Article

Impact of soil productivity on the growth of two Meyer lemon trees

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SUMMARY

Home gardening is one of the most popular activities in the United States. As participation increases, more homeowners are turning to home testing to solve gardening problems. We aimed to apply home soil testing to one such problem: identifying the cause of the growth differences between two lemon trees in our backyard. Since the two lemon trees are of the same species, were planted at the same time sideby-side, and given similar amounts of water and fertilizer, we hypothesized that differences in physical and chemical soil characteristics were influencing differences in soil productivity and plant growth. We tested five factors that affect soil productivity: soil composition, permeability, water-holding capacity (WHC), pH and free ions, and ion exchange capacity. We analyzed five soil samples with three trials each from the root spread perimeter of each tree, then analyzed variance of the results. We found that the soil samples from the lemon tree with higher growth had significantly higher WHC and permeability due to higher humus content and better anion exchange capacity (AEC) due to higher clay content. High sand content and low humus content reduced WHC, permeability, and AEC in the poor soil. All samples had sufficient nutrients and ideal pH, so the two soils did not differ obviously in the tested chemical characteristics. A clay and humus mixture can be added to improve soil productivity for the lowerperforming tree. Overall, our study demonstrated the effectiveness of home soil testing to characterize soils and help homeowners solve common gardening problems.

INTRODUCTION

Home gardening has increased by 200% since 2008, with 35% of households in the United States growing food at home or in a community garden (1). During the COVID-19 pandemic, even more people have begun home gardening and crowdfunding community gardens (2). This may be attributed to the higher emotional wellbeing and sense of connectivity that is associated with vegetable gardening in urban settings (3). In fact, more than half of all California residences have a citrus tree (4). By using two lemon trees as our experimental subjects, we studied the most popular crop

category in California residences.

The major factor affecting plant growth is soil productivity, defined as a soil's capacity to produce a certain crop yield under certain inputs of water and nutrients. Soil productivity is influenced primarily by the physical and chemical composition of soil (5). Physical soil composition consists of soil texture and structure; the former describes the amounts of sand, clay, and silt that make up a specific soil, while the latter refers to the arrangement of these particles to form distinctive geometries (6-7). In each soil sample, 40-80% of soil is made of sand, clay, and silt; different combinations result in different properties (8). Soils with a nearly equal balance of all three particles are described as loam soils (6). Prior studies have shown that soil water content decreases gradually as particle size increases and texture becomes coarser (9). Sand, the largest particle, increases permeability, water infiltration, capillarity, and density but does not retain water for long-term use. Silt, the second largest, increases aeration and water retention. Clay, the smallest, is critical for ion exchange and increases water retention but resists water infiltration.

Soil organic matter (SOM) is another important component of physical soil composition. SOM makes up only 1 - 6% of soil but has significant effects on physical properties and chemical composition (8). SOM contains live organisms, fresh residue, and stable, clay-bound matter called humus that contributes nutrients and increases water-holding capacity (WHC), permeability, and ion exchange capacity. WHC measures water absorption and retention, while permeability measures water infiltration and the speed of water movement through soil. Both are critical for plant growth in the dry, sunny Southern California climate. In fact, lemon trees grown in soils with low silt, clay, and SOM have lower WHC, permeability, and ion exchange capacity, resulting in significantly lower fruit yield (10).

lon exchange capacity is a chemical property that refers to a soil's ability to hold onto cationic (positively charged) and anionic (negatively charged) nutrients. Ion exchange capacity is an indicator of soil fertility; soils with poor ion exchange capacity suffer from leaching (9). Leaching then decreases WHC, as shown by a decrease in soil water content after leaching under consistent air pressure and water pressure of soil pores (11). Ion exchange capacity can be improved by clay and SOM because negatively charged sites on the surfaces of clay and SOM particles attract cations. Anions, however, travel with water and run out more frequently.

