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# Soil conditioning for enhancing plant growth using biochar and hydrochar under microgravity

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Cultivating plants in outer space is crucial for bioregenerative life support systems in human space exploration. This study aims to investigate the effects of soil conditioning with biochar and hydrochar on the growth and production of *Malabar Spinach* in microgravity conditions. Peanut shell biochar and wood hydrochar were applied at a 3% dosage by mass. Two gravity conditions were considered, including 1 g and microgravity simulated by a Random Positioning Machine (RPM). After an 18-day plant growth period, microgravity reduced the fresh biomass accumulation of *Malabar Spinach* by up to 71%. This reduction was attributed to inhibited leaf and root growth, which decreased light interception and nutrient uptake. In microgravity, biochar was more effective than hydrochar in enhancing plant production, mitigating the growth inhibition caused by microgravity. In the presence of biochar, microgravity significantly enhanced the biosynthesis of chlorophyll a and carotenoids by up to 36%. Furthermore, biochar and hydrochar treatments in microgravity conditions significantly increased the nutrient contents, such as K and P, in *Malabar Spinach* leaves. These findings indicate that biochar and hydrochar are promising soil conditioners for enhancing plant development in low-gravity conditions.

As humanity expands beyond Earth, the ability to cultivate plants becomes increasingly important for extended missions to outer space destinations<sup>1</sup>. Plants play a crucial role in life support systems by producing oxygen through photosynthesis and serving as a renewable food source during long-term space exploration, including human habitation and potential immigration to habitable exoplanets<sup>2</sup>. Given these functions, plants are considered a key component of bio-regenerative life support systems. However, plants growing in space face significant environmental challenges. For instance, nutrient deficiency, limited water and altered gravity in space induce harsh environments for plant cultivation<sup>3</sup>. Developing methods to condition the soil to help plants better adapt to these space conditions is crucial for supporting future space missions.

Techniques for applying soil conditioners to enhance soil quality and promote plant production have been developed. Biochar is produced through the pyrolysis of biomass, such as peanut shells, in the absence of oxygen. Previous studies have reported that biochar can improve soil fertility, enhance water retention, and promote beneficial microbial activity<sup>4</sup>. Its porous structure increases soil aeration and nutrient availability, making it an effective amendment for improving crop yields and soil quality<sup>5</sup>. Hydrochar is produced by hydrothermal carbonization. It involves treating

biomass with heat and pressure in the presence of water<sup>6</sup>. Hydrochar offers similar benefits to biochar, including improved soil structure and nutrient retention. Hydrochar typically has a lower pH than biochar<sup>7</sup>, which can enhance its effectiveness in certain soil types or conditions. Some studies have reported the potential contaminant leaching from biochar/hydrochar derived from sewage sludge and livestock manure<sup>8,9</sup>. In addition, the heavy metal immobilization capacity of biochar/hydrochar has also been reported due to its abundant functional groups and porous structure<sup>4,10</sup>. It seems that the variability of biochar/hydrochar properties depending on the feedstock used. Nevertheless, the effects of these soil conditioners on plant development in low gravity in space are still not well understood.

Previous studies have investigated the effects of low gravity on plants. Microgravity conditions were simulated using a 3D clinostat, resulting in increased chlorophyll loss, along with elevated levels of jasmonates (JAs) and abscisic acid (ABA) in oat leaves under microgravity<sup>11</sup>. These findings contrast with the results that chlorophyll content in rice seedlings increased under simulated microgravity<sup>12</sup>. These discrepancies suggest that the effects of microgravity on plant metabolism and physiological characteristics may vary depending on the specific plant species. Previous studies also discovered significant changes in the cell proliferation rate and regulation of

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cell cycle progression in *Arabidopsis thaliana* seedlings<sup>13</sup>. This aligns with the findings reporting increased cell proliferation but reduced cell growth in *Arabidopsis thaliana* under low gravity conditions simulated by RPM<sup>14</sup>. Additionally, several studies have explored the effects of low gravity on vegetables and crops. Lettuce was grown aboard the Tiangong Spacelab, where it was observed to exhibit increased height and a deeper green colour under microgravity conditions<sup>15</sup>. In contrast, an investigation of rice growth in a space environment reported that spaceflight resulted in decreased germination rates and plant height<sup>16</sup>. Overall, there remains a need for further research to enhance our understanding of the coupled effects of microgravity and soil conditioners (i.e., biochar, hydrochar) on the production of food plants, such as vegetables.

Therefore, this study aims to investigate the effects of biochar and hydrochar treatments on plant development under microgravity conditions. An RPM was utilised to simulate microgravity conditions. The tested plant species was *Malabar Spinach*, which was a commonly grown vegetable. The experiment duration was 18 days. Plant morphology, physiological properties, and productivity were measured to evaluate plant development. This study represents the first effort to enhance our mechanistic understanding of how biochar and hydrochar influence vegetable plant growth in microgravity.

## Results

### Plant morphological properties

Figure 1 illustrates the increase in leaf area per pot during the plant growth period under different gravity and soil conditions. At the midpoint of the growth period, which was 8 days, the gravity-induced changes in leaf area enlargement were not significant. Under microgravity conditions, leaf area enlargement was observed to decrease by 5% and 6% with biochar and hydrochar treatments ( $p > 0.05$ ), respectively. As the growth duration extended to 16 days, microgravity inhibited leaf area enlargement by up to 30% ( $p > 0.05$ ), regardless of the soil condition. This indicates that as the duration of plant growth increases, the inhibition of leaf development caused by microgravity becomes more pronounced. In this figure, both biochar and hydrochar significantly enhanced leaf area enlargement. Specifically, on the 8th day, biochar and hydrochar increased leaf area enlargement by up to 148% and 117% ( $p < 0.05$ ), respectively. By the 16th day, the application of biochar and hydrochar resulted in improvements of up to 109% ( $p < 0.05$ ) and 57% ( $p > 0.05$ ) in leaf area enlargement, regardless of gravity conditions. This suggests that peanut shell biochar is more effective than wood hydrochar in promoting leaf development in *Malabar Spinach*.

