THE EFFECTS OF WEAK MAGNETIC FIELD EXPOSURE ON PLANT DEVELOPMENT IN SIMULATED SPACE-LIKE CONDITIONS

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Abstract

Magnetic fields are a natural element of any planetary body with intensities that vary from the geomagnetic field to near-null magnetic fields. Living things are constantly affected by magnetic field intensities, so understanding these effects can further the knowledge in areas of science such as space exploration and pioneering. Plants are essential to any environment sustainable for human life to prosper. This study focuses on the effects that weak magnetic field (WMF) exposure and its interaction with changes in soil types has on *P. vulgaris* and *A. thaliana*. Seeds from both species are exposed to a WMF of 0.5 mT in groups of 0, 60, 120, 240 minutes for six days. Following the exposure, the seeds were randomized and planted across three mediums: soil, lunar regolith, and a 50/50 mix of both. Results suggest that WMF exposure had no significant effects on germination or growth. Changes in medium significantly ($p < 0.001$) affected stem height, wet aboveground biomass, and dry belowground biomass. These results provide more information on possible consequences that WMF exposure and changes in soil can have on plant development. Further investigation is required to better understand these effects for future eras of space exploration.

Introduction

The Earth's magnetic field (MF), or geomagnetic field (GMF), constantly affect all living things on the planet. The GMF changes over time and is currently the strongest it has been in the last 100,000 years. However, it has weakened over the past 200 years (Buis 2021). The measure of the GMF is often seen in Telsa (T), the SI unit of the induction vector *B*, or Gauss (G), the unit of measurement for induction in the cgs-emu system (Lanza and Meloni 2006). The vertical component of the GMF is ~67 µT and its horizontal component is ~33 µT (Maffei 2014). In this experiment, the GMF will be taken as an estimate of 50 µT as taken from the study done by Belyavskaya (2004).

Numerous studies have been conducted to better understand the importance that the GMF has on living things. Its effects on plant development and biological changes may potentially be positive or negative (Belyavskaya 2004; Silva and Dobránszki 2016; Radhakrishnan 2019). Understanding changes in MF intensity aids in gaining more knowledge on these effects. MFs in space and other planetary bodies, including Low Earth Orbit (LEO) conditions, have a big impact on the general knowledge of its effects on plant life. This is increasingly relevant as humans gradually introduce plant life to these space conditions.

Positive impacts on plant life are obtained under certain MF intensities and time exposures. Increased MF levels have been shown to improve crop productivity and development, seed germination, and more at MF levels ranging from 20 to 100 mT (Sarraf 2020; Maffei 2014; Radhakrishnan 2019). Galactic (<0.1 nT) and near null (~0 T) MFs have shown to negatively affect plant development with decreased reproduction growth, inhibition of overall growth, and alteration of metabolic activities being documented examples (Maffei 2014; Belyavskaya 2004). WMFs (100 nT to 0.5 mT), including the GMF, have shown to have a variety of impacts on plant development and their chemical makeup. Even with these significant impacts, Belyavskaya suggests that plants may adapt to WMFs through experimentation and exposure (Belyavskaya 2004). However, there is still little known about WMFs and their effects. It is necessary to further investigate this concept when delving deeper into the realm of space exploration and colonization.

Located in LEO, the International Space Station (ISS) – a key tool in space exploration – experiences a MF of ~30 µT - (Laurini and Gerstenmaier 2014; Califf et al. 2020). Systems like the Advanced Plant Habitat (APH) and Veggie on the ISS aid in exploring new technologies for crop production and food safety (NASA n.d.). However, the ability to grow plants in space-like conditions with a focus on the effects of WMF intensity still requires more research. This study will investigate how *Phaseolus vulgaris* (bush beans) and *Arabidopsis thaliana* respond to WMF exposures slightly higher than that of the GMF. By exploring potential negative or positive effects within this range, this study aims to expand our understanding of plant reactions to space-like conditions, potentially advancing future space exploration.

