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Valuing life in our soils: Effects of Microbial Activity in Vermicompost Tea on Sunflower Fitness

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Vermicompost tea (VCT) is a concentrated solution of microbes and nutrients that has been shown to increase plant growth. This study investigated the effects of microbes and nutrients in VCT on the growth (measured by plant biomass and rate of height increase) of sunflower (Helianthus annuus) under ideal-water and drought-simulated conditions. Three solutions (VCT, VCT without microbes, and water) were applied to groups of twenty greenhouse-grown sunflowers under ideal-water and drought-simulated conditions. Bacterial plates and carbon dioxide respiration tests measured soil microbial activity. We found that VCT increased plant heights and biomass under drought-simulated conditions and decreased plant heights and biomass under ideal-water conditions. VCT without microbes had the lowest level of growth throughout the study. Given that bacterial abundance was highest in soils with VCT added, the differing effects of VCT under ideal-water and drought-simulated conditions may have been due to the presence of different microbial communities. For example, certain microbes can increase drought-tolerance of plants by solubilizing limiting nutrients, while others can harm plants when water is in excess due to anaerobic processes. Plant-microbe symbiotic relationships, nutrient availability and hydrological factors need to be considered when evaluating the potential benefits of VCT application to agricultural crops.

Introduction

As the global population continues to increase at an accelerated rate, the demand and availability of food resources is becoming a greater cause for concern [1-2]. Although high crop yields are currently being maintained, soil in many areas is losing its ability to support agriculture without the addition of chemical fertilizers [3-4]. Healthy, productive soil requires not only water and nutrients, but also robust and diverse communities of micro- and macro-organisms [5]. Industrial agricultural practices tend to focus on maintaining sufficient water content and nutrient concentrations in the soil through irrigation and chemical fertilization, respectively, while largely disregarding the biological aspect. However, soil biology provides a number of agriculturally relevant ecosystem services such as the decomposition of organic matter, cycling of nutrients, soil rotation, and suppression of diseases and pests [5].

In the rhizosphere the zone of soil that surrounds plant roots microbe concentrations can be 10 to 1000 times greater than in surrounding soil due to relatively high amounts of available carbon from root exudates [6-7]. Certain bacteria in this rich community, known as plant-growth promoting rhizobacteria (PGPR), are particularly important to plant growth. Some of these bacteria increase nutrient availability for plants [7-8], while others suppress pathogens such as other bacteria, fungi, and viruses [9]. Certain PGPR can directly improve plant growth by supplying them with hormones and enzymes that stimulate plant growth or increase plant resistance to abiotic stresses such as drought [10]. Stressful growing conditions, including drought, can increase the importance of PGPR in aiding plant growth. Low water conditions can immobilize nutrients and make plants more susceptible to disease [11].

The importance of the biological component of the soil has been increasingly recognized in recent years, which has resulted in the development of a variety of agricultural techniques that attempt to introduce and sustain rich soil microbial communities. One such technique is the application of vermicompost tea (VCT). VCT is a highly concentrated solution of soil microbes, including active bacteria and fungi, and nutrients derived by soaking vermicompost in aerating water. Vermicompost consists of digested organic matter in the form of earthworm excrement (e.g., Lumbricus rubellus, Eisenia foetida) [12]. VCT has been shown to increase plant yield and health [13], which has been attributed to the high amounts of mineralized nutrients and to the presence of microbes that increase nutrient availability, suppress pathogens, and supply the plant with hormones and enzymes as described above [12-13].

Most research on VCT has focused on its ability to reduce the incidence of plant disease [14-15]. Of the few studies that have measured the effects of VCT application on plant growth, none have managed to determine whether the observed changes are a result of the nutrients or the microbes [12-13]. There has also been no research that has investigated whether VCT has differing effects when used with different types of moisture regimes. We aimed to address these research gaps by determining the effects that VCT, with and without microbes, had on the growth (defined as a collective measure of height and biomass) of sunflowers (Helianthus annuus) under both ideal and drought-simulated growing conditions. We selected sunflowers because they are an important agricultural crop [16], and are well suited to study due to their drought sensitivity, tall stature and rapid growth rate [17]. For reasons described above, we predicted that VCT would have a positive effect on plant growth, and that this effect would be greater in drought-simulated growing conditions.

