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RECENT PERSPECTIVES ON TEMPORARY RIVER ECOLOGY

Organic matter characteristics in a Mediterranean stream through amino acid composition: changes driven by intermittency

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Abstract Amino acid composition (quality) and abundance (quantity) of organic matter (OM) in an intermittent Mediterranean stream were followed during transitions from wet to dry and dry to wet conditions. Amino acids were analyzed in benthic material (epilithic biofilms, sand sediments, leaf material) as well as in the flowing water (dissolved organic matter, DOM). A principal component analysis and the estimation of the amino acid degradation index (DI) elucidated differences in amino acid composition and quality among the wet–drought–wet cycle. Amino acid content and composition were dependent on the source of OM as well as on its diagenetic state. The highest-quality OM (high DI and high N content) occurred on epilithic biofilms and the most degraded and lowest-quality OM occurred in sandy sediments. Differences between the pre- and post-drought periods were evident in DOM quality; autochthonous-derived material was predominant during the pre-drought (wet period preceding drying), while allochthonous inputs dominated during the post-drought period (wet period following drying). In contrast, benthic OM compartments were more stable, but benthic OM quality decreased continuously throughout the drought period. This study revealed that wet–drought–wet cycles resulted in subtle changes in benthic OM quality, and degradation of DOM was related to flow intermittency.

Keywords Amino acids · Biofilms · Intermittency · Mediterranean stream · Degradation index · Drought periods

Introduction

Organic matter (OM) is a primary source of carbon (C) and nitrogen (N) in many freshwater ecosystems worldwide (Fellman et al. 2009) and is a major component of the global carbon cycle (Porcal et al. 2009). OM in aquatic systems is a primary energy and nutrient source in food webs (Thingstad 2003), regulates the bioavailability of dissolved nutrients and metals (Dillon and Molot 2005; Shiller et al. 2006), and affects the optical properties of natural waters (Retamal et al. 2007). In streams, OM can accumulate in benthic compartments (e.g., biofilms growing on cobbles, wood, leaves or detritus) or be transported by stream flow. Biofilms play a key role in OM processing because they retain, decompose, and transform OM from the water column, while also producing additional OM. Bacteria are the main decomposers of both particulate and dissolved OM (POM and DOM, respectively; Meyer 1994; Amon and Benner 1996; Amon et al. 2001).

The OM that occurs in stream ecosystems is a heterogeneous complex mixture of carbohydrates, proteins, lipids, lignins, organic acids, and other less well-characterized compounds, such as humic substances (Thurman 1985). OM reactivity is largely dependent on its composition, which is controlled in part by source materials

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(Volk et al. 1997; Findlay and Sinsabaugh 1999). Local soil and plant litter inputs (France et al. 1996; Dalzell et al. 2005) as well as autochthonous material from instream primary producers (Webster and Meyer 1997) contribute to stream DOM. The arrival of OM to the stream and its subsequent degradation is related to local rainstorm events in the watershed (Da Cunha et al. 2001), but also to other hydrological episodes such as droughts and rewetting events. The source and diagenetic state of riverine OM are important factors determining its ultimate fate. Chemical biomarkers such as lignins, phenols, amino acids, sugars, and fatty acids are clues for identifying the sources and diagenetic pathways of OM (Dauwe and Middelburg 1998; Amon and Benner 2003). Of the many potentially informative biomolecules, carbohydrates and amino acids are dominant components of cells. Since proteins are ubiquitous components of all source organisms and degradation mixtures (Cowie and Hedges 1992), characterizing the composition of amino acids provide valuable insight into the origins and reactivities of OM in complex mixtures (Dauwe and Middelburg 1998; Dauwe et al. 1999; Chen et al. 1999; Wu et al. 2003). Amino acids (found in proteins or as free amino acids), are a major form of organic nitrogen and amongst the most labile fractions of bulk OM (Spitzy and Ittekkot 1991). Their degradation supports microbial production and enlarges the biologically available pools of mineralized NO_3, NO_2 and NH_4 (Stepanauskas et al. 1999). Amino acid composition has been extensively studied in bulk DOM and POM in marine environments (Mannino and Harvey 2000; Amon et al. 2001; Davis and Benner 2005), but few studies have focused on comparisons between particulate and dissolved fractions in fresh waters (Hedges et al. 2000; Wu et al. 2003; Duan and Bianchi 2007; Tremblay and Benner 2009).

The hydrology of Mediterranean streams is highly variable (Acuña et al. 2005) as evidenced by their extreme droughts and floods. Mediterranean streams are physically, chemically and biologically influenced by these episodes (Gasith and Resh 1999). In particular, the transport and degradation of OM in Mediterranean streams can be affected during the transition from wet-to-dry (drought) and dry-to-wet (flood or rewetting events) conditions (Ylla et al. 2010). The quality of the materials transported by the first rains after an extended drought is affected by processes occurring in the accumulated dry OM. Others have noted that the composition of dissolved organic carbon (DOC) changes drastically during rewetting (Butturini et al. 2003, Vázquez et al. 2007). The mobilized DOM increases biodegradable DOC (BDOC) content and microbial activity during rewetting (Romaní et al. 2006). On the other hand, OM degradation and microbial activity during drought is favoured by sediment moisture (Amalfitano et al. 2008) and responds to the existence of senescent algae that retard water loss from surface sediments (Claret and Boulton 2003).

Amino acid abundance and composition during these biogeochemically relevant periods (sensu “hot moments” in McClain et al. 2003) could be helpful for understanding the sources and biogeochemical cycling of labile OM in rivers (Thomas 1997). In this study, we analyzed amino acid composition and degradation of freshwater benthic OM and DOM in a stream reach during a typical Mediterranean drought and the subsequent rewetting period. Our hypothesis was that intermittency is a primary factor influencing the rate of OM decomposition. Our prediction was that OM would be degraded during the pre-drought period and after rewetting, mobilized OM will provide high quality OM to the recovering benthic biofilms. The main objectives of the study were (1) to compare amino acid composition (quality) and abundance (quantity) in the stream during the drying and rewetting in the different OM pools, and (2) to determine if changes in dissolved and benthic amino acid compositions are representative of the diagenetic state and quality of the OM during the pre- and post-drought phases.

