

ROOT NODULATION IN THE WETLAND TREE *PTEROCARPUS OFFICINALIS* ALONG COASTAL AND MONTANE SYSTEMS OF NORTHEAST OF PUERTO RICO

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ABSTRACT

In Puerto Rico, brackish water wetlands were dominated by *Pterocarpus officinalis* previous to extensive deforestation due to agriculture. Today remnant wetlands are limited to small areas that are threatened by rise in sea level. We examined the root nodules of *P. officinalis* in montane and coastal sites and at 0, 10, 20 cm from the surface to determine if site conditions relate to the formation and characteristics of nitrogen fixing nodules. We found no significant difference in nodule quantity between montane and coastal ecosystems. However, although all the montane sites experienced nodule growth, there were sites in the coast that did not. The montane sites had 96.3 percent of nodules active, whereas the coastal had 82.2 percent. Nodule mass and diameter were significantly larger near the surface of the soil and in montane sites. More research is needed to understand if nitrogen fixing and accumulation rates also differ between the coastal and montane sites where *P. officinalis* occurs. Studying the presence, distribution, and potential environmental conditions that are related to nodule formation will be helpful in developing management strategies and conservation initiatives to maintain this species and the wetland forests in which it occurs.

RESUMEN

Previo a la deforestación extensa debido a la agricultura, los humedales de agua salobre en Puerto Rico estaban dominados por *Pterocarpus officinalis*. Hoy en día estos humedales remanentes están limitados a pequeñas áreas de bosque amenazadas por el alza en el nivel del mar. Examinamos los nódulos fijadores de nitrógeno en las raíces de *P. officinalis* a 0, 10 y 20 cm de la superficie en sitios ubicados en la zona de la costa y la montaña para determinar si las características de los nódulos están relacionadas a las condiciones ambientales de los sitios donde ocurren. No encontramos diferencias significativas en la cantidad de nódulos entre los sitios de la costa y la montaña. Sin embargo, en todos los sitios muestreados en las montañas había nódulos, mientras que hubo sitios en la costa donde no se encontraron nódulos. La biomasa y el diámetro de los nódulos eran mayores cerca de la superficie y en los sitios de montaña. En los sitios de montaña un 96.3 por ciento de los nódulos encontrados estaban activos, mientras que en la costa fue 82.2 por ciento. Ahora es necesario llevar a cabo mas investigación para entender si la tasa de fijación y/o la acumulación de nitrógeno también difiere entre los sitios de la costa y la montaña donde *P. officinalis* está presente. Estudiar las características de *P. officinalis* a lo largo de su distribución en el paisaje es necesario para desarrollar estrategias de manejo e iniciativas de conservación para mantener a esta especie y los bosques de humedal donde habita.

INTRODUCTION

Wetlands are areas that flood routinely and their vegetation has evolved to survive those conditions (Brinson *et al.* 1981). Wetlands can be in coastal areas with salinity changes associated with tides and rises in sea level. The salinity ecotone determines plant growth and can affect the production of fruits and flowers (Eusse and Aide 1999). The biggest difference between freshwater montane and coastal wetlands is the hydroperiod (Brinson *et al.* 1981). The hydroperiod is the timing and duration of flooded conditions experienced by a wetland, and it is also influenced by the microtopography of the landscape, as with the presence of valleys and depressions (Lugo and Brown 1988). *Pterocarpus officinalis*, is a tree native to Puerto Rico that forms forested wetlands in brackish water with lower salinities than those tolerated by mangroves (Cintrón 1983, Weaver 2000).

In Puerto Rico, freshwater wetlands dominated by *P. officinalis* were common in the coastal plains before deforestation reduced them into small patches throughout the island near the extreme of their physiological tolerance (Cintrón 1983, Eusse and Aide 1999). Like many legumes, *P. officinalis* produces nodules that fix nitrogen, especially in flooded conditions (Fougnies *et al.* 2007), and has the capacity to lower its contact to high salinity by growing large buttresses and extending lateral roots (Loveless 1983). The buttress root system of this tree may reach 5 to 6 m in width and 5 meters in height (Lugo and Brown 1988). This buttress root system strategy allows *P. officinalis* to grow in various levels of salinity.

