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RECENT PERSPECTIVES ON TEMPORARY RIVER ECOLOGY

Preconditioning effects of intermittent stream flow on leaf litter decomposition

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Abstract Autumnal input of leaf litter is a pivotal energy source in most headwater streams. In temporary streams, however, water stress may lead to a seasonal shift in leaf abscission. Leaves accumulate at the surface of the dry streambed or in residual pools and are subject to physico-chemical preconditioning before decomposition starts after flow recovery. In this study, we experimentally tested the effect of photodegradation on sunlit streambeds and anaerobic fermentation in anoxic pools on leaf decomposition during the subsequent flowing phase. To mimic field preconditioning, we exposed *Populus tremula* leaves to UV–VIS irradiation and wet-anoxic conditions in the laboratory. Subsequently, we quantified leaf mass loss of preconditioned leaves and the associated decomposer community in five low-order temporary streams using coarse and fine mesh litter bags. On average, mass loss after approximately 45 days was 4 and 7% lower when leaves were preconditioned by irradiation and anoxic conditions, respectively. We found a lower chemical quality and lower ergosterol content (a proxy for living fungal biomass) in leaves from the anoxic preconditioning, but no effects on macroinvertebrate assemblages were detected for any preconditioning treatment. Overall, results from this study suggest a reduced processing efficiency of organic matter in temporary streams due to preconditioning during intermittence of flow leading to reduced substrate quality and repressed decomposer activity. These preconditioning effects may become more relevant in the future given the expected worldwide increase in the geographical extent of intermittent flow as a consequence of global change.

Keywords Anoxic pond · Drought · Mediterranean · Organic carbon dynamics · Temporary stream · UV radiation

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Introduction

Climate change and intensified water withdrawal for human use will dramatically increase the extent and duration of surface drying in Southern Europe. In the Mediterranean Basin, an increase in summer air temperature of up to 5.5°C, a decline in precipitation by 30–45%,

and more floods and droughts, are predicted by the end of the twenty first century (Giorgi et al. 2004). These changes are of particular consequence for temporary streams, which are found on every continent and climate, and which are the dominant freshwater type in Southern Europe (Tockner et al. 2009). Intermittent flow is expected to become more common in streams across the globe due to the effects of global change (Larned et al. 2010).

Seasonal reduction in surface flow disconnects a stream from its surrounding environment—longitudinally, laterally, and vertically (Lake 2003). This fragmentation typically leads to a distinct mosaic of terrestrial and aquatic habitat types. Initially riffles and runs dry out, exposing bare dry sediments, while stagnant pools may persist longer depending on river bed morphology and the permeability of the bed sediments (Boulton and Lake 1990; Stanley et al. 1997). In such persistent stagnant pools, organic material and nutrients accumulate, and environmental conditions become harsh because of rising temperature, oxygen depletion, and acidification (Boulton and Lake 1990; Lake 2003; von Schiller et al. 2011).

In continuously flowing, forested, headwater streams, the allochthonous input of terrestrial leaves serves as a pivotal energy source (Vannote et al. 1980). However, in temporary streams, water stress may shift the timing of leaf abscission, so that leaf fall coincides with ceased flow (Boulton and Lake 1992). As a consequence, the leaves accumulate in residual pools and/or on the surface of the dry streambed, and are only transported downstream upon the first flush events (Acuña et al. 2007). Hence, in temporary streams, organic matter dynamics are controlled through the seasonal alternation of terrestrial and aquatic phases.

Organic matter decomposition in temporary streams has been studied by, e.g., Boulton and Boon (1991), Maamri et al. (1997), and Datry et al. (2011), and in floodplains and wetlands by, e.g., Glazebrook and Robertson (1999), Battle and Golladay (2001), and Langhans et al. (2008). Decomposition was usually slower for non-submersed than for submersed leaves, due to higher decomposer activity in the presence of water. In addition, decomposition was slower in standing pools than in flowing channels, likely because leaching of leaf litter results in more toxic substances and more acidic and oxygen-depleted water, which in turn reduces the numbers and activity of decomposers such as aquatic hyphomycetes and macroinvertebrates (Lake 2003; Canhoto and Laranjeira 2007; Schlief and Mutz 2007).

Limited information is available on the effect of the preconditioning that takes place during the dry phase on leaf decomposition during the subsequent wet phase. A lack of moisture on dry sediment may result in intensive desiccation of leaves, enhancing subsequent leaching losses during the first water contact (Leopold et al. 1981; Gessner et al. 1999). Furthermore, leaf abscission due to water stress in the riparian vegetation reduces canopy cover, thus leaves remaining on the sediment surface are also exposed to high solar radiation, possibly leading to photodegradation of the leaf tissue (Austin and Vivanco 2006; Day et al. 2007; Henry et al. 2008). In contrast, leaves entering oxygen-depleted stagnant pools may be subject to leaching and fermentation during anaerobic degradation (Küsel and Drake 1996; Reith et al. 2002). Thus, leaves that enter the stream during cessation of flow might be preconditioned, prior to their further decomposition after recovery of stream flow. This preconditioning may change the chemical composition of the leaves. The latter is known to control their suitability as substrate for decomposers such as invertebrates and aquatic hyphomycetes, thereby affecting leaf decomposition rates (Leroy and Marks 2006; Yoshimura et al. 2008).

The aim of our present study was to examine how preconditioning, by photodegradation on sunlit streambeds and by anaerobic fermentation in anoxic pools, affects leaf decomposition during the subsequent flowing phase. We hypothesized that leaf preconditioning would alter leaf decomposition rates by modifying leaf chemical composition and thereby their suitability as substrate for leaf decomposers. Furthermore, we hypothesized that the effect of leaf preconditioning would be distinct for microbial and macroinvertebrate colonization of leaves because of the different sensitivity of both decomposer groups to changes in leaf chemical composition. Based on these hypotheses, we predicted differences between preconditioned and unconditioned leaves in leaf chemistry, decomposer communities, and decomposition rates mediated by microorganisms alone and together with macroinvertebrates.

