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Article

The Effects of Various Plastic Pollutants on the Growth of the Wisconsin Fast Plant

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SUMMARY

Plastic pollutants are known to cause problems in ocean basins; however, the effects of plastic pollutants on terrestrial life are relatively unknown. We performed this experiment to determine the effects of various plastic pollutants on the growth of *Brassica rapa*. Five plastic treatment groups, including compostable, biodegradable, and recyclable plastics, and one control group were prepared. At the end of the experiment the number of germinations, flowers, biomass, height, soil macronutrients nitrogen, phosphorus, and pH were measured. In the first trial, the height of the mushroom plastic variants was significantly shorter than the height of the polystyrene variants. The plants in the mushroom plastic had significantly lower biomass in both trials than all of the other variants including the control, other than HDPE milk jugs. There was no significant difference for flowering, germination, nitrogen, or pH in the soil. However, there was a significantly large amount of phosphates in the soil of the NaturBag compost bags. The significant decreases in height and biomass of the plants grown in the mushroom plastic show that they are impeding the growth, likely by physically blocking access to space and therefore nutrients. The increase in the NaturBag bags phosphorus levels indicates that the NaturBag is breaking down and releasing a large amount of phosphorus.

INTRODUCTION

Since the 1950s, 8.3 billion metric tons of plastics have been mass-produced. Only 30% of those plastics are still currently in use, with nearly 80% of all plastics ending up in landfills or ocean basins at the end of their lifespan (1). Only 9% of plastics are recycled, with 8% only being recycled once, ending up in landfills after they are discarded for the second time (1). Once in landfills, plastics can release toxins into the environment (2). Single-use packaging is the biggest conventional use of plastics, and most are not recycled when discarded. Recently, efforts have been made to produce more biodegradable plastics that will break down into smaller plastic polymers known as microplastics, and compostable plastics, which can be fully broken down into base elements (2). Both biodegradable and compostable plastics are thought to be more environmentally friendly than recyclable plastics, with compostable plastics having the least environmental impact (2).

This experiment used five different types of plastics and *Brassica rapa* as a model organism. *Brassica rapa*, known as the Wisconsin Fast Plant, was genetically engineered at the University of Wisconsin to have a rapid growth time of about 22 days, not including the time to produce seeds. They have been extensively researched and have ideal growing conditions that are well established (3). The plastics used in this study are some of the most common types of plastic, primarily plastics used for packaging (2). This includes recyclable polystyrene packing peanuts, high-density polyethylene (HDPE) milk jugs, biodegradable PET #1 water bottles, compostable NaturBag garbage bags, and EcoVation mushroom plastic packaging material. The recyclable plastics are known to last thousands of years before degrading, while biodegradable plastics may also last the same amount of time, however, they break down into much smaller polymers. Compostable plastics degrade in a range of weeks to months. These plastics, as well as a control group with no plastic, will be used to test the health of the Wisconsin Fast Plant in an environment containing these plastic variants. Additionally, a second control group with plants in ceramic pots will be used to grow the Wisconsin Fast Plants in an environment devoid of plastic, including the plastic seedling containers.

Marine-based plastic contamination has been studied due to the large plastic waste buildups in ocean basins (1). However, plastic buildups occur not only in oceans and lakes, but also throughout land ecosystems, and the effects of plastic on terrestrial plant and animal health are relatively unknown (1). Therefore, we designed this experiment as a starting point in researching the effects of plastic pollutants on terrestrial plant health. This research addresses the question, what is the effect of various plastic pollutants on the growth of the Wisconsin Fast Plant through multiple hypotheses. First, we hypothesize that if the plastic is recyclable then it will harm the plant health due to the toxins that can be released as the plastic breaks down in the soil (2). Second, we hypothesize that if the plastic is biodegradable, then there will be no effects on the plant health because no toxins or organic nutrients will be released, and microplastics will not be formed over the relatively short timeline of this experiment (1). Finally, we hypothesize that the compostable plastics will have a positive effect on plant health because of the helpful nutrients released, such as nitrogen (4, 5). This experiment aims to test this question and the hypotheses that accompany it, as well as determine what type of plastic is best, comparatively, for the plastics in terrestrial environments.

