.65	5.0 ± <0.1	7.24 ± 1.52	39.14 ± 8.15
P. australis				
Sierra Nevada	**9.00 ± 1.04**	**5.0 ± 0.0**	**2.31 ± 0.35**	**25.84 ± 3.63**
Aldeire	7.79 ± 0.03	5.0 ± <0.1	2.54 ± 0.18	32.56 ± 2.24
Jérez	8.13 ± 0.27	5.0 ± <0.1	1.63 ± 0.06	20.08 ± 0.07
Lanteira	11.08 ± 0.18	5.0 ± <0.1	2.76 ± 0.04	24.87 ± 0.51
Semiarid lowland	**11.28 ± 0.82**	**5.4 ± 0.3**	**3.28 ± 0.23**	**29.12 ± 0.78**
Aguas	12.81 ± 0.26	6.0 ± <0.1	3.73 ± 0.07	29.08 ± 1.07
Negras	9.99 ± 0.23	5.3 ± 0.3	3.04 ± 0.11	30.49 ± 0.89
Vícar	11.03 ± 0.19	5.0 ± <0.1	3.06 ± 0.13	27.79 ± 1.16
Comparison				
Region	4.5^{ns}	0.1^{ns}	12.0^{**}	4.4^{ns}
Species	3.4^{ns}	7.4^{*}	9.2^{*}	2.4^{ns}
Region × species	2.4^{ns}	7.4^{*}	6.9^{*}	1.1^{ns}

$^{**}P < 0.01$; $^{*}P < 0.05$; ns not significant ($P > 0.05$), Boldface = average value for each region.

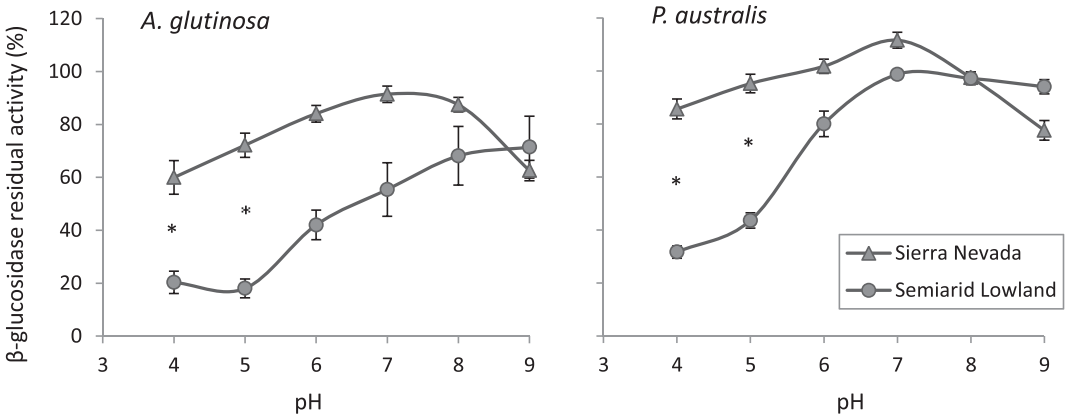

Figure 3. Enzyme stability measured as mid-term (4 h incubation) pH sensitivity of β-glucosidase activity ($n = 3$, mean ± SE). Statistically significant differences between regions for specific pH values are denoted with an asterisk (Tukey test, $P < 0.001$).

Ferreira and Chauvet 2011; Gonçalves, Graça and Canhoto 2013, 2015; Martínez *et al.* 2014), and may be due to a stimulation of fungal biomass and/or activity at higher temperatures (see review in Ferreira and Chauvet 2011). The higher decomposition rates and β-glucosidase efficiencies in lowland than in mountain streams, the leading role of temperature as predictor of rates and enzyme activity, and the fact that differences between regions vanished when rates were expressed on a thermal sum basis, strongly suggest that the thermal step of around 10°C between the two regions was the main driver of decomposition by stimulating fungal enzyme activity but not the increase in biomass. Certainly, β-glucosidase enzymes were closer to their thermal optimum in lowland than in mountain streams. This is not surprising since temperature greatly influences metabolic processes (Brown *et al.* 2004; Woodward, Penkins and Brown 2010) by driven enzyme kinetics and/or stimulating the rate of enzyme production (Wallenstein and Weintraub 2008), with the consequent increase in enzyme activity. Besides, accelerated degradation of recalcitrant substrates with temperature has been reported from soil (Trasar-Cepeda, Gil-Sotres and Leirós 2007; Keiblinger *et al.* 2012) and aquatic (Ylla, Romaní and Sabater 2012) environments.

