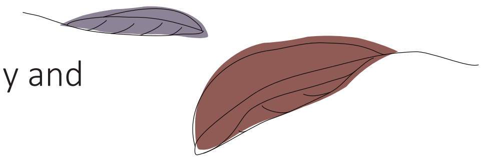
Effects of Plastic Pollution on Leaf Litter Decomposition in Swedish Freshwater Ecosystems



A field study addressing recently and globally emerging pollutants.

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EnvEuro- European Master in Environmental Science

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Effects of Plastic Pollution on Leaf Litter Decomposition in Swedish Freshwater Ecosystems. A field study addressing recently and globally emerging pollutants.

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Abstract

Plastic pollution is recognized as an increasing environmental problem, but few studies have addressed impacts on ecosystem functions, and very few have been conducted in the field. This study investigated the effects of polypropylene particles derived from disposable face masks on in-situ leaf litter decomposition processes in freshwater ecosystems. The field experiment followed a modified leaf litter bag protocol in a central-swedish urban pond and focussed on decomposition processes mediated by microbes and meiofauna. The litter bags with a mesh size of approximately 0.5mm in diameter were constructed from cotton and filled with 3.5g alder (*Alnus glutinosa L.*) leaves. Further, three different material treatments with different types of added material were applied to the litter bags: (1) plastic material was added, (2) saw dust as reference material was added and (3) no material was added (control group). Within the plastic treatment two different plastic particle size treatments (small/microplastic and big/macroplastic) and two leaching treatments (unleached and pre-leached) were included. The experiment period lasted seven weeks and included five timepoints at which subsamples of litter bags were retrieved. Three main response variables were quantified: (1) ecosystem respiration as an indicator for the metabolic activity of the organism community, (2) leaf mass loss and (3) tensile strength loss of the cotton bags. The latter two variables functioned as indicator for the decomposition potential of organic material in the studied ecosystem. The findings suggest that mixing a material resistant to biodegradation within a leaf litter patch acts as a physical barrier to microbes and thus slows down the decomposition, with this effect overall being stronger for the saw dust reference material than the plastic. The presence of unleached plastic was found to increase ecosystem respiration as well as decomposition of the cotton bags compared to the pre-leached treatment. Being one of very few field experiments this study provides valuable insights on how the pervasive extent of plastic pollution from face masks may affect key functions associated with carbon cycling in freshwaters. The obtained results further illustrate how complex biological stress responses to anthropogenic pollution might be on ecosystem level.

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Popular scientific summary

In the last years, the emergence of the COVID-19 virus globally shaped how our society works. Especially face masks became symbolic for our everyday lives. Not only were they present in grocery stores, public transport and cultural events, but we saw them discarded on the streets, sidewalks and in parks. The sudden spike in the use, combined with a lack of awareness, caused face masks to become a major environmental pollutant. In the course of my master's project I carried out a field experiment to see if face masks show effects on the natural breakdown of leaf litter in freshwaters. The decomposition of leaves is a complex interplay between many different organisms and their environment. It is also part of many processes that occur in water ecosystems, e.g., the cycling of carbon. This makes it a good indicator of how well an ecosystem functions.

The field experiment was designed as a litter bag study. I used cotton tea bags and filled them with alder leaves. To some of these leaf litter bags I added plastic particles in two different sizes cut from face masks. For both sizes I included an unleached group and a pre-leached group. For the pre-leached group the plastic was soaked in water for two days to release chemical compounds. To other bags I added saw dust as a reference material. Saw dust is a naturally derived material but hard for organisms to break down. Including saw dust as a reference material accounts for effects that are caused by having any breakdown-resistant material within the leaves. Differences between the saw dust and the plastic treatments then allow to see effects that arise because the added resistant material is plastic. Finally, I left some bags without any additional material as a control.

After preparation, I submerged the leaf litter bags in a pond on the university campus and left them exposed to the natural decomposing processes. At five timepoints I retrieved five bags of each treatment and measured three indicators for decomposition. First, I measured how much oxygen the microbes that colonized the leaf litter and cotton bags were consuming. Community respiration generally gives an idea of how active the organism community is. Secondly, I oven-dried the leaves of each litter bag and weighed them. The weight loss indicates how strongly the leaves have been decomposed. Finally, I measured how much tension the retrieved and dried cotton bags could withstand. The loss of tensile strength indicates how strongly the cotton bags have been decomposed.

The results showed that both plastic and reference material slow down the decomposition of leaves likely because the added material physically hinders microbes to access the leaves. This effect is stronger for smaller plastic particles than for bigger ones. I also found that chemical substances that are released from the plastic increases oxygen consumption and cotton bag decomposition. This finding is quite unexpected because other studies have shown that plastic often contains chemicals that are toxic to organisms. I suggest that this increase is a stress response of the microbes, similar to how our heartbeat increases when we feel stressed.

Concluding, my experiment is one of very few field studies regarding environmental plastic pollution and provides evidence that the pervasive face mask pollution might affect key ecosystem processes associated with carbon cycling in freshwaters. Further research on chemical compounds released from face masks and the microbial community that developed on the leaves would be very interesting and could help to interpret my results.

Introduction

Because human settlements have historically developed in the vicinity of rivers, lakes and oceans, aquatic ecosystems are particularly exposed to anthropogenic pollution. Indeed, the Millennium Ecosystem Assessment (2005) defines pollution as one of the most important drivers for changes in freshwater ecosystems. Further, the global hydrosphere is strongly connected and thus functions as a pathway for pollutants to reach even the most remote regions. Plastic debris as an environmental pollutant emerged only relatively recently but projected increases of the quantity of microplastics entering the environment are tremendous (Everaert et al., 2018; Lau et al., 2020; Lebreton and Andrady, 2019). Its full potential to affect ecosystem functioning is yet to investigate (de Sá et al., 2018; Koelmans et al., 2022; Ma et al., 2020). Ecosystem functioning results from the complex network of all ecosystem components, processes, properties and their maintenance (Reiss et al., 2009; Truchy et al., 2015). One of its core elements is organic matter decomposition as it forms the base of various biogeochemical cycles (Gessner and Chauvet, 2002). Therefore, this work studies effects caused by plastic pollution on leaf litter decomposition in an in-situ experiment.

Types of plastic are numerous and manifold (Rodriguez, 2022) but to address recent global events we decided to use disposable protective masks made from polypropylene in this study. With the COVID-19 pandemic this specific plastic product became a pervasive element in everyday life of the last three years globally. A lack of capacity and awareness for proper disposal made it inevitable that single-use face masks would enter natural ecosystems and thus intensify global plastic pollution.

Disposable protective equipment is characterized by its very short lifespan, and primary functioning is comprised after just a few hours (OECD, 2020). Still, the plastic material in face masks remains mostly unaltered when discarded and entering the environment with reduction to microplastics therefore occurring primarily in the environment. To enable comparisons among different stages of face mask breakdown in the environment, two different sizes of the plastic material were included in the study design as well as an unleached and a pre-leached treatment group for each size. Differences in size should reflect two temporal stages of pollution: recently disposed masks (bigger sized macroplastic) as well as masks that already experienced some kind of physical breakdown (smaller sized microplastic). We further expected a short-term effect of plastic leachates on the aquatic environment and thus included a pre-leached treatment level for both sizes. To account for effects due to the presence of physical particles but which may be independent of particle material, saw dust was included in the study as a reference material.

Hypotheses

Hypothesis 1: If non-leaf breakdown-resistant material is present, then the decomposition will slow down.

This hypothesis sees added material primarily as physical barrier to the food source which leaf-decomposing organisms must either circumvent or push through. In the context of microbial exploration of soil pore space Arellano-Caicedo et al. (2021) investigated potential effects of habitat geometry. Their findings suggest that more complex microhabitats decrease fungal and bacterial growth (Arellano-Caicedo et al., 2021). Combined with toxic compounds released from polymers, the plastic is expected to have a stronger effect compared to natural biodegradation-resistant material.

Hypothesis 2: If particles are smaller while the total weight of added material is held constant, then the negative impact of the material is greater.

As the weight of the added material is held constant, the number of particles increases with decreasing particle size. More of smaller sized particles are more dispersed within the leaf pack compared to less of the bigger sized particles. Once the big plastic particle is overcome, decomposers are physically undisturbed within the leaves while dispersed small plastic particles constitute a reoccurring hindrance.

Hypothesis 3: If the added plastic is pre-leached, then the effect of plastic on decomposition is smaller.

Various studies show that plastic pollution releases leachates that can be toxic to their environment (Capolupo et al., 2020; Gunaalan et al., 2020; Sullivan et al., 2021). Therefore, plastic is not only a physical obstacle, but also brings along a chemical effect. In line with these findings, it is hypothesized that pre-leached plastic has a less strong impact on aquatic decomposition processes. However, this difference is expected to cease with time as the leachates get washed away.

Literature review

Hazard: Plastic as an environmental pollutant

Current plastic production and usage is probably one of the few topics discussed by the public, politics, and researchers equally when it comes to environmental pollution. It is a controversial and emotionally loaded subject precisely because plastic nowadays is strongly integrated into every aspect of our everyday life. Modern communication, medicine, food packaging, clothing, and housing are only a few very vital things unimaginable without plastics. At the same time, it is inevitable that these synthetic polymers enter the environment. Studies have found traces of plastics in the most pristine and remote areas from the Arctic (Lusher et al., 2015) to the Antarctic Ocean (Cunningham et al., 2020).

Although European plastic production was strongly impacted by the COVID-19 pandemic, global production still grows rapidly (PlasticsEurope, 2021), fueling concerns about plastic pollution of natural ecosystems. In 2017 the investigative journalists Chris Tyree and Dan Morrison sampled tap water from eight distinct regions of the world and found microplastics in 83% of all samples (n=159) (Kosuth et al., 2017). Thus, research on occurrence, effects, and fate of microplastics gains attention even outside the scientific community. However, many questions remain unanswered (Akdogan and Guven, 2019).

Even if a standardized terminology has never been implemented, plastic pollutants are typically classified into macro-, meso-, micro-, and nanoplastic classes, based on their particle size (Chowdhury et al., 2021). Once discarded into the natural environment, various physical, biological and chemical processes on land and in water contribute to this fragmentation of macroplastics to microplastics and further to nanoplastics (Cole et al., 2011). However, synthetic polymers can have extremely slow degradation rates and complete decomposition of plastic in the environment can take years or centuries (Ioakeimidis et al., 2016).

Being the ultimate terminus of plastic litter transported from inland, the marine environment has been the main focus of research, while terrestrial and freshwater ecosystems received substantially less attention (Li et al., 2018; Wagner et al., 2014). Most plastic products are used on land and it is estimated that around 80% of marine plastic litter originates from land-based sources (Jambeck et al., 2015; Kibria et al., 2022), making rivers a key pathway for plastic pollution to our oceans. However, in recent years the perception of freshwaters solely as passageway shifted and researchers contribute increasing attention to occurrence and effects of plastics in these ecosystems. Several studies showed that freshwaters are subjected to high microplastic pollution levels (Horton et al., 2017; Li et al., 2018; Wu et al., 2019). Lechner et al. (2014) found for the Danube between Vienna and Bratislava in 2010 and 2012 more plastic items (> 20 mm) in the upper 0.5 m of the water column than drifting larval fish. Other studies show that microplastic concentration in river and lake sediments is comparable to findings on marine sediments, thus implicating that freshwater ecosystems also function as a sink for plastic particles (Cera et al., 2020; Horton et al., 2017).

Equally as the research on sources and occurrence of microplastic in freshwaters has grown, effects on organisms living in rivers and lakes have been increasingly studied in recent years. So far, most effort has been granted to impacts of microplastic on fish and macroinvertebrates under laboratory conditions (de Sá et al., 2018; Kallenbach et al., 2022). The main weaknesses of the current research in this field are the unrealistic environment and exposure rates in the laboratory. Studies on chronic toxicity have mostly used shapes, concentrations, and types of polymers differing from those predominant in natural ecosystems. In fact, the lowest reported Lowest/No Observed Effect Concentrations (LOECs/NOECs) of microplastic exceeded the highest measured environmental concentration by two orders of magnitude (Burns and Boxall, 2018). Further, single-organism studies are endpoint-oriented and thus are insufficient to allow estimates of microplastic effects on ecosystem processes and functioning such as organic matter decomposition (López-Rojo et al., 2020).