Materials and methods

Study sites

Field experiments were carried out in four Mediterranean temporary streams (Candelaro, Fuirosos, Taibilla, Vallcebre) and in one temperate (Demnitzer Mühlenfließ) temporary stream, which differed in catchment lithology, riparian vegetation cover, and physicochemical characteristics (Tables 1, 2). These diverse sites were chosen in order to replicate field experiments in a variety of temporary streams with different environmental conditions.

The Candelaro (study site at a headwater stream reach) is located in the Gargano promontory in Puglia, Italy, and carries high nutrient loads as a consequence of intensive fertilizer use and groundwater use for irrigation. The Fuirosos drains the Montnegre-Corredor mountain range in

Table 1 Geographic characteristics of five temporary stream sites

Catchment	Geographic location	Order	Elevation at study site (m a.s.l.)	Mean annual precipitation (mm)	Mean air temperature (°C)	Lithology	Riparian vegetation
Candelaro	41° 46′ N, 15° 18′ E	2nd	100	600	15.3	Dolomitic	Grass: *Phragmites australis, Cyperaceae*
Demnitzer	52° 21′ N, 14° 11′ E	3rd	40	480	9.0	Glacial sediments	Forest: *Que0rcus robur, Alnus glutinosa, Carpinus betulus, Fagus sylvatica*
Fuirosos	41° 42′ N, 2° 34′ E	3rd	150	750	16.5	Granitic	Forest: *Alnus glutinosa, Corylus avellana, Populus nigra, Platanus acerifolia*
Taibilla	38° 08′ N, 2° 13′ W	2nd	1,200	583	13.3	Limestone, sandstone	Grass, shrub: *Scirpus holoschoneus, Salix sp., Populus alba*
Vallcebre	42° 12′ N, 1° 49′ E	1st	1,200	924	7.3	Limestone	Grass, forest: *Populus nigra, Corylus avellana, Prunus spinosa, Pinus sylvestris, Quercus pubescens*

Table 2 Physical and chemical characteristics of five temporary stream sites during the leaf decomposition experiment (mean ± 1SD, n = 12 per stream, i.e. three sampling dates at each of four pool sites)

Parameter	Candelaro	Demnitzer	Fuirosos	Taibilla	Vallcebre
Water temperature (°C)[a]	12.0 ± 1.7	3.9 ± 3.2	11.8 ± 5.3	11.5 ± 1.4	5.6 ± 1.5
Current velocity (cm s^{-1})	40 ± 0	20 ± 9	22 ± 16	35 ± 20	13 ± 29
Depth (cm)	17 ± 2	33 ± 10	nd	20 ± 5	29 ± 6
Specific conductance (μS cm^{-1})	997 ± 49	903 ± 32	158 ± 23	759 ± 16	1,554 ± 106
pH	7.0 ± 0.4	8.2 ± 0.1	7.6 ± 0.1	8.6 ± 0.2	7.3 ± 0.9
O_2 (mg L^{-1})	10.9 ± 1.2	11.2 ± 1.3	11.2 ± 1.3	9.2 ± 1.5	12.7 ± 3.6
DOC (mg C L^{-1})	5.0 ± 1.0	13.5 ± 0.8	4.2 ± 1.1	4.0 ± 1.2	4.3 ± 1.0
NO_3^- (mg N L^{-1})	7.0 ± 0.7	9.4 ± 1.7	0.6 ± 0.5	1.2 ± 0.1	0.4 ± 0.3
SRP (μg P L^{-1})	<5	47 ± 3	8 ± 4	29 ± 1	41 ± 16

nd not determined

[a] Mean of data recorded at hourly intervals

Catalonia, Spain, and exhibits near-natural conditions. The Taibilla (Rambla de la Rogativa study site) lies at the eastern margin of the Baetic ranges in Murcia, Spain, and drains a highly erosive landscape, leading to high sediment and solute loads. The Vallcebre (Can Vila study site) lies at the southern margin of the Pyrenees in Catalonia, Spain, and also drains a highly erosive landscape. The Demnitzer Mühlenfließ (Demnitzer) is a temperate lowland stream in Brandenburg, Germany, with elevated nutrient loads during high flow due to upstream agricultural sites.

Leaf preconditioning

For our model species of leaf, we chose the European aspen (*Populus tremula*). *Populus* species are common in riparian forests throughout Europe. However, the European aspen was absent from all study sites, thereby avoiding adaptation effects of aquatic decomposer species.

Freshly fallen senescent leaves of European aspen were collected with nets from beneath a stand of trees in a forest (52° 27′ N, 13° 40′ E) close to Berlin, Germany, in autumn 2009. The leaves were air-dried and stored in the dark until used in the preconditioning experiments, which were performed in winter 2009/2010 at the Institute for Freshwater Ecology and Inland Fisheries in Berlin, Germany.

We mimicked conditions in the residual pools that occur during the dry season by incubating a set of 500 g of European aspen leaves in a water tank under anoxic (<0.1 mg DO L^{-1}) and acidic (pH 5.0) conditions at room temperature (20°C). We used artificial stream water (180 L,

prepared following Fischer et al. 2006) that was inoculated with 1 L of lake water to introduce aquatic microorganisms. After 21 days, the leaves were removed from the water tank and immediately air-dried using a fan (48 h). Two subsets of four replicate mesh bags, each bag containing approximately 4.0 g of leaves, were also submersed in the water tank and sampled to determine mass loss after 24 h (initial leaching) and 21 days (full leaching) of exposure, respectively.