Methods

Experimental Design

We conducted the experiment in a greenhouse, with full spectrum overhead lights used to simulate 16 hours of daylight. Although a greenhouse is limited by the fact that the plants within it are not subject to many of the abiotic and biotic stresses that they would normally endure in an agricultural environment, growing the plants in this way gave us greater control over the variables that we wished to manipulate, and as well also minimized many confounding factors (e.g. pests, disease heterogeneous soils, etc.). The sunflowers were grown for 63 days between January 8th and March 11th, 2013. Four sunflower seeds were planted in each 15 cm diameter pot filled with commercial potting soil. After the plants reached cotyledon the two shortest plants in each pot were removed, leaving two plants per pot.

Three different treatment solutions, each with 20 replicate pots, were applied directly after the plants were thinned. The first solution was VCT, brewed as per supplier recommendations. The materials and ingredients for VCT were provided by Living Soil Solutions of Calgary, AB. VCT was brewed on site by placing vermicompost in a mesh bag and suspending it in a bucket of deionized water in a 1:40 ratio (weight of vermicompost to water). Additional ingredients were added in accordance with the instructions for 20 L of water: 21 mL of kelp, 16 mL of fish extract, 21 mL of Soluplks (humic acid) and 60 mL of molasses. The mixture was then aerated for 24 hours, after which it was considered VCT. 200mL of VCT were applied to each pot of the treatment group titled vermicompost tea with microbes (VM) within four hours of brewing.

The second solution was autoclaved VCT. It was made by autoclaving VCT at 120C for 20 minutes to kill the microbes in the mixture. After autoclaving, the solution was placed in an ice bath to cool it to room temperature. 200mL of autoclaved VCT were applied to each pot of the treatment group titled vermicompost tea with no microbes (VNM) at the same time as the other solutions.

The third solution was deionized water. 200mL of deionized water were applied to each pot of the treatment group titled water only control (WOC) at the same time as the other solutions.

Regular watering was done using equal amounts of deionized water twice a week for half of each of the three treatment groups, to simulate ideal-water conditions. Watering was done once a week using the same amount for the other half of each treatment group to simulate drought conditions. In this way, the treatments under drought simulation received only half the water. The authors determined the watering regime arbitrarily.

Data Collection

The distance between the two plants in each pot was measured (hereafter termed distance) after the plants had first been thinned to account for differences in competition effects. Plant heights were measured weekly throughout the growth period.

We estimated bacterial abundances for the three treatment solutions pre-application using nutrient agar plates. We applied the three treatment solutions to nine nutrient agar plates (three for each solution), and had planned on counting bacteria colonies after a 40-hour period. Unfortunately, due to the high number of colonies it was impossible to determine the number of colonies at that time, and so instead the plates were visually analyzed to qualitatively determine whether there were morphological differences between them. The pH values of the treatment solutions were also measured.

We estimated soil bacterial abundances from 100 g soil samples 49 days after treatment application. Seven soil samples were taken from each of the six treatment group/water condition combinations. Soil samples were homogenized and 1 g of 2 mm sieved 99 mL of soil was suspended in distilled water using a VORTEX. This solution (100 L) was applied to nutrient agar plates and then left for one week before colonies were counted. We intended to estimate soil fungi abundances using a similar method unfortunately, the results had to be discarded due to experimental error.

We conducted carbon dioxide (CO2) respiration tests on one pot from each treatment group directly after application using a dynamic closed-chamber system. Each replicate was left inside the chamber for one hour; the methods were conducted as per Pumpanen et al. [18].