Figure 2 shows the root surface area of *Malabar Spinach* at different soil depths under the combined effects of microgravity and soil conditioners. Among all conditions, the root surface area was relatively large at a depth of 10–20 mm. However, as the depth increased beyond 30 mm, the root surface area decreased. Similar to the negative impact of microgravity on leaf area growth, the root surface area was also reduced under microgravity conditions, particularly in the control soil and biochar-treated groups at depths of 10–30 mm. For instance, at a soil depth of 10–20 mm, the root surface area was reduced by 26%, 29%, and 12% under control, biochar-treated, and hydrochar-treated conditions, respectively. When the soil depth exceeded 30 mm, the microgravity-induced changes in root surface area became less pronounced.

### Leaf pigment content

Figure 3 shows the contents of plant pigments, including chlorophyll a, chlorophyll b, and carotenoids in the leaves of *Malabar Spinach*, under the combined effects of microgravity and soil conditioners. These three pigments play essential roles in plant photosynthesis by capturing light energy and protecting plants from excessive radiation<sup>17</sup>. Microgravity conditions led to increases in chlorophyll a content of 4%, 36%, and 6% under control, biochar-treated, and hydrochar-treated conditions, respectively. The most significant enhancement in chlorophyll a content due to microgravity was observed in the biochar treatment ( $p < 0.05$ ). Chlorophyll b, an accessory pigment to chlorophyll a, also exhibited increases of 2%, 44%, and 6% under

microgravity conditions for the control, biochar-treated, and hydrochar-treated groups, respectively, compared to normal 1 g conditions on Earth ( $p > 0.05$ ). Similarly, microgravity improved carotenoid biosynthesis in the leaves of *Malabar Spinach* by 1%, 33%, and 8% under the control, biochar-treated, and hydrochar-treated conditions, respectively. Among the various soil conditions, only the presence of biochar resulted in a significant increase in carotenoid content under microgravity ( $p < 0.05$ ). The alterations in plant pigments in the leaves of *Malabar Spinach* induced by biochar and hydrochar were not significant under either microgravity or 1 g-gravity conditions ( $p > 0.05$ ).

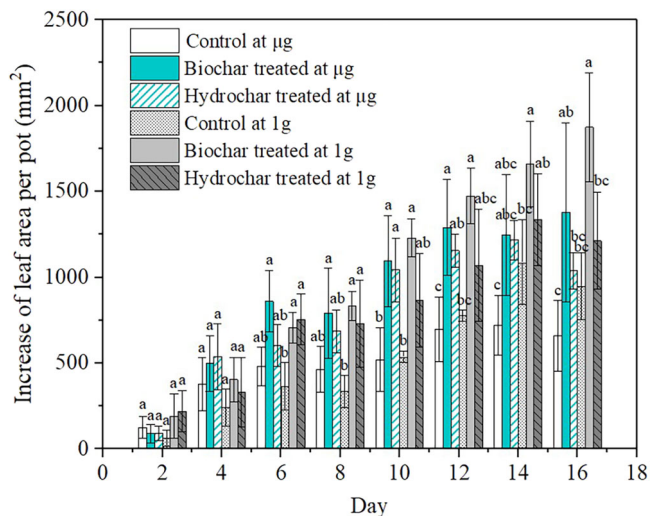
### Plant biomass and water use efficiency under microgravity

Figure 4 shows the increase in fresh biomass per pot during the plant growth period under the coupled effects of microgravity and soil conditioners. Compared with the control soil condition, the biomass accumulation of *Malabar Spinach* could be enhanced by 344% with biochar treatment ( $p < 0.05$ ) and by 65% with hydrochar treatment ( $p > 0.05$ ) in microgravity level. This suggests that biochar is more effective than hydrochar in enhancing the biomass accumulation of *Malabar Spinach*, which aligns with the observations of plant leaf and root development shown in Figs. 1 and 2. In comparison to 1 g normal gravity, microgravity induced a 69% reduction in fresh biomass accumulation for the control soil condition ( $p > 0.05$ ) and up to a 71% reduction for the biochar and hydrochar treated groups ( $p < 0.05$ ). This indicates a more statistically significant reduction in biomass accumulation due to microgravity in the presence of biochar/hydrochar, suggesting an interaction between microgravity and soil conditioner affecting plant biomass accumulation.

