Impacts of Long and Short-Term Antibiotic Exposure on *Arabidopsis thaliana* Fitness

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ABSTRACT

Antibiotic exposure and the development of antibiotic resistance have important implications for public and environmental health. *Arabidopsis thaliana* is an indicator of how other species and ecosystems will respond to environmental stresses, and its response to antibiotics can assist in understanding how increased antibiotic use is altering environmental health. I grew 105 *A. thaliana* plants in greenhouse soil, organic farm soil, and factory farm soil which had experienced long-term antibiotic exposure. I treated them weekly with 10 µg/ml, 100 µg/ml, and 500 µg/ml solutions of tetracycline or penicillin for 45 days before harvest, at which point I measured mortality rate, fruit production, and height. After drying the plants for three days I measured above ground dry biomass. I found that long-term exposure to agricultural runoff from the organic farm and the factory farm was detrimental to fruit production, height, and mass, and that short-term exposure to tetracycline decreased height and mass as well as increased mortality rate. The negative impacts that resulted from tetracycline exposure were somewhat mitigated by being grown in organic farm soil, and more significantly mitigated by being grown in factory farm soil. These results imply that certain antibiotics may be less environmentally impactful to use in agriculture than others and that higher antibiotic concentrations in agricultural runoff are harmful to plant fitness. The findings of this study also suggest that utilizing soils which have undergone long-term antibiotic exposure may be an effective approach in mitigating the negative effects of short-term antibiotic exposure on ecosystem success.

KEYWORDS

antibiotic resistance, animal agriculture, environmental toxicity, tetracycline, penicillin

INTRODUCTION

Antibiotic use has been steadily increasing in the modern world, and their overuse is creating substantial issues. Antibiotics are used in a variety of fields, ranging from medicine and agriculture to beekeeping, ethanol production, food preservation, and many others (Meek et al. 2015). In medicine, a study by Busfield (2015) found that antibiotic prescriptions around the world for conditions where they are not particularly effective have increased over time. This overuse is partially due to the classification of "rational" medicine used by the World Health Organization, which is defined as patients receiving appropriate medicines in doses and for time periods that meet their need, which cost the lowest amount for the patient and the patient's community ("The Pursuit" 2012). The cause of the issues antibiotic overuse results in are not only antibiotics themselves, but that as bacteria are repeatedly exposed to antibiotics, they develop resistance to them. This is done through the use of resistance genes passed to offspring and the horizontal transmission of genes between neighboring bacteria, which have led to the development of antibiotic resistance for every antibiotic that is currently in use (Davies and Davies 2010). If the trajectory of overuse continues, some studies have claimed that antibiotics will no longer be an effective form of treatment for even minor infections, and this decreased efficacy could result in devastating consequences (Meek et al. 2015). Antibiotics and antibiotic resistant bacteria result in a myriad of negative impacts, including public health risks and environmental damage (Hao et al. 2014). These impacts are not only dangerous, but also expensive, as antibiotic resistant infections cost the United States healthcare system more than 20 billion dollars each year (Hao et al. 2014). Antibiotic overuse has resulted in a variety of harmful consequences which will continue to spread unless restrictions on antibiotic use are put in place.

One of the main sources of antibiotics and ultimately antibiotic resistance is their widespread us in agriculture, particularly for animals. Antibiotics are used in medicine and agriculture as a form of treatment, but they are also widely used in intensive agricultural operations for purposes that are not medically necessary. For example, antibiotics in agriculture are used to prevent infections, which are a common issue when animals live in tightly packed areas, but they are also used consistently over long periods of time to promote animal growth (Khachatourians 1998). Antibiotic use in agriculture has led to the presence of bacteria with antibiotic resistance in soils from swine farms, dairy farms, and non-agricultural areas that utilize manure (Ghosh and

LaPara 2007). This is an issue that not only impacts the agricultural areas but also surrounding ecosystems as antibiotics and antibiotic resistant bacteria spread.

Not only is antibiotic overuse and resistance problematic for humans and animals, but it impacts plants as well. Antibiotics influence many crops, some of which are extremely economically important, and a great deal of research focuses on these species. For example, a study by Eluk et al. (2016) found that the concentrations of five antibiotics that the dairy industry is currently allowed to use put sorghum, corn, and soybean crops at risk in relation to the influence of the antibiotics on the crops' germination frequency and root elongation. Antibiotics and antibiotic resistance impact many species, but focusing on economically important crops neglects other species, such as *Arabidopsis thaliana*, also known as the thale cress or mouse-ear cress. Research focused on *A. thaliana* is important because *A. thaliana* is a model organism in the mustard family, and its responses to antibiotics could be indicative of the effects of antibiotics on many other species. *A. thaliana* also grows in a multitude of places, and it likely has important relationships with countless species. Additionally, it is a weedy, somewhat resilient plant, so if antibiotics impact its growth and reproductive success, they could be even more detrimental to species that are more sensitive. Although antibiotic and antibiotic resistant bacteria exposure in *A. thaliana* requires more research, some studies have examined aspects of this issue. For example, Gudiño et al. (2018) found that exposure to carbenicillin and penicillin led to a decrease in the size of the root apical meristem and exposure to carbenicillin resulted in increased levels of auxin and decreased levels of indole glucosinolates in *A. thaliana*. This study describes the root apical meristem as the area that is mainly responsible for cell division in the root, and therefore root growth, as well as auxin alterations leading to changes in the root length and lateral root formation, and the responsibility of indole glucosinolates in controlling fungal infections. In addition to these changes, Bardini et al. (2003) found that exposure to the antibiotic kanamycin resulted in many methylation alterations in the genome, both hypermethylation and hypomethylation, which can result in developmental abnormalities. Overall, studies have examined the impact of antibiotics on *A. thaliana* in the past and have found that a variety of antibiotics negatively influence several different traits. Although past studies have focused on the impacts of antibiotics and antibiotic resistant bacteria on the growth and reproduction of *A. thaliana*, this is an area that requires further research. Tetracycline and penicillin are the second and third most commonly used antibiotics in animal agriculture ("Food and Drug Administration" 2016), and I plan to use them both because of their widespread introduction into the environment as a result of their frequent use, and because there is a lack of research into their impacts on *A. thaliana*.

Additionally, there are several other characteristics that are indicators of growth and reproductive success that have not had their responses to tetracycline and penicillin studied or have not had their responses to any antibiotics studied. These characteristics are what I plan to focus on in this research. I will examine above ground dry plant biomass, plant height, and the number of plants that die prior to harvest as indicators of growth success, as well as the number of fruits produced as an indicator of reproductive success. Additionally, the response of *A. thaliana* to short-term antibiotic exposure in conjunction with the presence of long-term agricultural runoff and antibiotic resistant bacteria is an important aspect of this field of research that has not been considered, which I plan to examine as well. This area of study will require a great deal of research as the overuse and misuse of antibiotics continues to spread.