Wetland forests of *P. officinalis* have been found to store more organic matter in the soil than other terrestrial forests, and have an uneven canopy ranging from 17 to 30 m in height depending on its location (Álvarez López 1990). There are differences between montane (riverine or riparian) and coastal *P. officinalis* forests. In montane forests there is higher root biomass average than in coastal forests (Cintrón 1983, Álvarez López 1990). However, these studies did not describe root nodule presence in the root biomass.

In order to host nitrogen fixing bacteria in its roots *P. officinalis* produces nodules (Loveless 1983). A few species of bacteria have an enzyme that enables them to convert the nitrogen gas (a triple bond that is highly unreactive) into a more reactive and biologically useful substance through nitrogen fixation. However, bacteria such as *Rhizobium spp.* alone cannot fix nitrogen without legumes. Nitrogen fixation is important because it releases nitrogen in the form of ammonia and ammonium ions, which are then converted into amino acids and plant proteins. It is an essential nutrient for plant life especially in flooded wetlands where soils are under anoxic conditions (Loveless 1983, Fougnies *et al.* 2007).

Although *P. officinalis* forms these nitrogen fixing nodules, little is known about the distribution and abundance of this phenomenon along the continuum of locations where the tree occurs in Puerto Rico. Does *P. officinalis* produce nodules in all the environmental conditions where it grows? Do nodules occur at a particular depth from the surface? Do nodule characteristics such as mass and diameter differ between ecosystems (montane and coastal) and soil depths?

STUDY SITES

The study was conducted in coastal and montane regions of northeastern Puerto Rico. We sampled at three sites in each region (Table 1). The coastal region included the Natural Reserve of the Northeast Ecological Corridor (NEC), which is a 13 km² diverse tract of land between the towns of Luquillo and Fajardo, which includes forest, wetlands, beach, coral communities, and a bioluminescent lagoon (DRNA 2007, Junta de Planificación 2008). Samples were taken near the coastal red mangroves, in what was an active meander of the Juan Martín River, which is now a stagnant pool due to topographical alterations of the site by landfill and road construction (personal observation of the authors and accounts by a local fisherman). Other samples from the NEC were collected in a second site located near the San Miguel entrance, near the main road intersection

TABLE 1. Collection sites for *Pterocarpus officinalis* root nodules in northeast Puerto Rico.

	Site Code	Description	Location
Montane	Mameyes	At the end of the Bisley Experimental Watershed trail, Río Mameyes	Barrio Sabana, Luquillo
	Puente Roto	Intersection of road 988 and Río Mameyes	Barrio Sabana, Luquillo
	Espíritu Santo	Río Espíritu Santo along road 186, below Field Station	Sector El Verde, Río Grande
Coastal	Sabana Seca H	High salinity, adjacent to to <i>Langularia racemosa</i>	North Tract, Naval Base, Sabana Seca, Toa Baja
	Sabana Seca L	Low salinity, close to <i>Typha dominguensis</i>	North Tract, Naval Base, Sabana Seca, Toa Baja
	NEC	San Miguel-las Paulinas gate entrance, Río Juan Martín, Fajardo	Northeast Ecological Corridor

with the flowing and meandering section of the Juan Martín River. The third coastal site was in the North Tract of the Naval Security Group Activity, in Sabana Seca, Toa Baja, where there are *P. officinalis* and white mangrove (*Laguncularia racemosa*) forests. This Sabana Seca site was sampled at the area where the highest salinity levels occur, close to the white mangroves, and 200 m south at a lower salinity area close to *Typha dominguensis*. This *P. officinalis* forest has been described in detail by Eusse and Aide (1999) and Rivera Ocasio *et al.* (2007). The three coastal sites were at sea level, had few topographical differences, and were subject to seasonal changes in water level and in salinity levels.

In the montane region, at all three sites there was a continuous flow of water in the adjacent rivers. The montane sites were all located adjacent to or within the Luquillo Experimental Forest (LEF) at elevations lower than 500 m. The Mameyes site was a forest stand dominated by *P. officinalis*, and is described in detail by Álvarez López (1990). The Puente Roto and Espíritu Santo sites were tabonuco type forest with patches of individual *P. officinalis* trees. The Mameyes and Puente Roto sites were

along the Mameyes River, and the third site along the Espíritu Santo River (Table 1). The montane sites were similar in elevation.