Vulnerability: Ecosystem functioning and leaf litter decomposition

System sciences study the property of emergence, i.e., the development of features not present in any of the system's individual elements but arising through interaction of those (De Wolf and Holvoet, 2004; Goldstein, 2010). In ecology the emergence of such functional phenomena is driven by the interplay between organisms and their environment. Consequently, ecosystems develop self-sustaining processes like soil formation, water purification and energy cycling, which comprise ecosystem functioning. In this sense only the interaction of organisms with each other and with their physical environment shapes an ecosystem into a habitat for these very organisms.

As ecosystem functioning is an emerging phenomenon it cannot be understood by studying an ecosystem on its species-level alone. Indeed, taxonomic dynamics and functional changes can be independent from each other (Gessner and Chauvet, 2002; Matthews et al., 1982). Recently, ecosystem research progressively moves away from the traditional focus on taxonomic composition and makes efforts to place organisms and particular processes in a wider ecosystem and food web context (Reiss et al., 2009).

The challenges in inferring variation in ecosystem functioning from measures of biodiversity and community structure have led to an increasing focus on developing more specific indices and indicators for ecosystem functioning. For example, Gessner and Chauvet (2002) proposed leaf litter decomposition as an indicator for functional stream integrity to complement the traditional assessment of structural attributes. Their reasoning for choosing leaf litter decomposition is threefold: (1) it is a key process of ecosystems and biogeochemical cycles, (2) leaf litter breakdown processes in streams have proven to respond unambiguously to anthropogenic stresses, and (3) compared to other ecosystem processes the monitoring of leaf litter breakdown is relatively simple and cost-effective (Gessner and Chauvet, 2002).

Inland freshwater ecosystems are intricately tied to their terrestrial surroundings with a growing literature showing how terrestrial organic matter supports aquatic food webs (Cole, 2013). Webster and Benfield, 1986 highlight the importance of vascular plant matter as allochthonous carbon input to freshwater ecosystems and the decomposition of leaf litter in rivers and lakes is a complex interplay of various terrestrial and aquatic organisms. Despite some critics (Gessner et al., 1999), a three-phase concept for leaf litter breakdown has been established. When leaves first enter the water, leaching processes immediately start to wash out solutes. In this initial phase most soluble nutrients are rinsed from the often already nutritionally poor leaves, leaving only hardly digestible structural materials like cellulose and lignin of the cellular matrix behind (Benfield et al., 2017). Within the first days, microbes colonize the leaf litter and start the enzymatic cleavage which softens the leaves and breaks them into smaller particles (Benfield et al., 2017; Webster and Benfield, 1986). This process is widely known as microbial conditioning and is considerably important for leaf palatability and the third processing phase (Gessner et al., 2010; Pascoal et al., 2021). The first microbes to settle on tree leaves are fungi, typically hyphomycetes, progressively giving way to bacteria. For the conversion of coarse (CPOM) to fine particulate organic matter (FPOM) Wallace et al. (1982) have highlighted the importance of macro-invertebrates. Species associated with leaf litter shredding are mainly insects in the families of Diptera, Plectoptera, and Trichoptera but also crustaceans and molluscs.

Risk: Linking pollutants to ecosystem functioning

Studies on ecosystem functioning effects are vital for a sound risk assessment of environmental pollutants (Fleeger, 2020) which in turn forms the basis for control strategies. In the case of microplastics, the main barrier is that we still do not know their full impact as in-situ research is rare and laboratory studies do not replicate real-world conditions (Burns and Boxall, 2018; de Sá et al., 2018; Horton et al., 2017). Some studies have been suggesting that current pollution loads are unlikely to have significant environmental impacts (Backhaus and Wagner, 2020; Burns and Boxall, 2018; Ma et al., 2020), but whatever impacts occur are likely to increase with growing production and low recycling rates. Despite major uncertainties, societies of industrial countries perceive microplastic pollution as one of the most urgent environmental threats. Interestingly, the willingness to mitigate plastic pollution is particularly high while other pollutants and environmental problems (e.g., climate change) whose adverse effects are better proven remain little addressed.

The hypothesized effects of microplastics on the biosphere are manifold across all levels of ecological organization, ranging from (sub)lethal impacts on individual organisms and populations to process impairments on community level up to possible losses of ecosystem productivity due to nutrient cycling obstruction (Ma et al., 2020). By establishing manipulative experiments in micro- or mesocosms recent studies have aimed at assessing effects on community and ecosystem level. In their study with stream water-filled glass jars, López-Rojo et al. (2020) found an inverse linear relationship between leaf litter decomposition by microbes and detritivores (caddisfly larvae) and microplastic concentration in stream water while a similar trend for microbes-only decomposition of enclosed leaf litter was not significant. Seena et al. (2022) observed a decrease in decomposition rates by aquatic hyphomycetes when exposed to polystyrene micro- and nanoparticles. The effect was more pronounced for smaller particles in the nano-size range (Seena et al., 2022). In contrast, Silva et al. (2022) could not find cascading effects on leaf litter decomposition due to polyethylene (PE) microplastics exposure, but did observe a reduction of the abundance of benthic macroinvertebrates. However, Huang et al. (2021) showed that PE microparticle concentrations similar to those used in Silva et al. (2022) (but with a longer exposure period) affected the mediating role of benthic macroinvertebrates in the freshwater nitrogen cycle.

In regards to terrestrial ecosystems, Lozano et al. (2021) performed a large-scale greenhouse experiment on grassland plant species. They mixed polyester fibers into the soil and found that while litter decomposition decreased under drought stress it increased under well-watered conditions (Lozano et al., 2021). Similarly, Lin et al. (2020) found enhanced soil enzymatic activity after mixing polyethylene fragments into on-site forest soil. Yu et al. (2021) studied a soil mesocosm with a common wetland plant species and observed negative effects of different polymers on soil enzymatic activity as well as changes of soil properties and microbial community while Chen et al. (2020) found no significant effects of biodegradable polylactic acid (PLA) microplastics on soil enzymatic activity or microbial community. Finally, Boots et al. (2019) did not find any statistically significant alteration of soil organic matter after synthetic fibers were added to soil containing rosy-tipped earthworms and planted with perennial ryegrass.

The inconsistency of these results has multiple reasons. First, the study design and used microplastic concentration varies and thus makes comparisons difficult (Koelmans et al., 2022; Ma et al., 2020). Secondly, diverging results demonstrate the complexity of the interplay between single organisms, ecosystem processes and anthropogenic intrusions (Koelmans et al., 2022). Thirdly, microplastics are often seen as one type of pollutant when in reality the level of impact is likely to vary according to microplastic physicochemical properties, leading to differing research outcomes (Koelmans et al., 2022; Rochman et al., 2019). Relevant parameters are size, shape, density, color, functional group, crystallinity, stability, and surface charge (Ma et al., 2020). Together with various additives these characteristics form a continuum within which microplastic particles can oscillate when exposed to ecosystem processes (Koelmans et al., 2022).

Materials and Methods

Experimental design and preparation

The study employed a standard litter bag protocol to study the decomposition of alder (*Alnus glutinosa L.*) leaf litter, whereby mesh bags containing a known amount of organic material are deployed in the environment for a certain amount of time (Benfield et al., 2017). The mass loss from the organic material after retrieval allows to draw conclusions on the decomposition potential of the studied ecosystem (Bärlocher, 2020). In this study Belle Vous® cotton tea bags with an approximate mesh size of 0.5mm in diameter were filled with 3.5g of dried alder leaves collected in the Autumn 2017 from tree stands around Ultuna, Uppsala. The use of cotton bags instead of the commonly used nylon mesh bags was due to the need to use any synthetics but the studied plastic particles in the experiment. A laboratory pre-trial showed that the cotton bags would maintain enough structural integrity to hold plastic particles in for the entire study duration.

By virtue of their recent relevance as anthropogenic litter entering the environment, medical face mask of type IIR EN 14683:2019 manufactured by Verdent sp. z o.o. (Poland) were used to produce the micro- and macroplastic particles. To keep the studied material homogeneous, only the blue layer consisting of melt-blown polypropylene fibers was used as a source of plastics. The blue layers were removed from the masks and cut into rectangular particles of 3x3mm for the small size and 60x60mm for the big size using scissors and a scalpel. During the cutting process of the smaller size, particles were mixed and thus particles in one bag were made of multiple masks. The litter bags were subjected to a "material" treatment with three levels (plastic, reference, control). To the plastic treatment 0.2g of plastic particles were added while 0.2g saw dust was added to the reference treatment. Further, bags within the plastic treatment were subjected to a plastic size and a plastic leaching treatment. Altogether, this yielded six different treatment combinations: (1) small unleached plastic, (2) small leached plastic, (3) big unleached plastic, (4) big leached plastic, (5) reference, and (6) control.

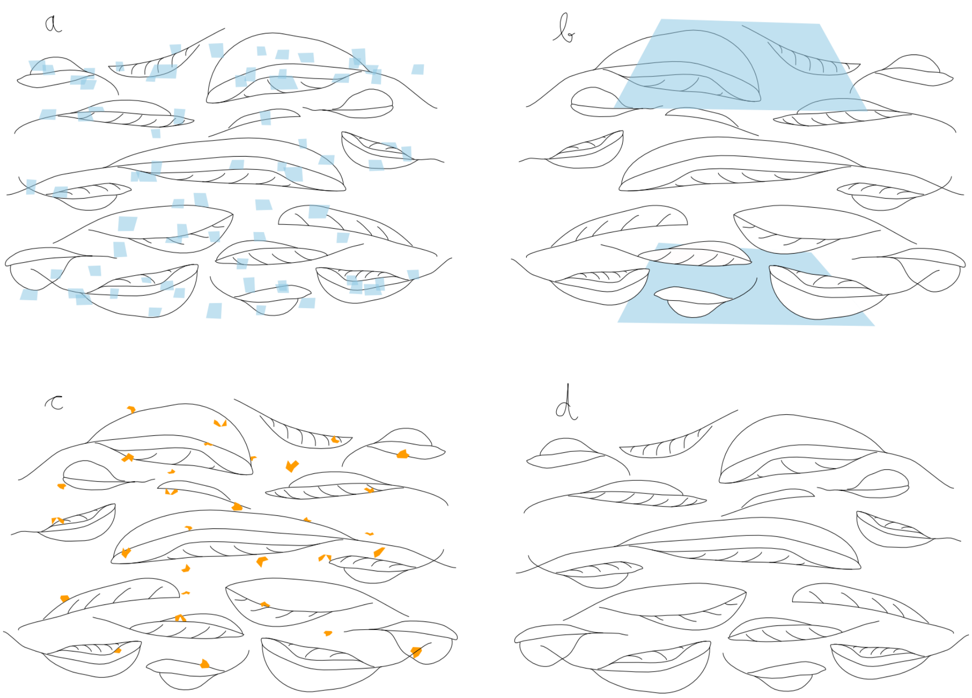

Figure 1: Sketch of how the added material was distributed within the leaves in the cotton bags. (a) illustrates the leached and unleached small plastic treatments, (b) shows the leached and unleached big plastic treatments, (c) illustrates the reference (saw dust) treatment, and (d) shows the control bags with no added material (not to scale).

The microplastic was put into the bags and scattered by rubbing both sides of the bag against each other before the leaves were added. While testing different options this way showed the highest degree of distribution of the particles. As much of the microplastic particles stuck to the sides of the cotton bags it was decided against putting the bigger sized sheets between the leaves. Instead, one was placed on each side of the inside of the bag. To enable a comparison between the potential effect of plastic and of any physical particle on leaf litter decomposition the subsample with 0.2g untreated saw dust per cotton bag was prepared. Fig. 1 illustrates how the added material and leaves were distributed within the cotton bags. As a final step in the sample preparation the filled bags were sealed by sewing them shut with an uncolored cotton thread.