This study seeks to answer the question: How does antibiotic exposure affect the success of *A. thaliana?* Multiple sub-questions will assist in answering this central research question, including: How does being grown in agricultural soil that has experienced long-term antibiotic exposure affect the growth and reproductive success of *A. thaliana*? How does short-term exposure to tetracycline and penicillin affect the growth and reproductive success of *A. thaliana*? How is the impact of short-term antibiotic exposure on *A. thaliana* influenced when the *A. thaliana* is grown in soil that has experienced long-term agricultural runoff which contains antibiotics? In terms of long-term agricultural antibiotic exposure, I hypothesize that being grown in agricultural soil that has experienced long-term antibiotic exposure will be detrimental to the growth and reproductive success of *A. thaliana.* I also hypothesize that short-term exposure to tetracycline and penicillin will be detrimental to the growth and reproductive success of *A. thaliana*. My final hypothesis is that exposure to tetracycline and penicillin will have a less detrimental impact on growth and reproductive success when *A. thaliana* is grown in soil that has experienced long-term antibiotic exposure through agricultural runoff. This study has multiple areas of focus, which will all be utilized to determine the impacts of antibiotic exposure on *A. thaliana.*

METHODS

Soil and seed selection

The subject of this study is Wild-Type Col-0 *Arabidopsis thaliana*, as it is the most commonly studied ecotype and has been used in comparable studies (Gudiño et al. 2018, Shi et al. 2016, Woodward and Bartel 2018). I grew *A. thaliana* in three different soil types: Sunshine Mix 4 (no antibiotic use, referred to as greenhouse soil), soil from McClelland's Dairy (no antibiotic use, referred to as organic soil), and soil from a large cattle ranch (antibiotic use, referred to as Cattle Ranch soil). 20 gallons of surface soil were collected from each farm, as close to the pens as possible as the soil there is most likely to have had the highest exposure to antibiotics from the feed given to the cows and their feces. Cattle Ranch is a large farm in Selma, CA, which previously used subtherapeutic antibiotics prior to the passage of California Senate Bill 27 on October 10, 2015, which, among other restrictions, banned the use of medically important antimicrobial drugs solely for increasing weight gain or improving feed efficiency ("S.B. 27" 2015). Cattle Ranch no longer uses subtherapeutic antibiotics but continues to use therapeutic antibiotics. McClelland's Dairy is a small dairy farm in Petaluma, CA, which has never used any antibiotics. In both sites, I collected soil roughly 100 feet from the cattle holding areas.

Growth protocol

I conducted the growth protocol using a combination of the methods of Gudiño et al. (2018), the protocol described by Russell et al. (2002), the protocol described by Eddy and Hahn (2011), and previous personal experience. After collecting the soil from the aforementioned sites, I filtered it through a sieve to remove soil clumps, plant debris, rocks, and other material. I then mixed the farm soils with 50% Turface by volume, and filled 105 3.5-inch square pots, with each type of soil in 35 pots. I placed the pots into trays, three trays with 17 pots and three trays with 18 pots, in an upper tray with holes and a lower tray with no holes to bottom water the plants. I then bottom watered the soil to saturation and added additional water to the bottom trays to keep the soil wet. To stratify the seeds, I divided 450 seeds roughly evenly onto damp filter paper in 15 petri dishes. I covered the petri dishes with parafilm and placed them in a fridge at 4°C for five days.

After the five-day stratification period, I made small divots in the surface of the soil in each pot, and planted four seeds in each divot. I covered the trays that held the pots with germination domes until cotyledons developed, then partially removed the germination domes until two sets of true leaves developed. True leaves had developed for most plants after one week of growth, at which point I fully removed the germination domes from all six trays. After one week of growth, I removed miscellaneous other plants that were growing in the soil, which were likely a result of unintentionally collected plant matter in the farm soils. For the entire growth period, I grew the plants in the Oxford Tract greenhouse at temperatures ranging from 21-24°C, with HID lights on for 16 hours each day and off for 8 hours each day. The Oxford Tract Greenhouse staff treated the plants with Peters Professional 20-20-20 fertilizer and YaraLiva calcium nitrate diluted to a solution of 82 parts per million of nitrogen by volume once per week for 45 days, as well as with Camelot O Fungicide/Bactericide once per week for 45 days. I watered the plants through the bottom of the pots as needed for the first week and the Oxford Tract Greenhouse staff watered the plants using the same method for the remaining 38 days. 14 days after planting, I thinned and transplanted the plants to have one plant in each pot. The plants were grown a total of 45 days before being harvested.

Antibiotic treatment

To conduct the antibiotic treatment of the plants, I used tetracycline and penicillin, as they are two of the most commonly used antibiotics in agriculture according to the Food and Drug Administration (2016). I based the antibiotic concentrations on a study conducted by Minden et al. (2017), as well as advice from Professor Federico Navarro-García of Complutense University of Madrid and advice from Professor Miguel Angel Blazquez of The Institute of Plant Molecular and Cellular Biology in Valencia (personal communication, F. Navarro-García and M. Blazquez). I used three concentrations of antibiotic solution: 10 μg/ml, 100 μg/ml, and 500 μg/ml of both antibiotics in addition to a control group with no antibiotic treatment. These concentrations were converted to 710 μg, 7100 μg, and 35500 μg of antibiotic per plant based on the average water retention of common loam soils (Vossen et al. 2016). During each antibiotic treatment, I ground the antibiotics to a fine powder with a mortar and pestle, mixed the total antibiotic amount for each treatment group with 75 ml of water, and poured five ml of this antibiotic solution at the base of each plant. I treated the plants with the antibiotic solution once per week, with the first treatment occurring one week after planting.

Data collection

To measure the impacts of antibiotic exposure on *A. thaliana*, I examined several characteristics as indicators of growth and reproductive success. To determine the impacts of antibiotics on the growth of *A. thaliana*, I measured the number of plants that died prior to harvest, the height of the plants before harvest, and the above ground biomass of the dried plants after harvest. The number of plants that died prior to harvest was determined by counting the number of plants that died during the growth process. I measured the plant height from the base of the stem to the end of the tallest point of the plant by straightening the plant against the edge of a ruler. To determine dry biomass, I harvested the above ground portion of the plants, dried the plants in paper bags in a drying room with low humidity at 40°C for three days, at which point I weighed them on a high precision balance scale. To determine reproductive success, I examined the number of fruits produced by the plants by counting the number of fruits on each plant after the harvest.

Data analysis

To analyze the broad array of data I collected I used several statistical tests, all of which were conducted in version 4.0.3 of R. I used a Kruskal-Wallis test to determine if there were differences in each variable between soil types, and pairwise comparisons using the Wilcoxon rank sum test to analyze differences between pairs of soil types. I also used a Kruskal-Wallis test to determine if there were differences in each variable between antibiotic concentrations or antibiotic types, and pairwise comparisons using the Wilcoxon rank sum test to analyze differences between pairs of antibiotic concentration groups or antibiotic type groups. P-values were corrected using the Holm method. Collectively, the results of these tests showed me if the traits that I examined showed significant differences with changes in soil type, antibiotic concentration, or antibiotic type.

RESULTS

Impact of soil type on growth and reproduction

I found that agricultural soils with potential antibiotic exposure reduced A. thaliana growth and fecundity but did not affect mortality. Changing the soil type led to significant differences in fruit number, plant height, and above ground dry biomass ($p<0.05$, Table 1). These significant differences also remained when examining changes in those three variables between any combination of the soil types, although there was no significant difference in mortality rate between soil types (Table 1). *A. thaliana* grown in the ranch soil had the lowest number of fruits produced, with a mean of 11.85 fruits per plant, while the greenhouse soil had the highest, with a mean of 374.32 fruits per plant (Figure 1A). That trend also applied to above ground dry biomass (Figure 1B) and plant height (Figure 1C). Plant growth at the end of the experiment is visually shown in appendix Figure A and appendix Figure B. *A. thaliana* grown in organic soil and greenhouse soil had similar mortality, two and four plants dying respectively, but ranch soil had a higher mortality of eight plants (Figure 1D). Collectively, these results reject the null hypothesis that soil type does not affect the growth and fecundity of *A. thaliana*, as three of the four examined variables showed significant differences by soil type ($p<0.05$, Table 1).

