Mine Water Environ (2017) 36:239–247
DOI 10.1007/s10230-017-0432-x

TECHNICAL ARTICLE

Do Mining Activities Significantly Affect Feeding Behavior of Freshwater Benthic Macroinvertebrates? A Case Study in South Sardinia (Italy)

Alberto Basset[1] · Maurizio Pinna[1] · Monia Renzi[1,2]

Received: 10 July 2015 / Accepted: 18 January 2017 / Published online: 25 March 2017

Abstract We investigated the cascading effects of chemical contaminants on alder leaf detritus quality and the exploitation of this feeding resource by benthic macroinvertebrates (isopods, gastropods, and mayfly nymphs). Trophic behavior and energetics of benthic macroinvertebrates were used to evaluate the effects of contaminants on detritus exploitation. A two-way factorial nested experimental design was used to statistically quantify how leaf detritus origin (polluted and unpolluted streams) and the pollution level of the site selected for microbial conditioning of the detritus could affect the feeding resource quality for aquatic benthic macroinvertebrates, as measured by their trophic behavior (i.e. food selection) and exploitation (i.e. food ingestion). Alder leaves collected from a polluted stream in a former mining area (South-Sardinia, Italy) had Cd, Pb, and Zn concentrations up to 10 times that of leaves collected from an unpolluted stream. When benthic macroinvertebrates were given the option to choose, they all selected leaves from the unpolluted stream and/or those conditioned in the unpolluted stream. Ingestion rates were also significantly affected by both considered factors: leaf origin and conditioning. In addition, synergistic effects strongly increased the chemical contaminant stress on the leaf detritus quality. These results show that the terrestrial component of land–water ecotones affected by mining can be a relevant indirect pathway of chemical stress to benthic macroinvertebrate species.

Keywords Water contamination · Chemical contaminants · Microbial conditioning · Alder leaves · Detritivore macroinvertebrates · Streams

✉ Monia Renzi
monia.renzi@bsrc.it

[1] Department of Biological and Environmental Sciences and Technologies, University of Salento, SP Lecce-Monteroni, 73100 Lecce, Italy

[2] Bioscience Research Center srl, via Aurelia Vecchia 32, 58015 Orbetello, Italy

Introduction

Mining can cause contaminants to leach into groundwater and discharge into streams (Cidu et al. 2009). Leaching from waste rock and tailings can continue long after mine closure; this leachate degrades the surrounding environment, as well as groundwater and stream water quality, and can even impact marine coastal areas (Cidu et al. 2007). Chemical contaminants affect freshwater ecological communities by direct toxicity (i.e. via zinc pollution; Zhang et al. 2012). As example, various ecotoxicological studies have evaluated the effects of direct exposure to chemical contaminants. Cadmium concentrations of 1–5 mg L^{-1} affect blood and kidney functions of vertebrates (Carlson and Zelikoff 2008), while effects on gills are recorded after 3 days of exposure to 5 mg L^{-1} (Gony 1990). Levels of Pb greater than 300 mg L^{-1} cause blood alterations after 30 days of in vitro exposure (Santos and Hall 1990). Furthermore, indirect effects, that modify the quality of feeding resources and animal behavior, could affect freshwater communities thereby acting on the detritus-based food web structures, i.e. smothering of benthic zones by iron oxyhydroxides (Mayes et al. 2008) or incorporation into food resources (Cobb et al. 2000). In landscapes severely perturbed by mining, contaminated leaves coming from the stream basin's riparian vegetation potentially represent an additional contaminant source that could impact the detritus-based communities of streams (Basset 1993). Inverse relationships between the concentrations of chemical contaminants, pollution in aquatic ecosystems, and some indicators of community health status as well as decomposition rates, species richness, and abundance have already been reported (Basset et al. 2013; Dadea et al. 1996; Pinna et al. 2013; Smolders et al. 2003; Vignes et al. 2012; Xu et al. 2014). Furthermore, a direct relationship between contaminant concentrations in water and species richness and body-size features suggests that chemical contaminants degrade trophic resources (Dadea et al. 1996). In fact, body-size, species richness, and abundance are principally affected by trophic resource availability and its quality.

