Breakdown rates and macroinvertebrate colonisation of alder (*Alnus glutinosa*) leaves in an acid lake (Lake Orta, N Italy), before, during and after a liming intervention

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ABSTRACT

To test the effectiveness of the liming intervention on Lake Orta, the speed of leaves decay and of colonisation processes by macrobenthonic fauna were studied on alder leaves (Alnus glutinosa) placed on the bottom of the lake and recovered after appropriate time intervals. Experiments were performed at two sites (North and South) and two depths (-3 and -18 m), during three successive winters: 1988-1989 (pre-liming), 1989-1990 (liming), 1990-1991 (post-liming). Two main results emerged: 1) alder leaves, which are known to have a medium to high decaying speed in a number of aquatic environments, behave in Lake Orta as a low speed species. Decaying processes in the three years are significantly different only in station N3, where the mean breakdown rate in 1988-1989 is more than twice that measured in the two subsequent winters. 2) The species richness of colonising benthic fauna is low: the community is made up almost exclusively of Chironomidae, which form 70 to 100% of the whole population; among them, the genus Phenopsectra is always present, while Tanytarsus was collected only during the first year and in the less deep sampling sites. The mean population abundances were higher before liming.

Key words: leaf processing, acidification, liming, Alnus glutinosa, macrozoobenthos

1. INTRODUCTION

The macrobenthic community of Lake Orta at the end of the 90s was characterised by very low abundance and species richness (Bonacina *et al.* 1988; Cattaneo 1988).

The liming intervention should have encouraged the recovery of the benthic community, and leaf decomposition processes were employed to assess indirectly the effectiveness of the neutralisation. Two aspects of leaf processes were considered: breakdown rates and leaf colonisation by macro-invertebrates. These aspects of the decomposition process are not separate, since invertebrate activity regulates the rate of detritus processing (Petersen & Cummins 1974; Iversen 1975; Pidgeon & Cairns 1981). This normally happens in unpolluted aquatic environments, although there is a significant reduction in the rate of breakdown processes in the bottom of lakes as compared with streams (Witkamp & Frank 1969; Webster & Simmonds 1978). In fact, decay of vascular plant detritus results from several interactions between the physical and chemical nature of specific leaves and the biotic and abiotic features of the environment (Petersen & Cummins 1974; Reice 1974; Webster & Benfield 1986).

Pre-, during- and post-liming experiments were therefore devised to test whether liming would enhance *Alnus glutinosa* leaf processing in Lake Orta.

2. METHODS

2.1. Experimental design

Two experimental sites were selected (N and S), and two depths (-3 and -18 m) were compared at each site (Fig. 1). Six series of five leaf-packs (dry weight = $5 \pm$ 0.1 g each) were placed by SCUBA divers on the bottom at each of the four experimental points during the winters 1988- 1989 (pre-liming), 1989-1990 (liming) and 1990-1991 (post-liming), that is 24 series or 120 leaf packs per year. The leaf packs were recovered (one line or five replicates at a time) in the following order: after 2 days (to measure the leaching) and subsequently after periods roughly corresponding to 150, 225, 300, 450 and 600 degree-days calculated as mean cumulated temperature. Temperature ranges were measured weekly at the experimental sites by means of min-max thermometers.

Leaf-packs were made up of decaying leaves taken from alders (*Alnus glutinosa*) which are very abundant along the lake shore. The leaves were collected from trees growing near the shore and dried in the air. Some of these were weighed before and after immersion in lake water for 24 hours at 5 °C, in order to measure weight loss due to leaching.

Before the 5-pack line was raised from the bottom, each pack was closed in a plastic bag, in order to avoid

loss of organisms during the lifting procedure. Each pack was then washed and the benthic organisms fixed with formaldehyde. Weight loss was measured after 48 hours at 50 $^{\circ}$ C.

Benthic invertebrates were determined following the taxonomic keys published by C.N.R. (1977-1986), while identification of trophic-functional groups was based on Merritt & Cummins (1984).



Fig. 1. Map of Lake Orta and sampling sites.

2.2. Data analysis

Leaf processing rates were estimated by linear regression, according to the one variable negative exponential model (Petersen & Cummins 1974):

$$W_t = W_0 e^{-kt} \tag{1}$$

were: *t* is time (in days); W_0 is the initial weight of the leaf pack; W_t is the weight of the leaf pack after t days; *k* is the leaf processing rate (in day⁻¹).

