UTILIZING NATURAL COMPONENTS TO COMBAT ANTHROPOGENIC EFFECTS: BIODEGRADATION OF SINGLE-USE PLASTICS BY WHITE-ROT FUNGI

by

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ABSTRACT OF THE THESIS

Utilizing natural components to combat anthropogenic effects: biodegradation of single-use plastics by white-rot fungi

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Plastic pollution is a recognized global threat that must be resolved in order to preserve and conserve natural ecosystems. Fungi are prime candidates for being one of the many solutions to reducing plastic waste since they are primary decomposers. In particular, white-rot fungi possess ligninolytic enzymes, which break down complex lignin molecules. The present study demonstrates that selected white-rot fungi are able to biodegrade certain single-use plastics. The selected fungi are the following: *Pleurotus ostreatus*, *P. ostreatus columbinus*, *Lentinula edodes*, *Ganoderma lucidum*, and *Trametes versicolor*. The selected single-use plastics for this study are the following: high-density polyethylene, low-density polyethylene, polypropylene, polystyrene, and polyethylene terephthalate.

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1. INTRODUCTION

One of the most distinguishing features of the Anthropocene Epoch is plastic (Waters *et al.* 2016; Avio, Gorbi, & Regoli 2017; Geyer *et al.* 2017). The accumulation of plastic waste has become a major environmental concern due to its recalcitrant nature (Barratt *et al.* 2003; Kathiresan 2003; Gautam *et al.* 2007; Shah *et al.* 2008; Ibrahim *et al.* 2009; Russell *et al.* 2011; Loredo-Treviño *et al.* 2012; Bhardwaj *et al* .2013; Ghosh *et al.* 2013; Krueger *et al.* 2015; Álvarez-Barragán *et al.* 2016; Gajendiran *et al.* 2016). In 2014 alone, the US created 33.25 million tons of plastic waste with 3.17 million tons recycled, 4.98 million tons combusted (incinerated) with energy recovery, and 25.1 million tons landfilled (EPA 2016). The amount of plastic waste in landfills continues to build at an average of 24.65 million tons per year (EPA 2016). This is in addition to the 140.19 million tons of plastic waste that is already sitting in the landfills from 1960-2013 (EPA 2016). Due to its economic value, production of plastics continues to increase (Shah *et al.* 2008; Russell *et al.* 2011) without proper plastic waste management in place. As a result, plastic pollution continues to accumulate in landfills and natural environments globally. Thus, there is a need to resolve this predicament, and some solutions have been formulated (i.e. oxo-biodegradable plastics, recycling, and biodegradation). The most promising solution is utilizing natural decomposers, mainly fungi, to biodegrade plastics (Barratt *et al.* 2003; Tokiwa *et al.* 2009; Bhardwaj *et al.* 2013; Krueger *et al.* 2015).

Numerous studies have shown that some plastics are susceptible to biodegradation by ascomycetes. Russell *et al* (2011) experimentally demonstrated that the endophytic fungus, *Pestalotiopsis microsporia*, from the Amazon were capable of decomposing polyester polyurethane (PUR) in both aerobic and anaerobic conditions by secretion of a serine hydrolase. Several strains of *P. microsporia* were capable of degrading PUR but one in particular, E2712A, displayed the highest degradation in both conditions and was even able to use PUR as a sole carbon source. Russell *et al.* (2011) claim this is the first time that endophytic fungi have been described as PUR degraders and they are confident that more exist. *Aspergillus niger* and *Cladosporium herbarum* were also known to utilize PUR as a sole carbon source but the process was slow and only possible in aerobic conditions (Filip 1979; Russell *et al.* 2011). Ma and Wong (2013) experimentally showed *Aspergillus flavus* was capable of biodegrading a water based PUR but not thermoplastic polyester polyurethane. Ibrahim *et al.* (2009) experimentally illustrated that six ascomycetes were capable of biodegrading PUR including two which were not previously listed before. Additionally, although it is not an ascomycete, Gusse *et al.* (2006) were the first to demonstrate that white-rot fungus, *Phanerochaete chrysosporium*, can biodegrade phenolic resins (PR). *P. chrysosporium* was the one of eleven fungi strains tested. The ascomycete *A. niger* and basidiomycete *Schizophyllum commune* were other strains tested and found to be incapable of biodegrading PR (Russell *et al.* 2006).

Most of the focus has been on ascomycetes biodegrading either PUR or PE (including HDPE and LDPE), but two studies showed that basidiomycetes were also capable of biodegrading plastics. Milstein *et al.* (1992) demonstrated that three white-rot fungi, *Phanerochaete chrysosporium*, *Pleurotus ostreatus,* and *Trametes versicolor*, were able to biodegrade PS. However, the PS was copolymerized with lignin and the amount of degradation was dependent on the concentration of lignin present. The second study was by Gusse *et al.* (2006), who were the first to demonstrate that *P. chrysosporium* can biodegrade phenolic resins (PR) but *P. ostreatus* could not. Furthermore, from our literature search, there is a lack of biodegradation studies on PP and PE using white-rot fungi.

Of all the various types of fungi, wood-decaying fungi are the promising decomposers for tackling the plastic pollution issue. Wood-decaying fungi are the only fungi capable of breaking down lignin, which is a highly complex molecule that varies in size and arrangement depending on plant species (Gusse *et al.* 2006; Russell *et al.* 2011; Krueger *et al.* 2015). Lignin is comprised of linked phenols, which contain aromatic hydrocarbon rings. There are two types of wood-decaying fungi: white-rot fungi and brown-rot fungi. White-rot fungi produce a variety of lignin peroxidases to breakdown lignin, along with laccases, and manganese peroxidases while brown-rot fungi breakdown the wood cellulose with another set of enzymes leaving behind lignin.

The problem with plastics is they are recalcitrant in nature, which means they are difficult to breakdown. The most problematic plastics are the single-use plastics, which are generally composed of polyethylene (PE), polyethylene-terephthalate (PET), polypropylene (PP), or polystyrene (PS). C—C bonds are stable, especially in aromatic rings, which is the reason why plastics are hard to breakdown in the environment. However, since white-rot fungi are capable of degrading lignin, it is hypothesized that they should be able to breakdown certain types of plastics. Plausibility is even more apparent when the molecule structures of PE, PET, PP, and PS are compared to an example of a lignin molecule, so long as the white-rot fungi possess the necessary extracellular enzymes.

Utilizing white-rot fungi as a source to biodegrade single-use plastics is a more practical way of naturally disposing of plastics, as opposed to incinerating, recycling, or landfilling, since white-rot fungi are already being grown commercially for human consumption. Thus, if it can be demonstrated that white-rot fungi are capable of biodegrading these problematic plastics, then it would be easier to implement a strategy for reducing global plastic waste. For instance, instead of using wood based resources to grow edible fungi, we could use plastic waste, or a mix of plastic waste and wood products, to grow them instead. This would save on resources and allow reduction of plastic waste in an environmentally friendly and sustainable way.

The purpose of this study is to determine if white-rot fungi are capable of biodegrading PS, PP, PET and PE, and to see if different soil communities are capable of degrading the same plastics. Three hypotheses are proposed: 1) white-rot fungi can biodegrade PS, PP, PE, and PET, 2) white-rot fungi can utilize PS, PP, PE, and PET as a main carbon source, and 3) the rates of biodegradation are dependent on three factors: type of plastic, white-rot fungal species, and soil quality.

2. MATERIALS AND METHODS

Basidiomycetes

Five species of white-rot fungi that are commercially grown for human consumption were used for this study: *Pleurotus ostreatus* (pearl oyster), *Pleurotus ostreatus* var. *columbinus* (blue oyster), *Lentinula edodes* (shiitake), *Ganoderma lucidum* (reishi), and *Trametes versicolor* (turkey tail). These fungal species were purchased from Fungi Perfecti in the form of 'plugs.' Pure cultures were created using malt extract agar (MEA), and subcultured as needed for experiments.

Plastics

Five plastic types were used in the present study: polystyrene (PS), polypropylene (PP), two forms of polyethylene (HDPE & LDPE), and polyethylene terephthalate (PET; aka PETE). These plastics were obtained from the Rutgers University—Camden cafeteria in their single-use consumable forms: clear food containers (PS), straws (PP), plastic bags (HDPE & LDPE), and water bottles (PET). The decision for this was to gain a more pragmatic result since plastics encountered by microorganisms in natural environments will be in consumable forms. Encountering a liquid plastic that is homogenized with nutrients (Russell *et al.* 2011, for example) is highly unlikely. The plastic will be dispersed unevenly in natural environments. Plastics were standardized, weighed, and sterilized with 95% ethanol before each experiment.

Media

Various media were used to grow fungi. For cultures and subcultures, malt extracted agar (MEA) was used since it is a selective media for fungi. During certain experiments, 25%

MEA was used to encourage fungi to search for other carbon sources (i.e. the plastic pieces). Lignin media, a mixture of hardwood chippings, was used to provide sustenance to the fungi, but also to potentially coax the fungi into using their ligninolytic enzymes. It was hypothesized that the necessary ligninolytic enzymes needed to break down the plastics would only be produced in the presence of lignin. Additionally, tap water agar was used during certain experiments to provide minimal nutrients, which would force the fungi to either utilize the plastic pieces as a carbon source, or perish.

<u>One-Month Biodegradation Assay</u>

Pure cultures of each fungus (N=3) were created in 60mm x 15mm petri plates using two different media: 25% MEA and lignin with distilled water (2.5g lignin/ 12.5mL diH2O/ plate). Plastic pieces were standardized using a single hole puncher (~6mm diameter). After one week, five pieces of plastic were added to the plates with a combined weight recorded after being sterilized with 95% ethanol. Plates were left undisturbed in the culture chamber for one month. After which, the plastic pieces were removed, rinsed in distilled water, air dried, and then the combined weight was recorded.

<u>Three-Month Biodegradation Assay</u>

As a result of minimal mass loss in the one-month degradation experiment, a second, and longer experiment was established. Pure cultures of each fungus (N=3) were created in 60mm x 15mm petri plates using two different media: 25% MEA and tap water agar. After one week, one plastic piece (1cm x 1cm) was inserted into each plate on the surface of the media after being weighed and sterilized in 95% ethanol. Samplings occurred at 2 and 3 months. Upon which, the plastic pieces were removed, rinsed in distilled water, air dried, and then weighed.

Plastic Preference Test

The plastic preference test was established to determine if the fungi preferred one plastic to another. Each fungi species was inoculated into the center of a standard size petri plate with 25% MEA. Four plastic pieces in total—one of each type of plastic were placed along the outer edge of the plate. One plastic piece at 12 o'clock, another at 3, 6, and 9. These were left to incubate for one week. This test was repeated following the same set-up but with tap water agar for media instead of 25% MEA. If there was a preference by one of the fungi for a type of plastic, most of the hyphal growth would be observed heading toward and around that type of plastic. If there was no preference, then the fungi would show an equal amount of hyphal growth in all directions in the plate.

Soil Experiment

Soil was collected in buckets and brought to the lab from three different locations—Crow's Wood, Pine Barrens, and Rutgers—Camden campus. Each location offers a unique assemblage of organisms due to their different soil types. The Pine Barrens are coniferous forest in southern New Jersey. Its soil is sandy, acidic, and nutrient poor. Crows Woods Nature Preserve is a small deciduous forest that has a fluvaquent (USDA 2017), loamy (USDA 2017), nutrient rich soil located in Haddonfield, NJ. Rutgers University—Camden campus is an urbanized area that has a nutrient rich, but stressed soil located in Camden, NJ. It has several trees, both coniferous and deciduous, but it is mainly comprised of heavily mulched areas and maintained grasses. Each soil was homogenized and placed into aluminum trays (N=3) in ambient temperature. Plastic pieces (1cm x 1cm; N=10) were added to each tray. Prior to this, the plastic pieces were weighed and an average weight was calculated for each plastic type. Samplings occurred at 1, 3, 6, and 12 months. After which, the plastic pieces (N=10) were removed, rinsed, air-dried, and then weighed. Once a month, during this experiment, each tray received 250mL of tap water.

Statistics

Data were initially entered into Microsoft Excel, and analyzed in SAS for factorial ANOVA. Factors were fungal species, plastic types, media types, and time. Percent mass loss was calculated using the following formula: $((mass_{before} - mass_{after})/mass_{before}) \times 100$. Four outliers were excluded from statistical analyses because their ‘results’ were a product of missing plastic pieces, which gave the perception of higher mass loss percentage, when in fact, it was irrelevant. Tukey’s Studentized Range was used to determined differences within factors signified by alphabetical letters. Different letters meant factors compared were significantly different.

3. RESULTS

One-month biodegradation assay

The one-month biodegradation assay shows the type of plastic presented to fungi is highly significant ($P < 0.0001$). In figure 4, all the data from the one-month assay is displayed, where an easy pattern can be determined. The five selected white-rot fungi are capable of biodegrading HDPE and LDPE at different rates, but are unable to biodegrade PP, PS, or PET. It is possible these fungi are still capable of biodegrading PP, PS, and PET, but more time is required. These particular plastics are known to be highly recalcitrant in nature. Thus, it would make sense that these would take longer to biodegrade.

The one-month biodegradation assay shows the type of media used to grow the fungi is significant ($P = 0.0396$). Figure 5 is the same data from figure 4, but separated by media. The biodegradation of plastic yielded higher mass loss percentage in the lignin media as opposed to the MEA media in regards to HDPE and LDPE. However, the results were the nearly the same for PP, PS, PET.

Pleurotus ostreatus (PO) displayed the greatest biodegradation ability with HDPE and the least with PP (figure 6). PO performed significantly better in lignin media than MEA media when dealing with HDPE, but LDPE, PP, PS, and PET were similar. *Pleurotus ostreatus columbinus* (POC) also displayed the highest biodegradation ability with HDPE although the overall mean percent mass loss between plastics was insignificant. However, looking at just the HDPE data between media types, there is a significant difference, with POC performing better in lignin media than MEA media. *Lentinula edodes* (LE), much like PO and POC, displayed the highest biodegradation ability with HDPE, than the other plastics, with PP being the least. POC performed significantly better in lignin media than MEA media in regards to HDPE and LDPE. *Ganoderma lucidum* (GL) displayed the highest biodegradation ability with both HDPE and LDPE, with slightly better results with LDPE although the difference is insignificant. Again, PP is shown to be the least capable of biodegradation. The only significance in media difference was seen with LDPE, where GL in lignin media performed significantly better than in MEA media. *Trametes versicolor* (TV) displayed the highest biodegradation ability with LDPE, although the overall difference is considered insignificant due to the highly variable results from HDPE. PP was the least biodegradable. TV displayed no significant difference in regards to media, although TV seems to do better biodegrading plastics in MEA media than in lignin media.

Overall, a pattern has emerged with only slight variability between HDPE and LDPE. PO and LE display abilities for biodegradation of plastics in a particular order: HDPE, LDPE, PET, PS, and PP; with HDPE being the most and PP the least. GL and TV display their biodegradation abilities in a similar order with only HDPE and LDPE switched: LDPE, HDPE, PET, PS, and PP; with LDPE being the highest and PP the lowest. POC was different from the other fungi because of its LDPE data, it was highly variable, but overall puts it closer towards PP. Thus, POC's order of biodegradation ability is HDPE, PET, PS, PP and LDPE; with HDPE being the highest and LDPE being the lowest. Despite this difference with LDPE, POC still fits within the overall pattern seen. PET, PS, and PP are consistently last and in that particular order, which suggests that PP is the most recalcitrant plastic in regards to biodegradation via white-rot fungi. Additionally, each fungus performed better in lignin media with the exception of TV who performed better in MEA media.

These results make sense since although each fungus is classified as a white-rot fungus, there is variability within the group. All white-rot fungi possess ligninolytic enzymes, such as, laccases, lignin peroxidases, and manganese peroxidases. However, the enzymes will be slightly varied, and each variation is crucial in chemistry.

Three-month Biodegradation Assay

The purpose of the three-month biodegradation assay was to see if the targeted white-rot fungi were capable of biodegrading the selected single-use plastics (MEA media), and to see if the fungi were able to utilize the plastic as a main carbon source (tap water agar media).

ANOVA analysis of the two-month samples across all fungi, plastics, and media shows a significant percent mass loss ($P < 0.0001$), which is dependent on all three factors. *Pleurotus ostreatus* (PO) again displayed the highest biodegradation abilities with HDPE, followed by LDPE, PET, PS, and PP. MEA and tap water agar media did not show a significant difference between the different types of plastics except for PP, which displayed a relatively large negative percent mass loss, indicative of mass gain. *Pleurotus ostreatus columbinus* (POC) displayed the same pattern as the one-month biodegradation assay, with the highest percent mass loss seen in HDPE, followed by LDPE, PET, PS, and PP. A significant difference in percent mass loss is seen in HDPE, with POC performing better in MEA media than tap water agar. For the other four plastics, although insignificant, POC performed better in tap water agar. *Lentinula edodes* (LE) performed best with PP, followed by HDPE, LDPE, PET, and PS. LE showed varied results for media comparison. LE in MEA media showed a slightly higher percent mass loss in HDPE, but was reversed for LDPE. For PP, little to no percent mass loss was seen in MEA media, but a relatively large and significant percent mass loss was observed in the tap water agar. LE performance was equal between media for both PET and PS. *Ganoderma lucidum* (GL) displayed the highest biodegradation abilities with HDPE, followed by PET, LDPE, PS, and PP. GL performed significantly better in tap water agar media than MEA media for HDPE. For the other four plastics, performance was near identical with a slight increase in tap water agar, which is insignificant. *Trametes versicolor* (TV) displayed the highest capabilities of biodegradation with HDPE, followed by PET, PS, PP, and LDPE. TV performed significantly better in tap water agar media than MEA media, which showed a mass gain. No other media difference was observed. Overall for the two-month samples across media, LE displayed the highest percent mass loss of HDPE, followed by POC, PO, TV, and GL; with TV and GL being near identical. All fungi were significantly different from the control. For LDPE, the order from highest to lowest is LE, POC, PO, GL, and TV with POC and PO being very similar and TV displaying a gain instead of a loss. LE, POC, and PO were significantly different from the control, as was TV but in the opposite direction (gain). Only LE displayed a percent mass loss for PP and PO showed a gain, both were significantly different from the control. Regarding PS, none of the fungi caused a significant percent mass loss in the plastic. Same with PET.

ANOVA analysis of the three-month samples across all fungi, plastics, and media shows a significant percent mass loss of plastics ($P < 0.0001$), which is dependent on all three factors. *Pleurotus ostreatus* (PO) showed the highest biodegradation ability with HDPE, followed by LDPE, PET, PS and PP; with PS and PS nearly identical. *Pleurotus ostreatus columbinus* (POC) and *Lentinula edodes* (LE) displayed the same order as PO. *Ganoderma lucidum* (GL) followed the same trend except HDPE and LDPE percent mass loss were near identical. *Trametes versicolor* (TV) again veered in a separate direction. TV performed the best with HDPE, followed by PET, PS, PP, and LDPE. Overall, across media, POC displayed the greatest ability to biodegrade HDPE followed by POC, and LE. PO performed the best for LDPE, followed by GL, LE, and POC. For, PP, PS, and PET, all fungi were equally unable to utilize the plastics. In regards to the 3-month samples in MEA media, LE performed the best with HDPE followed by PO and POC. The order for LDPE was the same for the overall LDPE fungi performance comparison, Same with PP, PS, and PET. However, with the three-month samples in tap water agar, the order of best to least performers changed. For HDPE, POC displayed the highest biodegradation abilities, followed by PO, TV, and GL. For LDPE, percent mass loss was not significant.

ANOVA analysis for percent mass loss of plastics by fungi in MEA across time shows percent mass loss was significant ($P < 0.0001$), and dependent on fungi, plastics, and time. Performance of PO over the three months showed different trends for each plastic except for PP and PS. Percent mass loss of HDPE by PO was initially negative and rose to a significantly positive mass loss the second month followed by roughly the same mass loss in the third month. LDPE percent mass lass by PO showed an upward trend of continuous mass loss over the three months. PET mimicked HDPE results, but to a smaller degree, while little to no activity occurred for PP and PS. Percent mass loss of HDPE by POC increased significantly between the first and second month, but decreased slightly in the third month, but remained significant from the first month and control. The same trend was seen with LDPE, but to a lesser degree. Little to no activity with PP, PS, and PET. LE displayed the same trends as PO. There was no significant percent mass loss of HDPE by GL. Although it is insignificant, there was an upward trend of percent mass loss in LDPE by GL, and little to no activity with PP, PS, and PET. There was a significant percent mass gain in HDPE during the third month by TV. First two months were insignificant. For LDPE, there was an initial mass loss during the first month by TV, but then reverse to mass gains for the second and third months. Again, little to no activity occurring with PP, PS, and PET.

ANOVA analysis of percent mass loss over time in tap water agar across fungi and plastics, shows the percent mass loss is significant ($P < 0.0001$), and dependent on all three factors. PO displayed an upward trend for HDPE. The same trend was seen in LDPE to a lesser and insignificant degree. For PP, there was a relatively large and significant mass gain at two months followed by a large mass loss in the third month, which ultimately negated one another. POC displayed the same trends as PO, except for the PP mass gain. LE displayed an initial percent mass loss in HDPE at two months, but then decreased for the third. This trend was also seen with LDPE. For PP, a relatively large and significant percent mass loss was seen in the second month, but was not seen in the third month. There was a mass gain in the third month, which negated the mass loss. This trend is similar to PO with PP, but in the opposite direction. HDPE percent mass loss by GL was higher in the second month, than the third, and both were significant. LDPE, was reversed and insignificant. HDPE percent mass loss by TV showed an upward trend over each month. LDPE percent mass loss by TV was insignificantly positive during the second month and went negative during the third month. Little to no activity occurred with PS, and PET across fungi. And no activity occurred with PP except for PO, and LE. Overall, the fungi displayed the highest biodegradation abilities with HDPE followed by LDPE. The fungi were not able to biodegrade PP, PS, and PET except for LE, which was able to biodegrade PP in tap water agar.

Plastic Preference Test

The purpose of the plastic preference test was to see if fungi favored one plastic over another. Hyphal growth was even in all directions of each plate, which signifies that there was no preference for a particular type of plastic despite different media.

Soil Experiment

The purpose of the soil experiment was to determine if different soil types were a factor in the biodegradation rate of plastics. Plastic biodegradation was insignificant in the Crow's Wood (CW) soil. However, an overall arching theme seen was a continuous fluctuation of mass percentage loss and gain. For instance, HDPE the first month shows a mass gain, but then a loss of that gain in the third month. At six months, the mass gain is nearly identical to the first month, which is then followed by a mass loss at twelve months, which is similar to the third month. This sine wave is seen again in PP and PS, with slight variations. However, with LDPE there is an initial mass loss followed by a mass gain, which then gradually returns to the initial mass loss. It is almost like the beginning of a learning curve, where if it were to continue, the mass loss would become greater and eventually plateau. However, this is only mere speculation since, the experiment ended at the twelve-month mark. Overall, LDPE showed the most promise of being able to biodegrade in the CW soil with PS and PP coming in last.

Plastic biodegradation was significantly different from one another ($P < 0.0001$) in the Pine Barrens (PB) soil. The beginning of a sine wave was seen in both HDPE and PP, but at a slower rate than the CW soil. HDPE was seen with an initial mass gain in the first month, which is lost during the third and sixth month, and is regained in the twelfth month. LDPE was seen to have a fairly consistent mass loss over the twelve-month period, with a slight dip during the third month. PS shows a small initial mass loss, which decreases over time.