An important effect of temperature on the degradation of complex crystalline polysaccharides consists in stimulating substrate accessibility to enzymes; marked increases with temperature of the relative adsorption of endoglucanases and cellobiohydrolases to cellulose have been, indeed, reported (Ooshima, Sakata and Harano 1983). A key trait differentiating litter

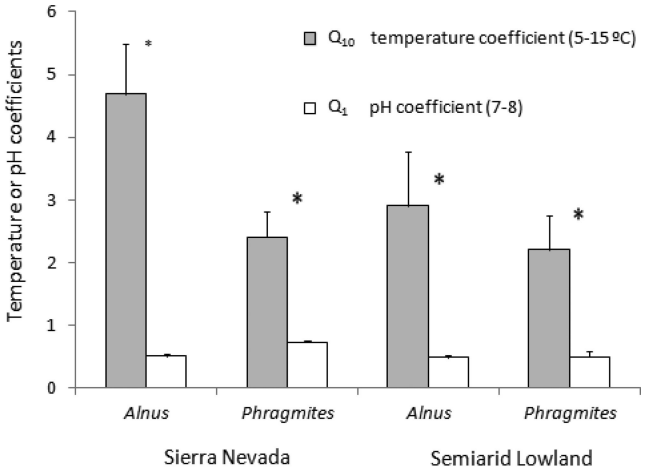

Figure 4. Apparent Q_{10} and Q_1 values for β-glucosidase activity from both leaf litters incubated in the two regions. An asterisk indicates significant differences ($P \leq 0.05$). Data are the mean of three replicates + SE.

chemistry of both species assayed here, is the higher concentration of Si in *Phragmites*, which strongly binds with cellulose in the form of polymerised silicic acid (silica gel), determining a silico-cellulose cell membrane (Ma and Yamaji 2006). This could limit the accessibility of enzymes to cellulose fibres, thus making decomposition of this substrate highly sensitive to temperature. This is in agreement with the differences we detected in decomposition rates (d^{-1}) between regions for the two species: the rate of *Phragmites* was on average 3.4 times (3.0 in terms of Q_{10})—in *Alnus* only 1.5 times (1.4 in terms of Q_{10})—higher in lowland compared to mountain streams. Furthermore, the >2 times higher cellulose concentration of *Phragmites* than of *Alnus*, and its lower concentration of non-structural carbohydrates used as fuel by degrading microbiota, could have exacerbated temperature sensitivity of decomposition of the former substrate. In fact, both substrate accessibility and availability to exoenzymes have been proposed as major constrains of temperature sensitivity of organic matter decomposition in soils (Gershenson, Bader and Cheng 2009; Dungait *et al.* 2012).

Interestingly, although a high concentration in lignin, or lignin-derived substances, has been linked to high sensitivity to temperature of decomposition (Davidson and Janssens 2006; Ylla, Romaní and Sabater 2012), this was not the case observed for *Alnus*, which presented more than 3-fold the lignin content of *Phragmites*. As pointed out by several authors (Fierer *et al.* 2005; Kleber *et al.* 2011; Blagodatskaya *et al.* 2016), the inherent activation energy of the substrate is not the sole factor influencing the temperature sensitivity of decomposition, and in field conditions the relationship between such sensitivity and C quality may be obscured by complex interactions between temperature and a range of other abiotic and biotic factors. For instance, marked alkaline conditions in our lowland streams could have interfered with temperature sensitivity of lignin decomposition in *Alnus*, since enzymes involved in this process (e.g. laccases) often have maxima of activity and stability at acidic pH (3–4) (Bollag and Leonowicz 1984; Fukushima and Kirk 1995).

Our findings, however, strengthen the argument of a major role of temperature on differences in litter decomposition between the two regions, since they greatly agree with results obtained in laboratory experiments, which reported similar values of temperature sensitivity for *Alnus* decomposition (Q_{10} *ca* 1.5) within the thermal range 5°C–15°C and standard pH conditions (Ferreira and Chauvet 2011; Martínez *et al.* 2014). Furthermore, Donnelly *et al.* (1990) have reported higher decomposition sensitivity to temperature of cellulose compared to lignin in soil organic matter. These results point out to a fundamental dependence on substrate chemistry of temperature effects: the lower temperature sensitivity in *Alnus* compared to *Phragmites* perhaps due to a higher concentration of non-structural carbohydrates in the former that might attenuate its whole recalcitrance (Hättenschwiler *et al.* 2011) and/or to the need of long-term experiments to detect effects of environmental factors on lignin degradation (e.g. Berg, Ekbohm and McClaugherty 1984; Di Nardo *et al.* 2004).