Plant detritus decomposition is an interactive process that involves heterotrophic microorganisms and detritivore macroinvertebrates (Borsodi et al. 2003; Lussenhopp 1992; Suberkropp and Klug 1976; Varga 2003). Leaf detritus decomposition supports stream food webs (Gessner et al. 1999; Petersen and Cummins 1974; Tank et al. 2010), although the efficiency of decomposition processes can depend on numerous factors such as spatial and temporal patterns (Fonnesu et al. 2004; Pinna et al. 2003; Sangiorgio et al. 2008), climate events (such as summer drought, which occurs commonly in the Mediterranean ecoregion; Di Sabatino et al. 2014; Pinna and Basset 2004; Pinna et al. 2004, 2016), nutrient availability, and other environmental factors (reviewed by Webster and Benfield 1986). In particular, the microbial conditioning process (sensu Menéndez et al. 2003) allows initial leaf detritus colonization by microorganisms, making the detritus more appealing for macroinvertebrates (Bärlocher 1981). During the conditioning (after at least 20 days of exposure), different nutrient loads in the water produce significant differences in macroinvertebrates feeding behavior (Menéndez et al. 2003). Nevertheless, the effects of chemical contaminants on the conditioning process have not yet been clarified. Chemical contaminants could affect trophic webs of aquatic ecosystems directly, affecting species density and species richness, or indirectly, affecting leaf detritus quality and its conditioning process. Therefore, some aspects such as palatability, nutrient content, attractiveness, and energy content of leaf detritus can be reduced indirectly by chemical contamination of the leaf origin sites and the quality of the conditioning streams.

For example, meiobenthic taxa, which are restricted to free-burrowing lifestyles in aerobic sediment horizons, are severely vulnerable to water and sediment pollution, especially metals that are bioavailable between the sediment–water interface and the redox boundary, due to their oxidation state (Hagopian-Schlekat et al. 2001). Despite exposure to Cd, Pb, and Zn levels in the abiotic compartments, this study has indicated that feeding resources could represent an important source of chemical contaminants for the meiobenthic communities in ecosystems stressed by mining activities. Furthermore, levels accumulated in tissues of these species could be transferred through the trophic web, with possibly important consequences to vertebrates and humans, and ecosystem health (Coull and Chandler 1992). In fact, copepods and nematodes are an obligate nutritional source for many estuarine juvenile fish and crustaceans (Coull 1990; Gee 1989). Recent research indicates that biological and geochemical characteristics play an important role in metal accumulation in benthic invertebrates, and consequently in metal transfer to higher trophic levels. Concentrations of metals in benthic organisms increase with both the metal content (with the exception of Cd), and the silt, refractory organic matter, and chlorophyll-*a* levels in the sediment, which in turn are influenced by the effects of sediment geochemistry on metal bioavailability (Kalantzi et al. 2014).

This research focused on indirect effects of chemical contaminants (i.e. Cd, Pb, Zn) leached from former mining sites on the feeding behavior of benthic macroinvertebrates. In particular, the research addressed three main questions: (1) can leaves from a mining basin accumulate chemical contaminants? (2) Can chemical contamination of leaves affect the attractiveness of the feeding resource and its palatability for benthic macroinvertebrates? (3) Can the microbial conditioning process of leaf detritus, carried out in streams with different concentrations of chemical contaminants in water, affect the feeding process and the feeding behavior of benthic macroinvertebrates?