METHODS

Field Sampling

Stratified samples of soil were collected using a bulk density soil corer (Model # HM-930, Gilson Company Inc.) at 0, 10, and 20 cm from the surface. Sample volumes ranged from 25 to 30 cm³ obtained near the buttress roots of adult *P. officinalis* trees, and trying to avoid roots from other vegetation. The systematic sampling design was based on Álvarez López (1990) and Saur *et al.* (1998) findings that most root nodules in *P. officinalis* occur close to the trunk and buttresses, and on average the root biomass and root nodules decline dramatically after 0.3 meters from the surface. The soil samples were kept refrigerated until processed in the laboratory.

Laboratory Sampling

In the laboratory, soil, roots and nodules were separated. The mass for the soil sample, the roots, rocks, and nodules were obtained using an analytical

balance (Model # E10640 Explorer Ohaus) and diameter for nodules was measured with a caliper (Model #31-415-3 Swiss Made SPI). Each nodule was visually inspected for signs of activity by slicing and inspecting the inside of the nodule for red stain implying the presence of leghemoglobin. All roots were washed with stream water, wet weighed and then placed in drying ovens at 65 C until constant weight was reached. Dry mass was determined for the roots only. All the samples are stored at the Analytical Laboratory of the USDA-FS International Institute of Tropical Forestry for future reference and further analyses.

Data Analysis

Root biomass, along with quantity, diameter, and biomass of nodules were compared among categories of depths from soil surface, and between coastal and montane sites. Statistical analyses of category means was done with an Analysis of Variance (ANOVA) conducted using SAS software (SAS Version 9.1, SAS Institute 2003, Cary, North Carolina, U.S.A.). All statistics were considered significant at a level of 0.05.

RESULTS

Nodule characteristics and root biomass between forests

There was no difference in quantity of nodules between coastal and montane sites (Fig. 1) ($F_{1,63}=1.97$, $p=0.1654$). However, the highest values of nodules per cm^3 of sampled soil were from a coastal site (Fig. 2). The North East Ecological Corridor (NEC) was the coastal site that had the highest nodules per cm^3 but also had a tree without nodule growth; on the other hand, at all montane sites nodule growth was observed. Montane sites had 96.3 percent active nodules while the coastal had 82.2 percent. There were differences found in the characteristics of *P. officinalis* nodules between the coastal and montane sites. Nodule mass differed between the two ecosystems ($F_{1,144}=8.68$, $p=0.0037$), montane sites had heavier nodules ranging from 0.05 to 0.75 g and coastal sites ranged from 0.028 to 0.17 g (Fig. 3). The Mameyes River site had the highest nodule mass while the Sabana Seca site had the lowest ($F_{5,140}=3.95$, $p=0.0022$) (Fig. 4). The same pattern was observed for nodule diameter ($F_{1,144}=5.64$, $p=0.0189$), with greater