Plastic biodegradation was significantly different ($P = 0.0106$) in the Rutgers University—Camden (RUC) soil. The most promising plastic was HDPE, which started out with a mass gain in the first and third months, and turned into mass loss during the sixth and twelfth months. Overall, the plastic type was insignificant, but again this sine wave pattern was seen in LDPE, PP, and PS.

When comparing HDPE across all three soil types there was no significant difference. However, RUC soil seems to be the most promising since at the twelfth month mark it displayed the highest mass loss percentage for HDPE. In regards to LDPE across soil types, although insignificantly different from one another, LDPE showed the highest amount of percent mass loss of all the types of plastics and across soil. Potentially, the biodegradation of LDPE could happen in all soil types with PB soil being the most promising. PP across soil types was insignificant and displayed various rates of sine waves over the 12-month time period. There is a potential for PP biodegradation in PB and RUC since there was a little bit of mass loss, but not in the CW soil. In regards to PS across soil types, there was a significant difference in mass loss percentage. The PB soil displayed an interesting decline in mass loss over the twelve-month period, while CW showed a typical sine wave. RUC also displayed a potential sine wave, but it appeared to be shifting towards more mass loss over time instead of remaining fairly consistent. PB and RUC soils displayed the most potential for biodegradation of PS.

When all the soil experiment data is statistically analyzed together a significant difference in mass loss is seen ($P = 0.0001$), but more importantly a significant soil*plastic*month interaction ($P = 0.0139$) is seen, which means that there are other factors contributing to the mass loss percentages of the various plastic types within the different soils.

4. DISCUSSION

The purpose of this present study was to determine if selected white-rot fungi were capable of biodegrading single-use plastics in order to help reduce plastic waste. It was also an attempt to fill in a knowledge gap regarding fungi biodegrading plastics since most of the focus has been on the ascomycetes group. The selected white-rot fungi species were ultimately chosen because they are grown on a commercial scale for human consumption, which makes it possible for a potentially large scale plastic waste reduction operation.

High-density polyethylene (HDPE)

Pleurotus ostreatus (PO), *P. ostreatus columbinus* (POC), *Lentinula edodes* (LE), and *Trametes versicolor* (TV) displayed the ability to biodegrade HDPE. Rates varied depending on the fungal species, growth media, and time. PO, POC, and LE displayed the highest biodegradation abilities of HDPE. Additionally, PO, POC, and LE performed the best in the lignin media. TV was only able to biodegrade HDPE in tap water agar. *Ganoderma lucidum* (GL) results were insignificant. Thus, the current conclusion is GL is not able to biodegrade HDPE. However, GL has potential to still biodegrade HDPE, but at a much slower rate than the other fungi.

Low-density polyethylene (LDPE)

PO, POC, LE, and GL displayed the ability to biodegrade LDPE. Rates varied depending on fungal species, growth media, and time. PO performed the best of all the fungi. LE and GL performed significantly better in lignin media than MEA and tap water agar media. TV was not able to biodegrade to LDPE. PO, LE, and GL displayed increasing trends of percent mass loss of LDPE over a three-month period.

<u>Polypropylene (PP)</u>

Lentinula edodes (LE) was the only fungi capable of biodegrading PP, and it only occurred during the second month using tap water agar. Additionally, this is the first time that a fungus has been documented biodegrading PP. *Pleurotus ostreatus* (PO) showed a mass gain during the second month with tap water agar, which suggests there is potential with PO. However, PO was grouped with POC, LE, and GL as being unable to biodegrade PP.

<u>Polyethylene terephthalate (PET) & Polystyrene (PS)</u>

All five fungi were unable to biodegrade PET and PS despite various media and time.

<u>Soil Experiment</u>

The purpose of this study was to determine if different soil types, which house different soil communities, would play a factor in biodegradation of HDPE, LDPE, PP, and PS. The experiment displayed some potentials for biodegradation of certain plastics. Crow's Wood and Pine Barrens soils showed an upward trend and consistency, respectively, of percent mass loss with LDPE. Rutgers University-Camden soil showed an upward trend in percent mass loss of HDPE. The rest of the data resulted in various forms of sine waves.

There is no hard evidence to explain the sine waves. However, there are a few hypothesized factors that have not been tested in this experiment. An organism may possess the needed enzyme to biodegrade a plastic, but the by-product could be toxic or harmful to the organism, thus causing them to cause an initial mass loss, but then stop. Another factor is competition. A fungus may colonize a plastic that it is capable of breaking down, but competition who cannot, may out compete the other fungi, for instance, causing the biodegradation to stop. This could also happen in the reverse situation. Competition with other organisms may be a factor. The soil was not sterilized, and other organisms could have preyed upon potential biodegrading fungi. Ultimately, this experiment turned into a pilot study and needs to be repeated to isolate potential fungi, and then designed for a more controlled study.

In conclusion, *Pleurotus ostreatus*, *P. ostreatus columbinus*, *Lentinula edodes*, *Ganoderma lucidum*, and *Trametes versicolor* are capable of biodegrading single-use plastics. Biodegradation rates varied with fungi species, plastic type, growth media, and time. Additionally, the rates of biodegradation are relatively slow; then again so is fungal growth. With further studies, rates can be maximized and potentially applied to the long-term goal of developing a process to utilize these plastics as a growth medium for commercially grown white-rot fungi, which would help to reduce plastic waste.

5. FUTURE DIRECTIONS

Despite this present study, currently, the best way to reduce plastic waste pollution to natural environments is through education and single-use plastic bans around the world. However, even if a global single-use plastic ban was achievable, there is still the issue of millions of tons of present plastic waste to be dealt with.

For continuation of this study, several things need to occur next. A color clearance test is needed in order to confirm which of the various ligninolytic enzymes are being used in the biodegradation of the selected plastics and under which conditions of nutrient and alternate carbon supplies. All five fungi need to be placed into long-term biodegradation assays. Although, three-month biodegradation assays showed significant plastic decomposition rates, they were relatively small. A twelve-month biodegradation assay would suffice. Microscopic investigation, including SEM imaging, are needed to determine the cause for percent mass gain, which was seen frequently in the present study. Identification and production of the plastic biodegrading, ligninolytic enzymes would be beneficial, if possible. Eventually, if everything pans out, the fungi need to be tested to ensure that the selected white-rot fungi are still edible after they have consumed plastic waste. The soil experiment needs to be repeated to isolated potential biodegrading fungi. Those fungi then need to go through biodegradation assays. Those found capable should then also go through the other steps listed above in this section.

The discovery of potential plastic biodegraders is still in its infancy. In the coming years, many new and exciting discoveries will be made on how to utilize natural components to combat anthropogenic issues such as plastic pollution.

6. FIGURES

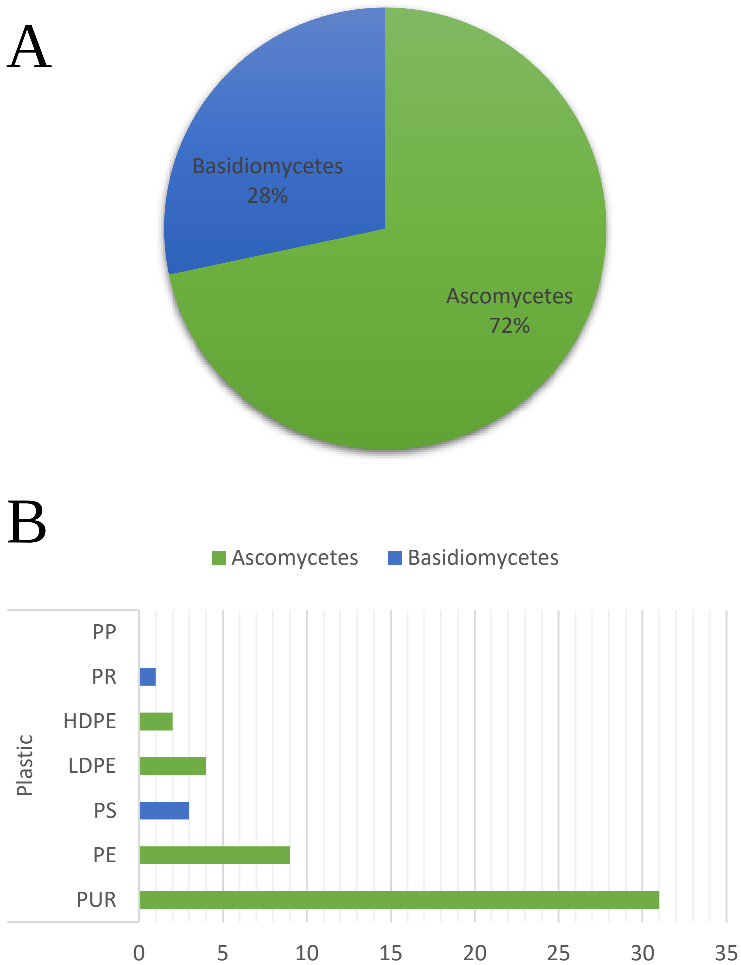


Figure 1: (A) A pie chart displaying the percentage of fungi tested for plastic degradation split into two categories: basidiomycetes and ascomycetes. (B) A bar graph displaying the types of plastics tested and how often. PP = polypropylene; PR = phenolic resins, HDPE = high-density polyethylene; LDPE = low-density polyethylene; PS = polystyrene; PE = polyethylene; PUR = polyester polyurethane.

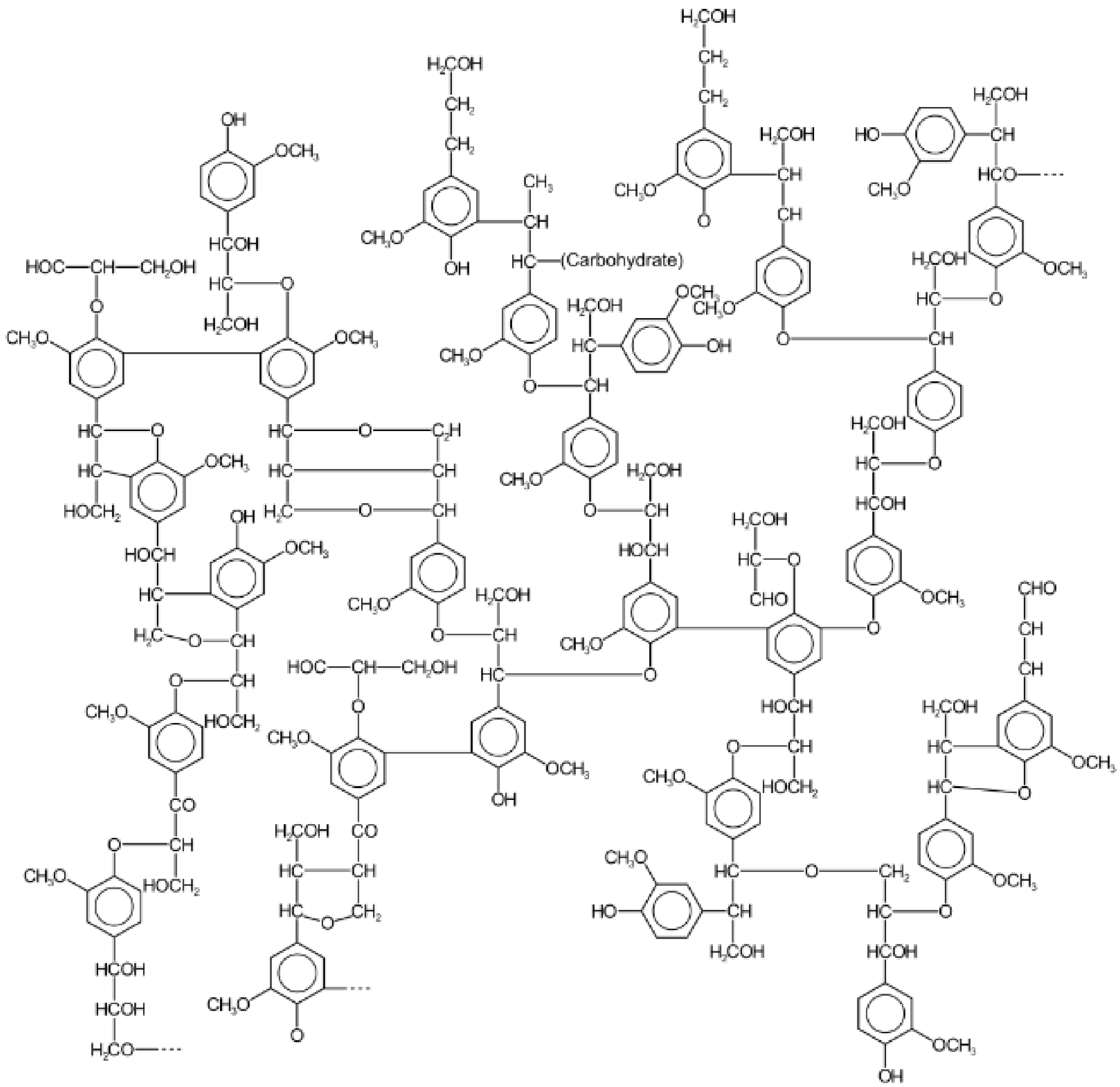


Figure 2: An example of lignin molecule (Glazer and Nikaido 1995).

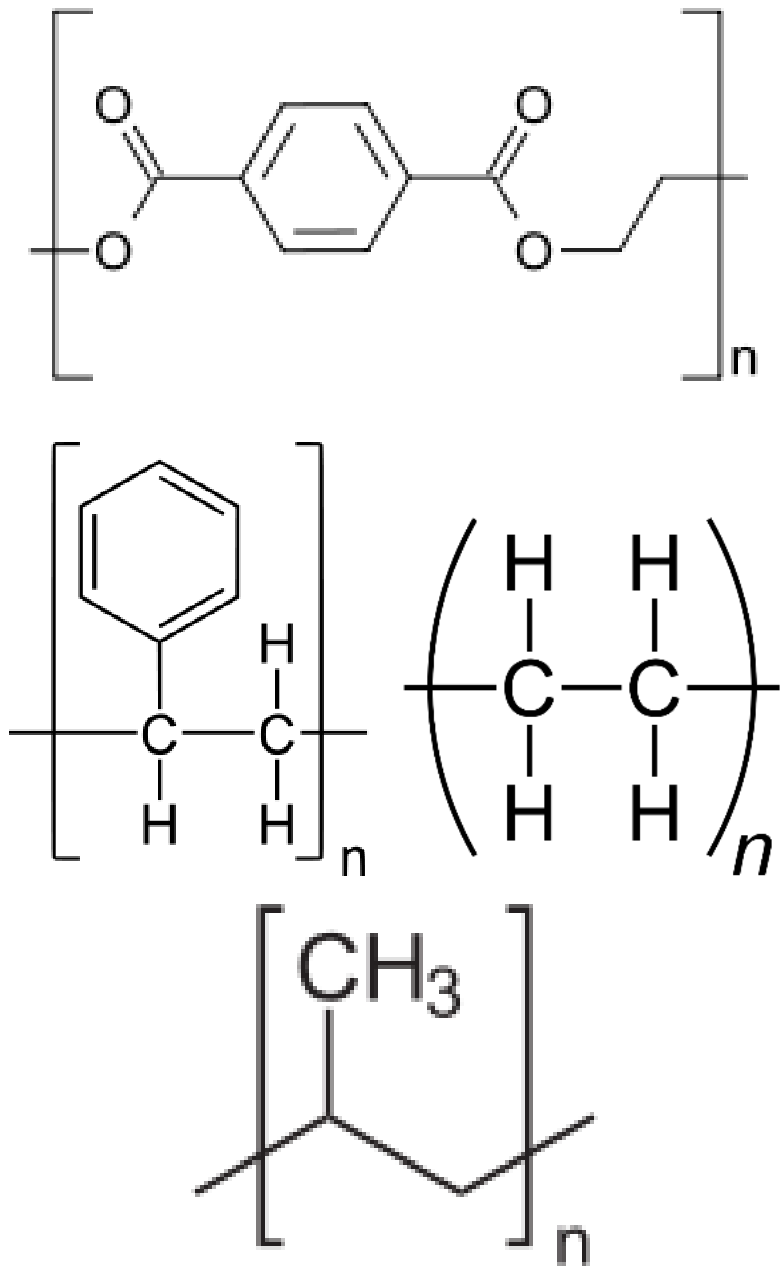


Figure 3: Molecules of targeted plastics for present study (from left to right; top to bottom): polyethylene terephthalate (PET), polystyrene (PS), polyethylene (PE), and polypropylene (PP).

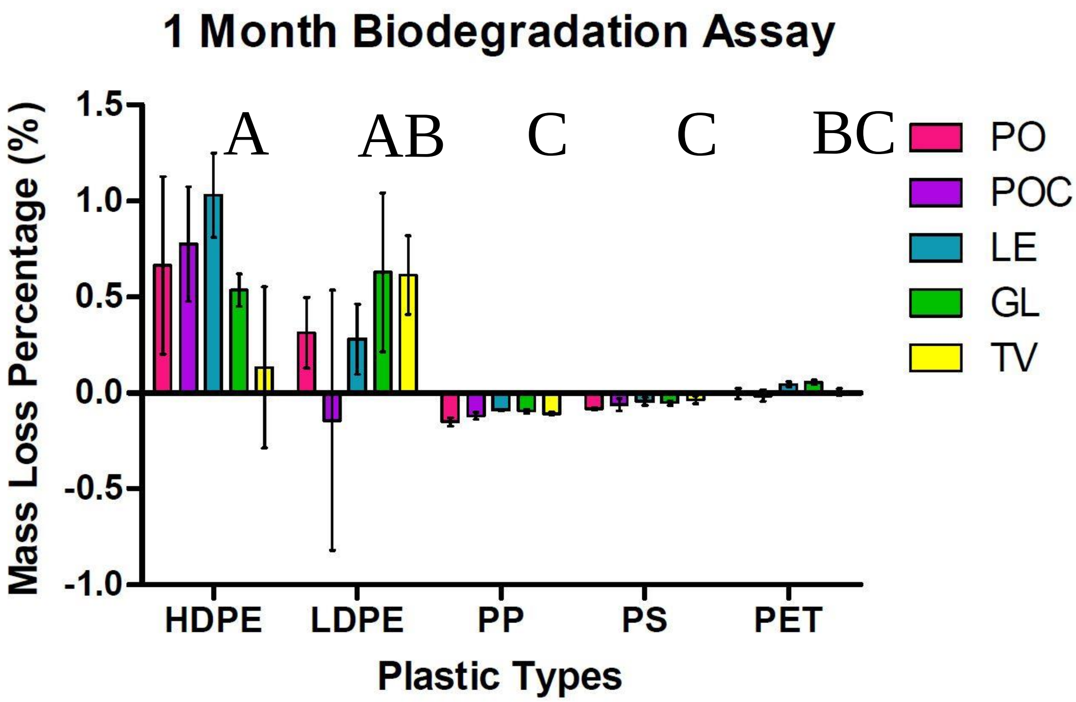
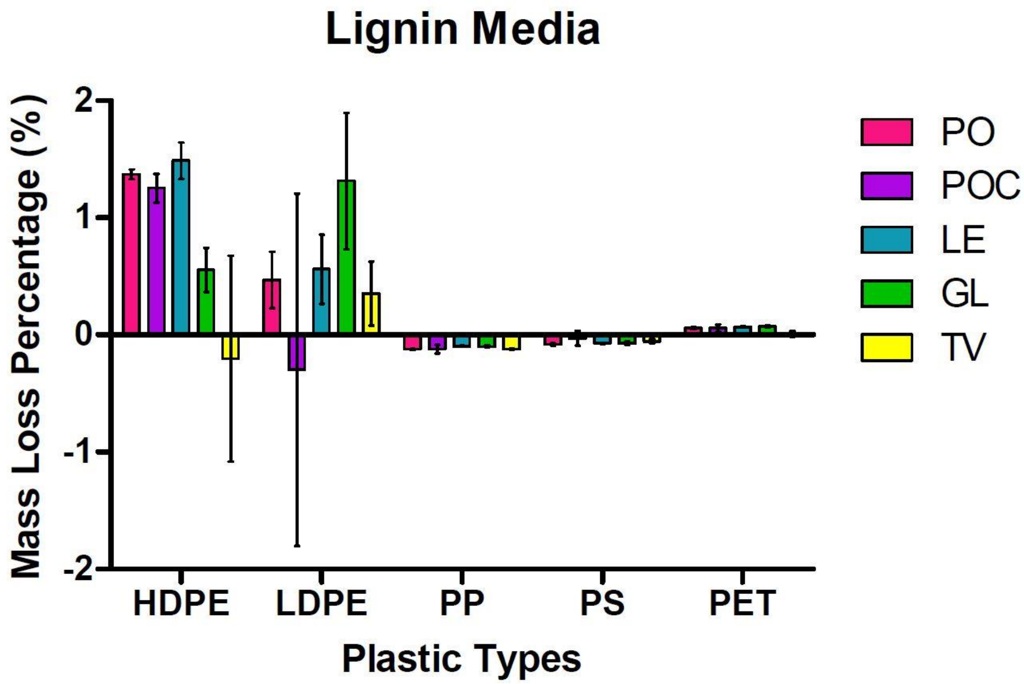


Figure 4: Mean percent mass loss ($\pm SE$) of all plastics by all fungal species from the one-month biodegradation assay across media. GLM analysis shows percent mass loss is significant (P = 0.0008) and dependent on the type of plastic present (P < 0.0001), but not dependent on the fungal species (P = 0.7070). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). PO = *Pleurotus ostreatus*; POC = *Pleurotus ostreatus columbinus*; LE = *Lentinula edodes*; GL = *Ganoderma lucidum*; TV = *Trametes versicolor*; HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.0008 ***
Fungi	P = 0.7070
Plastic	P < 0.0001 ***
Fungi*Plastic	P = 0.3855
Media	P = 0.0396 ***
Fungi*Media	P = 0.1272
Plastic*Media	P = 0.1785
Fungi*Plastic*Media	P = 0.1149