Since microbial decomposition rates do vary over time multiple retrieval dates can add valuable information to the study results. Therefore, groups of bags with five replicates of each treatment (control, reference, non-leached small plastic, leached small plastic, non-leached big plastic, leached big plastic) were retrieved from the aquatic ecosystem on five retrieval dates, namely 2, 7, 14, 21, and 34 days after installment. Thus, the study design amounted to 150 bags in total, 25 bags per treatment and 30 bags per retrieval date respectively.

For the pre-leached treatment of the plastic the blue sheets were separated from the face masks and, before cutting, placed into beakers with 800 ml MilliQ water. The leaching lasted 48 hours at room temperature with occasional stirring. Afterward, the masks were air-dried for 4 hours and further processed in the preparation of the litter bags. During the preparation process, only equipment of metal, glass or wood and cotton clothing was used. All surfaces were regularly wiped with a 70% ethanol solution and the working area included an extraction fan to keep the contamination with other plastic particles as low as possible.

Study site

The study was conducted in a pond at campus Ultuna of the Swedish University of Agricultural Sciences in Uppsala. The pond is part of an ornamental garden and is surrounded by high grass on the northern and eastern shore and rich shrub vegetation along the south-western shore. It was most likely formed as a depression in an outwash plain of glaciofluvial sediments (so called kettle hole) when the last deglaciation of the Scandinavian ice sheet occurred about 21.000 – 13.000 years ago (Cuzzone et al., 2016). In 1990 it was last dredged and the bottom sediments now have a thick soft upper layer of dead organic material. The water depth increases rapidly from the shore with a maximum depth of around 1.7m in most parts of the pond. Fig. 3 shows a map of the pond area overlaid with a satellite picture. Standard water chemistry values for N, P and C for samples taken before and during the experiment period are shown in Table 1.

Table 1: Pond water values for total nitrogen, total phosphorus, dissolved organic carbon, nitrite + nitrate nitrogen, phosphate phosphorus and ammonium nitrogen for two sample dates before and during the experiment period. Data from the SLU Geochemical laboratory and the LEAF-PAD project was kindly provided by Michael Peacock.

Sampling date	Tot-N_TNb µg/l	Tot._P µg/l	DOC mg/l	NO2+NO3_N µg/l	PO4_P µg/l	NH4_N µg/l
19-May-22	788	183	11.3	<1	19	7
15-Jun-22	1020	134	11.1	1	48	26

Only a relatively small fraction of the total nitrogen is bioavailable as inorganic N of nitrite, nitrate and ammonium. Further, a low TN:TP ratio of 4.3:1 (May) and 7.6:1 (June) compared to the Redfield ratio (16:1) for May and June respectively indicate a nutrient limitation of N. The pond might also be affected by dystrophia as high dissolved organic carbon (DOC) levels cause a high degree of humic color (brownification) and makes light likely a limiting factor for primary production (Seekell et al., 2015).

Installment and retrieval

For the installment in the pond the prepared cotton bags were attached to five stainless-steel chains of 5m length each. The chains helped to weigh down the otherwise floating cotton bags to the ground and made sure that the bags would stay in place and fully submerged. By attaching the bags tightly to the chains and omitting two links between neighboring bags, the horizontal spread was secured, and they could not lie on top of each other. There was no evidence of rusting of the chains over the study period, and all bags had the same level of exposure to the chains.

Onto every chain one bag for each treatment and retrieval date was tied using a cotton thread in a randomized order. To keep the retrievals fast and easy, cable ties were used to color-code the bags according to their retrieval date. For example, all bags that were planned to be retrieved on the first date were marked by a red cable tie. Thus, the disturbance for the other bags caused by the retrieval could be kept as short as possible. In advance, the cable ties had been leached with MilliQ water to limit interference with leachates from the cable ties during the experiment.

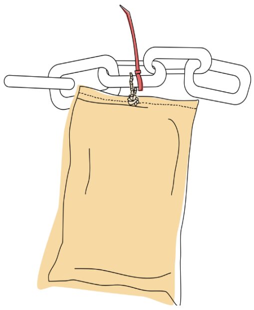

Figure 2: Sketch illustrating how bags were attached to the chains including cable tie (not to scale).

On May 31st, 2022 all chains were placed in the near-shore, shallow water region of the pond. The distribution of shallow water habitat was not even around the pond, and hence chains were deployed in two distinct areas: three chains were installed close to the southeastern shore and two to the northwestern shore of the pond (see Fig. 3). The water table of the pond is quite sensitive to the weather conditions, causing the depth at which the samples were installed to fluctuate around 1 to 1.5m. For extra security, the chains were fastened to the ground by metal poles on both ends.

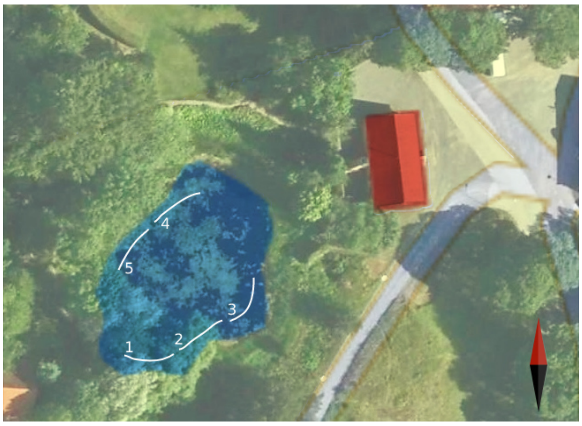

Figure 3: Map of the study area overlaid with a satellite photo. White lines indicate the locations of chains 1-5. Map and photo taken from eniro.se.

Subsamples of litter bags were retrieved 2, 7, 14, 21 and 34 days after installment. Due to the depth and opacity of the water the chains had to be lifted to the water surface to identify and recover due bags. Once out of the water, the bags were carefully cleaned from adhering vegetation and biofilm and immediately placed into corresponding pre-leached plastic bags for respiration measurement. The experiment terminated with the last retrieval on July 4th, 2022.

Ecosystem respiration

Ecosystem respiration represents the total cellular respiration of all organisms in an ecosystem, and thus provides a measure of the metabolic activity of organisms in an ecosystem. In this study, macroconsumers were excluded from the litter bags, and thus quantification of ecosystem respiration represents the activity of micro-organisms and meiofauna, with the microbial fraction expected to be dominant. Quantification of respiration as an ecosystem process is based on the simple correlation that the more metabolically active organism communities are, the more they will respire and thus cause the level of dissolved oxygen in the water to drop over time. Ecosystem respiration was measured by the means of a closed dissolved oxygen measurement system. To keep the disturbance of the decomposing community low, the whole cotton bag was incubated for three hours in filtered, oxygen-saturated pond water. O_2 measurements were taken before and after incubation to calculate the consumption rate (mg O_2 per hour and liter) of the heterotrophic organisms.

The cotton bags were incubated in Toppits Safeloc® 3L Freezer Bags. The incubation water was taken from the pond on the day before the retrieval of the subsample, filtered through a 36µm sieve and stored in a 20°C temperature room. Prior to the first retrieval, the plastic bags were pre-leached for 48h in MilliQ water to prevent leaching of any chemicals during the incubation. These bags were tested during the experiment preparation period on their air-tightness and proved sufficiently sealed for usage during the respiration incubation. Although the use of plastic containers for the respiration trial was not ideal, impacts were minimized by pre-leaching and a relatively short exposure time. Further, all samples were equally exposed and thus any arising error from the freezer bags would have affected all treatments in the same way.

Immediately after their retrieval, the cotton bags were put into their corresponding freezer bags and brought to the 20°C temperature room. For the measurement setup two FireSting®-O2 optical oxygen meter (Pyroscience, Aachen) with four channels each were available. Thus, all six retrieved bags from one chain could be measured at once, leaving one probe for a blank with only O_2-saturated incubation water and one for a control with only O_2-saturated, deionized tap water. To each cotton bag one liter of O_2-saturated incubation water was added. After the first oxygen measurement the plastic bags were sealed airtight and stored for three hours in the dark at constant 20°C air temperature. After incubation the plastic bags were reopened and the second oxygen measurement was taken. Care was taken to ensure that the same oxygen probe was used for both measurements on one bag.

O_2 consumption for each bag was calculated as follows:

$$R = \left[\frac{DO_{S(start)} - DO_{S(end)}}{t_S}\right] - \left[\frac{DO_{B(start)} - DO_{B(end)}}{t_B}\right]$$

where
R = O_2 consumption in mg/L/h
$DO_{S(start)}$ = first reading of dissolved oxygen of the incubation water for the sample bag in mg/L
$DO_{S(end)}$ = second reading of dissolved oxygen of the incubation water for the sample bag in mg/L
t_S = incubation time of the sample bag in h
$DO_{B(start)}$ = first reading of dissolved oxygen of the incubation water for the blank in mg/L
$DO_{B(end)}$ = second reading of dissolved oxygen of the incubation water for the blank in mg/L
t_B = incubation time of the blank in h

Following respiration measurements, the freezer bags containing the cotton bags were stored overnight in the dark in a fridge at 8°C for continuing processing on the next day.

Leaf mass loss determination

The day after respiration measurements, the retrieved cotton bags were carefully opened along the seam and both leaves and cotton bag were placed into separate aluminum trays.

The trays with the bags and leaves were placed in an oven and dried at 50°C for 48 hours. After drying the leaves were carefully cleaned from the added plastic and reference material and weighed in their trays to determine dry weight and total leaf mass loss.

$$DW = w_{LT} - w_T$$

$$LML = \frac{DW}{w_{ini}}$$

where
DW = remaining dry weight of leaves in g
LML = leaf mass loss
w_{LT} = weight of tray with dried leaves
w_T = weight of tray
w_{ini} = initial leaf weight

Tensile strength loss determination

Since cotton strips are often used to investigate decomposition rates in various ecosystems (Colas et al., 2019; Tiegs et al., 2013), changes in tensile strength of the cotton bags were measured as another indicator for decomposition. To that end 80x25mm sized strips were cut off the retrieved and dried bags. Approximately, a 10mm portion of the ends of the strips was then placed into the grips of a Mark-10© motorized force tester (Mark-10 Corporation, Copiague). The strips were pulled with a fixed speed of 20mm/min and peak tension was measured with a Mark-10© digital force gauge (Mark-10 Corporation, Copiague). Peak tension measurements were converted into tensile strength loss by subtracting the value from the baseline, where the baseline is the average across peak tension recordings of 30 unused cotton bags.

$$TSL = \frac{PT^* - PT}{PT^*}$$

where
TSL = tensile strength loss
PT^* = baseline in N
PT = measured peak tension after incubation and drying in N

Decay rates

To measure water temperature one SmartButton© temperature logger (ACR Systems Inc., Surrey) programmed to record water temperature in a 30 minutes interval was attached to each end of each chain. The resulting data sets were used to calculate degree days for each chain:

$$DD = \overline{T} \times IT$$

where $\overline{T}$ is the daily average temperature.
IT = incubation time in days
DD = incubation time in degree days

The calculated degree days for each chain and incubation duration were used to calculate decay rates (k values) of leaf litter and cotton bags:

$$k_{LML} = \frac{ln(LML)}{DD} \qquad k_{TSL} = \frac{ln(TSL)}{DD}$$

with k_{LML} being the decay rate of leaves and k_{TSL} the decay rate of cotton bags.

