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Can terrestrial isopods (*Isopoda*: *Oniscidea*) make use of biodegradable plastics?

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ABSTRACT

Biodegradable plastics more and more replace conventional plastics, because they are considered environmentally friendly. Soil macro-invertebrates have been demonstrated to consume some of these biodegradable plastics, but studies usually do not go beyond notice of consumption and possible short-term ecotoxicological effects on organisms. This study uses the terrestrial isopod *Porcellio scaber* as a soil detritivore model and three biodegradable plastics (starch-, cellulose- and poly(3-hydroxybutyrate) (PHB)-based films) to evaluate both the contribution of isopods to the disintegration of biodegradable plastics and the effects of plastic-feeding on isopod ecology. Consumption rate of starch-based plastic was similar to that of leaf litter (mainly beech) and on average higher than those of the other two plastic types. Digestibility, however, was highest for cellulose-based plastic. HPLC results show that isopods break down starch-based plastic into maltose and glucose, and cellulose-based plastic into cellobiose. No glucose was present in feces of isopods having fed cellulose-based plastic, either for inability of breaking down cellobiose into glucose, or due to a rapid uptake of the glucose by isopods. Growth rates were negative, but not significantly different from zero, for all food sources; cellulose-based plastic caused the highest biomass loss to isopods. Toughness of starch-based plastic diminished over time when litter and/or isopods were present. Cellulose-based plastic increased in toughness over the disintegration experiment, possibly affecting its consumption by isopods. Overall, isopods increased the disintegration rates of starch- and cellulose-based plastics, but no PHB film was consumed, and its disintegration rate was low. We conclude that starch-based plastic is comparable to a natural low-quality food source (e.g., beech litter), and isopods would probably consume starch- and cellulose-based plastics in the field.

1. Introduction

1.1. Plastics and fauna

Plastic litter is hazardous to a variety of living creatures (Scott, 2000; Stevens, 2002). Large plastic items have been shown to cause suffocation or entanglement, or disrupt digestion in birds, fishes, turtles, mammals and invertebrates (Browne et al., 2008; Derraik, 2002; Laist, 1987, 1997; Murray and Cowie, 2011; Quayle, 1992; Ryan, 1988). Over 180 species are known to ingest plastic fragments (Derraik, 2002), and some animals even feed selectively on plastic particles with specific shapes and colors mistaking them for potential prey items (Moser and Lee, 1992).

The potential ingestion of plastics by animals increases with increasing fragmentation of plastic debris. For instance, fragments smaller than 1 mm (microplastic fragments) have been accumulating for several years in the marine water column and can now represent up to 85% of the stranded plastic debris in some places ashore (Browne et al., 2007; Hildago-Ruz and Thiel, 2013). These microplastics are ingested by animals and their effects on marine invertebrates have received increasing attention over the last decade (Browne et al., 2008; Teuten et al., 2007). Once ingested, they might be retained in the digestive track, egested through feces or transferred through the epithelial lining of the gut into body tissue (translocated), and long-term toxicological effects cannot be excluded (Browne et al., 2008).

1.2. Biodegradable plastics

The substitution of conventional plastics by biodegradable ones would reduce environmental problems (Gross and Kaira, 2002; Ren,

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2003; Rosa et al., 2004a). These polymers undergo biodegradation, that is, chemical degradation through the action of naturally occurring microorganisms (Stevens, 2002). Although microorganisms are key for chemical decay, macroorganisms can also eat and even digest biodegradable polymers contributing to their mechanical, chemical or enzymatic ageing upon decomposition (Albertsson and Karlsson, 1994).

A readily degradable plastic retains all properties expected by its design during its lifetime, but rapidly disintegrates and is assimilated by microorganisms returning it to the ecosystem in a harmless manner (Stevens, 2002).

Unlike conventional plastics that are based on non-renewable sources, biodegradable plastics are made from naturally occurring polymers, such as starch, cellulose or bacterial energy stores, and therefore can (1) be degraded by naturally occurring organisms (see above), and (2) be considered sustainable.

Starch is a renewable and widely available material that can be converted to thermoplastic using a swelling or plasticizing agent. Blends and composite materials can be produced by processing starch with biodegradable polymers (Rudnik, 2008). Such a thermoplastic can undergo up to 97.5% biodegradation after 44 days in controlled composting conditions (Degli-Innocenti et al., 1998).

Cellulose thermoplastic materials can be produced through esterification of cellulose. The raw material can be derived from cotton, recycled paper or many other sources (Rudnik, 2008), and cellulose-based plastics are being used for various commercial applications such as food packaging. In controlled composting conditions, up to 96.8% biodegradation of cellulose film was observed after 47 days (Degli-Innocenti et al., 1998).