Our data on β-glucosidase activity as a function of pH also point out to a relatively weak effect of this factor, compared to temperature, on interregional differences in litter decomposition, at least regarding the cellulosic fraction. Overall, % efficiency of β-glucosidases as a function of pH was relatively low (<40%) in the two regions. Furthermore, Q_1 values (pH range 7–8) were below 1 in all cases, suggesting that the prevailing alkaline conditions in lowland streams could have depleted the final step of cellulose hydrolysis. This is in sharp contrast to values of Q_{10} determined within the temperature range 5°C–15°C, indicating that this activity, at least doubled with a 10°C temperature increase regardless of the origin of the enzyme.

Activity at the stream pH was, nonetheless, significantly higher in lowland streams, despite their alkaline pH, and this factor emerges in PLS models with a significant VIP score to the enzyme's activity. This apparent conflict with results for optimum pH, might be driven by higher enzyme concentration (more than accelerated activity) in lowland compared to

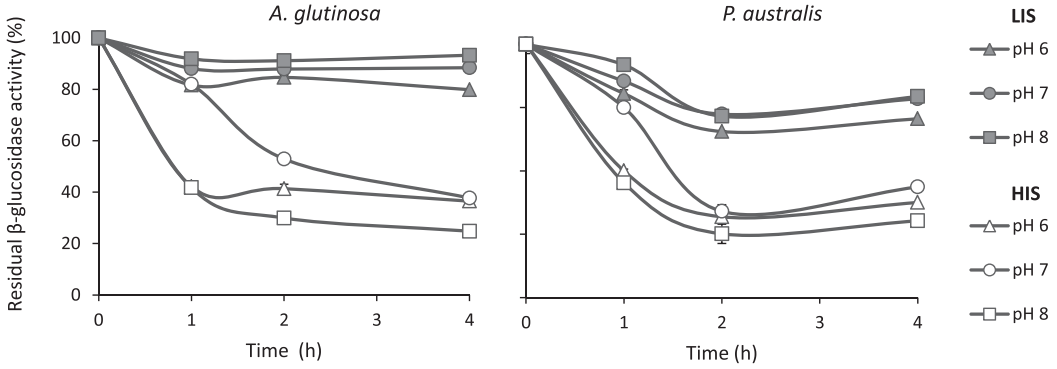

Figure 5. Effects of IS on β-glucosidase activity in enzymatic extracts of *Alnus* and *Phragmites* leaf litter, from the stream with higher electric conductivity (Río de Aguas, semiarid lowland). Buffers with low ionic strength (LIS) and with high ionic strength (HIS) were evaluated at different pH values ($n = 3$, mean ± SE).

