Leaf litter decomposition in Torna stream before and after a red mud disaster

running title: Leaf litter decomposition in Torna stream

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Abstract

The aim of the study was to estimate the breakdown of the allochtonous litter in an artificial stream running in an agricultural area and compare it with the same values following a toxic mud spill into the same stream. Litter bags were filled with three types of leaves (*Quercus robur*, *Populus tremula* and *Salix alba)* and placed to the bottom of the river. Ergosterol was used to detect fungal biomass. We supposed the absence of fungi and the retardation of leaf litter decomposition. Only pH and conductivity increased significantly. Leaf mass loss after the catastrophe was much slower than in 2009 and the decay curves did not follow the exponential decay model. Prior to the catastrophe, leaf mass loss was fast in Torna, compared to other streams in the area. The reason is that the stream is modified, the bed is trapezoid and covered with concrete stones. Fungal biomass was lower, than in the pre-disaster experiment, because fungi did not have enough leaves to sporulate. Leaf mass loss followed the

exponential decay curve before the disaster, but after that it was possible only after a non-change period.

1. Introduction

Deciduous leaf litter is a major energy source for the food webs in woodland streams (1; 14; 6; 18). The mechanism of leaf litter breakdown consists of three parts after entering streams: (I) leaching of soluble compounds; (II) microbial colonization and degradation (conditioning) and (III) fragmentation by physical abrasion and invertebrate shredding. Fungi, especially aquatic Hyphomycetes, are regarded as the primary decomposers of leaf litter in streams. Leaf mass loss follows the exponential decay curve (8). Ergosterol content is widely used to estimate fungal biomass because of the strong correlation between these two variables (12; 3; 4). Ergosterol is present in fungi but absent from plants and animals (27). Fungi associated with decomposing leaf litter in streams are affected by a number of factors, which is turn determine their abundance, development and activity (4; 5; 6). Chemical parameters of water, especially pH, alkalinity and nutrient availability, significantly affects aquatic hyphomycetes and thus their role in decomposition, resulting in slower breakdown rates in acidic than in circumneutral streams (29).

On 23 October 2000, the "Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy" or, in short, the EU Water Framework Directive (WFD) was finally adopted (10). The aim of the directive is to reach a “good” overall quality of all waters. The directive, extending to 27 countries, initiated an important trend towards an ecosystem-based approach for water policy and water resource management (22). Our laboratory carried out a number of basic researches on Hungarian midland streams and their ecological processes to support the implementation of the directive (23; 30; 24; 33). Many of our study sites are located in the area of Bakony Mountain, like the area of Kolontár and Devecser, and some of them were impacted by a red mud disaster. In 2009, an experiment was carried out to study leaf litter decomposition in three Hungarian midland streams. Our original aim was to study leaf litter decomposition and leaf mass loss in natural and strongly modified streams.

On the 4th of October 2010, the western dam of cassette X of an alumina plant (Hungarian Alumina Co.) broke and alkaline, corrosive red mud escaped. Due to the ruptured dam, a mixture of ca. 1700 thousand m^3 of red mud and liquid inundated the lower sections of the settlements of Kolontár, Devecser and Somlóvásárhely via the Torna creek (7). The red mud moved following the course of the local (Torna) stream. The pollution impacted an about 40 km^2 area. In Torna stream almost all organisms died and the ecosystem of river Marcal was also seriously damaged. The pollution almost reached the Danube.

After the disaster, a similar experiment as in 2009 was conducted at Torna stream with the same leaf species. The aim of the study was to estimate the breakdown of the allochtonous litter in an artificial stream running in an agricultural area and compare it with the same values following the toxic mud spill into the same stream.

2. Materials and Methods

Leaf litter decomposition experiments (pre-disaster experiment) were carried out in 2009 in the Torna stream (47º6.367'N, 17º26.090'E). This stream is located in the area of Bakony Mountains. Our sampling point was a heavily modified section of the stream, where the bed is strengthened and fortified by trapezoid concrete blocks. The river bed is characterized by microlithal (Ø>2 cm to 6 cm) and akal (Ø>2 mm to 2 cm) (24). There is no natural tree vegetation, because the stream flows through an agricultural area, but some *Populus tremula* and Salicaceae (*Salix viminalis*, *Salix caprea*, *Salix alba*) were present (24).