Chemical soil properties are influenced by a soil's chemical composition, including pH and nutrient levels. pH indicates the acidity of a soil and affects solubility and availability of nutrients, microorganism activity, and crop yield (12-13). Lemon trees have an ideal pH range of 5.5 - 6.5. To maintain this pH range, the mixture of soil components can be altered. Clay and SOM increase the buffering capacity of soils, which is important because soil pH fluctuates as environments inevitably change. In contrast, sandy soils often have low SOM and are more vulnerable to acidification (13).

Nitrogen, potassium, and phosphorus are the three macronutrients most critical for productive soils. They are needed in large quantities and found in soil as mobile nitrate (NO_3^{-}) , immobile phosphate (PO_4^{3-}) , and immobile potassium ions (K^*) (14). Nitrogen, which is not retained long in the root zone, is a component of chlorophyll and nucleic acids (15). Sufficient nitrogen is vital for high rates of photosynthesis, vigorous plant growth, and dark green leaf color (8). Potassium plays a key role in osmoregulation, enzyme activation, pH neutralization, and energy production. Phosphorus is a component of nucleic acids and is important for cell division, tissue development, and regulation of protein synthesis (16). Lemon trees require double the amount of nitrogen needed by other citrus species, potassium for high-quality fruit, and phosphorus for flowers to bloom (16-17).

We purchased two Meyer lemon tree seedlings about ten years ago and planted them side by side at the same time. One lemon tree consistently produces more fruit and visibly has higher biomass, while the other tree has lower fruit yield (**Figure 1**). We conducted this project to find why these two trees differ so significantly in growth despite growing in similar environments and receiving similar amounts of water and fertilizer. Access to and retention of water and nutrients play critical roles in soil productivity and, consequently, plant growth. Thus, we hypothesized that the growth differences between the two lemon trees in our backyard were due to differences in soil productivity. We specifically hypothesized that differences in soil productivity resulted from differences in physical and chemical soil composition. Our results show that higher clay and humus levels in the good soil led to higher permeability, WHC, and ion exchange capacity. However, the two soils did not differ obviously in pH and macronutrient content. Like my family, many homeowners are amateur gardeners who encounter problems that they do not know how to characterize and address. The methods and results from this study can serve as an accessible example.

RESULTS

We took five evenly spaced soil samples from around the root spread of each lemon tree. We conducted three trials per sample for a total of 15 trials per tree for soil composition, WHC, soil permeability, and ion exchange capacity. The soil samples from the lemon tree with good growth were labelled G1, G2, G3, G4, and G5 for "good" growth. The samples from the lemon tree with poor growth were labelled B1, B2, B3, B4, and B5 for "bad" growth. We extended this labelling system to include trial numbers during the experiments (i.e. G1.1, G1.2, G1.3 for the 3 trials of sample 1 with good growth, B1.1, B1.2, B1.3 for sample 1 with bad growth, etc.).

We analyzed the results from each experiment by comparing the B and G samples to the controls to determine the specific factors influencing each property. Overall, the two soils showed significant differences in physical characteristics but no obvious differences in the tested chemical characteristics. Results are summarized in **Table 1**.

We tested soil composition to analyze particle distribution. The B samples contained 51% sand, 22% clay, and 27% silt. The G samples contained 45% sand, 24% clay, and 31% silt (**Table 1**, **Figure 2**). According to the USDA Soil Texture Triangle, the B samples are on the border between sandy clay loam and loam, and the G samples are loam (8). While preparing the soil samples, we noticed that the G samples were darker in color and had a chunkier texture. Then, in the WHC experiment, we observed that the control sample of humus (9.33 g) weighed less than the same volumes of sand (20.36 g) and clay (14.93 g). Likewise, the ANOVA analysis showed that the G samples (average weight 14.56 g) weighed significantly less (p < 0.001) than the B samples of the same volume (average weight 16.41 g) (**Table 2**). Together, these



Figure 1. The two Meyer lemon trees in the Southern California backyard of this study. The left tree exhibits improved growth, as evident from the higher leaf density and number of fruits.