Methods

Materials

The Triaxial Helmholtz Coil System (HHC) was utilized to manipulate the MF, producing a B-field up to 7.5 G (0.75 mT). Located at Old Dominion University, the HHC was designed around a 150 cm triaxial square Helmholtz C-spin coil system with a volume of 27,000 cm^3 (30 cm x 30 cm x 30 cm) and a field uniformity of 99%. Its control software facilitates ambient-field-cancellation, creating a near-zero MF environment within 2-3 mG (0.2-0.3 µT). This software specifies the intensity, frequency, and direction of the magnetic field in real time. A growth chamber was utilized to store the seeds/plants in conditions similar to the environment on the ISS. This environment was created within the chamber's capabilities, i.e., excluding anti-gravity. A constant temperature was set at 22℃ and CO_2 levels remained ambient, ranging from 400 to 600 ppm. Each day, the chamber cast white light for 16 hours and went dark for the remaining 8 hours.

Experimental Design

P. vulgaris and *A. thaliana* were exposed to a constant WMF for six days. *P. vulgaris* are an antioxidant-rich food that could provide space radiation protection for crew members if consumed (NASA n.d.), and *A. thaliana* is the model plant used for many experiments including those aboard the ISS (NASA n.d.; Paul, Elardo, and Ferl 2022). The seeds of each species were divided into four separate exposure groups with one being a control. After six days of exposure, the seeds were planted and divided across three groups of soils. Four weeks were allocated for the plants to grow where growth measurements were taken twice weekly. After the four-week growth period, all plants were harvested for data collection.

Exposure Period

In this experiment, 360 seeds were exposed to a WMF of 5 Gauss (0.5 mT) in the x-, y-, and z-directions. Using the HHC system, the machine was calibrated prior to every exposure session following the cancellation of the local GMF to its near-zero intensity (0.2-0.3 μT). Exposure groups were distinguished by time: 0, 60, 120, and 240 minutes with 0 minutes as the control. *P. vulgaris* seeds were separated into 9 Petri dishes amongst the exposure groups. Each exposure group had 9 dishes of 5 seeds totaling 180 seeds being exposed. Similarly, *A. thaliana* seeds were separated into 5 dishes of 9 seeds per exposure group still with 180 seeds total. Prior to exposure, seeds were rinsed with deionized (DI) water to aid in fungus and mold prevention and Petri dishes were lined with filter paper. 5 mL of DI water were added to each dish prior to exposure and as needed over the six-day period. Seed-filled Petri dishes were placed 2-3 ft above the HHC's platform level and rotated constantly during exposure at a rate of 0.005 rev/s. Seeds were stored in a growth chamber at all times excluding their exposure sessions. Seeds were exposed per their designated exposure groups every morning for six days, ending the exposure period on the seventh day.

Growth Period

Seeds were planted on the seventh day following the end of the six-day exposure period. Per each species, 60 seeds were planted across three primary support matrices: soil, lunar regolith, and a mix of both. The soil used was organic with no fertilizers added; this was considered the control. The lunar regolith was a lunar stimulant developed by and obtained from Exolith Labs. The stimulant was mineral based with a particle size distribution similar to that of Apollo soils. Seeds were randomized prior to being planted, and 5 seeds from each exposure group were separated into each matrix, or medium. This totaled 20 plants per medium, 60 plants per species, and 180 plants total. Seeds were planted with 500g of soil, with the soil/regolith mix having 250g of each, in small pots. Potted plants were stored in the growth chamber and allowed a growth period of four weeks. Tap water was used to water the plants and drainage holes prevented the plants from being overwatered. Stem height and diameter measurements were taken twice weekly for data collection. Leaf width measurements were also collected as the plants grew over time. After the four-week exposure period, all plants were harvested for final data collection.