Table 1. P-values of variables studied by soil type. G represents greenhouse soil, which was used as a control, O represents organic soil, A represents ranch soil, and All is an analysis of differences between all soil types. The hyphenated column heads are comparisons of two soil types. Overall comparisons were done using Kruskal-Wallis tests, pairwise comparisons were done using Wilcoxon rank sum tests, p-values were corrected using the Holm method, differences were considered significant when $p<0.05$, and $n=105$.

Variable	All	G-O	G-A	O-A
Mass	3.44E-14	2.40E-10	1.20E-10	0.0045
Height	1.69E-13	4.20E-09	2.70E-10	0.0027
Mortality	0.1017	1	0.64	0.13
Fruit Number	1.32E-14	9.00E-10	2.40E-10	8.10E-05

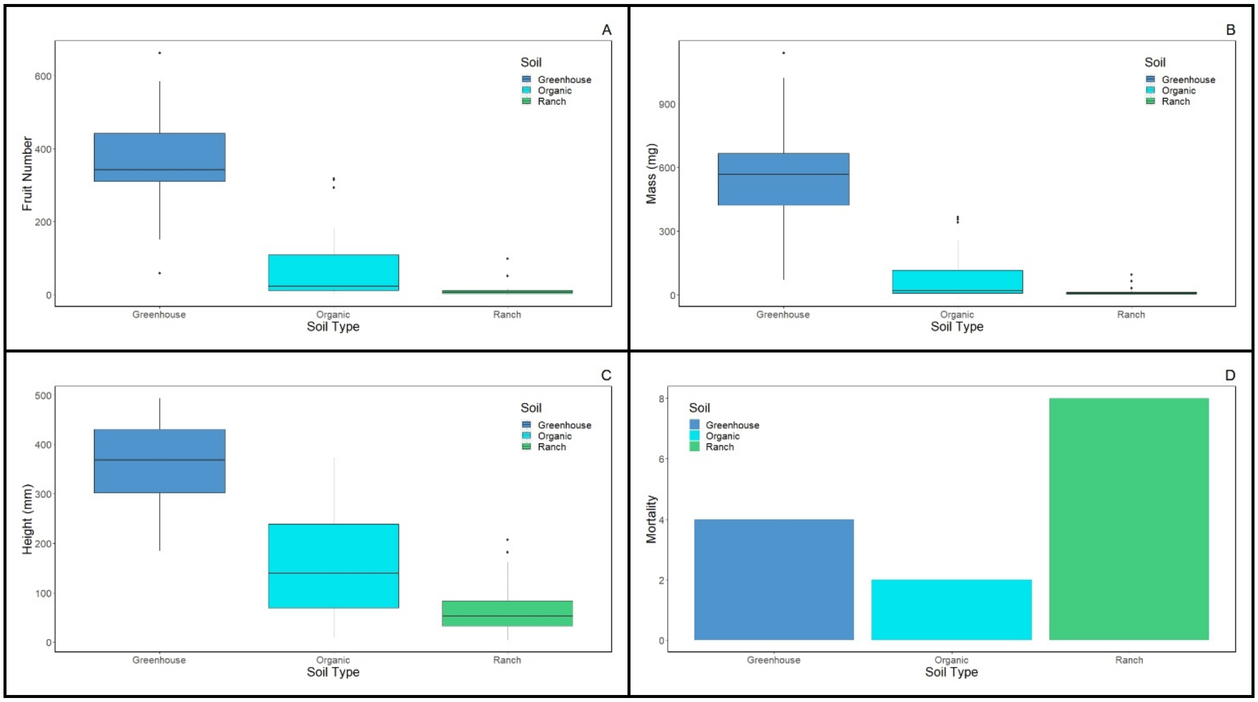


Figure 1. The impact of soil type on *A. thaliana*. Figure 1A: the number of fruits produced in each soil type, Figure 1B: the above ground dry biomass in mg of *A. thaliana* grown in each soil type, Figure 1C: the height in mm of *A. thaliana* in each soil type, Figure 1D: the number of plants that died in each soil type. Greenhouse soil was used as a control, in Figures 1A-1C the median is denoted by the bold horizontal line, the box delimits the interquartile range, and the whisker lines extend to the observed maxima and minima, except for the outliers symbolized by points, and n=105. There were overall significant differences ($p<0.05$) in mass, height, and fruit number by soil type.

Impact of short-term antibiotic exposure on growth and reproduction

I found that short term exposure to antibiotics decreased the success of *A. thaliana*, with these detrimental impacts being more pronounced for tetracycline at higher antibiotic concentrations. Short term antibiotic exposure impacted growth and potentially impacted fecundity, but there were no significant differences when comparing plants treated with the two antibiotic types for any of the variables examined except for mortality (Table 2). Unlike tetracycline, there were no significant differences in any variables when comparing the penicillin groups between themselves or to the control (Table 3). There were no significant differences between any pairs of groups in the number of fruits produced, although there was a difference overall ($p<.05$, Table 3). Additionally, the 500 µg/ml tetracycline group produced the fewest fruits by far, with a mean of 11 fruits per plant compared to an overall mean of 158.08 fruits per plant (Figure 2A). Plants treated with tetracycline showed a significant decrease in mass and height and an increase in mortality in the 500 µg/ml group in comparison to the control group (0 µg/ml) ($p<0.05$, Table 3). Most of the mortality was also seen in *A. thaliana* treated with tetracycline, with 10 out of the total 14 plants that died being in that group (Figure 2D). Mortality was particularly high at higher concentrations of tetracycline, with the group that was treated with 500 µg/ml of tetracycline experiencing seven out of 14 total deaths (Figure 2D). Additionally, there were significant differences when comparing all the treatment groups for each variable that was studied. These results reject the null hypothesis that short-term antibiotic exposure does not impact growth and reproduction, as there were significant differences between groups in height, mass, mortality, and fruit number ($p<0.05$, Table 3).

Table 2. P-values of the variables studied by antibiotic type. P represents penicillin, T represents tetracycline, and N represents a control group with no antibiotic treatment. All is a comparison of all the groups at once, and the hyphenated column heads are comparisons of two types of antibiotics. Overall comparisons were done using Kruskal-Wallis tests, pairwise comparisons were done using Wilcoxon rank sum tests, p-values were corrected using the Holm method, and differences were considered significant when $p<0.05$.

Variable	All	P-T	P-N	T-N
Mass	0.1885	1	0.34	0.17
Height	0.5085	1	1	0.53
Mortality	0.01251	0.11	0.96	0.055
Fruit Number	0.1239	1	0.1	0.36

Table 3. P-values of the variables studied by antibiotic concentration and type. P represents penicillin, T represents tetracycline, 0, 10, 100, and 500 represent the concentrations of the antibiotic solution in µg/ml which was applied to the plants weekly, with 0 µg/ml as the control group. All is a comparison of all the groups at once, and the hyphenated column heads are comparisons of two specific treatment groups. Overall comparisons were done using Kruskal-Wallis tests, pairwise comparisons were done using Wilcoxon rank sum tests, p-values were corrected using the Holm method, and differences were considered significant when $p<0.05$.