Polyhydroxyalcanoates (PHAs) form a group of biodegradable thermoplastics that are extracted from the intracellular carbon energy store that is accumulated as granules within cells by a wide variety of bacteria, being the poly(3-hydroxybutyrate) (PHB) one of the most studied plastics from this group (Bucci et al., 2005; Maehara et al., 2001; Wang et al., 2005). PHB has been used in small disposable products and packaging materials (Rosa et al., 2004b). Tansengco and Dogma (1999) observed up to 91% degradation of PHB films in burial after four weeks, and Krupp and Jewell (1992) demonstrated complete degradation within 60 days at 37 °C under laboratory conditions.

1.3. Biodegradable plastics and the environment

In large scale, the degradation of biodegradable plastics involves composting sites and landfills, places in which the activity and efficiency of microorganisms is affected by interactions with other organisms. Several studies have tested the microbial degradation of biodegradable plastics in sludge, soil or composting conditions (Kim et al., 2000; Rosa et al., 2004a; Körner et al., 2005; Wang et al., 2005).

Some biodegradable plastics are developed for agricultural purposes, undergoing degradation in the same environment and becoming available to a variety of soil invertebrates. The use of biodegradable materials is thought to be already an improvement in relation to petroleum-based plastics. However, their effects on the environment and wildlife are not yet known, risking the substitution of one problem for another.

1.4. Biodegradable plastics and soil fauna

Invertebrates have been recorded to consume some of these polymers that present enhancing agents (such as starch) (Anderson et al., 1995; Fritz et al., 2003; Rudnik, 2008; Tsao et al., 1993; Wool, 1988), but these studies rarely go beyond notice of consumption.

Studies using macroinvertebrates that consume biodegradable plastics mainly focus on possible toxicological effects of one type of biodegradable plastic, while their ecological effects on the fauna are usually not approached. Fritz et al. (2003) approached further potential toxic effects by applying ecotoxicological tests with biodegradable plastics using earthworms, the water flea *Daphnia* and some plant seeds and found no inhibition in growth of earthworms as well as possible benefits from the consumption and digestion of the undegraded organic matter. Similar results have been also reported by Rudnik (2008). Nonetheless, earthworms cannot actively tear plastics into pieces like soil arthropods.

Terrestrial isopods, or woodlice, have been shown to ingest certain biodegradable plastics under artificial laboratory conditions (Anderson et al., 1995; Fritz et al., 2003; Rudnik, 2008; Tsao et al., 1993; Wool, 1988) and they fragment their food sources prior to ingestion. As terrestrial isopods play an important role in decomposition processes in many ecosystems (Hassall et al., 1987; Kautz and Topp, 2000; Sousa et al., 1998; Zimmer and Topp, 1999) and are among the most abundant groups of macro-decomposers in many communities (Hassall and Dangerfield, 1990; Zimmer, 2002), they are appropriate model organisms to understand the effects of biodegradable plastic consumption by the soil fauna.

This study uses the well-studied woodlouse species *Porcellio scaber* Latreille 1804 (Crustacea: *Isopoda*: *Oniscidea*) as a soil detritivore model and three biodegradable plastics (starch-, cellulose- and PHB-based) to answer the following questions: (1) do terrestrial isopods have the potential to promote disintegration rate of biodegradable plastics in the soil; (2) how does the consumption of different biodegradable plastics affect isopod's feeding and growth rates; and (3) would isopods feed on biodegradable plastics if another food source was available? We hypothesize that woodlice feeding on biodegradable plastics would increase the disintegration rate of plastics from materials that occur in their natural food source (starch and cellulose). We also hypothesize that biodegradable plastic consumption when another food source is available would indicate that animals might partially digest it. In addition, this would suggest that isopods can feed on them in natural conditions.

2. Material and methods

2.1. Isopods and biodegradable plastics

Specimens of *P. scaber* were collected in home compost in Salzburg-Nonntal, Thumegger Bezirk (13.05°E, 47.79°N), and kept under laboratory conditions for three to four weeks prior to experiments. Ovigerous females were excluded in the disintegration and feeding rates experiments.

Three different biodegradable plastics were used in the experiments: starch-, cellulose- and PHB-based. The starch-based plastic was bought commercially as doggy bags (BioBag Dog, made in Norway) and made from Mater-Bi granules (Novamont, Italy). The cellulose-based films were kindly supplied by Innovia (England) (Nature Flex NE30, suitable use for food packaging). PHB powder was kindly supplied by Biomer (Germany) and transformed into films in the laboratory by pouring 3 mL of 3% chloroform solution in a Petri dish (7 cm diameter) over a hotplate at 120 °C in a hood, until chloroform had evaporated completely. All films were cut into squares of 4 cm^2 for the experiments.

2.2. Biodegradable plastic disintegration (mass loss)

Starch-, cellulose- and PHB-based plastics were used as treatments, along with conventional plastic films with comparable presentation to the three biodegradable plastics (color and hand-felt texture). Experimental units containing ca 10 g of soil, one 4 cm^2 piece of plastic and two *P. scaber* were kept in a temperature-controlled chamber at 15 °C and 12:12 h photoperiod. The soil was taken from abandoned agricultural grassland with pH of 5.5 ± 0.2, water holding capacity of 0.51 ± 0.03 g of water per g soil (dry mass) and organic matter content of 17 ± 4%. Water was sprinkled every two days to keep soil moist but with no accumulation of water droplets in units. Animal-free controls consisted of units with soil and plastic (N = 7 for both treatments and controls).