Figure 2. Physical composition of soil samples. The B samples (n = 15) were between sandy clay loam and loam and contained more sand than the G samples (n = 15). The G samples were loam and contained higher levels of clay and silt than the B samples.

Sample	Soil Composition			Soil Permeability						Ion Exchange Capacity		
	% Sand	% Clay	% Silt	WHC***	Dry Samples (s)**	Wet Samples (s)*	рН	N	Р	ĸ	CEC	AEC
B1	51	19	30	0.36	334	1379	6	4	3	3	High	Low
B2	48	25	27	0.31	340	3190	6	4	4	2	High	Medium
B3	46	22	32	0.35	309	1483	6	3	3	3	High	Low
B4	53	26	21	0.36	244	1312	6	3	3	3	High	Low
B5	57	17	26	0.38	609	2198	6	4	2	2	High	Low
B Mean	51	22	27	0.35	367	1912	6	3.6	3	2.6	High	Low
Standard Deviation (SD)	6	6	4	0.12	175	774	0	0.5	0.7	0.5	N/A	N/A
G1	49	22	29	0.47	145	1354	6.5	4	4	2	High	Medium
G2	47	20	33	0.49	70	644	6.5	4	4	2	High	Medium
G3	41	29	30	0.46	160	1036	6.5	4	4	3	High	Medium
G4	42	25	33	0.47	112	1102	6.5	4	4	3	High	Medium
G5	44	25	31	0.47	70	911	6.5	4	4	3	High	Medium
G Mean	45	24	31	0.47	112	1009	6.5	4	4	2.6	High	Medium
SD	4	4	2	0.01	51	296	0	0	0	0.5	N/A	N/A
p-value (ANOVA)	e N/A 1			1.21e-05	0.00455	0.04280	N/A					

Table 1. Summary of results from the five main experiments.

Note: Nutrient levels: 4 = surplus, 3 = sufficient, 2 = adequate, 1 = deficient, 0 = depleted. Significance: *** = 0.001, ** = 0.01, * = 0.05.

factors indicated higher humus content in the G samples.

We calculated the mean WHC for the 15 B samples and the 15 G samples (**Table 2**). B samples had low average WHC (0.35) and G samples had medium average WHC (0.47) according to the WHC classification in the *Carolina Physical and Chemical Properties of Soil* kit manual. A mixed model ANOVA indicated that such a difference in WHC between B samples and G samples was statistically significant (p < 0.001). Among the control samples of humus, clay, and sand in this experiment, humus had the highest WHC (0.47), followed by clay (0.37) and sand (0.24). Therefore, higher humus levels in the G samples appear to associate strongly with higher WHC.

We tested permeability in both wet and dry conditions. The mean time for the 15 dry B samples was 367 s, which was significantly longer than the average 112 s for the 15 dry G samples (p = 0.00455 from the ANOVA analysis). The mean time for the 15 wet B samples was 1,912 s, which was significantly longer than the average 1,009 s for the 15 wet G samples (p = 0.0428 from the ANOVA analysis) (Figure 3). For controls, water traveled slowest through clay (dry: 365 s, wet: 6,965 s) and fastest through humus (dry: 37 s, wet: 239 s).





Figure 3. Soil permeability of samples. In both experiments, G samples (n = 15) took significantly less time than B samples (n = 15) and thus had higher permeability. The larger SD of the B samples indicates less uniformity within the poorer soil. Significance: *** = 0.001, ** = 0.01, * = 0.05.

The permeability of sand was between clay and humus (dry: 190 s, wet: 327 s). Humus was the most permeable, followed by sand and clay. Although the G samples had 2% more clay, the decrease in permeability from this small amount of clay was insignificant compared to the increase in permeability from the large amount of humus.