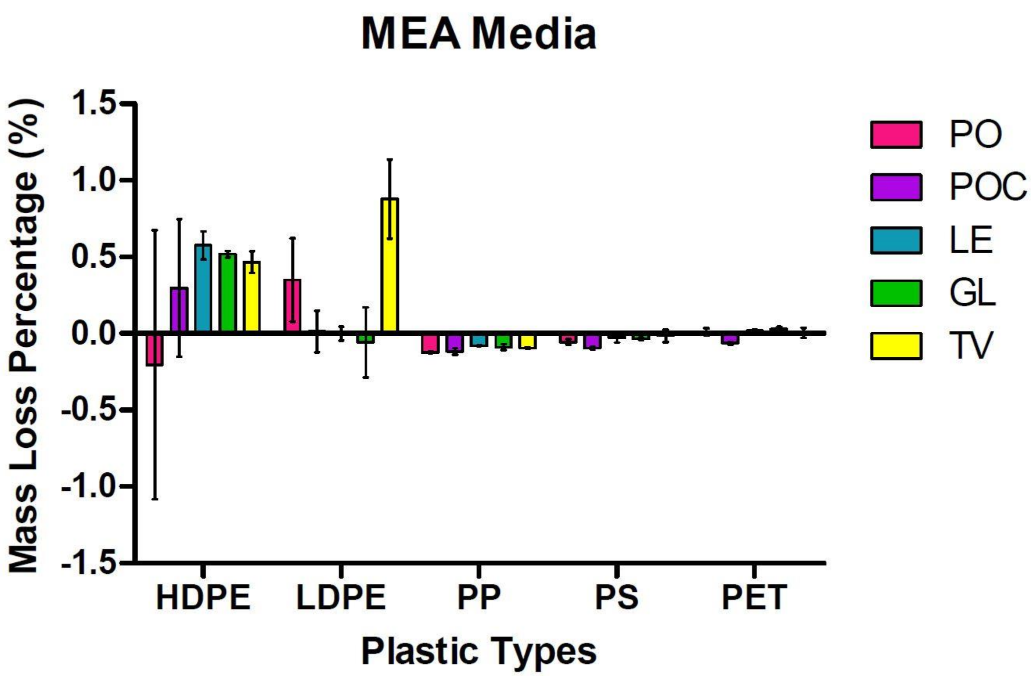


Figure 5: Mean percent mass loss ($\pm SE$) of all plastics by all fungal species from the one-month biodegradation assay separated by media. GLM analysis shows percent mass loss is significant (P = 0.0008) and dependent on the type of media used (P = 0.0396). PO = *Pleurotus ostreatus*; POC = *Pleurotus ostreatus columbinus*; LE = *Lentinula edodes*; GL = *Ganoderma lucidum*; TV = *Trametes versicolor*; HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

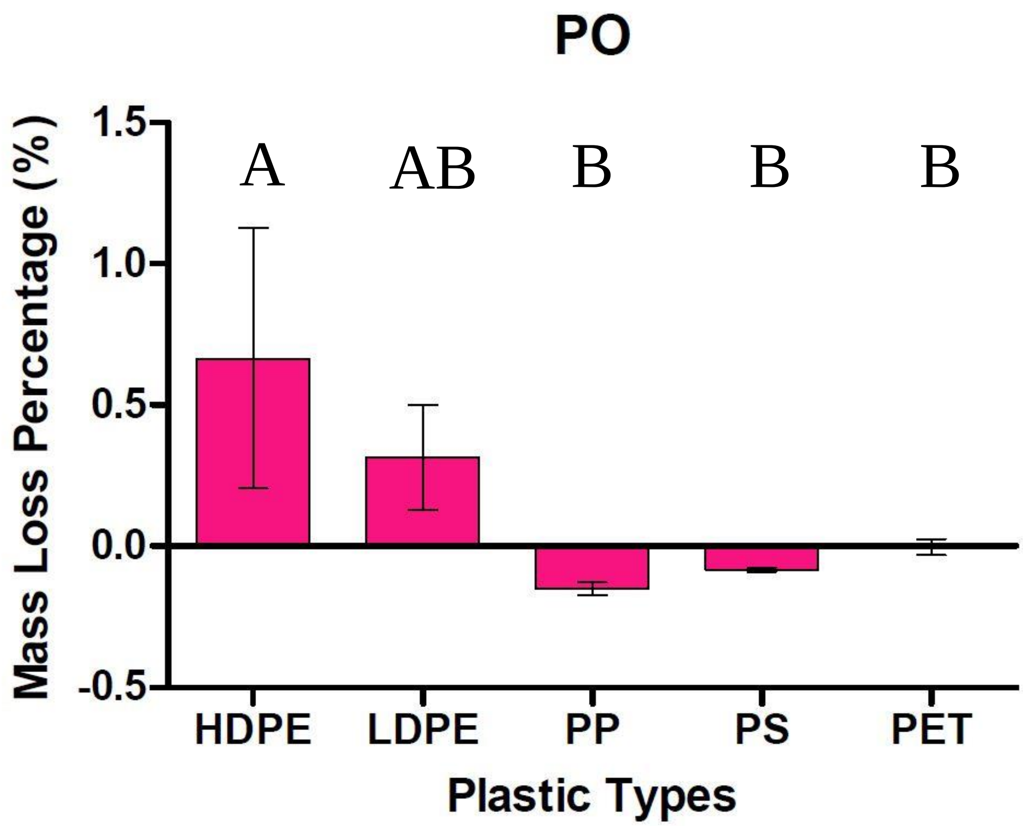


Figure 6: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus* (PO) from one-month biodegradation assay, across media. Significant differences between plastics (P = 0.0005) is indicated by different letters (Tukey's Studentized Range). There is a significant interaction between Plastic*Media at P = 0.0002. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P = 0.0005 ***
Media	P = 0.0010 ***
Plastic*Media	P = 0.0002 ***

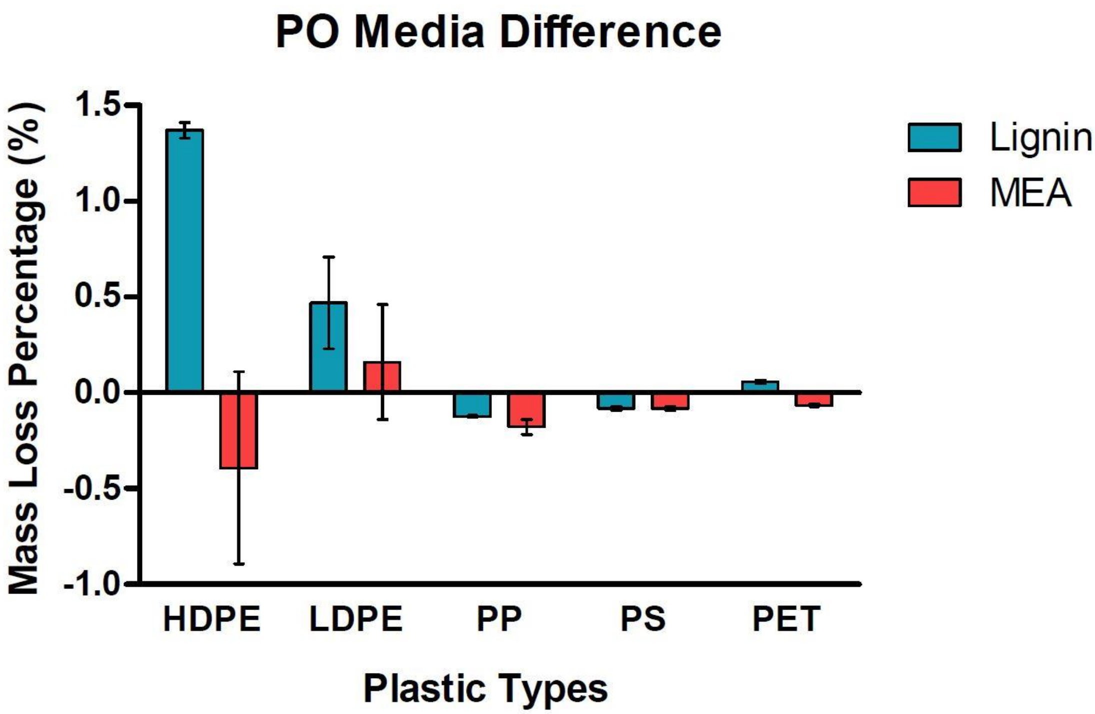


Figure 7: Mean percent mass loss ($\pm$ SE) of plastic types influenced by *Pleurotus ostreatus* (PO) separated by media. GLM and Tukey's Studentized Range showed a significant difference between media (P = 0.0010). There was a significant interaction between Plastic*Media at P = 0.0002. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

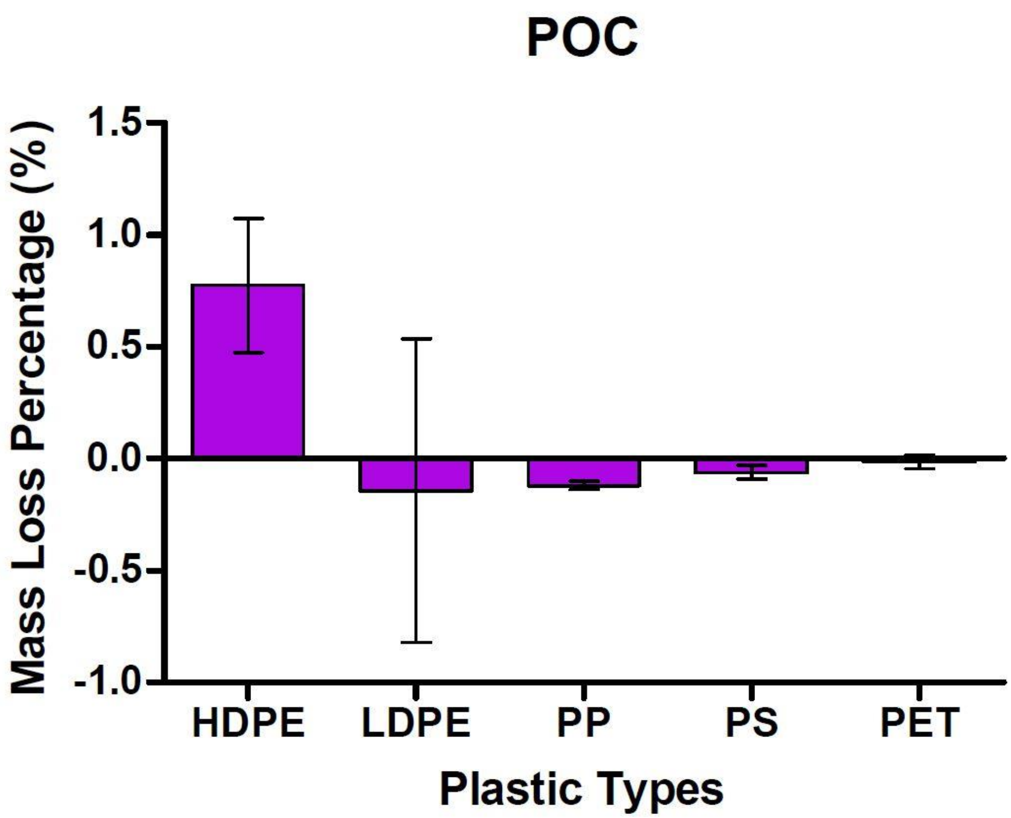


Figure 8: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus columbinus* (POC) from one-month biodegradation assay, across media. GLM and Tukey's showed that mass loss percentage is not significant (P = 0.6837), nor dependent on the type of plastic (P = 0.3693). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.6837
Plastic	P = 0.3693
Media	P = 0.6196
Plastic*Media	P = 0.7890

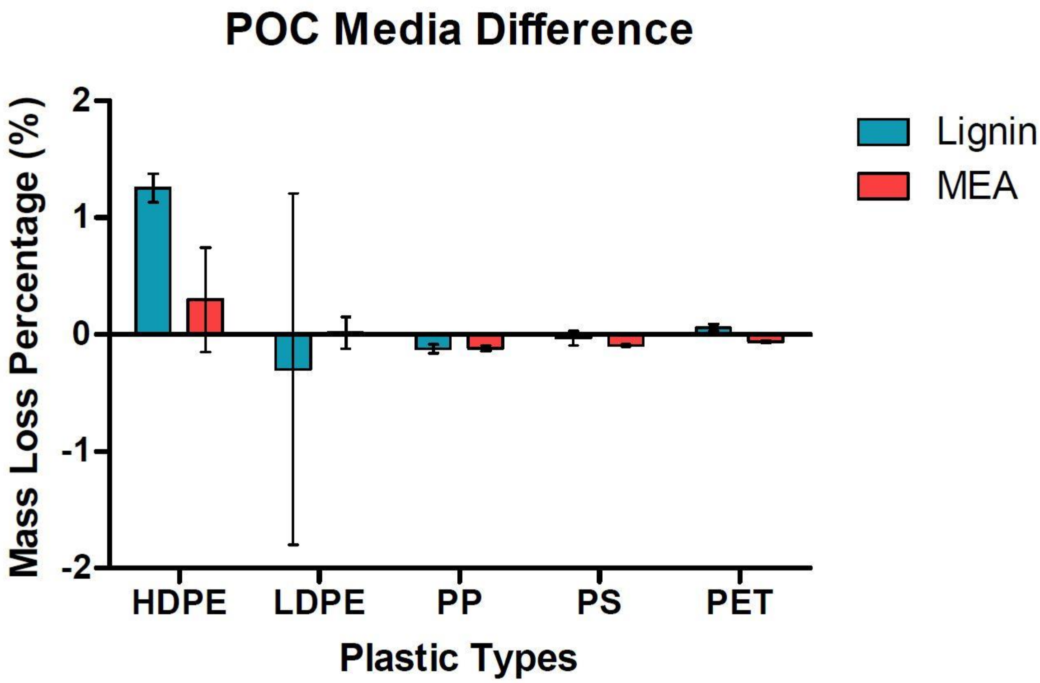


Figure 9: Mean percent mass loss ($\pm\ SE$) of each plastic type influenced by *Pleurotus ostreatus columbinus* (POC) from one-month biodegradation assay. GLM and Tukey's showed that mass loss percentage is not significant (P = 0.6837), nor dependent on the type of media (P = 0.6196). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

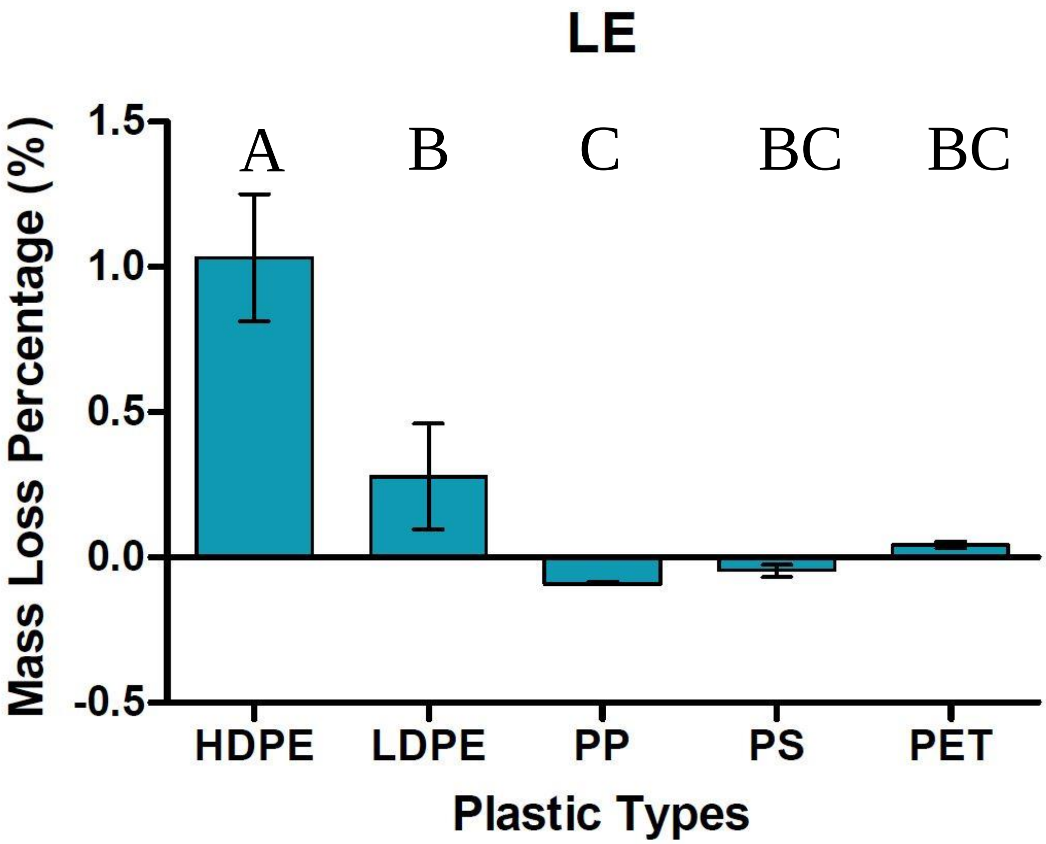


Figure 10: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Lentinula edodes* (LE) from one-month biodegradation assay, across media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P < 0.0001). Significant differences between plastics (P < 0.0001) are indicated by different letters (Tukey's Studentized Range). There was a significant interaction between Plastic*Media at P = 0.0014. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P < 0.0001 ***
Media	P = 0.0005 ***
Plastic*Media	P = 0.0014 ***

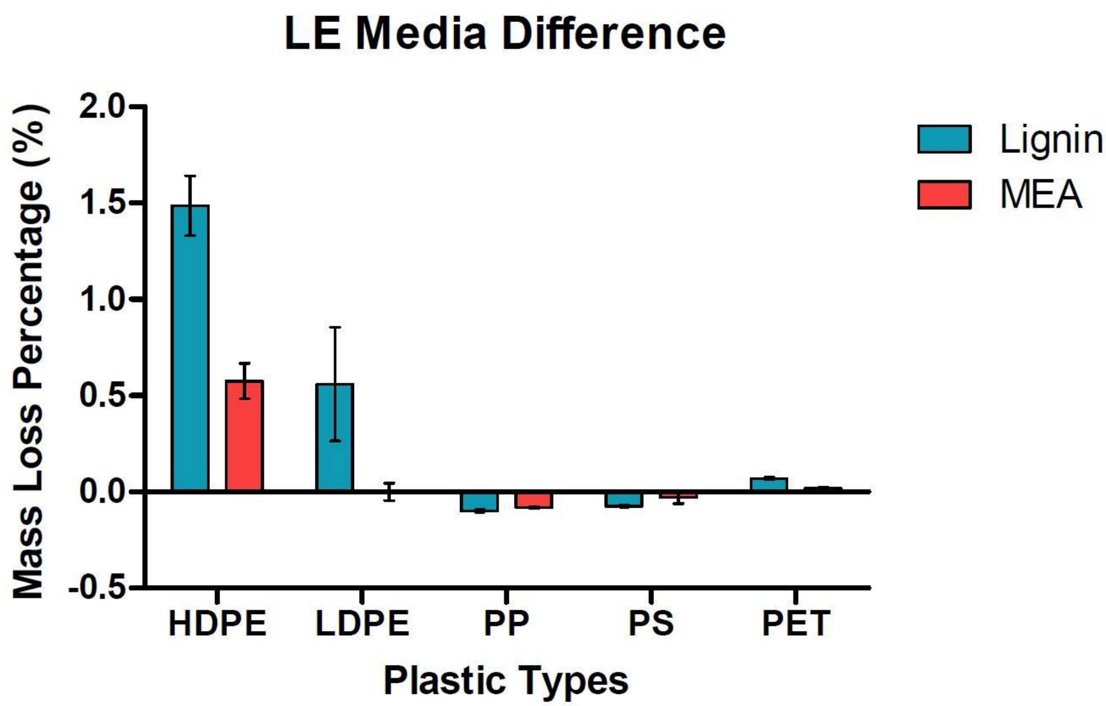


Figure 11: Mean percent mass loss ($\pm SE$) of plastic types influenced by *Lentinula edodes* (LE) separated by media. GLM and Tukey's Studentized Range showed a significant difference between media (P = 0.0005). There was a significant interaction between Plastic*Media at P = 0.0014. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

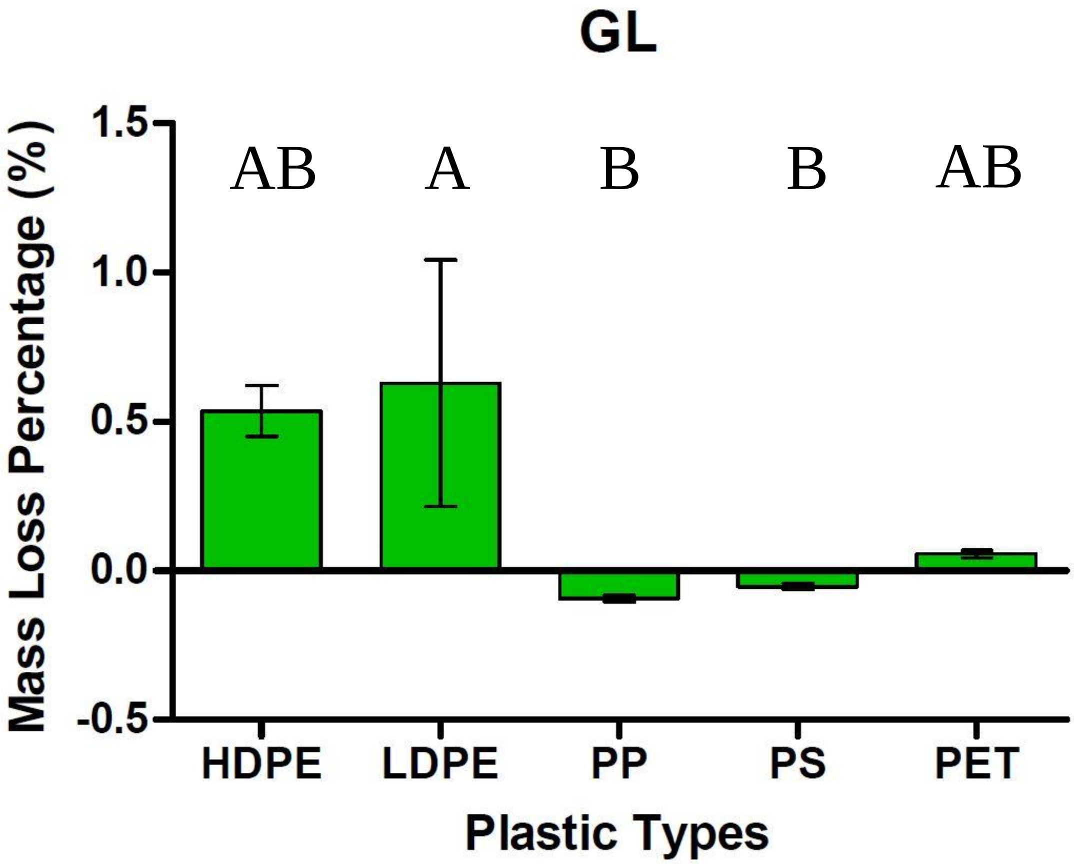


Figure 12: Mean percent mass loss ($\pm SE$) of plastic types influenced by *Ganoderma lucidum* (GL) from one-month biodegradation assay, across media. GLM showed that mass loss percentage is significant (P = 0.0024). Significant differences between plastics (P = 0.0055) are indicated by different letters (Tukey's Studentized Range). There was a significant interaction between Plastic*Media at P = 0.0144. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.0024 ***
Plastic	P = 0.0055 ***
Media	P = 0.0472 ***
Plastic*Media	P = 0.0144 ***