Statistical Analysis

In this study, variation in the placement of chains with the experimental system (pond) is assumed to be associated with background variation in environmental characteristics, such as differences in water depth, vegetation, etc. that differ depending on chain location within the pond. Such background variation has potential to affect the studied parameters of the litter bags, increases noise in the data and possibly reduces the capacity to detect significant effects of the main experimental factors. To account for this influence of location, the chains can be used as a blocking factor. Using a blocking factor controls for intrinsic heterogeneity among experimental units (blocks) by splitting the deviations between model predictions and observations into among-block variability and the sampling error (Kanters, 2022). This reduces the error mean square of the statistical model and thus increases statistical power (Zar, 1999). Since each chain contained a complete factorial (treatment x incubation time) set of bags where the exact location of each bag on the chain was assigned randomly, the study employed a randomized complete block design (RCBD). The statistical analysis of this study was performed as a non-additive linear mixed model testing for differences in means of LML, TSL and O_2 consumption between treatments. The model included chain as a random block effect, and two fully crossed fixed factors: material and exposure time. Additionally, two further fixed factors were nested within material: leaching and plastic particle size. Reflecting the hierarchical nature of the design, different error terms incorporating the random effect were used to test the fixed effects. Full model specification and an in-depth explanation of the underlying statistical theories is provided in the supplementary material.

For the statistical analysis JMP® Pro (version 16.0.0, SAS Institute AB, Solna) was used for all response variables, some with log(y+1) or square root transformation when necessary. As estimation method restricted maximum likelihood (REML) was deployed with $\alpha = 0.05$ as conventional confidence level. On significant main effects a Tukey's pairwise comparison was run as post-hoc analysis. Due to its rapidly increasing weakness with increasing number of factor levels, Tukey's was not used on significant nested or interaction terms. Instead, differences were graphically explored. Explanatory variables were type of material (M), size within material (S), leaching within material (L), interaction of S x L, incubation time (IT), interactions of IT x M, IT x S, IT x L, and IT x S x L, of which all were nominal categorical variables. Response variables were O_2 consumption in mg/h/L, percentage leaf mass loss (%LML), leaf decay rate (k_{LML}), percentage tensile strength loss (%TSL), and cotton bag decay rate (k_{TSL}).

Results

Ecosystem respiration

Exposure days significantly affected ecosystem respiration (Table 2). O_2 consumption increased steadily from a mean value of 0.29mg O2 per hour and liter on day two to 0.49mg/h/L on the last retrieval day (Fig. 4). Leaching also had a significant effect on ecosystem respiration (Table 2). Respiration rates were highest when unleached plastic was present and lowest for the control bags (Fig. 5) with sample means of 0.40mg/h/L and 0.35mg/h/L respectively. Values for leached plastic and the reference material fell in between with the leached plastic being closer to the control group and the reference material being closer to the upper end.

Table 2: Nested, mixed model ANOVA analyses testing fixed effects of material (M), size (S), leaching (L) and incubation time (IT) on ecosystem respiration. The influence of chain (C) was modeled as a random effect, with random effect error terms indicated in italic. Statistically significant effects are highlighted in bold.

		O2 consumption		
Source	df	dfDen	F ratio	Prob > F
C				
M	2	20.51	2.20	0.137
S[M]	1	18.52	0.20	0.662
L[M]	1	18.52	5.33	**0.033**
SxL[M]	1	18.52	1.10	0.307
CxSxL[M] resid 1				
IT	4	94.18	9.17	**<.001**
ITxM	8	94.00	0.96	0.473
ITxS[M]	4	92.48	0.45	0.774
ITxL[M]	4	92.48	0.51	0.726
ITxSxL[M]	4	92.48	0.17	0.954
CxITxSxL[M] resid 2				

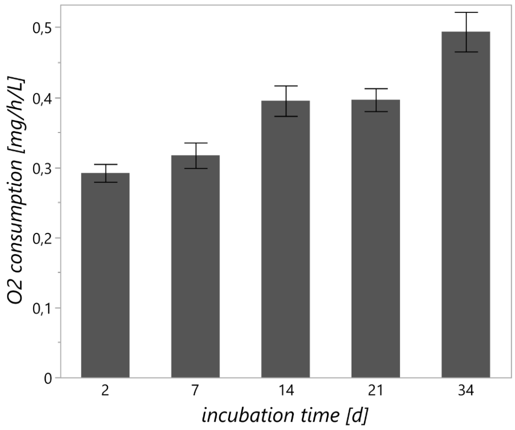

Figure 4: Effect of incubation time on ecosystem respiration (mean ± SE plotted).

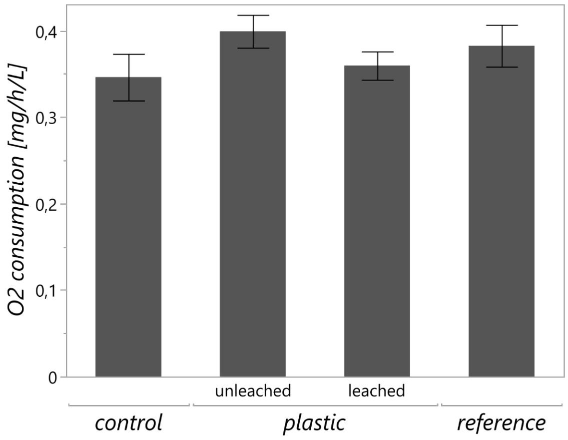

Figure 5: Effect of leaching nested within material on ecosystem respiration (mean ± SE plotted).

Leaf decomposition

Table 3: Nested, mixed model ANOVA analyses testing fixed effects of material (M), size (S), leaching (L) and incubation time (IT) on leaf mass loss and leaf decay rate. The influence of chain (C) was modeled as a random effect, with random effect error terms indicated in italic. Statistically significant effects are highlighted in bold.

		Sqrt(%LML)			Log(-kLML)		
Source	df	dfDen	F ratio	Prob > F	dfDen	F ratio	Prob > F
C							
M	2	19.34	7.90	**0.003**	19.26	6.80	**0.006**
S[M]	1	19.71	6.96	**0.016**	19.67	7.21	**0.014**
L[M]	1	19.71	2.08	0.165	19.67	1.93	0.180
SxL[M]	1	19.71	0.60	0.449	19.67	0.54	0.471
CxSxL[M] resid 1							
IT	4	94.77	769.27	**<.001**	94.8	2496.54	**<.001**
ITxM	8	94.79	1.94	0.063	94.83	1.47	0.177
ITxS[M]	4	95.16	2.73	**0.033**	95.24	2.74	**0.033**
ITxL[M]	4	95.16	0.18	0.946	95.24	0.12	0.975
ITxSxL[M]	4	95.16	0.51	0.727	95.24	0.41	0.800
CxITxSxL[M] resid 2							

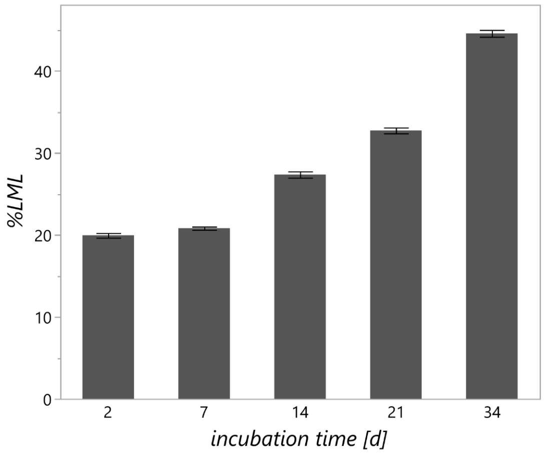

Figure 6: Effect of incubation time on percentage LML (mean ± SE plotted).

The model outputs on leaf mass loss and leaf decay rates were very similar to each other and resulted in the same significant terms (Table 3). Therefore, it was decided to display and discuss only leaf mass loss. Graphical results of k_{LML} can be found in the supplementary material. As expected, leaf mass loss increased over time. 20% of initial leaf mass was lost after the first 48 hours. Another 24% were lost throughout the following month. Very small standard errors indicate a high degree of clarity of the time pattern among the LML data.

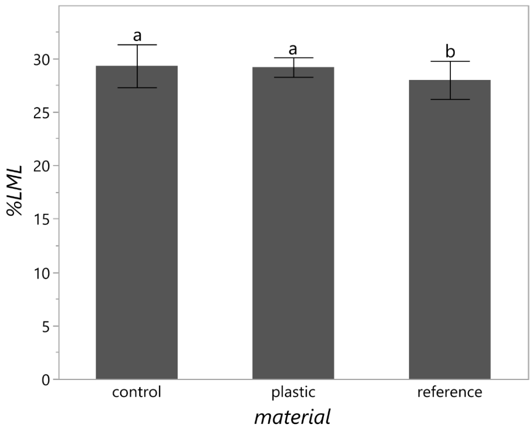

Figure 7: Effect of material on percentage LML (mean ± SE plotted). Lettering above the bars indicate homogenous subsets from the post-hoc Tukey's test.

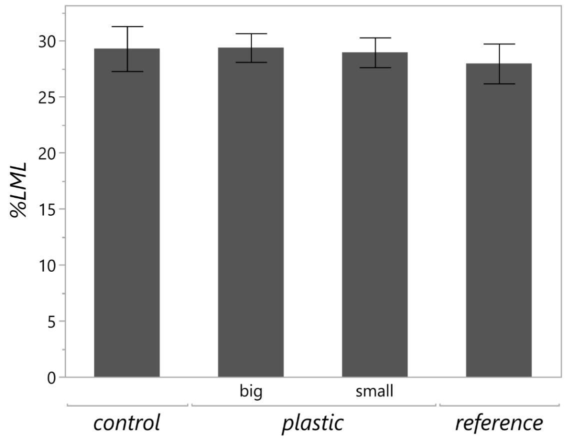

Figure 8: Effect of size nested within material on percentage LML (mean ± SE plotted).

Material was another significant factor for leaf decomposition (Table 3). While control bags and bags containing plastics were similar (29.3% and 29.2% respectively), the reference material significantly decreased mean LML to 28.0% (Fig. 7). The post-hoc Tukey's test showed a significant difference between control and the reference material ($t_{19.3}$ = 2.85, p = 0.026) and between the plastic and the reference material ($t_{19.3}$ = 3.95, p = 0.002). Note that the Tukey's test was performed on square-root transformed data to comply with underlying statistical assumptions. Note also that the standard error bars grow larger in Fig. 7 and Fig. 8, indicating that the effects of material and size are not as clear as the effect of incubation time.

Fig. 8 shows a similar pattern for size as for material, which was also significant (Table 3). Mean leaf mass losses for the control group and the big plastic were 29.3% and 29.4% respectively. The presence of small plastic particles decreased the mean value to 29.0%. A decrease to 28.0% was observed in the presence of the reference material.

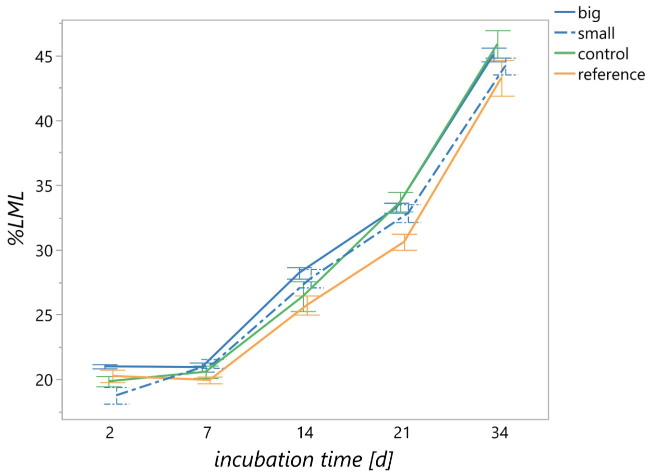

Figure 9: Effect of the interaction between time and size nested within material on percentage LML (mean ± SE plotted).Note that the y-axis does not start at 0.

Not only the main effect of size but also its interaction with time was significant in the leaf mass data (Table 3, Fig. 9). Initially, the small and big sized plastic treatments were on opposite ends of the range at day 2. However, the two size treatments had converged by day 7, after which the difference increases again. The slowing effect of small plastic and saw dust compared to the control is most pronounced on the day of the last retrieval, indicating that the effect becomes stronger with time.