Methods

Stream water samples and material from benthic habitats (e.g., cobbles, sand sediment, decaying leaf material) were collected from Fuirosos stream (third order). The Fuirosos is situated in Montnegre-Corredor Natural Park in the north-east of Spain (latitude 41°42′N, longitude 2°34′E). The area has a Mediterranean climate, with precipitation mostly in autumn and spring and occasional summer storms. During most years, water flow in the channel progressively decreases during summer and hydrological connectivity between stream habitats is eventually interrupted. The studied stream reach is 3–5 m wide and 10 m in length. The riparian vegetation is made up of alder (*Alnus glutinosa*), hazelnut (*Corylus avellana*), poplar (*Populus nigra*) and plane trees (*Platanus acerifolia*). Sample collection took place during drying and rewetting periods in spring, summer, and autumn.

Physical and chemical characteristics of the stream water (i.e., dissolved oxygen, water temperature, and conductivity) were measured in the field. Carbon and nitrogen content in DOM were analyzed as DOC, DON (dissolved organic nitrogen), and the fraction of BDOC (Ylla et al. 2010). DOC and total nitrogen concentrations were determined using a Shimadzu TOC-VCS coupled to a total nitrogen analyzer after filtration of the samples (0.2 µm nominal porosity). DON was determined as the difference between total nitrogen and the sum of inorganic nitrogen forms (nitrate, nitrite, and ammonia).

We started sampling the drying period (pre-drought period) on 8 May 2006, when stream flow was at a baseflow (7.7 L s^{-1}). Sampling occurred five more times during the ensuing ~1.5 month drying period (25 May and 12, 15, 19, 26 June 2006). The last two sampling events occurred just after stream flow had ceased, the streambed was exposed to drying, and benthic material was drying out (subsurface layers were still wet). The stream remained completely dry for nearly 3 months until 13 September 2006 (rewetting day), when water flow resumed. We sampled the post-drought period six times until 4 December 2006, when we recognized that autumnal rains were continually providing streamflow. During the drying period the reach consisted of approximately 60% riffle habitat (with cobbles and rocks covering 70% of the riffle area) and 40% pool habitat (with sandy sediment covering 80% and patches of deposited leaves covering 5% of the pool habitat). Upon re-wetting in September, the heavy rains flushed the fine sediment and remaining OM from the pool habitat, thus homogenizing the stream bed within the study reach (i.e., pool became similar to the riffle area and was dominated by cobbles and rocks at ~70%; Ylla et al. 2010).

Amino acid content

Amino acid concentration and composition was determined from both the benthic material (leaf material, biofilms growing on rocks and cobbles, biofilm and fine sediment material) and from bulk water samples (i.e., as a proportion of DOM). Four replicate samples of benthic material in each category (specified above) were randomly collected during each sampling event. Sand and leaf samples were obtained from the streambed by coring a defined area (4.3 cm^2). Sediment samples were obtained from the stream bed between 0 and 5 cm deep. Cobbles were collected directly from the streambed. Stream water (approx. 8 L) was also collected for analysis. Upon return to the laboratory, stream water was filtered through precombusted GF/F filters (0.7 μm nominal porosity) to separate DOM from POM. The OM from the cobbles (from the upper and lower sides, so including both autotrophic and heterotrophic organisms) and sand was detached by immersion in distilled water and sonication (3 min, Selecta sonication bath at 40 W and 40 kHz). Leaf material was not separated from the biofilm growing on it, and it was homogenized with a mixer for analysis (Ylla et al. 2010).

The collected benthic material and filtered water samples were freeze-dried and then hydrolyzed in sealed vials with 6 M HCl at 110°C for 20 h. Subsamples of the material were then analyzed (5 mL for DOM and 100 μL of material extracted from sand, leaves and cobbles). The HCl remaining in the sample after hydrolysis was removed by a nitrogen flush. The residue was derivatized with a fluorescent reagent (AccQ Fluor reagent, Waters®) according to the manufacturer's instructions. After these preparation steps, all of the derivatized samples were filtered using centrifuge filters (micro-spins, 0.45 μm) to remove particles and prevent problems with the HPLC column. Afterwards, amino acids were analyzed using high performance liquid chromatography (HPLC, Waters). The HPLC system included a Waters AccQ Tag column for separation of amino acids, a Waters 2475 fluorescence detector (excitation wavelength 250 nm; emission wavelength 395 nm; gain 10), a 717 plus auto-sampler and a 1525 Waters binary pump. The eluents were an acetate-phosphate buffer (Waters® AccQ·Tag eluent A concentrate) and a 60% acetonitrile HPLC grade solution. The gradient profile was provided in the manufacturer's instructions. The run time for each sample was 50 min. The injection volumes were 5 μL for benthic samples and 10 μL for water samples. Content of each amino acid in each analyzed sample was calculated in moles (%), in μg g DW^{-1}, in mg cm^{-2} in benthic materials and in μg L^{-1} for amino acids in DOM.

The validity of the method was checked by the addition of an internal standard (50 pmol of α-aminobutyric acid), which was recovered at nearly 100% (50 pmol ± 5 for DOM and 50 pmol ± 10 for benthic samples) during the treatment and analysis of the standards and samples. Amino acids were identified by retention times and quantified by comparison between standard and sample peaks. The amino acid standards came from a hydrolyzed protein that contained a mixture of 17 primary amino acids (Table 1). All of them could be quantified except cysteine, due to analytical problems.

Amino acids were classified as essential or non-essential and by their functionality. Essential amino acids cannot be synthesized de novo by the organism and must be supplied by the diet. Essential amino acids have been basically described for humans and humans and other mammals have the same requirements. It is particularly striking that fish, many insects, and even protozoans have practically overlapping needs for essential amino acids (Taylor and Medici 1966; Dauwe and Middelburg 1998).

Essential amino acids (Table 2) were isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, arginine and histidine. Amino acid classification into functional groups (Dauwe and Middelburg 1998; Mannino and Harvey 2000; Wu et al. 2003; Table 2) separated basic (histidine, arginine, lysine), acidic (aspartic acid, glutamic acid), hydroxylic (serine, threonine), neutral (glycine, alanine, proline, valine, isoleucine, leucine), sulfuric (methionine) and aromatic (tyrosine, phenylalanine) amino acids. The amount (concentration) of each functional group as well as the percentage of essential versus non-essential amino acids were calculated for all samples.