We analyzed the pH and nitrogen, phosphorus, and potassium levels of the soil samples using the color comparators of the *Rapitest* soil test kit included in the *Carolina* kit. B samples had mean pH = 6 and G samples had mean pH = 6.5, both in the ideal range for a lemon

tree. All samples had adequate potassium and surplus or sufficient nitrogen and phosphorus **(Figure 4)**. Thus, there was no obvious difference between the two soils in pH and macronutrient levels.

While the pH and free ions analysis measured the availability of essential nutrients, ion exchange capacity was tested to determine the ability of the samples to hold onto those nutrients. Both B and G samples had high cation exchange capacity (CEC) for Crystal Violet. For Eosin Y, G samples had medium anion exchange capacity (AEC) and B samples had low AEC **(Table 3)**. The movement of positively

Sample		Weight of Empty Column (g)	Weight of Column + Dry Soil (g)	Weight of Column + Saturated Soil (g)	Weight of Water (g)	Weight of Soil (g)***	WHC***		
	Clay	3.25	18.18	23.66	5.48	14.93	0.37	Low	
Control	Sand	3.14	23.50	28.34	4.84	20.36	0.24	Low	
	Humus	3.20	12.53	16.90	4.37	9.33	0.47	Medium	
	B1.1	2.88	19.74	25.64	5.90	16.86	0.35	Low	
B1	B1.2	2.90	19.16	25.16	6.00	16.26	0.37	Low	
	B1.3	2.92	19.54	25.45	5.91	16.62	0.36	Low	
B2	B2.1	2.93	19.00	24.85	5.85	16.07	0.36	Low	
	B2.2	2.90	19.55	24.43	4.88	16.65	0.29	Low	
	B2.3	2.93	19.50	24.18	4.68	16.57	0.28	Low	
	B3.1	2.91	18.67	24.78	6.11	15.76	0.39	Low	
B 3	B3.2	2.87	18.97	24.08	5.11	16.10	0.32	Low	
	B3.3	2.88	18.52	24.03	5.51	15.64	0.35	Low	
	B4.1	2.92	19.08	24.73	5.65	16.16	0.35	Low	
B4	B4.2	2.88	18.98	24.77	5 79	16.10	0.36	Low	
	B4.3	2.87	19.28	25.26	5.98	16.41	0.36	Low	
	B5.1	2.94	20.60	26.50	5.90	17.66	0.33	Low	
B5	B5.2	2.96	19 72	27.54	7.82	16.76	0.47	Medium	
	B5.3	2.89	19.41	25.14	5.73	16.52	0.35	Low	
B Mean		2.91	19.31	25.10	5.79	16.41	0.35	LOW	
SD		0.11	1.94	2.24	1.08	1.98	0.12	N/A	
	C1.1	2.04	17.44	24.20	6.04	14.50	0.49	Modium	
G1	G1.1	2.94	17.44	24.30	6.71	14.50	0.46	Medium	
91	G1.2	2.50	18.16	25.55	7.30	15.26	0.40	Medium	
	G2.1	2.30	17.16	23.96	6.80	14.27	0.40	Medium	
G2	62.1	2.05	17.10	25.03	7.27	14.27	0.40	Medium	
01	62.2	2.50	17.70	25.00	7.49	14.00	0.43	Medium	
	62.5	2.52	16.70	23.00	7.40	12.77	0.46	Medium	
C 2	63.2	2.33	16.04	24.40	7.05	14.05	0.40	Medium	
30	63.2	2.03	17.54	24.03	8.02	14.00	0.47	Medium	
	63.5	2.92	17.04	25.00	0.02	14.02	0.40	Medium	
G4	G4.1	2.92	17.10	25.52	0.22	14.10	0.40	Medium	
	G4.2	2.93	17.39	20.07	0.20	14.40	0.40	Medium	
	G4.3	2.92	19.00	20.00	0.03	14.30	0.40	Medium	
G5	05.1	2.90	17.76	20.30	7.09	11.30	0.46	Medium	
	G5.2	2.93	17.50	24.04	7.00	14.03	0.40	Modium	
	65.3	2.91	17.55	24.02	7.09	14.62	0.40	wedium	
G Mean		2.92	17.47	24.94	7.47	14.56	0.47	MEDIUM	
SD	SD		0.42	0.53	0.53	0.42	0.01	N/A	
p-value	p-value (ANOVA)			N/A	5.35e-05	1.21e-05	N/A		