Tensile strength loss

Peak tension recordings varied strongly across all treatments which is reflected by the relatively large standard errors especially in the first retrievals of the study (Fig. 10 and Fig. 11).

Table 4: Nested, mixed model ANOVA analyses testing fixed effects of material (M), size (S), leaching (L) and incubation time (IT) on tensile strength loss and cotton bag decay rate. The influence of chain (C) was modeled as a random effect, with random effect error terms indicated in italic. Statistically significant effects are highlighted in bold.

		Sqrt(%TSL)			-k(TSL)		
Source	df	dfDen	F ratio	Prob > F	dfDen	F ratio	Prob > F
C							
M	2	18.03	5.69	**0.012**	16.84	3.28	0.063
S[M]	1	19.08	1.55	0.228	17.7	4.01	0.061
L[M]	1	19.08	6.06	**0.024**	17.7	6.26	**0.022**
SxL[M]	1	19.08	12.20	**0.002**	17.7	25.33	**<.001**
CxSxL[M] resid 1							
IT	4	92.99	69.86	**<.001**	91.31	6.81	**<.001**
ITxM	8	93.07	2.36	**0.023**	91.38	1.72	0.104
ITxS[M]	4	94.13	0.88	0.476	92.38	5.69	**<.001**
ITxL[M]	4	94.13	2.92	**0.025**	92.38	7.35	**<.001**
ITxSxL[M]	4	94.13	7.89	**<.001**	92.38	17.37	**<.001**
CxITxSxL[M] resid 2							

The significant increase in TSL with time is visible in Fig. 10. High initial decomposition rates for the cotton bags led to a percentage TSL of almost 15% after incubation for 48 hours. Subsequently, k_{TSL} was cut in half by the time of the second retrieval (Fig. 11). For the last two retrievals after 21 and 34 days respectively, the decay rate per degree day picked up again. Mean tensile strength loss was already exceeding 90% on the day of the last retrieval (Fig. 10).

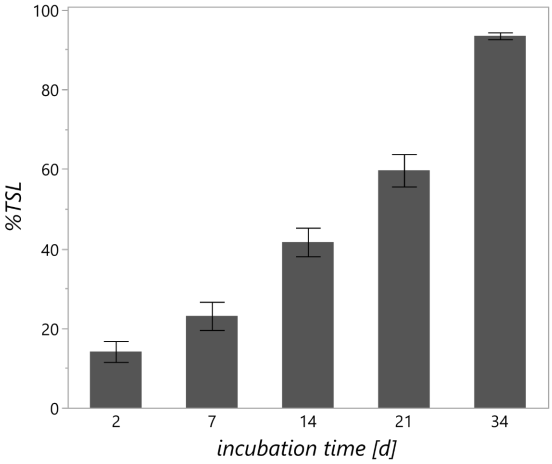

Figure 10: Effect of incubation time on percentage TSL (mean ± SE plotted).

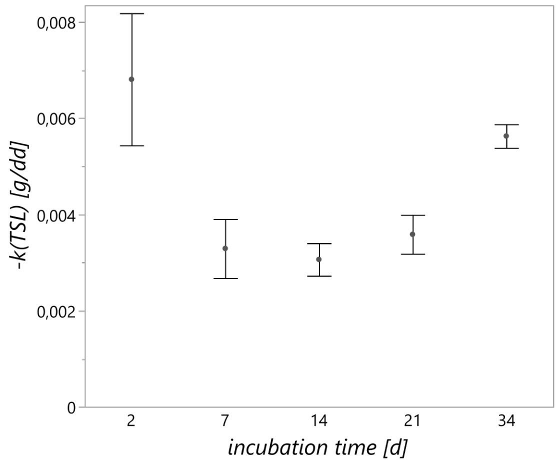

Figure 11: Effect of incubation time on k_{TSL} (mean ± SE plotted). Note that the y-axis does not start at 0.

No effect of material in the model with k_{TSL} as response variable could be detected, whereas material was significant for percentage TSL (Table 4). The post-hoc Tukey's test on the square root transformed data showed a significant difference in TSL between the plastic treatments and the control bags ($t_{18} = -3.01$, $p = 0.0198$). Contrary to what was seen in the LML data, the presence of plastic increased mean TSL to 47.6% compared to 43.9% of the control group (Fig. 12). The reference treatment however lowered TSL to 40.4%.

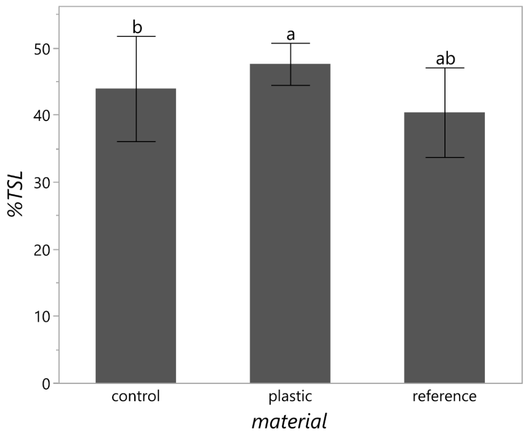

Figure 12: Effect of material on percentage TSL (mean ± SE plotted). Lettering above the bars indicate homogenous subsets from the post-hoc Tukey's test.

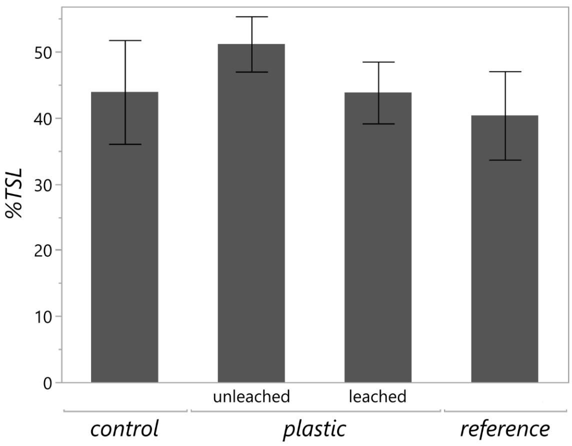

Figure 13: Effect of leaching nested within material on percentage TSL (mean ± SE plotted).

Leaching had a significant effect on both response variables (Table 4). The results of both models are very similar and thus only %TSL is displayed in Fig. 13. Corresponding graphs for k_{TSL} can be found in the supplementary material. Litter bags containing unleached plastic had the highest response values with a sample mean of 51.2% for TSL (Fig.13). This means a surge by more than 7 percent compared to the control (43.9%). With a mean TSL of 43.8% the addition of leached plastic did not significantly affect the strength loss of the cotton bags.

Significant Interactions

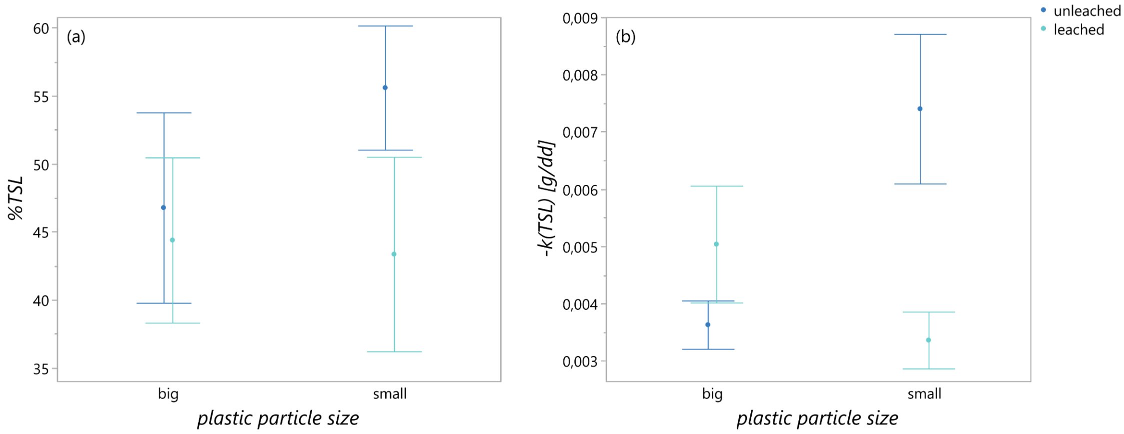

Figure 14: Effect of the interaction between size and leaching nested within material on (a) percentage TSL and (b) k_{TSL} (mean ± SE plotted). Note that y-axes do not start at 0.

Fig. 14 shows that the main effect of leaching also interacted with the two different sizes of the studied plastic particles for both response variables. TSL between unleached and leached plastic only deviated by about two percent (46.8% and 44.4% respectively) for the big sized treatment. In the small sized treatment however TSL values were on average more than a quarter higher when the particles were unleached compared to pre-leached plastic (55.6% compared to 43.3%).

For k_{TSL} Fig. 14 shows that the main effect of leaching was reversed for big sized plastic with a higher mean for leached (0.005g/dd) than for unleached big plastic (0.004g/dd). Results for the small plastic treatment were consistent with the main effect of leaching. On average, k_{TSL} more than doubled in the presence of unleached small plastic compared to leached small plastic (0.007g/dd compared to 0.003g/dd).

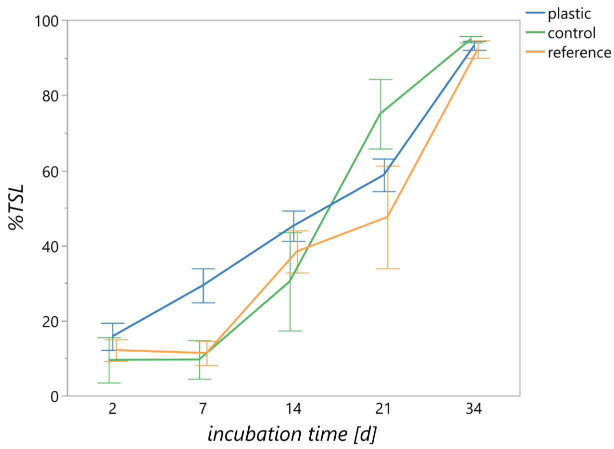

Figure 15: Effect of the interaction between material and time on percentage TSL (mean ± SE plotted).

The interaction between material and time was significant for %TSL (Table 4) but not for the decay rate. Fig. 15 reveals that strength loss rose more steeply when plastic is present only for the first three retrievals. By day 21, the mean values of the control group exceeded both the plastic and the reference treatment. The biggest difference was observed after seven days, where mean TSL was 29.4% for plastic, 11.3% for saw dust and 9.6% for the controls. On day 21 values deviated again strongly with now the controls having the highest TSL at 75.1%, followed by plastic at 58.8% and saw dust at 47.6%. As the experiment terminated, all treatment results converged again as they all just exceeded 90% strength loss.

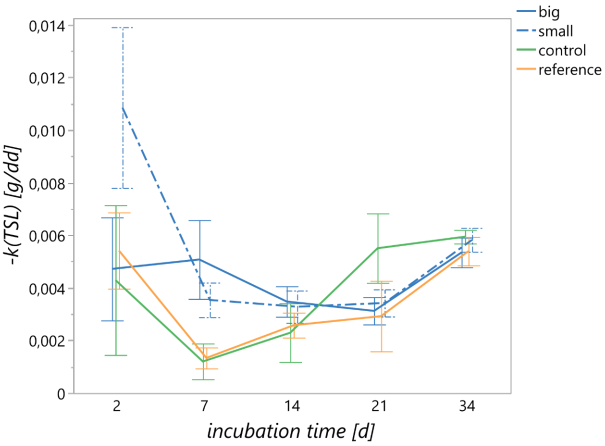

Figure 16: Effect of the interaction between size and time nested within material on k_{TSL} (mean ± SE plotted).