Methods

Experimental Design

Laboratory experiments were developed to reduce Type I and Type II errors following a logic model based on a nested hierarchical design developed on fixed vs a priori randomly defined factors (Benedetti-Cecchi 2004; Underwood 1994, 2003). Two nested-design experimental sets were performed. The first experimental set was developed using a two-way factorial nested experimental design (Fig. 1). In the first set, the origin of leaf detritus (leaf origin, *LO*, with two fixed levels: polluted and unpolluted river basin) and the contamination of the stream used for the microbial conditioning process of leaves [conditioning site (*CS*), with three fixed levels: unpolluted (UP), slightly polluted (SP), highly polluted (HP) stream] were combined ($n = 6$). Four benthic macroinvertebrate species were tested separately for each $LO \times CS$ combination leaf detritus condition. Leaves from unpolluted and polluted river basins were acclimatized in the field for 20 days in UP, SP, and HP streams. Each leaf detritus condition was offered to benthic macroinvertebrates separately. The second experimental set (Fig. 1) was developed with the same design as the first. However the six feeding resource combinations ($LO \times CS$) were offered together, giving the animals of each species the option to choose a resource combination. Mean frequency of individuals and average consumption rates were calculated for each species.

Origin of Feeding Resource

In this study, alder leaves (*Alnus glutinosa*; Gaertn 1790) were collected from riparian trees in a mine water polluted river basin, Rio Ingurtosu (39.525334 N, 8.474588E), located inside the Sardinian Geomining Park, and in an unpolluted river basin, Rio de Su Casteddu (39.270788 N, 8.832089 E), located outside the Geomining Park, in an area that has not been mined. Alder leaves were used as the feeding resource for benthic invertebrates in both experimental sets because it is widely distributed along the riparian zone of freshwater ecosystems in the Mediterranean area and contributes significantly to the food resources available to the detritus-based food webs in streams. The field microbial conditioning process was carried out in three streams with different levels of contamination (UP = Rio de Su Casteddu; SP = Rio Naracauli (39.518684 N, 8.4847182 E); HP = Rio Piscinas (39.547203 N, 8.466305 E; Table 1; Dadea et al. 1996).

Benthic Macroinvertebrate Species

Four species were chosen from the most diffuse benthic macroinvertebrates in freshwater ecosystems of Sardinia: two gastropods, *Physa acuta* (Draparnaud 1805) and *Bithynia tentaculata* (Linnaeus 1758), one crustacean isopod, *Proasellus coxalis* (Dollfus 1892), and one insect, *Caenis macrura* (Stephens 1835) at nymph stage. The selected gastropods are scraper-grazer species; isopods and insect are shredders (Goodyear and McNeill 1999). Benthic macroinvertebrates were all collected from the unpolluted stream (Rio de Su Casteddu) and were held at 15 °C in the laboratory for 20 days before the experiments started.

Sampling and Conditioning Sites

Alder leaves and benthic macroinvertebrate were collected in southwestern Sardinia (Italy), which has been widely exploited for mineral extraction since the Roman age. In this area, the principal minerals exploited were sphalerite and galena, with variable pyrite content and, in some mines, barite and fluorite. Ore bodies near the surface are generally oxidized and consist mostly of smithsonite, hydrozincite, and cerussite (Boni 1994). In the Montevecchio-Ingurtosu area, lead and zinc ores were exploited until 1991 (Dadea et al. 1996), but the industrial exploitation peak was during the 1950s (Cidu et al. 2009). The entire area is part of the Sardinian Geomining Park (*Parco Geominerario Storico e Ambientale della Sardegna*); Sardinian Regional Authority planned the environmental reclamation and economic development of the impacted area (RAS 2003).