Table 1 Amino acid concentrations during pre-drought (PRE, $n = 6$ for the benthic materials and $n = 4$ for the water fraction), rewetting (REW, $n = 1$) and post-drought (POST, $n = 5$) periods

	Epilithic biofilms (μg g DW^{-1})			Sand sediment (μg g DW^{-1})			Leaves (μg g DW^{-1})			Water dissolved (μg L^{-1})		
	PRE	REW	POST	PRE	REW	POST	PRE	REW	POST	PRE	REW	POST
ASP	160 (40)	70	140 (30)	70 (20)	50	50 (10)	100 (10)	90	120 (40)	8.44 (2.3)	10.53	10.20 (0.74)
SER	170 (40)	70	130 (20)	60 (10)	50	50 (10)	110 (10)	90	120 (40)	12.19 (2.23)	37.59	13.21 (1.35)
GLU	230 (60)	60	140 (30)	60 (10)	50	50 (10)	110 (20)	80	140 (60)	26.92(3.12)	68.16	15.58 (4.04)
GLY	140 (40)	70	100 (30)	80 (20)	40	40 (10)	120 (10)	100	120 (60)	9.41 (1.54)	24.49	21.50 (2.34)
HIS	190 (90)	20	100 (50)	20 (0.0)	40	60 (20)	40 (10)	30	40 (10)	31.99 (5.67)	80.45	9.90 (5.12)
ARG	300 (90)	80	160 (30)	80 (20)	40	50 (10)	110 (10)	70	90 (20)	11.94 (2.04)	20.09	6.55 (0.55)
THR	220 (60)	80	160 (30)	70 (10)	50	60 (10)	130 (20)	100	140 (50)	17.87 (2.89)	42.11	13.40 (0.45)
ALA	200 (70)	70	140 (30)	60 (10)	40	40 (10)	130 (10)	110	160 (60)	18.53 (3.54)	49.16	11.52 (1.48)
PRO	160 (50)	60	100 (20)	40 (10)	30	30 (0.0)	110 (20)	80	130 (50)	29.33 (13.43)	53.68	9.28 (1.73)
TYR	120 (30)	30	30 (10)	0.0 (0.0)	20	20 (10)	80 (10)	50	60 (10)	8.77 (1.99)	13.40	3.43 (0.57)
VAL	190 (60)	70	140 (30)	50 (10)	40	40 (10)	130 (20)	110	140 (50)	14.36 (1.97)	32.78	10.79 (0.35)
MET	20 (0.0)	10	10 (0.0)	0.0 (0.0)	10	0.0 (0.0)	10 (0.0)	10	10 (0.0)	8.97 (4.13)	0.84	7.54 (1.09)
LYS	100 (30)	20	60 (20)	20 (10)	10	10 (0.0)	60 (10)	40	60 (20)	8.54 (1.88)	12.82	2.06 (1.64)
ILE	140 (40)	60	100 (20)	40 (10)	20	20 (0.0)	110 (10)	80	110 (40)	6.41 (1.94)	13.30	4.68 (0.27)
LEU	170 (50)	70	130 (20)	60 (20)	40	40 (0.0)	130 (20)	100	140 (50)	11.12 (2.8)	21.62	6.89 (0.24)
PHE	190 (70)	70	100 (10)	30 (10)	30	20 (10)	100 (10)	70	90 (20)	11.63 (2.28)	19.85	6.64 (0.77)
Total aa	2710 (810)	910	1750 (330)	740 (170)	540	610 (90)	1600 (180)	1210	1660 (250)	236.43 (47.7)	500.87	153.17 (13.15)

Reported values are averages and standard errors (in parentheses)

ASP aspartic acid, *SER* serine, *GLU* glutamic acid, *GLY* glycine, *HIS* histidine, *ARG* arginine, *THR* threonine, *ALA* alanine, *PRO* proline, *CYS* cysteine, *TYR* tyrosine, *VAL* valine, *MET* methionine, *LYS* lysine, *ILE* isoleucine, *LEU* leucine, *PHE* phenylalanine

Table 2 Factor loadings, eigenvalues and cumulative variance of the principal components analysis (PCA) of amino acid concentrations from epilithic biofilms, sand sediment, leaf material and DOM during the wet–drought–wet period

	PCA		Amino acids features	
	First axis	Second axis	Functional group	Essential/non-essential
ASP	**−0.750**	−0.295	Acidic	Non-essential
SER	**−0.719**	0.078	Hydroxylic	Non-essential
GLU	**0.643**	0.138	Acidic	Non-essential
GLY	**−0.762**	**0.581**	Neutral	Non-essential
HIS	**0.873**	0.299	Basic	Essential
ARG	−0.147	−0.481	Basic	Essential
THR	−0.273	0.045	Hydroxylic	Essential
ALA	0.231	−0.371	Neutral	Non-essential
PRO	**0.674**	0.051	Neutral	Non-essential
TYR	0.458	0.095	Aromatic	Non-essential
VAL	−0.308	**−0.617**	Neutral	Essential
MET	0.188	**0.780**	Sulfuric	Essential
LYS	0.395	**−0.603**	Basic	Essential
ILE	−0.356	**−0.784**	Neutral	Essential
LEU	−0.364	**−0.865**	Neutral	Essential
PHE	0.077	**−0.608**	Aromatic	Essential
Eigenvalue	0.372	0.238		
Cumulative variance (%)	37.2	61.0		

The highest loading factors (larger than 0.5) at extremes of the axes (positive and negative) are marked in bold. The amino acids functional groups as well as their classification as essential/non-essential amino acid are also shown. Amino acid abbreviations are given in Table 1

Amino acid degradation index

The amino acid degradation index (DI; Dauwe et al. 1999) was derived from biogeochemistry studies on marine sediments to determine the diagenetic state of OM. This index links the amino acid composition to OM diagenesis and is used to quantify the quality of the OM based solely on its chemical composition. The index considers the whole suite of amino acids in the calculation and allows different samples to be directly and quantitatively compared. The DI of our samples was calculated following the empirical formula of Dauwe et al. (1999) and the molar percentage of amino acids in the samples. Large negative DI values are indicative of severely degraded samples, whereas positive DI values are indicative of fresh materials.

Data analysis and statistics

Differences between the pre- and post-drought periods in DI, essential amino acids, and amino acid functional groups for benthic materials and DOM were analyzed using a one-way repeated measures analysis of variance (RM-ANOVA). Probabilities within groups (day and day × substrata) were corrected for sphericity using the Greenhouse-Geisser correction. All probabilities were adjusted using the Dunn-Sidak correction. A post hoc multiple comparison test (Tukey HSD) was used to examine the differences between substrata. Normality and homogeneity of variances were tested before applying the parametric test.

The percentage of DOC represented by amino acids in units of C (AA-C/DOC) and the percentage of DON represented by amino acids in units of N (AA-N/DON) were calculated. AA-C/DOC was calculated as the hydrolysable amino acids in units of C (AA-C) in total DOC. Similarly, AA-N/DON was calculated as the hydrolysable amino acids in units of N (AA-N) in total DON.