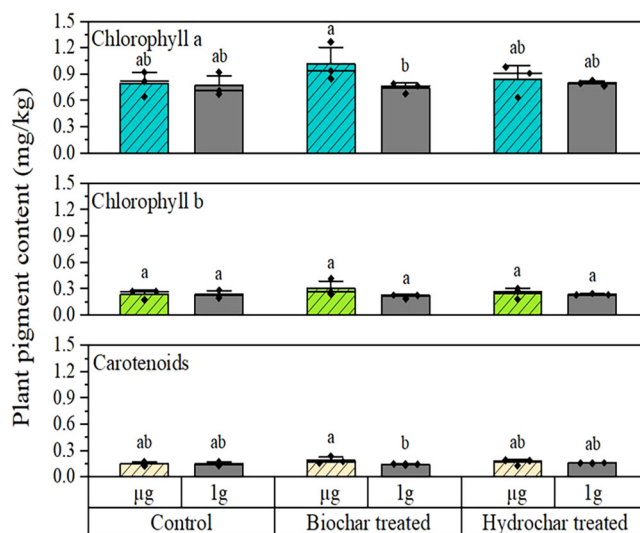
Figure 5a shows the transpiration rate of *Malabar Spinach* under coupled effects of microgravity and soil conditioners. In microgravity, the transpiration rates decreased by 31%, 24%, and 1% for the control, biochar, and hydrochar-treated groups, respectively. However, these changes were not statistically significant ( $p > 0.05$ ). Compared to the control soil condition, biochar and hydrochar significantly reduced the transpiration rates by 47% and 49% in microgravity, respectively ( $p < 0.05$ ). Figure 5b shows the water use efficiency (WUE) of *Malabar Spinach* under coupled effects of microgravity and soil conditioners. WUE is defined as the ratio of biomass produced to the amount of water consumed during plant growth, reflecting how effectively a plant utilises water for growth and productivity<sup>18</sup>. Compared to control soil, biochar increased WUE by 278% ( $p < 0.05$ ), while hydrochar increased it by up to 67% ( $p > 0.05$ ) in microgravity condition. The significant increase in WUE with biochar treatment was attributed to its promotion of plant productivity and a slight decline in transpiration (as shown in Figs. 4 and 5a). In microgravity, *Malabar Spinach* experienced a 65–68% reduction in WUE compared to 1 g gravity conditions. This significant decrease was particularly evident in the biochar and hydrochar-treated groups, resulting from a notable decline in biomass accumulation and a slight increase in transpiration due to microgravity (as shown in Figs. 4 and 5a). Consequently, this led to a significant inhibition of water use efficiency.

### Plant nutrient uptake and potentially toxic metal accumulation

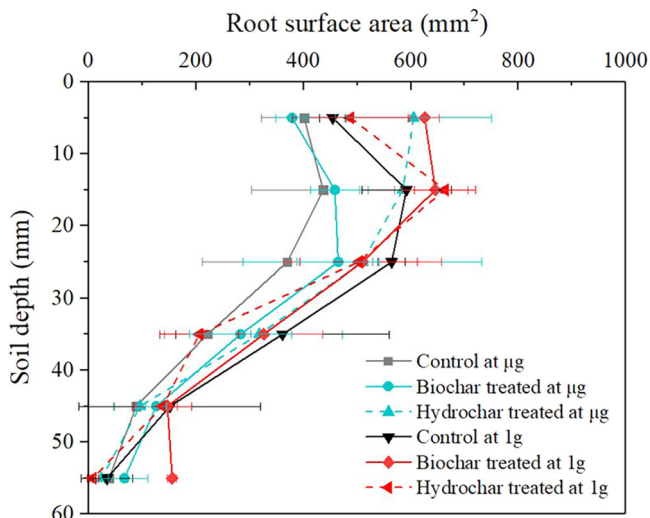
The concentrations of nutrient elements, including K, P, Ca, Mg, N, and C, in *Malabar Spinach* leaves are presented in Table 1. Under biochar treatment, K content in the leaves increased by up to 174%, while hydrochar treatment resulted in a 55% increase ( $p < 0.05$ ). Microgravity further enhanced plant K uptake by 22% and 25% in the presence of biochar and hydrochar, respectively. Additionally, the application of soil conditioners, particularly hydrochar, significantly increased plant P content, resulting in a 38–52% higher leaf P concentration ( $p < 0.05$ ). Microgravity also improved leaf P levels, especially under hydrochar treatment, by 31%. Regarding Ca, its concentration in *Malabar Spinach* leaves increased by up to 73% and 26% under biochar and hydrochar treatments, respectively. Significant increases in plant Ca content were observed only in the biochar-treated condition ( $p < 0.05$ ). Unlike K and P, the Ca concentration in plant leaves was not significantly affected by changes in gravity levels. For the other three



**Fig. 1 | Variations in the increase of leaf area under coupled effects of microgravity and soil conditioners.** Data are presented as mean value ± standard deviation ( $n = 3$ ). Different letters above the bars indicate significant differences ( $p < 0.05$ ) between the groups under different treatments.



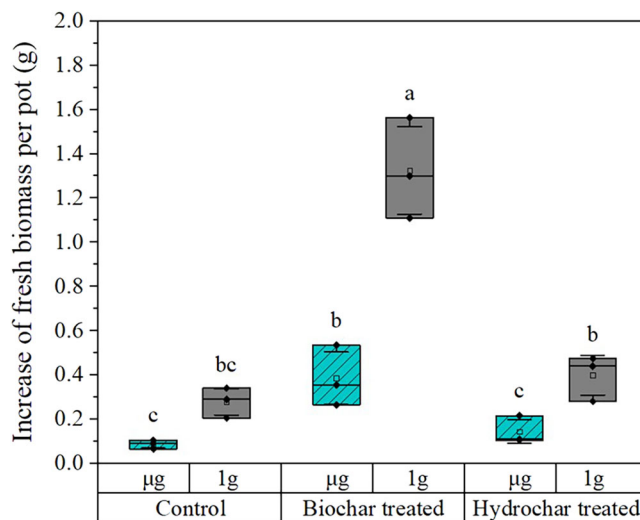
**Fig. 3 | Variations in plant pigment contents, including chlorophyll a, chlorophyll b, and carotenoids, under coupled effects of microgravity and soil conditioners.** Data are presented as mean value ± standard deviation ( $n = 3$ ). Different letters above the bars indicate significant differences ( $p < 0.05$ ) between the groups under different treatments.



**Fig. 2 | Variations in root surface area with depth under coupled effects of microgravity and soil conditioners.** Data are presented as mean value ± standard deviation ( $n = 3$ ).

elements (i.e., Mg, N and C), their concentrations in *Malabar Spinach* leaves were not significantly influenced by either soil conditioner (biochar or hydrochar) or microgravity conditions.