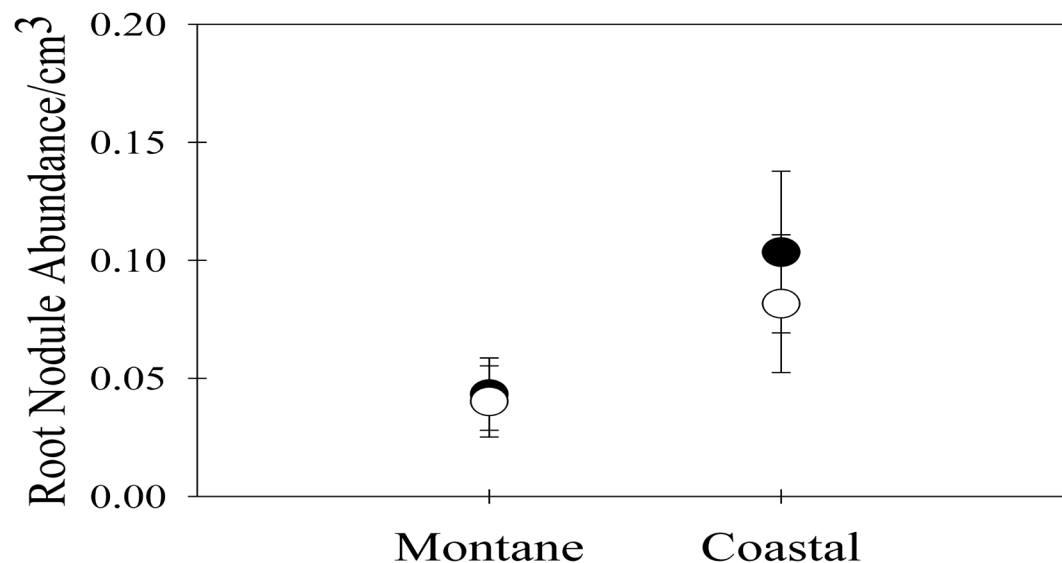


FIGURE 1. Coastal and montane root nodules per cm^3 of sample, both the total and amount active.

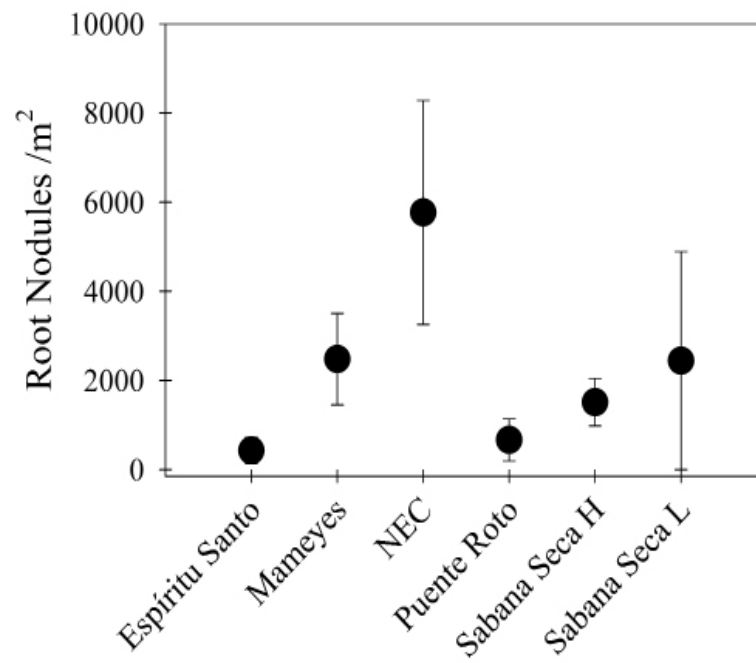
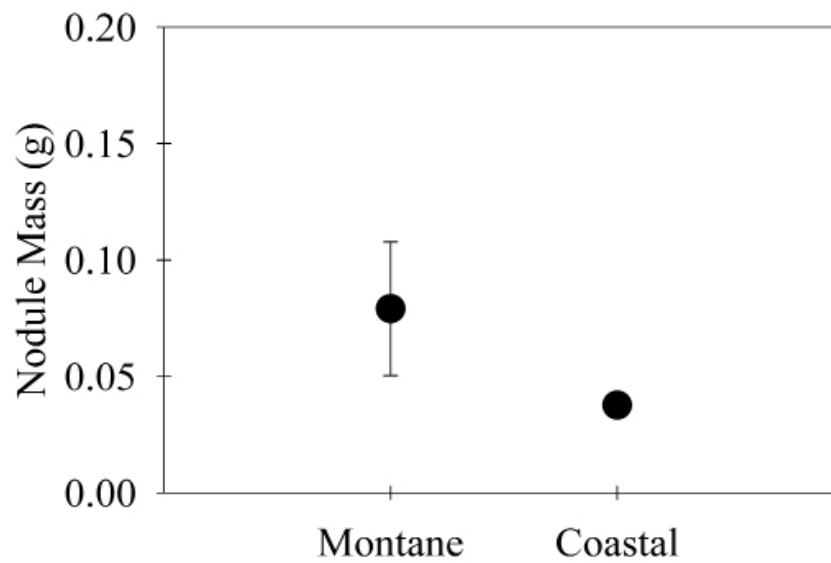
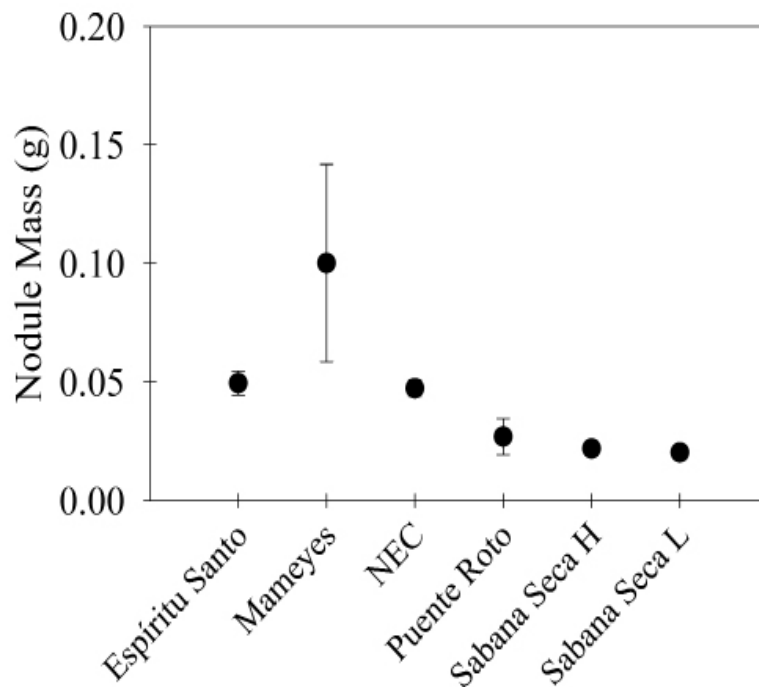
FIGURE 2. Abundance of root nodules per cm^3 from all sites.**FIGURE 3.** Root nodule mass (g) between the montane and coastal ecosystems.