Photodegradation of a second set of leaves was mimicked by irradiation for 12 h per day over a period of 21 days with a combination of UV and daylight fluorescent lamps (covering wavelengths from 300 to 700 nm; UV: Cosmedico Arimed B6 [with 31% UVB of total UV], daylight: Osram Biolux 965; 50 W m^{-2} total radiation, 17 W m^{-2} UV radiation; measured with LiCor 1800 spectroradiometer). A control set of leaves was stored in the dark under dry conditions.

For the irradiation treatment and for the control, two subsets of 4 replicate mesh bags each were used to determine mass loss during the irradiation and storage in the dark for 21 days. Another two subsets were used to determine mean leaching loss by submersion in artificial stream water for 24 h under laboratory conditions, with a leaf mass to water volume ratio equal to that of the anoxic pretreatment.

To characterize the effect of preconditioning on the chemical composition of leaves, four leached leaf subsamples from the two treatments and the control were oven-dried (40°C, 48 h), milled to a fine powder, and analyzed for five parameters: (a) percentage of organic compounds (loss on ignition at 450°C), (b) total carbon and (c) nitrogen content (Elementar vario EL C/N elemental analyzer, Hanau, Germany), (d) fiber contents (gravimetric lignin and cellulose determination, Gessner 2005b), and (e) phenolics (detection with a spectrophotometer following extraction with 70% acetone, Bärlocher and Graça 2005).

In-stream leaf decomposition

For the field experiments, fine (0.5 mm mesh size) and coarse mesh (8 mm) nylon bags were used to differentiate between microbial- and invertebrate-driven decomposition, respectively (Bärlocher 2005). Each bag was filled with approximately 4.0 g dry mass (DM) of either control leaves or preconditioned leaves (anoxic or irradiated). Field decomposition experiments were conducted concomitantly at all 5 study sites in March/April 2010 when stable flow conditions were re-established and the aquatic communities had recovered from flow cessation at all study sites. Bags containing leaves from the different treatments (control, irradiated, anoxic) were tied to an iron rod anchored in the streambed. Four pool sites per stream, located within a few hundred meters, gave quadruplicate samples for each treatment.

Field samples were collected at two time points to determine if the hypothesized effect of leaf preconditioning persisted at early and late stages of decomposition. A first set of bags was retrieved from the streams after 10 days of exposure, and a second set was retrieved after approximately 45 days, depending on the observed progress of leaf decomposition in the particular stream (38 days in Demnitzer, 40 days in Candelaro, 47 days in Vallcebre, 48 days in Fuirosos, 50 days in Taibilla). Bags were cut from the iron rods, and stored in individual plastic bags on ice in a cooler during transport. A total of 240 bags were used (5 stream sites, 4 pool sites, 3 treatments, 2 mesh sizes, 2 sampling dates), only 1 of which was lost (a coarse mesh bag from Demnitzer).

Hand held sensors were used, adjacent to the iron rods, to measure dissolved oxygen (mg DO L^{-1}), pH, specific conductance (μS cm^{-1}), and current velocity (cm s^{-1}). Measurements were made at each of the four pool sites, at three dates (date of leaf bag exposure, first sampling date, second sampling date), in each of the 5 streams (therefore $n = 12$ per stream). In addition, surface water was collected, filtered (0.7 μm glass fiber filters, Whatman GF/F), and stored in polyethylene bottles until analysis for dissolved nutrients. One iButton temperature logger (iBCod 22L, Alpha Mach Inc., Mont St-Hilaire, Canada) was fixed to an iron rod at each of the four pool sites to record the temperature at hourly intervals.

Laboratory analyses of field samples

Immediately upon arrival at the local laboratory, leaves were removed from the bags and carefully rinsed with tap water to remove any adhering debris. The remaining slurry from the coarse mesh bags was passed through a 500 μm mesh screen to retain macroinvertebrates, which were preserved in 70% ethanol until the individuals were identified, counted, and assigned to functional feeding groups (Schmidt-Kloiber et al. 2006). Invertebrate abundance was expressed as number of individuals (Ind) per g leaf ash free dry mass (AFDM).

Two discs were cut out from each of 5 randomly selected leaves from every bag (10 discs per bag) using a cork borer (11 mm diameter). One set of 5 discs was placed in an individual small polyethylene bag and sent to the IGB (kept frozen at −20°C) for analysis of ergosterol content (see below). The second set of 5 discs was placed into an aluminium pan, oven-dried (40°C, 48 h), and weighed to the nearest 0.0001 g to obtain DM. This set was subsequently ignited at 450°C in a muffle furnace to obtain the percentage of organic compounds.

Following quick drying using a fan, the bulk of leaves from each bag was oven-dried (40°C, 48 h) before

weighing to the nearest 0.01 g DM. Total DM of leaves per bag was then calculated as the sum of DM of the bulk of leaves and the sets of leaf discs. The total AFDM remaining was obtained by multiplying the total DM with the percentage of organic compounds determined with the set of 5 discs. To determine the mass loss due to handling of bags, extra control leaf bags were carried to the field and back, and remaining mass was defined as 100% AFDM remaining (Benfield 2006). In addition, the initial weights of the control and irradiation samples were corrected for leaching losses occurring within the first 24 h of submersion, since leaves from the anoxic pretreatment had already been leached during 21 days of incubation in the water tank prior to initial weight determination for the field experiment. Each correction factor was determined from the mean leaching loss during 24 h as explained earlier.

The total content of ergosterol (a proxy for fungal biomass) in decomposing leaves was measured from a freeze-dried and weighed (DM) set of 5 leaf discs from each bag (see above), by microwave-assisted liquid phase extraction (Young 1995) and detection with HPLC and UV spectrometry (Gessner 2005a; Dionex UltiMate 3000 LC, Sunnyvale CA, USA). The ergosterol concentration in leaves was expressed in μg g^{-1} AFDM of leaves.