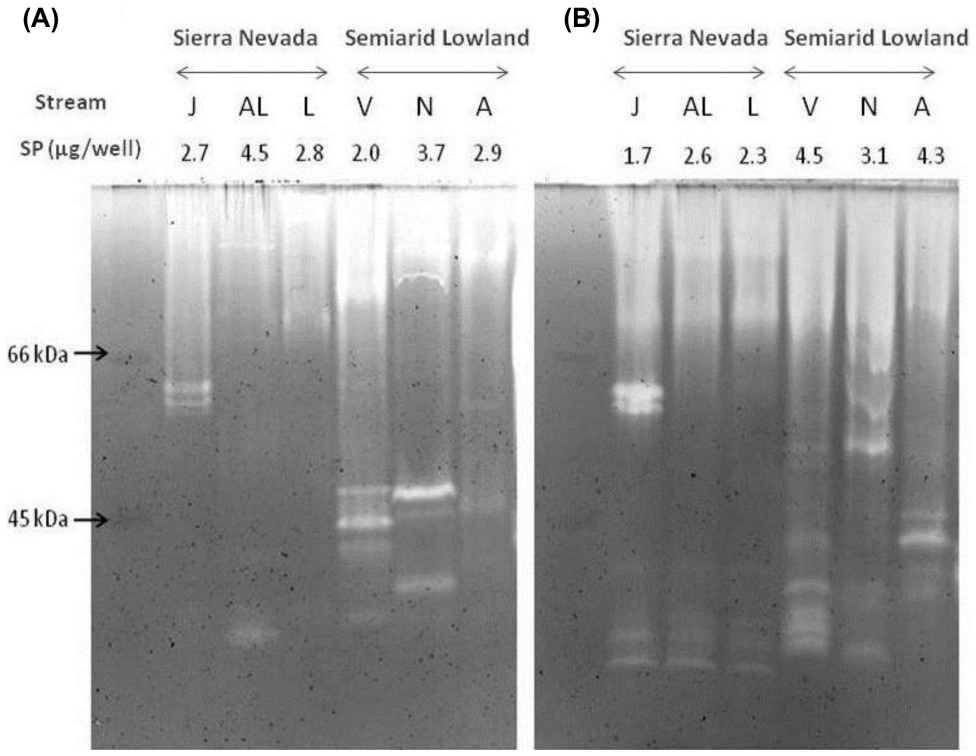

Figure 6. Zymograms for endo-β-1,4-glucanase activity in a concentrated enzyme solution of *Alnus* (**A**) and *Phragmites* (**B**). The first lane contains the molecular weight marker (MWM). Numbers under code in each lane indicate the soluble protein (SP) content in extracts (μg/well).

Table 5. Model parameters of PLS regression with model's predictive ability (Q^2), variance of dependent variable explained by predictor variables (R^2 [Y]), variable importance on projection (VIP) and standardised PLS partial coefficients. In all models only one PLS component was selected by the autofit function. Models with significant predictive power ($Q^2 > 0.097$) are in bold.

Dependent variable	Q^2	R^2 [Y]	Predictor	VIP[a]	Standardised coefficient
Decomposition rate (k)					
Alnus	–0.22	0.54	Temperature	1.43 ± 0.23	0.22 ± 0.15
			$N\text{-}NO_3$	1.18 ± 1.04	0.18 ± 0.21
			Conductivity	1.02 ± 0.65	0.16 ± 0.15
			pH	1.02 ± 0.39	0.16 ± 0.11
Phragmites	**0.63**	**0.79**	Temperature	**1.34 ± 0.13**	**0.21 ± 0.04**
			pH	**1.19 ± 0.09**	**0.19 ± 0.04**
			Alkalinity	**1.18 ± 0.14**	**0.19 ± 0.03**
			Conductivity	**1.09 ± 0.28**	**0.17 ± 0.06**
β-glucosidase activity (stream temperature)					
Alnus	0.01	0.31	pH	1.15 ± 0.26	0.14 ± 0.25
			Alkalinity	1.15 ± 0.21	0.13 ± 0.24
			Temperature	1.10 ± 0.31	0.13 ± 0.24
			$P\text{-}PO_4$	1.06 ± 0.66	0.12 ± 0.30
Phragmites	**0.24**	**0.74**	Conductivity	**1.37 ± 0.31**	**0.23 ± 0.30**
			Temperature	**1.15 ± 0.36**	**0.19 ± 0.25**
			pH	**1.05 ± 0.24**	**0.17 ± 0.23**
β-glucosidase activity (stream pH)					
Alnus	**0.51**	**0.81**	Conductivity	**1.36 ± 0.29**	**0.23 ± 0.07**
			Temperature	**1.28 ± 0.18**	**0.21 ± 0.05**
			$NO_3\text{-}N$	**1.13 ± 0.48**	**0.20 ± 0.27**
			pH	**1.05 ± 0.08**	**0.18 ± 0.01**
Phragmites	**0.41**	**0.66**	Conductivity	**1.23 ± 0.29**	**0.18 ± 0.09**
			Temperature	**1.21 ± 0.22**	**0.18 ± 0.08**
			pH	**1.15 ± 0.17**	**0.17 ± 0.03**
			$NO_3\text{-}N$	**1.06 ± 0.78**	**0.16 ± 0.13**

[a]Only the predictors having a VIP score greater than 1 are shown.

mountain streams, perhaps induced by higher enzyme production under the elevated temperatures in the former region (Conant *et al.* 2011; Blagodatskaya *et al.* 2016). Results for enzyme stability revealed some functional adaptation of enzymes to the pH regime of their environment of origin, but, overall, enzyme stability from mountain streams was slightly higher at pH 7, or very similar at pH 8, compared to lowland streams. This result adds some support to the notion that differences in pH between the two regions played a minor role on the final step of cellulose degradation compared with that of temperature.