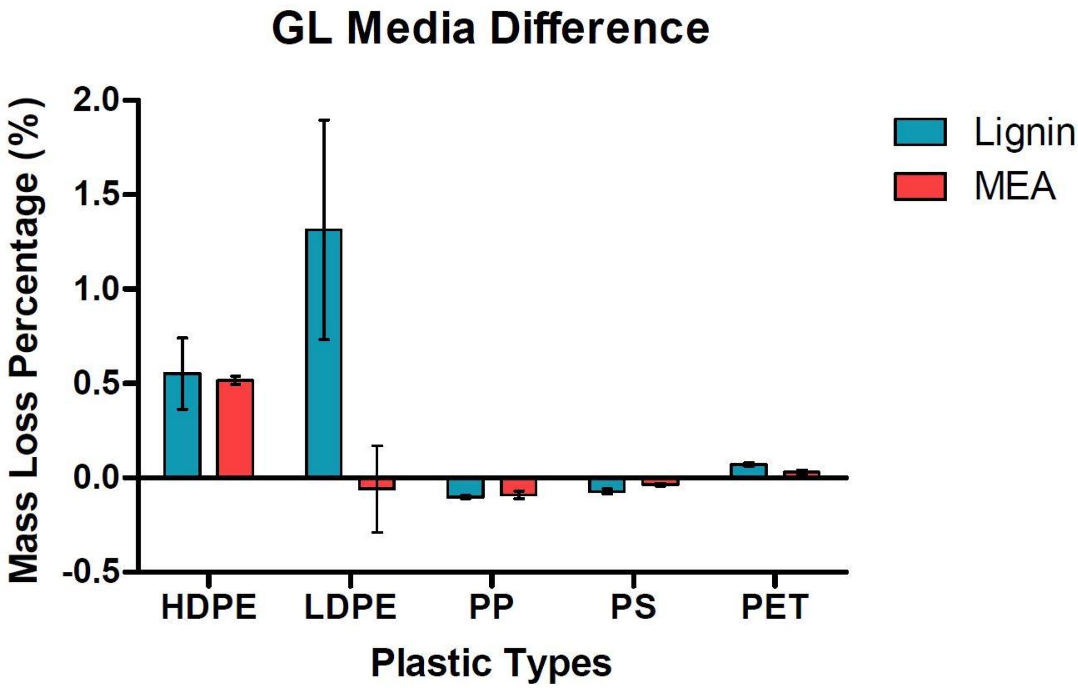


Figure 13: Mean percent mass loss ($\pm\ SE$) of plastic types influenced by *Ganoderma lucidum* (GL) separated by media. GLM and Tukey's Studentized Range showed a significant difference between media (P = 0.0472). There was a significant interaction between Plastic*Media at P = 0.0144. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

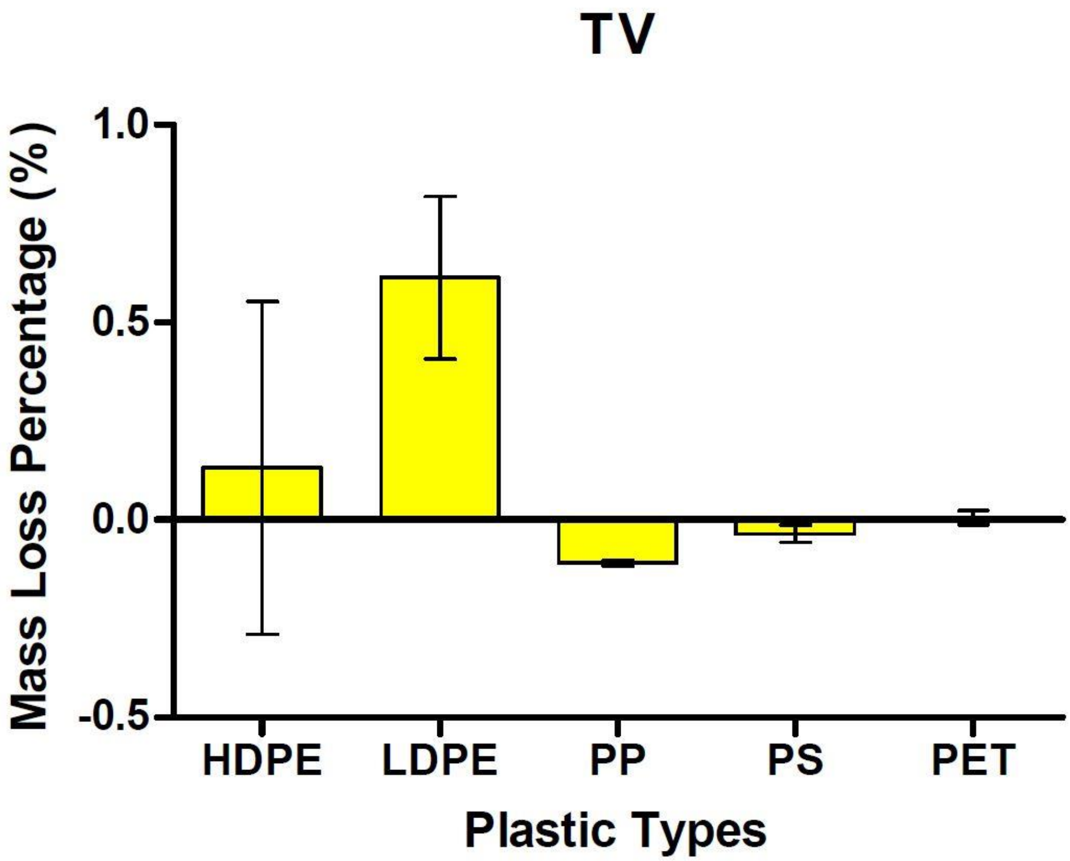


Figure 14: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Trametes versicolor* (TV) from one-month biodegradation assay, across media. GLM and Tukey's showed that mass loss percentage is not significant (P = 0.3230), nor dependent on the type of plastic (P = 0.1658). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.3230
Plastic	P = 0.1658
Media	P = 0.2033
Plastic*Media	P = 0.6943

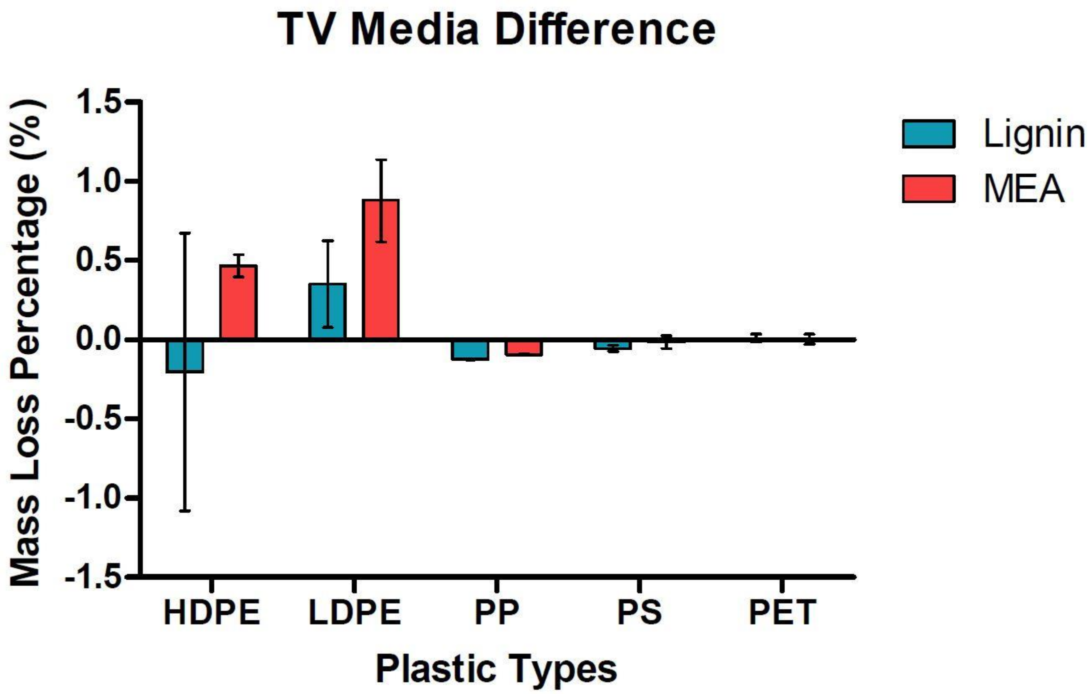


Figure 15: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Trametes versicolor* (TV) from one-month biodegradation assay. GLM and Tukey's showed that mass loss percentage is not significant (P = 0.3230), nor dependent on the type of media (P = 0.2033). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

Three-Month Biodegradation Assay

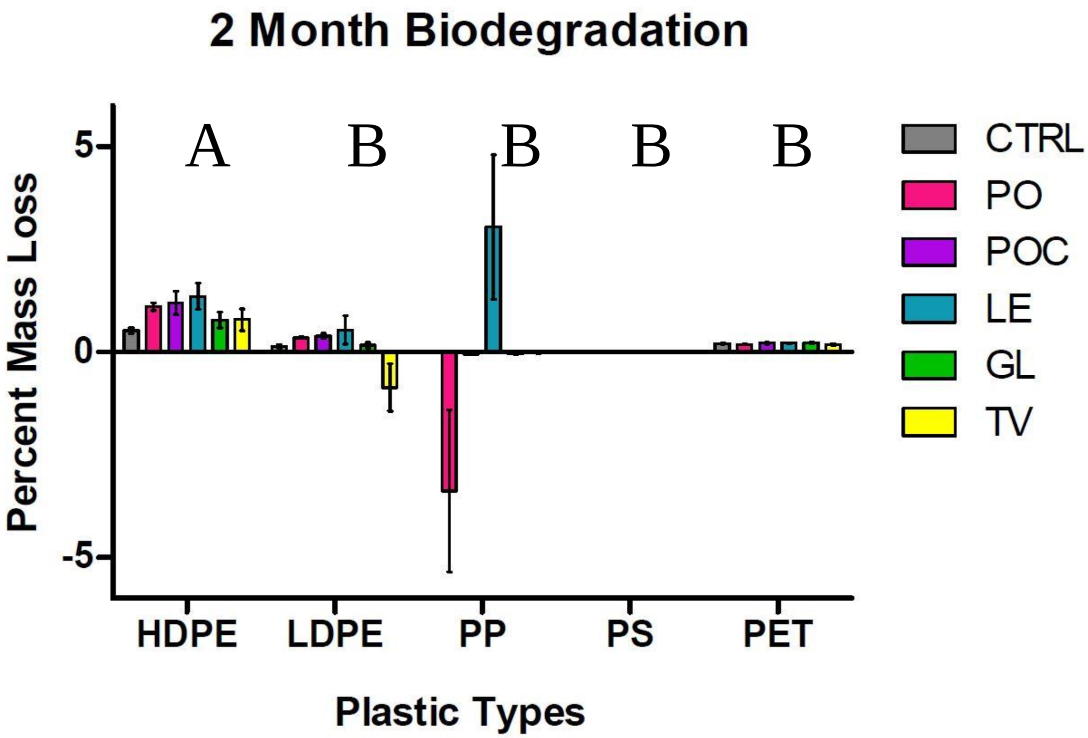


Figure 16: Mean percent mass loss ($\pm SE$) of all plastics by all fungal species from the two-month samples across media. GLM analysis shows the percent mass loss is significant (P < 0.0001), and is dependent on the fungal species (P < 0.0001) and the type of plastic (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). PO = *Pleurotus ostreatus*; POC = *Pleurotus ostreatus columbinus*; LE = *Lentinula edodes*; GL = *Ganoderma lucidum*; TV = *Trametes versicolor*; HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P < 0.0001 ***
Plastic	P < 0.0001 ***
Fungi*Plastic	P < 0.0001 ***
Media	P = 0.6411
Fungi*Media	P < 0.0001 ***
Plastic*Media	P = 0.5395
Fungi*Plastic*Media	P < 0.0001 ***

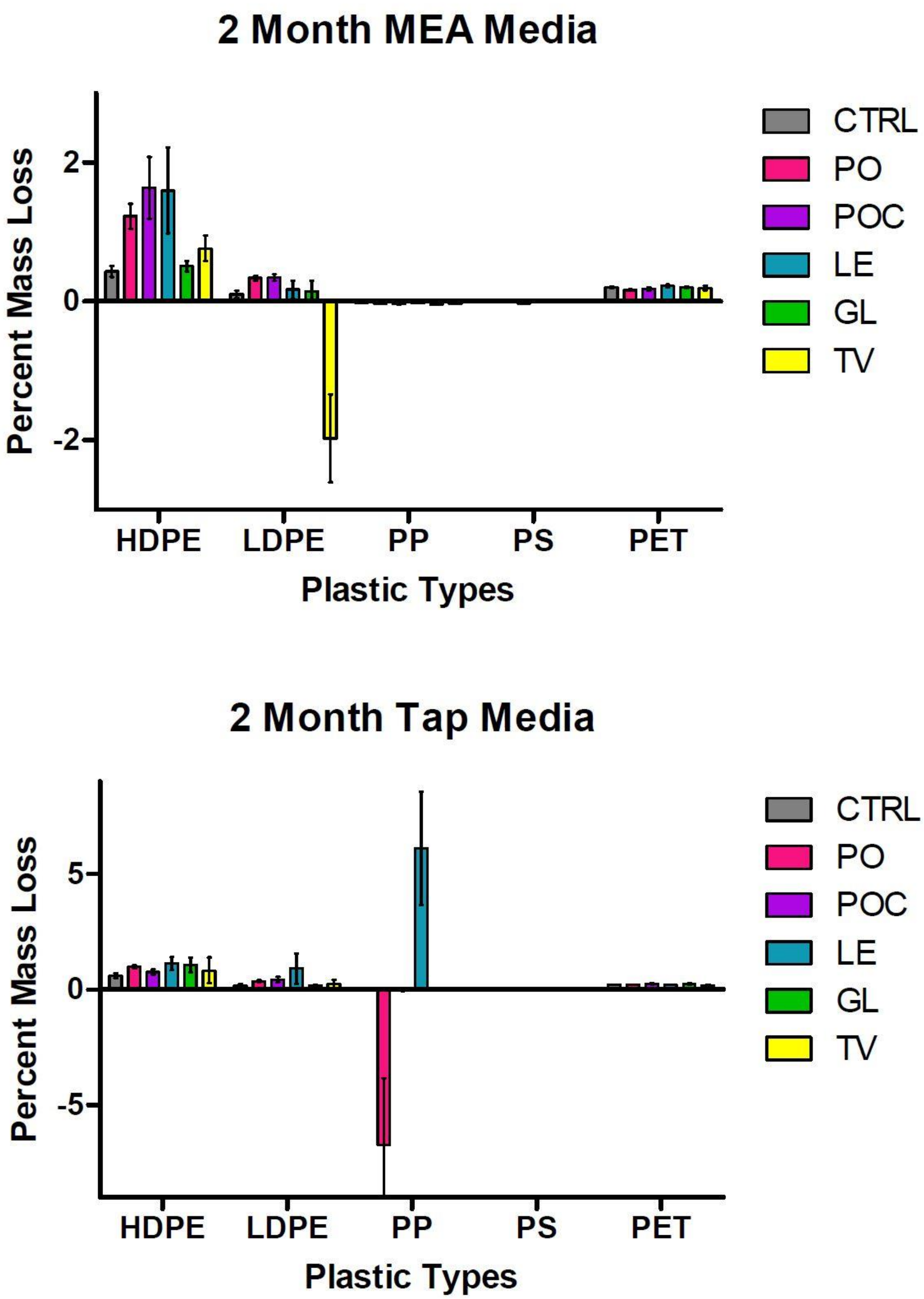


Figure 17: Mean percent mass loss ($\pm$ SE) of all plastics by all fungal species from two-month samples separated by media. GLM analysis shows the percent mass loss is significant (P < 0.0001), but not dependent on the type of media (P = 0.6411). PO = *Pleurotus ostreatus*; POC = *Pleurotus ostreatus columbinus*; LE = *Lentinula edodes*; GL = *Ganoderma lucidum*; TV = *Trametes versicolor*; HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

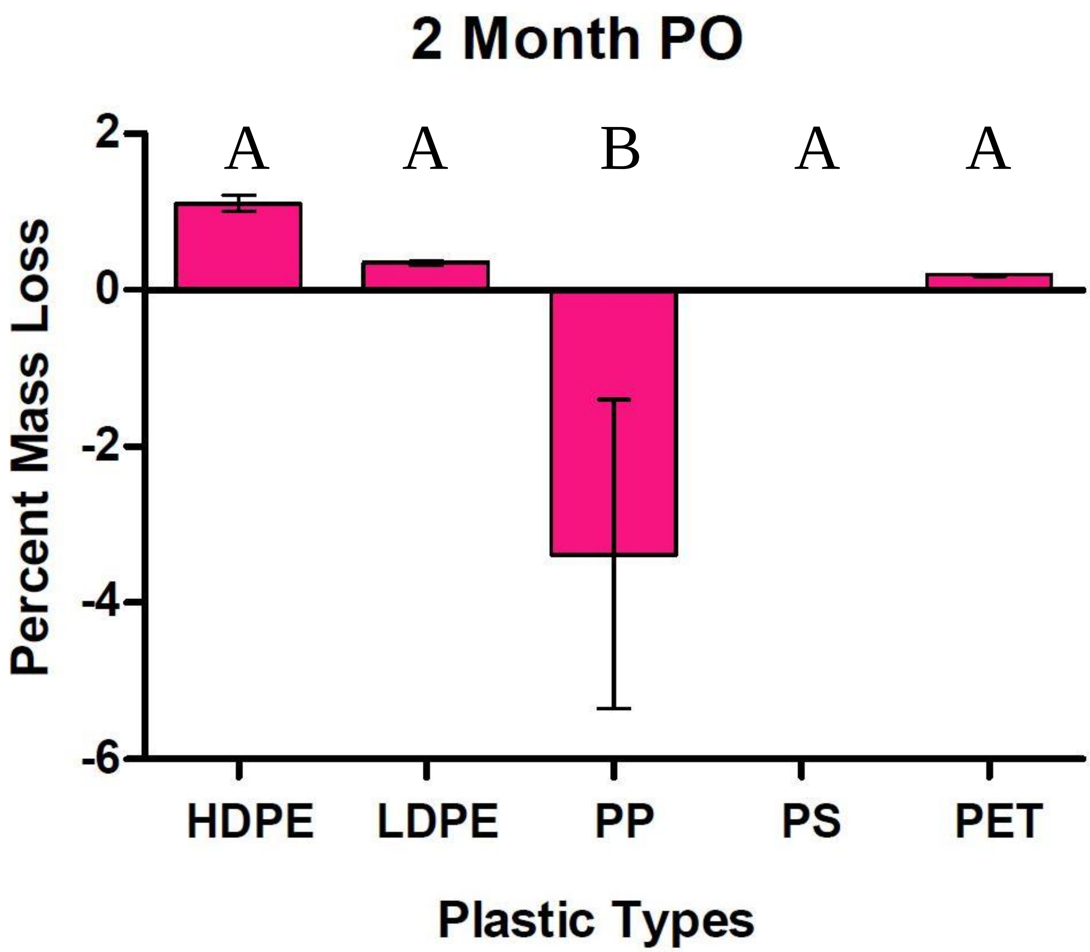


Figure 18: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus* (PO) from two-month samples, across media. GLM analysis shows that the percent mass loss is significant (P = 0.0003), and is dependent on the type of plastic (P = 0.0008). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). There is a significant interaction between Plastic*Media at P = 0.0044. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.0003 ***
Plastic	P = 0.0008 ***
Media	P = 0.0268 ***
Plastic*Media	P = 0.0044 ***

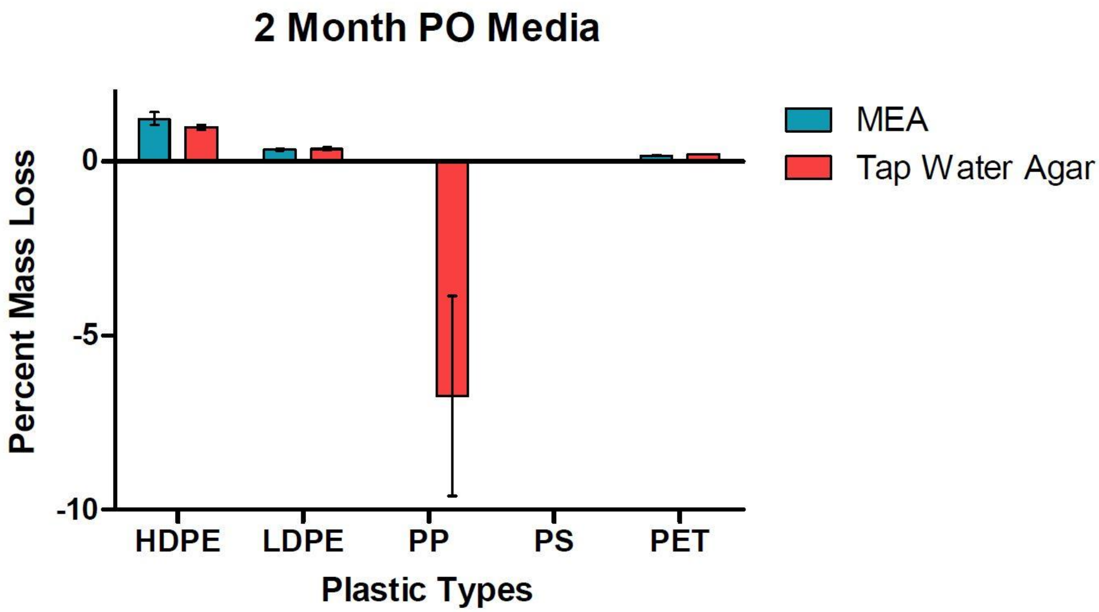


Figure 19: Mean percent mass loss ($\pm SE$) of plastic types influenced by *Pleurotus ostreatus* (PO) from two-month samples separated by media. GLM analysis shows percent mass loss is significant (P = 0.0003), and dependent on media (P = 0.0268). There was a significant interaction between Plastic*Media at P = 0.0044. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

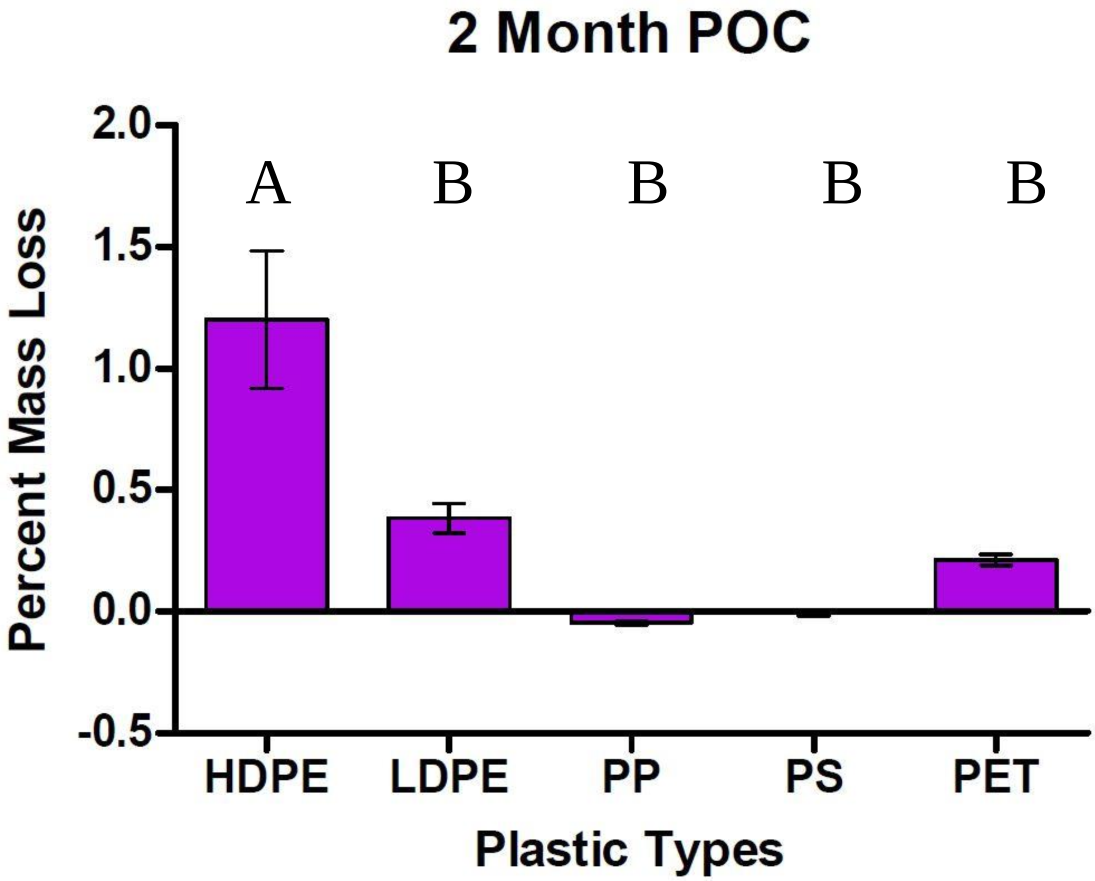


Figure 20: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus columbinus* (POC) from two-month samples, across media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). There was a significant interaction between Plastic*Media at P = 0.0207. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P < 0.0001 ***
Media	P = 0.1592
Plastic*Media	P = 0.0207 ***