Units were maintained for 14 days or until only 10–20% of the plastic remained visible. After the experiment, soil was thoroughly searched for plastic remains, and water was added to retrieve lost plastic that could not be collected during visual search. Plastics were washed, air-dried and reweighed for plastic mass loss calculation in mg per day.

Biodegradable plastic mass loss without and with isopods was compared by one-sided t-test (normal distribution) or Mann–Whitney (non normal distribution) for each plastic type in order to access the effect of isopods on plastic disintegration.

2.3. Feeding and growth rates of isopods on biodegradable plastics

Experimental units (N = 11 for each treatment and corresponding isopod-free controls) containing a bottom of plaster of Paris (to keep humidity), one 4 cm^2 piece of the biodegradable plastic (or leaf litter (*Fagus sylvatica*) fragment of approximate size) and one *P. scaber* were kept at 15 °C and 12:12 h photoperiod for 15 days. After the experiment, isopods were kept for another day with a piece of carrot as food source in order to make them egest all consumed biodegradable plastic (Wood et al., 2012). Feeding and growth rates from feeding on biodegradable plastic were compared to mixed leaf litter (dominated by beech, *F. sylvatica* L.) collected at Kapuzinerberg in Salzburg, Austria (oven-dried at 40 °C for 48 h before being offered to the isopods). We used a low-quality natural food source in order not to underestimate consumption of plastics in comparison to a high-quality leaf litter. Isopod weight was recorded every three days, and linear regression was used to calculate initial and final mass (to account for weight fluctuation due to change in body water content). Three isopods of each treatment were sacrificed and oven-dried overnight at 60 °C after the experiment in order to correct wet weight to dry weight. Fecal pellets were collected every two days, kept frozen during experiment, and oven-dried overnight at 60 °C at the end of the experiment. Consumption of plaster of Paris happened on occasion and feces were visually distinguished and eliminated from units. Biodegradable plastic remains were washed and air-dried (and leaf remains oven-dried) and reweighed.

Relative consumption rate (RCR), approximate digestibility (AD) and relative growth rate (RGR) were calculated as proposed by Waldbauer (1968). Data are presented as RCR, AD and RGR, but Analysis of Covariance (ANCOVA) was used to analyze feeding and growth data, as suggested by Raubenheimer and Simpson (1992) provided that statistical problems may occur from the analysis of indices that are obtained as ratios. Consumption was analyzed through the amount of ingested food, using the duration of the experiment and initial isopod mass as covariates. Digestibility was analyzed through the mass of egested feces, using the ingested amount, the duration of the experiment and initial isopod mass as covariates. Growth was analyzed through final isopod mass, using the duration of the experiment and initial isopod mass as covariates.

2.4. Quasi-natural conditions: Simulation of biodegradable plastic in the soil

Abundant soil (enough to cover the base of the experimental units, ca 350 g) and mixed leaf litter (same type from experiment above; ca 4 g) were placed in 20 × 20 cm^2 experimental units containing ten (starch- and cellulose-based) or six (PHB film) 4 cm^2 pieces of biodegradable plastic, and ten *P. scaber* (N = 5 for each treatment and the corresponding controls). After 28 days at 15 °C and 12:12 h photoperiod, plastic was retrieved, washed and reweighed and isopods counted. Plastic mass loss was calculated for each treatment and compared as described in the disintegration experiment.

Consumption rates of plastics could be calculated for all experiments and were also compared for each plastic type using ANCOVA as described in the feeding rates experiment.

Table 1
Biodegradable plastic mass loss (mg per day) in units with one piece of starch- cellulose- or poly(3-hydroxybutyrate) (PHB)-based plastic, soil and two *Porcellio scaber*, and in control units without isopods for 4–14 days.

Plastic type	Without isopods (control)	With isopods
Starch-based	0.06 ± 0.01 (0.5 ± 0.1%)*	1.4 ± 0.4 (11 ± 4%)*
Cellulose-based	0.16 ± 0.01 (0.99 ± 0.05%)	0.17 ± 0.01 (1.03 ± 0.09%)
PHB-based	0.01 ± 0.01 (0.05 ± 0.03%)	0.010 ± 0.003 (0.05 ± 0.01%)

Numbers are expressed in mean ± SE in mg per day and percentage per day in parenthesis and * indicates significant difference (p < 0.05).

2.5. Biodegradable plastic toughness

Biodegradable plastic toughness was measured with a penetrometer (Graça and Zimmer, 2005; Williams, 1954) using the weight necessary to break through the plastic with a steel stick of 5 mm in diameter. Pieces of starch- and cellulose-based plastics from all experiments were measured, as well as the initial condition of the plastics, in order to access indirect effect of isopods and time on biodegradable plastic disintegration.