A Pearson correlation analysis was performed to determine the potential relationship between DI and the percentage of N in amino acids (% AA-N, calculated as the sum of the hydrolysable amino acids in units of N divided by the total amount of amino acids in each sample). The SPSS software package for Windows (Version 14.0.1, SPSS Inc., 1989–2005) was used.

The amino acid composition of the stream compartments during drying and rewetting was examined by means of multivariate analysis. Data were based on the relative abundances (mol%) of amino acids in the four analyzed compartments (three benthic compartments and DOM) for the 12 sampling campaigns. An arcsine squareroot transformation was applied to normalize the variables. Amino acid data were first analyzed with detrended correspondence analysis (DCA; Hill and Gauch 1980) to determine the length of the gradient for the first two axes. DCA indicated that the maximum gradient length was shorter than 3 SD units (0.548). Therefore, the use of linear ordination techniques was appropriate (ter Braak and Smilauer 2002). PCA was performed using CANOCO version 4.5.

Results

Physical and chemical parameters

Physical and chemical characteristics of the stream water differed between the drying and rewetting periods (Ylla et al. 2010). During the pre-drought period, there was a progressive increase in stream water conductivity (from 229 to 329 μS cm^{-1}) and dissolved oxygen decreased to a very low concentration (1.6 mg O_2 L^{-1}). Maximum water temperature (19.5°C) was observed in the pool immediately before stream flow cessation. Increased DOC (6.7 mg L^{-1}), BDOC (1.5 mg L^{-1}) and DON (0.5 mg L^{-1}) in the stream water on 13 September was associated with an increase in stream flow (36.8 L s^{-1}). During this peak-flow episode, dissolved oxygen increased (9.8 mg O_2 L^{-1}) and conductivity declined to pre-drought concentrations (206 μS cm^{-1}).

Amino acid composition

The concentration of amino acids in epilithic biofilms and sand sediments (μg g DW^{-1} and μg L^{-1} summarized in Table 1) were higher during the pre-drought compared to the post-drought period. Amino acid concentrations in leaf material was similar between the two periods. Most amino acid concentrations decreased during the rewetting event, especially amino acids in epilithic biofilms. However, amino acid concentrations in DOM peaked at this same time.

The amino acid concentration per cm^2 of streambed surface area in benthic materials was higher during the pre-drought than during the post-drought period. Amino acid concentrations in sand were 1.22 ± 0.52 mg cm^{-2} and on cobbles were 0.05 ± 0.013 mg cm^{-2} during the pre-drought period, and were 0.52 ± 0.17 and 0.03 ± 0.008 mg cm^{-2}, respectively, after drought. In leaf material, amino acid concentrations were similar before and after drought (2.22 ± 0.49 and 2.52 ± 0.69 mg cm^{-2}, respectively).

The composition spectra of individual amino acids (Fig. 1) differed between benthic materials and DOM. Methionine was nearly absent in benthic substrata but relatively abundant (3.5% of the total amino acids) in DOM. A peak of glycine (25% of total amino acids) was recorded in DOM during the post-drought period. On average, glycine, alanine, serine, threonine and valine were the most abundant amino acids and accounted for over 50% of the total amino acid concentrations in benthic materials. DOM was dominated by glycine, alanine, glutamic acid and proline (accounting for 43.4% of the total amino acids).

The PCA yielded two significant ordination axes or factors (Fig. 2; Table 2). The first axis (37.2% of the total variance) contained positive loadings of the amino acids histidine, proline, glutamic acid and tyrosine and negative loadings of glycine, aspartic acid and serine. The former occurred in DOM during the pre-drought period, as well as during first day of rewetting (13 September) and during stable winter flow conditions (last sampling date). Glycine, aspartic acid and serine were observed in greater concentrations in post-drought water samples (with the exception of the initial day after the water return and the last sampling day). Amino acid composition of benthic substrata did not change considerably between pre- and post-drought periods. Compared to other benthic compartments, epilithic biofilms had higher concentrations of histidine, proline, glutamic acid, tyrosine, leucine, isoleucine, valine, phenylalanine, lysine, arginine and alanine, while sand sediment samples had higher concentrations of methionine, glycine, aspartic acid, serine, leucine, isoleucine, valine and threonine.

The second PCA factor or axis (23.8% of the total variance) separated DOM from benthic materials based on several amino acids. Amino acids most influential for separating DOM samples were methionine and glycine, whereas benthic substrata had higher leucine, isoleucine, valine, phenylalanine, lysine, arginine and alanine concentrations.

Functional groups and essential amino acids

The relative abundances of amino acid functional groups in benthic material did not change between pre-drought and post-drought periods (RM-ANOVA, day effect, $P > 0.05$). However, a continuous decline in essential amino acids was observed during the pre-drought period (RM-ANOVA, day effect, $P = 0.043$, $F_{2.7,24.5} = 4.4$). Significant differences were observed among benthic substrata. Epilithic biofilms had the highest percentage of essential amino acids (49%), followed by sand sediment and leaf material (44%) (Tukey's test, $P < 0.05$). Sand sediments contained a lower proportion of aromatics and a higher proportion of hydroxylic amino acids than epilithic biofilms and leaf material (Fig. 3). Lower percentages of acidic, basic and hydroxylic amino acids and a higher percentage of neutral amino acids characterized the leaf material (RM-ANOVA, substrata effect, $P < 0.05$, Fig. 3).

Temporal changes in functional amino acid groups were particularly pronounced for DOM during the post-drought period (RM-ANOVA, day effect, $P < 0.05$, Fig. 4a). On the first day of rewetting (13 September), the sulfuric group