Variable	All	0-P10	0-P100	0-P500	P10-P100	P10-P500	0-T10	0-T100	0-T500	T10-T100	T10-T500
Mass	0.04749	1	1	1	1	1	1	1	0.048	1	0.096
Height	0.02137	1	1	1	1	1	1	1	0.019	1	0.05
Mortality	0.00219	NA	1	1	1	1	1	0.678	0.033	1	0.16
Fruit Number	0.04784	0.621	1	0.976	1	1	1	1	0.051	1	0.318

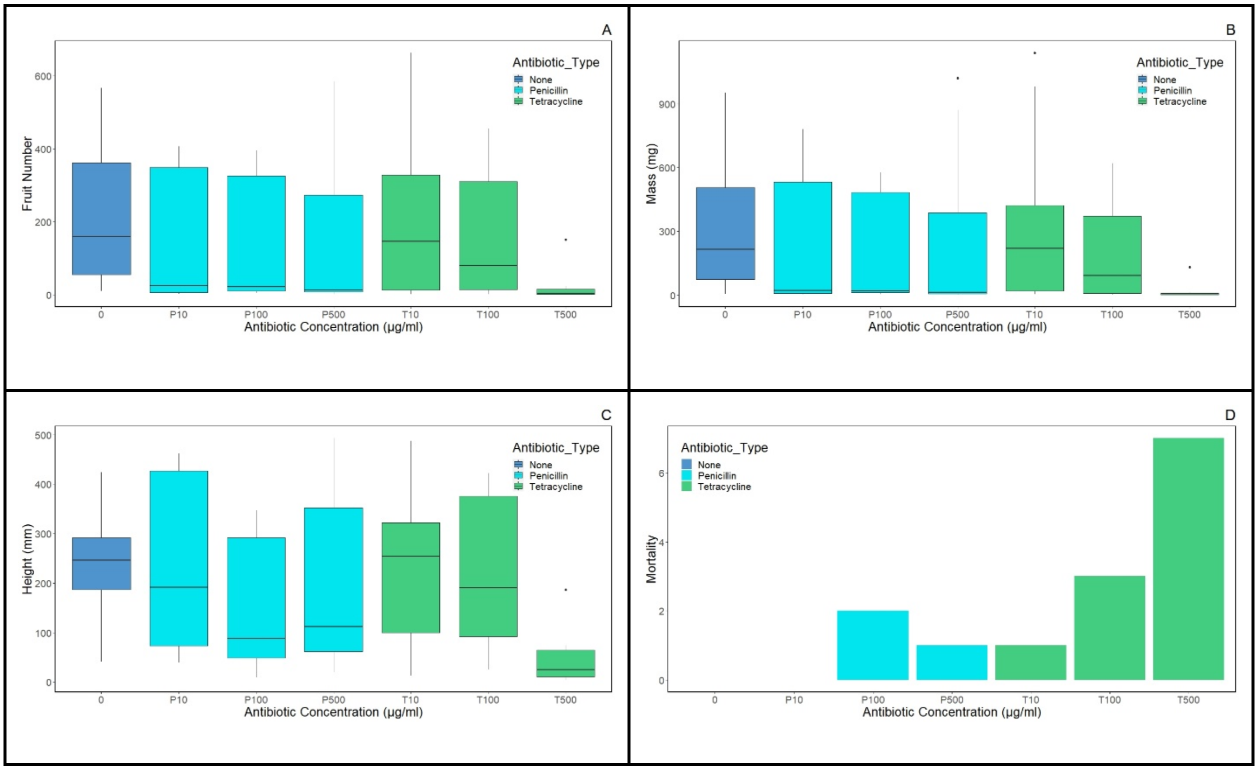
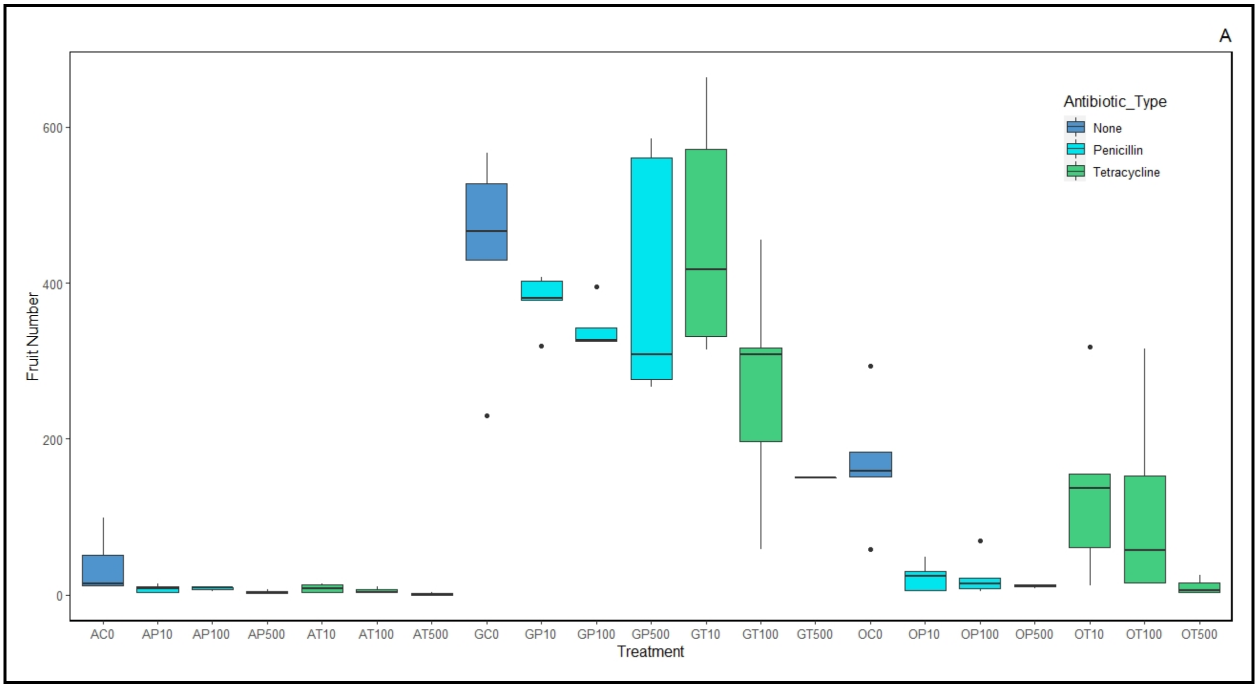
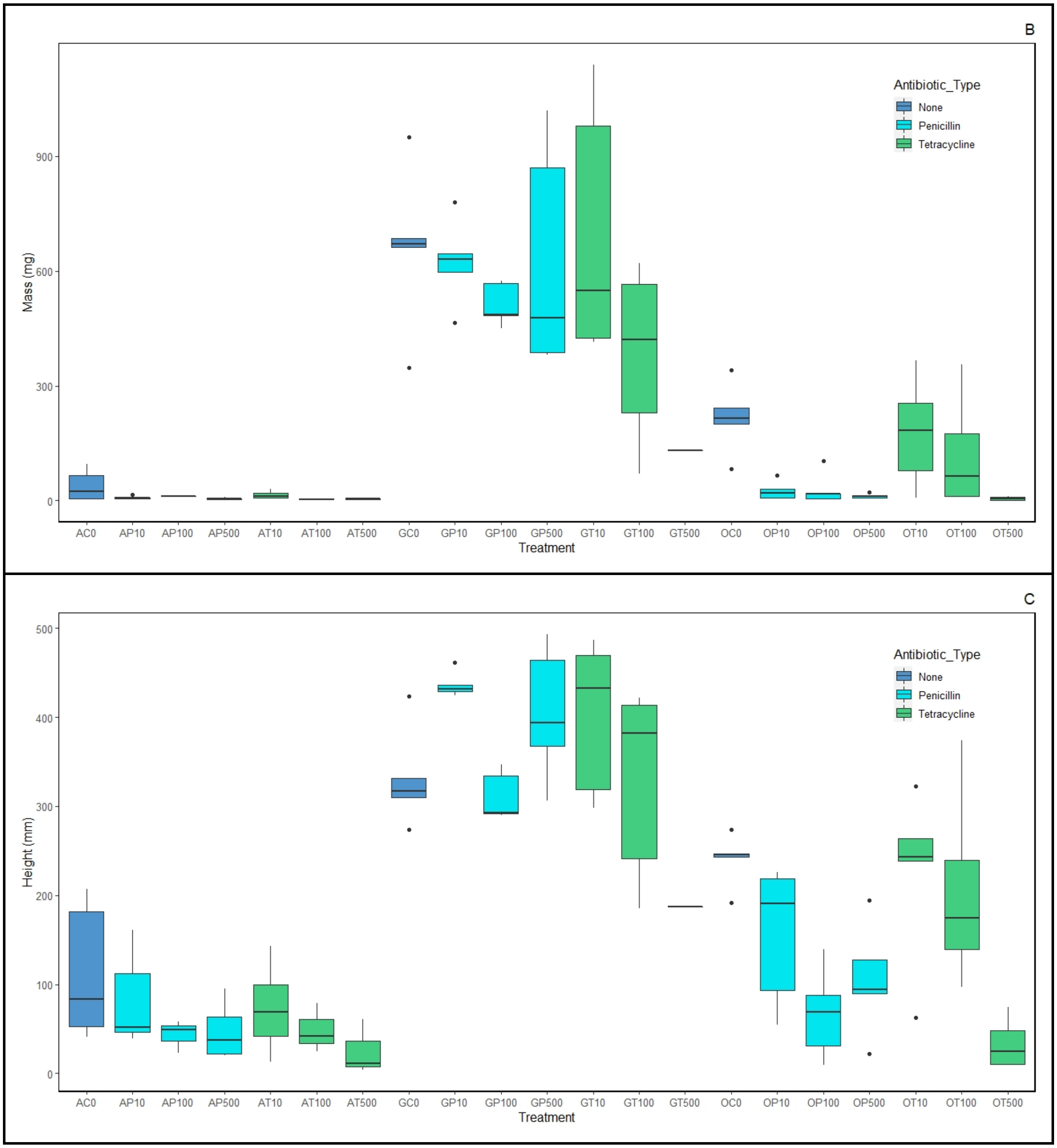