Figure 6 shows the accumulation of soil pollutants (i.e., heavy metal Cu and Cd) in the leaves of *Malabar Spinach* under coupled effects of microgravity and soil conditioners. The heavy metal content in vegetation is a crucial factor for assessing plant quality. Under microgravity conditions, plant Cu concentration increased by up to 24%, regardless of soil conditions. A significant increase in plant Cu uptake due to microgravity was observed in the non-biochar/hydrochar-treated groups ( $p < 0.05$ ). The application of biochar and hydrochar reduced plant Cu concentration by 7% ( $p > 0.05$ ) and 17% ( $p < 0.05$ ), respectively, under microgravity. Compared with Cu, Cd poses greater ecotoxicity and requires particular attention. Microgravity significantly enhanced plant Cd accumulation by up to 114% ( $p < 0.05$ ), regardless of soil conditions. Under microgravity level, biochar reduced plant Cd accumulation by 9% ( $p > 0.05$ ), while hydrochar decreased it by 36% ( $p < 0.05$ ).



**Fig. 4 | Variations in the increase of fresh biomass per pot under coupled effects of microgravity and soil conditioners.** Data are presented as mean value ± standard deviation ( $n = 3$ ). Different letters above the bars indicate significant differences ( $p < 0.05$ ) between the groups under different treatments.

### Multivariate analysis of variance

Table 2 shows the results of multivariate analysis of variance (MANOVA) for the plant characteristics related to the morphology, physiology and production of *Malabar Spinach*. Among these characteristics, gravity significantly reduced plant biomass and water use efficiency (WUE) ( $p < 0.05$ ), regardless of soil conditions (as shown in Figs. 4 and 5). This reduction was attributed to altered metabolism and decreased levels of carbon fixation and photosynthetic capacity in plants under microgravity<sup>19</sup>. Conversely, the accumulation of heavy metals, including Cu and Cd, in plants significantly increased under microgravity ( $p < 0.05$ ) (as shown in Fig. 6). This increase was due to microgravity-related changes in uptake kinetics and enhanced membrane fluidity under low gravity<sup>19,20</sup>. Biochar significantly increased leaf area, plant biomass, and WUE ( $p < 0.05$ ), while hydrochar did not have a significant impact on these parameters ( $p > 0.05$ ) (as shown in Figs. 1, 4, and 5). This effect was attributed to the abundant mineral nutrients provided by

biochar (Table S2). In comparison, hydrochar demonstrated higher efficiency in soil Cd immobilisation, thereby reducing plant Cd accumulation (as shown in Fig. 6). An interaction of gravity level and soil condition was observed on the biomass and WUE of *Malabar Spinach* ( $p < 0.05$ ) (as shown in Figs. 4 and 5). Microgravity could not significantly affect the increase in biomass and WUE when soil conditioner was absent ( $p > 0.05$ ). In the presence of biochar or hydrochar, microgravity significantly reduced the biomass accumulation and WUE ( $p < 0.05$ ). Nevertheless, peanut shell biochar could significantly enhance biomass accumulation and WUE in microgravity conditions.

### Discussion

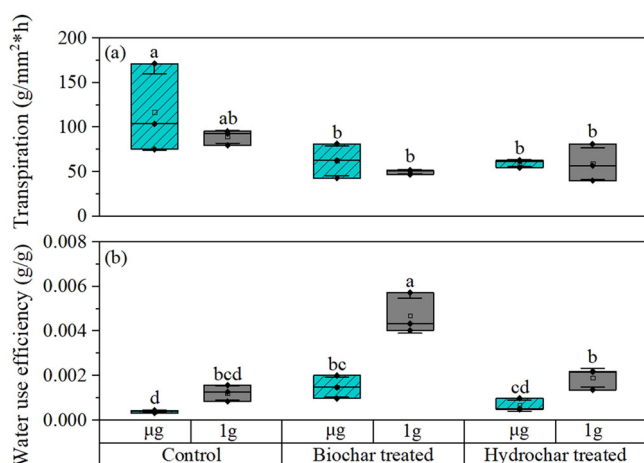
The reduction in leaf area enlargement due to low gravity might be attributed to decreased plant cell growth<sup>14</sup>. Additionally, microgravity during spaceflight could hinder  $Ca^{2+}$  utilization, impacting processes such as the formation of plant cell wall architecture and lignin biosynthesis<sup>21</sup>. Furthermore, the negative impact of microgravity on signal transduction might cause the reduction of leaf development<sup>22</sup>. Both biochar and hydrochar demonstrate greater efficiency in stimulating leaf growth during the relatively early stages of plant development. This can be explained by the fact that although wood hydrochar contains higher organic matter, peanut shell biochar has greater levels of soil-available nutrients such as K, P, N, Mg, and Ca (as shown in Table S2). These mineral nutrient contents contribute to improved leaf growth and larger leaf area. The inhibition of root growth in microgravity was attributed not only to decreased plant cell growth but also to hampered signalling via  $Ca^{2+}$  in physiological processes<sup>14,21</sup>. Low gravity could decrease the water infiltration rate, thus reducing water loss through leaching<sup>3</sup>. Consequently, relatively high soil moisture could sometimes hinder root system development<sup>23</sup>, leading to a reduced root surface area

under microgravity conditions. Furthermore, compared to the control, peanut shell biochar and wood hydrochar increased the root surface area, indicating enhanced root growth and development. This improvement was attributed to the enhanced soil mineral nutrients and organic content, respectively (as shown in Table S2).