2.6. High-performance liquid chromatography (HPLC)

Feces from starch- and cellulose-based plastic consumption were prepared for high-performance liquid chromatography analysis (HPLC) by adding 100 μL of distilled water and shaking at 80 °C for 2 h. Feces collected during the feeding and growth rates experiment were used for starch-based plastic consumption characterization (n = 7), while isopods from laboratory culture were fed on cellulose-based plastics in order to collect enough material for characterizations of feces from cellulose-based plastic consumption (n = 4).

Chromatograms were obtained using the ICS300 (Dionex), with an AS50 autosampler, ED50 electrochemical detector, using quadruple wave for detecting carbohydrates, and a PA20 column (150 × 3 mm with a PA20 precolumn).

3. Results

3.1. Biodegradable plastic disintegration (mass loss)

The highest plastic mass loss per day in isopod-free control units was observed for cellulose-based plastic, while starch-based plastic lost mass at about half this rates. PHB-based plastics had negligible mass loss under the same conditions.

When isopods were present the highest mass loss rates were observed for starch-based plastics. This loss was significantly higher than in the controls (t_6 = 2.912; p = 0.0135). Cellulose-based plastic mass loss was not significantly different from controls (t_{12} = 1.006; p = 0.1672), although there were visual signs of consumption by isopods. PHB films showed no sign of consumption by isopods or difference in mass loss from control units (U' = 28.500;

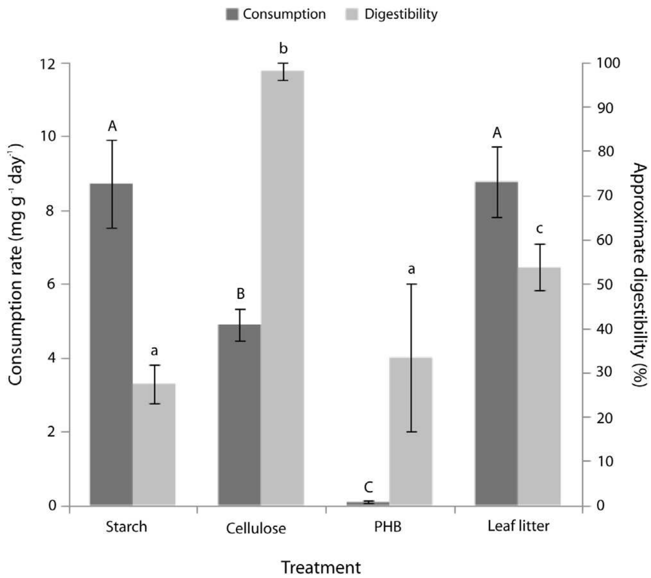

Fig. 1. Consumption rate (left) and approximate digestibility (right) of biodegradable plastics or mixed leaf litter by *Porcellio scaber* after 15 days. Data are mean ± SE; letters indicate significant difference of both parameters among treatments.

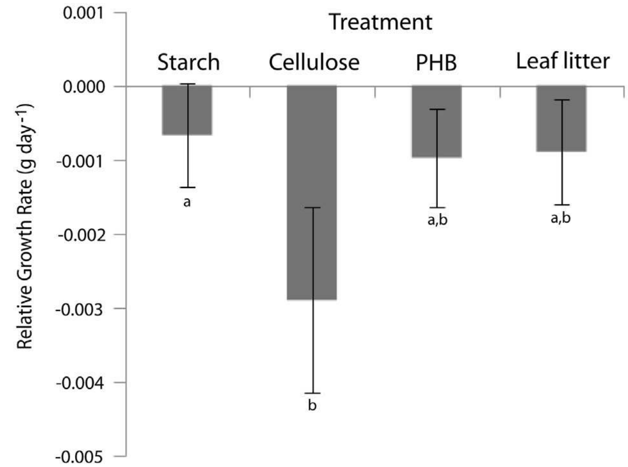

Fig. 2. Relative growth rates (dry weight) of *Porcellio scaber* feeding on biodegradable plastics or mixed leaf litter for 15 days. Data are mean ± SE; letters indicate significant difference among treatments.

$p = 0.318$) (Table 1). The conventional plastic films had no signs of consumption by isopods in any presentation.

3.2. Feeding and growth rates

Consumption rates were significantly higher when isopods fed on leaf litter or starch-based plastic ($F_{3,34} = 19.77$; $p < 0.001$) than on cellulose- or PHB-based plastics. Approximate digestibility was significantly different between treatments ($F_{3,33} = 39.29$; $p < 0.001$), being highest for cellulose-based plastic followed by leaf litter, and with no significant difference between PHB- and starch-based plastics (Fig. 1).