Quercus robur, *Populus tremula* and *Salix alba* leaves were collected shortly after the fall (September, 2008). They were put into big paper boxes to avoid breaking and transported to the laboratory. The leaves were dried in at 70 °C to constant mass then 10 g of leaves were measured and put into 10 cm x 10 cm leaf-litter bags. The mesh size was 5 mm x 2 mm. The top of the bags were closed carefully with hot glue to keep the litter inside. Leaf-litter bags were transported back to the sites. They were fixed onto a fence grid (mesh size about 5 cm). Bags were sprinkled to avoid leaf-litter break when they were placed horizontally into the water at the bottom of the stream, parallel to the flow. The fence grids were stably fixed with wooden and metal stakes and rocks close to the main stream.

Bags were collected from the 13th of January 2009 on every 28th day during 140 days. Each time three bags were removed for analyses.

To estimate the fungal biomass, the following extraction and HPLC procedures were used:

sets of five leaf discs were preserved in 10 ml of KOH–methanol (8 g KOH per liter). Lipids were extracted and saponified at 80°C for 30 minutes. The extracted lipids were partitioned into a non-polar phase and ergosterol was purified by solid-phase extraction (SPE). A final purification and quantification of ergosterol was achieved by HPLC: eluent : 100% methanol, flow: 1 ml min^{-1}, gas: He, pump: Waters 600, UV lamp (waters 490), time const.: 0,1 , AUFS:1, λ=282 nm, thermostat (Waters 410), temperature 33°C, column: Nova-Pak C18; 3,8x150 mm; 4 µm. HPLC grade methanol and isopropanol were used for sample preparation and HPLC analysis. Analytical grade KOH pellets and analytical grade HCl were used for solution preparation. Sigma, purum; ≥95% (HPLC) ergosterol standard was applied to prepare standard curves.

Fungal biomass was estimated from ergosterol content (17) of leaf litter following the procedures described by Gessner (12). To convert ergosterol content to fungal biomass, a factor of 5.5 mg of ergosterol g^{-1} of fungal biomass (15) was used.

Temperature, dissolved oxygen, oxygen saturation, pH and conductivity were measured in the field with a multiparameter field sensor (HQ40d multimeter). The main chemical variables (NO_2^-, NH_4^+, NO_3^-, soluble reactive phosphorus: SRP, TP, SO_4^{2-}, soluble reactive silica: SRSi,

Cl^-, chemical oxygen demand with permanganate method: COD_{PS}) were analyzed in the laboratory (2; 36).