While an interaction of material and time was not significant, size interacted significantly with time for cotton bag decay rates (Table 4). The significance of this interaction term is mostly driven by the first two retrievals as can be seen in Fig. 16. While mean decay rate is considerably elevated in the presence of small plastic after the first 48h (0.01g/dd compared to 0.005g/dd of the big plastic and 0.004g/dd of the control), this effect drops rapidly thereafter. On day 7, k_{TSL} for the small plastic has decreased to 0.004g/dd and was thus lower than for the big plastic. While all treatments resulted in a decrease of k_{TSL} between the first two retrievals, it was stable around 0.005g/dd in the presence of big plastic particles during the same period. Decay rates were still elevated for both plastic size treatments when compared to the other treatments on day 14 but were exceeded by the control group on the following two retrievals. From day 14 onwards the size of the plastic particles did not show any differing effects on k_{TSL}.

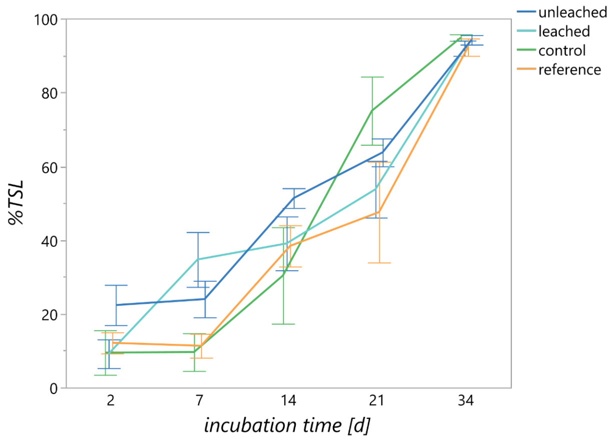

Figure 17: Effect of the interaction between leaching and time nested within material on percentage TSL (mean ± SE plotted).

The interaction between leaching and time showed significance for both response variables (Table 4). Both results were fairly similar, hence from an elaboration on k_{TSL} was abstained (a corresponding graph can be found in the supplementary material). Fig. 17 shows that TSL was elevated when the present plastic particles were unleached compared to the pre-leached treatment, except for day 7. On this day, results were on average considerably higher for the leached treatment (34.7%) compared to the unleached group (24.0%).

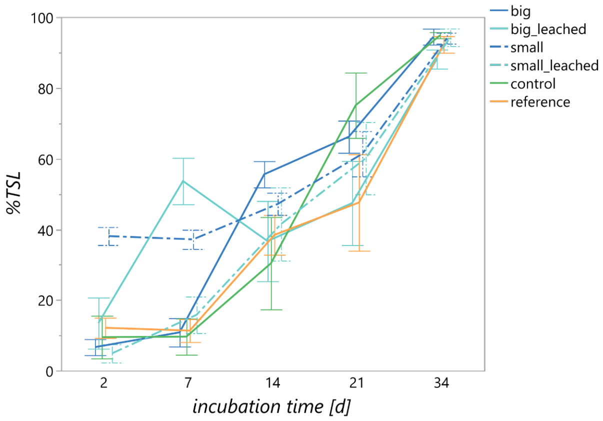

Figure 18: Effect of the interaction between size, leaching and time nested within material on percentage TSL (mean ± SE plotted).

The full interaction between size, leaching and time was significant for both response variables (Table 4). Again, due to redundancy only figures for %TSL are discussed.

In Fig. 18 four main points need to be mentioned: (1) small unleached plastic elevated strength loss substantially for the first two retrievals. (2) big leached plastic resulted in a local peak of %TSL on the second retrieval but was among the lower values obtained on day 14. (3) the presence of unleached big plastic resulted in a steep increase of %TSL after the second retrieval and was only exceeded by the control group on day 21. (4) Standard error bars are partly very large, indicating a large variation within the data.

Discussion

The in-situ experiment showed complex effects of plastic presence on aquatic decomposition processes and ecosystem respiration, whereby these effects vary with the particle size of the plastic and whether the plastic was pre-leached. The significance of time on all response variables and its effect direction was generally expected and is straightforward. All observed values naturally increased with time. Interestingly, some other significant effects varied over time, which is discussed further down in this chapter.

Effect of material

The main effect of material was significant for k_{LML}, %LML and %TSL. For both responses of leaf litter decomposition, the effect of material stems from the reference material being significantly different from the control and plastic treatments. The hypothesis that the added material acted as a physical barrier might be visible in these findings. The saw dust as reference material was much thicker than the plastic and thus harder to overcome for the colonizing organisms. Further, the saw dust as barrier was more complex since it varied substantially in size, shape and thickness. Studies on micro-structured soil chip systems have found fungal litter decomposing species to respond diversely and not predictable to obstacles in terms of their foraging behavior (Aleklett et al., 2021). Further, fungal foraging responses to obstacles may have negative impacts on bacterial substrate degradation as branching hyphae around barriers can block the dispersal of bacteria (Arellano-Caicedo et al., 2021). However, it must be kept in mind that the total number of particles per litter bag was considerably lower for saw dust than for small plastic. The amount of plastic added to the litter bags was approximately half of a face mask which provides considerably more surface area for biofilm to grow on. This is not true for the reference material as the saw dust was heavier and resulted in much lower added surface area. Increased area for microbial growth might counteract the negative effect of increased habitat complexity in the presence of plastic.

Effect of size

The significance of size for LML was mostly driven by a difference between the big plastic treatment and the reference treatment, with the reference treatment resulting in an impaired decomposition. The obtained value for smaller sized plastic treatment was located in between. Due to the different sizing the big plastic particles could not be distributed the same way within the leaf pack as the reference material and the small plastic particles. While the small plastic and saw dust particles were similarly dispersed within the leaves, the big plastic sheets were placed on the sides of the leaf pack (two sheets in each bag), i.e., between leaves and the inner side of the cotton bag. Once the microbes found their way into the leaf pack the big plastic did not constitute an obstacle anymore. The smaller plastic particles as well as the saw dust though did hinder microbes to access leaves within the pack. These particles also hampered access to both, the leaves underneath and the leaves above them whereas only one side of the big plastic sheets had contact to leaf matter.

Effect of leaching

Contrary to hypothesis 3, leaching had no significant effect on leaf litter decomposition. Regarding tensile strength loss even an opposing effect to what was anticipated was observed. Compared to the control and leached treatment, unleached plastic enhanced TSL.

Non-leached plastic increased O_2 consumption of the studied system and stimulated tensile strength loss of the cotton bags as well. A very recent study by Sheridan et al. (2022) showed that plastic leachates strongly promoted bacterial growth in Scandinavian surface waters. Although they used low-density polyethylene plastic bags while face masks deployed in this study consisted of melt-blown polypropylene fibers, their conclusions can help to interpret the results at hand. By higher lability and an observed increase of bacterial growth efficiency, they reason that plastic-derived dissolved organic matter (DOM) is more readily bioavailable for use by microbial communities than natural DOM (Sheridan et al., 2022). This might as well be an argument for the stimulation of ecosystem respiration in the presence of unleached plastics found in this study. Enhanced bacterial growth due to plastic leachates could also explain the strongly accelerated k_{TSL} in the unleached plastic treatment for the first days of the experiment. As leachates get transported away, diluted and chemically changed this effect naturally decreased after a few days. For cotton bag decomposition the effect of leaching thus varied with time. Interestingly, for the respiration data a time x leaching interaction was not significant.

Another approach to the increased respiration rates and tensile strength loss under the plastic treatment would be to see it as a stress response. Similar as our heart beat increases under stress it may be that decomposing microbes and microfauna consume more oxygen in the presence of a stressor. Plastic leachates contain harmful compounds like catalyst remnants, dyes, softeners, etc. that can be toxic to organisms (Gunaalan et al., 2020). Sullivan et al. (2021) showed that disposable face masks release heavy metals like Cu and Pb which are known for their environmental impact and adverse effects on ecosystem functioning (Peters et al., 2013; Sridhar et al., 2001; Sullivan et al., 2021). At this point it is important to stress that only the blue layer of the face masks was used in this study, whereas others used whole masks including ear straps and nose wires.

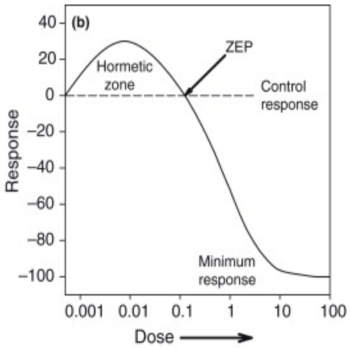

Figure 19: Typical β-curve describing the hormesis of a low-dose stimulating-high-dose inhibiting effect. ZEP = zero equivalent point marks when the response leaves the hormetic zone. Taken from Ray et al. 2014.

The theory of chemical hormesis argues that biological responses to chemical stressors are often observed to be biphasic, meaning that biological growth and respiration can be stimulated when exposed to low doses of a chemical while impaired at high doses (Calabrese and Baldwin, 1998; Ray et al., 2014). Calabrese and Blain (2011) had found approximately 9000 observations of hormetic dose responses (82% followed an inverted U-curve, i.e., β-curve as seen in Fig. 19), out of which 2000 were metabolic responses, including oxygen uptake. Important to note here is that the stimulating low-dose effects, although historically considered as being beneficial, do not necessarily indicate benefits for the studied organisms. A modern approach to hormesis sees it as a result of fundamental reparative processes in response to a disturbance of homeostasis (Ray et al., 2014). A biphasic response behavior might be able to explain the observed increases in respiration and cotton bag decomposition under the presence of unleached plastic.

Interaction Effects

Effects of size x leaching

For tensile strength loss the effect of leaching interacted with size. The unleached plastic increased the decomposition of the cotton bags and this effect was more pronounced for small plastic particles. This interaction may be counterintuitive at first sight. As described in the methods section the big plastic sheets had much more contact to the cotton bags than the smaller sized particles (see Fig. 1). Thus, any stimulating effect of plastic on TSL would be expected more eminent for the bigger sized treatment. Of the theories discussed for the main effect of leaching only the theory of hormesis can provide some explanation of the observed interaction. With the big plastic sheet adjacent to the canvas the dose of plastic leachates might have exceeded the peak of the response curve in Fig. 19 and thus led to decreasing responses.

On the contrary, one could argue that the small plastic particles exhibit a more broken structure, which might release leachates more easily. Because of the cutting the small plastic treatments exhibit more broken polypropylene fibers. Breakage of plastics into smaller particles has been found to accelerate leaching kinetics for some compounds in previous studies (Do et al., 2022).

Interaction effects with time

The interaction plots in the results section reveal a general turning point of effects after the third retrieval for differences between the plastic treatments and the control group. Decreasing effects of plastic on LML became visible after 14 days of incubation. This indicates that plastic pollution expresses its full effect on leaf litter decomposition with progressing exposure time. Increased habitat complexity due to the presence of added particles might be the effect driver instead of short-term leachate effects.

In contrast, enhancing effects on TSL were pronounced up until day 14 and all treatments converged after day 21. The study duration covered a much bigger period of the endpoint in TSL then in LML. Maximum values ranged around 90% for TSL on the last retrieval day whereas leaf masses were only halved by the end of the study. That the observations for TSL in all treatments converged on the last retrieval might therefore be due to the convergence to the endpoint (100% TSL). However, it is interesting that the control group exceeded all other treatments on day 21 while it was the lowest on the foregone dates.

Conclusion, limitations and further research

The data generated through this field study provides some first insights into effects of plastic pollution on leaf litter decomposition in freshwater ecosystems. The litter bag experiment investigated how polypropylene interacts with decomposing processes in an anthropogenic impacted pond for two plastic particle sizes, below and above the generally adopted threshold for microplastic. Further, pre-leached and unleached plastic treatment levels were included in the study design. This enabled to discuss potential impacts of plastic leachates on the ecosystem. Additionally to the obligatory control group, a reference treatment using saw dust was included to account for effects of biodegradation-resistant material as physical barrier.