Although all growth rates were negative, they were not significantly different from zero in any treatment (starch: $t_{10} = 1.05$; $p = 0.318$; cellulose: $t_8 = 2.12$; $p = 0.066$; PHB: $t_8 = 1.43$; $p = 0.19$; leaf litter: $t_9 = 1.51$; $p = 0.166$). The highest isopod mass loss occurred when isopods fed on cellulose, followed by leaf litter, PHB and starch. There was no significant difference ($F_{3,33} = 1.888$; $p = 0.151$), although pairwise comparison showed significant difference between starch- and cellulose-based treatment ($p = 0.024$) (Fig. 2).

3.3. Quasi-natural conditions

Isopods consumed starch- and cellulose-based plastic even when (low quality) litter was available. Both starch-based ($U' = 25.000$; $p = 0.0047$) and cellulose-based plastics ($t_4 = 2.501$; $p = 0.0333$) lost significantly more mass when isopods were present; PHB plastics did not (Table 2).

Consumption rates on starch- and cellulose-based plastics were significantly higher than that from PHB based plastic ($F_{2,11} = 22.580$; $p < 0.001$) (Table 3). Survivorship was high for all treatments (86%, 84% and 76% for cellulose-, PHB- and stach-based plastics, respectively).

3.4. Biodegradable plastic toughness

Toughness of starch-based plastics was higher at initial condition and lower at the end of the quasi-natural experiment. Toughness of plastic from units with isopods was slightly lower than from controls in all experiments, and toughness of plastics from the control of feeding rate experiment was similar to initial toughness of the plastic (Fig. 3).

Toughness of cellulose-based plastics was higher after the disintegration experiment and lower after the quasi-natural experiment. Isopods also influenced toughness of the plastic compared to control units in all experiments (Fig. 4). No statistical analysis was used due to insufficient number of remaining plastic pieces at the end of the experiments.

3.5. High-performance liquid chromatography (HPLC)

Based on peaks of glucose, maltose and maltodextrines standard solution, degradation products (mono- and oligo-saccharides) were identified in the feces of isopods fed on starch-based plastic. In the feces of isopods fed on cellulose-based plastic, cellobiose was identified but no glucose was detected (Fig. 5).

The concentration of glucose and maltose in the feces of animals fed starch-based plastic was 31.3 ± 13.0 and $1.4 \pm 0.5\ \mu M\ mg^{-1}$, respectively. The concentration of cellobiose in the feces of animals fed cellulose-based plastics was $2.4 \pm 0.3\ \mu M\ mg^{-1}$.

4. Discussion

In this study, isopods consumed two types of biodegradable plastics and had an effect on the plastics disintegration rate; their

Table 2
Plastic mass loss in quasi-natural conditions for 28 days with enough soil to cover the base of the experimental units, leaf litter, 10 pieces of biodegradable plastic and 10 *Porcellio scaber* (~300 mg, total mass of isopods) per unit.

Plastic type	Without isopods	With isopods
Starch-based	0.13 ± 0.01 (0.11 ± 0.01%)**	1.7 ± 0.2 (1.5 ± 0.2%)**
Cellulose-based	1.16 ± 0.05 (0.66 ± 0.03%)*	1.6 ± 0.2 (0.9 ± 0.1%)*
Poly(3-hydroxybutyrate)-based	0.008 ± 0.004 (0.007 ± 0.004%)	0.005 ± 0.002 (0.005 ± 0.002%)

Data is expressed as mean ± SE in mg per day and in percentage of plastic mass loss in parenthesis. Significant difference is expressed by * $p < 0.05$) or ** $p < 0.01$.

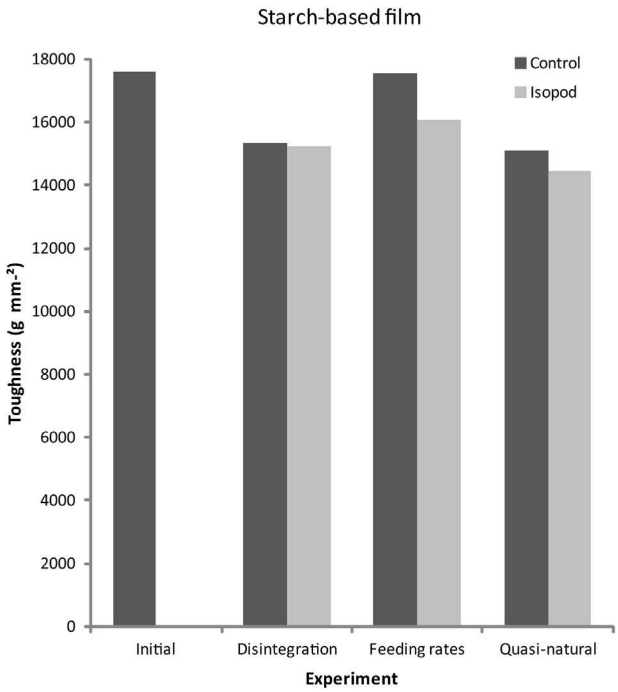

Fig. 3. Starch-based film toughness (g mm^{-2}) in animal-free control units and units with *Porcellio scaber*, from the different experiments conducted in this study and plastic on its initial condition. Data is represented as mean; SE is not shown since it could not be calculated due to low number of remaining plastic pieces in some experiments.