Figure 2. The impact of short-term antibiotic exposure on *A. thaliana*. T represents tetracycline, P represents penicillin, and 0, 10, 100, and 500 represent the concentrations in µg/ml of antibiotic solution that was applied to the plants weekly, with 0 µg/ml as the control group. Figure 2A: the number of fruits produced in each treatment group, Figure 2B: the above ground dry biomass in mg of *A. thaliana* grown in each treatment group, Figure 2C: the height in mm of *A. thaliana* in each treatment group, Figure 2D: the number of plants that died in each treatment group. In Figures 2A-2C the median is denoted by the bold horizontal line, the box delimits the interquartile range, and the whisker lines extend to the observed maxima and minima, except for the outliers symbolized by points. There were overall significant differences ($p<0.05$) in mass, height, mortality, and fruit number by antibiotic treatment.

Combined impacts of short-term and long-term antibiotic exposure on growth and reproduction

I found that increased concentrations of short-term antibiotic exposure and long-term antibiotic exposure led to decreased success in *A. thaliana*, but the short-term effects on fruit number, mass, and height appear to be less extreme in soils that have experienced long-term antibiotic exposure. Higher concentrations of tetracycline treatment were detrimental to *A. thaliana* for most of the variables examined. Additionally, being grown in agricultural soil was detrimental to the success of *A. thaliana* for all the variables examined except mortality, with agricultural soil that experienced long-term antibiotic exposure resulting in the least success. However, the differences in the variables examined, except for mortality rate, within treatment groups exposed to short-term antibiotics appear to be the most severe in the greenhouse soil, less severe in the organic soil, and the least severe in the ranch soil. Short-term antibiotic exposure effects appeared to be moderately lessened when organic soil was used and substantially lessened when ranch soil was used (Figure 3). The decreases in fruit number, mass, and height are more rapid in greenhouse soil than in organic soil, and more rapid in organic soil than ranch soil (Figure 3). The differences in plant growth between and within treatment groups is visually shown in appendix Figure 2. For all the variables examined except for mortality rate, the null hypothesis that the detrimental effects of short-term antibiotic exposure will not be less extreme in plants grown in soils that have experienced long-term antibiotic exposure can be rejected.

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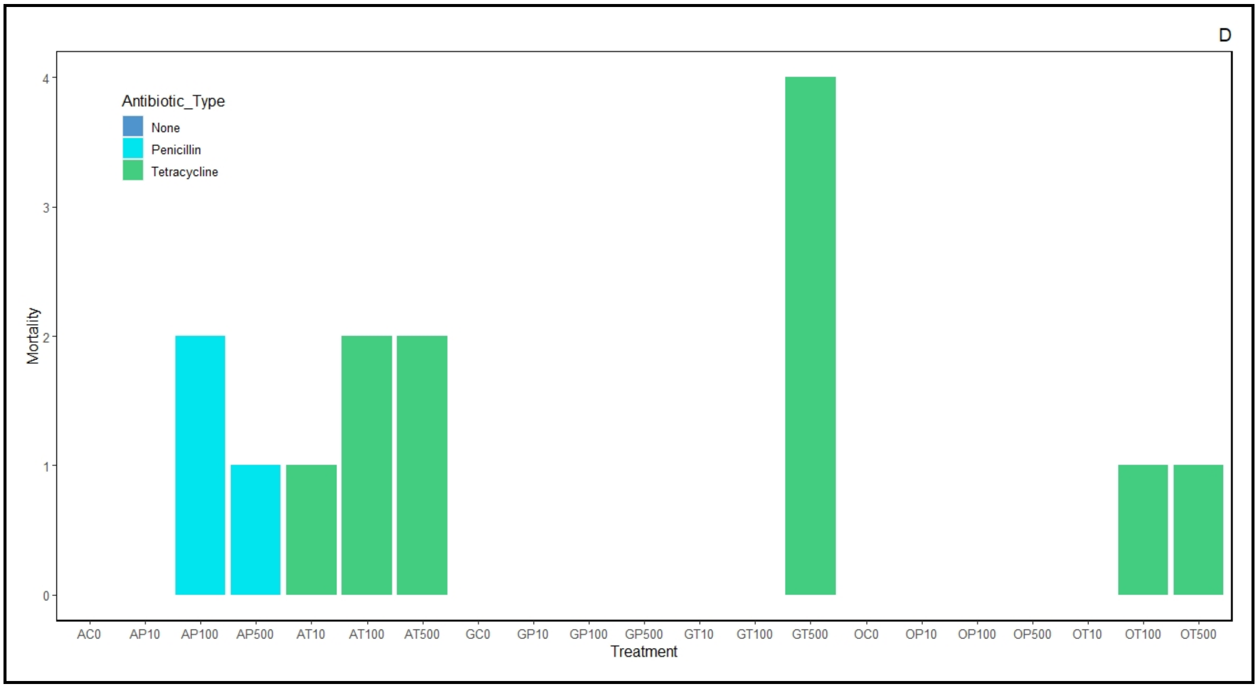