Data Collection and Statistical Analysis

Percent germination was measured after the six-day exposure period for each Petri dish of both *P. vulgaris* and *A. thaliana*. Using a two-way analysis of variances (ANOVA) via RStudio, the effects that exposure and species type had on total percent germination were tested. Per species, a one-way ANOVA was also conducted to test the effects of exposure on percent germination. A Tukey post-hoc test, also referred to as a Tukey Honest Significant Difference (HSD) test, was conducted following the one-way ANOVA to test for any significant differences amongst the exposure groups. Similar to Carr et al. in their analysis (2023), Kaplan-Meier estimates were produced to analyze rate of germination as a time-to-event. Using the death and survival probabilities given by Goel et al. (2010), the survival equation is given below:

$$S_t = \frac{\begin{matrix}\text{Number of subjects} \\ \text{living at the start}\end{matrix} - \begin{matrix}\text{Number of subjects} \\ \text{died}\end{matrix}}{\text{Number of subject living at the start}} \quad (1)$$

The death probability is the total death (*d*) over the total alive at the start (*n*). Kaplan-Meier probabilities refer to events as "death", where in this study the event is germination and *n* is total ungerminated seeds at the start. Kaplan-Meier estimates and survival functions were done for each exposure group for both species. Any seeds lost during the six-day period were recorded and censored in this analysis. A log-rank statistic was conducted to test if the germination rate was significantly different between exposure groups (Goel, Khanna, and Kishore 2010). This equation is as follows:

$$Log - rank\ test\ statistic = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2} \quad (2)$$

Here *E* is the total number of expected events and *O* is the total number of observed events.

Harvesting data includes stem height, stem diameter, wet and dry aboveground biomass (AGBM), and dry belowground biomass (BGBM). Leaf area, length, average width, max width, and weight were also taken for each leaf on each plant. The averages of the leaf measurements were taken. Wet biomass is referred to as the biomass recorded from the plant immediately after being extracted from its soil. Dry biomass is that which was recorded after being placed in an oven to dry out its water. The AGBM included all contents of the plant above its soil, including its leaves, and the BGBM included everything below, meaning its roots. A two-way ANOVA was conducted to test the effects of exposure, medium type, and their interaction. This was done for each harvesting parameter listed. A Tukey HSD post-hoc test was conducted following this ANOVA to test for significant differences amongst the exposure groups and the medium types.

Results

Germination

At the end of the six-day exposure period, the two-way ANOVA shows that exposure to a WMF of 0.5 mT had no significant effect on percent germination amongst both species. Similarly, species type showed no significance either. Within each species, a one-way ANOVA shows that exposure had no significant effect on percent germination for both *P. vulgaris* and *A. thaliana*. The Tukey HSD post-hoc test shows no significant difference amongst the exposure groups for both species as well. The average percent germination for *P. vulgaris* was ~92% and ~91% for *A. thaliana*. All exposure groups exceeded an average percent germination of over 90%. Kaplan-Meier curves show the decrease in ungerminated seeds, and subsequently the increase of percent germination, over the six days (Fig. 1). Day three shows a noticeable increase in germination for all exposure groups in both species. The 120-minute group produced the most germinated seeds on day three with ~66% germinated for *P. vulgaris* and ~51 for *A. thaliana*. However, log-rank tests show no significant difference between any of the exposure groups, compared against the control, for both species.

Growth and Harvesting

After the four-week growth period, only one of the *A. thaliana* plants grew out of the sixty. Any analysis done from the harvesting data excluded this one plant, i.e., only the bean plants were considered. Following the two-way ANOVA, exposure nor its interaction with the change in the medium had any significant effects on the harvesting parameters listed prior. Medium type shows a significant effect on stem length, stem diameter, wet and dry AGBM, dry BGBM, average leaf area, and average leaf length (Table 1). Stem length, wet AGBM, and dry BGMB were affected the most with P-values less than 0.001. Tukey HSD plots show that regolith was significantly different compared to soil and the 50/50 mix for most significantly affected parameters (Fig. 2). Stem diameter was the only parameter that shows a significant different between the soil and the 50/50 mix with a P-value of ~0.01361. Plants grown in regolith show to have the least growth compared to the soil and 50/50 mix (Fig. 3). Stem diameter was the only parameter where regolith, although still lower than soil, had a higher max value and median than that of the 50/50 mix (Fig. 3). Soil and the 50/50 mix had the most growth amongst most of the parameters with max values and higher medians being interchangeable between the two (Fig. 3). The 50/50 mix shows a median either larger than or similar to that of the soil in most cases, showcasing that plants were able to grow well in this medium when compared to the organic soil.