At each participating laboratory, filtered water samples from the field were analyzed for the concentration of soluble reactive phosphorus (SRP), nitrate (NO_3^-), and dissolved organic carbon (DOC) using standard colorimetric methods and a TOC analyzer.

Data analysis

The tissue constituents of preconditioned (i.e. incubated in anoxic water tank or UV–VIS irradiated and leached) versus leached control leaves were compared using the Wilcoxon rank sum test.

The overall effects of stream (n = 5) and treatment (control, irradiation, anoxic; n = 3) on % AFDM remaining, ergosterol content, and invertebrate abundance, were tested using the Kruskal–Wallis rank sum test. Subsequently, differences in % AFDM remaining, ergosterol content, and invertebrate abundance between the two sampling dates, mesh sizes (coarse and fine), and between the treatments and the control, were estimated using the Wilcoxon signed rank test. Samples were therefore paired so that they matched in all factors (stream, sampling date, mesh size, treatment, and replicate pool site) except for the factor tested. Relations among variables were assessed using Spearman rank correlations.

Non-parametric tests were chosen throughout, because not all data met normality requirements, even after data transformations. All statistical analyses were performed using *R* statistical software (version 2.12.0, Ihaka and Gentleman 1996, http://www.r-project.org) with a significance level set at $\alpha = 0.05$ for all tests.

Results

Environmental characteristics of the study streams

The five streams differed substantially in water quality (Table 2), reflecting the different land use types in their respective catchments. The temperate Demnitzer was the coldest and most nutrient-rich stream, while Fuirosos had the lowest nutrient content. Specific conductance differed by one order of magnitude among streams (158–1554 μS cm^{-1}). Current velocity showed high within-stream variability and peaked in Taibilla at 85 cm s^{-1}. No flood or dry events occurred during the field experiments.

Leaf preconditioning

Leaves incubated under anoxic conditions lost 18.5 ± 1.2% AFDM (mean ± 1SD, n = 4) during the first 24 h, and an additional 7.8 ± 1.5% AFDM after 21 days (the end of the experimental exposure). Leaves preconditioned by UV–VIS irradiation lost 3.7 ± 1.1% AFDM after 21 days, and an additional amount of 19.2 ± 1.1% AFDM during subsequent leaching (24 h exposure). In comparison, control leaves lost only 1.9 ± 0.6% AFDM after 21 days (stored dry and dark), and subsequent leaching led to an additional loss of 16.8 ± 0.7% AFDM.

Incubation under anoxic conditions led to an increase in relative fiber content and a decrease in the C:N ratio and phenolic content (Table 3). In contrast, in the irradiated leaves, these components did not differ significantly from the controls after leaching (Table 3).

In-stream leaf decomposition

The % AFDM remaining after the decomposition experiments varied significantly between the five streams (Kruskal–Wallis, $\chi^2 = 30.94$, $P < 0.001$, n = 239) (Fig. 1). The mean % AFDM remaining at the second sampling date was 33% lower than at the first sampling date (Wilcoxon signed rank, $V = 6896$, $P < 0.001$, n = 119). The mean % AFDM remaining was 11% lower in coarse than in fine mesh bags (Wilcoxon signed rank, $V = 5969$, $P < 0.001$, n = 119). However, a few fine mesh bags contained lower % AFDM than did the corresponding coarse mesh bag (regardless of stream type, treatment, sampling date, or site). Remarkably, some fine mesh bags from Demnitzer and Taibilla in the first sampling and from Vallcebre in the second sampling had an increase in % AFDM remaining, instead of a decrease.

Table 3 Tissue components (mean ± 1SD, n = 4) of air-dried *P. tremula* leaves (initial) and comparison of tissue components of leaves stored under dark and dry conditions and leached for 24 h (control) with preconditioned leaves that were (a) exposed to irradiation with UV–VIS light and leached for 24 h (irradiated), and (b) incubated and leached in anoxic water for 21 d

	Initial	Control	Irradiated	Anoxic
AFDM (%)	89.8 ± 0.4	91.9 ± 0.3	90.1 ± 0.8*	88.8 ± 3.1
Lignin (%)	14.5 ± 0.6	18.1 ± 0.5	17.4 ± 0.7	21.0 ± 1.0*
Cellulose (%)	24.1 ± 0.6	30.4 ± 0.6	30.2 ± 0.2	32.6 ± 0.8*
Phenolics (%)	3.2 ± 0.3	1.5 ± 0.1	1.6 ± 0.3	1.3 ± 0.1*
C:N ratio	61.3 ± 2.6	58.3 ± 2.1	56.3 ± 2.4	51.4 ± 2.2*
Lignin:N ratio	19.0 ± 0.6	22.3 ± 0.8	20.7 ± 1.5	22.7 ± 0.6

AFDM ash free dry mass

Asterisks indicate a significant effect of preconditioning treatment (irradiation, anoxic pool environment) compared to control (Wilcoxon rank sum test, $*P < 0.05$)

Overall, anoxic incubation and irradiation inhibited loss of leaf mass (Fig. 1; Table 4). For the anoxic treatment, the inhibition was significant for both sampling dates and mesh sizes, with 7% more AFDM remaining on average. For the irradiation treatment, the inhibition was only significant for fine mesh bags (Table 4), with 4% more AFDM remaining on average. Significant preconditioning effects were detected for all study streams except for Vallcebre.

Leaf-associated fungal biomass

The concentration of ergosterol (a proxy for fungal biomass) in decomposing leaves differed significantly among the five streams (Kruskal–Wallis, first sampling $\chi^2 = 13.64$, $P < 0.01$, second sampling $\chi^2 = 27.48$, $P < 0.001$, n = 60). In addition, ergosterol concentrations increased significantly between the two sampling dates (Wilcoxon signed rank, $V = 0$, $P < 0.001$, n = 60). Mean concentrations were 51 ± 18 μg g^{-1} AFDM (mean ± 1SD, n = 60) at the first sampling and 160 ± 53 μg g^{-1} AFDM at the second sampling (Fig. 2).