We measured wet and dry aboveground and root biomasses after the plants were harvested. We measured root biomass by removing the soil from roots manually and rinsing clean using tap water. The roots of the two different plants in each pot could not be separated therefore, we measured the root biomasses for the entire pot (reducing the number of replicates from 20 to 10). Finally, we measured dry biomasses for both aboveground and roots after the plants had been dried at 65C for 72 hours. It is important to note that the weights that were being measured (especially for the plants under drought-simulated conditions) were so small that the measured values approached the precision limit of the weight scale that was used.

Data Analysis

We analyzed data using the statistical program JMP, version 10.0.2, 2012. A repeated measures multiple regression was conducted to determine the effects of treatment solution, water condition, and distance on mean plant heights individual plant and pot were included as random variables within the regression, and the effects of the cofactors were nested within day of measurement. Two variables were used from this analysis to compare treatment groups growth rates (i.e. slope of the change in height at the mean day of the experiment), and least squared mean (LSM) heights over the course of the experiment. These two variables were used because they both have their advantages and disadvantages growth rates are more sensitive to differences between treatment groups than least squared mean heights, but also can be misleading when growth is not linear.

Further multiple regressions were conducted to determine the effects of treatment, water condition, and distance on dry aboveground, belowground, and total plant biomass. A two-way ANOVA was conducted to determine the effects of treatment and water condition on soil bacterial abundance, while a one-way ANOVA was conducted to determine the effect of treatment on the carbon dioxide respiration flux of soil microbes. An alpha value set as 0.05, and differences between treatment groups were determined by comparing 95% confidence intervals. Data were reported as mean 95% confidence intervals, unless otherwise noted.

All data used for ANOVAs and ANCOVAs were analyzed to determine whether they met assumptions of homogeneity and normality of variances using Levennes and Shapiro-Wilks tests respectively. All data met the assumptions except for soil bacterial abundances, which met neither. Logarithmic and square root transformations were attempted but made no differences on whether either assumption was met. Non-parametric tests such as the Mann-Whitney U and Kruskal Wallis were considered but rejected because they assume homogeneous variances as well, and ANOVAs are relatively robust to violations of assumptions [19].

Results

Height

All of the plants steadily increased in height over the 55-day period of measurement, although rates appeared to slow for the plants under drought simulated conditions after approximately day 35 (Fig. 1). Mean heights differed among treatment and water condition, after adjusting for day, when the repeated measures of individual plants and pots and the effect of time were taken into account as random effects and the covariate day (i.e., slope for this factor is an estimate of growth rate) was nested within each level of treatment and water condition (overall model $R^2=0.98$, $p<0.001$). Distance had no effect on either model, and so was removed from the analysis . Inspection of Fig. 1 shows non-linear growth, but we used a linear term in the model because a quadratic term (day2) was a weaker predictor of plant height when combined in the same model (F=276 for day2 versus F=703 for day). At the mean day of the experiment, the growth rate (i.e. the slope of the change in height) of the plants in VM was higher than that of the plants in either WOC or VNM under drought-simulated conditions, while the growth rate of the plants in WOC was higher than that of the plants in either VC or VNM under ideal water conditions (Table 1).

There was also a significant interaction between treatment and water condition at explaining least squared mean heights ($F(2,54.08)=3.30$, $p=0.04$), meaning that the strength of the treatment effect differed between water conditions. The least squared mean (LSM) heights of the treatment groups followed a similar pattern to growth rates, except that differences were less pronounced VCT and WOC were statistically similar under both water conditions (Table 1).

Biomass

Treatment solution and water conditions had interactional effects on aboveground and total plant dry biomass, as well as the belowground:aboveground dry biomass ratio (Table 2). Under drought simulated conditions, mean aboveground, belowground, and total dry biomasses were the same for all treatments, while the belowground:aboveground ratio was higher for WOC (Fig. 2). Under ideal water conditions, WOC had higher aboveground and total mean dry biomasses than VM and VNM, while the belowground biomass and belowground:aboveground ratio were the same for all treatments. Distance was positively related to belowground and total plant dry biomass.