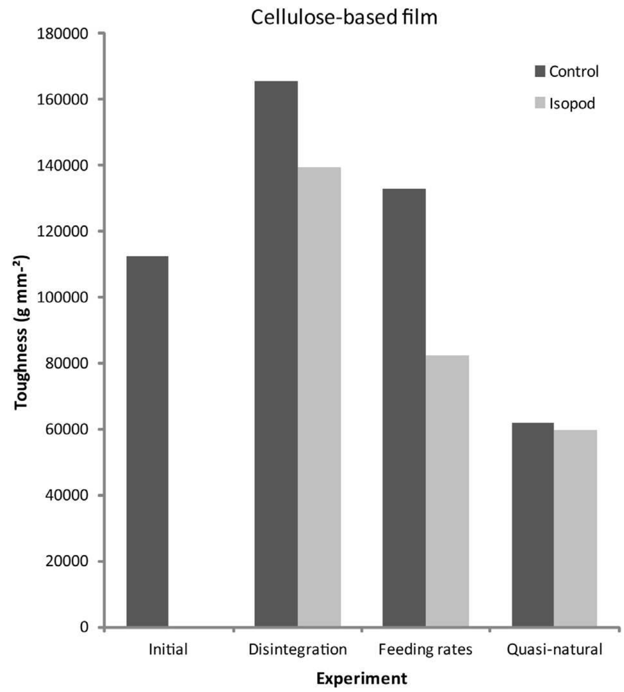

Fig. 4. Cellulose-based film toughness (g mm^{-2}) in animal-free control units and units with *Porcellio scaber* from the different experiments conducted in this study and plastic on its initial condition. Data is represented as mean; SE is not shown since it could not be calculated due to low number of remaining plastic pieces in some experiments.

consumption had different effects on the animals. We will discuss in turn the effects of the isopods on the plastic disintegration and the effects of plastic consumption to the animals.

4.1. Effects of terrestrial isopods on biodegradable plastic disintegration

Physical disintegration is an essential part of the biodegradation process and the starch-based plastic presented increased disintegration rates upon isopod activity. The direct consumption of starch-based plastics even when litter was available indicates that isopods have a potential to increase disintegration rate of starch-based plastics if disposed in environments where animals are present. Wool (1988) and Tsao et al. (1993) have also shown the involvement of certain soil macroinvertebrates such as cockroaches and crickets in the primary biodegradation of starch-enhanced polyethylene (PE) films. However, they have only visually evaluated consumption in categories (no consumption, less than 50%, or greater than 50%) and the effect of animals in this process was not quantified.

Cellulose-based plastic consumption could also be noticed in all conditions, although in smaller proportion than that of starch-based plastic. Provided that the toughness of the cellulose-based plastic had increased by the end of the disintegration experiment, isopods did not affect the disintegration rate of this plastic in plastic-only treatment. This might have been due to interactions with the water from the soil, since cellulose fibers tend to form a network upon wetting that can entrap water making it thicker and less flexible (Lee and Fan, 1982). This increase in toughness might have hindered cellulose plastic consumption by isopods in the disintegration experiment. Such increase in toughness did not occur during the experiment with cellulose based-plastic and leaf litter, and isopods could consume the plastic, contributing to an increase in disintegration rate of plastic films. This suggests that isopods can also speed up the disintegration of cellulose-based plastic, depending on presentation attributes such as toughness.

The effect of the presence of litter on the increased disintegration rate of the plastics can be attributed to its higher microbial activity in relation to other soil layers as previously shown in the literature (Andersson et al., 2004). Alternatively, plastic pieces may have been attached to the leaf litter and were then simply co-consumed along with the latter (authors' pers. obs.: see below).

The lack of consumption by isopods and low disintegration rates of PHB films in the experiments might have been due to the preparation method that requires dissolution of PHB powder in chloroform, and perhaps PHB films retained traces of the chemical that could be avoided by isopods. We might have obtained different results, had we used commercially available PHB films.

Table 3
Consumption rate of *Porcellio sacber* on starch-, cellulose- or poly(3-hydroxybutyrate) (PHB)-based plastic under different experimental conditions.

Experiment	Conditions	Consumption rate (mg g^{-1} day^{-1})		
		Starch	Cellulose	PHB
Feeding and growth rates (15 days)	Plastic and isopod	9 ± 1	4.9 ± 0.4	0.08 ± 0.04
Plastic disintegration (4–14 days)	Plastic, isopod and soil	12 ± 4	1.4 ± 0.2	0.11 ± 0.03
Quasi-natural conditions (28 days)	Plastic, isopod, soil and litter	4.8 ± 0.7	4.1 ± 0.5	0.02 ± 0.01

Data expressed as mean ± SE.