RESULTS

Given Immediate Plastic Exposure

In trial one, the seeds were planted in the soil at the same time as the plastics, and then grown for 22 days within controlled conditions. There were 36 plants per group. We recorded data for germination, the number of flowers, height, biomass, as well as nitrogen, phosphorus, and pH in the soil. We performed ANOVAs and t-tests to determine whether or not there were significant changes between treatment groups for germination, number of flowers, height, and biomass. We also recorded changes in nitrogen, phosphorus, and pH in the soil.

The mushroom plastic was well ahead of the rest of the plastics during its growth stages, ending up roughly 5-8 days ahead of the typical growth time (**Figure 1**). The mushroom plastic variants reached the stage of life where they were able to seed and drop all of their flowers, while all other treatment groups were still in the flowering stages of the life cycle.

Figure 1: Experimental setup. Left column is compost bags, the middle column is ice mountain water bottles, and the right column is mushroom plastic. Three days after planting, buds in the mushroom plastic are widely seen, with one or two sprinkled throughout the compost bags and ice mountain water bottles.

While we were not able to determine whether or not the number of flowers remaining on the plants was statistically significant, the amounts of flowers were proportional to the number of germinations, with the very slight exception of the mushroom plastic. This means that roughly the same number of flowers were being produced per plant, and the differences were insignificant. While the mushroom plastic did not have as many flowers on the plants compared to the peanuts, which had the same germination, this was to be expected, seeing as the plants had started to drop their flowers at the end of the growth period. Additionally, there was no significant difference in germination rate between any groups (**Figure 2**). Any differences in germination rates were due to chance, and were not attributed to the various treatments.

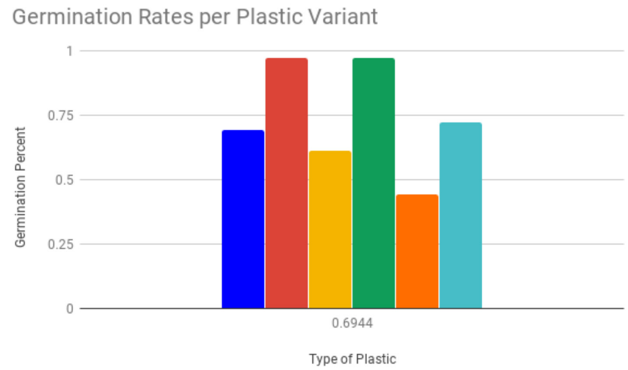

Figure 2: Germination rates per plastic variant. Germinations for each soil type were obtained using a chi-square test comparing germination rates to the control group (p=0.9978). This suggests the differences in germination between treatment groups were due to chance.

There were significant changes in both mean height and biomass in trial one. The heights across all treatment groups were not significant; however, the difference in height between the polystyrene packing peanuts and the mushroom plastic was statistically significant (**Figure 3,** t-test p=0.0466). All other combinations were insignificant. The mean plant height for the polystyrene condition was 5.8 cm, while the mean height for mushroom plastic was 4.9 cm. Although NaturBag compost bags had a mean height of 4.5 cm, these plants did not have enough germinations, resulting in a larger margin of error and no significant difference compared to any of the other plastics. The difference in mean biomass between mushroom plastic and every other treatment group, except for HDPE, was significant (**Figure 4,** ANOVA and individual t-tests). The mean biomass of the mushroom plastic was 0.057g. Additionally, HDPE had significantly less biomass than PET #1, with a mean biomass of 0.062g compared to 0.084g, respectively (**Figure 4**, t-test p=0.0315). The mushroom plastic had significantly less biomass compared to the control, which means that the mushroom plastic variant significantly harmed the biomass of the Wisconsin Fast Plant.

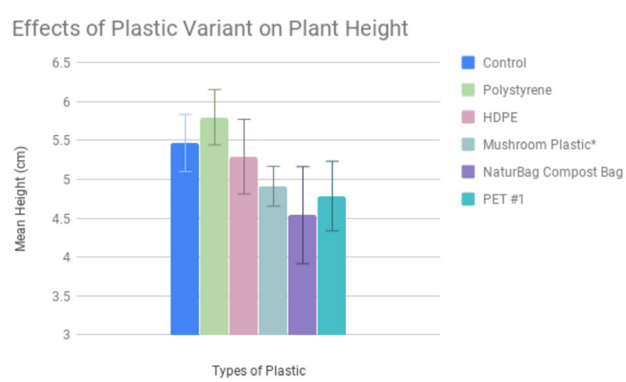

Figure 3: The average height depending on the plastic variants in the soil in trial one. The ANOVA is insignificant, p=0.2211, but the t-test between polystyrene and mushroom plastic shows mushroom plastic is significantly shorter p=0.0466. The error bars are the standard error of the mean height in trial 1 (ANOVA).