Our results also suggest that β-glucosidase activity could have been hindered by the high ionic concentration of the water in lowland streams, since increasing IS, regardless of pH conditions, reduced this activity even in extracts from the lowland stream with higher mineral concentration. In contrast, high litter decomposition has been detected in hardwater compared to softwater streams, which was related to the role of Ca^{2+} and Mg^{2+} as activating cations in hydrolytic enzyme reactions (Chróst 1991; Romaní and Sabater 2000), including β-glucosidase activity (King 1986). Nevertheless, high ionic concentration is not always ruled by alkalinity in inland waters, since ions others than bicarbonate, Ca^{2+} and Mg^{2+}, may be present, potentially affecting decomposers activity differently than alkalinity. Our lowland streams showed, indeed, higher concentrations of chloride and sulphate than the mountain ones, and leaf mass loss and activity of hydrolytic enzymes (Roache, Bailey and Boon 2006), cellulose decomposition (Mendelssohn *et al.* 1999) and β-glucosidase activity (Saibi, Amouri and Gargouri 2007) have been reported to be negatively affected by increasing NaCl concentration, salinity or IS, respectively.

As litter decomposition is fundamentally driven by the interaction between resource chemistry and decomposers, both controlled by the environment, specific adaptations of microbial communities to be particularly capable of degrading the type of litter they encounter most often under the environmental conditions of their native range are to be expected. This 'HFA' of litter decomposition (Hunt *et al.* 1988) did not find substantial support from our data, since decomposition rates of both species were faster in lowland than in mountain streams and both, rates and enzyme activity, showed similar patterns of variation with environmental factor in the two species. A recent meta-analysis of HFA quantification of litter decomposition in forest soils indicates that litter mass loss was 4.2% faster on average at the home environment (Wang, Zhong and He 2013), notably in undisturbed forest soils (Austin *et al.* 2014). However, this percentage is relatively low compared to ~70% of variation of leaf litter decomposition in forest soils explained by chemical characteristics of litter and climate (temperature and precipitation), particularly when studies embrace large regional scales (Gholz *et al.* 2000; Parton *et al.* 2007). Thus, given the ample differences in environmental conditions, certainly temperature, between the two regions studied here, it is possible that the hypothetical HFA of *Alnus* in mountain streams may have been overridden by the thermal step between regions.

Fungal assemblages, major drivers of leaf litter decomposition, were species poor and compositionally different in lowland compared to mountain streams (Casas *et al.* 2011). Thus, differences in decomposition rates between regions cannot be attributed to fungal diversity, in agreement with studies suggesting considerable functional redundancy among aquatic hyphomycete species (Duarte *et al.* 2006; Gonçalves, Graça and Canhoto 2015). Moreover, despite lower species richness, the number of endoglucanase isoenzymes detected in samples of *Alnus* was higher in lowland than in mountain streams and, overall, higher in *Phragmites* compared to *Alnus*. These results suggest a key control of environment and, particularly, substrate on gene expression of enzymes involved in cellulose degradation. Accordingly, results from forest soils suggest that microorganisms respond quickly to litter chemistry modifying gene expression of enzymes involved in decomposition (Kourtev, Ehrenfeld and Huang 2002). However, the observed variations in the number of isoenzymes were not correlated to interregional or between-substrates differences in decomposition rates. Functional enzyme parameters did not reveal major adaptations to perform better under the prevailing conditions (i.e. temperature and pH) of their environments of origin.

Overall, our results suggest that interregional differences in decomposition rates and cellulose degradation fundamentally depended on temperature that accelerated enzyme kinetics, and perhaps enzyme production, in lowland streams, largely overriding effects from other environmental factors or potential HFA. This temperature effect was highly dependent on substrate chemistry: the higher temperature sensitivity of decomposition in *Phragmites* was probably caused by its silico-cellulose cell membrane that might hinder accessibility of enzymes to cellulose fibres. The strong influence of leaf traits on temperature sensitivity of decomposition has been noted in recent studies from forest soils (Salinas *et al.* 2011) and headwater streams (Gonçalves, Graça and Canhoto 2013; Ferreira, Chauvet and Canhoto 2015). Nevertheless, our knowledge on how specific leaf traits respond under global warming scenarios remains still fragmentary.

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Conflict of interest. None declared.

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