Figure 3. The impact of short and long-term antibiotic exposure on *A. thaliana*. A represents ranch soil, O represents organic farm soil, and G represents greenhouse soil, which was used as a control. T represents tetracycline, P represents penicillin, and 0, 10, 100, and 500 represent the concentrations in µg/ml of antibiotic solution that was applied to the plants weekly. Plants treated with 0 µg/ml were used as a control group. Figure 3A: the number of fruits produced in each group, Figure 3b: the above ground dry biomass in mg of the *A. thaliana* in each group, Figure 3c: the height in mm of the *A. thaliana* in each group, Figure 3d: the number of *A. thaliana* that died in each group. In Figures 3A-3C the median is denoted by the bold horizontal line, the box delimits the interquartile range, and the whisker lines extend to the observed maxima and minima, except for the outliers symbolized by points.

DISCUSSION

The extensive use of antibiotics in animal feed is a relatively new phenomenon, as this practice has only been utilized since about 1950 (“National Research” 1980). Therefore, the impacts of this widespread antibiotic use on the ecosystems surrounding agricultural operations are largely unknown and require research to elucidate them. The results of this study support that both long-term and short-term exposure to antibiotics, particularly in the context of agricultural runoff, are detrimental to the growth and fecundity of *A. thaliana*. I also found that the negative impacts of short-term exposure were somewhat mitigated by previous long-term exposure to the soils the plants were grown in. I found that long-term exposure to agricultural runoff containing antibiotics negatively impacted fruit production, height, and mass in *A. thaliana*. Additionally, my results showed that short-term exposure to tetracycline negatively impacted mass and height, and increased mortality. Short-term exposure to penicillin did not significantly impact any of the traits that I examined. Additionally, the detrimental effects on fruit production, mass, and height of short-term tetracycline exposure were reduced in plants grown in soils exposed to long-term agricultural runoff, with an increase in harm mitigation with runoff that contained antibiotics. These results suggest that antibiotic exposure is harmful to *A. thaliana*, but if the soils that *A. thaliana* are grown in are exposed to long-term agricultural runoff, particularly runoff that contains antibiotics, they have a greater capacity to withstand the negative impacts of concentrated short-term antibiotic exposure. This resilience as a result of agricultural runoff and long-term antibiotic exposure could provide insight into potential ways crops can be protected from short-term antibiotic damage, particularly as agricultural antibiotic use and introduction into the environment becomes an increasingly important issue.

Impact of soil type on growth and reproduction

The detrimental effects of agricultural runoff on *A. thaliana*, particularly from farms that use subtherapeutic antibiotics, suggest that agricultural runoff is harmful to the growth and reproduction of *A. thaliana*. This harm is likely due to the introduction of fertilizers, pesticides, manure, and other factors into the ecosystem through agricultural runoff, and is compounded by the introduction of antibiotics into the runoff. Although long-term antibiotic exposure negatively impacted three out of the four variables I examined by decreasing height, biomass, and fruit production, it did not affect mortality rate. This may be the result of the negative impacts decreasing fitness in *A. thaliana*, but the concentration of toxic compounds still being sublethal. The soil was collected roughly 100 feet from the cattle holding areas, and therefore likely contained lower concentrations of toxic compounds and resulted in less significant damage to *A. thaliana* than soil from closer to or inside the cattle holding areas would have. This result is supported by exposure to cow manure leading to increases in available heavy metal content in soils which can be toxic to plants, as well as increases in soil pH which can disrupt plant processes (Zhao et al. 2014). Additionally, Guala et al. (2010) found that low heavy metal concentrations in soil had only a moderate impact on the mortality of alfalfa, lettuce, radish and *Thlaspi caerulescens*, but the mortality increased nonlinearly at higher concentrations. These results suggest that *A. thaliana* may have experienced exponential increases in mortality if it were exposed to higher concentrations of heavy metals than those in the soils I used. Exposure to cow feces at both farms used in this study may also play a role, as it has been found to immobilize nitrogen critical to *A. thaliana* fitness (Patten et al. 1980).

My results suggest that *A. thaliana* grown in the ranch soil had the least success, potentially due to harmed microbial processes in the soil as a result of exposure to manure with antibiotics, in comparison to the organic farm soil which contained manure without antibiotics, and the greenhouse soil which contained no manure or antibiotics. This theory is supported by Kumar et al. (2005), who found that because the concentrations of antibiotics in manure are low, they are unlikely to result in direct toxic effects on the soil microflora and microfauna. However, these concentrations can still negatively affect soil microbial populations, which decreases food sources for other soil organisms and therefore can disrupt the soil microbial processes which *A. thaliana* relies on. Both the evidence from this research and previous studies suggests that agricultural runoff, particularly that which contains antibiotics, is harmful to *A. thaliana*. Not only is long-term antibiotic exposure detrimental to the success of *A. thaliana*, but short-term antibiotic exposure is as well.

Impact of short-term antibiotic exposure

The results of this study suggest that short term antibiotic exposure may reduce growth and fecundity in *A. thaliana* in some cases. One explanation for this finding could be that the detrimental effects on growth are a result of damage caused by tetracycline to the microbial community. This is supported by Chessa et al. (2015), who exposed soil to tetracycline solutions at similar concentrations as the ones that I used. They found that higher tetracycline concentrations harmed the soil microbial community structure and function, but the effects decreased after seven days. However, weekly antibiotic application in my study did not allow the effects to decline. The impact of tetracycline could also be not only due to its alterations to the soil microbiome, but its impacts on *A. thaliana* itself. For example, Bowman et al. (2011) found that exposure to tetracycline results in decreased intracellular calcium levels in *A. thaliana*, which alters protein synthesis and accumulation and is ultimately detrimental to plant growth and reproduction. My results are supported by a variety of studies, including Liu et al. (2017), who found that 1 mg/L exposure to tetracycline reduced *A. thaliana* biomass by 33.3%.

My results suggest that penicillin was not as harmful to *A. thaliana* as tetracycline, potentially due to their different levels of soil persistence. Penicillin's half-life in soil is less than seven days, while tetracycline's is more than 20 (Tasho and Cho 2016), and the impact of tetracycline was likely compounding throughout the experiment with multiple applications compared to penicillin which degrades faster. The variation in short-term antibiotic effects on *A. thaliana* between antibiotic types could also be a result of differences between which bacteria tetracycline and penicillin are effective against, as well as their modes of action. For example, tetracycline is a broad-spectrum antibiotic effective against gram-positive and gram-negative bacteria, and functions by inhibiting bacterial protein synthesis through preventing aminoacyl-tRNA from attaching to the ribosomal acceptor (A) site. Tetracyclines are bacteriostatic, halting the growth of bacteria but not killing it, and therefore halting its multiplication (Chopra and Roberts 2001). Alternatively, penicillin is effective against gram-positive and most gram-negative cocci, as well as gram-positive rods and most anaerobic bacteria. Penicillin works as a bactericidal antibiotic which hampers the cross-linking of peptidoglycan in the bacterial cell wall (Yip and Gerriets 2021). Thus, the different bacteria these antibiotics impact as well as their different modes of action may explain the variation in their impacts on *A. thaliana*. The concentrations of antibiotic solution I used in this study are likely higher than most plants would experience in nature, but their impacts illustrate that short-term antibiotic exposure to tetracycline does influence the success of *A. thaliana*. It is important not only to understand the long-term and short-term effects of antibiotic exposure on *A. thaliana*, but also to understand their combined influence.