Table 2. Water Holding Capacity (WHC) of the soil samples.

Note: WHC less than 0.4 is classified as low capacity, and WHC between 0.4 and 0.6 is classified as medium capacity according to the kit manual. Significance: *** = 0.001, ** = 0.01, * = 0.05.

charged Crystal Violet and negatively charged Eosin Y mimicked the movement of cationic and anionic nutrients through soil, respectively. For Eosin Y, the controls sand and sand + humus had low AEC, while sand + clay had medium AEC. This displays the negative effects of sand on AEC and how clay increases AEC. The G samples contained 2% more clay and 6% less sand than the B samples (Figure 1). Therefore, the higher AEC of the G samples was consistent with its higher clay levels, and the low AEC of the B samples was consistent with its higher sand content and lower clay content.

DISCUSSION

The higher permeability and WHC of the G samples mean that water travels to the roots significantly faster in the G soil and is retained significantly better. The G soil also provides better aeration and resists nutrient leaching due to its more compact, fine texture. The organic matter in the humus balances the effects of higher clay and silt levels by increasing drainage speed and binding particles into stable clumps. In contrast, the sandy texture of the B soil means that while water drainage is efficient, water retention and AEC are low. Thus, while both soils hold onto cation nutrients well, the good soil retains mobile anion nutrients like NO₃⁻ more effectively.

An unexpected result was that the soils did not differ obviously in the measured chemical characteristics. Nutrient deficiencies were expected for the poor soil, but both soils had an ideal pH for lemon trees and contained sufficient nitrogen, phosphorus, and potassium. Even the difference in ion exchange capacity, a chemical property, was likely due



Qualitative Analysis of pH and Free Ions

Figure 4. pH, nitrogen (N), phosphorous (P), and potassium (K) levels of soil samples. Both B samples (n = 5) and G samples (n = 5) had an ideal pH for lemon trees, adequate potassium, and sufficient or surplus amounts of nitrogen and phosphorus. All three ions are dimensionless.

to differences in physical composition. Thus, our hypothesis that the growth differences between the two lemon trees was being caused by differences in both physical and chemical soil composition was partially supported. The soil productivity of the poor soil can be improved by the addition of a clay and humus mixture.

In addition, the B samples had a larger standard deviation (SD) in physical characteristics compared to the G samples, suggesting an uneven distribution of soil composition, permeability, and WHC in the poor soil (Table 1). Likewise, the B samples had more variation in nitrogen and phosphorus levels (Figure 4). The relative chemical inconsistency of the B samples could be impactful despite the B and G samples showing little differences in pH and free ions. Overall, such

		Cry	stal Violet		Eosin Y			
5	ample	Average Amount of Water (mL)	Color Intensity	CEC	Average Amount of Water (mL)	Color Intensity	AEC	
	Sand	6	Dark	Low	5	Dark	Low	
Controls	Sand + Clay	15	Light	High	6	Light	Medium	
	Sand + Humus	18	Light	High	6	Medium	Low	
	B1	10	Light	High	6	Medium	Low	
В	B2	13	Light	High	6.3	Light	Medium	
	B3	14	Light	High	6	Medium	Low	
	B4	15	Light	High	6	Medium	Low	
	B5	15	Light	High	6.3	Medium	Low	
B Mean		13.40	Light	High	6.12	Light	Low	
SD		3.2	N/A		0.4	N/A		
G	G1	17	Light	High	7.7	Light	Medium	
	G2	17	Light	High	6.7	Light	Medium	
	G3	17	Light	High	5	Light	Medium	
	G4	16	Light	High	6.7	Light	Medium	
	G5	16	Light	High	7	Light	Medium	
G Mean		16.60	Light	High	6.62	Light	Medium	
SD		3.2	N/A		1.1		N/A	

Table 3. Ion Exchange Capacity of the soil samples.