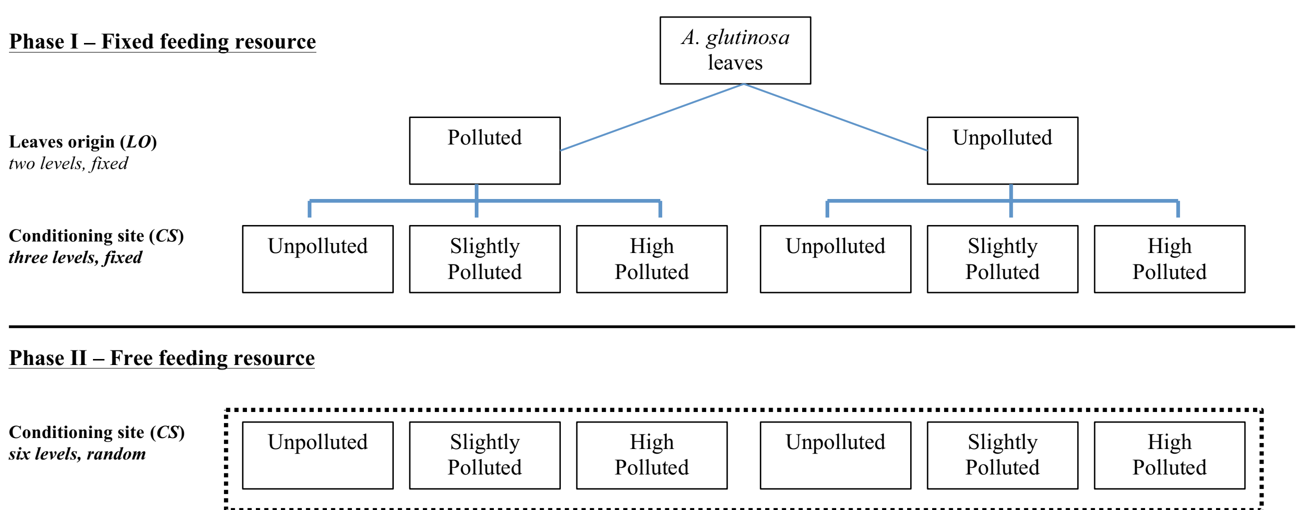

Fig. 1 Experimental design. The hierachical model of the two-way factorial nested experimental design: the origin of the leaf detritus (leaf origin, *LO*, two levels: polluted and unpolluted river basin) and the stream of the in-field leaf microbial conditioning process (conditioning site, *CS*, three levels fixed: unpolluted, slightly polluted, high polluted streams) were combined ($n = 6$). During Phase I, feeding resources were given separately, while in Phase II, each species could choose from among the six different resources

Table 1 General features of the sampling sites

River	Pollution	Zn (mg L^{-1})	Pb (mg L^{-1})	Cd (mg L^{-1})	*LO*	*CS*
Rio De Su Casteddu	UP	<3.00	<0.050	<0.005	Yes	Yes
Rio Naracauli	SP	16.67	0.015	0.210	Yes	Yes
Rio Piscinas	HP	54.14	0.066	0.577	No	Yes

Pollution categories: *UP* unpolluted, *SP* slightly polluted, *HP* highly polluted

Levels of chemical contaminants in water were reported from the literature (Dadea et al. 1996). Leaves were collected in unpolluted and polluted sites by a mining area (Montevecchio-Ingurtosu basin, South-Eastern Sardinia, Italy). Stream flows for the most of their course on tailings, since stream floods were used in the past to move tailings materials towards the sea. The mining area is divided in two streamed basins: Rio Roiacani-Piscinas and Rio Naracauli. This site was exploited since the Roman age (probably the first Century B.C.) up to about 50 years ago

The occurrence of auto-depuration, where the concentrations of chemical contaminants are reduced in the water by biomineralization, was reported for the study area (Wanty et al. 2013). The former mining activities that affected the Montevecchio-Ingurtosu area produced large quantities of dust, debris, and sludge that impacted the quality of water courses (Di Gregorio and Massoli-Novelli 1988). As a consequence of mining, watercourses have been highly polluted, particularly by Cd, Pb, and Zn (Dadea et al. 1996; Medas et al. 2013). In this environmental context, *LO* and *CS* sites were selected based on the chemical contaminant concentrations in the water (Table 1).

Chemical Analyses of Feeding Resources

Five chemical contaminants (Cd, Cu, Mn, Pb, and Zn) were quantified in the leaves of *A. glutinosa* after mineralization in a microwave oven with an acid solution and quantified by Inductive Coupled Plasma—Optical Emission Spectroscopy (ICP-OES, model ARL 3520). Cd, Pb, and Zn are chemical tracers of mining activities, whereas Cu, and Mn were used as indicators of natural levels (not linked to mining). In fact, Cu and Mn are not typically associated with the geological mineralization reported for this mining area (Cidu et al. 2007). Recovery and reproducibility of the quantification method were tested using opportune standard reference materials (NIST). Observed recoveries were higher than 95% for all tested substances. The quantification limits were: 0.0001 mg kg^{-1} (dry weight, dw) for Cd, Cu, and Mn; 0.001 mg kg^{-1} (dw) for Pb; and 0.01 mg kg^{-1} (dw) for Zn.