Combined impacts of short-term and long-term antibiotic exposure

My results suggest that *A. thaliana* grown in soil exposed to agricultural runoff and antibiotics over long periods of time may be resistant to the short-term effects of antibiotics. The results of this study support that the negative effects of short-term tetracycline exposure on fruit production, mass, and height were somewhat mitigated by long-term agricultural runoff exposure, and this mitigation was bolstered when the agricultural runoff the plants were exposed to over a long period of time contained antibiotics. This may be due to long-term exposure to agricultural runoff which contains antibiotics resulting in the development of antibiotic resistant bacteria, as well as both types of agricultural runoff benefiting the soil microbial community. These findings are supported by Wichmann et al. (2014), who examined manure from cattle that had been treated with antibiotics similar to those at Cattle Ranch and found 80 different antibiotic resistance genes in their fecal samples. Their research suggests that a variety of different antibiotic resistance genes are present in agricultural runoff from farms that treat their cattle with antibiotics. Additionally, once these genes are released into the environment, antibiotic resistance levels are sustained for up to 18 months in the soil, as far as 100 meters from animal holding pens (Ghosh and LaPara 2007). These studies support my findings and may provide an explanation as to why long-term antibiotic exposure mitigated the negative effects of short-term antibiotic exposure in *A. thaliana*.

In addition to antibiotic resistance, agricultural soils may be more resilient to the damage done by exposure to harmful compounds than greenhouse soil due to variations in their composition. For example, factors such as aggregation, substrate quality, and the structure and diversity of the microbial community contribute to the resilience of a soil microbiome (Griffiths and Philippot 2014). Soils that were exposed to agricultural runoff would likely have a higher diversity of microbes than soil kept in a greenhouse and therefore may be more resilient to disturbances such as short-term antibiotic exposure, as agricultural greenhouse soils have been shown to possess lower soil microbial diversity than agricultural open field soils (Sun et al. 2018). Additionally, Chessa et al. (2015) found that the addition of tetracycline to soil negatively impacts the soil microbial communities, but the addition of cow manure mitigates these inhibitory effects and contributes to the recovery of the soil. This supports my findings that cattle farm soils experienced less detrimental effects as a result of short-term antibiotic exposure than the control soil. My results as well as previous research suggest that although cattle farm soil is harmful to *A. thaliana*, it is also beneficial to the resilience of *A. thaliana* in mitigating the harm caused by short-term antibiotic exposure, through the addition of antibiotic resistant bacteria and increased microbial diversity in the soil.

Themes and synthesis

Antibiotics are a necessary component of human and veterinary medicine and they have many positive uses, but an increased reliance on them threatens fragile ecosystems. The development of antibiotics and their subsequent widespread use has resulted in many beneficial contributions, including the prevention of diseases and improved trauma recovery (Chandel and

Budinger 2013). Additionally, antibiotics have the capacity to mitigate disease in livestock, as well as promote animal growth, which can increase the success of agricultural operations (van den Bogaard & Stobberingh 1999). However, both short-term and long-term antibiotic overuse can be harmful to the environment. Short-term antibiotic use can result in damage to the soil microbial community and acute toxicity, and long-term antibiotic use has led to the development of antibiotic resistant bacteria. Both of these impacts influence many aspects of ecosystem success, including the growth and fecundity of *A. thaliana*. My results suggest that long-term antibiotic exposure resulted in significant damage to both the growth and fecundity of *A. thaliana*. Additionally, short-term exposure to tetracycline caused damage to *A. thaliana* potentially through both direct toxicity and causing damage to the soil microbiome *A. thaliana* grows in. Not only does antibiotic exposure harm *A. thaliana*, but it can alter many facets of an ecosystem. The presence of antibiotics in the environment can lead to antibiotic resistance, ecological function disturbance, changes in the microbial community structure, and alterations to nitrogen fixation, sulfate reduction, and methanogenesis (Ding and He 2010). These impacts cause serious ecological damage to many species, and even though my research suggested that the harm caused by short-term antibiotic exposure in *A. thaliana* was somewhat mitigated by being grown in agricultural soils that had experienced long-term antibiotic exposure, plants that experienced either length of antibiotic exposure were still overall less successful than the control plants that did not. Although the results of this experiment point to the conclusion that antibiotics are detrimental to *A. thaliana*, their use in agriculture is necessary in many ways, so the goal should not be to eradicate them entirely but to determine which concentrations they should be used at to minimize the amount of antibiotics needed to achieve optimal agricultural results.

Limitations and future directions

There are still aspects of this experiment which could be improved, and many ways in which the results of this study could be expanded upon through further research into *A. thaliana* and antibiotic exposure. For example, some extraneous variables were not completely controlled for, as there could be many components of agricultural runoff and variations in soil type that influence the success of *A. thaliana*, including differences in nutrient concentration, soil particle size, and more. The main unanticipated result of this study was the lack of significant differences in mortality rate, but this is likely due to detrimental but ultimately sublethal soil conditions and antibiotic concentrations. A useful future study could increase the antibiotic solution concentrations to determine the concentrations necessary to cause significant mortality. Future research could use many variations of antibiotic concentrations, both higher, lower, and less disparate than the ones that this research used. This study only tested a few antibiotic concentrations and further research could examine the antibiotic concentration cutoff that would result in significant mortality. Future studies could also find a way to control for more extraneous variables, including potentially sterilizing each soil type and growing plants in the sterilized soil as well as the unsterilized soil, which would allow more direct comparisons between the groups. This would assist in determining if the impacts of soil type on *A. thaliana* are a result of the soil microbial community or physicochemical traits of the soil.

There are many potential future studies that could expand upon concepts that this research focused on. For example, further research could take soil samples from various locations on and around farms, test the soils for antibiotic resistant bacteria, and then grow the plants in those soils to ensure antibiotic resistance is present and examine the effects of different levels of antibiotic resistant bacteria. This would test to what extent the areas that surround agricultural operations develop antibiotic resistant bacteria, and how proximity to agricultural operations influences their impacts on the areas around them. Further research could also focus on finding what realistic concentrations of antibiotics in agricultural runoff are, as this study based those values on previous research and not actual findings. This study focused on two of the most common antibiotics used in agriculture and one model organism, but there are many species which are consistently exposed to several kinds of antibiotics, and a variety of variables that could be used to measure their success. Any combination of those could be the subject of a future study, as well as research that focuses on exposure to multiple antibiotics, which could have negative, positive, or additive interactions. Overall, other research could certainly be done to expand upon and improve the results that this study found through examining other organisms, other antibiotics, or variables that were not the focus of this research.