Note: AEC = *anion exchange capacity, CEC* = *cation exchange capacity.*

uneven physical and chemical distribution may have been another factor negatively affecting lemon tree growth.

In conclusion, there are few differences in the measured chemical characteristics but several significant differences in the tested physical characteristics, especially those related to water retention. Considering California's particularly dry climate, poor water retention was likely the most influential factor behind one lemon tree's poorer growth.

In addition, one confounding factor that could have influenced the growth differences was differing amounts of reflected sunlight due to different physical backgrounds. Though the two lemon trees were planted side by side, the tree with poor growth is located closer to the corner and in front of a white wall, while the tree with better growth is in front of a brown brick wall (Figure 1). The former's shaded corner location may prevent it from attaining sunlight for photosynthesis during dimmer days, while its white background reflects more heat and light during the hot summers compared to the brown brick background of the other tree. This may exacerbate the water burden already placed on the lemon tree with poor growth due to its soil composition. In the future, we can measure the amount of reflected light and the average temperature near the two trees during different times of the day to better characterize this potential factor.

Limitations that could have affected the results include imprecise timing and subjective color assessment. During the permeability experiment, five samples had to be observed simultaneously, so timing precision may have been compromised. In the ion exchange capacity experiment, color intensity of filtered water was assessed through comparison to surrounding samples. These aspects can be improved by incorporating slow-motion recordings and color references into the experimental design. Slow-motion video could be used for rewind purposes to determine more precisely when specific moments occurred. Ideally, a colorimeter would be used to determine color intensity of the filtered water. From a realistic homeowner perspective, however, a color scale or examples of what should be considered light, medium, and dark water would improve the color assessment.

Further research on soil structure, porosity, and nutrient distribution would also contribute to this study by addressing the variability in the B samples and the effects of WHC and permeability on soil aeration and nutrient availability. Testing soil uniformity would enable more concrete conclusions to be drawn about soil consistency. Testing micronutrients such as iron, zinc, and manganese would also contribute to the comprehensiveness of the chemical analysis.

In total, our results provide valuable insight to homeowners and gardeners who are having difficulties raising healthy plants. Applying these experiments to home soils can pinpoint problems and determine what specific particles, nutrients, or fertilizers to add to improve soil productivity. Potential applications of this study include soil testing for gardens, backyards, and parks to characterize and study soil quality at the household, community, and city levels. An increased accessibility to soil testing would help people correct common errors like overwatering and using the wrong fertilizer.

MATERIALS AND METHODS Preparing the soil samples

500 g of soil from 8 in below the ground was retrieved from each of five evenly spaced sites around the perimeter of each lemon tree's root spread. Each sample was placed on separate 18" x 20" sheets of Nalgene Versi-Dry surface protectors from ThermoFisher Scientific. Rocks, stones, and branches were taken out, clumps of soil crushed, and soil spread out evenly. Samples were placed in an open area and dried under natural sunlight for 1 day before being transferred into labelled 10" x 14" Ziploc bags for storage.