At the first sampling, there were no significant treatment effects on the ergosterol concentration. At the second sampling, the ergosterol concentration in leaves from the anoxic preconditioning was on average 22 μg g^{-1} AFDM lower than in the control leaves and 33 μg g^{-1} AFDM lower than in the irradiated leaves (Wilcoxon signed rank, control $V = 159$, $P < 0.01$, irradiated $V = 175$, $P < 0.001$, n = 20).

Lower concentrations of ergosterol generally corresponded to higher percentages of AFDM remaining

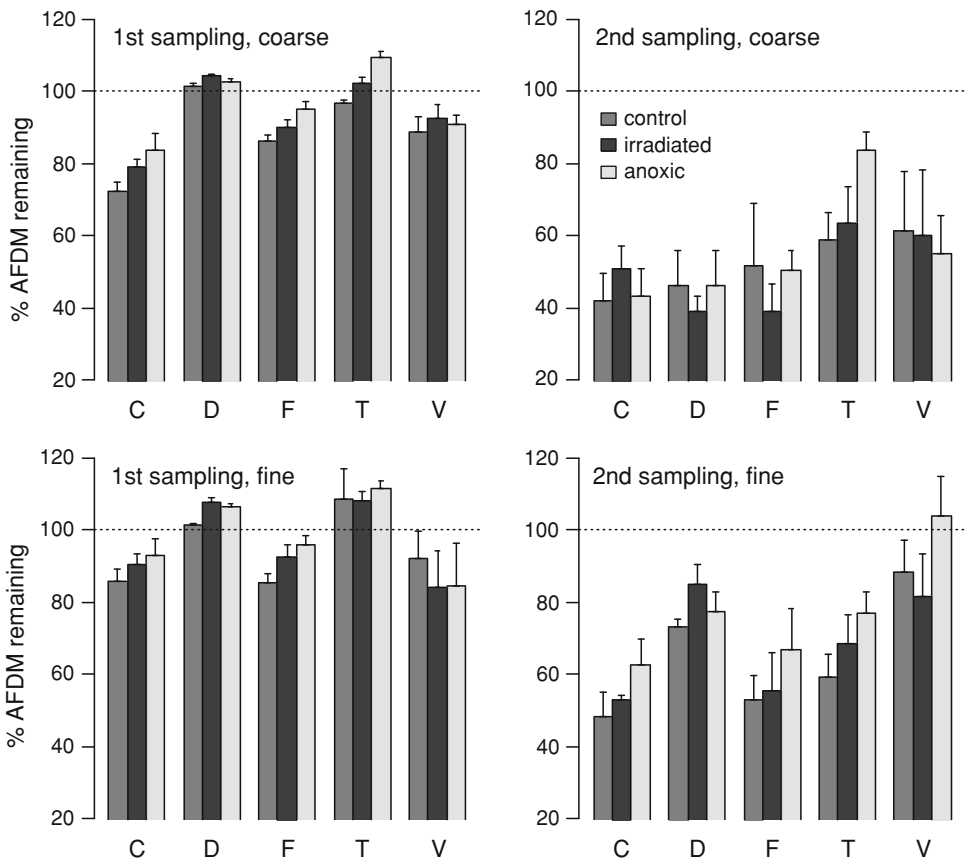


Fig. 1 Percentage of remaining ash free dry mass (AFDM) of control and preconditioned (irradiation with UV–VIS light, anoxic pool environment) *P. tremula* leaves in coarse mesh (8 mm) and fine mesh (0.5 mm) litter bags after 10 days (1st sampling) and approximately 45 days (2nd sampling) in five temporary streams (*C* Candelaro, *D* Demnitzer, *F* Fuirosos, *T* Taibilla, *V* Vallcebre; mean ± 1SD, n = 4)

Table 4 Paired Wilcoxon signed rank tests on the effect of preconditioning (irradiation with UV–VIS light, anoxic pool environment) on leaf mass loss of *P. tremula* leaves for two sampling dates (10 days and approximately 45 days) and two mesh sizes in five temporary streams

	Total (n = 80)	1st sampling (n = 40)	2nd sampling (n = 40)	Coarse mesh (n = 40)	Fine mesh (n = 40)
Control versus irradiated	2,154**[a]	626**	476[a]	506[a]	587*
1st sampling (n = 20)				174	195
2nd sampling (n = 20)				91[a]	110*
Control versus anoxic	2,557***	688***	618**	615**	679***
1st sampling (n = 20)				187	227*
2nd sampling (n = 20)				120**	128**
Anoxic versus irradiated	2,263***[a]	574*	188**[a]	541*[a]	605**
1st sampling (n = 20)				112	198
2nd sampling (n = 20)				135***[a]	115*