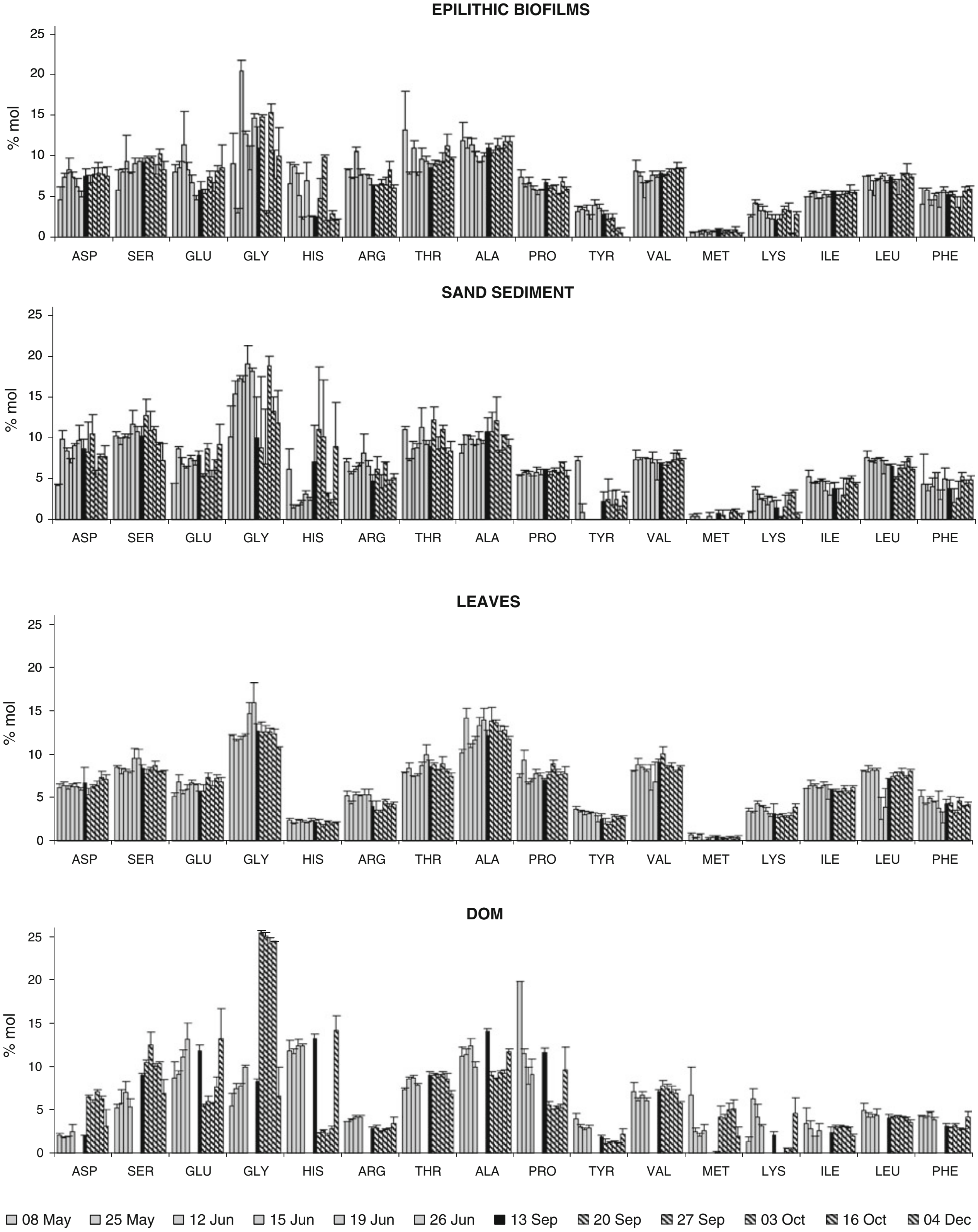
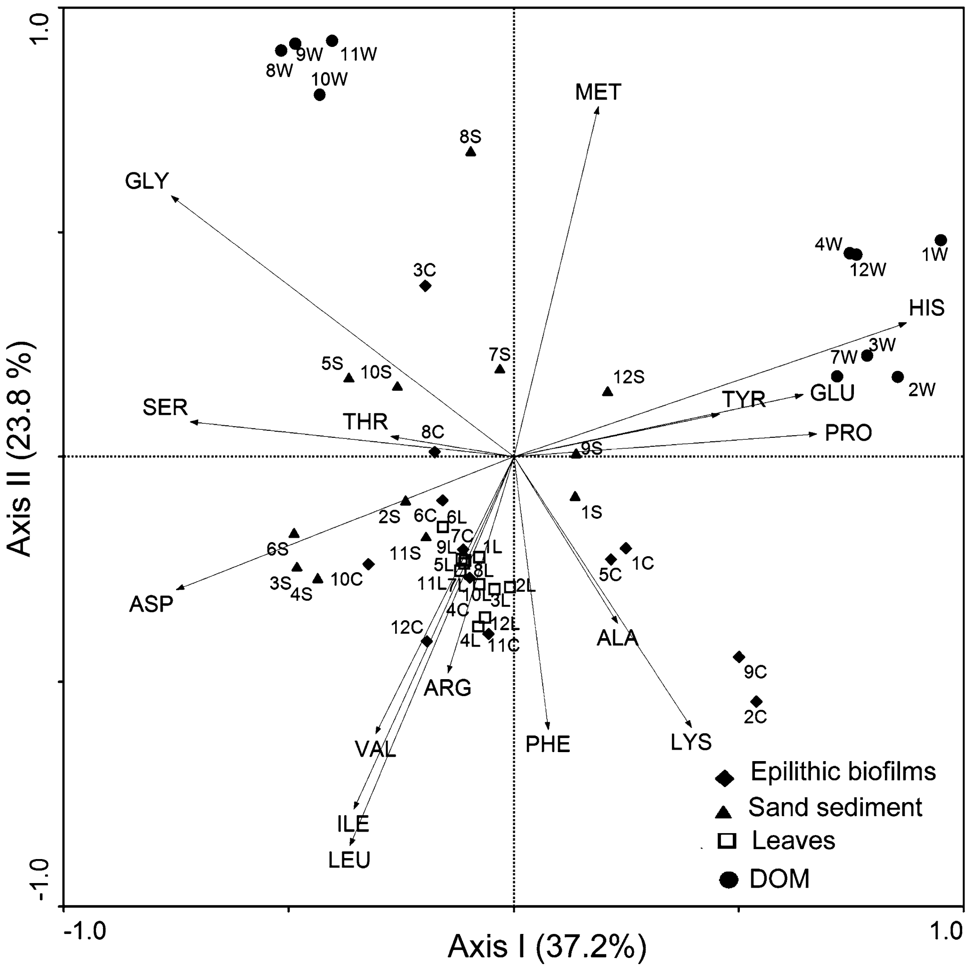


Fig. 1 Amino acid spectra (mol%) in epilithic biofilms, sand sediment, leaf material and dissolved in stream water throughout the wet–drought–wet process (12 campaigns). From 8 May 2006 to 26 June 2006 corresponds to the pre-drought period (*grey bars*), the rewetting day occurred on 13 September 2006 (*black bar*) and the post-drought period occurred from 20 September 2006 to 4 December 2006 (*diagonal pattern bar*). Mean values and standard errors are shown ($n = 4$). Amino acids abbreviations are given in Table 1

Fig. 2 A bi-plot of the first and second components/axes of the principal components analysis (PCA) of amino acid concentrations from epilithic biofilms (*C*), sand sediment (*S*), leaf material (*L*) and DOM (*W*) during the wet–drought–wet period (*numbers* indicate the sampling campaign; from *1* to *6* corresponds to the pre-drought period, *7* to the rewetting day, and *8* to *12* the post-drought period). Amino acid abbreviations are given in Table 1