Table 1

Results of two-way ANOVA for soil types

Parameter	*F Value*	*Pr(>F)*
Stem length (cm)	31.63	< 0.0001 ***
Stem diameter (mm)	4.788	0.0145 *
AGBM - Wet (g)	9.787	0.0004 ***
AGBM - Dry (g)	3.452	0.0428 *
BGBM - Dry (g)	15.635	< 0.0001 ***
Avg. Leaf Area (mm^2)	4.386	0.0228 *
Avg. Leaf Length (mm)	6.951	0.0038 **
Avg. Average Leaf Width (mm)	1.023	0.374
Avg. Max Leaf Width (mm)	2.339	0.116
Avg. Leaf Weight - Dry (g)	2.440	0.106

Discussion

The results in this study give a deeper insight into the effects that changes in MF exposure may or may not have on plant development. In this case, no significant data was found that implies any effects were made by WMF exposure on plants. However, with a MF intensity lying on the brink of the WMF range adopted for this study combined with it being higher than the GMF, the results give more information on when effects can be expected to occur. Studies have shown that negative effects for extremely weak MFs (0 nT – 100 nT) and positive effects for strong MFs (>1 mT) may occur during periods of exposure (Maffei 2014; Silva and Dobránszki 2016). The WMF range (100 nT – 0.5 mT) varies in effects with some being positive, others being negative, and in some cases none at all. The results in this study show that plants can withstand WMF conditions without any inherently negative or positive consequences. Being able to survive with changes in MF intensities gives reason to further investigate their adaptability and to discover the range of MF intensity that one could expect effects on development to occur.

Promotion of germination and growth were expected prior to conducting this study. Studies have shown promotion in germination for plants exposed to MF ranges of 500 µT to 750 µT as well as positive effects on growth and photosynthesis from ranges 0.0005 T to 0.1 T (Maffei 2014; Radhakrishnan 2019). Considering the increase in intensity compared to that of the GMF, results mimicking studies showing positive effects in development when MF intensities were increased were expected. Research has shown reduction in CO_2 uptake (~500 µT) and regression in growth and germination (0.4 mT - 0.5 mT) (Maffei 2014; Silva and Dobránszki 2016). This variation in results showcases the lack of uniformity and reproducibility of data that imply expected effects of WMF exposure. The results from this study, although unexpected, were not unwarranted.

Implications for Space Exploration

Information from lunar and Martian soil compositions, gathered through exploration, reveals that while both contain essential minerals for plant growth, they lack nitrogen (Wamelink et al. 2014). Previous studies have shown that plants are capable of growing in lunar soil and lunar stimulant (Paul, Elardo, and Ferl 2022; Wamelink et al. 2014), although their growth seems to be stunted or underdeveloped in these conditions. The results of this study align with what has been seen in similar experiments. The significant differences between the lunar regolith and the organic soil (Fig.3) indicates that Earth's soils provide the resources necessary to promote plant development that lunar regolith cannot. Lacking biotic activity and organic matter, contributing to the lack of nitrogen, lunar soils cannot provide the micro and macronutrients plants needs for optimal growth (Duri et al. 2022). No further nutrients were added to the regolith medium used in this study, apart from the minerals from tap water that could be accounted for. Noting this, it seems likely that the significant differences found between the regolith and the 50/50 mix could be attributed the addition of organic materials from the organic soil in the mix, thus promoting plant growth in this medium compared to that of the regolith.