Soil microbial activity

VCT increased mean soil CO2 respiration flux immediately after it was applied to the pots ($F(2,12591)=68319.10$, $p<0.001$). Over the course of the hour of measurement, VM had a mean respiration flux of 191.18±0.19m/m, which was higher than that of WOC (93.97±0.19m/m). The mean respiration flux of VNM could not be calculated due to experimental error. Based on colony counts of bacteria one week after soil samples were applied to agar plates (Table 3), treatment and water condition were found to have no effect on bacteria abundance in the soil 49 days after treatment ($R^2=0.13$, $F(5,34)=1.01$, $p=0.429$).

Treatment Solution Tests

Based on visual analysis, agar plates that were treated with VCT had higher bacteria abundances than those treated with autoclaved VCT or distilled water 40 hours after treatment (Fig. 3). The three treatments had similar pH measurements VCT had a slightly higher pH at 7.37, followed by deionized water at 7.18, followed lastly by autoclaved VCT at 7.15.

Water condition	Plant Group	Growth rate			Height		
		Mean (cm/day)	95% confidence interval (±cm/day)	Letters indicating significant difference*	Least squared mean (cm)	95% confidence interval (±cm)	Letters indicating significant difference*
Ideal-water	VM	0.71	0.02	A	23.94	1.34	AB
	VNM	0.69	0.02	B	21.92	1.34	A
	WOC	0.75	0.02	B	25.22	1.34	B
Drought simulated	VM	0.23	0.02	C	15.10	1.35	C
	VNM	0.19	0.02	D	12.19	1.34	D
	WOC	0.18	0.02	D	13.05	1.3	CD

Table 1: *Mean growth rates, least squared mean heights, and variances of Helianthus annuus over 55-day period of measurement. Vermicompost tea (VCT), autoclaved VCT, and deionized water were applied to VCT with microbes (VM), VCT with no microbes (VNM) and water only control (WOC) plant groups, with 20 plant replicates per group, under drought simulated and ideal-water conditions. A repeated measures linear regression was conducted with individual plant and pot as random variables, treatment group and water condition nested in day of measurement, and (R^2=0.98, $p<0.001$) (n=20). Significant differences between treatment groups (shown by letters) were determined by comparing 95% confidence intervals..*

Dry Biomass Parameters	Overall Model				Treatment solution and water condition solution			Distance effects		
	R^2	DF	F	*p*	DF	F	*p*	DF	F	*p*
Aboveground	0.88	5,114	161.14	<0.001	2,114	24.2	<0.001	-	-	-
Belowground	0.59	9,50	7.84	<0.001	-	-	-	1,50	4.02	0.05
Belowground: Aboveground	0.47	5,54	9.63	<0.001	2,54	4.88	<0.001	-	-	-
Total Plant	0.93	9,50	70.13	<0.001	2,50	12.92	<0.001	1,50	4.94	0.03

Table 2: *Model summary statistics for variance in dry biomass parameters. The R^2, degrees of freedom (DF), F statistic (F) and p value for the overall model as well as the treatment and water interaction and the effect of distance are presented.*

Water Condition	Treatment group	Mean number of bacterial colonies	95% confidence interval (±cm)
Ideal-water	VM	22.00	41.70
	VNM	8.14	41.70
	WOC	33.57	41.70
Drought-Simulated	VM	2.00	41.70
	VNM	49.00	41.70
	WOC	4.14	41.70

Table 3: *Summary statistics for colony counts of soil bacteria one week after solutions containing diluted soil samples were applied to nutrient agar plates. Seven soil samples were taken each from the VCT with microbes (VM), VCT with no microbes (VNM) and water only control (WOC) plant groups, under both drought simulated and ideal-water conditions. There were no significant differences between the six treatment groups, as determined by comparing 95% confidence intervals.*