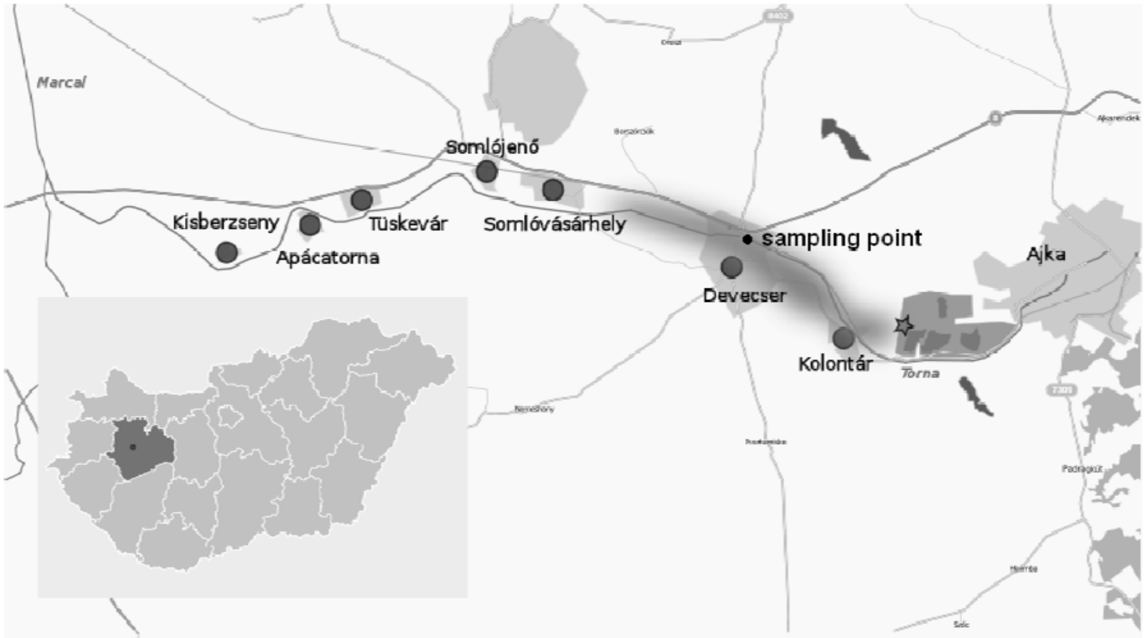

Figure 1. The area of the disaster. ☆ = place of dam collapsing (http://en.wikipedia.org/wiki/File:Ajkai_v%C3%B6r%C3%B6siszap-katasztr%C3%B3fa_v%C3%A1zlat_2010-10-04.svg)

On the 4th of October the dam of an industrial sewage reservoir in Kolontár was broken and a huge amount of red mud covered the area (Fig. 1). All fish and macroivertebrates were killed by the alkaline mud. Later gypsum was added to the water of the stream to neutralize the alkaline mud, but neutralizing effect of this treatment failed to be proven in laboratory experiments. No macroinvertebrates, but some fungi and algae were found on February, 2011, indicating beginning recovery.

An experiment similar to the one described above was conducted in 2011 at Torna stream (post-disaster experiment) after the red mud disaster. The same three species of litter were used and bags were installed in the stream on the 17th of February 2011. In the first month samples were collected weekly, later twice a month, for 140 days. Sample collection and measurements were the same as mentioned before.

3. Results

3.1 Water chemical parameters of Torna stream

The NO_2^-, NO_3^-, NH_4^+, SRP, TP, Cl^-, SO_4^{2-}, COD values did not differ significantly from the pre-disaster (2009) results (Table 1). The average pH of Torna stream was 7.92 and the average conductivity was 1234 μS cm^{-1} during the pre-disaster experimental period.

Table 1.The annual averages of the physical and chemical parameters of Torna stream in 2009 and 2009

		DO	DO	Cond	pH	T	NH_4-N	NO_3-N	COD_{PS}	TP	SO_4^{2-}
		mg L^{-1}	%	μS cm^{-1}		°C	mg L^{-1}	mg L^{-1}	mg L^{-1} O_2	mg L^{-1}	mg L^{-1}
2009	Avg	9.80	95.82	1234.23	7.92	12.64	0.06	3.60	10.87	150.12	593.23
	StdDev	0.83	2.77	13.81	0.08	4.24	0.16	0.54	7.89	55.24	124.75
2011	Avg	9.65	96.32	769.32	7.97	13.21	0.19	4.00	8.32	173.05	638.24
	StdDev	0.75	3.20	22.14	0.06	3.25	0.13	0.21	2.24	99.60	233.40

After the disaster dissolved oxygen concentrations and oxygen saturation did not differ significantly from the previous results, while pH and conductivity (Fig. 2) increased significantly.

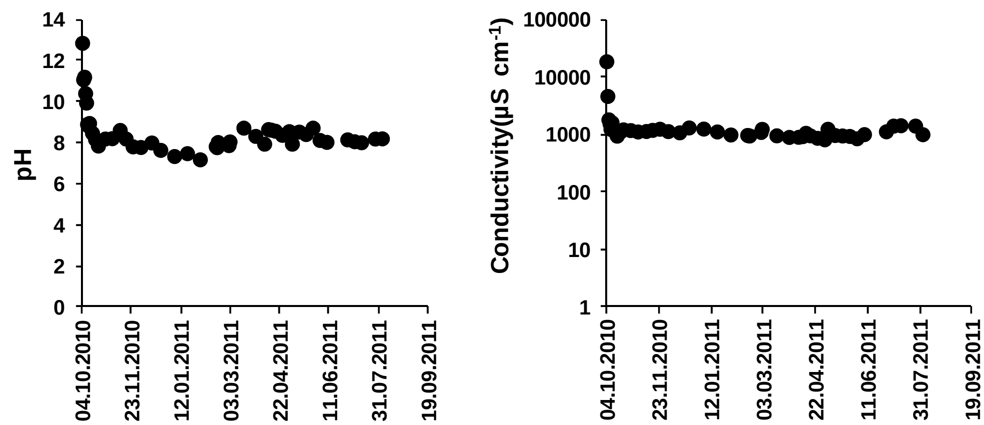

Figure 2.The pH and conductivity values of Torna stream between 4 of October 2010 (the day of the disaster) and 19 September 2011

On the day of the disaster (4th of October 2010) the pH was 12.87 and the conductivity was 18,860 μS cm^{-1} and these high values prevailed for many hours. By the next day both parameters decreased and 10 days after the disaster the pH was 8.4 and the conductivity was 1,196 μS cm^{-1}, both close to normal.

