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Influence of soil pH on abundance of mycorrhizae in a coffea arabica (Rubiaceae) plantation

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Influence of Soil pH on Abundance of Mycorrhizae in a *Coffea arabica* (Rubiaceae) Plantation

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ABSTRACT

Soil pH can have a large effect on the growth and fitness of plants. *Coffea arabica* (Rubiaceae) is an extremely important crop for the economy of Costa Rica. Coffee plants become more susceptible to the detrimental infection of *Mycena citricolor*, a fungus, with lower pH. pH can also have an effect on Vesicular-Arbuscular Mycorrhizae (VAM) amounts in the roots, therefore limiting the mutualistic fungus's ability to help increase plants growth. Previous studies have also shown that *M. citricolor* increases closer to the forest, while mycorrhizae decrease slightly. This study, in Cañitas, Costa Rica, shows that pH increased significantly with distance from the forest but never exceeding 6.5. Mycorrhizae showed only a slight trend towards increased abundance in the center of the plot. This suggests that increased pH creates a better environment for mycorrhizae and decreased *M. citricolor* infection. Common garden seedling experiment also supports this: pH 7 is the optimal pH growth for the coffee plants. pH 7 had a larger increase diameter growth and a higher number of mycorrhizae per root than acidic and basic conditions, however, all seedling heights were similar. These results are helpful to coffee farmers because it indicates that by increasing the soil pH of coffee plots to 7 they can have a better crop growth.

RESUMEN

El pH de los suelos puede tener un gran efecto en el crecimiento y el exito reproductive de las plantas. *Coffea arabica* (Rubiaceae) es un cultivo muy importante en la economía de Costa Rica. Las plantas de café, estas se vuelven más susceptibles a la infección por el hongo *Mycena citricolor* a bajos pH. El pH puede también tener un efecto en la cantidad de Vesículas-Arbusculares de Micorrizas (VAM) en las raíces, limitando así la capacidad mutualística de ayudar en el crecimiento de la planta. Estudios previos han demostrado además que *M. citricolor* aumenta en la cercanía al bosque, mientras que las micorrizas disminuyen un poco. Este estudio realizado en Cañitas, Costa Rica muestra que existe un aumento significativo en el pH al aumentar la distancia al bosque, pero nunca superior a 6.5. Las micorrizas muestran solo una leve tendencia a aumentar su abundancia en el centro de la cuadrícula. Esto sugiere que un aumento en el pH crea un mejor ambiente para las micorrizas y disminuye las infecciones por el hongo. Un experimento con plántulas también apoya esto: pH de 7 es optimo para el crecimiento de plantas de café. pH de 7 tiene un mayor diámetro de crecimiento y un mayor número de micorrizas por raíz que suelos en condiciones ácidas o básicas, sin embargo, el tamaño de las plántulas es similar. Estos resultados son de importancia para los caficultores ya que indica que al aumentar el pH de los suelos a 7 ellos pueden tener una mejor cosecha.

INTRODUCTION

Coffea Arabica (Rubiaceae) is one of the largest agricultural exports in Costa Rica exporting 1,210,000 bags in 2009/2010 (Gonzalez *et al.* 2010). A major problem for coffee growers is *Mycena citricolor*, a fungus that causes American leaf spot disease and can be very detrimental to plants and its economic value. In Costa Rica, American leaf spot disease or ojo de gallo was reported to cause an average annual crop yield loss up to 20% (Rae & Tewari 1986). *M. citricolor* causes brownish spots to occur on leaves and fruits causing them to fall off the tree. Farmers have noticed that the fungus favors more acidic soils so they try to inhibit fungal growth by altering the pH using chemicals or lime spray (Waller *et al.* 2007). Ojo de gallo is a large problem in tropical areas because tropical soils are poor and usually very acidic (Cardoso 2006).