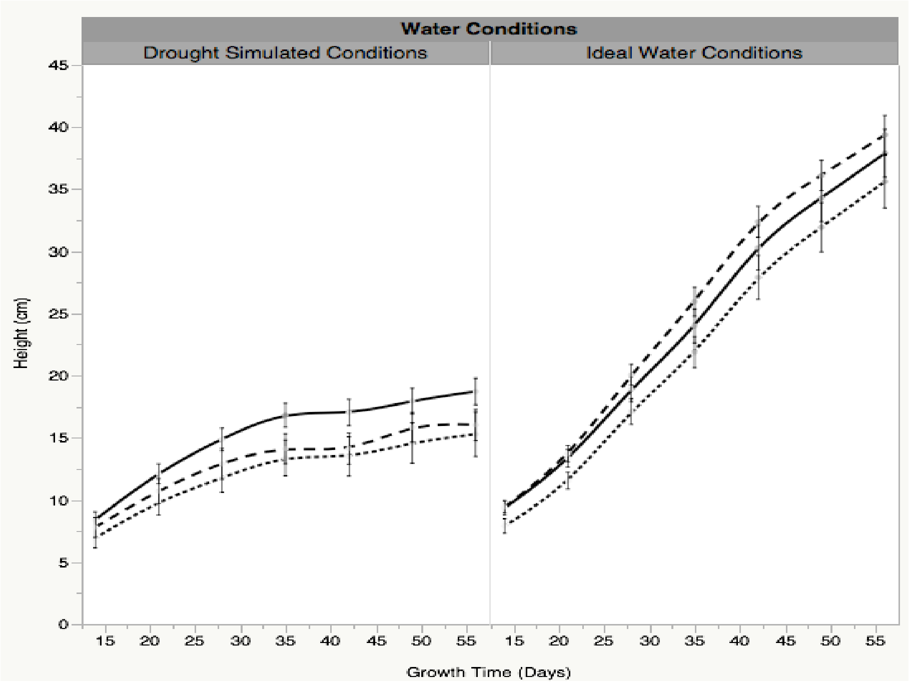

Figure 1:
Mean heights of Helianthus annuus over a period of 55 days (plants were grown in a greenhouse for 63 days in total). Vermicompost tea (VCT), autoclaved VCT, and deionized water were applied to VCT with microbes (VM ( )) VCT with no microbes (VNM (—)) and water only control (WOC ()) treatment groups, with 20 plant replicates per treatment group, under drought simulated and ideal-water conditions. Error bars indicate standard error (95% confidence intervals are not used here to increase clarity, as it is difficult to distinguish between them on the figure, n=20 for all treatment groups).

Discussion

Pattern 1: VM plants had higher growth than WOC and VNM plants in drought-simulated conditions

The first main pattern observed in this experiment was that plants with VCT applied (VM) had higher growth (shown most clearly in Table 1 by mean growth rate) than the control group of plants that only received water (WOC) and the group of plants that received autoclaved VCT (VNM) in drought-simulated conditions. Although the results for least squared mean (LSM) heights appear to follow this pattern, VM and WOC were not significantly different (Table 1). As noted in the methods, this lack of significance could be due to the fact that least square mean height is relatively insensitive to differences between treatment groups. There were also no significant differences between the aboveground biomasses of the treatment groups (Fig. 2). Mean biomass of VM plants were trending towards being the largest in both water regimes, however the results were not significant, possibly due to limitations of the weight scale that was used.