The changes in nitrogen or pH were not significant for any of the treatments (**Table 1**). However, for the phosphorus tests, all treatments, including the control, had 5ppm except for NaturBag, which had 25ppm. This significant change indicates that NaturBag compost bags are causing some significant difference in the soil phosphates.

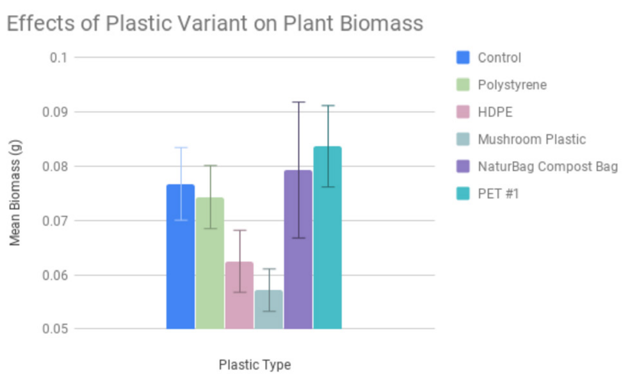

Figure 4: The average biomass depending on plastic variants in the soil in trial 1 (ANOVA P=0.0315). The following t-tests between mushroom plastic were significant for all other treatments other than HDPE (P<0.05). HDPE compared to PET#1 was also significant (t-test, P=0.0315). The error bars are the standard error of the mean biomass in trial 1.

	Nitrogen (ppm)	Phosphorus (ppm)	pH
Before	5ppm	5ppm	6.5
After	Control: 10ppm Polystyrene: 5ppm HDPE: 10ppm Mushroom: 5ppm NaturBag: 10ppm PET#1: 5ppm	Control: 5ppm Polystyrene: 5ppm HDPE: 5ppm Mushroom: 5ppm NaturBag: 25ppm PET#1: 5ppm	Control: 6 Polystyrene: 6 HDPE: 6 Mushroom: 6 NaturBag: 6 PET#1: 6

Table 1: The results of soil test kits in trial 1. Each trial was repeated once per box, or three times per plastic type. The most notable difference is a 25ppm phosphorus in the NaturBag soil, compared to 5ppm in all other soils.

Pre-Incubation of the Plastics

In trial two, we implanted the plastics in the soil 18 days ahead of the seeds with the assumption that they would degrade more and therefore have more noticeable effects on the plants. Once again, there were 36 plants per group. The changes in the number of flowers per plant, depending on the plastic in the soil, were insignificant for both the first second trial. The germination rates were not affected by the plastics in the soil for either trial one or two. Any variation in the germination rates was due to chance.

Once again, there were significant changes in the mean heights and biomass'. The heights across all plastics were decreased compared to the first trial (**Figure 3, Figure 5**). The mean heights were 1.26cm and 1.63cm for the polystyrene and mushroom plastics, respectively. They were both significantly shorter than all other plastics variants ($p<0.05$). The mean mass of the mushroom plastic was much lighter than other plastic groups, at 0.0126g (**Figure 6**, $p<0.05$). This trial corroborates the first trial in that the mushroom plastic impeded the growth and biomass of the Wisconsin Fast Plant.

We did not observe significant changes in nitrogen or pH for any of the treatments (**Table 2**). However, for the phosphorus tests, all treatments, including the control, had 12.5ppm except for NaturBag, which had 37.5-50ppm. This is a significant change, meaning NaturBag compost bags are causing some significant difference in the soil phosphate.

The biomass of Wisconsin Fast Plants was significantly decreased by the presence of mushroom plastic in the soil, and the phosphorus content in the soil was significantly increased in the presence of NaturBag compost bags.