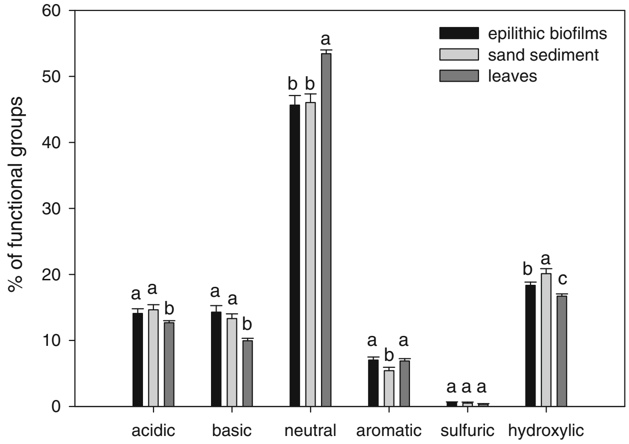


Fig. 3 Functional groups of protein amino acids (%). Acidic (aspartic acid, glutamic acid); basic (histidine, arginine, lysine); neutral (glycine, alanine, proline, valine, isoleucine, leucine); aromatic (tyrosine, phenylalanine); sulfuric (methionine) and hydroxylic (serine, threonine). Mean values and standard errors are shown ($n = 12$ corresponding to the 12 mean sampling values). Significantly different groups as determined by Tukey's test ($a > b > c$, $\alpha = 0.05$) are also shown

disappeared and the aromatics declined. The sulfuric group reappeared during the following days (RM-ANOVA, day effect, $P < 0.05$, $F_{1.7,5.3} = 6.2$), whereas basic amino acids declined rapidly. Concentrations of functional groups were similar to those observed during the drought once basal flow was re-established (last sampling day; Fig. 4a). The proportion of essential amino acids in DOM decreased during the pre-drought period and increased to 44% during the rewetting period (Fig. 4b).

Organic matter diagenesis indicators

Sand sediment accumulated more degraded material during the pre-drought period compared to other benthic material, while epilithic biofilms contained the least amount of degraded material (Tukey's test, $P < 0.001$). The DI of amino acids decreased in benthic materials during the pre-drought period (RM-ANOVA, day effect, $P = 0.005$, $F_{2.4,21.4} = 9.5$, Fig. 5), especially in sand and leaves. The DI increased immediately after rewetting in sand and leaf material. However, after rewetting the DI was similar to

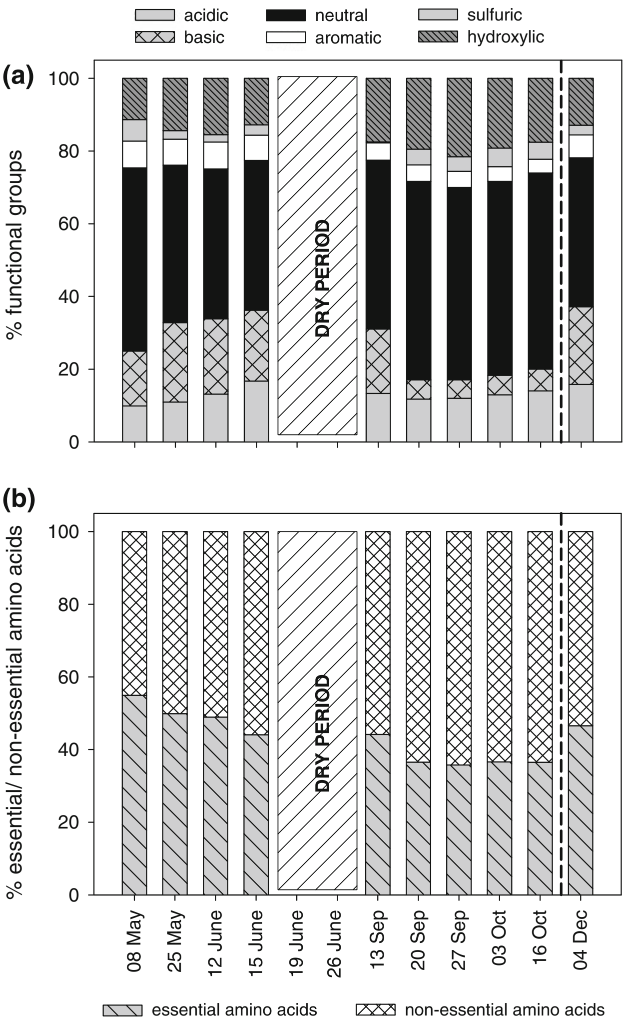


Fig. 4 Functional groups of protein amino acids in dissolved organic matter throughout the wet–drought–wet process (**a**; *upper panel*) and the evolution of the essential and non-essential amino acids during the same period (**b**; *lower panel*). The *dashed line* indicates when the basal hydrological conditions were re-established

pre-drought conditions in the epilithic biofilm. The DI of DOM increased abruptly when discharge was highest (Fig. 5).

There was a positive relationship between DI and the contribution of hydrolysable amino acids in units of C (AA-C) in total DOC. Higher DI values were related to higher percentages of AA-C/DOC during the pre-drought period (Fig. 6a). However, the DOM was more degraded during the post-drought period, as indicated by lower DI values and lower percentages of AA-C/DOC. During rewetting (13 September) and when the stream's winter stable basal conditions were re-established (4 December), the DI and the percentages of AA-C/DOC were close to pre-drought values. Analogous relationships between DI and the contribution of hydrolysable amino acids in units of N (AA-N) in total DON were observed for pre- and post-drought periods (Fig. 6b).

The positive correlation ($r = 0.539$; $P < 0.001$) between the DI and the percentage of N in amino acids (% AA-N) suggests that higher DI values are linked to higher percentages of AA-N. The highest DI and % AA-N in DOM and epilithic biofilms were recorded during the pre-drought period and contrasted to values observed in the post-drought period. The lowest DI values occurred in sand biofilms during the pre-drought period, indicating that benthic OM was rapidly degraded. There was no difference in leaf material between pre- and post-drought periods, which always had low percentages of AA-N. Upon the first day of rewetting, DOM had high DI values and % AA-N, while benthic substrata had the lowest percentage of AA-N (Fig. 7).