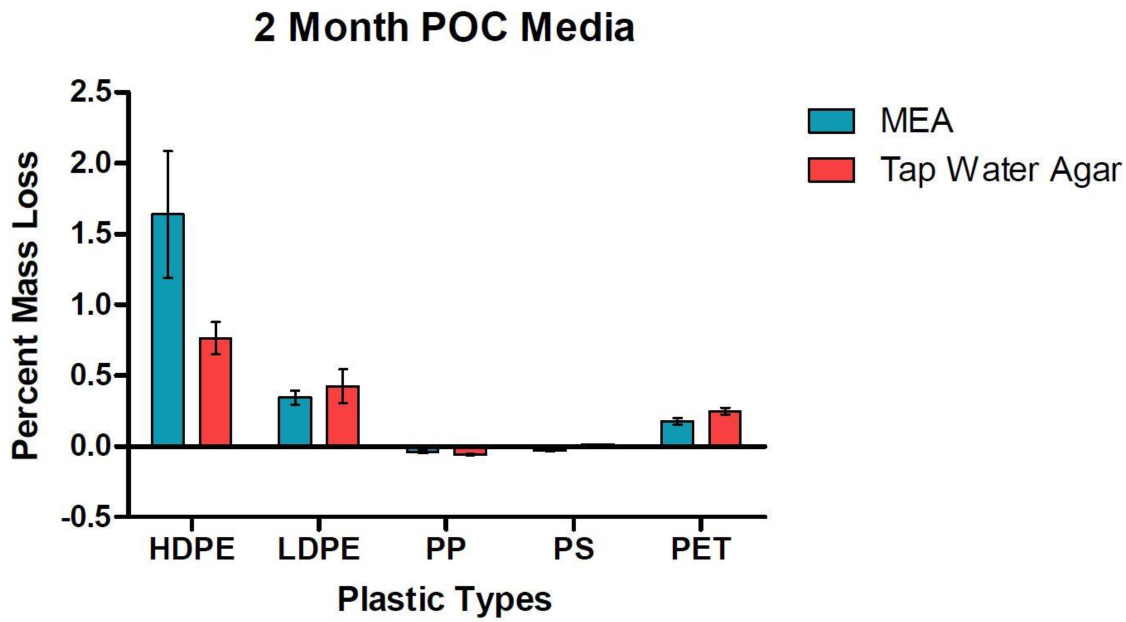


Figure 21: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus columbinus* (POC) from two-month samples separated by media. GLM analysis shows percent mass loss is significant (P < 0.0001), but it not dependent on media (P = 0.1592). There was a significant interaction between Plastic*Media at P = 0.0207. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

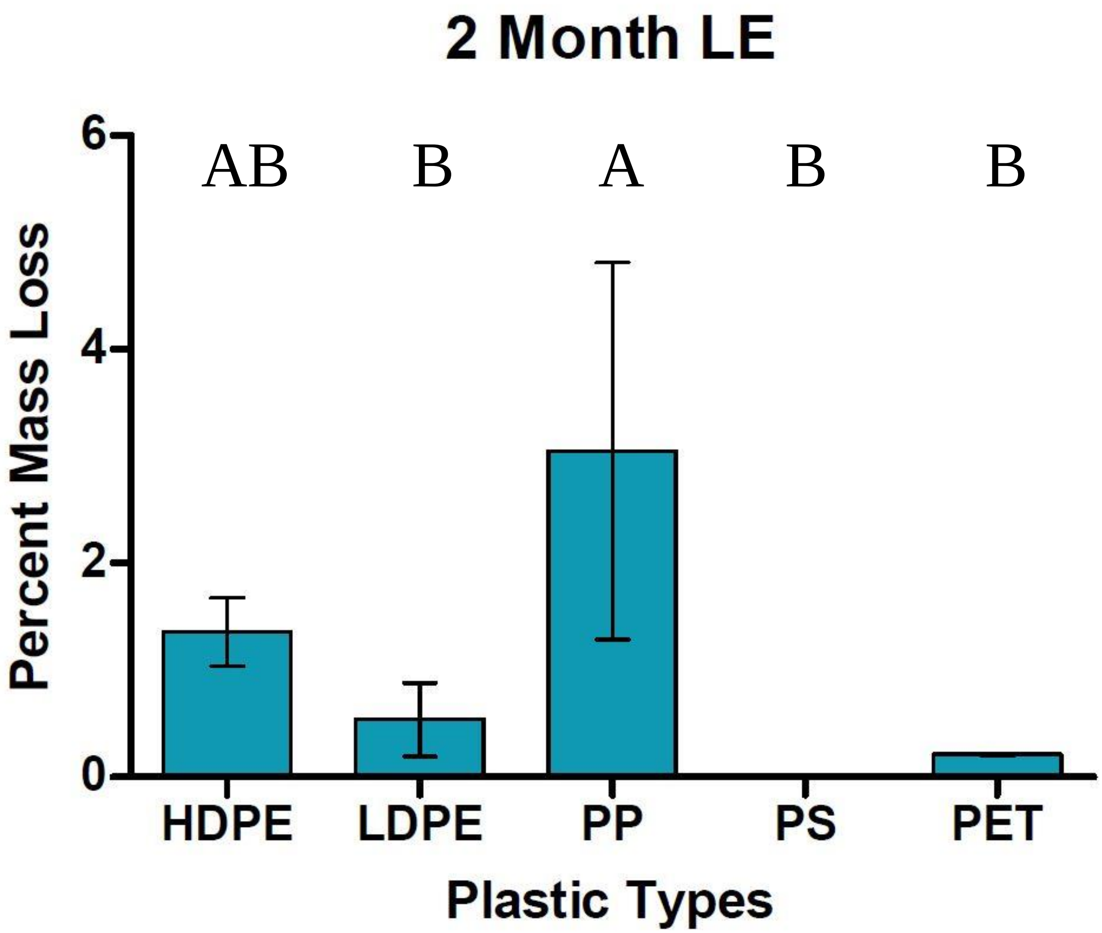


Figure 22: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Lentinula edodes* (LE) from two-month samples, across media. GLM analysis shows percent mass loss is significant (P = 0.0013) and is dependent on the type of plastic (P = 0.0103). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). There was a significant interaction between Plastic*Media at P = 0.0039. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.0013 ***
Plastic	P = 0.0103 ***
Media	P = 0.0258 ***
Plastic*Media	P = 0.0039 ***

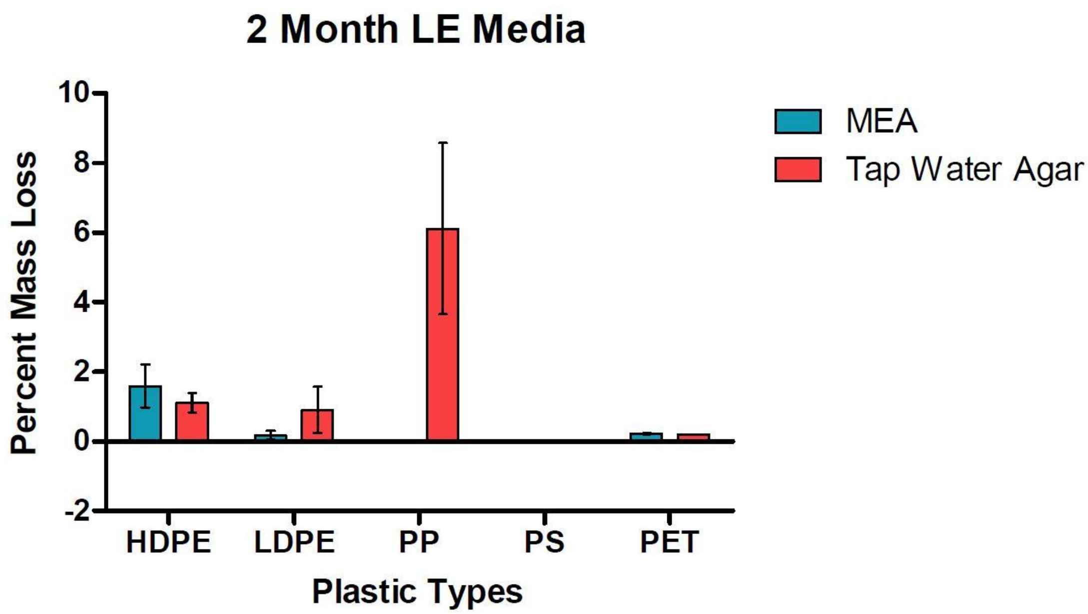


Figure 23: Mean percent mass loss ($\pm$ SE) of plastic types influenced by *Lentinula edodes* (LE) from two-month samples separated by media. GLM analysis shows percent mass loss is significant (P = 0.0013) and is dependent on the type of media (P = 0.0258). There was a significant interaction between Plastic*Media at P = 0.0039. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

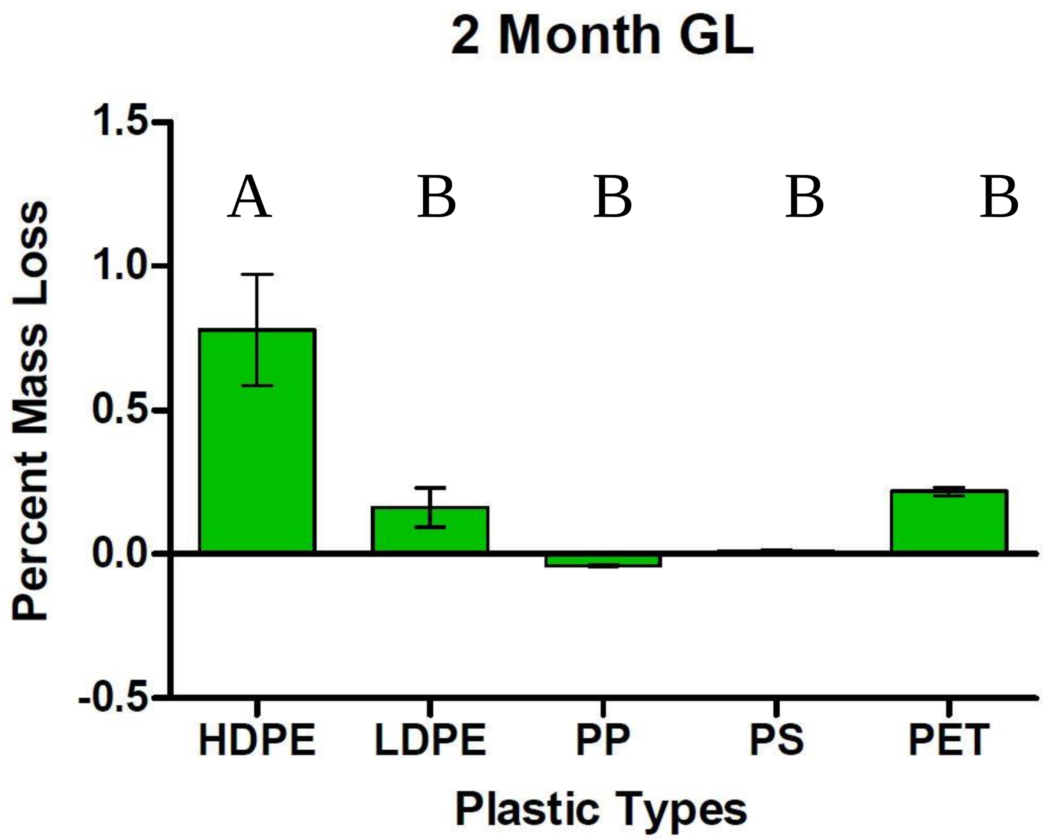


Figure 24: Mean percent mass loss ($\pm SE$) of plastic types influenced by *Ganoderma lucidum* (GL) from two-month samples, across media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P < 0.0001 ***
Media	P = 0.1059
Plastic*Media	P = 0.1303

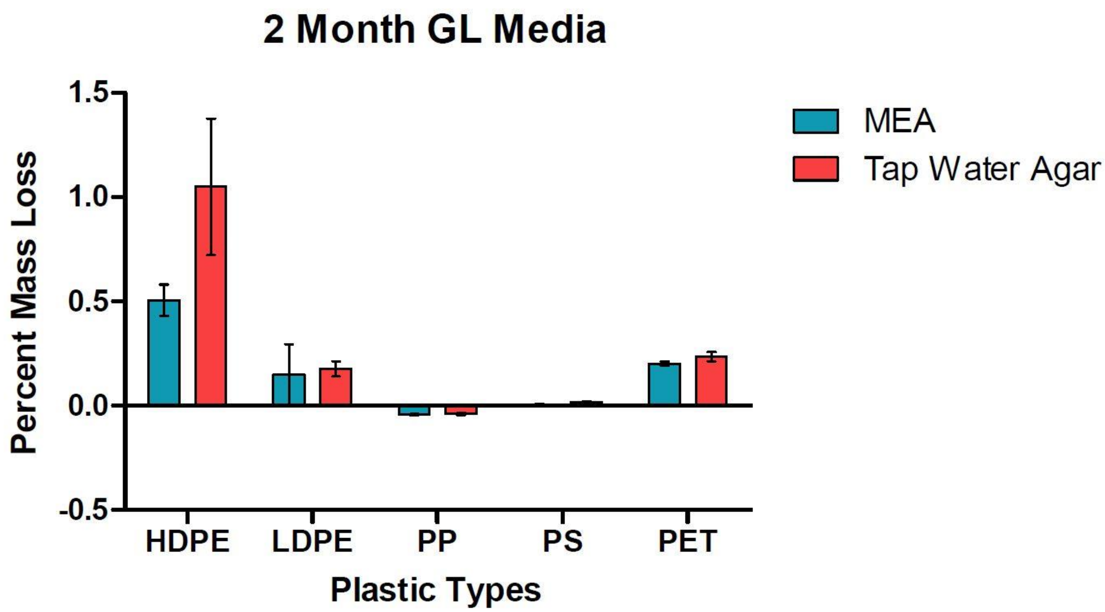


Figure 25: Mean percent mass loss ($\pm SE$) of plastic types influenced by *Ganoderma lucidum* (GL) from two-month samples separated by media. GLM analysis shows percent mass loss is significant ($P < 0.0001$) but is not dependent on the type of media ($P = 0.1059$). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

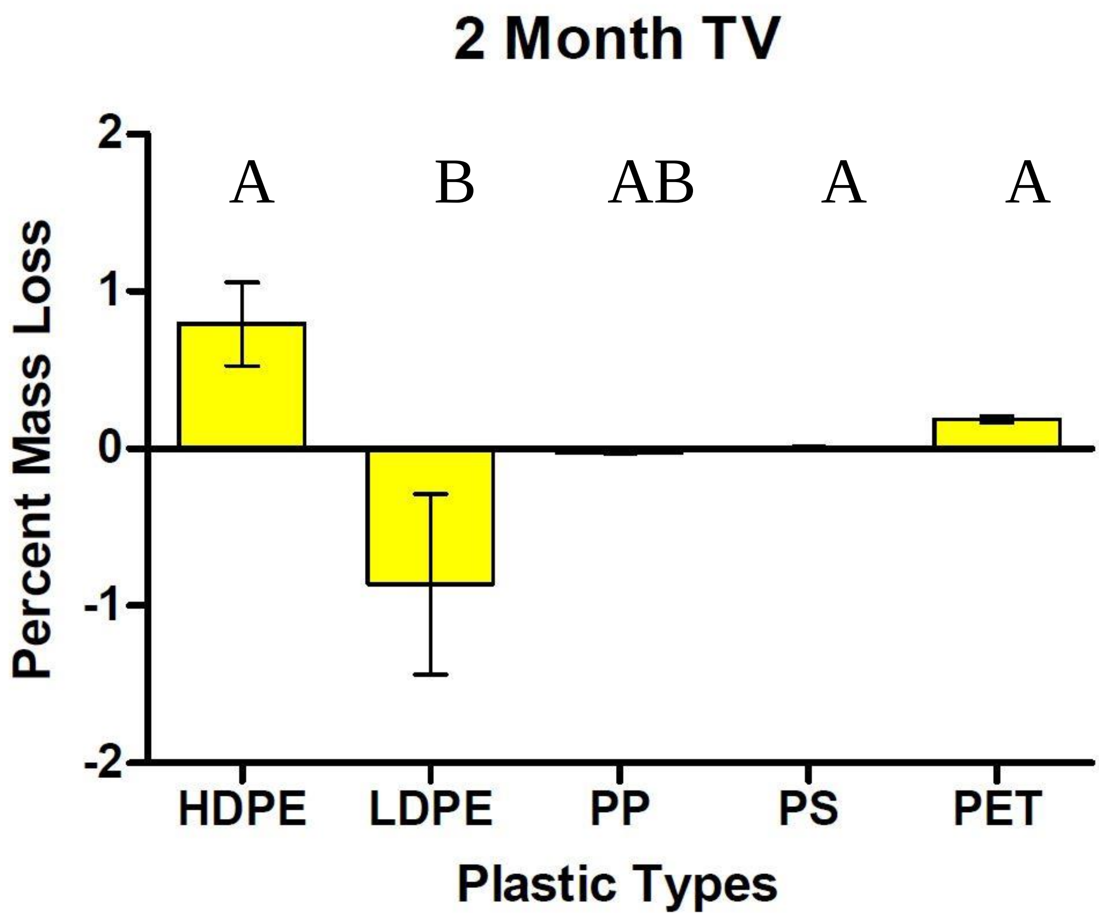


Figure 26: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Trametes versicolor* (TV) from two-month samples, across media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P = 0.0003). There was a significant interaction between Plastic*Media at P = 0.0020. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P = 0.0003 ***
Media	P = 0.0184 ***
Plastic*Media	P = 0.0020 ***

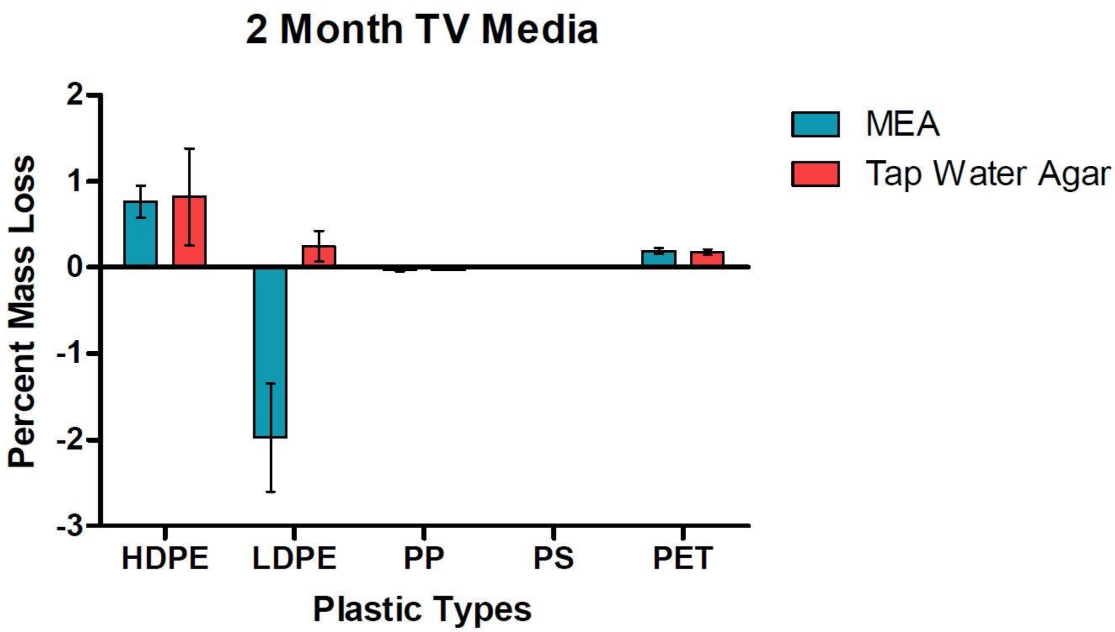


Figure 27: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Trametes versicolor* (TV) from two-month samples separated by media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of media (P = 0.0184). There was a significant interaction between Plastic*Media at P = 0.0020. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

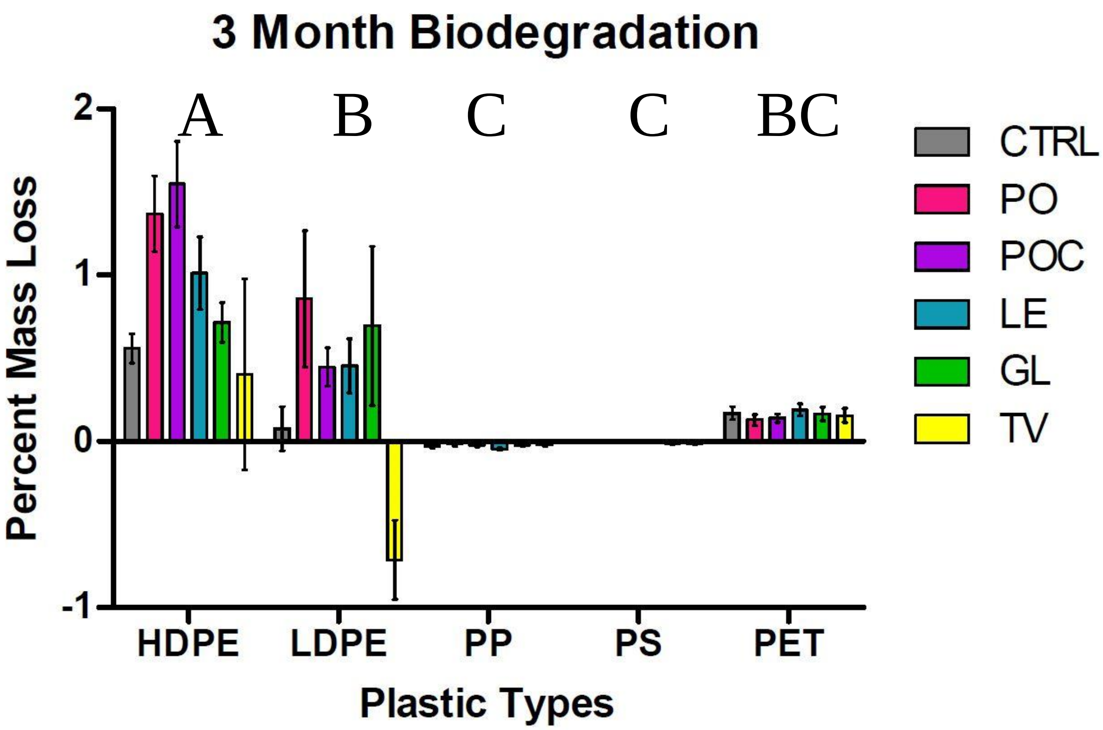


Figure 28: Mean percent mass loss ($\pm SE$) of all plastics from the three-month samples across all fungi and media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the fungal species (P < 0.0001) and plastic type (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). PO = *Pleurotus ostreatus*; POC = *Pleurotus ostreatus columbinus*; LE = *Lentinula edodes*; GL = *Ganoderma lucidum*; TV = *Trametes versicolor*; HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P < 0.0001 ***
Plastic	P < 0.0001 ***
Fungi*Plastic	P < 0.0001 ***
Media	P = 0.6368
Fungi*Media	P = 0.0286 ***
Plastic*Media	P = 0.0169 ***
Fungi*Plastic*Media	P = 0.0128 ***