Features of Behavioral Experiments

Freshly fallen *A. glutinosa* leaves were collected with nets from beneath a stand of trees in polluted and unpolluted sites. The leaves were air-dried and stored in the dark room until the microbial conditioning process, which was performed in spring. Leaves were put in fine (0.5 mm mesh size) mesh nylon bags that were tied to an iron rod anchored in the streambed. After 20 days of in field preconditioning, leaves were removed from the water and air-dried using a fan for 48 h, and then used as a food source for in vitro testing. The in vitro experiments were performed under controlled conditions. The animals collected in the unpolluted stream were preconditioned in the laboratory for a week and not fed for 1 day before the experiments. Unpolluted water was pre-filtered before the experiment. Exposure time was fixed to 16 days. During the experiments with animals, temperature was continuously monitored and maintained at 15 °C, and the light/dark photoperiod set to 12 h. To obtain more quantitative assessments of benthic macroinvertebrates densities, pre-weighted leaf-bags leaves were used. After 1 month, leaves were retrieved and the four species were identified and counted.

Energetics

The animals were dried individually in an oven at 60 °C for 72 h and weighed on a microbalance to the nearest 0.1–1.0 pg. Ash content was weighed after combustion of all specimens of each taxon in a muffle furnace at 500 °C for 6 h. Large individuals were combusted individually; smaller individuals were gathered into groups of 10–50 individuals, based on their dry weight. Individual body weights were expressed as ash free dry weight (AFDW) per the literature (Barbone et al. 2007; Marini et al. 2013; Pinna et al. 2004; Sangiorgio et al. 2014).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, http://www.graphpad.com). Graphics and statistics (ANOVA) were used to evaluate significant differences between treatments and controls (*t* student test). A specific-sized GraphPad Prism routine was used to plot and interpolate LC_{50}, LC_{20}, and their 95% confidence intervals. The results were statistically analyzed to calculate the average values (±standard deviation) and significance of observed differences between the experimental conditions.

Results

Chemical Analyses of Feeding Resources

Concentrations of chemical contaminants (mg kg^{-1} dw) in the collected *A. glutinosa* leaves in polluted and unpolluted areas are reported in Table 2. Leaves from the polluted area had accumulated high levels of Cd, Pb, and Zn. Their Pb and Cd concentrations were 3–18 times higher than those in leaves from the unpolluted stream. Chemical contaminants not considered to be linked to mining activities in this area (Cu, Mn) evidenced quite similar levels in leaves from the polluted and unpolluted areas, with ratios closed to 1. The ANOVA test performed on these data on the *LO* factor confirmed a significant difference for Cd, Pb, and Zn ($p < 0.01$) and the absence of significant differences for Cu and Mn. Enrichment factors ($BEF_{w/o}$) calculated for chemical contaminants showed enrichment in leaves from polluted sites compared to unpolluted stream. $BEF_{w/o}$ (polluted vs unpolluted sites) were: 1.52 vs 0.70 (Zn), 0.30 vs 0.68 (Cd), 101.3 vs 9.3 (Pb).

Behavioral Experiments

To distinguish the contribution of direct toxicity of the feeding resource from the contribution of the conditioning process, animals were allowed the option of choosing among feeding resources. The mean frequency of individuals from considered species relative to leaf origin and conditioning site are reported in Fig. 2. Leaves conditioned in the control stream (Rio de Su Casteddu, unpolluted), had a proportionally greater number of individuals colonizing the unpolluted leaves with time, going from a frequency of about 0.30 individuals after 2 h, to 0.40 after 96 h (data not shown). The mathematical regression describing this process is: $y = 0.301 + 0.0197 \ln(x)$ (associated statistics is: d.f. $= 6$, $r = 0.761$, $p < 0.05$).