Past studies have determined that ojo de gallo is more common on the edge of the fields closest to forests or shady areas (Gallagher 2009, Peterson 2010). It could be because closer to the forest has increased shade, higher diversity types of plants surrounding, and more leaf litter, which could make it more acidic (Finzi *et al.* 1998). Higher diversity of plants and increase leaf litter can lead to altered pH acidity environments because changes in the ration of acid vs. base cations available (Finzi *et al.* 1998). Acidic environments not only facilitate the growth ojo de gallo but studies have shown that it also negatively affects the interactions with mycorrhizae (Graw 1979). Kopp (2010) observed that mycorrhizae abundance is lower at the edge of the plot implying that increased presence of ojo de gallo has a large effect on mycorrhizae and overall plant fitness, which may be attributed to soil pH.

Vesicular-Arbuscular Mycorrhizae (VAM) provides hope for *C. arabica* growth in these poor acidic tropical soils (Cardoso 2006). Mycorrhizae are very helpful to plant growth because it enhances the physical, chemical and biological soil quality and helps with nutrient acquisition in agriculture (Cardoso 2006). Farming processes can become more sustainable and potentially decrease the need for chemicals by taking advantage of naturally occurring mycorrhizae (Johnson and Pfleger 1992). Many factors can affect the soil and mycorrhizae like application of fungicides and pesticides so it is important to study and understand factors that are affecting them to enhance mycorrhizae (Johnson *et al.* 1992, Johnson & Pfleger 1992). Fungicides have been shown to sometimes enhance mycorrhizae because it removes the antagonistic fungus. However, it is hard to observe the overall effect of the fungicides (Johnson & Pfleger 1992).

This study investigates change in pH and mycorrhizae growth in relation to distance from the forest. It also analyzes the effect of altered pH environments on the growth and development of seedlings and their mycorrhizal fungus. Based on past studies showing less *M. citricolor* (Gallagher 2009, Peterson 2010) and more mycorrhizae in the roots of plants in the center of the field (Kopp 2010) suggests that there may be some connection with ojo de gallo infestation and mycorrhizae. I predicted that the further from the forest the soil will have a higher pH and there will be more mycorrhizae present.

METHODS

Study Site

This study took place in Cañitas, Costa Rica from April 6 to May 3, 2011 on the LIFE Monteverde farm. It has 5.7 hectares of non-organic coffee fields, which are broken into 33 separate coffee plots surrounded by naturally regenerated forest (total 8 hectares). Elevation of the farm is between 1200 to 1300m. LIFE Monteverde produces approximately 16,000 lbs of coffee per year (Kopp 2010). Fungicides and chemical fertilizers are applied sparingly and only 3 times a year. They also take other precautions like pruning older branches and leaving plant rubbish on top of the soil to help protect it. This study used the same plot that Peterson (2010) and Kopp (2010) utilized (Fig 1).

FIGURE 1. Map of LIFE Monteverde farm where study was conducted in Cañitas, Puntarenas, Costa Rica. Plot used is identified with yellow box. Forest surrounding plot identified with arrow.

Field Experiment

To study soil acidity, I measured pH from soil below a total of 60 plants, these plants were 0-23m from the edge of the plot by the forest. pH was measured using electronic pH meter. Edges had more shade and leaf liter surrounding than the center with full sun and not as much debris cover. Each tree was in the range of 1.3-2 meters tall. To study mycorrhizal formation, I took 10 samples from the edge (0-6m) and 10 from the center (6-23m) of the plot.

Common Garden Seedling Experiment

To measure mycorrhizae formation as a function of pH environment, seedlings were planted in different soil environments prepared with chemicals that are commonly used in farming practice (Rae & Tweari 1986, Vaast *et al.* 1992). Sulfur was used to make the soil more acidic (pH≈5.5). Calcium carbonate was used to make the soil more basic (pH≈10) and unmodified soil was used as a control (pH≈7). Sulfur was slow acting so it took awhile to lower the soil pH from 7 to 5.5. Ten seedlings were in each of the three treatments for 30 seedlings total. I took initial and final mycorrhizae samples from three randomly selected seedlings in each treatment for a total of 18 samples. I looked at the height and diameter growth over the month to observe change. All initial seedlings were of similar size (average size was 31cm and 1.1cm diameter). Seedlings were all placed in same area where they got full sunlight for at least 5 hours a day.