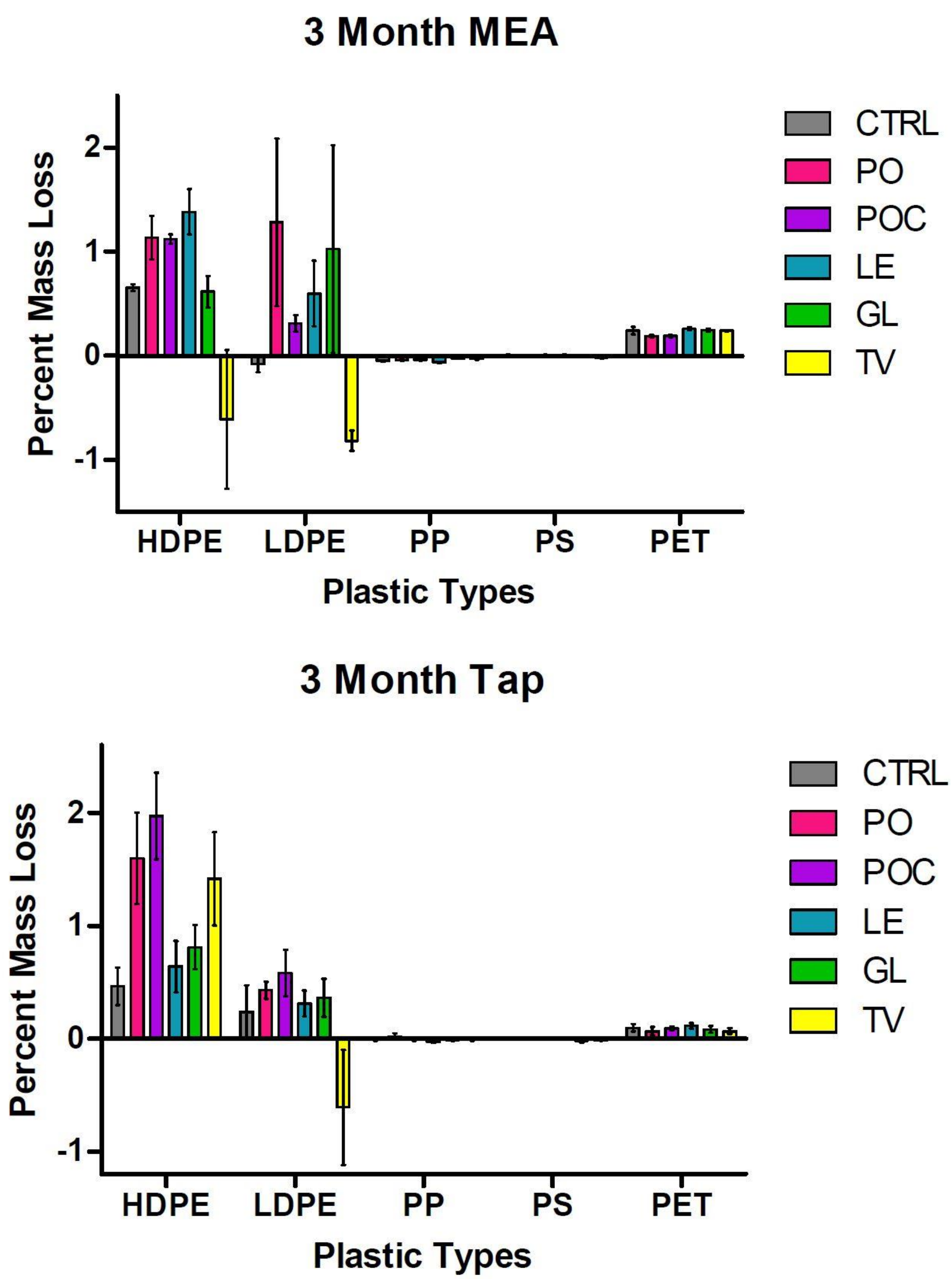


Figure 29: Mean percent mass loss ($\pm SE$) of all plastics by all fungi from three-month samples, separated by media. GLM analysis shows percent mass loss is significant ($P < 0.0001$) but is not dependent on the type of media ($P = 0.6368$). PO = *Pleurotus ostreatus*; POC = *Pleurotus ostreatus columbinus*; LE = *Lentinula edodes*; GL = *Ganoderma lucidum*; TV = *Trametes versicolor*; HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate.

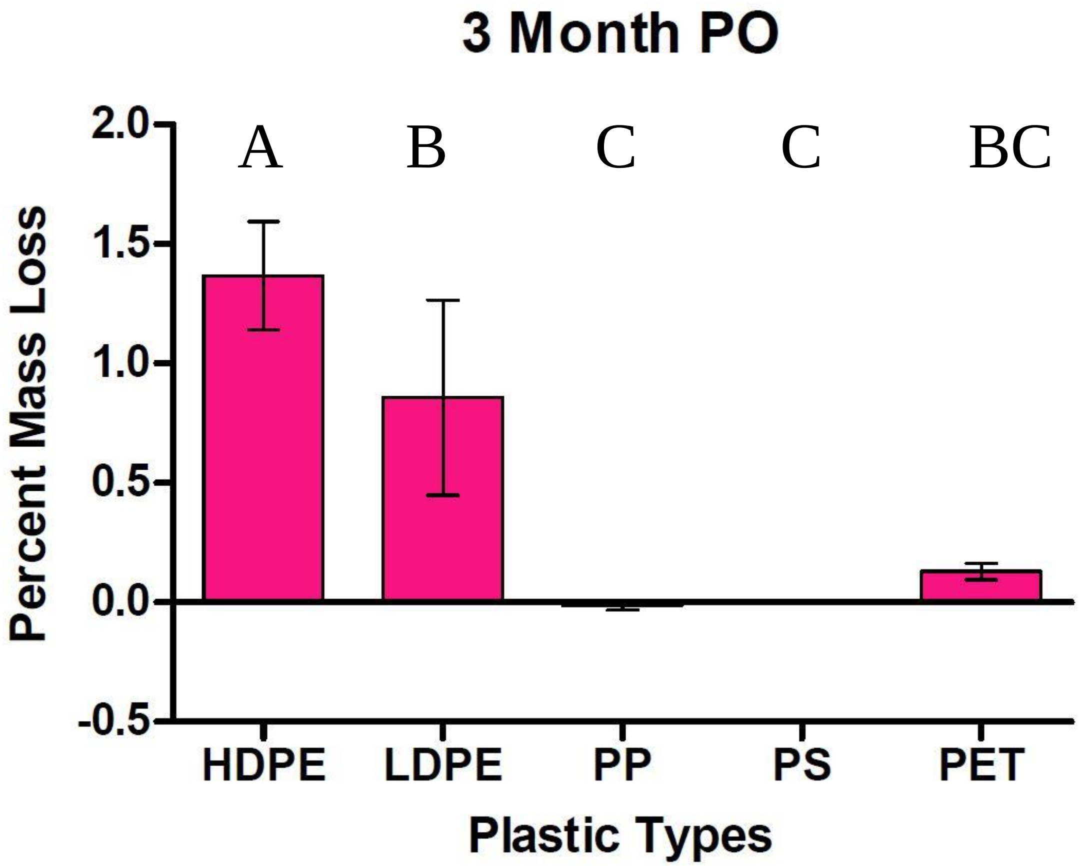


Figure 30: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus* (PO) from three-month samples, across media. GLM analysis shows the percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P < 0.0001 ***
Media	P = 0.6448
Plastic*Media	P = 0.7150

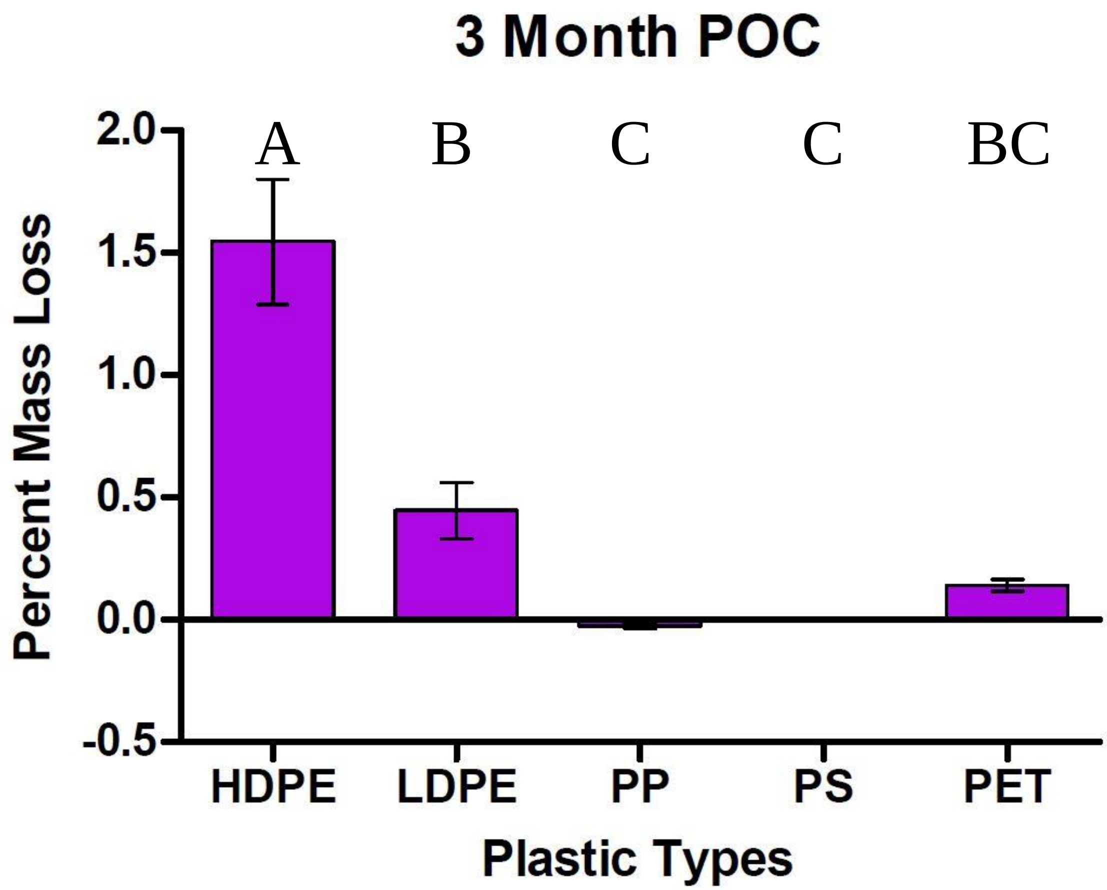


Figure 31: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus columbinus* (POC) from three-month samples, across media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P < 0.0001 ***
Media	P = 0.0594
Plastic*Media	P = 0.0452 ***

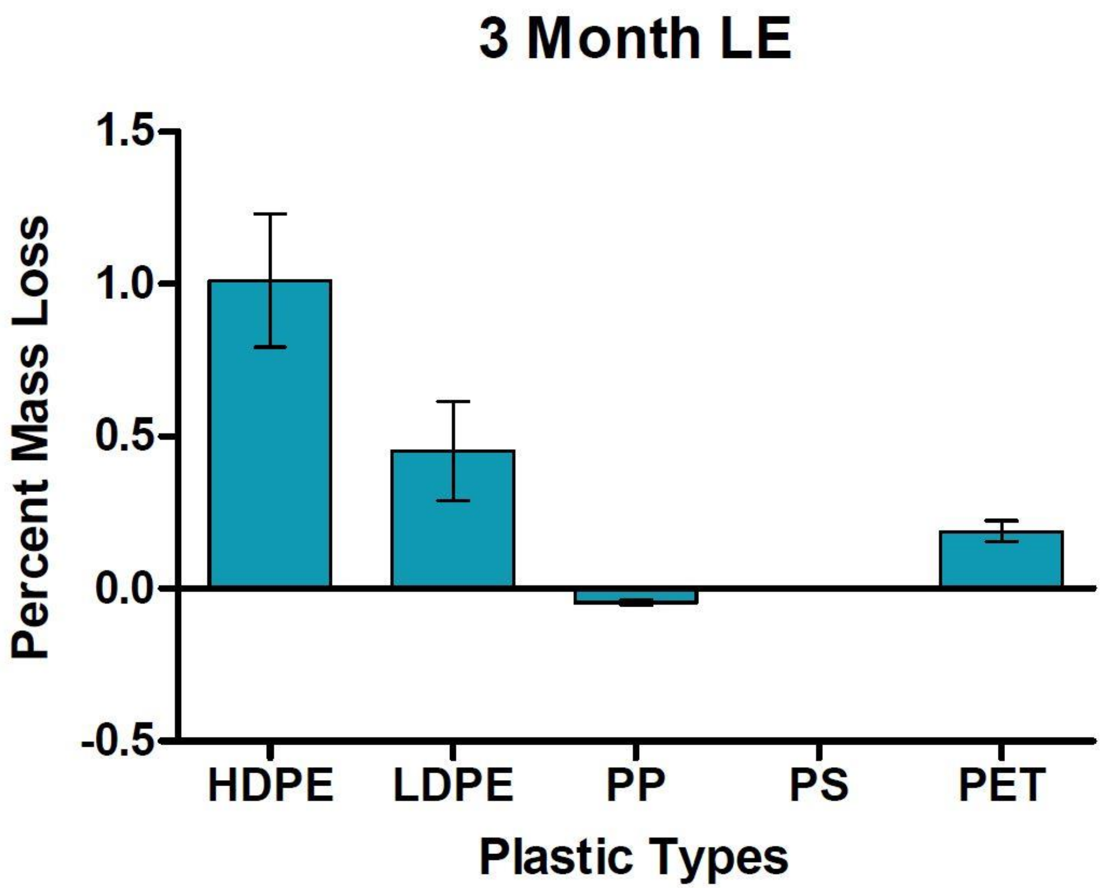


Figure 32: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Lentinula edodes* (LE) from three-month samples, across media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P < 0.0001 ***
Media	P = 0.0452 ***
Plastic*Media	P = 0.0346 ***

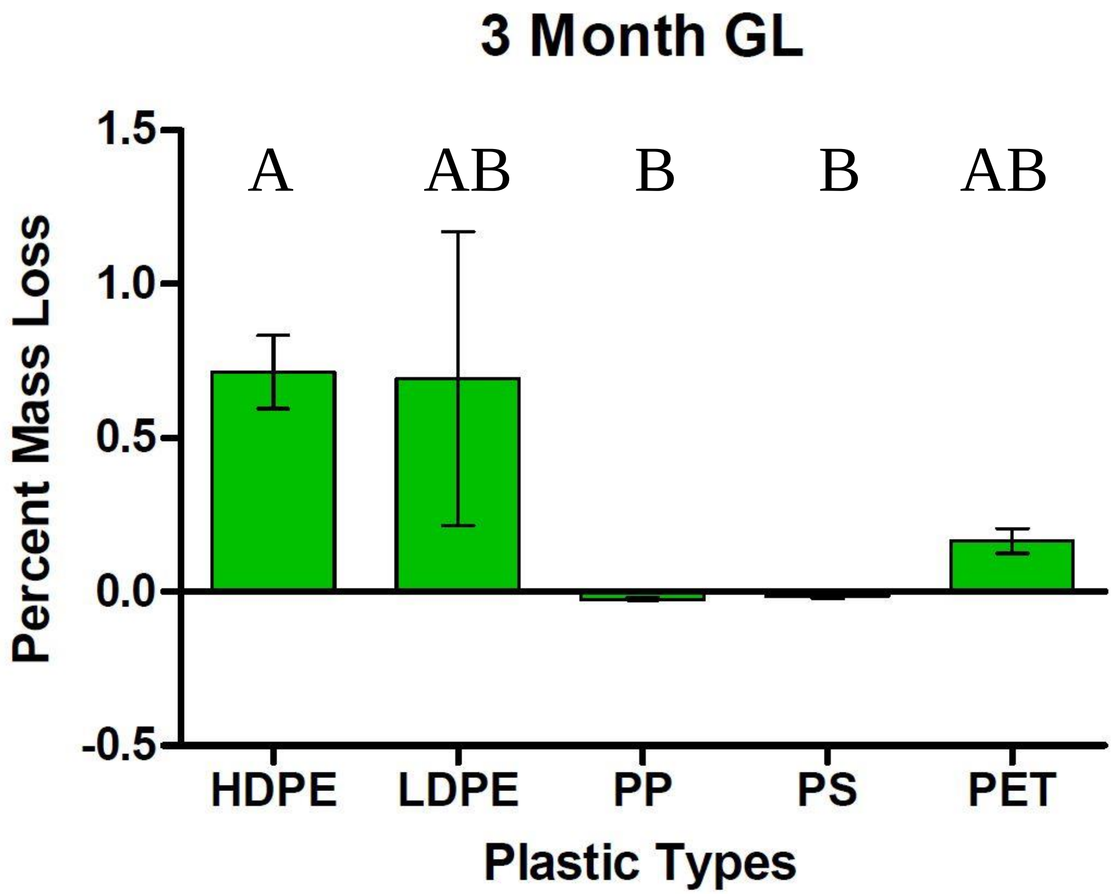


Figure 33: Mean percent mass loss ($\pm SE$) of plastic types influenced by *Ganoderma lucidum* (GL) from three-month samples, across media. GLM analysis shows percent mass loss is not significant (P = 0.0625), but is dependent on the type of plastic (P = 0.0016). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.0625
Plastic	P = 0.0016 ***
Media	P = 0.5685
Plastic*Media	P = 0.9601

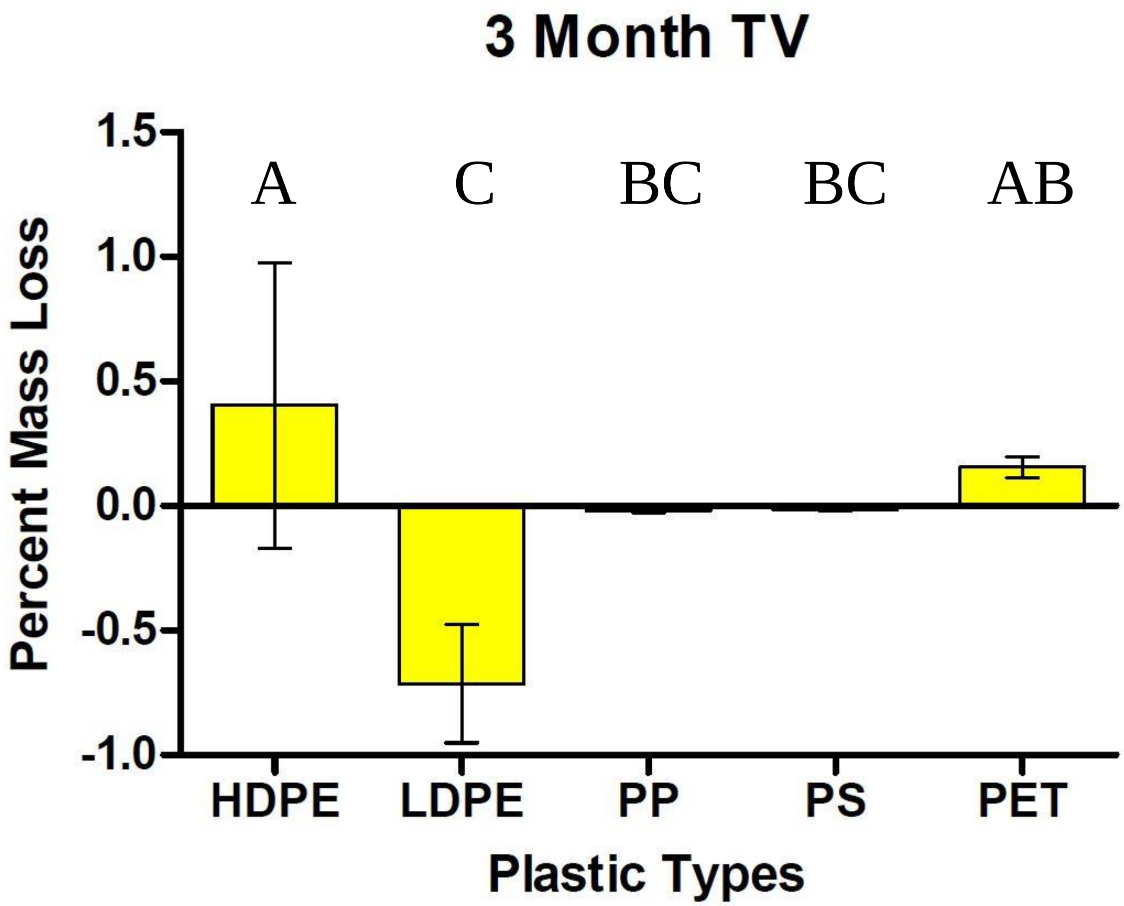


Figure 34: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Trametes versicolor* (TV) from three-month samples, across media. GLM analysis shows percent mass loss is significant (P < 0.0001) and is dependent on the type of plastic (P = 0.0002). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Plastic	P = 0.0002 ***
Media	P = 0.0407 ***
Plastic*Media	P = 0.0122 ***