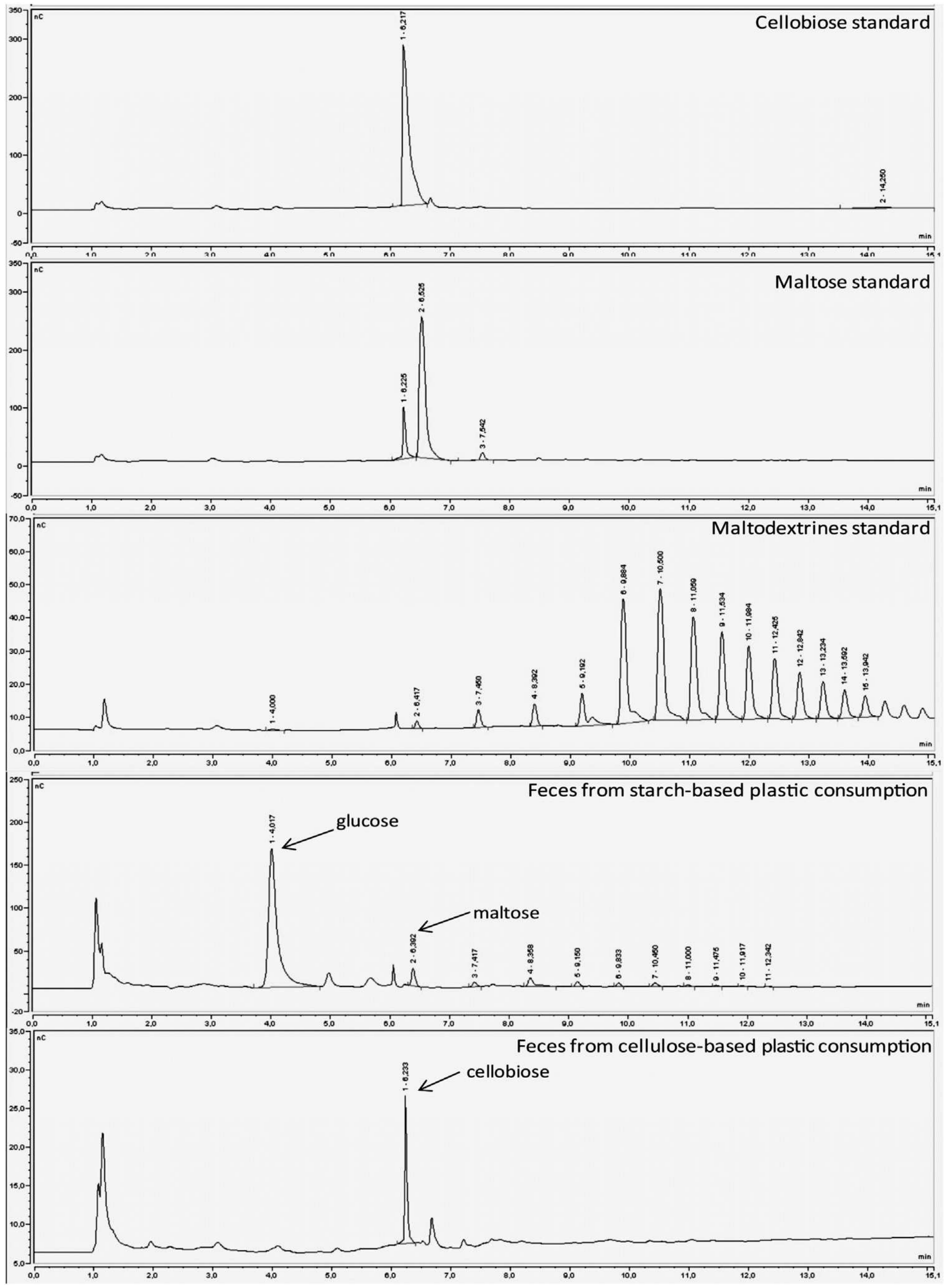

Fig. 5. Chromatograms from cellobiose, maltose and maltodextrine standards, and from the fecal pellets from consumption of starch- or cellulose-based plastics by the terrestrial isopod *Porcellio scaber*.

4.2. Effects of biodegradable plastic to terrestrial isopods

The isopod's consumption of and growth rates from starch-based plastic were comparable to those of animals fed leaf litter. Both the leaf litter used in this study and starch-based plastic can be considered low-quality food sources with low nitrogen content and animals were not expected to gain weight on a strict biodegradable plastic diet.

Although the digestibility of starch-based plastic was lower than that of leaf litter, growth rates were not significantly different. This is an indication that despite its low digestibility, isopods could utilize the digested portion (i.e., glucose) of the starch-based plastic. HPLC analysis indicated that isopods break down the starch into maltose and glucose. Obviously the amount of maltose and glucose digestively released from starch-based plastic exceeded the ability to absorb these nutrients from the food bolus inside the gut, and excess of maltose and glucose were egested. These smaller molecules egested in the feces should be rapidly used by microorganisms in the soil or by other coprophagous animals.