The model was linearized applying the logarithm transformation:

$$\log W_t = \log W_0 - k t \tag{2}$$

The processing rate was then estimated as the slope of the regression line of $\log W_t$ on $\log W_0$.

The test for the equality of the slopes of the regression lines (Sokal & Rohlf 1995) was used to assess the differences of the rates at each site and depth over the three years.

Abundance of each taxa was expressed as number of individuals per leaf pack and a multivariate analysis of abundance data was carried out, following the approach of Clarke (1993) and Clarke & Warwick (1994). The starting point was a taxa by samples data matrix, whose entries are the mean abundances of each taxon in each sample of three leaf packs.

The dissimilarity between every pair of samples was measured using the Bray-Curtis coefficient:

$$\delta_{jk} = 100 \frac{\sum_{i=1}^{p} |y_{ij} - y_{ik}|}{\sum_{i=1}^{p} (y_{ij} + y_{ik})}$$
(3)

where: δ_{jk} is the dissimilarity between the *j*th and *k*th sample; y_{ij} and y_{ik} are the mean abundances of the *i*th taxon in the *j*th and *k*th year, respectively; *p* is the total number of taxa. Abundance data were 4th root transformed before applying the formula, to down-weight the importance of the very abundant species so that the less dominant, and even the rare species, play some role in determining dissimilarity values. The dissimilarity values were set in a samples by samples dissimilarity matrix, which was the basis of an ordination by nonmetric multidimensional scaling (MDS). MDS was preferred to other ordination methods because it is conceptually simple, does not make assumptions on the distribution of the original data or on the proprieties of the dissimilarity measure and is not negatively affected by the presence of many zeroes in the original data matrix. MDS, like all the ordination methods, produces a map of points, each representing an sample. The distances between the points on the map are selected, by an iterative procedure, to match the dissimilarities among objects: if object 1 has higher dissimilarity to object 2 than to object 3, it should be placed farther on the map from bject 2 than from object 3. The final configuration of points is achieved by minimisation of the stress value that is a measure of the mismatch between the original dissimilarity values and the distances in the map. Since the match is never perfect, the stress value associated with the selected configuration of points is a measure of the success of the MDS in representing the relationships among objects.

The contribution of each species to the separation between groups formed by clustering was assessed using the SIMPER procedure. The contribution from the ith taxon to the Bray-Curtis dissimilarity between two samples j and k can be defined as:

$$\delta_{jk}(i) = 100 \frac{|y_{ij} - y_{ik}|}{\sum_{i=1}^{p} (y_{ij} + y_{ik})}$$
(4)

				$k (\text{day}^{-1})$		
site	depth (m)	year	estimate	confidence interval	n	r^2
N	3	1988-89	4.5 10 ⁻³	6.3 10 ⁻³ - 2.8 10 ⁻³	25	0.55
Ν	3	1989-90	$2.0\ 10^{-3}$	2.5 10 ⁻³ - 1.5 10 ⁻³	25	0.75
Ν	3	1990-91	$2.2 \ 10^{-3}$	2.5 10 ⁻³ - 1.8 10 ⁻³	25	0.86
Ν	18	1988-89	3.7 10 ⁻³	5.8 10 ⁻³ - 1.7 10 ⁻³	20	0.46
Ν	18	1989-90	$3.2 \ 10^{-3}$	3.7 10 ⁻³ - 2.6 10 ⁻³	25	0.87
Ν	18	1990-91	$3.8 \ 10^{-3}$	5.5 10 ⁻³ - 2.0 10 ⁻³	25	0.47
S	3	1988-89	3.4 10 ⁻³	4.2 10 ⁻³ - 2.6 10 ⁻³	25	0.77
S	3	1989-90	$2.1 \ 10^{-3}$	2.9 10 ⁻³ - 1.3 10 ⁻³	25	0.56
S	3	1990-91	3.4 10 ⁻³	5.0 10 ⁻³ - 1.8 10 ⁻³	25	0.45
S	18	1988-89	$2.6 \ 10^{-3}$	4.0 10 ⁻³ - 1.3 10 ⁻³	25	0.43
S	18	1989-90	$2.4 \ 10^{-3}$	3.0 10 ⁻³ - 1.9 10 ⁻³	25	0.77
S	18	1990-91	2.7 10 ⁻³	$3.1\ 10^{-3} - 2.3\ 10^{-3}$	25	0.89

Tab. 1. Breakdown rates (*k*) of alder leaves in Lake Orta.