Discussion

Amino acids are informative biomolecules by which OM diagenesis can be measured (Ittekkot et al. 1984; Cowie and Hedges 1994; Dauwe and Middelburg 1998). In this study, the composition of specific amino acids and the differences in amino acid degradation indices over time were characterized for dissolved and benthic OM collected from drying and rewetting phases in a Mediterranean stream, the Fuirosos River in Spain. Drying and rewetting episodes differentially affected the degradation of OM and this difference was more noticeable for DOM collected from the flowing water column compared to the OM collected from benthic compartments.

The most abundant amino acids in the Fuirosos were glycine and alanine. The predominance of these two amino acids has already been described in dissolved and particulate OM of aquatic systems (Mannino and Harvey 2000; Duan and Bianchi 2007) and in aquatic sediments (Keil et al. 1998). The relatively conservative behavior of glycine may be related to its abundance in the structural matrices of diatoms and bacteria as well as to its comparatively minor food value to micro- and macro-consumers because of its short chain length (Dauwe and Middelburg 1998).

Detailed analyses of amino acid compositions revealed strong differences between benthic substrata (epilithic biofilms, sandy deposits, and leaves) and DOM in the water column and highlighted that different processes take place in the two compartments. OM being either produced, retained and/or accumulated for longer time periods in these benthic compartments gradually degrades as it ages. In contrast, DOM in flowing water is primarily exported from watershed soils and the short residence time along the stream-channel flow path probably limits its degradation in

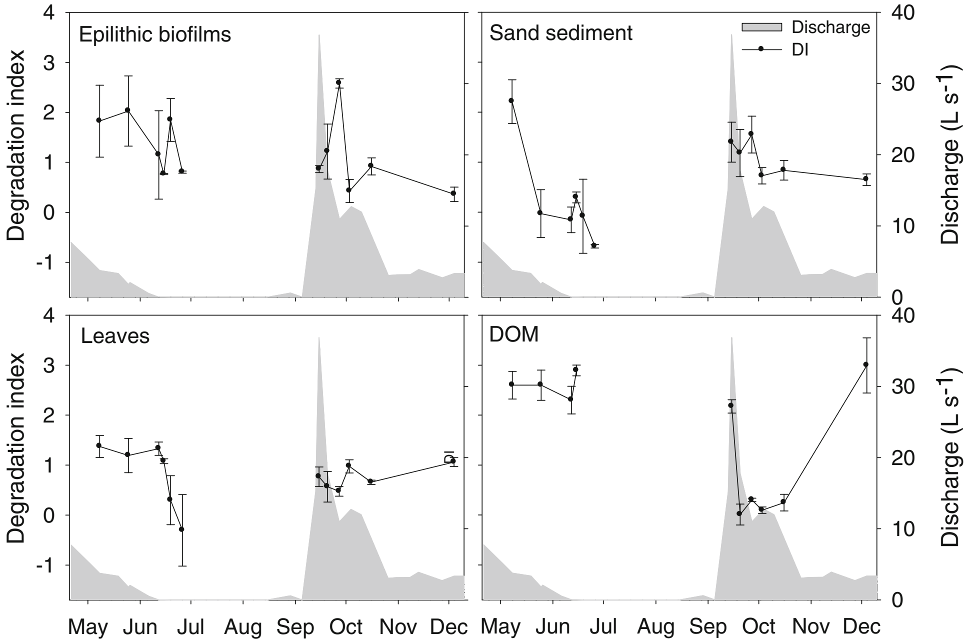


Fig. 5 Evolution of the degradation index (DI) and the water discharge throughout the wet–drought–wet period in epilithic biofilms, sand sediment, in leaf material and in dissolved organic matter. Mean values and standard error are shown for the degradation index (*n* = 4)

these low order streams and quality changes might be linked to hydrology. Amino acid concentrations in benthic compartments per streambed area in this temporary Mediterranean forested stream were greater in sand and leaves than in epilithic biofilms. However, epilithic biofilms were rich in amino acid content per biofilm DW and composition amino acids was dominated by less degraded amino acids (i.e., diagenetically younger than leaves and sand substrate). The most degraded and lowest quality OM was observed in sand sediments. This compartment contained the highest concentration of hydroxylic acids (Ser and Thr) and the lowest concentration of aromatics (Tyr and Phe). Amino acids such as glycine, serine and threonine preferentially accumulate during decomposition (Lee and Cronin 1984; Muller et al. 1986) and are considered to be highly recalcitrant and indicative of old OM (Chen et al. 2004). In contrast to OM associated with sand, epilithic biofilm contained high concentrations of histidine, alanine and glutamic acid, as well as tyrosine and phenylalanine. These amino acids are indicative of less degraded, fresher OM. Histidine and alanine usually occur in fresh materials (Jennerjahn and Ittekkot 1997) and glutamic acid, tyrosine and phenylalanine are considered the most labile and easily degradable species (Cowie and Hedges 1992; Cowie et al. 1992). These last three amino acids (Glu, Tyr and Phe) are concentrated in cell plasma (Hecky et al. 1973) and are quickly consumed, leading to their low concentrations in decomposing material (Dauwe and Middelburg 1998; Lomstein et al. 2006). Also, the highest amino acid concentrations per units of DW measured in epilithic biofilms was indicative of fresh, labile organic material derived from autotrophic organisms like algae. Algal biomass provides an important source of labile OM (Mannino and Harvey 2000) and epilithic biofilms have higher concentrations of autotrophs than sediments (Romaní and Sabater 2001), though heterotrophic bacteria can also add significant N content to the OM pool. In comparison with the other benthic compartments, leaf material was rich in leucine, isoleucine, valine and phenylalanine; all common in fresh organic material (Yamashita and Tanoue 2003). Leaf material was also rich with structural amino acids such as glycine, serine and threonine (Hecky et al. 1973). The mixed occurrence of the former with the latter indicated that both benthic microorganisms and plant material occurred together in the leaf litter we sampled (Artigas et al. 2008).