3.2 Leaf mass loss

In the pre-disaster experiment decay curves were well characterized by exponential decay models for all leaf species (Fig. 3.a), as described in the literature (12). *Populus tremula* was the less resistant and *Quercus robur* was the most tenacious leaf. At the end of the experiment all leaves were shredded and emptied from the litter bags, so leaf litter breakdown was fast and means that leaf litter decomposition followed the exponential decay curve.

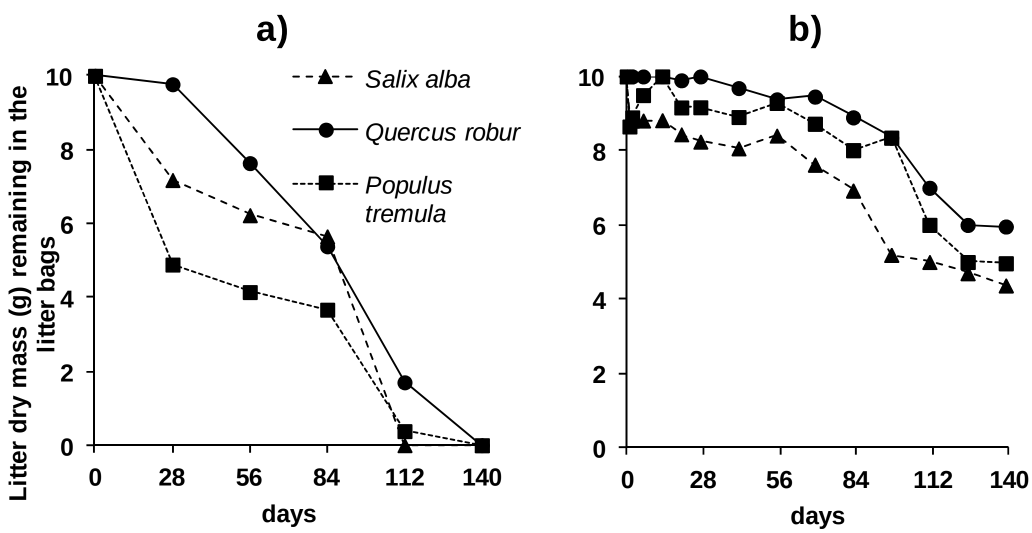

Figure 3. The litter dry mass (g) remaining in the leaf-litter bags a) between 3 of January and 2 of June 2009 and b) between17 of February and 7 July 2011 in the Torna stream

In literature it is common to describe litter decay with an exponential equation (12, 14, 18), but not in the case described. Leaf mass loss (Fig. 3.b) after the catastrophe was much slower than in 2009. The whole length curves do not follow the exponential decay model: after an initial no-change period leaf litter loss appeared quasi-linear (more details in discussion). Mass loss of leaves started later and lasted much longer. Before the disaster all leaves were shredded from the litter bags, but after the disaster only about the 50% of them.

3.3 Ergosterol concentrations

As regards the ergosterol concentrations *Quercus robur* reached the highest and *Populus tremula* the lowest values before the disaster. Salix and Populus curves reached their maximum during the first month,while *Quercus* in the fourth month (Fig. 4.a). Fungal biomass decreased the slowest in the case of *Quercus robur*.