When comparing two groups of samples, δ_{jk} is averaged over all pairs (j, k) of years with j in the first and k in the second cluster, to give $\overline{\delta}$, the mean dissimilarity between the clusters. The same for $\delta_{jk}(i)$ to give $\overline{\delta}_i$, the average contribution from the *i*th species to the overall dissimilarity between the two years. The values of $\overline{\delta}_i$ reported below are rescaled as percentages of $\overline{\delta}$.

Three different analyses were carried out. In the first all the macrobenthic organisms found in the leaf packs and identified to family level were considered (Trichoptera, which were rather rare, were not divided beyond the order level). In the second analysis only chironomids, identified to the genus level, were considered. For the third analysis, all the organisms found in the leaf packs were classified according to their trophic-functional groups, independently from their taxonomic position (Merritt & Cummins 1984) and the abundance of the trophic-functional groups was used in the analyses instead than abundance of taxa.

3. RESULTS

3.1. Leaf breakdown

Plant detritus processing in freshwater is the result of two successive steps: an initial, rapid leaching of water-soluble substances, directly affected by abiotic factors, and a subsequent weight-loss due to micro- and macro-invertebrate activity (Reice 1974).

The estimates of the processing rates (k) for each site, depth and year, together with confidence limits and the determination coefficient of each regression are reported in table 1. Even though in some cases the values of the determination coefficient (r^2) seem rather low, the regressions are always highly significant ($\alpha < 0.001$), due to the fairly high number of degrees of freedom.

All the estimates are included within the interval $4.5 \ 10^{-3} - 2.1 \ 10^{-3} \ day^{-1}$, which are slow rates according to the classification of Webster & Benfield (1986). On the other hand, studies carried out in freshwater habitats

with pH near to neutrality found for *Alnus glutinosa* medium (Triska 1970) or fast processing rates (Cummins *et al.* 1989). In particular the processing rates estimated in the present study are markedly lower than those measured for example by Fano *et al.* (1985) in a lentic habitat.

Processing rates are significantly different among the three years only at N3 ($\alpha < 0.001$). Here the rate estimated for 1988-1989 is more than two times higher than the rates of the following years. At S3 the rate estimated for year 1989-1990 is lower than the rates of both the previous and the following years, however the difference is not significant ($\alpha = 0.18$), and the confidence intervals largely overlap. At the depth of 18 m, processing rates at both sites are virtually unchanged over the three years.

3.2. Colonisation

Table 2 shows the list of the taxa recorded over the three years. The highest abundance values (annual mean) for the community were recorded during the first year (1988-1989): in three of the four sampling sites, namely N3, N18, S18, a mean of 35 individuals per leaf-pack was found; in the site S3 the mean value was 58.

In the second year, a marked reduction in the number of colonising individuals was recorded. This decrease was particularly evident in sites N18 and S3. During the third winter a further, though less marked, reduction was noted in sites N18 and S18, whereas in site N3 mean abundance values were double those of previous years (Tab. 3).

Chironomids are by far the most important representatives of the community (70 to 100% in each leafpack, Tab. 3). Mean abundance values referring to all the samples collected in the four stations (SIMPER procedure analysis) show for the group a decrease as high as 50% from the first to the second winter (34.76 ind./pack *versus* 17.63 ind./pack) and a partial recovery from the second to the third winter (26.0 ind./pack).

DIPTERA	ODONATA
Chironomidae	Coenagrion sp.
Phaenopsectra sp.	
Dicrotendipes nervosus group	MEGALOPTERA
Chironomus thummi group	
Polypedilum sp.	Sialis sp.
Tanytarsus sp.	
Demicryptochironomus vulneratus	CRUSTACEA
Orthocladius sp.	
Chaetocladius sp.	Asellus sp.
Procladius sp.	
Ablabesmyia sp.	OLIGOCHAETA
Undetermined Pentaneurinae	
	Naididae
Ceratopogonidae	Vejdovskyella comata
Undetermined Ceratopogonidae	Nais variabilis-elinguis
	Pristina longiseta
Athericidae	Dero digitata
Undetermined Athericidae	Stylaria lacustris
	Undetermined Naididae
TRICHOPTERA	
	Tubificidae
Mystacides azurea	Bothrioneurum vejdovskyanum
Ecnomus tenellus	Undetermined Tubificidae
Agrypnia varia	
Oecetis sp.	Lumbriculidae
	Undetermined Lumbriculidae
COLEOPTERA	
	HIRUDINEA
Dytiscidae	
	<i>Erpobdella</i> sp.

Tab. 2. List of macroinvertebrate taxa found in the leaf packs.