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

[a] Due to one missing value, n is reduced by 1

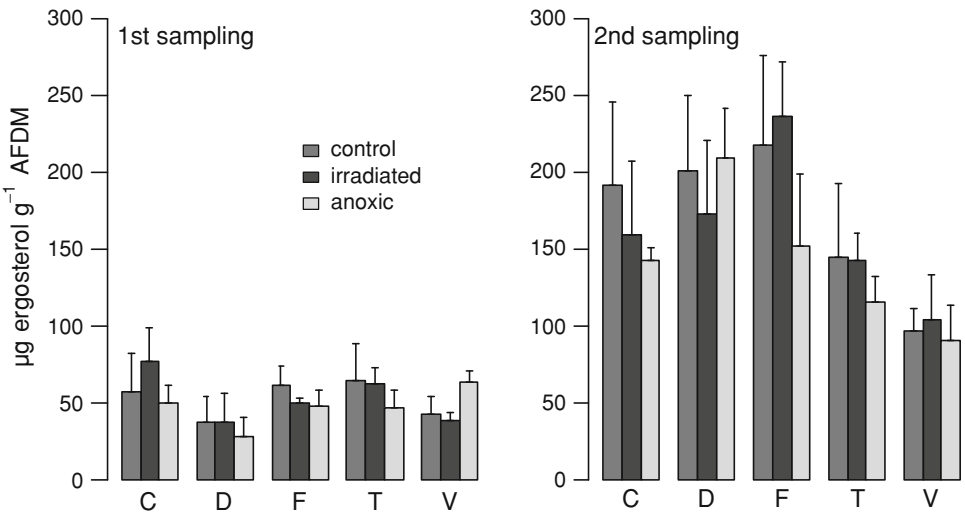


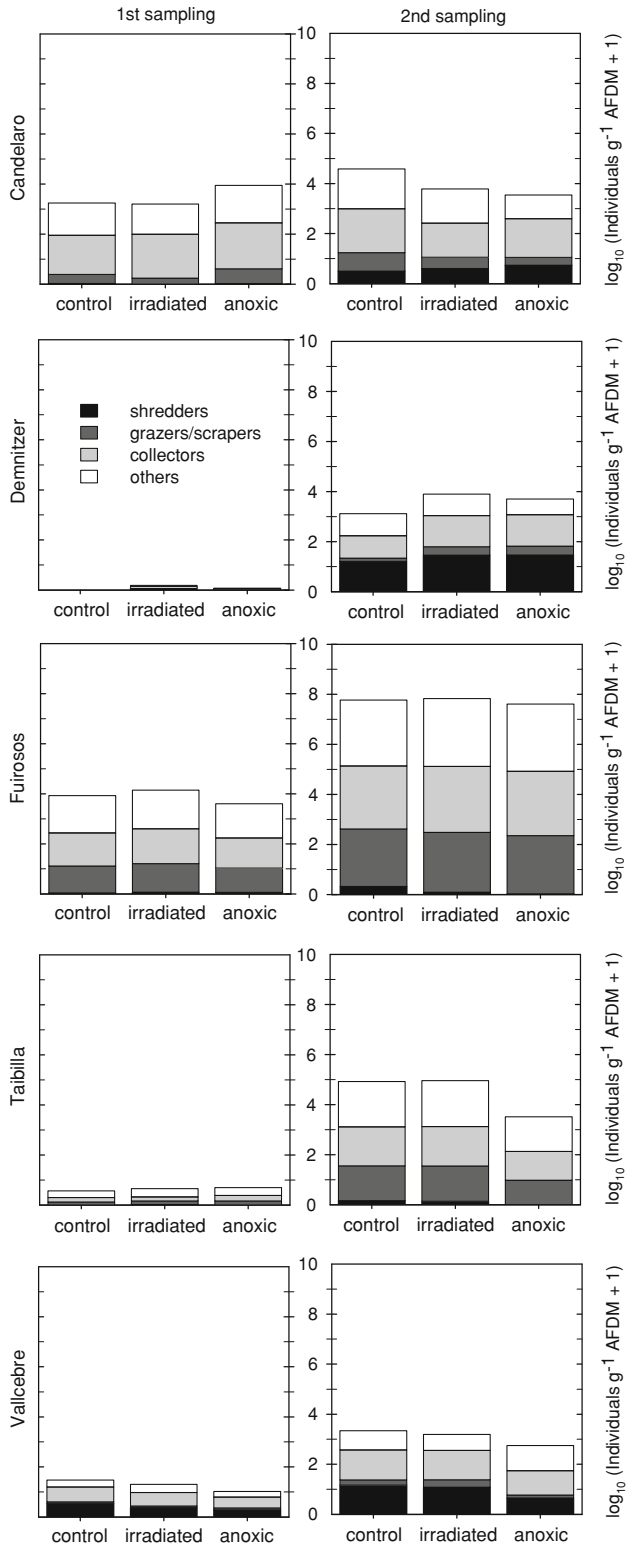
Fig. 2 Concentration of ergosterol in remaining ash free dry mass (AFDM) of control and preconditioned (irradiation with UV–VIS light, anoxic pool environment) decomposing *P. tremula* leaves in fine mesh (0.5 mm) litter bags after 10 days (1st sampling) and approximately 45 days (2nd sampling) of exposure in five temporary streams (*C* Candelaro, *D* Demnitzer, *F* Fuirosos, *T* Taibilla, *V* Vallcebre; mean ± 1SD, n = 4)

(Spearman rank correlation, $\rho = -0.56$, $P < 0.001$, n = 60). In particular, the difference in the ergosterol concentration between the anoxic and control leaves, and between the anoxic and irradiated leaves, corresponded to the difference in % AFDM remaining (Spearman rank correlation, control $\rho = -0.57$, $P < 0.001$, irradiated $\rho = -0.36$, $P < 0.05$, n = 20), i.e. less ergosterol was associated with slower leaf mass loss for the anoxic preconditioning.

Leaf-associated macroinvertebrates

The invertebrate community associated with the leaf packs consisted of 36 families (95% of all individuals were *Insecta*, for taxa list see Online Resource 1). Abundances ranged from 0 Ind g^{-1} AFDM in the Demnitzer to 2,870 Ind g^{-1} AFDM in Fuirosos (Fig. 3). Mean values at the second sampling were 66 ± 43 Ind g^{-1} AFDM (mean ± 1SD, n = 12) in Candelaro, 43 ± 35 Ind g^{-1} AFDM in Demnitzer, 1,069 ± 969 Ind g^{-1} AFDM in Fuirosos, 99 ± 68 Ind g^{-1} AFDM in Taibilla, and 28 ± 17 Ind g^{-1} AFDM in Vallcebre.