FIGURE 4. Root nodule mass (g) among the sites.



values at montane sites, ranging from 2.2 to 7.9 mm, while coastal sites ranged from 1.6 to 7.0 mm (Fig. 5). Once again, Mameyes River site had the largest diameter of root nodules and Sabana Seca had the lowest ($F_{5, 140} = 7.67$, $p=0.0001$) (Fig. 6). Overall, montane root nodules showed high variation of mass and diameter values, while the coastal sites had much less variation (Fig. 3 and 5).

The biomass of roots differed between sites, with coastal samples having more grams per cm^3 for fresh (wet) roots ($F_{1, 63} = 13.19$, $p=0.0006$) and dry roots ($F_{1, 63} = 4.38$, $p=0.0404$) than montane sites. The montane sites had lower root biomass, and this may be associated with a larger amount of rock mass found in the soil compared with the coastal samples ($F_{1, 61} = 5.69$, $p=0.0201$) (Fig. 7).

Nodules and Roots in Relation to Soil Depth

There was no difference in quantity of nodules among soil depths of the montane and coastal

sites combined ($F_{2, 62} = 1.01$, $p=0.3694$) (Fig. 8). The amount of active nodules also did not show a significant pattern among sampled soil depths. However, there were differences between nodule characteristics among soil depths. Diameter of the root nodules from both montane and coastal sites was higher at the surface and decreased with greater depth (Fig. 9). The coastal sites showed differences in nodule diameter ($F_{2, 116} = 13.32$, $p=0.001$) and nodule mass ($F_{2, 166} = 22.80$, $p=0.0001$) among depths, having larger and heavier nodules at the surface. Montane nodules showed similar patterns, however due to the higher variation there were no differences for diameter ($F_{2, 24} = 1.78$, $p=0.1908$) or mass ($F_{2, 24} = 1.58$, $p=0.2260$) with depth.