The higher growth of the VM under drought conditions may have been due to the presence of plant-growth promoting rhizobacteria (PGPR) in VCT. Previous research has shown that vermicompost has high amounts of PGPR [20] while the results of our soil treatment tests indicate that there were high amounts of microbes present in VCT and that at least initially, this increased soil respiration. PGPR can directly enhance the drought-tolerance of plants by supplying them with enzymes such as 1-aminocyclopropane-1-carboxylic acid (ACC) deanymase [10]. ACC deanymase reduces the level of ethylene in plants, which inhibits plant growth, especially in water-stressed conditions [21]. In addition to this, PGPR also play a role in solubilizing nutrients such as phosphorus and making them available for plant uptake within the rhizosphere [20].

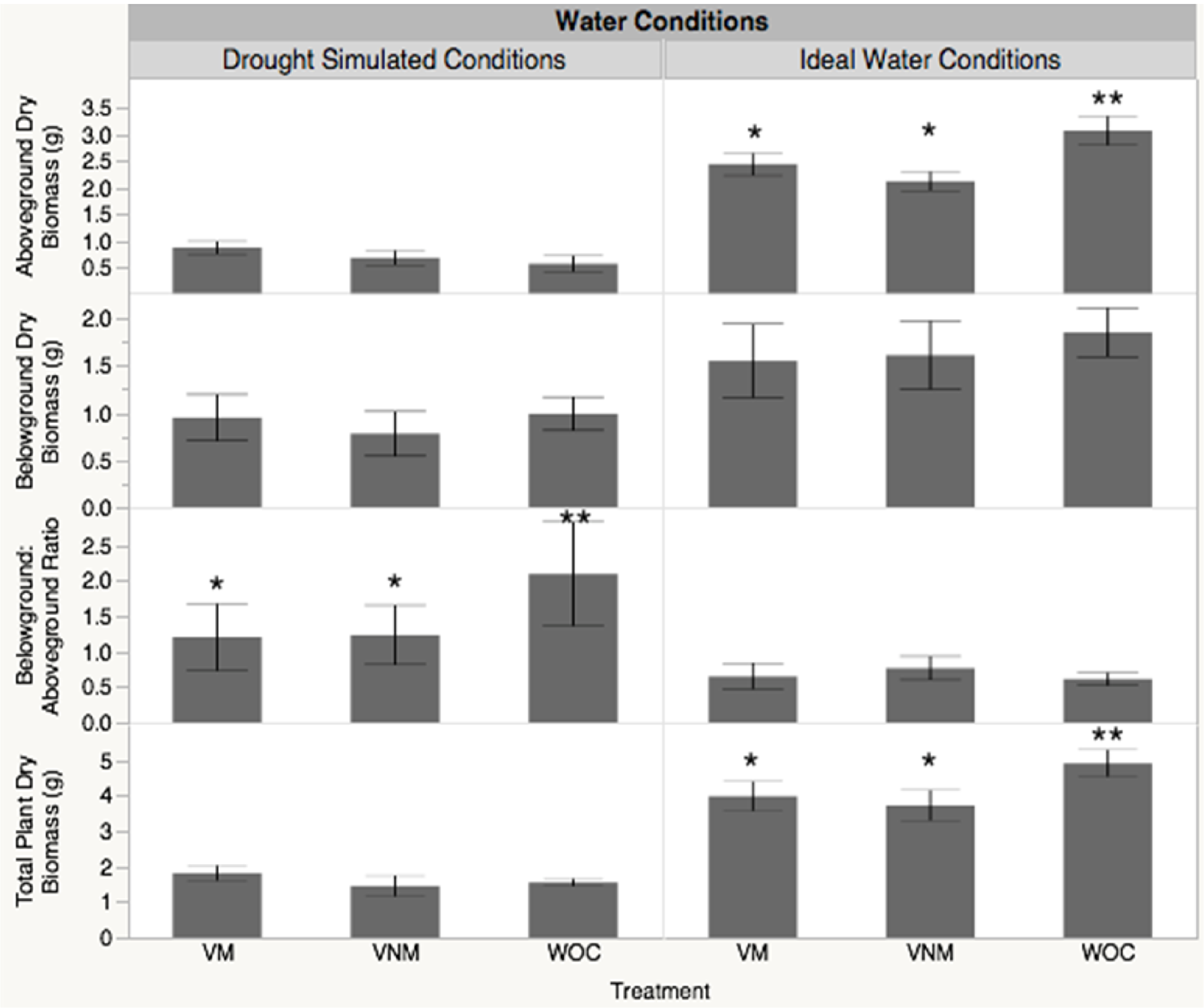

Figure 2:
Aboveground (upper), belowground (upper middle), belowground:aboveground ratio (lower middle) and total pot (lower) biomass of Helianthus annuus after 63 days. Vermicompost tea (VCT), autoclaved VCT, and deionized water were applied to VCT with microbes (VM) VCT with no microbes (VNM) and water only control (WOC) treatment groups, with 20 plant replicates per treatment group, under drought simulated and ideal-water conditions. Error bars indicate 95% confidence intervals. Multiple linear regressions were conducted Aboveground: R^2=0.88, F(5,114)=161.14, $p<0.001$. Belowground: R^2=0.59, F(9,50)=7.84, $p<0.001$. Belowground:Aboveground ratio: R^2=0.47, F(5,54)=9.63, $p<0.001$.$Totalpot : R^2$=0.93, F(9,50)=70.13, $p<0.001$. Significantly different groups within each water condition (determined by comparing 95% confidence intervals) are noted with asterisks. n=10 for treatment groups when belowground biomass and total plant biomass was measured; n=20 for treatment groups when aboveground biomass was measured.

Figure 3:
Pictures of agar plates 40 hours after they were treated with one of three treatments. Plates in the top row were treated with vermicompost tea (VCT) that was autoclaved; plates in the middle row were treated with VCT; plates in the bottom row were treated with deionized water. Plates that are darker yellow in colour have more bacterial colonies.