Table 2 Concentration of chemical contaminants (mg kg^{-1} dw) in *A. glutinosa* leaves collected from both polluted and unpolluted streams (**significant differences, ANOVA test, $p < 0.01$)

LO	Cd (**)	Cu	Mn	Pb (**)	Zn (**)
Polluted	0.0630	0.0089	0.0082	1.520	25.27
Unpolluted	0.0034	0.0051	0.0072	0.464	2.11
Ratio	18.53	1.75	1.14	3.28	11.98

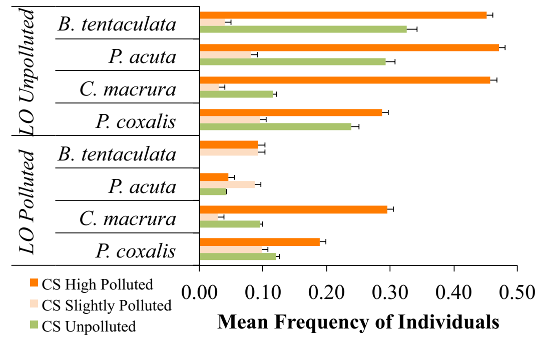

Fig. 2 Mean frequency of individuals for each species related to the two factors considered. Mean frequencies of individuals per species are reported as percentages (+SD) for each experimental condition. Data are grouped according to both leaf origin, *LO* (polluted vs unpolluted) and microbial conditioning site (*CS*): unpolluted, slightly polluted, and highly polluted) of the leaves ($n = 6$)

Table 3 shows the distribution of individuals among the resources, evidencing the role of bottom-up pathways of stress on the trophic behavior of benthic detritivores. Both *LO* and *CS* factors were significant sources of variation in the distribution of individuals. Analyzing the variance components of the mean number of individuals per resource in sets with gastropods and arthropods (Table 4) showed that almost 90% of the variance of gastropod distribution is explained by *LO*, while 57% of the variance of arthropod distribution is explained by *CS*.

Table 3 Two-way ANOVA performed on the role of bottom-up pathways of stress on the trophic behavior of benthic macroinvertebrates when they have the possibility to choose among feeding resources (**$p < 0.01$, *$p < 0.05$)

Sources of variation	SS	d.f.	MS	F
LO	26.96	1	26.96	25.97**
CS	94.48	2	47.24	45.50**
LO × *CS*	18.08	2	9.04	8.71**
Error	741.2	714	1.04	

Data related to the distribution of individuals among resources

Table 4 Variance components of the mean number of individuals per resource in sets with gastropods and arthropods

Sources of variation	Gastropods	Arthropods
LO	2.22 (89.43%)	11.25 (29.32%)
CS	0.02 (0.97%)	25.71 (67.09%)
LO × *CS*	0.06 (2.42%)	0.07 (0.17%)
Error	0.18 (7.18%)	1.35 (3.52%)

Energetics

In the first series of experiments, consumption rates simply followed the behavioral selection of the leaves, showing that it was actually a trophic behavior. Consumption rates in different sites relative to the *LO* are reported in Fig. 3; data are pooled for all species. On average, consumption rates of control leaves conditioned in the control stream were twice that observed for the contaminated resources. Concerning consumption rates, the two factors that could represent a significant source of variation ($LO \times CS$) caused significant heterogeneities in the data set. Significance of observed results were tested by two-way ANOVA (Table 5). Consumption rates are also reported for each species separately (Fig. 4).