The samples had no significant differences in fresh root biomass among soil depths ($F_{2, 62} = 1.58$, $p=0.2150$) (Fig. 10A). However, dry root biomass was different among soil depths ($F_{2, 62} = 3.50$, $p=0.0362$), the surface had the highest values of dry root biomass per cm^3 , but there was no difference

FIGURE 5. Root nodule diameter (mm) between the montane and coastal ecosystems.

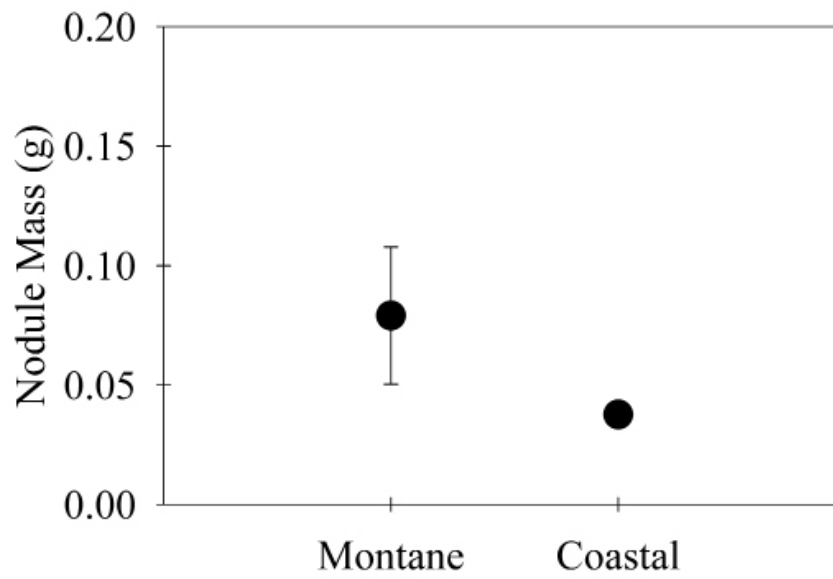


FIGURE 6. Root nodule diameter (mm) along the six sites.

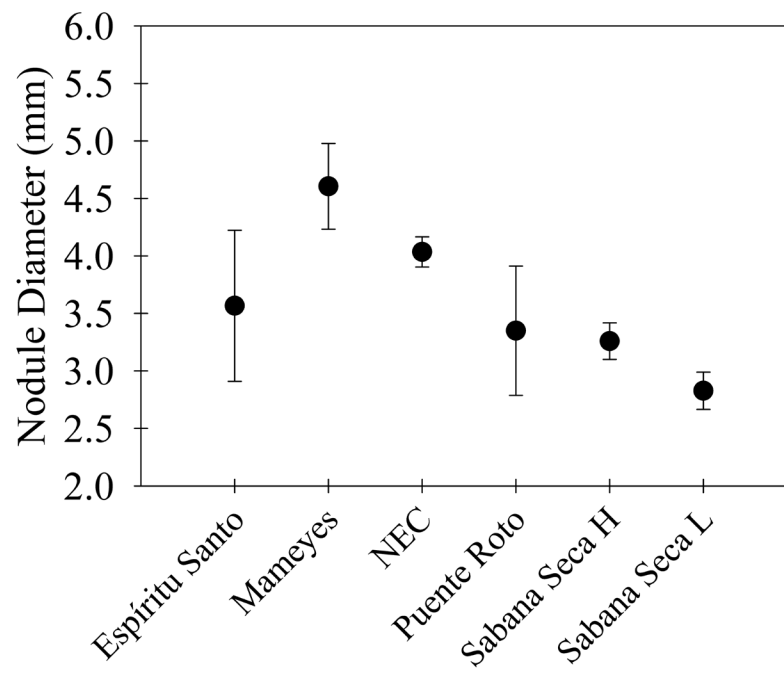


FIGURE 7. Coastal and montane mass (g) of soil sample components of fresh/wet roots in solid circles, dry roots in open circles, and rocks in inverted triangle between montane and coastal ecosystems.