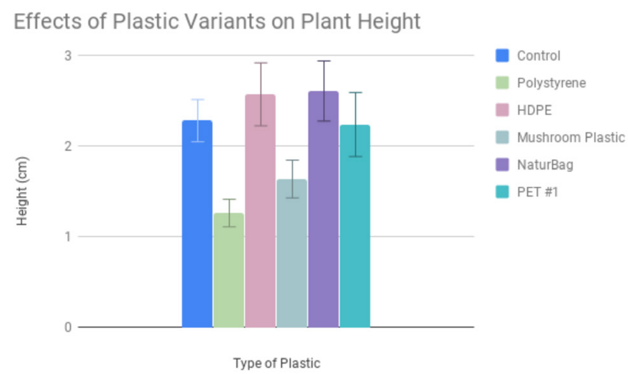

Figure 5: The average height depending on the plastic variants in the soil in trial 2. The heights between the various plastic groups were significant in trial two (ANOVA p=0.00175). The polystyrene packing peanuts and the mushroom plastic are both significantly shorter than all other plastics (t-tests p<0.05), but not each other. The error bars are the standard error of the mean height in trial 2.

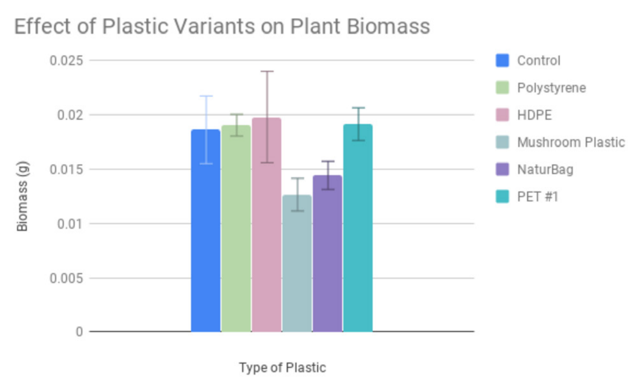

Figure 6: The average biomass depending on plastic variants in the soil in trial 2. The ANOVA of biomass in trial two between all of the groups was insignificant (P=0.249). However, t-tests between the mushroom plastic and all other groups other than HDPE showed significant differences (p<0.05). The error bars are the standard error of the mean biomass in trial 2.

DISCUSSION

The biomass of Wisconsin Fast Plants was significantly lower in the presence of mushroom plastic in the soil. The mushroom plastic is significantly lighter than all plastics and the control except the HDPE milk jugs (**Figure 4**, $p=0.0315$). These results were also corroborated in trial two, indicating that this trend was truly due to the plastics. One possible reason for this is that the loss of the flowers in only the mushroom plastic group caused the loss of biomass. Additionally, the mushroom plastic plants also were shorter than the control and other groups. Another possible reason for the lack of biomass for the mushroom plastic group is that the mushroom plastic broke down, resulting in it taking up more volume. By increasing the volume of the plastic in the relatively small area, the mushroom plastic could have been impeding growth more than the benefits were helping the growth (6). By having something hard within the soil, the ability for the roots to grow and intake the required nutrients becomes more difficult (6). Additionally, the packing peanuts rose to the top of the soil when watered, therefore removing them from the soil and out of the path of the roots. A future experiment regarding these two plastics would be to grow them in much larger containers and compare the results between the different size containers.

	Nitrogen (ppm)	Phosphorus (ppm)	pH
Before	5ppm	5ppm	6.5
After	Control: 5ppm Polystyrene: 10ppm HDPE: 5ppm Mushroom: 5ppm NaturBag: 5ppm PET#1: 5ppm	Control: 12.5ppm Polystyrene: 12.5ppm HDPE: inconclusive. Test was too green to determine on the color chart, which included only ranges of blue. Mushroom: 12.5ppm NaturBag: 37.5-50ppm PET#1: 12.5ppm	Control: 6 Polystyrene: 6 HDPE: 6 Mushroom: 6 NaturBag: 6 PET#1: 6

Table 2: The results of soil test kits in trial 2. Each trial was repeated once per box, or three times per plastic type. The most notable difference is 37.5-50ppm phosphorus in the NaturBag soil, compared to 12.5ppm in all other soils.