Mycorrhizae Measurements

To measure the mycorrhizae I use a modified procedure from Bagyaraj & Sturmer (2008). I collected root samples and put them in separate labeled plastic bags. The next day I washed the samples with tap water and cut them into smaller pieces, then placed them in 2% KOH for 90 min in a 90°C water bath. After rinsing samples with tap water 3 times and soaking the roots in alkaline H_2O_2 solution (3ml 20% Nh4OH, 30ml 3% H_2O_2, 567ml tap water) for 1 hr; then rinsed again with tap water 3 times. Next I acidified roots in 1% HCL solution for 15 min. Last I stained the roots using a 4:1 staining solution of acidic glycerol (500mL glycerol, 450mL water, 50mL 1% HCL) and 0.05% trypan blue by putting the stain solution in tubes and placing in a 90°C water bath for 1 hr. After 1 hr I removed the stain solution and cut 3 samples from each root piece. By observing under a 40X compound light microscope I counted the number of vesicles and determined average presence for each plant.

RESULTS

Field Experiment
Soil pH differed significantly with increasing distance from the forest from 0-23m ($F_{(1,60)}$=5.78, p=0.019, Fig 2.). pH increased with distance from the forest increasing. However, There was no significant difference in the number of mycorrhizae per root with pH increase ($F_{(1,18)}$=2.94, p=0.104, Fig 3.). Mycorrhizae amounts/root ranged from 19-56. Soil pH doesn't reach 7 in this plot. The pH of the edge ranged from 4.6-6.5 while the pH in the center was between pH 6-6.5

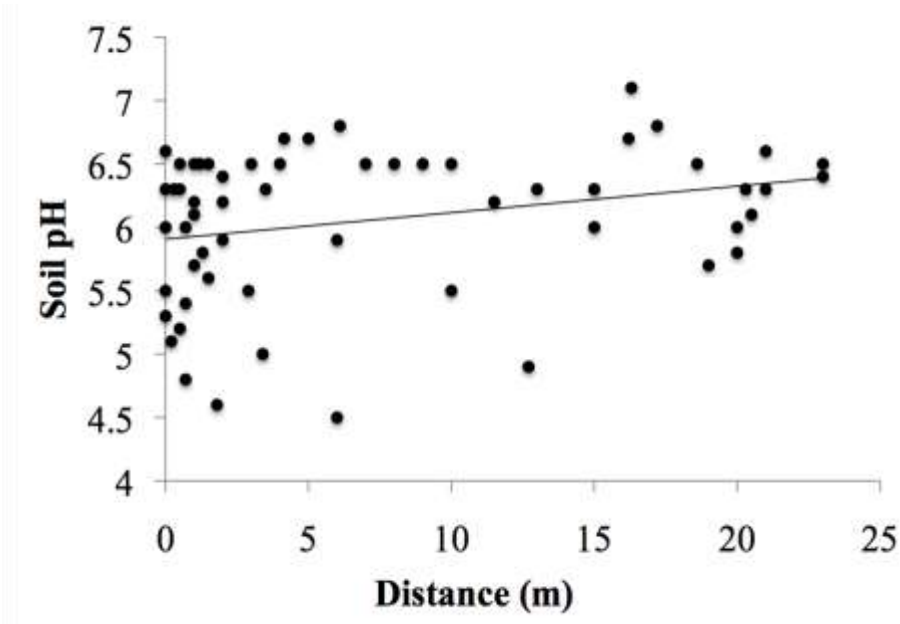

FIGURE 2. Linear regression of soil pH compared with distance (meters) from the forest edge in a single plot of *C. arabica* at LIFE Monteverde farm. pH increased with increasing distance from the forest. Equation:Y=0.021x + 5.9064, R^2= 0.0879, p< 0.05.