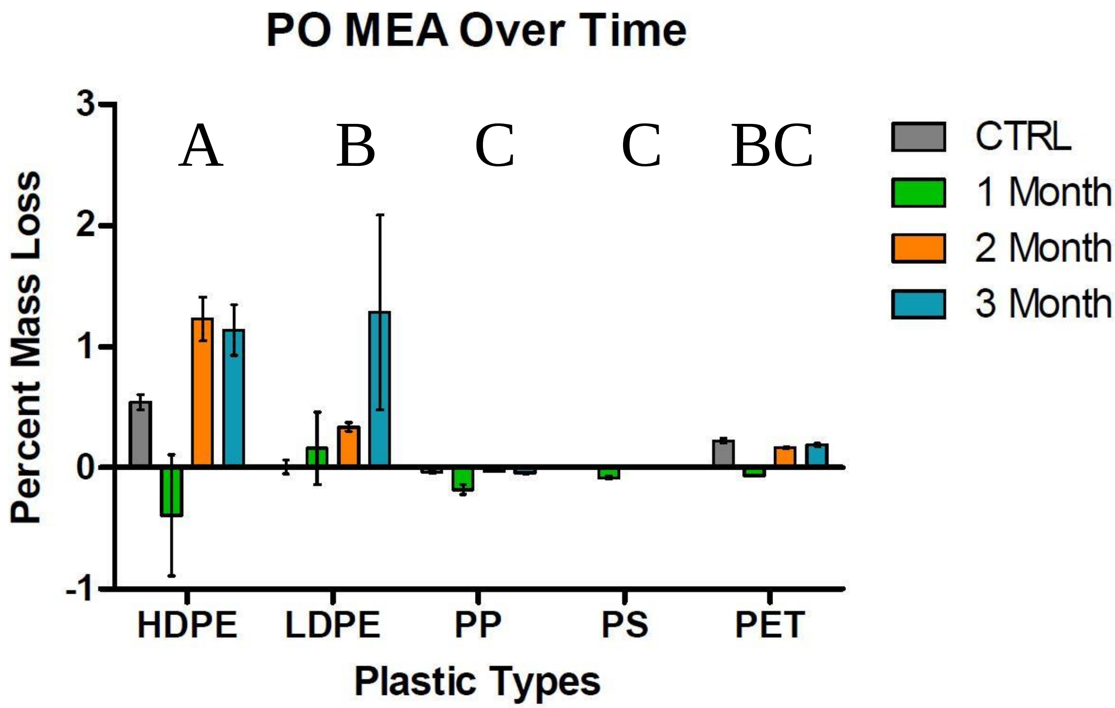


Figure 35: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus* (PO) in MEA media over three months. GLM analysis shows percent mass loss is significant (P = 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P = 0.0025 ***
Plastic	P < 0.0001 ***
Fungi*Plastic	P = 0.0043 ***
Month	P = 0.2966
Fungi*Month	P = 0.3514
Plastic*Month	P = 0.5687
Fungi*Plastic*Month	P = 0.0985

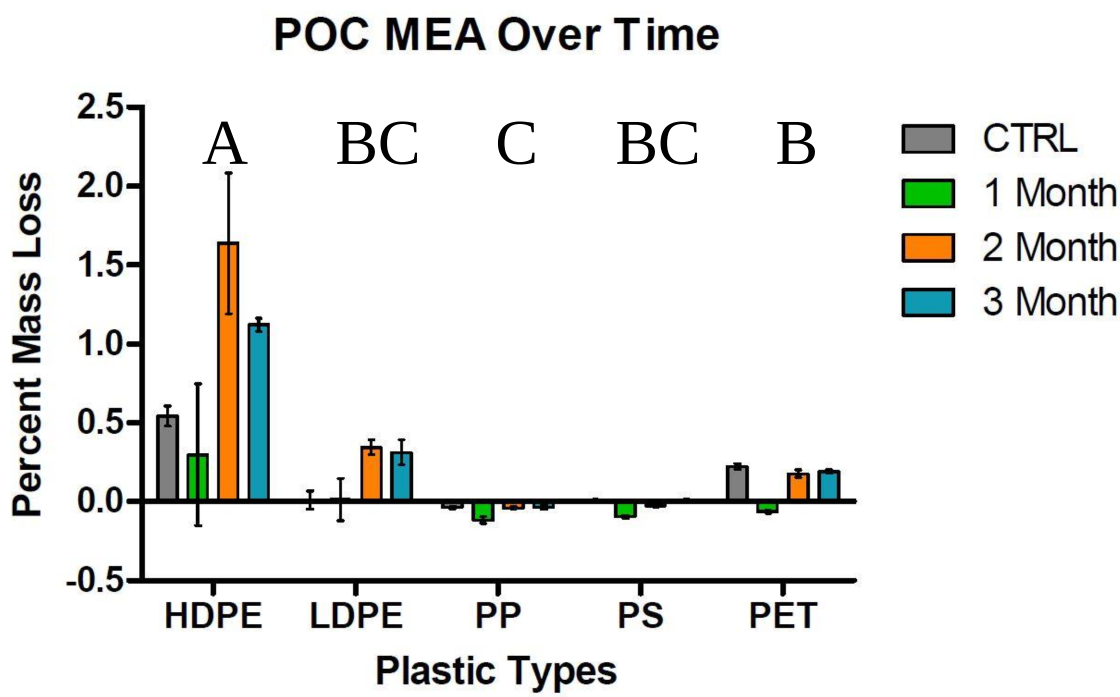


Figure 36: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus columbinus* (POC) in MEA media over three months. GLM analysis shows percent mass loss is significant (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P < 0.0001 ***
Plastic	P < 0.0001 ***
Fungi*Plastic	P < 0.0001 ***
Month	P = 0.3466
Fungi*Month	P = 0.2529
Plastic*Month	P = 0.7169
Fungi*Plastic*Month	P = 0.0367 ***

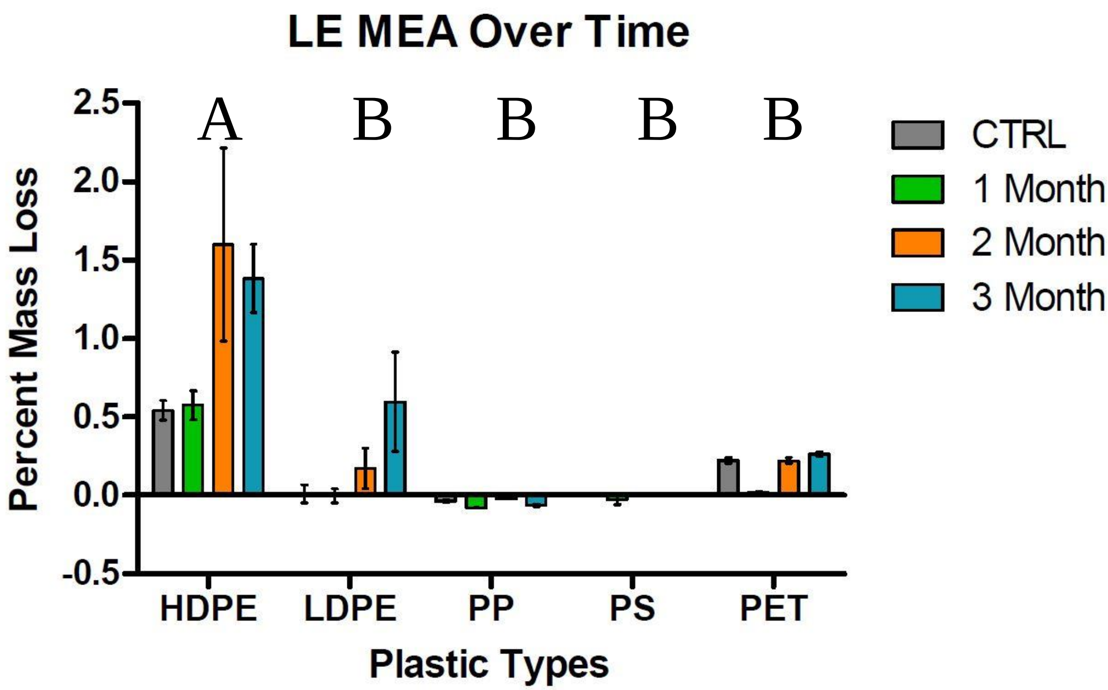


Figure 37: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Lentinula edodes* (LE) in MEA media over three months. GLM analysis shows percent mass loss is significant (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P = 0.0001 ***
Plastic	P < 0.0001 ***
Fungi*Plastic	P = 0.0006 ***
Month	P = 0.7381
Fungi*Month	P = 0.8389
Plastic*Month	P = 0.9718
Fungi*Plastic*Month	P = 0.3063

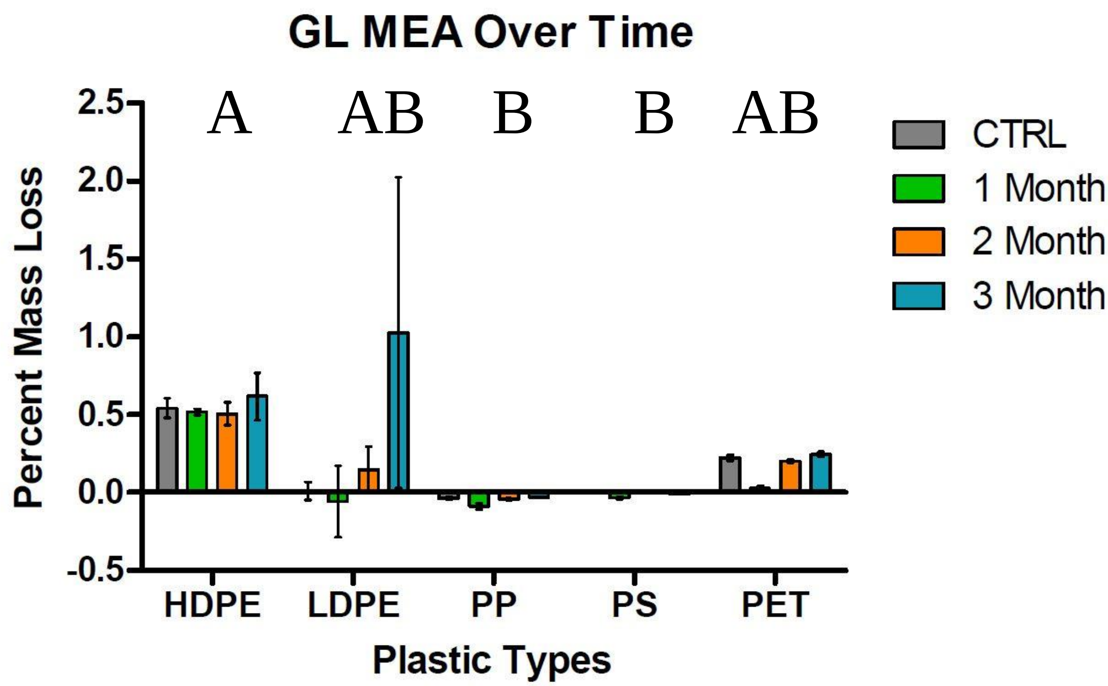


Figure 38: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Ganoderma lucidum* (GL) in MEA media over three months. GLM analysis shows percent mass loss is not significant (P = 0.0870). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.0870
Fungi	P = 0.2612
Plastic	P = 0.0056 ***
Fungi*Plastic	P = 0.3056
Month	P = 0.3000
Fungi*Month	P = 0.3457
Plastic*Month	P = 0.7849
Fungi*Plastic*Month	P = 0.3650

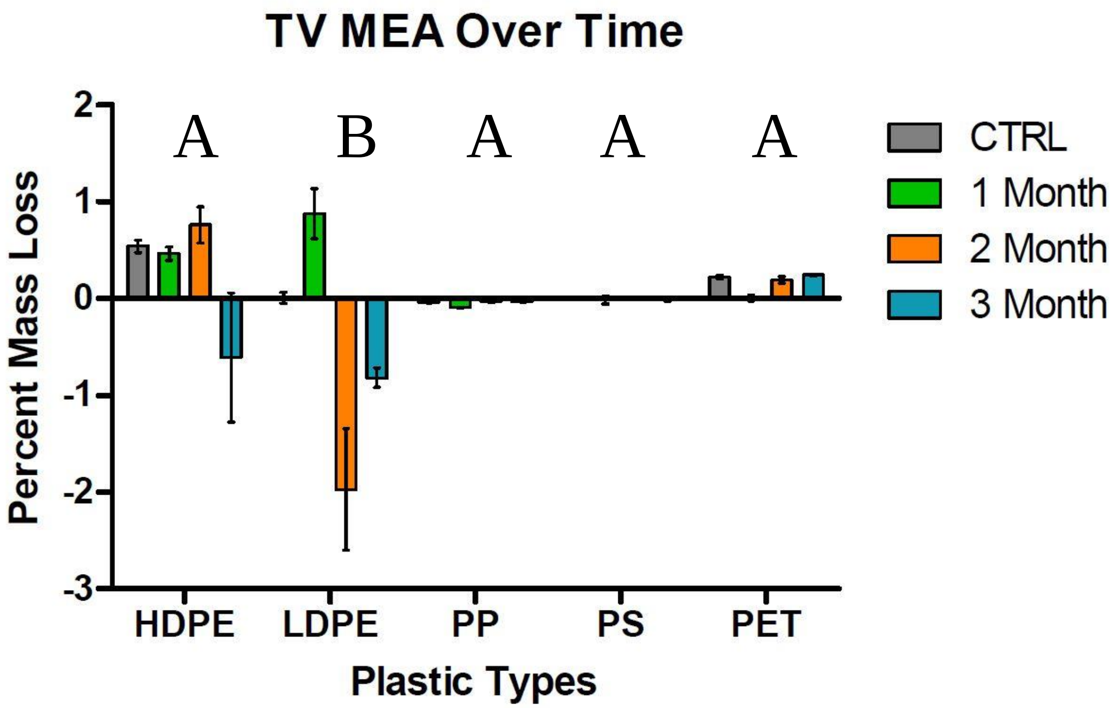


Figure 39: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Trametes versicolor* (TV) in MEA media over three months. GLM analysis shows percent mass loss is significant (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P = 0.0003 ***
Plastic	P < 0.0001 ***
Fungi*Plastic	P < 0.0001 ***
Month	P = 0.8753
Fungi*Month	P = 0.7948
Plastic*Month	P = 0.0245 ***
Fungi*Plastic*Month	P = 0.0007 ***

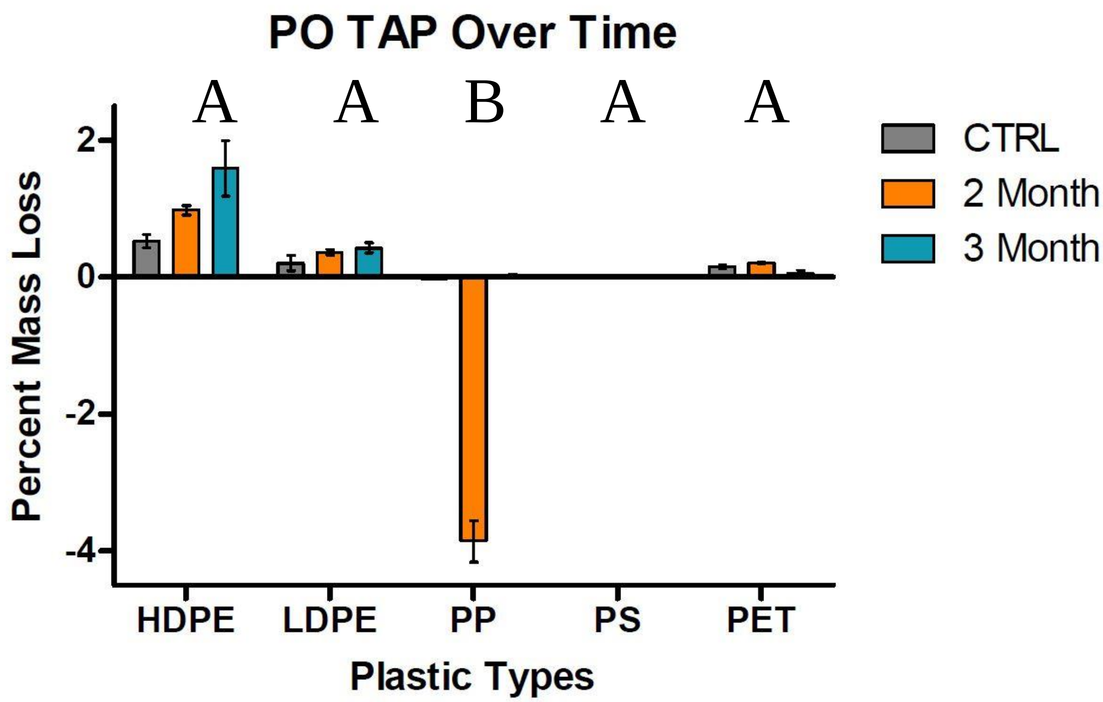


Figure 40: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus* (PO) in tap water agar media over three months. GLM analysis shows percent mass loss is significant (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P = 0.1063
Plastic	P < 0.0001 ***
Fungi*Plastic	P = 0.0006 ***
Month	P = 0.0197 ***
Fungi*Month	P = 0.0146 ***
Plastic*Month	P = 0.0017 ***
Fungi*Plastic*Month	P = 0.0022 ***

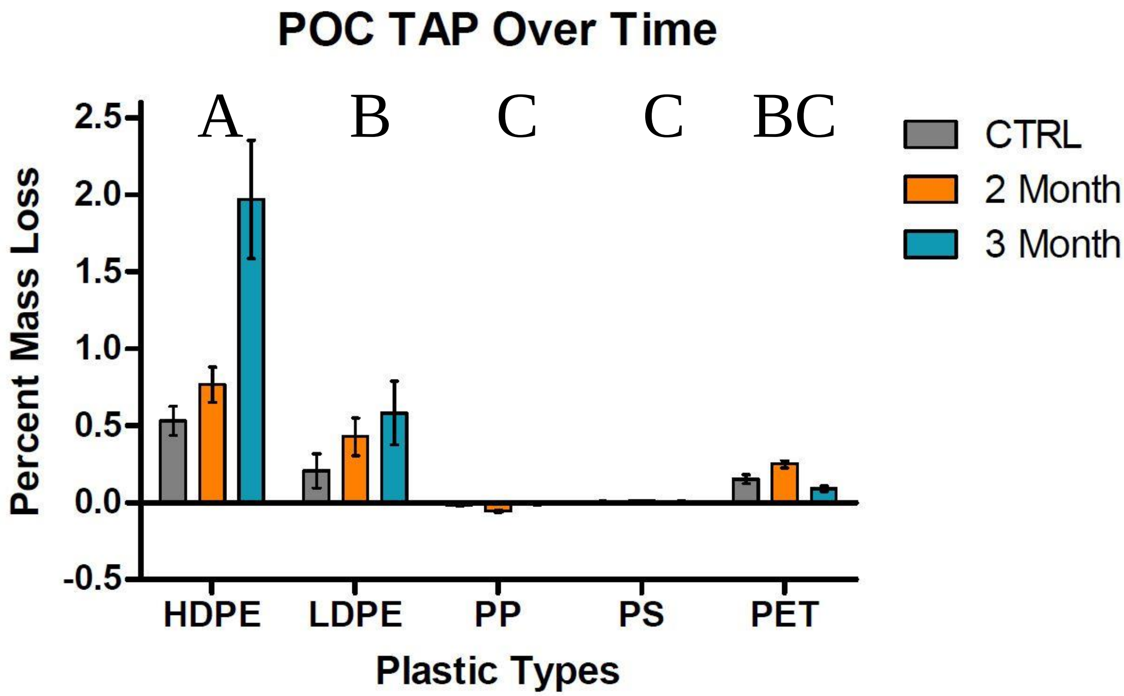


Figure 41: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Pleurotus ostreatus columbinus* (POC) in tap water agar media over three months. GLM analysis shows percent mass loss is significant (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P = 0.0003 ***
Plastic	P < 0.0001 ***
Fungi*Plastic	P < 0.0001 ***
Month	P = 0.0702
Fungi*Month	P = 0.0169 ***
Plastic*Month	P = 0.0071 ***
Fungi*Plastic*Month	P = 0.0013 ***

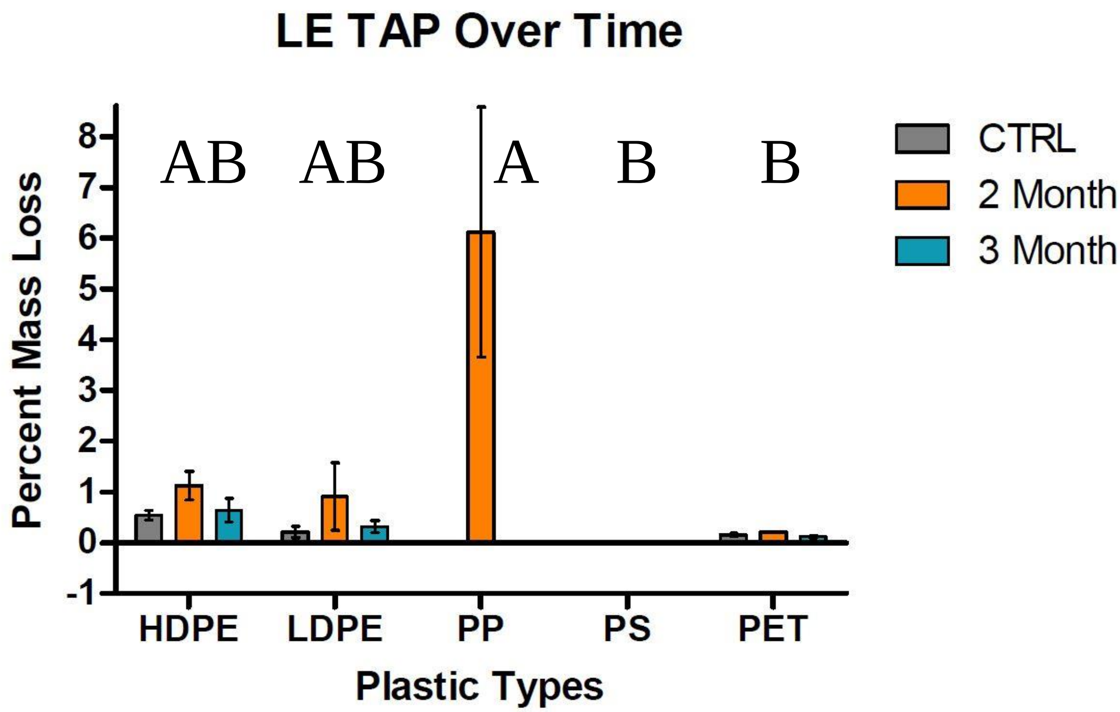


Figure 42: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Lentinula edodes* (LE) in tap water agar media over three months. GLM analysis shows percent mass loss is significant ($P < 0.0001$). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P = 0.0055 ***
Plastic	P = 0.0059 ***
Fungi*Plastic	P = 0.0023 ***
Month	P = 0.0063 ***
Fungi*Month	P = 0.0090 ***
Plastic*Month	P = 0.0023 ***
Fungi*Plastic*Month	P = 0.0017 ***