5					I			U	5				
		198	8-89			198	9-90		1990-91				
	N3	S3	N18	S18	N3	S3	N18	S18	N3	S3	N18	S18	
Chironomidae	42	46	24	25	21	15	9.9	25	48	21	14	22	
Athericidae			0.1						0.1		0.1		
Trichoptera	1.4	3.5	0.3	0.5	1.5	0.5	0.1	0.1	1.8	1.1	0.3		
Dytiscidae						0.1			0.1	0.1			
Odonata		1.2	0.1		0.1	0.2			0.1	0.1			
Megaloptera	0.3		0.1		0.5	0.1		0.1		0.3			
Asellidae			0.2								0.1		
Naididae	1.6	8.9	4.4	2.0	1.1	1.9	4.7	0.5	3.9	0.2	0.1	0.3	
Tubificidae	0.1	0.3	0.6	1.4	0.1	0.7		0.2	0.1	0.1			
Lumbriculidae	0.2	0.1		8.4		0.3						0.1	
Hirudinea		0.1				0.2							

Tab. 3. Abundance of families of macroinvertebrates at each site and depth in the three years of the study. Each value is the mean of all the leaf packs collected during one year.

Oligochaets were also present in all the sampling sites. Specimen of three families were collected: Naididae, Tubificidae, Lumbriculidae. During the first year, the highest mean abundances were recorded in S18 (12 ind./pack) and N3 (9 ind./pack), whereas in winter 1989-1990 significant abundances were recorded only in N18 (Tab. 3). The decrease particularly involved the Naididae, whose abundance underwent a reduction of 50% for each year (4.19 ind./pack in 1988-1989, 2.05 ind./pack in 1989-1990, 1.12 ind./pack in 1990-1991)

The trichopteran community was very poorly represented, specimen being collected almost exclusively at N3 and S3.

Coenagrion sp. (Odonata) and *Sialis* sp. (Megaloptera) were sporadically collected only in N3 and S3.

MDS analysis results in two clusters (Fig. 2) which split the sublittoral (N18 and S18) and littoral (N3 and S3) sites, indicating differently structured communities: Trichoptera, Naididae, Chironomidae, Sialidae and Odonata reach higher densities in littoral stations, whereas in sublittoral sites Tubificidae and Lumbriculidae are the dominant groups.

If the time factor (in years) is superimposed on the clusters (Fig. 2) it can be demonstrated that only in the sublittoral stations (N18, S18) does there exist a temporal evolution in the community structure.



Fig. 2. MDS plot of leaf pack samples based on: all the colonizing invertebrates identified to the family level (a, b); chironomids identified to the genus level (c, d); all the invertebrates classified according to their trophic-functional group (e, f).

During the pre-liming period, total biomass values (Fig. 3) as measured at S18 from the 50th day are far and away higher than those measured in remaining sampling stations. During the following years the differences in total biomass values were less pronounced, but there is an evident tendency towards higher values in littoral sites (Fig. 3).

3.3. Chironomidae

The genus *Phaenopsectra* is always present in the samples, reaching in times considerable abundances. However, a tendency towards lower and lower values is undoubtely present (23.26 ind./pack in 1988-1989; 11.55 in 1989-1990; 8.12 in 1990-1991, mean annual

values), and is much more evident at station S3 (from 34 to 12 ind. pack). The same trend is present in the genera Tanytarsus, collected almost exclusively during 1988-1989 in N3 and S3, and Chironomus, present with significant densities only at S18 in the same year (mean value: 9 ind./pack). On the other hand, Procladius sp. and Orthocladius sp. show increasing, although always low, densities. The same is true for the tribe Pentaneurinae, whose densities increase from 0.62 ind./pack in 1988-1989 to 11.1 ind./pack in 1990-1991. During this last period, much lower average abundance values were recorded only at site N18 (3 ind./pack). Only Dicrotendipes shows first a decrease and then a considerable increase. Specimens of this genus were collected in all sites, but abundances were higher in littoral stations N3 and S3 (Tab. 4).



Fig. 3. Variation of total biomass of macroinvertebrates in the leaf packs over the exposure period at each site and depth in the three years of the study. Biomass is expressed as mg of invertebrate (dry weight) per g of leaf (dry weight).

MDS reveals a clear temporal trend: in particular it is possible to make a distinction between the samples collected during the first year and those collected in the following two years. Again in the first year, sublittoral and littoral sites are clearly differentiated: in the sublittoral sites intra- and inter specific diversity is higher than that present in the littoral sites.