The role of PGPR may be especially important in dry soils, where nutrients have a low mobility due to limited solubilization and transport from water. This can result in local nutrient deficiencies around the roots of plants in dry soil, even in relatively nutrient rich soils [22]. Indeed, the belowground:aboveground dry biomass ratio of WOC was higher than the other treatment groups, which could indicate that these plant had relatively low access to nutrients (Fig. 2) [23]. Overall, the ability of PGPR to supply enzymes that increase drought resistance, and as well solubilize and transport nutrients, may have increased their value in drought-simulated conditions, and explained why VM outperformed WOC and VNM.

Pattern 2: VNM plants had equal growth to WOC plants in drought-simulated conditions

Another observed pattern in drought-simulated conditions was that there were no differences between the heights (Table 1) and between the aboveground and total plant biomasses (Fig. 2) of the VNM and WOC treatment groups. This pattern can potentially be explained by the absence of PGPR in the autoclaved VCT solution, resulting in VNM plants being unable to uptake the nutrients present in autoclaved VCT. As explained above, because dry soil causes nutrients to have a low mobility and solubility, local nutrient deficiencies could form around the roots, even when the rest of the soil was nutrient rich [22]. PGPR can therefore be especially beneficial in dry soil because they solubilize nutrients and make them available for root uptake. The fact that VCT, which contains PGPR, appeared to benefit plants in drought-simulated conditions is further evidence for this explanation.

Pattern 3: VM and VNM plants had lower growth than WOC plants in ideal-water conditions

The third main pattern observed in this experiment was that VM and VNM plants had lower heights (especially growth rates, as shown in Table 1) and aboveground and total plant biomass (Fig. 2) than WOC plants in ideal-water conditions, in contrast to drought conditions. It is possible that there are different reasons for why VM and VNM plants follow this pattern.