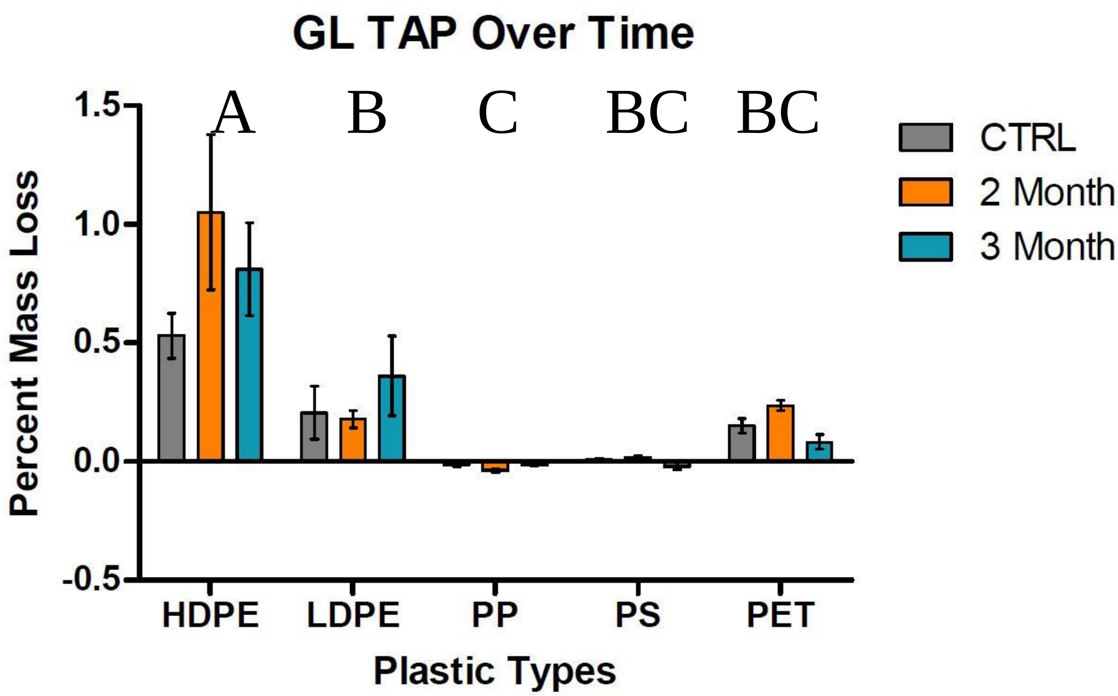


Figure 43: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Ganoderma lucidum* (GL) in tap water agar media over three months. GLM analysis shows percent mass loss is significant (P < 0.0001). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P < 0.0001 ***
Fungi	P = 0.0933
Plastic	P < 0.0001 ***
Fungi*Plastic	P = 0.0861
Month	P = 0.4452
Fungi*Month	P = 0.9273
Plastic*Month	P = 0.3716
Fungi*Plastic*Month	P = 0.9742

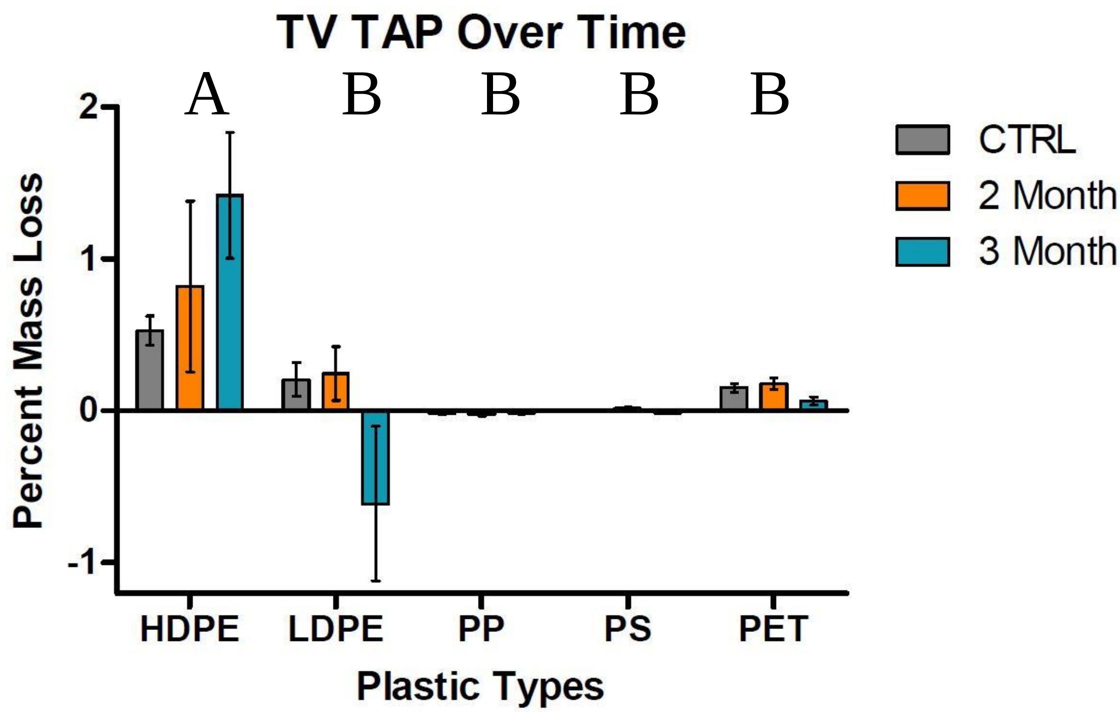


Figure 44: Mean percent mass loss ($\pm SE$) of each plastic type influenced by *Trametes versicolor* (TV) in tap water agar media over three months. GLM analysis shows percent mass loss is significant (P = 0.0002). Percent mass loss between plastics is not significant where plastics show the same letter (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate. Below is the ANOVA summary; *** = significant P value.

Percent mass loss	P = 0.0002 ***
Fungi	P = 0.7275
Plastic	P < 0.0001 ***
Fungi*Plastic	P = 0.0398 ***
Month	P = 0.5486
Fungi*Month	P = 0.8247
Plastic*Month	P = 0.3355
Fungi*Plastic*Month	P = 0.1226

<u>Soil Experiment</u>

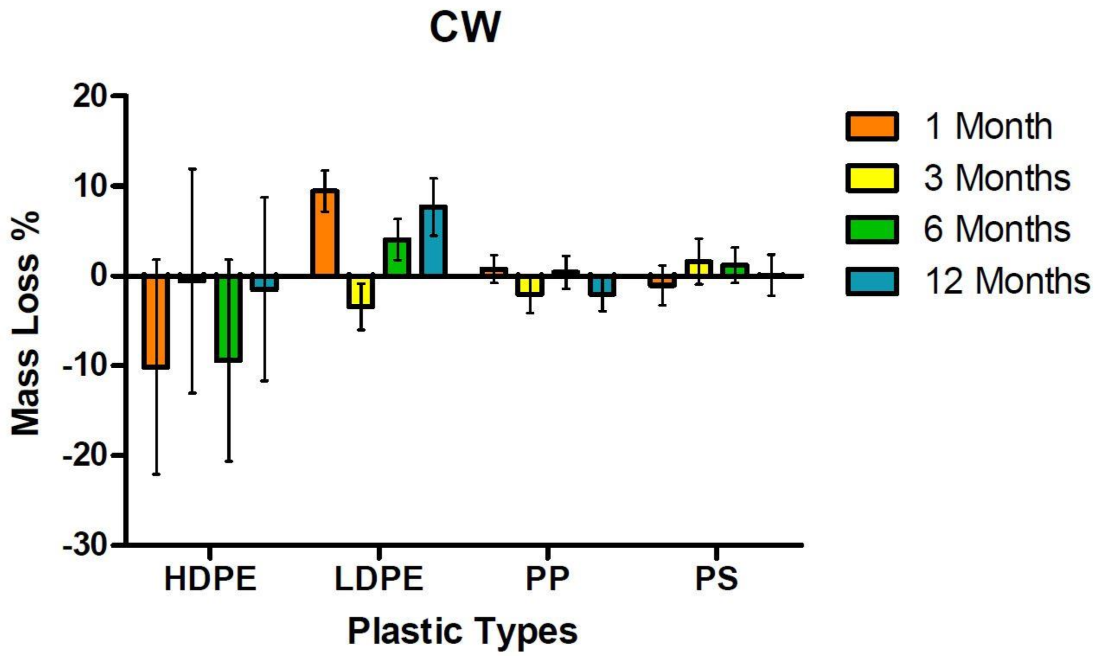


Figure 45: Mean percent mass loss ($\pm SE$) of different plastics in a homogenized Crow's Wood (CW) soil over a 12-month period. GLM analysis showed mass loss percentage is not significant (P = 0.4953), nor dependent on the type of plastic (P = 0.0691) or time (P = 0.9837). Tukey's analysis showed a significant difference between HDPE & LDPE. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene.

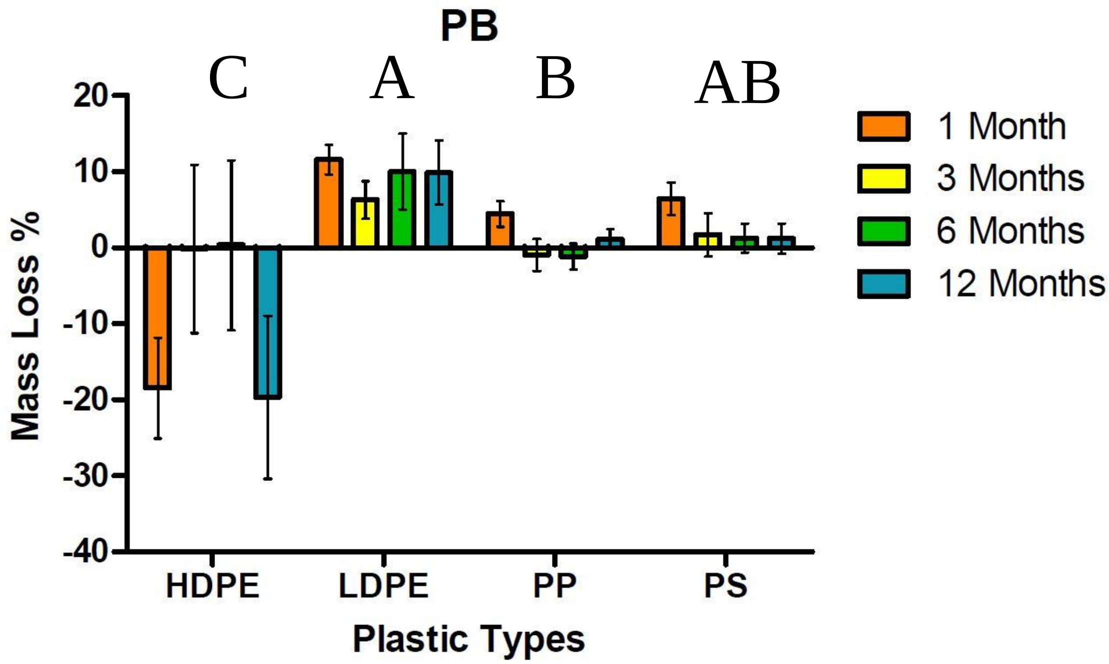


Figure 46: Mean percent mass loss ($\pm SE$) of different plastics in homogenized Pine Barrens (PB) soil over a 12-month period. GLM analysis showed mass loss percentage is significant ($P < 0.0001$) and dependent on the type of plastic ($P < 0.0001$), but not time ($P = 0.6533$) although there was a significant interaction of Plastic*Month ($P = 0.0353$). Significant differences between plastics are indicated by different letters (Tukey's Studentized Range). HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene.

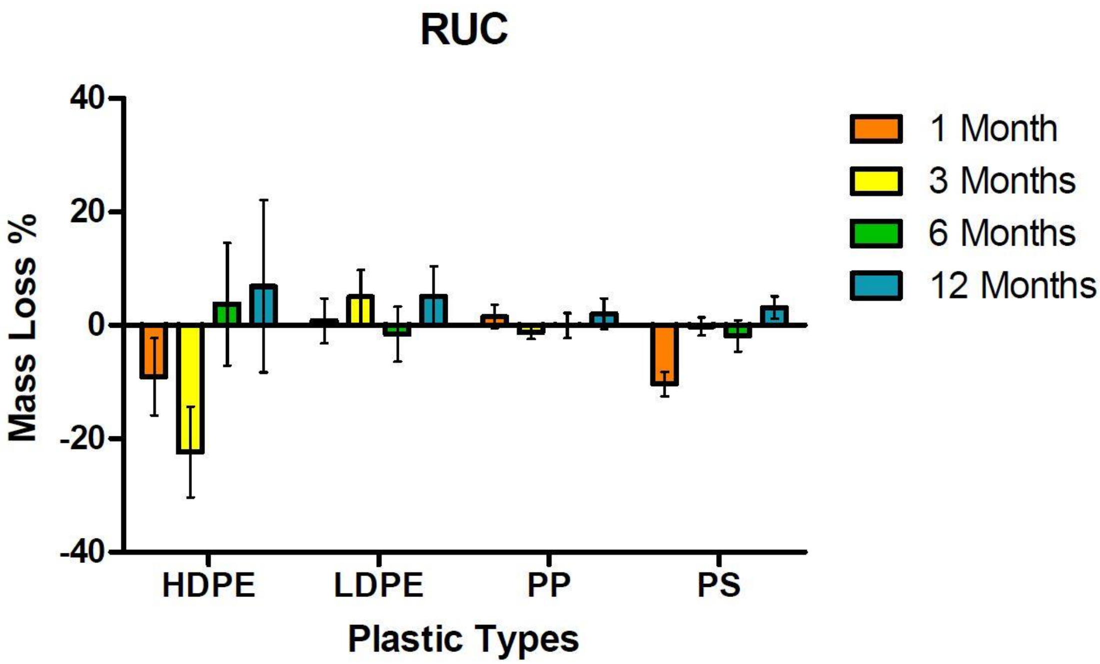


Figure 47: Mean percent mass loss ($\pm SE$) of different plastics in homogenized Rutgers University—Camden (RUC) soil over a 12-month period. GLM analysis showed mass loss percentage is significant (P = 0.0106) and dependent on time (P = 0.0464), but not the type of plastic (P = 0.1434) although there is a significant interaction of Plastic*Month (P = 0.0328). Tukey's Studentized Range showed no significant difference between plastics nor time. HDPE = high-density polyethylene; LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene.

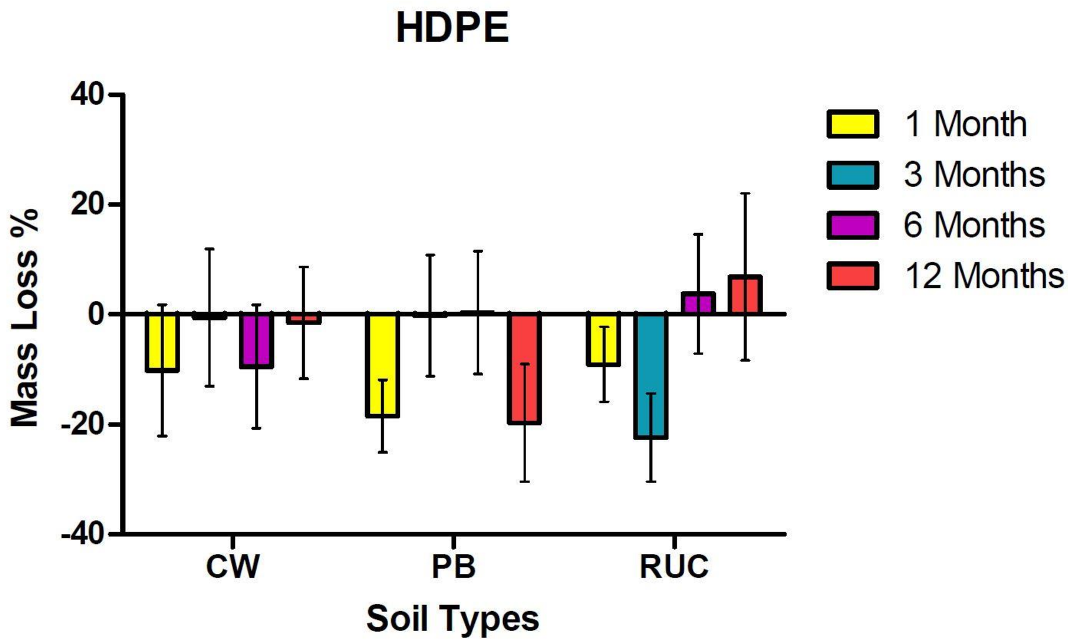


Figure 48: High-density polyethylene (HDPE) mean percent mass loss ($\pm SE$) across different soils over a 12-month period. GLM and Tukey's Studentized Range showed mass loss percent was not significant, nor reliant on soil or time. CW = Crows Woods; PB = Pine Barrens; RUC = Rutgers University—Camden.

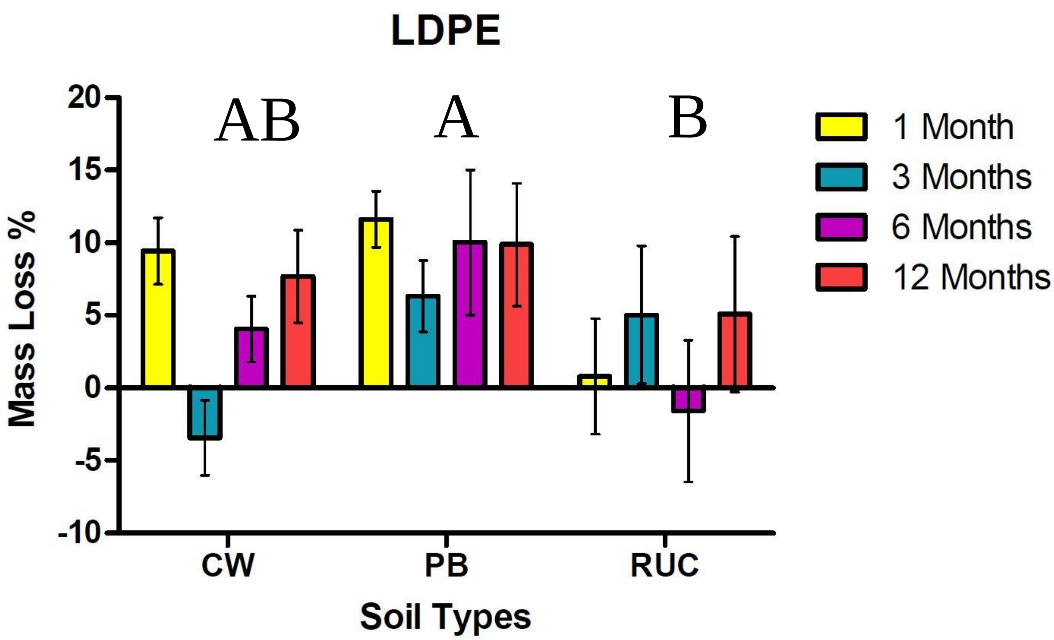


Figure 49: Low-density polyethylene (LDPE) mean percent mass loss ($\pm SE$) across different soils over a 12-month period. GLM showed mass loss percentage was not significant overall (P = 0.1623), but the type of soil was a factor (P 0.0423). Significant differences between soils are indicated by different letters (Tukey's Studentized Range). CW = Crows Woods; PB = Pine Barrens; RUC = Rutgers University—Camden.

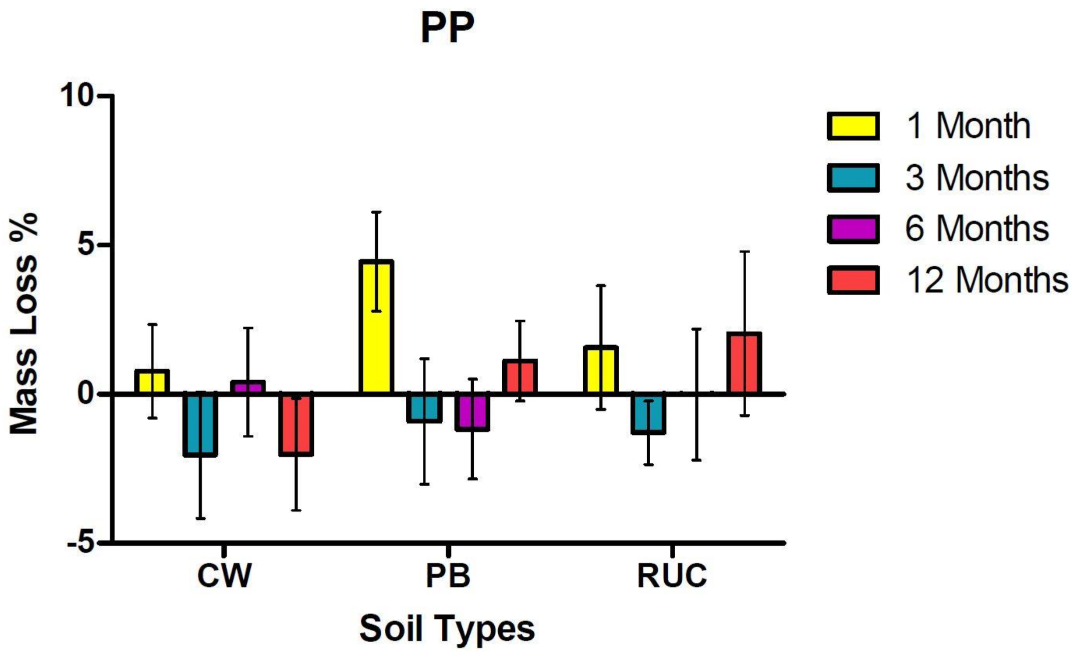


Figure 50: Polypropylene (PP) mean percent mass loss ($\pm SE$) across different soils over a 12-month period. GLM and Tukey's Studentized Range showed mass loss percentage was not significant, nor reliant on soil or time. CW = Crows Woods; PB = Pine Barrens; RUC = Rutgers University—Camden.

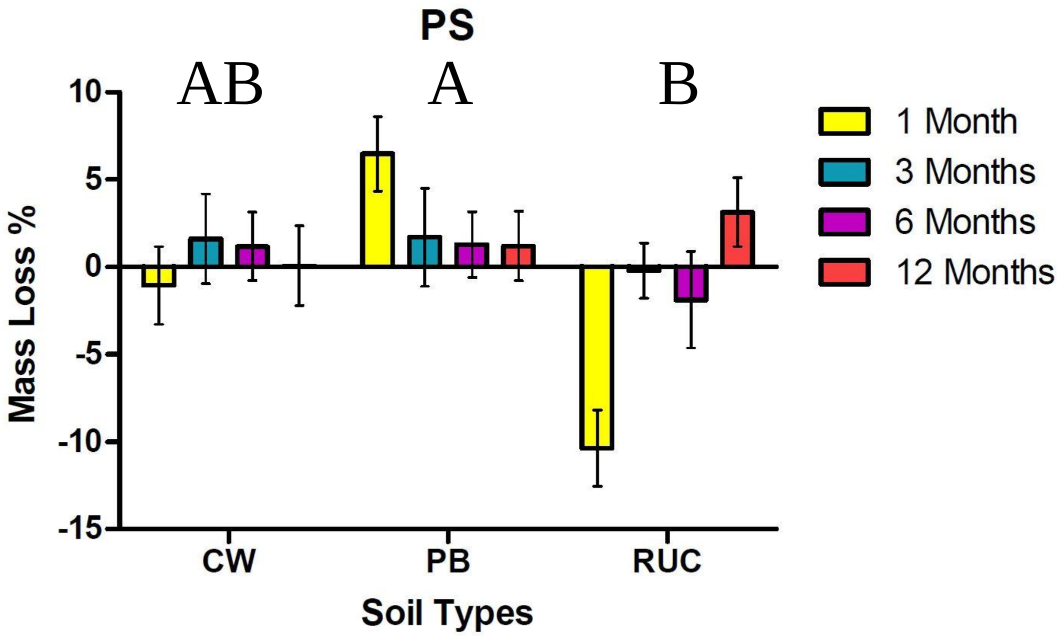


Figure 51: Polystyrene (PS) mean percent mass loss ($\pm SE$) across different soils over a 12-month period. GLM showed mass loss percentage was significant (P = 0.0017), and dependent on soil type (P = 0.0120), but not time (P = 0.4022) although there was a significant interaction of Soil*Month (P = 0.0037). Significant differences between soils are indicated by different letters (Tukey's Studentized Range). CW = Crows Woods; PB = Pine Barrens; RUC = Rutgers University—Camden.

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