Broader implications

Given the widespread importance of antibiotic use, antibiotic resistant bacteria, and the utility of *A. thaliana* as a model organism, the results of this study suggest that *A. thaliana* could be useful in determining the minimum dosage of antibiotics needed for agricultural economic benefit and the maximum dosage that does not harm plant fitness. Additionally, with its status as a model organism, the knowledge that is produced through research with *A. thaliana* can often be utilized to understand other organisms that may be more difficult to study. This knowledge could be beneficial to studies into crops with more economic significance than *A. thaliana* which have been shown to experience negative impacts due to antibiotic exposure, including carrot, lettuce, alfalfa, rice, barley, corn, and wheat, as well as crops that bioaccumulate antibiotics, such as corn, carrot, pea, and others (Minden et al. 2017). Its importance is illustrated by the fact that *A. thaliana* is one of the most cited organisms, it was the first plant genome to be sequenced, and it is now widely used in plant research as its responses to environmental factors can provide insight into other organisms (Edison et al. 2016). In addition to the importance of understanding *A. thaliana's* response to environmental factors, it is also crucial to understand how ecosystems will respond to their exposure to the increased use of antibiotics in agriculture and other fields. The release of antibiotics into ecosystems can alter soil and water quality, biogeochemical cycling, organic contaminant degradation, and more (Grenni et al. 2017), so understanding how they interact with different components of the ecosystem is necessary in mitigating these alterations. Banning antibiotic use is not a realistic solution, but it is crucial that the impacts of antibiotics on the environment are better understood.

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APPENDIX A: *A. thaliana* growth images

Figure A1. Ranch soil, penicillin treatment.

Figure A2. Ranch soil, tetracycline treatment.

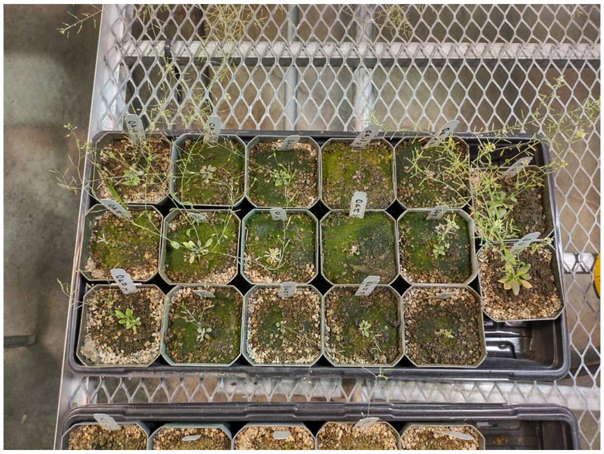

Figure A3. Organic soil, penicillin treatment.

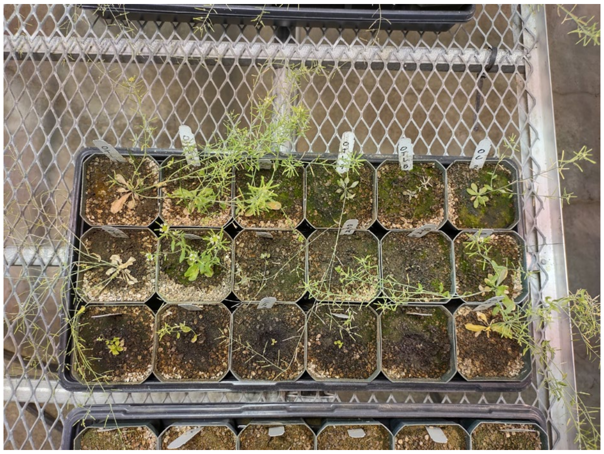

Figure A4. Organic soil, tetracycline treatment.

Figure A5. Greenhouse soil, penicillin treatment.

Figure A6. Greenhouse soil, tetracycline treatment.

Figure A. ***A. thaliana*** **after 45 days of growth, organized by soil type and antibiotic type.** From the top row to the bottom row, the soil types are ranch soil, organic soil, and greenhouse soil. The plants treated with penicillin are in the left column, and the plants treated with tetracycline are in the right column.

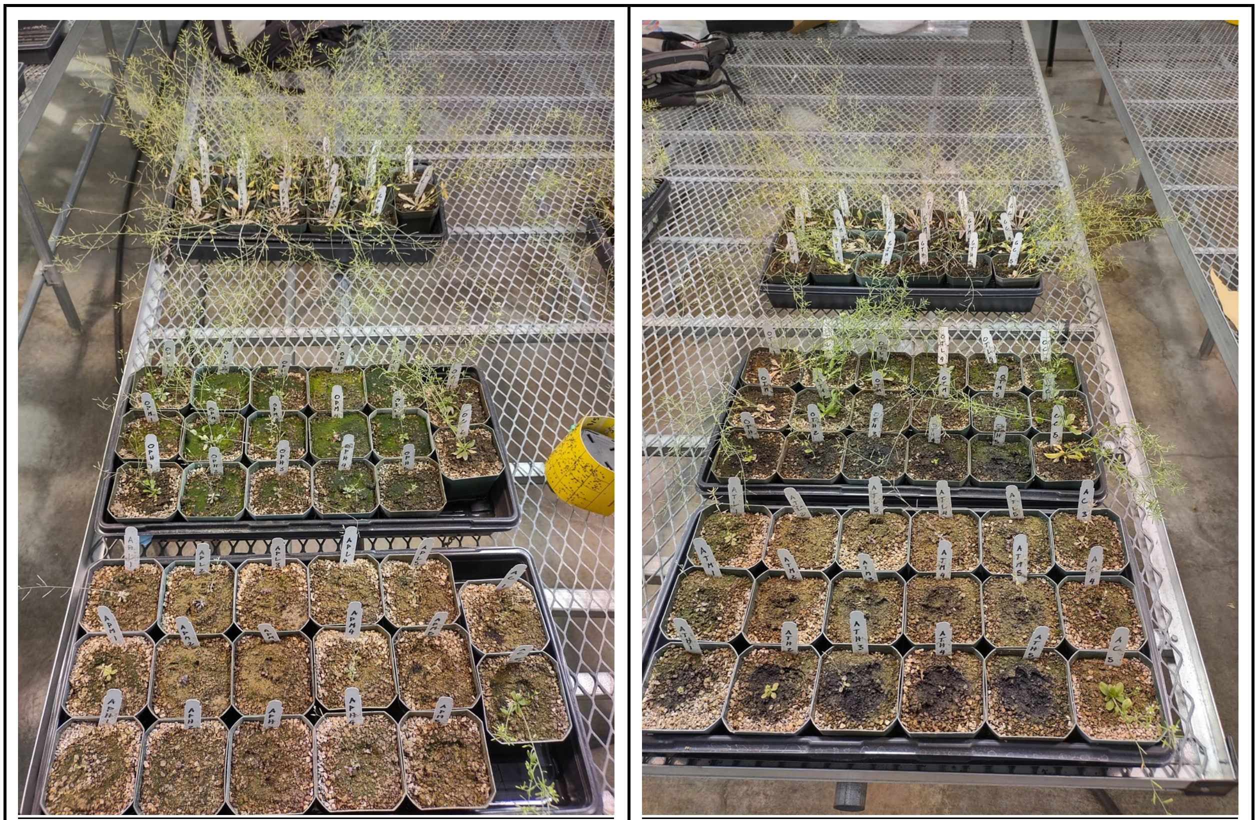

Figure B1. Penicillin treatment.

Figure B2. Tetracycline treatment.

Figure B. ***A. thaliana*** **after 45 days of growth.** Figure B1: the group treated with penicillin, Figure B2: the group treated with tetracycline. From the top row to the bottom row in Figure B1 and B2, the soil types are greenhouse soil, organic soil, and ranch soil.