Though amino acids in the benthic compartments were similar during the pre- and post-drought periods, there was a progressive degradation of benthic OM (progressive decrease in the DI) through time during the pre-drought period, particularly in sand sediments and leaf material. That temporal trend suggested that essential amino acids were preferentially consumed as degradation proceeded, which could have implications for consumers of this material. Lower concentrations of essential amino acids (e.g., Met) may limit macroinvertebrate growth (Phillips 1984; Dauwe and Middelburg 1998). This might be particularly relevant in sand where the DI was low (DI = −0.7), indicating that the most degraded material accumulated in sand and less so in leaf material (DI = −0.3). The OM in sediments is generally more degraded than materials on superficial substrata (like epilithic biofilms; Dauwe et al. 1999). The very low DI in sand sediments is indicative of longer accumulation and degradation processes occurring in the epipsammic biofilms. It is also possible that water

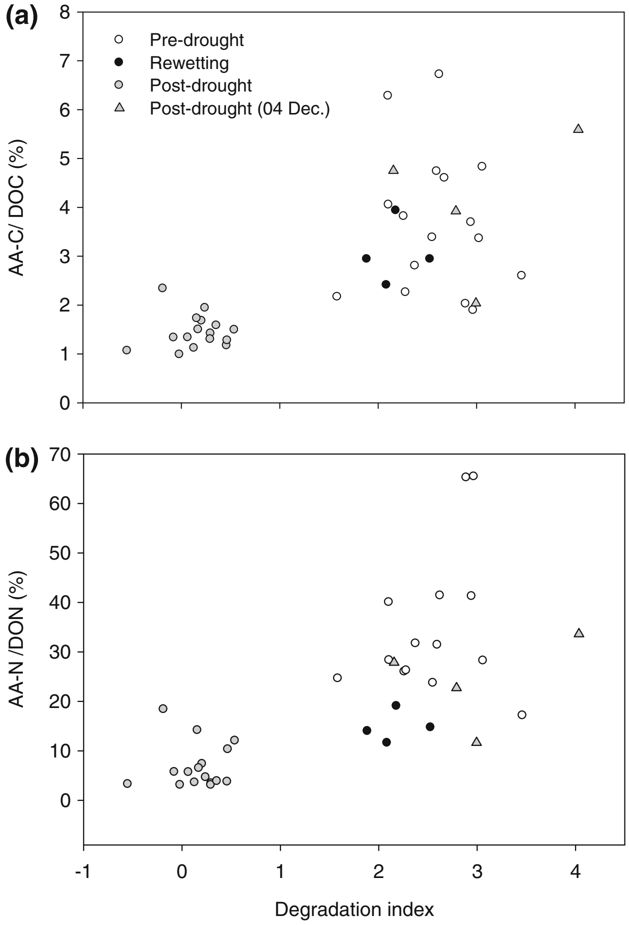


Fig. 6 The relationship between the degradation index and the contribution of amino acid-carbon to DOC (AA-C/DOC) (**a**; *upper panel*) and the relationship between the degradation index (DI) and the contribution of amino acid-nitrogen to DON (AA-N/DON) (**b**; *lower panel*). The pre- and post-drought periods are shown in both panels and the rewetting day (13 September) and the last sampling day (4 December) when the stable winter flow conditions were reestablished are also highlighted. In total, ten sampling campaigns were carried out, and four replicates per sampling campaign are represented

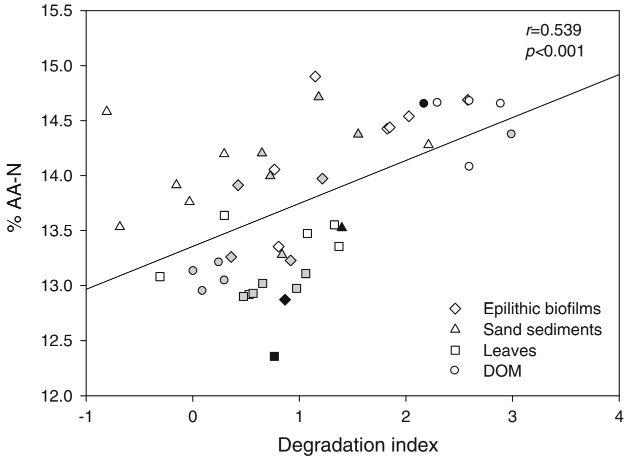


Fig. 7 Relationship between the degradation index and the %AA-N of epilithic biofilms, sand sediment, leaf material and dissolved organic matter (DOM) in the pre-drought (*white dots*), rewetting (*black dots*) and post-drought periods (*grey dots*)

that upwells towards the surface delivers recalcitrant OM to the first cm of sediments, where it accumulates, although ground water upwelling in such an intermittent stream is not common (Butturini et al. 2002).

While changes in amino acid composition were subtle in all benthic compartments throughout the wet–dry–wet period, changes were more pronounced in flowing water DOM. The pre-drought DOM amino acid signature indicated that it was primarily microbially derived OM and this was in contrast with the post-drought DOM signature that was indicative of terrestrially derived and more degraded OM. The pre-drought DOM water samples also had a greater percentage of amino acids relative to both the total DOC and the total DON (i.e., AA-C/DOC, AA-N/DON) compared to the post-drought period. This difference indicated that OM was more labile during the pre-drought period and that the supply of fresh labile OM to the system was interrupted by the dry phase. This temporal change in the average diagenetic state of OM in the various habitat compartments studied here was observable in the changes in the proportions of amino acid functional groups. Aromatic and basic amino acids in DOM were significantly reduced in the post-drought period, their decomposition being preferentially relative to that of neutral amino acids during DOM degradation (Wu et al. 2003). The decrease in basic amino acids (positively charged) can also be related to their sorption to aluminosilicate clay minerals (negatively charged) (Meier et al. 1999; Aufdenkampe et al. 2001). This process can enrich the amino acid content in the POM and reduce them in DOM. When the reconnection between the flowing water and the benthic compartments recovered, high-quality OM accumulated in the streambed mobilized and an ephemeral peak of labile DOM occurred (DI = 2.2). At that moment, resuspended material could be rapidly used by organisms (Romaní et al. 2006; Ylla et al. 2010). After this pulse, only the resuspended older and highly degraded sediment particles occurred in transport. The peak of glycine found in DOM during the post-drought period could be related to the mobility of older sand sediments, where glycine accounted for 20% of all amino acids.

Lower percentages of AA-N occurred in largely altered samples and higher amounts of AA-N occurred in fresh OM samples. The DOM collected from the pre-drought period was younger and of higher quality (higher N) than that found in benthic compartments. During the drying period, both the DI and the AA-N content of the DOM decreased and rapidly recovered on the first day of the rewetting. The low percentage of AA-N is diagnostic of the potential use of N as a key nutrient (Cowie and Hedges

1994) that is utilized by microbial processes during the pre-drought period.

We postulate that changing source inputs and the influence of hydrology (and perhaps sediment transport/scour) between the pre- and post-drought periods strongly influenced the observed temporal variation of DOM (evident in both the amino acid quantity and quality). However, amino acid composition of the benthic OM compartments were more stable over time and experienced only subtle changes in amino acid composition during both pre- and post-drought periods compared to water-column DOM. Therefore, our study revealed that flow intermittence in this Mediterranean stream had a stronger influence on the quality and amino acid composition of water-column DOM, and more limited influence on benthic OM compartments.

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Conflict of interest None.

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