Tab. 4. Abundance of genera of Chironomidae at each site and depth in the three years of the study. Each value is the mean of all the leaf packs collected during one year.

	1988-89				1989-1990				1990-91			
	N3	S3	N18	S18	N3	S3	N18	S18	N3	S3	N18	S18
Phaenopsectra sp.	22	34	21	16	12	12	8.4	15	9.9	7.1	6.7	9.0
Dicrotendipes nervosus group	8.5	5.4	1.1	0.4	0.2	0.7	0.1	0.5	15	1.3	0.7	0.3
Chironomus thummi group		0.1	0.9	8.7	0.1	0.2	0.2	1.1	0.2	0.1		0.1
Polypedilum sp.									0.1			
Tanytarsus sp.	10.6	3.0	0.7	0.1	0.4	0.5	0.1	0.1	0.7	0.1	0.1	0.1
Demicryptochironomus vulneratus						0.1						
Orthocladius sp.						0.1			0.7	0.6	0.1	
Chaetocladius sp.											0.1	
Procladius sp.	0.5	1.7		0.1	1.6	0.2	0.3	3.9	2.8	0.4	0.5	0.7
Pentaneurinae tribe	0.5	1.5	0.3	0.2	6.7	2.1	0.9	4.6	17	11	5.5	11

Tab. 5. Abundance of trophic-functional groups at each site and depth in the three years of the study. Each value is the mean of all the leaf packs collected during one year.

		1988-89					-1990		1990-1991			
	N3	S3	N18	S18	N3	S3	N18	S18	N3	S3	N18	S18
Collectors	43	52	28	37	13	16	13	17	30	8.7	7.6	9.8
Predators	1.2	4.5	0.5	0.3	9.1	2.9	1.1	8.6	21	13	5.9	12
Scrapers						0.1			0.7	0.6	0.1	
Shredders	1.4	3.5	0.4	0.5	1.4	0.4	0.1	0.1	1.5	0.5	0.3	

3.4. Trophic-functional groups

Collectors are by far the most abundant trophic functional group; they also present a clear density decrease, particularly evident when comparing first and second year (ind./pack: 36 in 1988-1989; 15 in 1989-1990; 13 in 1990-1991).

Predators are the second most numerous group and their density shows an opposite temporal evolution; as a consequence during 1990-1991 in sites S3 and S18 benthic community is formed by 50% of collectors and 50% of predators.

The other trophic-functional groups (scrapers, shredders) are nearly absent (Tab. 5).

From MDS analysis, littoral stations N3 and S3 make up one and the same cluster, although there is some temporal variation. In contrast, in sublittoral stations N18 and S18 trophic functional structure is highly diversified from year to year in the same site and from site to site in the same year (Fig. 2).

4. DISCUSSION

It appears from the results of this study that the liming did not favour either micro-organisms like bacteria and fungi, which are known to take part in decomposition processes, or the epi-benthic macro-invertebrated which colonise alder leaves. On the contrary, it seems to have made slightly worse the precarious, though stable, situation both of the communities and the decaying pro??cesses.

A post-liming study of only one year is not nearly enough to allow us to arrive at any reliable conclusions, but it does seem that a real recovery, at least in terms of community diversity , has followed the initial stress caused by the change in pH values (Calderoni & Tartari 2001). However, our results are confirmed by Nocentini *et al.* (2001) for 1993-1994, Baudo *et al.* (2001a) for 1996 and Bielli & Tesauro for 1998, albeit the last paper deals only with samples collected on the shore line.

All these authors point out the paucity of the benthonic biocoenosis both in quantity and in quality. It is significant that the same species, or groups of species, are recorded in the four periods under study.

An analysis of colonisation processes yelded scanty and fragmentary results: some light was thrown on situations which were already known, e.g. the quali- and quantitative differences caused by the different depths of sampling sites (see for example Nocentini et al. 2001), but our data did not point to a connection between action (the liming) and reaction (species diversity and population abundances). We agree with the major criticism that could be made about this research, which is that there was too short an interval between the end of the liming and the start of the experiments. However, in the light of the researches quoted above, we now know that much more time is necessary for the recovery of the benthonic fauna, mainly because of sediment toxicity (Burton et al. 2001; Baudo et al. 2001b). Considering the low sedimentation rate measured in the lake (0.22 cm y⁻¹, Guilizzoni et al. 2001) and the well known burying ability of benthonic animals, it is unlikely that experiments performed in the next few years will reveal more profound changes in the composition of colonising populations.

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