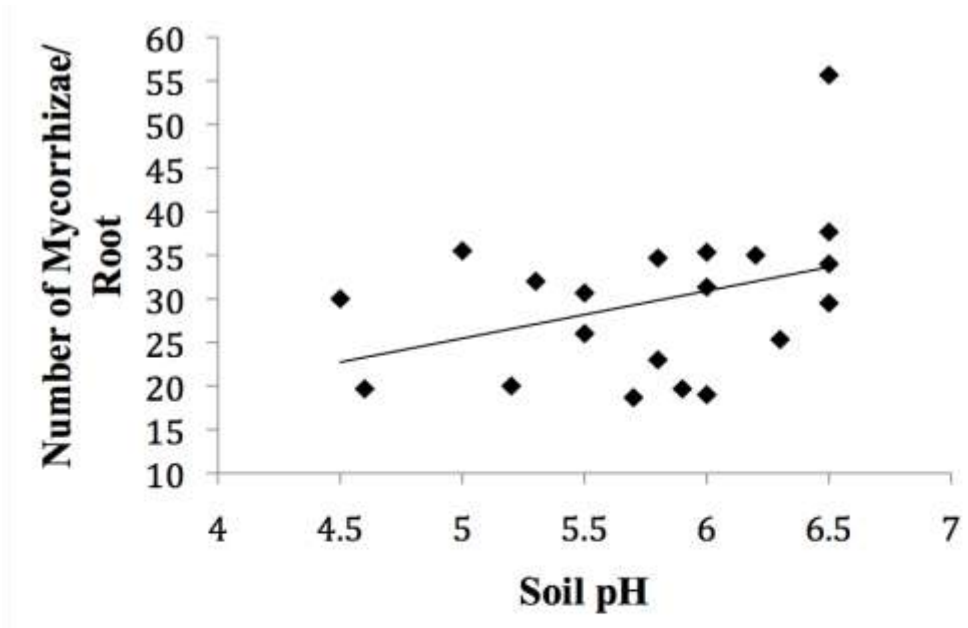

FIGURE 3. Linear regression of number of mycorrhizae in roots of *C. arabica* with increasing soil pH from a single plot in LIFE Monteverde farm. No significant relationship is observed, but there is a slight trend showing more mycorrhizae in higher pH. Equation:Y=5.4541x – 1.8094, R2=0.1404., p>0.05.

Common Garden Seedling Experiment

No difference was observed in the height gain by seedlings between treatments ($F_{(2,27)}$=0.021. p=0.98, Fig 4.) All treatment seedlings had an initial average height of 31cm. Acid treatment had the tallest final average height with 39cm, while base and control averaged 37cm in heights. Diameter growth of the control seedlings was significantly higher than diameters of the other treatments, ($F_{(2,27)}$=4.09, p=0.0281, Fig 4.). Control seedlings in pH 7 had a 6-fold increase in diameter growth than the other two treatments. There was no significant correlation between mycorrhizae amounts and seedling treatment (Kruskal-Wallis, X^2=2.49, Df=2, p= 0.29 Fig 5.). The control treatment of pH 7 had the most mycorrhizae growth compared to acid and base treatments, but seedlings in acid treatment had highest variation. Highest average of mycorrhizae per root in acid were 52, while control was steady 40 mycorhizae/root and base was significantly lower with 30. Seedlings across all samples were not in the best health; most had brown leaves except for a few control seedlings.