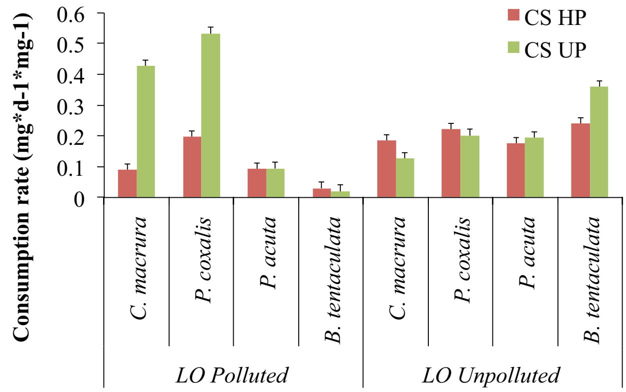

Fig. 4 Consumption rates for each species related to the two factors considered. Total average consumption rates are reported as consumption per each species to compare the effect due to the leaf origin (*LO*) with the effect due to the conditioning site (*CS*) and to assess the combined effects ($LO \times CS$) on the consumption of the feeding resource by each species. Data are expressed as mg day^{-1} per mg of biomass of individual. *HP* means highly polluted, *UP* means unpolluted

Discussion and Conclusions

Feeding Resource Features

Alder leaves collected in watercourses contaminated by former mining activities (Dadea et al. 1996) contained more Cd, Pb, and Zn than those collected in unpolluted streams.

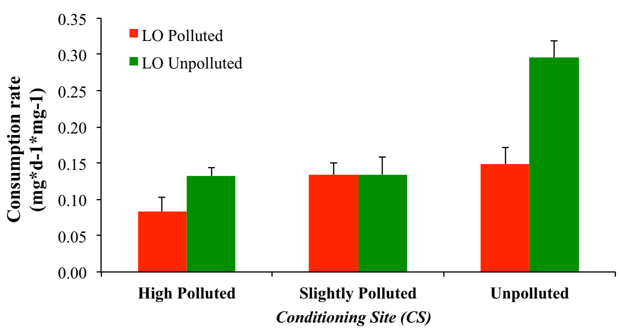

Fig. 3 Total average consumption rates related to the two factors considered. Total average consumption rates are reported as cumulative consumption to compare the effects due to leaf origin (*LO*) with the effect due to the conditioning site (*CS*), and to assess the combined effects ($LO \times CS$) on total consumption

Table 5 Two-way ANOVA performed on the role of bottom-up pathways of stress on the trophic behavior of benthic detritivores when they have the possibility to choose among feeding resources (**p < 0.01, *p < 0.05)

Sources of variation	SS	d.f.	MS	F
LO	0.82	1	0.82	10.42**
CS	1.53	2	0.77	9.77**
LO × *CS*	0.64	2	0.32	4.11**
Error	53.1	678	0.08	

Data related to the consumption rate per unit of biomass (mg d^{-1} mg^{-1})

Alder has a high survival rate and growth and can adapt to severe habitat conditions (Krzaklewski et al. 2012). It can survive in areas that are highly polluted by chemical contaminants, which accumulate in its leaves (Lorenc-Pulcinska et al. 2013). In this study, average levels of Cd and Pb measured in leaves collected from the polluted site were higher than values reported by the previously cited research, while Zn levels were similar (Lorenc-Pulcinska et al. 2013). Leaves from Rio Naracauli (polluted site) had higher concentrations of Cd, Pb and Zn than those from Rio de Su Casteddu (unpolluted site). A high bioaccumulation of Pb and Zn in leaves was recorded in the polluted site, along with an associated high $BEF_{w/o}$, evidence that the concentrations in the water were efficiently transferred into the riparian plants. The absence of significant differences (ANOVA, $p > 0.01$) between the tested sites confirmed that Cu and Mn are not commonly present in the mineralization of Sardinia's mining area (Boni 1994). Results confirm both that contaminants were efficiently transferred from the abiotic compartments to the biota (*A. glutinosa*) and that the selected chemicals were good tracers for the pollution at this mining site.