Hinderance on growth for plants in the regolith medium was expected prior to experimentation. These results are similar to the results of other experiments testing the effects lunar regolith may have on plant development. Paul et al. conducted an experiment where lunar samples from Apollo missions were used to grow *A. thaliana* (Paul, Elardo, and Ferl 2022). Even with the addition of a nutrient solution, root development was inhibited and growth was stunted. Wamelink

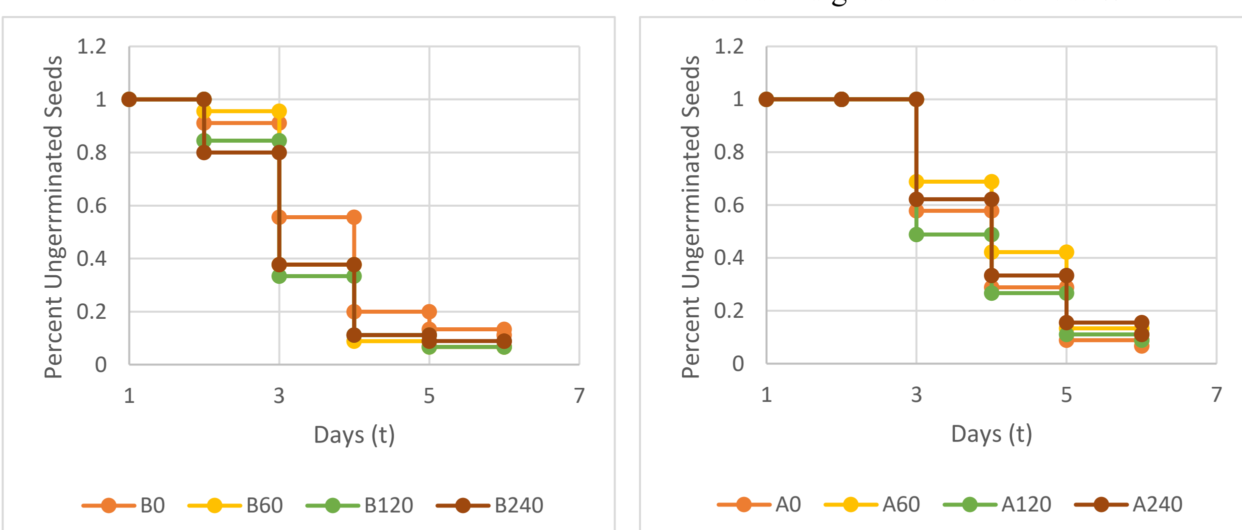

Fig. 1: Kaplan-Meier survival functions showing the percent decrease of ungerminated seeds, i.e., the increase in percent germination. (A) *P. vulgaris* seeds with exposure groups 0, 60, 120, and 240 minutes. (B) *A. thaliana* seeds with exposure groups 0, 60, 120, and 240 minutes.

et al. (2014) discovered a significant difference in biomass between organic soil and lunar stimulant, aligning with the significances found in this study. These discoveries lead to the belief that although plants can survive in lunar soils and conditions, this environment is not optimal for plant development. When considering the possibility of growing plants in space-like environments, much needs to be discovered before future pioneering and provision of a reliable food source.

An unexpected result obtained from this study was the data found from the 50/50 mix. In addition to providing a steady growth that was comparable to soil in all four growth parameters, the medians and maximum values of most of the harvest parameters were comparable if not larger than that of soil (Fig. 3). This indicates that it may be possible to regulate growth in lunar soils with an addition of certain nutrients that regolith may be missing. These additions might compensate for the deficiencies in regolith and counteract the previously observed developmental setbacks. This insight may lead to an improved understanding of plant adaptability in space-like conditions and aid in developing strategies for controlled plant growth.