Microgravity conditions could induce changes in biological systems related to chlorophyll biosynthesis and upregulate plastid-encoded transcript expression, leading to increased levels of pigments in plant leaves<sup>24,25</sup>. Research has demonstrated that microgravity enhances plant sensitivity to various environmental cues<sup>26</sup>. Additionally, nutrient supply (e.g., N, K, P) from biochar could influence the biosynthesis of chlorophyll and carotenoids<sup>27</sup>. Therefore, when microgravity occurred in conjunction with biochar treatment, plant sensitivity was enhanced, leading to an amplified stimulation of plant pigment biosynthesis. The reduced leaf area and root surface area observed under microgravity conditions (as shown in Figs. 1 and 2) limited the light interception and nutrient uptake of *Malabar Spinach*, resulting in lower biomass accumulation. Additionally, microgravity could alter plant metabolism and inhibit carbon fixation<sup>19</sup>. Plant photosynthesis was also restricted during spaceflight in microgravity<sup>1</sup>, contributing to the decline in biomass accumulation. These results highlight concerns about reduced vegetable production when growing plants in space. Biochar remains a recommended soil conditioner in microgravity conditions to enhance plant production.

The reduction in transpiration rate under microgravity contrasts with some previous findings<sup>2</sup>, reporting increased leaf temperatures in microgravity leading to suppressed leaf transpiration due to lower free air convection. Conversely, some other studies observed that enhanced transpiration rates in microgravity were associated with faster plant water uptake, likely due to the upregulation of genes related to water uptake<sup>28</sup>. Additionally, plants might maintain auxin levels that keep stomata open under microgravity<sup>28</sup>. The water movement in the soil-plant-atmosphere system is governed by Darcy's law. Therefore, differences in hydraulic heads (i.e., controlled by total suction) between the plant and soil could affect the plant water uptake<sup>4</sup>. The abundant ions released from the surface of biochar and hydrochar into the soil water (as indicated by the EC value in Table S1) could lead to increased soil osmotic suction and total suction. Consequently, this resulted in reduced plant water uptake and transpiration. The reduced WUE under microgravity conditions highlights the importance of effective irrigation and soil water retention for plant growth and productivity in space. Biochar presents a promising soil amendment to enhance plant WUE under microgravity conditions in space.

The notable enhancement of mineral nutrients (K, P, and Ca) in the leaves due to biochar was attributed to the abundance of mineral nutrient ions supplied by biochar when mixed with soil (as shown in Table S2), leading to improved nutrient uptake. Microgravity can positively affect plant uptake of nutrient elements such as K, P, and Ca, but does not significantly influence the content of N and C in the leaves. The increase in nutrient element concentration of plant leaves induced by microgravity is highly dependent on the specific type of nutrient. The reduced concentrations of Cu and Cd in plant leaves with biochar/hydrochar application were due to the immobilisation of heavy metals in the soil (as shown in Table S3),



**Fig. 5 | Variations in a transpiration and b water use efficiency under coupled effects of microgravity and soil conditioners.** Data are presented as mean value ± standard deviation ( $n = 3$ ). Different letters above the bars indicate significant differences ( $p < 0.05$ ) between the groups under different treatments.

**Table 1 | The concentrations of nutrient elements in plant leaves**

Plant growth condition		Nutrient concentration in plant leaves					
Gravity	Soil treatment	K (g/kg)	P (g/kg)	Ca (g/kg)	Mg (g/kg)	N (%)	C (%)
µg	Control	11.7 ± 1.7d	2.5 ± 0.3bc	7.7 ± 0.6c	5.6 ± 0.6b	1.4 ± 0.3a	45.4 ± 1.0a
	Biochar treated	32.0 ± 3.4a	2.9 ± 0.4b	13.3 ± 1.5ab	7.6 ± 0.9ab	1.3 ± 0.5a	45.9 ± 2.5a
	Hydrochar treated	18.1 ± 1.8c	3.8 ± 0.4a	9.7 ± 2.9bc	7.6 ± 1.4ab	1.7 ± 0.5a	44.3 ± 2.7a
1g	Control	11.8 ± 1.4d	2.1 ± 0.4c	8.6 ± 1.1c	6.2 ± 0.2ab	1.7 ± 0.3a	44.3 ± 1.3a
	Biochar treated	26.3 ± 2.5b	2.5 ± 0.0bc	13.9 ± 1.4a	8.3 ± 1.2a	1.2 ± 0.4a	46.9 ± 2.2a
	Hydrochar treated	14.5 ± 1.6cd	2.9 ± 0.1b	10.1 ± 1.7bc	6.1 ± 0.9b	1.5 ± 0.2a	45.6 ± 0.6a

Data are presented as mean value ± standard deviation ( $n = 3$ ). Different letters (e.g., a, b, c) indicate significant differences ( $p < 0.05$ ) between the groups under different treatments.

which decreased their bioavailability and subsequent plant uptake. The improved heavy metal uptake by *Malabar Spinach* under microgravity was attributed to altered uptake kinetics<sup>19</sup>. Additionally, the enhanced water uptake (shown in Fig. 5a) under microgravity resulted in the greater absorption of heavy metal ions dissolved in the soil water, leading to elevated Cd content in plant leaves<sup>20,28</sup>. This indicates that compared with Earth gravity, partial gravity and microgravity in space facilitate the accumulation of heavy metals in plant tissues. Therefore, it is essential to select higher-quality, less contaminated soil for planting and agriculture in space. Future studies are encouraged to explore the mechanisms by which recycled materials, such as biochar and hydrochar, for conditioning lunar or Martian regolith simulants in a low-gravity environment in space. This research could provide valuable insights into agricultural development and habitat construction on extraterrestrial bodies.

## Methods

### Soil conditioning

The soil tested in this study was laterite, collected from Bijie City, Guizhou Province, China (27°24'N, 105°20'E). The soil in Guizhou Province is suitable for plant growth due to its relatively high water retention capacity. A 2 mm mesh was used to sieve the soil from the field prior to testing. After sieving, the pH of the laterite soil was measured using a pH metre according to ASTM D4972-01 (2007)<sup>29</sup>, yielding a result of 4.45. Electrical conductivity (EC) was determined<sup>30</sup>, resulting in a measurement of 113 µS/cm. The total organic carbon content of the tested soil sample was analysed using a total

organic carbon analyser (Shimadzu, TOC-VCPH), which indicated a value of 0.88%.