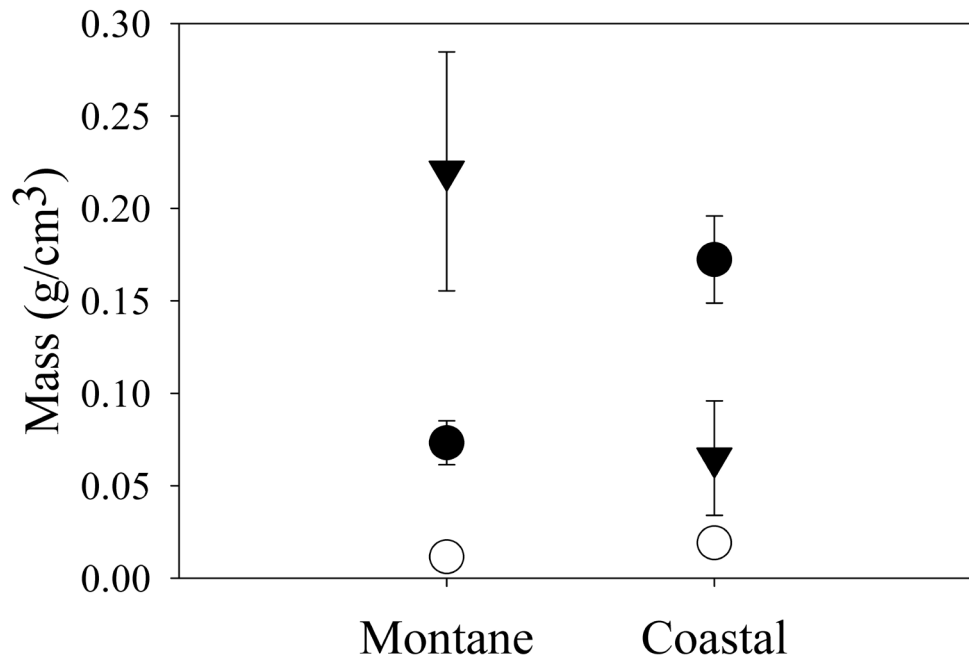
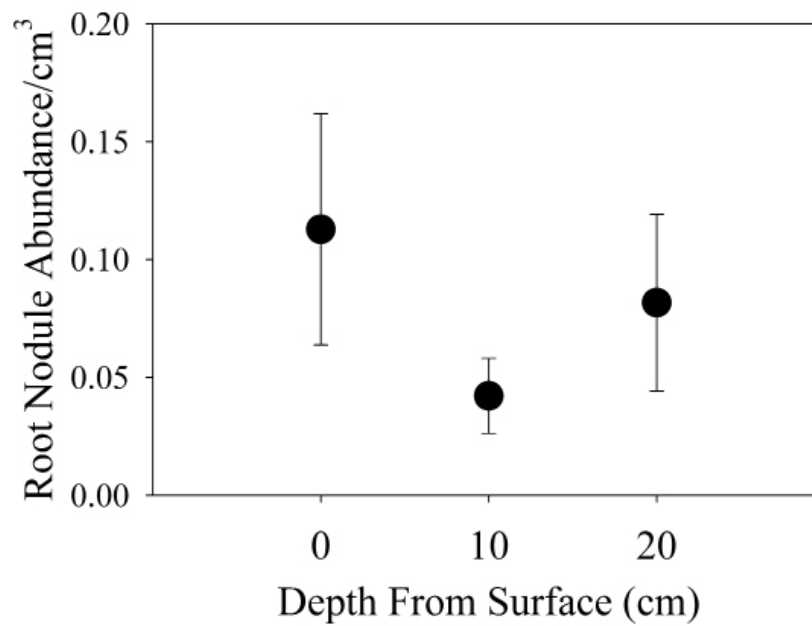


FIGURE 8. Root nodule abundance per cm³ from different depths from surface.



between the samples from 10 cm and 20 cm (Fig. 10B).