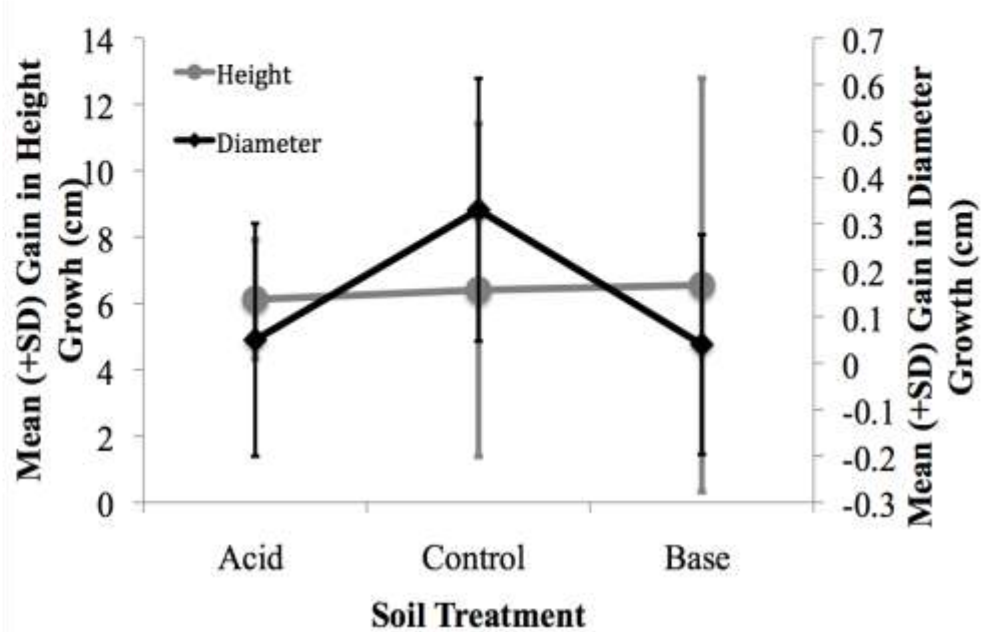

FIGURE 4. Mean (+ SD) gain in height and diameter growth (cm) in seedlings from three controlled pH environments: acid (pH=5.5-6), base (pH=8-10) and control (pH=7). Height showed no difference between treatments but control had significantly larger diameter growth than acid or base treatments. N=10 for each treatment.

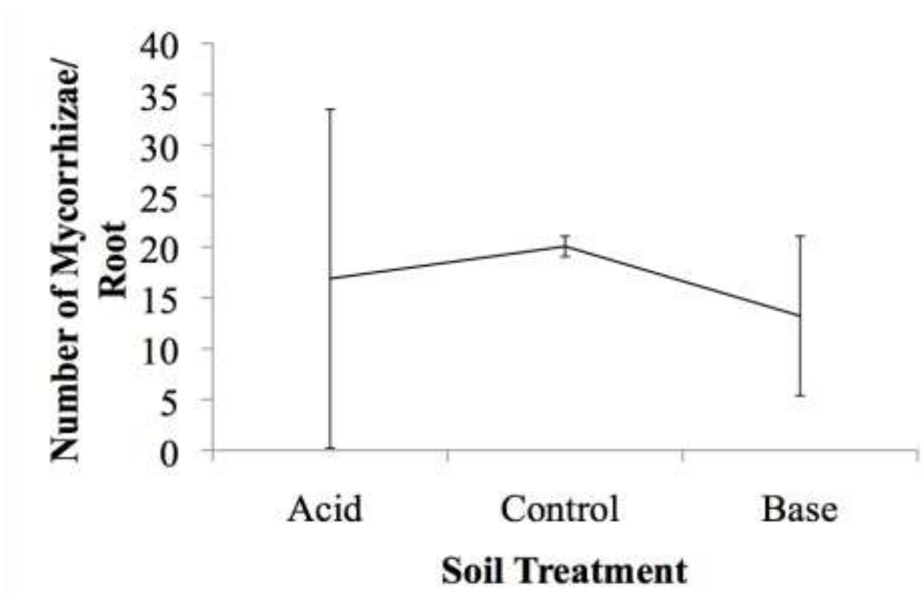

FIGURE 5. Average (+SD) number of mycorrhizae in roots of *C. arabica* seedlings from three different soil treatments, acid (pH=5.5-6), base (pH=8-10), control (pH=7). N=10 in each treatment.