The total abundance of invertebrates and the abundance of each of the three feeding groups (shredders, grazers, collectors) were significantly different among the five streams (Kruskal–Wallis, total abundance $\chi^2 = 38.53$, shredders $\chi^2 = 57.44$, grazers $\chi^2 = 58.01$, collectors $\chi^2 = 45.06$, $P < 0.001$ for all, n = 119). Shredders were only dominant in Demnitzer (up to 58 Ind g^{-1} AFDM or up to 69% of all individuals) and were rare in the other streams, where collectors dominated (38% of all individuals on average).



◀ **Fig. 3** Relative abundance (individuals g^{-1} leaf ash free dry mass (AFDM)) of invertebrate functional feeding groups in coarse mesh bags (8 mm) associated with control and preconditioned (irradiation with UV–VIS light, anoxic pool environment) decomposing *P. tremula* leaves in five temporary streams (mean of four replicate pool sites) after 10 days (1st sampling, *left panel*) and approximately 45 days (2nd sampling, *right panel*). Data were $\log_{10}(x + 1)$ transformed to adjust scales

Abundances of each of the three feeding groups were significantly higher at the second sampling date than at the first sampling date, except in Candelaro (significantly lower at the second date) (Wilcoxon signed rank, total abundance $V = 208$, shredders $V = 33$, grazers $V = 108$, collectors $V = 278$, $P < 0.001$ for all, n = 59). Preconditioning of leaves did not significantly affect the total invertebrate abundance, or feeding group abundance (Kruskal–Wallis, $\chi^2 \leq 0.68$, $P > 0.05$ for all, n = 119).

The mean contribution of macroinvertebrates to total mass loss (based on the difference in AFDM lost between coarse and fine mesh bags) was 32%. The percentage of invertebrate contribution to mass loss was not significantly affected by preconditioning treatment (Kruskal–Wallis Test $\chi^2 = 1.14$, df = 2, $P > 0.05$, n = 119).

Discussion

In this study, we experimentally tested the effects of preconditioning by photodegradation on sunlit streambeds and by anaerobic fermentation in anoxic pools of drying streams on leaf decomposition during the subsequent flowing phase. Overall, results supported our initial hypotheses. We found changes in leaf chemistry, mainly due to anoxic preconditioning, and significant decay-retarding effects of preconditioning on European aspen leaves in four of the five temporary streams. Preconditioning effects on decomposer communities, however, were only observed for fungal biomass.

Preconditioning effects on leaf chemistry and leaf mass loss

The effect of preconditioning on leaf chemistry differed between the two types of treatments. Anoxic preconditioning increased the proportion of less palatable refractory organic compounds (lignin, cellulose, and phenolics), which was accompanied by a decrease in the C:N ratio of the leaf tissue. In previous studies, in contrast, the leaching of nutrients led to an increase in the C:N ratio, which in turn reduced the subsequent leaf mass loss rate (Webster and Benfield 1986; Hladyz et al. 2009). In our study, the observed decrease in the C:N ratio was probably due to the intensive leaching and breakdown of labile carbon compounds, which increased the proportion of the remaining refractory carbon compounds (Boulton and Boon 1991). This most likely led to the observed retardation in mass loss, indicating that the quality of carbon may be more important for leaf mass loss than the C:N ratio per se.

Although we detected no effect of irradiation on leaf tissue components, there was a significant decrease in % AFDM. Nonetheless, irradiated leaves tended to lose more nutrients than control leaves during subsequent leaching (D. Dieter, unpublished data), suggesting that in addition to leaf desiccation (Leopold et al. 1981) photodegradation may also favor the release of nutrients from leaves during inundation, causing a reduced mass loss rate.

The mass loss we observed was comparable to previous reports for congeneric species in permanent streams (e.g. *P. nigra*: Langhans et al. 2008; *P. tremuloides*: Royer and Minshall 2001; see also summary by Casas and Gessner 1999). Unexpectedly, leaves from the first samples in Taibilla and Demnitzer and leaves in fine bags from Vallcebre gained AFDM over time. In Taibilla and Vallcebre, mass loss was probably restricted due to extensive carbonate deposition on leaf surfaces, especially in fine mesh bags, which was consistent with an increase in ash content (i.e. decrease in loss on ignition of leaf material down to 38% LOI in Vallcebre). Although a lower ignition temperature was chosen (450°C instead of 550°C) to avoid carbonate combustion, part of the carbonate could have been volatilized and therefore increased the calculated portion of organic compounds, leading to an overestimation in remaining AFDM. However, carbonate coating was not observed in Demnitzer. In this stream, biofilms may have developed on the surface of the leaves, contributing to an increase in AFDM together with organic deposition.

The effect of preconditioning on leaf mass loss also differed between the two types of treatments. Mass loss after approximately 45 days was reduced by 7% AFDM and 4% AFDM when leaves were preconditioned by anoxic incubation and irradiation, respectively. The effect of preconditioning on leaf mass loss was consistent over four of the five selected streams. The exception was Vallcebre, where we did not detect any effects. This inconsistency suggests that the effect of preconditioning may be mediated by characteristics of the stream water other than concentrations of nutrients, for example carbonate. In streams, where significant effects were detected, the retardation of leaf mass loss after preconditioning was most likely due to the observed modification in leaf tissue composition.

The effect of anoxic preconditioning was more pronounced than was the effect of irradiation. This effect was consistent for both sampling dates, and for fine and coarse mesh bags, indicating that preconditioning can affect leaf decomposition by microorganisms and invertebrates at early and late stages of decomposition.

Preconditioning effects on decomposer communities

The effect of preconditioning on leaf chemistry and leaf mass loss was only partially reflected in the abundance and composition of decomposer communities, with an overall stronger effect on fungi than on invertebrates.