VM plants may have had lower growth than that of WOC due to the formation of anaerobic conditions around plant roots. Anaerobic soil conditions can form when excess water limits the amount of oxygen diffusion occurring between the atmosphere and soil pores. Oxygen is essential to soil respiration, and even temporary waterlogging has been shown to slow leaf and shoot growth, cause wilting and increase incidence of disease [24]. VM plants may have been especially susceptible to anaerobic conditions because of the high concentration of microbes and nutrients initially supplied by the VCT. This influx would have resulted in high levels of microbial respiration, potentially depleting oxygen in the soil. Potted plants may be especially at risk to anaerobic conditions due to compacted soils, high water content and the contained nature of the root system.

Anaerobic conditions could also have reduced plant growth by changing the composition of soil microbes. Certain microbes produce chemicals that are toxic to plants. In aerobic soils these chemicals are usually metabolized by other microorganisms and therefore do not accumulate. [24]. However, in oxygen-deficient conditions, anaerobic microbes can outcompete aerobic bacteria such as PGPR. This can result in microbes beginning to reduce Fe3+ and Mn3+, which act as alternative electron acceptors to oxygen. Where anaerobic bacteria are abundant, Fe2+ and Mn3+ can accumulate in the soil. These elements are toxic to plants at high concentrations. If pockets of anaerobic soils formed near the sunflower roots, this resulting toxicity could have contributed to the observed reduced growth in VM plants.

A possible explanation why VNM plants had lower growth than WOC is the soils of the VNM treatment group may have supported communities of toxigenic bacteria, which have been found to decrease both plant growth and seed weight [25]. Autoclaving may have disturbed the natural balance of the microbial community, removing PGPR and allowing for harmful bacteria to take over. Indeed, our results showed that after 49 days there were no differences between bacterial abundances of the three treatments (Table 3), suggesting that a recolonization may have occurred, however we are unable to determine which types of bacteria were dominant. Beneficial bacteria such as PGPR normally suppresses the growth of parasitic and toxigenic bacteria, its absence in the autoclaved VCT may have exasperated the proliferation of harmful bacteria colonies [25].

Conclusion

Vermicompost tea (VCT) increased plant growth in drought-simulated conditions and decreased sunflower growth in ideal-water conditions. Furthermore, the nutrients in VCT alone did not benefit sunflower growth, which may suggest that soil biology can significantly influence agricultural yield. It is proposed that plant-growth promoting rhizobacteria may play an important role in increasing plant growth and help to explain the observed results. The results of this study also suggest that soil microbial health is influenced by many factors including plant-microbe symbiotic relationships, nutrient availability and hydrological factors. With proper consideration of these factors, VCT has the potential to play an important role in increasing agricultural sustainability at a time when climate change, overpopulation and land degradation demand it.

Implications for future research

Based on this study and similar research that has been conducted, the influence that microbes have on plant health and productivity should continue to be investigated. Of particular importance is the observed beneficial effect that VCT had on sunflower growth under drought-simulated conditions. We recommended that future research be conducted on the influence and possible benefits of VCT on crop production in water stressed regions. This topic is especially pertinent to regions where climate change is expected to increase the incidence of drought. The effect of VCT on other agricultural crops, especially those that are of agricultural importance and grown without irrigation, should also continue to be investigated. It is believed that initial soil quality may influence the effect of VCT, so caution should be used when generalizing the results of this study. The commercial potting soil used in this study was relatively nutrient rich to begin with, and VCT may have different effects on crop production in soils that are nutrient poor. Additionally, previous studies have shown that adding PGPR into heavily used industrial agricultural soils has beneficial effects [26]. It is therefore important to further examine the effect that synthetic fertilizers and industrial agricultural practices have on soil microbial health because VCT application may be an option for restoring overworked soils. Finally, a small but growing portion of the global food supply is produced in greenhouses under ideal-water conditions. Based on the results of this study, VCT application may actually be harmful to plants under these conditions. Further research is recommended to investigate whether this effect holds true for other plants that are grown under ideal-water conditions, both inside and outside the greenhouse.

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