Observations of ecosystem respiration, leaf mass loss and tensile strength loss of the cotton litter bags were statistically analyzed with a mixed model approach. This resulted in several interesting insights. The first hypothesis that added material would slow down decomposition was backed up by the data on leaf mass loss. Both materials, plastic and saw dust reduced average leaf mass loss with a greater reduction for small compared to big plastic particles. Regarding tensile strength loss results were mixed with a higher mean loss in the presence of plastic but lower average values for the reference material.

Evidence could not be found for the assumption that plastic would reduce decomposition more than the reference material. In fact, the contrary was observed for both, leaf and cotton bag decomposition. Finally, leaching had interesting effects on ecosystem respiration and tensile strength loss. Both response variables were significantly elevated in the presence of unleached plastic, which contradicts the hypothesis that leachates would impair decomposition processes.

The study at hand exhibits some limitations regarding the spatial and temporal extent as well as its design. First, the study is limited to the one pond used in the study and lacks spatial scalability. Still, findings are relevant for ponds in urban and garden settings with high carbon contents. Ponds located in close proximity to human activities are likely to be major entry points for plastic pollution into the hydrosphere. To employ several ponds of a similar type would increase the validity of such an experiment. Further, as seen in Fig. 9, the effect of material on leaf decomposition became more pronounced with progressing incubation time. Thus, studies on leaf decomposition should be designed in a way that enables longer incubation periods. Here, the progressing decomposition of the cotton bags did not allow further prolongation. Using metal mesh bags instead could tackle the time limit but exhibits other drawbacks (e.g., metal leachates).

In the study design some trade-offs had to be made which diminish its ability to represent reality. Gessner et al. (1999) point out that initial leaf mass loss for freshly shed alder leaves behaves differently than dried leaves, commonly used in field experiments. The pre-study drying facilitates the measurement of initial leaf mass but results in damages of the leaf tissue. Moreover, the use of litter bags with small mesh sizes excludes many of the otherwise contributing decomposers. This limits the study to decomposition processes mediated by microbes and meiofauna ignoring any possible interactions with detritivore macrofauna.

Regarding the data on tensile strength two main limitations exist. First, the cotton bags used in this study are no standardized research equipment and consequently vary in their size, mesh size, thread thickness and initial tensile strength. Second, the baseline used to determine tensile strength loss is only an average of 30 bags, which were never used as litter bags. Initial tensile strengths of the used bags may deviate from this average as the production and raw material is not standardized. Only the assumption that these deviations are random and zero on average makes the TSL data valid.

Despite the mentioned limitations this study is one of very few field experiments on environmental plastic pollution and thus provides valuable in-situ insights. For freshwaters close to urban areas such as garden ponds these insights have a great relevance as they constitute main entry points for plastics into the hydrosphere. The results indicate that increased habitat complexity due to anthropogenic pollution can alter ecosystem processes. Further, the findings show that leachates released from plastic during exposure to breakdown processes can trigger complex biological responses. These responses are not straightforward and more research on plastic leachates potentially reveal important details to enable a better understanding of the observed results. During this study leaf discs were sampled and frozen to sequence the microbial community. Linking the obtained data with information on types of organisms present would be another interesting topic for a follow-up study.

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Supplementary material

1. Statistics

To explain the underlying statistical theory the model is simplified by condensing all treatments into one pooled factor. This pooled factor has six treatment levels reflecting the different bags: “small plastic + unleached”, “small plastic + leached”, “big plastic + unleached”, “big plastic + leached”, “reference”, and “control”. Hence, the pooled factor model has two fixed effects (pooled factor, incubation time) and one factor controlling for random effects (chain). The actual model used in the data analysis is a more complex nested model and thus reflects the structure of the experiment design more accurately. It will be discussed later in this chapter.

The pooled factor model

The hypothesis testing for differences in means is known as Analysis of Variance (ANOVA), where the dependent variable is continuous and all independent ones are categorical. The special case which combines fixed and random effects is also known as Model III ANOVA and goes as a synonym for mixed models (Zar, 1999). This model is expressed as

$$y_{ijk} = \mu + \pi_i + \tau_j + \gamma_k + \varepsilon_{ijk} \tag{1}$$

where:
y_{ijk} = the observation within the kth level of factor C within jth level of factor T within the ith level of factor P
μ = the overall mean
π_i = the effect due to the ith level of the pooled factor P and i = 1, 2, …, p
τ_j = the effect due to the jth level of the time factor T for incubation days and j =1, 2,…,t
γ_k = the effect due to the kth level of the chain factor C and k = 1, 2, …, c
ε_{ijk} = the residual error of observation y_{ijk}

Factors P and T in Eq. (1) are fixed factors. Levels of fixed factors are seen as a full representation of all levels that the model attempts to draw statistical inferences about. If another study wished to repeat the experiment the levels of fixed effects would be exactly the same (Newman et al., 1997). In contrast to fixed effects which levels are chosen specifically, levels of random effects are supposed to be a random sample from a bigger population. Factors drawn randomly from a large population like five randomly chosen locations out of all possible locations are assumed to have a normal distribution of the form

$$c_k \; i.i.d. \sim N(0, \sigma_c^2).$$

The same assumptions are typically made for the error term:

$$\varepsilon_{ijk} \; i.i.d. \sim N(0, \sigma_\varepsilon^2).$$

The intent of including the random effect is to enable a generalization across all possible levels of the random effect (Zar, 1999). The random variable introduces another variance component into the model and hence reduces the variance of the residual (Meier, 2022).

The mixed effects model assesses if the fixed effects have a statistically significant effect on the overall mean of the dependent variable. Thus, with Eq. (1) the following null hypotheses are tested:

1. H_0: mean y is the same for all levels of the pooled factor
2. H_0: mean y is the same for all incubation durations

Hence, for the fixed effect it applies that by definition

$$\sum_{i=1}^{p} \mu_i - \mu = 0 \; and \; \sum_{j=1}^{t} \mu_j - \mu = 0$$

where μ_i-μ and μ_j-μ are the deviation from the mean caused by level i of the pooled factor and level j of the time factor respectively (Newman et al., 1997). The testing of null hypotheses in mixed models is based on the F ratio, which is calculated with the sum of squared deviations (SS) from the sample mean for any two independent samples $X = x_1, x_2, \dots, x_m$ and $Y = y_1, y_2, \dots, y_n$, one from each population

$$\hat{F} = \frac{SS_x/(m-1)}{SS_y/(n-1)} = \frac{MS_x}{MS_y}$$

Newman et al., 1997 show that $\hat{F}$ follows an F distribution only if the variance of X is the same as the variance of Y. The expected mean squares of fixed effects are partitioned into the variance due to the factor and the variance due to everything else in the experiment. For ANOVAs the denominator of the F ratio is the expected mean square of the error term, i.e. the variance due to everything else but the controlled factor. For the pooled factor model two F ratios must be calculated:

$$\frac{E[MS_P]}{E[MS_\varepsilon]} = \frac{(variance\ due\ to\ the\ treatment) + (variance\ due\ to\ everything\ else)}{(variance\ due\ to\ everything\ else)}$$

$$\frac{E[MS_T]}{E[MS_\varepsilon]} = \frac{(variance\ due\ to\ incubation\ time) + (variance\ due\ to\ everything\ else)}{(variance\ due\ to\ everything\ else)}$$

where

$E[MS_P]$ = expected mean squared difference between each P marginal mean and the overall mean,

$E[MS_T]$ = expected mean squared difference between each T marginal mean and the overall mean,

$E[MS_\varepsilon]$ = expected residual mean square

The F ratio in an ANOVA model therefore tests that the variance due to the fixed effect is zero (hence the name Analysis of Variance). If for example the variance due to the pooled treatment factor is zero, then $E[MS_P] = E[MS_\varepsilon]$. Then $\hat{F} = 1$ and $H_0(1)$ cannot be rejected. It is important to note that the variance due to everything else is the same on both sides of the fraction line. Otherwise, the F statistic would not allow to draw conclusions for the fixed effect.

It is a reasonable assumption that the factors used in the model are not independent of each other but rather that interaction exists, i.e. the presence of a particular level of one factor influences the effect that other factors have on y (Zar, 1999). A multi-factor model therefore often asks for interaction terms. For the pooled factor model, an interaction term for the fixed effects is introduced expanding Eq. (1) to the non-additive linear model:

$$y_{ijk} = \mu + \pi_i + \tau_j + (\pi\tau)_{ij} + \gamma_k + \varepsilon_{ijk} \tag{2}$$

The model of Eq. (2) tests for another hypothesis:

3. H_0: Differences in mean y among the levels of treatment are the same for all incubation durations and vice versa

However, there is another factor for which interactions may be present: γ_k. Two more interaction terms are included:

$$y_{ijk} = \mu + \pi_i + \tau_j + (\pi\tau)_{ij} + \gamma_k + (\pi\gamma)_{ik} + (\pi\tau\gamma)_{ijk} + \varepsilon_{ijk} \tag{3}$$

Interaction terms between fixed and random are considered random effects which are independent and identically distributed around zero (Quinn and Keough, 2002). Now that interaction terms between the fixed effects and the random effects are included the model uses interaction mean square in the hypothesis tests to calculate the F statistics for the fixed effects. As stated above, the introduction of random effects adds new variance terms to the model. If the experiment includes interaction between the fixed effect and the random effects the expected mean square of the pooled factor would change to

$E[MS_P]$ = (variance due to factor P) + (variance due to interaction of P and C) + (variance due to everything else).

Hence, the residual mean square (i.e., variance of everything else) in the denominator of the F ratio does not isolate the variance component of interest anymore. Conveniently, the expected mean square of the interaction term between the fixed effect and random effects is defined as

$E[MS_{PC}]$ = (variance due to interaction of P and C) + (variance due to everything else)

Eq. (3) makes $E[MS_{PC}]$ the proper error term for the F statistic on the fixed effects. Further, using the mean square error in the F statistic despite the presence of random interactions would lead to unjustifiable high degrees of freedom in the denominator, as shown by (Janky, 2000) amongst others. This is because samples from the same block lack independence, i.e. they are pseudo-replicates (Newman et al., 1997). This dependence also reasons to not include a separate interaction term for T x C as it would overestimate the number of replicates per level of incubation time. Instead, $E[MS_{PTC}]$ is now used as denominator in the F statistic for T.

The nested model

The models above validly display the different treatments used in the study. However, it compresses the hierarchical structure of the study design. Another way of modeling the experiment is to use a hierarchical mixed model in which factors are nested within others. This approach changes the fixed effects of the model. Factor T stays but instead of the pooled factor P the model now includes the material factor M (levels are “none”, “reference”, “plastic”), the size factor S (levels are “small” and “big”) and the leaching factor L (levels are “unleached” and “leached”). The nested model for this study is expressed by Eq. (4):

$$y_{ipqjk} = \mu + \alpha_i + \beta_{p(i)} + \delta_{q(i)} + \tau_j + \gamma_k + \varepsilon_{ipqjk} \tag{4}$$

where

y_{ipqjk} = the observation within the kth level of factor C within jth level of factor T within the qth level of factor L pth level of factor S both nested in the ith level of factor M

μ = the overall mean

α_i = the effect due to the ith level of the material factor M and i = 1, 2, 3

$\beta_{p(i)}$ = the effect due to the pth level of the size factor S nested within the ith level of factor M and p = 0, 1

$\delta_{q(i)}$ = the effect due to the qth level of the leaching factor L nested within the ith level of factor M and q = 0, 1

τ_j = the effect due to the jth level of the time factor T for incubation days and j =1, 2,…,t

γ_k = the effect due to the kth level of the chain factor C and k = 1, 2, …, c

ε_{ipqjk} = the residual error of observation y_{ipqjk}

The subscript p(i) and q(i) for β and δ in Eq. (4) emphasizes that the factors size and leaching are nested in the factor for material. This means for an observation to be assigned to a level of size or leaching, it must be assigned to a level of material (Grace-Martin, n.d.). Following the discussion about interaction term above the model in Eq. (4) is expanded to

$$\begin{aligned} y_{ipqjk} = \mu + \alpha_i + \beta_{p(i)} + \partial_{q(i)} + (\beta\partial)_{pq(i)} + \tau_j + (\alpha\tau)_{ij} + (\beta\tau)_{pj(i)} + (\partial\tau)_{qj(i)} \\ + (\beta\partial\tau)_{pqj(i)} + \gamma_k + \varepsilon_{ipqjk} \end{aligned} \tag{5}$$

and further to

$$\begin{aligned} y_{ipqjk} = \mu + \alpha_i + \beta_{p(i)} + \partial_{q(i)} + (\beta\partial)_{pq(i)} + \tau_j + (\alpha\tau)_{ij} + (\beta\tau)_{pj(i)} + (\partial\tau)_{qj(i)} \\ + (\beta\partial\tau)_{pqj(i)} + \gamma_k + (\beta\partial\gamma)_{pqk(i)} + (\beta\partial\tau\gamma)_{pqjk(i)} + \varepsilon_{ipqjk} \end{aligned} \tag{6}$$

Eq. (6) is the full model specification used in this study for all response variables. With the inclusion of the random interaction terms in Eq (6) the model uses $(\beta\partial\gamma)_{pqk(i)}$ and $(\beta\partial\tau\gamma)_{pqjk(i)}$ as denominator for the F statistic. All results discussed in the main body of this study are generated based on Eq. (6). Unfortunately, the presence or absence of interactions between the fixed effect and the random effects can never be proven. Hence, inclusion of random interaction terms is a matter of underlying assumptions discussed lengthily in (Newman et al., 1997). To address this lack of proof model results of Eq. (5), where F statistics are calculated based on the model residuals, are provided in the following section.