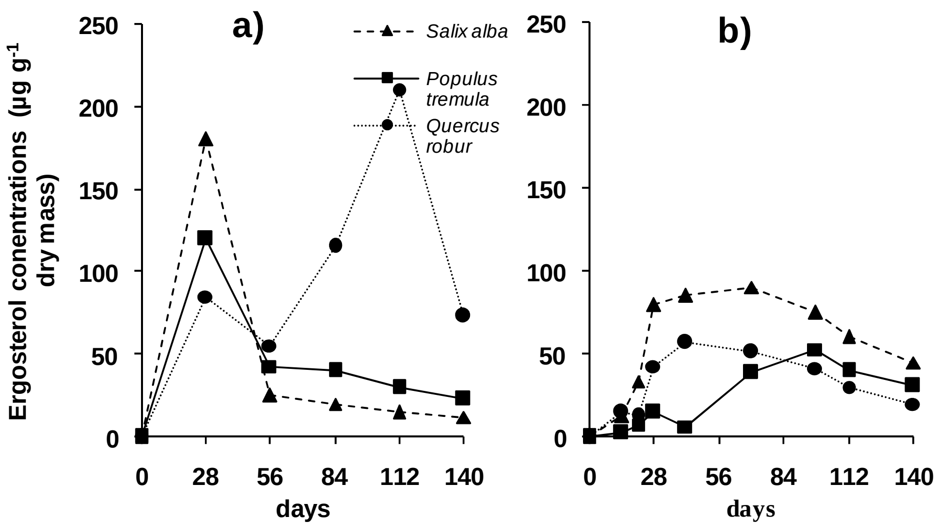

Figure 4.The ergosterol concentrations (µg g^{-1} dry mass) in the leaf-litter bags a) between 3 of January and 2 of June 2009 and b) between 17 of February and 7 of July 2011 in the Torna stream

In the post-disaster experiment ergosterol concentrations (Fig. 4.b) were much lower and reached their maximum values later in Torna stream. The highest amount of fungal biomass was recorded on Salix (*Salix alba*) leaves. Fungal biomass in *Quercus* leaves was much lower, than in 2009. Ergosterol concentrations started to increase much slower in the case of *Populus tremula* leaves.

4. Discussion

4.1 Water chemical parameters of Torna stream

Before the disaster, the annual average values of the water chemical variables at the sampling locations corresponded, at average, to similar values from streams in the Carpathian Basin (23; 24; 33). pH of the Torna stream was slightly alkaline (group mean: pH 7.9±0.2). After the disaster, pH increased seriously, killing all fish and macroinvertebrates in the stream. The corrosive alkaline mud swept over the stream and had serious effects on the downstream sections as well. In the first four months no macroinvertebrates were found, because not only they themselves and even their habitats disappeared.

4.2 Leaf mass loss

Before the disaster natural allochtonous litter load at Torna stream was negligible because the stream was surrounded by agricultural or urbanized areas. Leaf mass loss in the bags in 2009 was fast (Fig. 3.a) for double reasons: on one hand there is a shortage of food and on the other the flow velocity is much faster (24; 33) in the artificial trapezoid bed than at the other streams investigated in 2009. Therefore, the leaf litter fragments cannot remain in the bags for a long time, instead, they drift away. Whiles *et al.* (37) also observed unusually rapid litter decomposition rates in regulated streams. In contrast, Hladyz *et al.* (21) described faster alder breakdown in deciduous woodland streams in Ireland and Romania, than in pasture streams. In Switzerland oak broke down was faster in pasture streams than in woodland streams (21). Tuchman and King (31) assessed, that shredders and microbial processes control breakdown rates in woodland streams, whereas abrasion was thought to be the major process causing faster breakdown in pasture reaches because of increased surface run-off and variation in discharge (21).

In morphologically modified streams, like Torna stream, e.g. when the bed is evened, the flow velocity increases, and, as a consequence, other species will populate the stream than under natural conditions, and the leaf drifts away fast, as there are no parts of the stream that would function as accumulation zones. In this way, even though the quantity of leaf litter decreases quickly, it is not decomposed and utilized locally.

Webster and Waide (35) also described the effect of missing coastal vegetation which is relevant for Torna stream. According to this study, the decomposition rate of even the most resistant leaf litter species increased due to the lack of alternative food source of shredders and high flow velocity. Tuchman and King (31) observed higher litter decomposition but lower shredder biomass in litterbags in Michigan headwater stream sections in agricultural areas, compared to streams with an undisturbed forest environment, as in the case of Torna stream, located in an urbanized area. This implies that not only bed modifications, but also the alteration of riparian zones influences differentially stream systems (20).