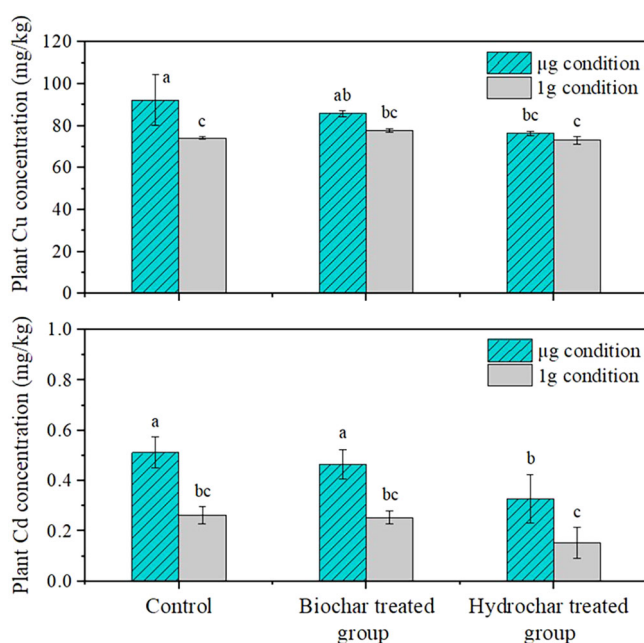
The biochar analysed in this study was derived from peanut shells commercially provided by Sanli New Energy Co., Ltd. in Shangqiu, Henan province<sup>4</sup>. It was produced at a pyrolysis temperature of 500 °C with a resident time of 1 h. X-ray photoelectron spectroscopy (XPS) analysis was performed using an Axis Ultra DLD instrument from Kratos Analytical Limited. The results determined its chemical composition, consisting of 36.9% C, 33.6% O, 8.0% Si, 5.2% Ca, 4.1% Al, 3.2% N, 3.1% Cl, 2.1% Fe, 1.9% K, 1.1% Mg and 0.8% Na. The hydrochar utilised in this research was produced from wood sawdust of an apple tree through Hydrothermal Carbonization (HTC) at a temperature of 200 °C, with a residence time of 1 h and a feedstock-to-deionized water ratio of 1:5 (w/w). Following the pyrolysis process, the reactor was allowed to cool to room temperature. The hydrochar was then collected after undergoing filtration, washing, and oven-drying at 100 °C<sup>6</sup>. The XPS analysis of the wood hydrochar revealed a chemical composition of 66.6% C, 31.8% O, 0.61% N, 0.45% Ca, 0.29% Si, and 0.15% Al. Peanut shell biochar and wood hydrochar were each uniformly mixed with soil at a dosage of 3% by mass. Deionized water was then added to achieve a soil water content of 40%. The treated soil was subsequently incubated until it was ready for testing.

### Set up of plant experiment

The trapezoidal pots featured an upper diameter of 50 mm and a bottom diameter of 20 mm. The height of the pots was 55 mm. Healthy seedlings of *Malabar Spinach*, approximately 40 days old and commercially purchased, were selected for the tests based on similar size and biomass. Each seedling was planted in an individual pot. The selected seedlings were planted in soil that contained either peanut shell biochar or wood hydrochar at a dosage of 3%. Control seedlings were planted in soil without any biochar or hydrochar for comparison. Six pots were prepared for each soil condition. Throughout the 18-day growth period, each pot was irrigated with a pipette every two days to maintain a water content of approximately 40%.

### Set up of microgravity system

The simulated microgravity condition was created using a Random Positioning Machine (RPM, yuri GmbH, Germany). This machine operates through three main components (as shown in Fig. S1): the RPM instrument, a computer with RPM control software, and a controller box. The RPM instrument features a sample container connected to two independent axes, allowing it to rotate at random speeds in all directions. This random motion causes the sample to experience gravitational forces from every direction, resulting in an average gravitational effect of approximately 10<sup>-3</sup> g over time. When the changes in direction of the object on the RPM are faster than the response of the object to gravity, it produces effects similar to those of true microgravity in space<sup>14</sup>. Half of the prepared pots containing *Malabar Spinach* seedlings, under three soil conditions (non-conditioner as control, 3% biochar, and 3% hydrochar), were fixed to the RPM instrument to simulate microgravity condition. Meanwhile, the other half of the pots were placed on a test table under 1 g of Earth gravity for comparison. To prevent soil particles from spilling during the rotation of the RPM instrument, the pots with *Malabar Spinach* seedlings were wrapped in plastic membranes with small holes. Each condition included three replicates.



**Fig. 6 | Variations in the concentrations of plant Cu and Cd under coupled effects of microgravity and soil conditioners.** Data are presented as mean value ± standard deviation ( $n = 3$ ). Different letters above the bars indicate significant differences ( $p < 0.05$ ) between the groups under different treatments.

**Table 2 | MANOVA of gravity and soil treatment on plant characteristics ( $p$ -value)**

Factor	Plant characteristics							
	Leaf area	Biomass	WUE	Chlorophyll a	Chlorophyll b	Carotenoids	Plant Cd	Plant Cu
Gravity	0.443	0.000	0.000	0.116	0.171	0.121	0.000	0.005
Soil treatment	0.003	0.000	0.000	0.502	0.736	0.464	0.011	0.068
Gravity×soil treatment	0.816	0.000	0.003	0.297	0.336	0.313	0.689	0.144

WUE stands for water use efficiency.