The cellulose-based plastic was highly digestible but had lowest consumption rate and caused the highest isopod mass loss (significantly different only from growth on starch-based plastic). HPLC analysis of feces from cellulose-based plastic consumption showed that cellulose was broken into cellobiose, but no glucose peak was observed. This lack of glucose in the feces might mean that animals do not have cellobiases to break the cellobiose into two molecules of glucose, or that glucose is taken up by isopods at the same rate as it is released from cellobiose. Breaking down a complex molecule like cellulose and not being able to break the molecule down to glucose would not be advantageous to isopods, meaning to have the cost of reducing cellulose to a smaller molecule, but not the benefit of utilizing the glucose to produce biomass. Therefore, it is likely that isopods rapidly use the available glucose and thus the glucose peak could not be detected. Furthermore, the conversion of cellubiose to glucose might be slower than the breakdown of maltose into glucose due to lower enzymatic activity for cellobiose substrates. Earthworms have been shown to have symbiotic bacteria that break down cellulose and then use endogenous cellobiases to break down the cellobiose, and this enzymatic activity was lower than when consuming maltose-based substrates (Lattaud et al., 1999). Thus, the process of reducing cellulose might be more time consuming, affecting the feeding rates on cellulose-based plastic and causing biomass loss to isopods.

The low consumption of PHB-based plastic was probably due to preparation (with addition of chloroform). However, weight loss not significantly higher than other plastics tested or leaf litter might be due to slow activity of animals in conditions where there is no adequate food source. Moreover, the lower consumption of starch plastic in units without soil in comparison to units with soil might also be related to activity since the animals were not provided with any option to shelter in our experiments.

Anderson et al. (1995) observed that radio-labeled starch-enhanced PE films consumed by terrestrial isopods appear to have been metabolized to some degree from the presence of degradation products of PE and radioactivity levels above background. However, since radioactive levels could have been found in the isopods due to the retention of PE/starch blends in the gut, it is debatable if the isopods could use these plastics to produce or maintain biomass. In this study, isopods were not able to gain biomass but maintained weight while feeding on low-quality food sources. As no single food source is sufficient for terrestrial isopods if eaten alone (Rushton and Hassall, 1983a), and growth rates of terrestrial isopods are greatly affected by the available food source (Rushton and Hassall, 1983b; Kautz et al., 2000), biodegradable plastics alone are not sufficient for positive growth. Although N was lacking on the diet, high survivorship indicated that animals did not obtain N from cannibalism. As isopods fed on starch and cellulose plastics even when litter was available, they may use these plastics as a complementary food source, at least if the available litter is low quality.

Under natural conditions, the water in the soil may cause the adherence between biodegradable plastic pieces and leaf litter (as it happened in the quasi-natural condition experiment: authors' pers. observation) raising the question whether isopods consumed the plastic "by mistake" while feeding on leaf litter. Nonetheless, in some cases, the only part of the leaf consumed was the part with plastic attached. Then, it is possible that the isopods selectively fed on that specific area that might represent an 'enhanced' leaf due to higher starch or cellulose content or possibly different local microbial community. More detailed studies on natural conditions would be necessary to clarify this issue

4.3. Further considerations

This study showed that terrestrial isopods have the potential to increase disintegration of starch- and cellulose-based plastics and that they can break down the plastic to smaller molecules. In cases where the amount of maltose and glucose digestively released from plastic exceeded the animal's ability to absorb these nutrients, the excess of maltose and glucose was egested. Isopods are known to contribute directly to leaf litter decomposition by consumption and indirectly by returning great amounts of the consumed litter as feces (Quadros and Araujo, 2008) that are rapidly colonized by microorganisms due to its increased surface (Hassall et al., 1987; Loureiro et al., 2006). Hence, these smaller molecules egested in the feces should be rapidly used by microorganisms in the soil or by other coprophagous animals producing an indirect effect on the plastic disintegration rate beside the direct consumption. Studying the degradation rate of these feces and if coprophagy of these feces occur would provide further evidence of the indirect effects of isopods on biodegradable plastic disintegration.

Isopod consumption of biodegradable plastics might change with increasing biodegradation stage and long-term ecological effects are not yet known. At later stages of biodegradation, a noticeable growth of microorganisms on the film surface is observed and some of the natural components from the plastic are still available (Bastioli et al., 1993). Later biodegradation stages are also associated with thinner and therefore less tough films. Therefore, contribution of animals is expected to change throughout the decomposition of the plastic as well as the nutritional value of the plastic to the animals. Studying the feeding and growth rates of plastic films that have decomposed for different time periods also would help to better understand these interactions of animals and plastics. More detailed studies on natural conditions would be necessary to determine whether similar results could be expected when high quality food sources were available to isopods and longer studies to see effects on reproduction would help understand long term effects to individuals and populations.

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