The *Carolina Physical and Chemical Properties of Soil* kit and accompanying manual were adapted for use in the following experiments:

Soil composition

Thirty-three plastic jars (30 samples, 3 controls: clay, silt, and sand) were labelled and marked at the halfway line with a black china marker pencil. Each jar was filled with the corresponding sample to the halfway mark. Tap water was added to the lower rim of the jar before each jar was shaken for 30 seconds. 1 drop of dish detergent was added to each jar to help the layers settle clearly overnight. Settled layers were labelled (from top to bottom): humus, clay, silt, and sand. The thickness of every layer except humus, an organic component, was measured and recorded. The thickness percentages of clay, silt, and sand for the samples were then calculated, averaged, and compared to the USDA Soil Texture Triangle in the *Carolina* kit manual to determine soil composition.

WHC and capillary action

Thirty-three plastic columns (30 samples, 3 controls: sand, clay, humus) were used. A black china marker pencil was used to mark a line 7 cm from the bottom end of each column. Two 1" x 1" cheesecloth pieces were secured over each bottom end using a rubber band. A balance scale was used to determine the weight in grams of each column before and after filling to the 7 cm mark with soil. The weight of soil was calculated by subtracting the weight of the empty column from the weight of the column and dry soil. A plastic bin was filled with water to the 1 cm mark and all 33 columns were secured into a holder and placed into the bin overnight with the cheesecloth ends submerged. The following day, the weight of absorbed water was calculated by subtracting the weight of the saturated column from the weight of the unsaturated column. The weight of absorbed water was divided by the weight of soil and the resulting value compared to the manual's standards to determine WHC.

Soil permeability

Each saturated column from the WHC experiment was

suspended using 2 twist ties above a vial. 10 mL of water was poured into each wet sample using a measuring cup. A timer was used to record when the first drop of water passed out of the bottom of each column and when all the water above each sample was absorbed. 30 dry soil samples and 3 control samples were prepared using the same procedure from the WHC experiment and the timing process was repeated. The time it took for 10 mL of water to travel through each wet and dry saturated column was used to measure soil permeability.

Analysis of pH and free ions

For pH, soil samples were filled to the bottom tester line and chemical reagents from a pH indicator capsule were added. Water was added using a pipet to the top tester line. The tester was shaken vigorously by hand for 1 minute and allowed to settle for another 1 minute. The color of the solution was compared with the pH chart and pH was recorded.

For nitrogen, phosphorus, and potassium, the 30 jars from the soil composition experiment were used. For each sample, 10 mL of soiled water was pipetted from the top of the 3 trial jars and mixed in a cup. The mixed water was added to each tester to the marked line and shaken for 1 minute with the powder from 1 corresponding indicator capsule. Testers settled for 5 minutes before colors of the solutions were compared to the corresponding charts.

Qualitative analysis of Ion Exchange Capacity

Thirty-three centrifuge tubes (30 samples, 3 controls: sand, sand + clay, sand + humus) were labelled. A line 15 cm from the bottom of each tube was marked with a black china marker pencil. Two rubber bands were bound at the line to help secure the centrifuge tube above the vial. Corresponding soil samples were filled to the 15 cm mark and 20 drops of 1% Crystal Violet were added evenly across the top of each sample. Water was pipetted in increments of 1 mL into each sample until water began to pass out the bottom of the centrifuge tube. The amount of water added and the color intensity of the filtered water relative to the other samples was recorded. Tubes and vials were rinsed and dried before the entire process was repeated with Eosin Y. Results were compared to the manual's Exchange Capacity Chart.

Statistical Analysis

Charts from the kit were used to analyze the data for WHC, analysis of pH and free ions, and qualitative analysis of ion exchange capacity. The linear mixed model of $y_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk}$ was used in the statistical analysis, where y_{ijk} is the observed response variable, such as WHC, μ is the grand mean, α_i is the fix effect for tree location with i = 1 or 2, β_{ij} is the random effect for the *j*th sample collected from the *i*th tree location with j = 1, 2, 3, 4, or 5, and ε_{ijk} is the random error of the *k*th measurement for the *j*th sample from the *i*th tree location with k = 1, 2 or 3. The ANOVA for the linear mixed effects model was performed using R package 'ImerTest' (18).

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