### Measurements of plant characteristics

During the 18-day growth period, leaf area was measured every two days to monitor the development of the *Malabar Spinach* leaves under different gravity and soil conditions. Leaf area was calculated using ImageJ based on high-resolution photographs. Prior to harvesting, a few fresh leaves were collected to assess plant pigment content, including chlorophyll a, chlorophyll b, and carotenoids<sup>31</sup>. At harvesting, the plants were gently washed with Milli-Q water, and the roots of the *Malabar Spinach* were scanned using an EPSON scanner (model: STD4800) to obtain the root surface area along the soil depth<sup>32</sup>. The increase in fresh biomass was calculated by subtracting the initial seedling biomass from the biomass at harvest. Fresh and dry biomasses were weighted using an electric balance with a precision of 0.0001 g, both before and after an oven-drying process at 60 °C for 24 h<sup>4</sup>. Transpiration of *Malabar Spinach* during the 18-day growth period was estimated<sup>33</sup>. Water use efficiency (WUE) was calculated as the ratio of dry biomass to water loss through transpiration<sup>18</sup>. Mineral nutrients (K, P, Ca, Mg, etc.) in plant tissues were extracted using 65% nitric acid (HNO<sub>3</sub>)<sup>34</sup>. Heavy metals (Cu, Cd) in *Malabar Spinach* tissues (0.1–0.5 g) were extracted with concentrated nitric acid (5 mL) and allowed to cold digest in a fume cupboard overnight. The temperature was then increased to 140 °C for digestion until only 1 mL remained<sup>35</sup>. Subsequently, the extracted elements from plant samples were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) after dilution.

### Measurements of soil property

After harvesting, soil samples were collected after the removal of any root residues. The samples were air-dried, ground, and sieved through a 2 mm mesh. Subsequently, pH and electrical conductivity (EC) were measured according to ASTM D4972-01 (2007)<sup>29</sup> and the methods outlined by Chowdhury et al.<sup>30</sup> (results are presented in Table S1). Plant available N and dissolved organic carbon (DOC) in the soil were extracted by the methods reported by Carter and Gregorich<sup>36</sup>, Jones and Willett (2006)<sup>37</sup>. Then they were determined using a TOC/TN analyser (Shimadzu, TOC-VCPH). The plant available concentrations of K, P, Mg, Ca, Cu and Zn in the vegetated soil after harvest were extracted using the Mehlich 3 Extraction Method<sup>38</sup>. The concentrations of plant available heavy metals (Cu and Cd) were extracted following the protocol established by Park et al.<sup>35</sup>. Subsequently, they were analysed by ICP-OES. The available concentrations of soil nutrients and heavy metals in the root zone soil after planting are summarised in Tables S2 and S3, respectively.

### Statistical analysis

Statistical analyses were performed using SPSS 20 (2011). One-way analysis of variance (ANOVA) was employed to assess statistical differences among the various treatments, with post-hoc Tukey's honestly significant difference (HSD) test applied. Multivariate analysis of variance (MANOVA) was conducted to evaluate the significance of the combined effects of gravity and soil conditions on plant development. Results were deemed statistically significant when the *p*-value was less than 0.05, corresponding to a 95% confidence interval. Different letters (e.g., a, b, and c) were used to indicate statistically significant differences (*p* < 0.05) among groups.

### Data availability

All data generated or analysed in this study are included in this published article and its supplementary information files.