Living fungal biomass was in the lower range of values previously reported (Gessner 2005a; Langhans and Tockner 2006). Anoxic preconditioning significantly reduced fungal biomass in decomposing leaves. The most likely explanation was the inhibition of fungal colonization because of the reduced quality of the carbon substrate. An alternative explanation is that terrestrial fungi, which colonize the leaves when they are attached to the tree, and which are among the first to grow on submerged leaves (Albrectsen et al. 2010), might have been killed during preconditioning under anoxic, acidic conditions. This may have retarded the growth of fungal biomass until re-colonization. Moreover, the concentration of ergosterol in fungal biomass may differ among fungal species and among their specific physiological states (Salmanowicz and Nylund 1988), which may lead to differences in ergosterol content despite similar biomass. Mass loss in fine mesh bags was significantly reduced by both irradiation and anoxic preconditioning. However, the ergosterol content was only significantly reduced in the anoxic preconditioned leaves. The fungal biomass in irradiated leaves may be similar to control leaves, but a reduced fungal activity may have resulted in slower mass loss.

Although anoxic preconditioning significantly reduced mass loss in coarse mesh bags, there was no effect on macroinvertebrate abundance. Invertebrates feeding on leaves may indeed have consumed less because of the low chemical quality and reduced fungal colonization of the leaf material, but they may have used the leaf packs not only as a food source but also as a habitat, which might have concealed the feeding effect (Richardson 1992; Dudgeon and Wu 1999). We believe that data on invertebrate biomass could have provided additional information, but assumed that abundance and biomass often follow the same pattern (Dangles et al. 2004).

Differences in mass loss between coarse and fine mesh bags are assumed to result from the different contributions of microbial versus invertebrate communities to total mass loss (Webster and Benfield 1986; Boulton and Boon 1991). We found that invertebrates contributed 32% to total mass loss, with values within previously reported ranges (Graça 2001; Langhans et al. 2008). The highest contribution of invertebrates to mass loss was found in Demnitzer and Vallcebre, which may be due to the higher abundance of shredders, compared to the other streams. In the other streams, collector-gatherers were dominant, while crustacean shredders, in particular, were rare because they are less adapted to intermittent stream flow (Maamri et al. 1997; Langhans and Tockner 2006; Datry et al. 2011). Intermittent stream flow favors organisms with drought resisting strategies, such as diapause and terrestrial stages

(Williams 2006), which is supported by the high proportion of insects found in our study.

Implications for organic matter dynamics in temporary streams

Ecosystem processes (such as organic matter decomposition, stream metabolism, and nutrient uptake) occurring during the dry phase in temporary streams have rarely been investigated, and have only recently been incorporated into conceptual models of temporary streams (Larned et al. 2010). Larned et al. (2010) proposed that the organic matter is continuously processed during downstream transport in permanent streams. This longitudinal pathway is interrupted by terrestrial processing modes during intermittence of water flow in temporary streams. Organic matter deposited during intermittence of flow is subject to a diversity of processing modes, including decomposition by aquatic and terrestrial communities, anaerobic and aerobic microbial metabolism, leaching, and material degradation by solar radiation and desiccation. Larned et al. (2010) consequently predicted that temporary streams will exhibit (a) longitudinal gradients in processing rates of organic matter with (b) higher processing rates during flow periods than during dry periods and (c) an increasing processing efficiency with increasing number of drying and re-flooding cycles due to the high diversity of processing modes.

Our study provides empirical evidence that photodegradation on sunlit stream beds, and the anaerobic processes in residual pools, modify leaf chemistry, which in turn reduces fungal biomass and decelerates leaf decomposition rates after flow recovery. These effects are likely to be enhanced by extended dry events and repeated drying and re-flooding cycles. Supporting the main conceptual ideas of Larned et al. (2010), this indicates that during dry phases, leaf processing is not only reduced but a preconditioning of leaves occurs, which has consequences on their further decomposition during wet phases. Therefore, the temporal and spatial heterogeneity of drying and re-flooding cycles should be taken into account when examining organic matter dynamics in temporary streams. Corroborating this, some previous studies reported decomposition of leaves to be slower at sites with low inundation permanence (e.g. Maamri et al. 1997; Datry et al. 2011), but without any acceleration of the decomposition rate at a higher frequency of drying and re-flooding cycles (Langhans and Tockner 2006). The effect was generally attributed to the scarcity of leaf-shredding invertebrates under temporary wet conditions, leading to a decrease in invertebrate-driven leaf processing (Maamri et al. 1997; Langhans and Tockner 2006; Datry et al. 2011; our study). Our study adds the effect of leaf preconditioning during dry phases reducing leaf decomposition rates during wet phases due to leaf chemical modifications. Consequently, the efficiency of organic matter processing in temporary streams is likely low, and extended no flow periods may lead to an increase in organic matter of poor quality being intermittently transported downstream.

These findings are especially relevant if we consider that the geographical extent of temporary streams is expected to increase worldwide due to the effects of climate and land use change. We expect that in permanent streams that will develop intermittence of flow in the future, the organic matter dynamics will change towards lower decomposition efficiency and discontinuous processing. The accumulation and preconditioning of organic matter during the intermittence of flow will result in major nutrient and carbon pulses at first flush events, and a decrease of substrate quality leading to retardation of organic matter decomposition.

In the present study, we empirically quantified the preconditioning effects on leaf litter decomposition and leaf-associated decomposers in temporary streams. Further studies are needed to understand the underlying biogeochemical mechanisms during preconditioning, the effect of preconditioning on microbial community structure and function, the interactions with other in-stream processes such as nutrient uptake and respiration, as well as the consequences for organic matter balances at the stream network and catchment scale.

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