The differences in flowering and germination were due to chance, rather than the plastics in the soil (p=0.9978 for trial one, p>0.99 for trial two). While the significance for the flowering was not able to be determined in trial one, the proportions of flowers to germinations were nearly the same (**Figures 2 & 3**). In trial two, there was no effect of the plastic on flowering (p=0.278). Given that the number of flowers is reduced by stress put on a plant, an equal proportion of flowers help to indicate the conditions are putting an equal amount of stress on each different treatment group (7). The largest change between the germination rate and flowers was the mushroom plastic. However, this change was seen to be farther in its life cycle. The plants have the capability to seed between 22 and 40 days, with most being around 30 days (3). This puts the mushroom plastic at about 5-8 days ahead of the typical growth expectancy, while the others were around the 21-22-day mark as expected. One possible reason for this is that if a plant is stressed it may attempt to flower and reproduce more quickly and focus energy on reproduction rather than growth. Again, growing the plants in larger containers may eliminate the space issues.

In trial one, an ANOVA showed that there was no significance in mean height across all of the six different treatment groups, however, a t-test showed a significant difference in height between polystyrene and mushroom plastic, with mean heights of 5.8 and 4.9 cm, respectively (p=0.0466). The difference between polystyrene and mushroom plastic is the only significant difference between all of the treatment groups, and it indicates that it is likely the difference between the heights was due to the plastic in the soil. However, in trial two, the results were drastically different (p=0.00175). The mushroom plastic was once again the smallest. However, the polystyrene was very short as well. Similar to the changes in biomass, it is possible this change was due to the mushroom plastic impeding the growth of the Wisconsin Fast Plant by getting in the way of the roots (6). However, because the polystyrene floated, there must be some other reason that the packing peanuts were harming the height of the plants in trial two. It is important to note that while the packing peanuts and the mushroom plastic may have been significantly different from each other in trial one, there were no significant changes in height compared to the control group, indicating that neither one deviated significantly from growth with no additional plastics. Additionally, the results from trial two are opposite of trial ones, and therefore another trial should be run to see which is more accurate.

There were no significant changes in the nitrogen or pH in the soils of all of the treatment groups for either trial one or two. A couple of possible reasons for this are that the plastics may not have had enough time to release any nutrients or H+ ions into the soil, or that the changes were not detectable with the equipment used. Another possibility is that if there were significant changes in a nutrient such as nitrogen, it could have been absorbed by the Wisconsin Fast Plants, and therefore not be detectable in the soil. A possible future experiment would be to measure the nitrogen in the plants after they were uprooted. While there were no significant differences between nitrogen or pH, NaturBag compost bags had a phosphorus level of 25ppm, while every other treatment group had 5ppm in trial one. A possible reason for this is that the NaturBag is composed of more phosphorus than any other plastics, and releases it when it degrades. Natural polymers, which are what the NaturBag compost bag is composed of, are known for fast degradation and could possibly have released more phosphates in the course of the trial (8). The second trial had similar results on a larger scale. The NaturBag had levels of between 37.5-50ppm, while the others had phosphorus levels around 12.5ppm. These higher levels of phosphorus are approaching a point where they may be dangerous if they get into a water supply, because the phosphates may cause algal blooms (9). The next step in these sets of experiments will be to isolate the NaturBags and test the water runoff under similar conditions as the previous trials. This will determine whether or not the degradation of the NaturBag has risk of being harmful to the environment even though it is a compostable plastic.

The differences in plastics in the soil of the Wisconsin Fast Plant resulted in many significant changes of mean biomass, with mushroom plastic and HDPE being significantly lighter than most of the other plants in both trials. The changes in the number of flowers and the germination rates were due to chance. There was one significant change in mean height, which was between mushroom plastic and polystyrene, however, these changes were not significant compared to the control group and because of the differences in the two trials, it is unclear whether or not the polystyrene is affecting the height of the plants. Finally, the soil macronutrients saw a significant rise in phosphorus for the NaturBag compost bags, indicating something about the bags causes increased phosphorus in the soil. Future experiments could be attempting the same experiment in a larger growing container, to lower the likelihood of the plastics impeding growing space. While the plastics overall seemed to have very few negative impacts on growth, this does not imply that they do not have negative impacts on the health of the plants, with the color of leaves possibly indicating a less healthy plant. Additionally, there may be problems later in life that were not addressed. A possible future experiment to test the long term effects would be to carry the tests through multiple generations. Additionally, it would be important to test these effects with even smaller plastics, such as microplastics. Another important note is that while the plastics were not stuck in the soil, many of them did float when watered. In a real-world situation, this would mean the plastics would be carried into water supplies, and eventually out to oceans, where their negative impacts have been well documented and explored.