Future Inquiry Opportunities

Throughout the experiment, potential future inquiries have been discovered. One being the specified range of MF intensity that require more research. It is recognized that a better general knowledge of the effects that changes in MF have on plants is necessary in many realms of science, space-exploration being the primary focus in this study. However, discovering the range in which both negative and positive effects occur can give insight into what is to be expected in certain environments. The levels between the 100 nT - 0.5 mT range of WMF intensity gives varied results on the impacts it has on plants, especially the intensities within this range that are lower and higher than that of the GMF. Future research should test intensities at both ends of the WMF spectrum as well as much

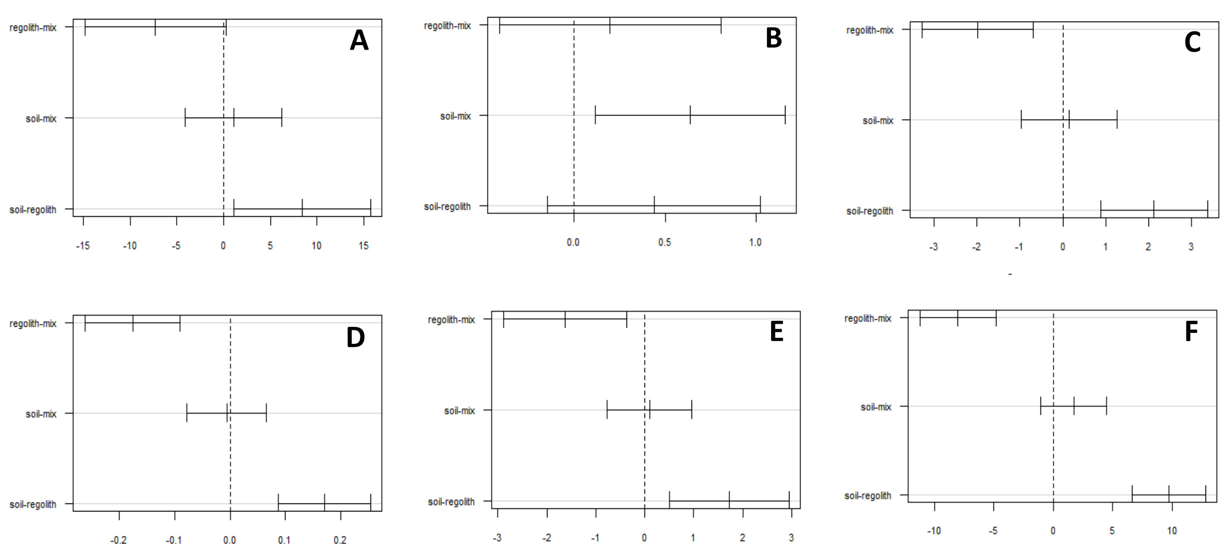

Fig. 2: Results of Tukey HSD analysis showing significant groupwise differences between soil types anywhere in the 95% confidence interval that does not include zero ($p < 0.05$). (A) Average leaf area (B) Stem diameter (C) Wet AGBM (D) Dry BGBM (E) Average leaf length (F) Stem height

lower intensities, including NNMF levels, to search for effects in conditions similar to those in space or LEO.

Another inquiry would be to test the effects of exposure on gene expression and ionic content. Gene expression can indicate ionic stresses in plants (Paul, Elardo, and Ferl 2022), and these alterations in nutrient ionic imbalances can affect plant development (Ravishankar et al. 2018). Although this effect was not tested in this study, multiple experiments have shown that decreased MF intensity has significantly affected ionic content of nutrient ions. (Ravishankar et al. 2018; Belyavskaya 2004) Alterations in these ionic balances indicate that the plant is under stress while developing in different MF environments as well as in lunar soil conditions (Paul, Elardo, and Ferl 2022). Future experimentation in this area would expand the understanding of these stresses and what particular ions are affected in manipulated MF environments.

Lastly, changes in experimental design would potentially better the final results and make-up for the plants lost during this study. Losing all but one *A. thaliana* plant by the end of the four-week growth period took half of the data that could have been analyzed. Growing the *A. thaliana* in better conditions, i.e. smaller pots and less soil per pot, could provide a more preferable environment for the plants to grow. Examples would be experimental designs similar to Paul et al. (2022) where *A. thaliana* was grown in cell culture trays or small pots. Increasing the different species type would also promote variety in results as well as testing the types of plants that NASA considers for future space exploration. Species that are nutritious for an astronaut's diet, such as antioxidant rich foods, would be an example of species to use.