Most studies use exponential decay curves or linear regression of ln(x)-transformed data for leaf mass loss (21; 25; 38; 32). We used the same curves in the pre-disaster experiment. There was no statistically significant difference between measured and fitted leaf mass loss as determined by one-way ANOVA ($F(1,14) = 4.6$, $p = 0.99$). In the post-disaster experiment fitting exponential decay curve was not possible. Leaf mass loss was not exponential, only if the non-change period is neglected. The reason of difference between pre- and post-disaster decay curves might be that gypsum was added to the water of the stream to neutralize the alkaline mud, which settled on the surface of the leaves making them hardly suitable for fungal colonization. Later gypsum addition was terminated and the gypsum moved to the lower and deeper parts of the stream so fungi had enough surface to colonize.

4.3 Ergosterol concentrations

Fungi were the first organisms to be present in Torna stream after the disaster but they had no surface to colonize. Macroinvertebrates were absent for several months, because they did not have any food (among others leaves) or habitats. The first macroinvertebrates were detected in April 2011 (half year after the disaster). Gypsum covered the sediment (or covered concrete stones), interfering macroinvertebrate settling. Additionally, dense green algal film grew up on the surface of gypsum, but it was absent from the red mud. Gypsum particles provide larger, while red mud particles a smaller surface for microalgae to be colonized. On the other hand red mud particles move faster so they are not able to afford a stable bed.

Pre-disaster ergosterol concentrations varied between 0-210 µg g^{-1}. About twice as high values (437 µg g^{-1}) were measured by Gessner and Chauvet (14) in *Fagus sylvatica* in a soft water mountainous stream. Suberkropp (29) determined higher values (704 µg g^{-1}) on *Liriodendron tulipifera* in a hard water lowland stream and Gessner et.al. (13) published a bit higher concentrations (316 µg g^{-1}) on *Alnus viridis* in a soft water, in a glacial stream. Ergosterol synthesis requires molecular oxygen (34) and low oxygen tensions can dramatically reduce ergosterol content. In our study oxygen levels in the stream and over the study period were similar and close to 100%. Therefore we conclude that the difference in ergosterol content is due to the different amount of leaves and the input of other leaves. One leaf litter bag contained only one kind of leaf. But because of natural input and drift, some leaves may have attached to the outer surface of the bags. Recent research shows that mixed leaf species accelerate leaf litter decomposition (26).

Many studies showed that aquatic fungi prefer slightly acidic to neutral conditions (5; 28). Low pH has harmful effects on aquatic hyphomycetes, in terms of species richness (28) and (11), leaf maceration activity (11) or conidial production (19). Leaf decomposition is significantly faster in the circumneutral streams (pH 6.4–7.1), when compared to acidic streams (pH 4.7–4.9) (8) but so highly alkaline waters (pH=12.87) have not been investigated. Dangles and Chauvet (9) observed, that fungal biomass shows no particular trend along the acidification gradient except that it peaks earlier in the stream closest to neutrality and ergosterol concentration increases and levels off or even increases further in the slightly acidic stream. Gessner and Chauvet (15) observed a similar pattern of fungal biomass in beech leaves exposed in a Pyrenean softwater stream unaffected by acidic deposition. Similar data within strongly alkaline conditions have not been described yet. This case shows that strong alkaline load (very high pH) has serious effects on Ingoldian fungi, in terms of fungal biomass. Ergosterol concentrations show clearly that the amount of fungi was much lower in the post disaster water. In terms of species richness or conidial production the same conclusions can be drawn.

4.4 Conclusion

In conclusion, water chemical parameters suggested that water quality of Torna stream was good and medium before the disaster, but after it pH and conductivity increased dramatically, killing almost all the biota in the stream. These parameters decreased a few days after the catastrophe, but biota could not follow the regeneration of physical and chemical parameters. Prior to the catastrophe, leaf mass loss was fast in Torna, compared to other streams in the area. The reason is that the stream is modified, the bed is trapezoid and covered with concrete stones. Fungal biomass was lower, than in the pre-disaster experiment, because fungi did not have enough leaves to sporulate. Leaf mass loss followed the exponential decay curve before the disaster, but after that it was possible only after a non-change period. Leaves were covered with gypsum, revoking the colonizable surface for fungi. The effect of acids is pretty well described in the literature, but less is known about alkaline mud in water.

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Legend for tables and figures

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