2. Model results of Eq. 5

Ecosystem Respiration

Table: Nested, mixed model ANOVA analyses testing fixed effects of material (M), size (S), leaching (L) and incubation time (IT) on ecosystem respiration. The influence of chain (C) was modeled as a random effect. The error term is indicated in italic. Statistically significant effects are highlighted in bold.

		O2 consumption		
Source	df	dfDen	F ratio	Prob > F
C				
M	2	111	1.30	0.277
S[M]	1	111	0.12	0.726
L[M]	1	111	2.76	0.099
SxL[M]	1	111	0.63	0.429
IT	4	111	9.48	**<.001**
ITxM	8	111	1.05	0.401
ITxS[M]	4	111	0.52	0.723
ITxL[M]	4	111	0.54	0.705
ITxSxL[M]	4	111	0.19	0.941
Residual	111			

Leaf Mass Loss

Table: Nested, mixed model ANOVA analyses testing fixed effects of material (M), size (S), leaching (L) and incubation time (IT) on leaf mass loss and leaf decay rate. The influence of chain (C) was modeled as a random effect. The error term is indicated in italic. Statistically significant effects are highlighted in bold.

		Sqrt(%LML)			Log(-kLML)		
Source	df	dfDen	F ratio	Prob > F	dfDen	F ratio	Prob > F
C							
M	2	115	7.17	**0.001**	115	5.29	**0.006**
S[M]	1	115	6.35	**0.013**	115	5.69	**0.019**
L[M]	1	115	1.87	0.175	115	1.46	0.230
SxL[M]	1	115	0.55	0.459	115	0.44	0.507
IT	4	115	784.55	**<.001**	115	2613.81	**<.001**
ITxM	8	115	1.98	0.055	115	1.54	0.152
ITxS[M]	4	115	2.79	**0.030**	115	2.86	**0.027**
ITxL[M]	4	115	0.18	0.947	115	0.12	0.977
ITxSxL[M]	4	115	0.53	0.714	115	0.44	0.778
Residual	115				115		

Tensile Strength Loss

Table: Nested, mixed model ANOVA analyses testing fixed effects of material (M), size (S), leaching (L) and incubation time (IT) on tensile strength loss and cotton bag decay rate. The influence of chain (C) was modeled as a random effect. The error term is indicated in italic. Statistically significant effects are highlighted in bold.

		Sqrt(%TSL)			-k(TSL)		
Source	df	dfDen	F ratio	Prob > F	dfDen	F ratio	Prob > F
C							
M	2	114	4.21	**0.017**	114	3.00	0.054
S[M]	1	114	1.29	0.259	114	3.78	0.054
L[M]	1	114	4.82	**0.030**	114	5.89	**0.017**
SxL[M]	1	114	8.97	**0.003**	114	23.22	**<.001**
IT	4	114	73.29	**<.001**	114	6.91	**<.001**
ITxM	8	114	2.50	**0.015**	114	1.76	0.093
ITxS[M]	4	114	0.91	0.458	114	5.78	**<.001**
ITxL[M]	4	114	3.03	**0.020**	114	7.45	**<.001**
ITxSxL[M]	4	114	8.38	**<.001**	114	17.71	**<.001**
Residual	114				114		

3. Figures of k_{LML}

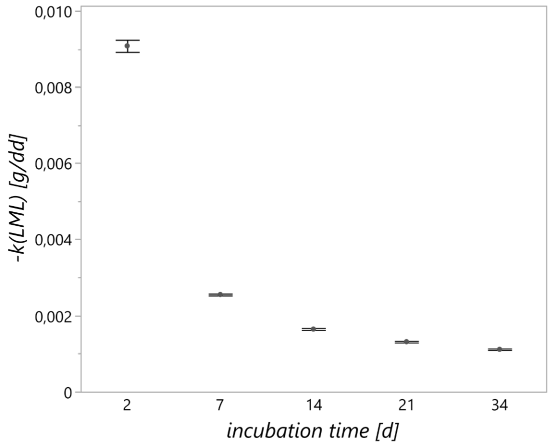

Figure: Effects of incubation time on k (mean ± SE plotted).

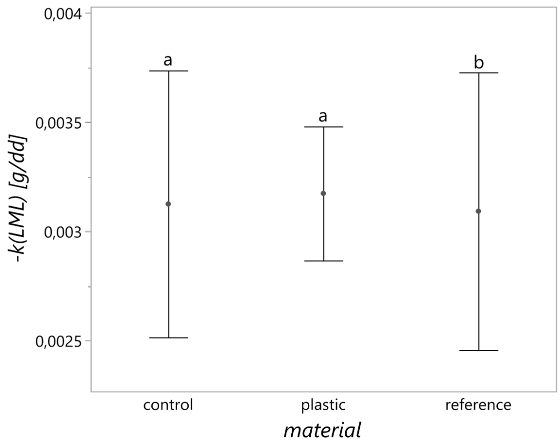

Figure: Effects of material on k_{LML} (mean ± SE plotted). Lettering above the bars indicate homogenous subsets from post-hoc Tukey's tests. Note that the y-axis does not start at 0.

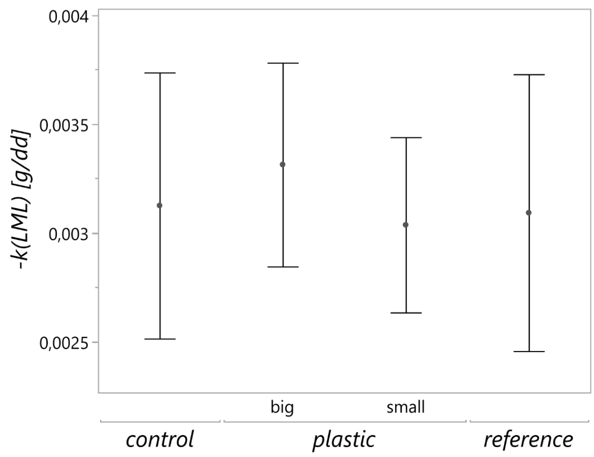

Figure: Effects of size nested within material on k_{LML} (mean ± SE plotted). Note that the y-axis does not start at 0.

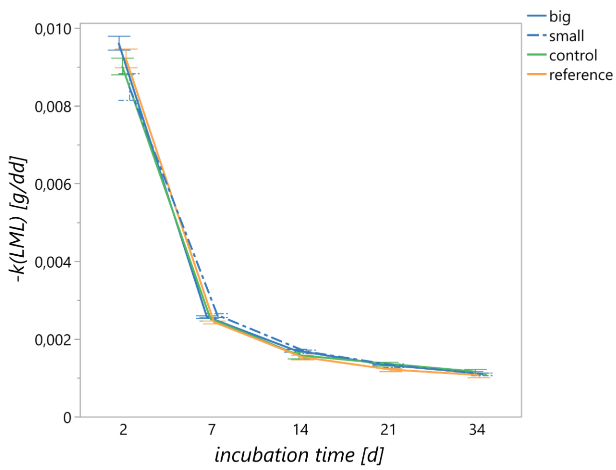

Figure: Effect of the interaction between time and size nested within material on k_{LML} (mean ± SE plotted).

4. Figures of k_{TSL}

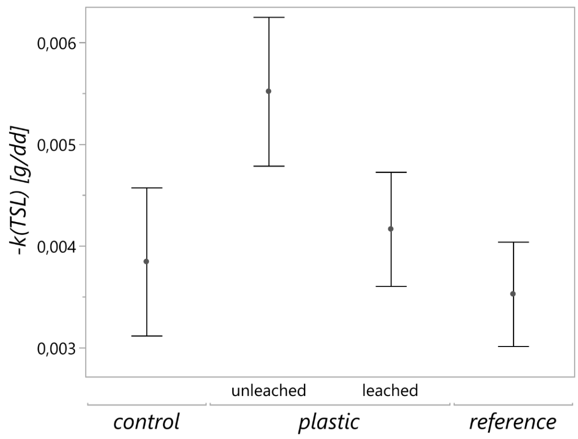

Figure: Effects of leaching nested within material on k_{TSL} (mean ± SE plotted). Note that the y-axis does not start at 0.

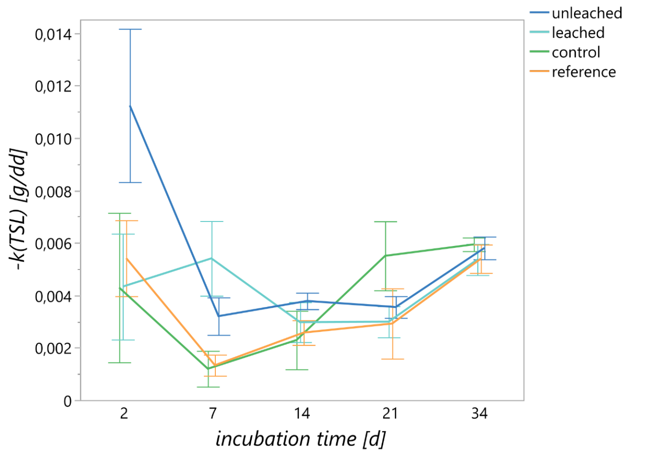

Figure: Effect of the interaction between leaching and time nested within material on k_{TSL} (mean ± SE plotted).

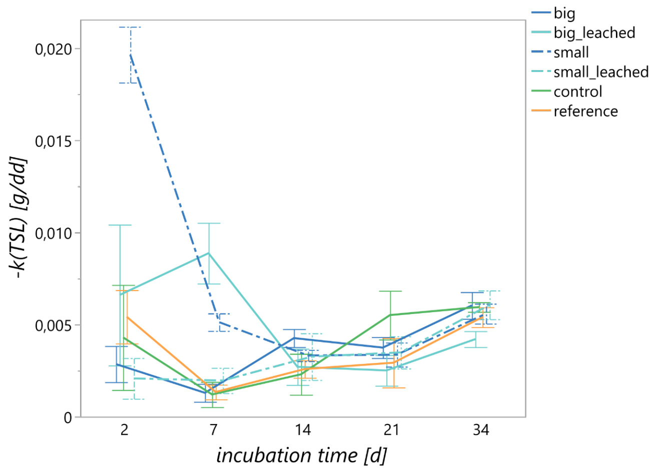

Figure: Effect of the interaction between size, leaching and time nested within material on k_{TSL} (mean ± SE plotted).