Conclusion

Changes in MF exposure have been shown to affect plant development. Data in this study suggest that further investigation on WMF intensities are required to obtain a better

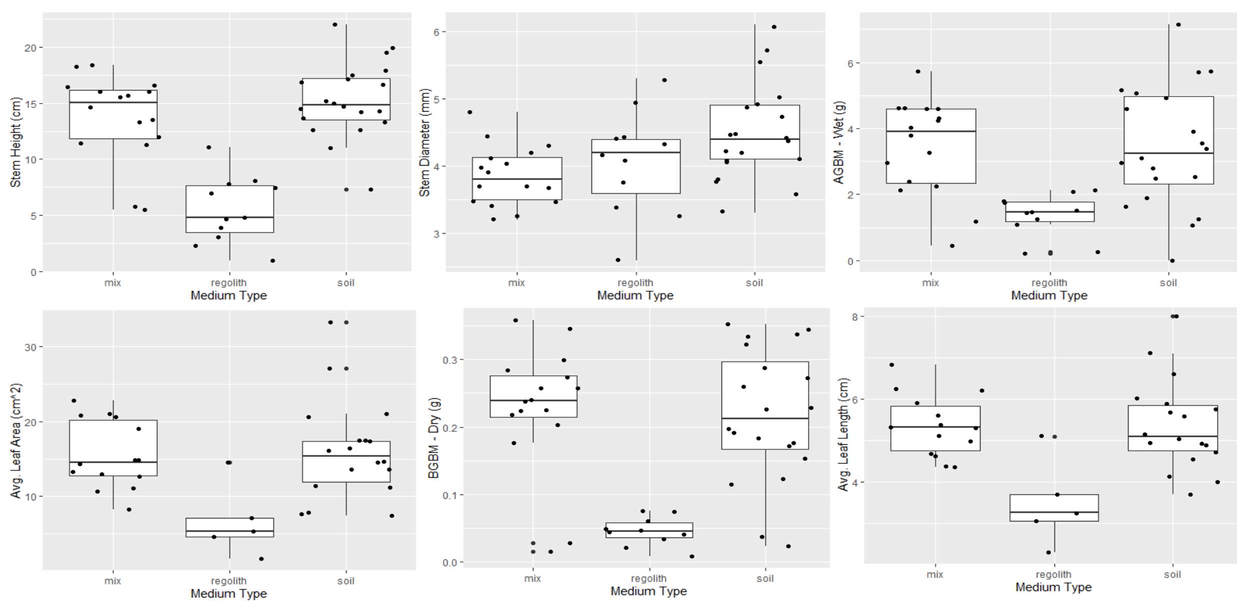

Fig. 3: Shows the distributed data and its outliers for the significant harvesting parameters across the three soil types.

understanding of these effects and when they may occur. Various results in numerous studies have shown effects that are both negative and positive for plants exposed to WMFs. Yet, this variability of results, including the lack of significance found in this study and others, calls for further experimentation on this subject matter. Furthermore, exposure to a wider range of MF intensities within the WMF range could potentially provide more insight into the potential effects. Results in this study, also, share similarities to others in which plants have the ability to grow in lunar regolith (Paul, Elardo, and Ferl 2022; Wamelink et al. 2014). However, this ability does not result in a promotion of plant development and growth. Lunar soils lack the organic materials required for plants to survive (Duri et al. 2022), but understanding which components of plants and their growth are affected could pave the way for resolutions to this potential problem. Experimentation that analyzes WMF exposure on a larger variety of species in soils similar to those in space are necessary for future space exploration. To prepare for the increasingly inevitable chance of life in space, research in this area of study could further expand on this limited knowledge.

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