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### References

- Hirai, H. & Kitaya, Y. Effects of gravity on transpiration of plant leaves. *Ann. N. Y. Acad. Sci.* **1161**, 166–172 (2009).
- Karahara, I. et al. Vegetative and reproductive growth of *Arabidopsis* under microgravity conditions in space. *J. Plant Res.* **133**, 571–585 (2020).
- Maggi, F. & Pallud, C. Martian base agriculture: The effect of low gravity on water flow, nutrient cycles, and microbial biomass dynamics. *Adv. Space Res.* **46**, 1257–1265 (2010).
- Ng, C. W. W., Wang, Y. C., Ni, J. J. & So, P. S. Effects of phosphorus-modified biochar as a soil amendment on the growth and quality of *Pseudostellaria heterophylla*. *Sci. Rep.* **12**, 7268 (2022).
- Wang, Y. C., Ng, C. W. W. & Ni, J. J. Interactive effects of ultraviolet irradiation and biochar treatment on the growth and active ingredients of *Pseudostellaria heterophylla*. *J. Plant Growth Regul.* **42**, 7660–7672 (2023).
- Kravchenko, E., Cruz, T. L. D., Sushkova, S. & Rajput, V. D. Effect of wood and peanut shell hydrochars on the desiccation cracking characteristics of clayey soils. *Chemosphere* **358**, 142134 (2024).
- Sun, Y. et al. Effects of feedstock type, production method, and pyrolysis temperature on biochar and hydrochar properties. *Chem. Eng. J.* **240**, 574–578 (2014).
- Liu, H., Lyczko, N., Nzihou, A. & Eskicioglu, C. Phosphorus recovery from municipal sludge-derived hydrochar: Insights into leaching mechanisms and hydroxyapatite synthesis. *Water Res.* **241**, 120138 (2023).
- Zhang, P., Zhang, X., Li, Y. & Han, L. Influence of pyrolysis temperature on chemical speciation, leaching ability, and environmental risk of heavy metals in biochar derived from cow manure. *Bioresour. Technol.* **302**, 122850 (2020).
- Xia, Y., Liu, H., Guo, Y., Liu, Z. & Jiao, W. Immobilization of heavy metals in contaminated soils by modified hydrochar: Efficiency, risk assessment and potential mechanisms. *Sci. Total Environ.* **685**, 1201–1208 (2019).
- Miyamoto, K., Yuda, T., Shimazu, T. & Ueda, J. Leaf senescence under various gravity conditions: relevance to the dynamics of plant hormones. *Adv. Space Res.* **27**, 1017–1022 (2001).
- Jagtap, S. S., Awhad, R. B., Santosh, B. & Vidyasagar, P. B. Effects of clinorotation on growth and chlorophyll content of rice seeds. *Microgravity Sci. Technol.* **23**, 41–48 (2011).
- Boucheron-Dubuisson, E. et al. Functional alterations of root meristematic cells of *Arabidopsis thaliana* induced by a simulated microgravity environment. *J. Plant Physiol.* **207**, 30–41 (2016).
- Manzano, A. et al. Novel, Moon and Mars, partial gravity simulation paradigms and their effects on the balance between cell growth and cell proliferation during early plant development. *npj Microgravity* **4**, 9 (2018).
- Shen, Y. et al. Research on lettuce growth technology onboard Chinese Tiangong II Spacelab. *Acta Astronaut.* **144**, 97–102 (2018).
- Zeng, D. et al. Proteomic analysis in different development stages on SP0 generation of rice seeds after space flight. *Life Sci. Space Res.* **26**, 34–45 (2020).
- Sharafzadeh, S. H. & Alizadeh, O. Chlorophyll a, Chlorophyll b and carotenoids of garden thyme (*Thymus vulgaris* L.) as affected by nutrients. *Adv. Environ. Biol.* **5**, 3725–3728 (2011).
- Angus, J. F. & Van Herwaarden, A. F. Increasing water use and water use efficiency in dryland wheat. *Agron. J.* **93**, 290–298 (2001).
- Wolff, S. A., Coelho, L. H., Zabrodina, M., Brinckmann, E. & Kittang, A. I. Plant mineral nutrition, gas exchange and photosynthesis in space: A review. *Adv. Space Res.* **51**, 465–475 (2013).
- Polinski, E. et al. 2-D clinorotation alters the uptake of some nutrients in *Arabidopsis thaliana*. *J. Plant Physiol.* **212**, 54–57 (2017).
- Vandenbrink, J. P., Kiss, J. Z., Herranz, R. & Medina, F. J. Light and gravity signals synergize in modulating plant development. *Front. Plant Sci.* **5**, 563 (2014).
- Paulsen, K. et al. Microgravity-induced alterations in signal transduction in cells of the immune system. *Acta Astronaut.* **67**, 1116–1125 (2010).
- Hu, X. T., Hu, C., Jing, W., Meng, X. B. & Chen, F. H. Effects of soil water content on cotton root growth and distribution under mulched drip irrigation. *Agr. Sci. China* **8**, 709–716 (2009).

24. Medina, F. J., Manzano, A., Villacampa, A., Ciska, M. & Herranz, R. Understanding reduced gravity effects on early plant development before attempting life-support farming in the Moon and Mars. *Front. Astron. Space Sci.* **8**, 729154 (2021).
25. Oveneri, A. et al Simulated microgravity, Indole-3-Acetyl Acid, and cow dung influence on Zea Mays plant growth in contaminated soil. *App. Environ. Res.* **46** (2024).
26. Manzano, A. et al. Light signals counteract alterations caused by simulated microgravity in proliferating plant cells. *Am. J. Bot.* **108**, 1775–1792 (2021).
27. Wu, C., Wang, Z., Sun, H. & Guo, S. Effects of different concentrations of nitrogen and phosphorus on chlorophyll biosynthesis, chlorophyll a fluorescence, and photosynthesis in *Larix olgensis* seedlings. *Front. China* **1**, 170–175 (2006).
28. Setiawan, G. D., Thiravetyan, P. & Treesubuntorn, C. Gaseous toluene phytoremediation by *Vigna radiata* seedlings under simulated microgravity: Effect of hypocotyl, auxin, and gibberellic acid. *Acta Astronaut* **211**, 88–96 (2023).
29. ASTM D4972-01. Standard Test Method for pH of Soils. American Society for Testing and Materials, West Conshohocken, PA (2007).
30. Chowdhury, N., Marschner, P. & Burns, R. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. *Plant Soil* **344**, 241–254 (2011).
31. Dumbrava, D. G., Moldovan, C., Raba, D. N. & Popa, M. V. Vitamin C, chlorophylls, carotenoids and xanthophylls content in some basil (*Ocimum basilicum* L.) and rosemary (*Rosmarinus officinalis* L.) leaves extracts. *J. Agroaliment. Process. Technol.* **18**, 253–258 (2012).
32. Karimzadeh, A. A., Leung, A. K., Gao, Z. Shear strength anisotropy of rooted soils. *Géotechnique*, 1-14 (2022).
33. Garg, A., Leung, A. K. & Ng, C. W. W. Comparisons of soil suction induced by evapotranspiration and transpiration of *S. heptaphylla*. *Can. Geotech. J.* **52**, 2149–2155 (2015).
34. Xing, K. et al. Relationships between leaf carbon and macronutrients across woody species and forest ecosystems highlight how carbon is allocated to leaf structural function. *Front. Plant Sci.* **12**, 674932 (2021).
35. Park, J. H., Choppala, G. K., Bolan, N. S., Chung, J. W. & Chuasavathi, T. Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant Soil*, **348**, 439–451 (2011).
36. Carter, M. R., Gregorich, E. G. Soil sampling and methods of analysis. CRC Press (2007).
37. Jones, D. L. & Willett, V. B. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biol. Biochem.* **38**, 991–999 (2006).
38. Mehlich, A. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* **15**, 1409–1416 (1984).

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## Author contributions

Charles W.W. Ng: Conceptualization, Methodology, Supervision, Writing-Review & Editing. Y.C. WANG: Investigation, Resources, Writing-Original Draft, Visualization.

## Competing interests

The authors declare no competing interests.

## Additional information

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