DISCUSSION

As expected, I found that soil pH increased with increasing distance from the edge. The pH of the soil closer to the forest was significantly more acidic than soil at the center of the plot. The pH of the edge had a much larger range (4.6-6.5) while the pH in the center was between pH 6-6.5. This supports the idea that soil pH plays a role in ojo de gallo distribution since ojo de gallo was observed in higher amounts by the edge and decreased with distance further into the plot like found in Peterson (2010). I assume that the edge has a high pH because it has higher diversity of plants surrounding and more leaf litter and than the center. Studies have shown that with increased diversity of trees in the forest, soil acidity and cation cycling can be altered (Finzi *et al.* 1998). Because of the higher diversity of trees on the edge there may be an imbalance of acid vs. base cation availability for uptake and mess with soil acidity. Increase leaf litter or debris can lead to slower decomposition time, which has been linked to having more acidic soil environments (Finzi *et al.* 1998). Since farmers often leave leaf debris throughout the plot it may explain why the pH of the plots was over all 6.5. Further studies could collect leaf litter and measure the effect on increase leaf litter amounts on pH. This could also help determine if the center has a higher pH because of less dense leaf litter or if it is because the center is only coffee plants and a few bananas plants leading to less diversity than the edge or because of increase sunlight.

It is particularly interesting that the pH of the field never reaches 7, as optimal pH for plant growth of coffee plants is usually between pH 6.5-7 (Cardoso 2006). Results from the common garden seedling experiment support this because the control treatment (pH 7) provided the best environment for growth. Diameter increased the most in the control and mycorrhizae per root was the highest, although not significantly. Having a higher diameter growth implies that the seedlings were thriving in neutral pH while the acid or base treatment was harsher so the seedling put its energy into height growth rather than increasing its diameter. The trend of more mycorrhizae in the control treatment also support that pH 7 is optimal. Number of mycorrhizae in the control sample doubled over the month from 20-40 mycorrhizae/root while acid and base had higher variation in amounts. One sample in acid increased from 16-52 giving an increase of 35 mycorrhizae/root but other samples from acid only increase by 4 mycorrhizae/root. Similar occurrence happened in the base treatment where seedlings increased from 13-32 in one samples but only 13-17 in another. This can be explained by the simple fact that altered soils had a difficult time adjusting to the desired pH because the chemicals took a while to activate. The acid treatment had the longest adjustment time changing from pH 7 to 6 and finally 5.5 over the month. This may be the reason that the acidic treatment did not have a huge effect on seedling height and mycorrhizae amounts compared to the others. The large standard deviations are most likely because some parts of the soil were more acidic or basic than others allowing for some of the roots to have optimal pH 7 soil available to grow in.

Since the environment in the center was not as acidic as the edge, mycorrhizae amounts should be higher. Graw (1979) observed a significant increase in root mycorrhizae in less acid soils with pH 6.0 than in soils of pH 4.0. I observed no significant statistical difference in the number of mycorrhizae present in the roots with increasing pH in the plot. However, there was a slight trend that more mychorrhizae was present above pH 6. The highest number of mycorrhizae per root was observed in pH 6.5 again implying neutral pH is best. Kopp (2010) also carried out his experiment from April to May and only found slightly more mycorrhizae in the center of the plots. It would be interesting to look at mycorrhizae amounts in the rainy season when ojo de gallo is actually present on the plants to observe the direct effects on the pH and mychorrhizae.

The results of this study suggest that neutral pH has the potential for mycorrhizae presence to be higher along with better growth of seedlings. Farmers can take this as evidence that to maximize the fitness of their coffee plants it is best to make the pH of the plots closer to neutral and even throughout with light debris cover. This will potentially help minimized the infestation of ojo de gallo while increasing mycorrhizae. An occasional application of a liming agent is a very simple and not too expensive way to maintain a high pH (Conyers *et al.* 1995), which could potentially create a larger output of coffee creating a larger profit for the farmer.

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