Direct Toxicity of Chemical Contaminants on Tested Species

Bioaccumulation of chemical contaminants in aquatic organisms is a well-documented field, even if little research has been performed on freshwater organisms (Goodyear and McNeill 1999). Despite that, insects at their larval stages could represent good biomonitoring species for the following reasons: (1) widespread frequency in different freshwater habitats, (2) fairly sedentary habits, (3) bioaccumulation and metal tolerance at low-moderate pollution levels, (4) proportional relationships between environmental levels and tissue levels of metal, (5) lifespans of several months to years, which allows the integration of responses for a reasonable period of time while not being too long to detect changes, (6) in larvae or early life stages, levels are not affected by sexual differences or reproduction, and (7) represent the base of food webs, allowing risks to be evaluated for upper trophic levels. Copepods are commonly used in ecotoxicological tests to predict the impact of chemicals on early development and reproduction in crustaceans (e.g. Dahl et al. 2009) while gastropods and isopods are used less frequently. A recent study demonstrated high Cd bioabsorption rates for shell dusts of *Physa acuta* (Hossain and Aditya 2013). Collector gatherers are highly affected by Cd and Zn in sediments rather than in the water, and by Pb in the water rather than that in sediments. In contrast, scraper grazers are equally affected by Zn in the water and sediments, and by Cd and Pb in water rather than in sediments (Goodyear and McNeill 1999).

Behavior vs Pollution

During the conditioning process, biotic (i.e. bacteria, fungi; Gessner et al. 1999) and abiotic (i.e. temperature, water flow, soil/water chemistry; Allard and Moreau 1986) factors affect the decomposition of leaves. A recent study indicated a species-specific pathway of bacteria on the leaves of different species (Krevs et al. 2013). When animals were given the option to choose among feeding resources, the conditioning process strongly affected species behavior, probably changing both palatability and nutrient bioavailability. Looking at the behavioral responses in our first series of experiments, from 30 to 40% of the individuals on leaf discs were found on discs from the unpolluted site conditioned in the unpolluted stream. It was also possible to observe an increase in the proportion of individuals colonizing the unpolluted leaves over the first few hours, suggesting that animals collect more information on their environment as their selectivity on unpolluted resources increases. Considering the entire dataset, there were three patterns of individual distribution. First, the provenance of the leaves is relevant; individuals of all species visited the leaves from the unpolluted site more than those from the polluted site. Second, the site of leaf conditioning was also relevant; individuals of the four tested species visited the leaves conditioned in an unpolluted stream more than those conditioned in the polluted one. Taken together, there was close to a four-fold difference between polluted leaves conditioned in polluted streams vs unpolluted leaves conditioned in unpolluted streams. Third, the species responded differently to the perturbations: gastropods were very infrequent on leaves from the polluted sites, independently of where they were conditioned, while arthropods were more sensitive to the microbial conditioning site than to the origin site.

Energetics

Since gastropods are larger than arthropods, they were more likely to be food limited; their strict preference for leaves from the unpolluted site suggests that, probably, the accumulation of Cd, Pb, and Zn in leaves inhibited the microbial colonization of the leaves. On the other hand, the site's influence on the leaf conditioning process could have affected the qualitative composition of the microbial communities. When each food resource was offered separately, the three patterns observed in the previous experiment were maintained, but the most relevant observation was that both arthropod species consumed more of the leaves from the polluted sites conditioned in the unpolluted stream. In fact, in this case, the animals consumed more detritus but produced less biomass than animal feeding on detritus from the unpolluted site conditioned in the polluted stream. The most likely explanation is that microbial conditioning on leaves was sparse in the former case, and denser in the latter case. This hypothesis is supported by the fact that leaves from the polluted area supported less microbial biomass than leaves from the unpolluted site. In conclusion, the observed results show that mining landscapes represent an indirect pathway of stress, as the land–water ecotone can strongly affect resource availability to benthic detritivores, which could affect their ability to colonize the area.

This research indicates that the microbial conditioning process is a fundamental step in plant decomposition in freshwater ecosystems, and that it significantly affects feeding behavior. Levels of Cd, Pb, and Zn in leaves were not found to directly interfere with the palatability of trophic resources, and the microbial conditioning process had the greatest effect on the feeding resource chosen. Furthermore, a species-specific (arthropods vs gastropods) feeding behavior of the considered species was observed.

Acknowledgements This research was developed within the Italian MIUR - PRIN projects, BIOforIU and LifeWatch-ITA. We are grateful to the University of Cagliari and to Dr. C. Dadea, Dr. C. Solla and Dr. A. Ponti for experimental and field activities, and to S. Janzen for language review.

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