A third trial has been started which is focusing on the phosphorus from the NaturBag, and the possible effects that come through water runoff. It will use a spectrophotometer to more accurately measure the phosphorus content.

Between trials one and two, the significant changes in mean biomass and the amount of phosphorus in the soil showed the same trends, despite the incubation period. The mean biomass of the mushroom plastic was less than the others, indicating that something about the compostable mushroom plastic caused a decrease in growth. Similarly, the NaturBag released substantially more phosphorus in both trials, although the impacts of that phosphorus on the plants' growth were not noticeable.

MATERIALS AND METHODS

Standard Growth Conditions

The growing conditions for this experiment were closely controlled through the use of a Conviron growth chamber, which controls temperature, humidity, and light levels in an enclosed environment. Wisconsin Fast Plants were grown in 1" by 1" by 2.25" plastic planting containers, as well as a control group in a similar-sized ceramic pot. These pots were filled with 26.5 to 28.5 grams of untreated topsoil. The plants were watered three times a week, typically Monday, Wednesday, and Friday, with 10 mL of tap water per container. There were two Wisconsin Fast Plant seeds per container, which were planted about 1 cm under the soil. The plants were grown for 22 days in a Conviron growth chamber, which maintained a constant temperature of 22°C and a constant 24-hour light on the brightest available setting. The Conviron chamber was also supposed to keep the relative humidity constant, however, the sensor did not work and the chamber was kept at around 35--55% humidity. These growing conditions were recommended by the University of Wisconsin (3).

Growth Conditions with Plastics

To test the effects of various plastic pollutants, the varying types of plastics were placed in the growing containers. There were 6 groups with 18 growing containers each or 36 seeds per group. The first group was a control group with no additional plastic. The second and third groups were the recyclable plastics: polystyrene packing peanuts and HDPE milk jugs. The fourth group was a semi-biodegradable plastic: the PET #1 Ice Mountain water bottles. The fifth and sixth groups were the compostable plastics: the mushroom plastic and the NaturBag compost bags. One additional group with the same amount of seeds was grown with no plastics in small clay pots. All plastics were cut into two 1 cm by 1 cm squares, with as close to 1 cm of depth as possible. The plastics were inserted about the same depth as the seed to maximize contact with the seed; however, many of the plastics came loose and rose to the top of the soil during watering.
For the second trial, the soil and plastics were mixed and then kept damp, with the same conditions as the Conviron chamber, for 18 days. The seeds were then planted, and the trial was completed as before.

Metrics for Growth

The Wisconsin Fast Plants growth was measured by counting the flowers and germination rates, measuring the biomass at the end of the 22-day trial, measuring the final height, and measuring the soil nutrients before and after the trial. To measure the final height, the plants were measured on the twenty-second day from the base of the soil to the top of the tallest shoot in centimeters. To measure the biomass, the plants were uprooted, the soil was washed off; they were dried, and then massed in grams. To take measurements of the soil nutrients, nitrogen, and phosphorus, as well as the pH, LaMotte soil macronutrient test kits were used following the manufacturer's instructions. These tests were taken once from the soil before the test and then once per box, totaling three times from each plastic, from the soil after the test.

Statistical Analysis

To determine if there were statistically significant differences in biomass, plant height, flowering, germination, and soil macronutrient levels, ANOVAs, t-tests, and Chi-squares were run using Google sheets. Chi-squares were run using the actual number of germinations compared to the control group, but are represented in Figure 2 as percentages. Changes were considered significant at $p<0.05$.

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agriculture and soil nutrient cycling, and her advice has been invaluable over the course of my experiment. She was the one who suggested the idea behind my second trial, pre-incubating the plastics to allow the soil microbes to start degrading the plastics.
A link to her faculty page on the University's website: https://horticulture.umn.edu/people/faculty/juliegrossman

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