DISCUSSION

Montane and coastal forests did not differ in root biomass, but the characteristic *P. officinalis* nodules were affected by the environment where they grew. There appears to be a negative effect on the amount of active nodules and in their size from being in the coastal environment, as there were smaller and less active nodules at coastal sites, and one sample tree had no nodule growth. In contrast, all samples from montane sites had

nodule growth and most nodules were active and larger. We suggest that coastal conditions, such as salinity, may be having the greatest influence on the growth of root nodules. As Medina *et al.* (2007) state, *P. officinalis* may be adapted to grow in saline environments, however it is not a halophyte and will not thrive in these environments, but can survive. Eusse and Aide (1999) found that flower and fruit growth in *P. officinalis* was roughly 10 times greater in low to intermediate salinity than in high salinity. Additionally, *P. officinalis*' seedlings had higher mortality rates and slower growth in 5 percent and 10 percent salinity levels than in 0 percent (Rivera Ocasio *et al.* 2007). In our study, we observed a

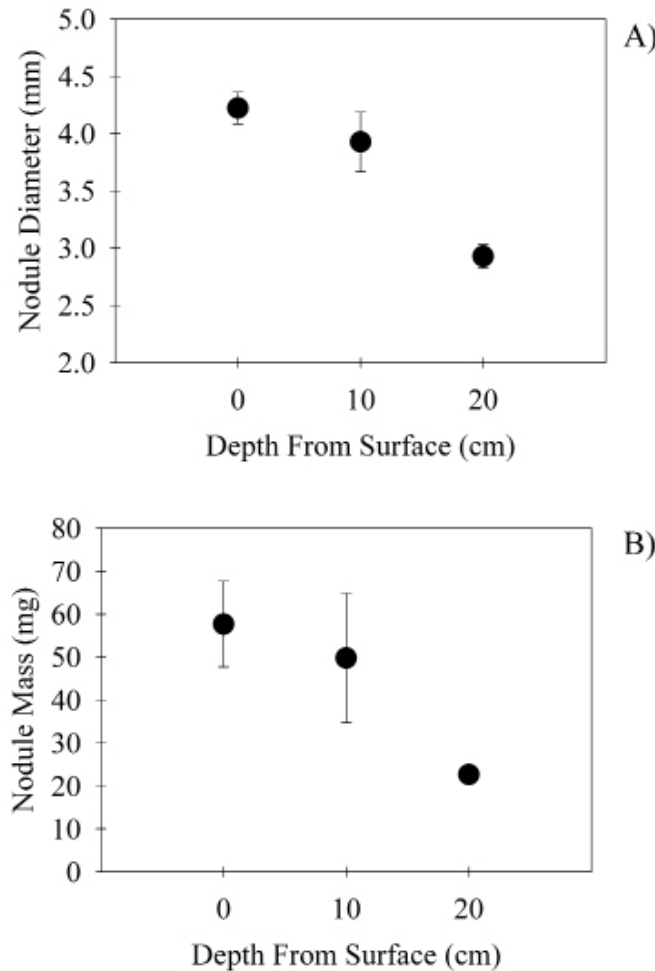


FIGURE 9. Mean root nodule a) diameter (mm) and b) mass (g) and depth from the surface.

coastal site without nodule growth and more non-active nodules, which affect the nitrogen intake of *P. officinalis*.

Coastal sites had greater root biomass than the montane ones. However, our technique was biased in its inability to collect larger roots, which were often seen in montane sites. In addition, more rocks were found in the montane sites, which also decreased our opportunity to sample larger roots. Further studies should be done controlling the amount of root biomass along the montane and coastal sites. The higher amounts of nodules found near the surface of *P. officinalis* roots may help with obtaining nitrogen gas from the environment, and have been also observed in other coastal site studies (Saur *et al.* 1998). Furthermore, with more root biomass near the surface there are more opportunities for root nodule growth at the soil

surface. Because we found no difference among soil depths and nodule activity, we suggest soil depth (less than 20 cm from surface) is not an obstacle for nodules to be active.

An increase in sea level as well as groundwater pumping and land use change that alters flow of freshwater may increase salinity in many of these remnant forests which may further affect the population and growth of this species (Eusse and Aide 1999). *Pterocarpus officinalis* plays a major role in the environment where it grows providing structure and resources for associated wetland flora and fauna (Cintrón 1983). Studying the presence, distribution and potential environmental conditions that are related to nodule formation will be helpful in developing management strategies and conservation goals to maintain this species and the wetland forests in which it occurs.

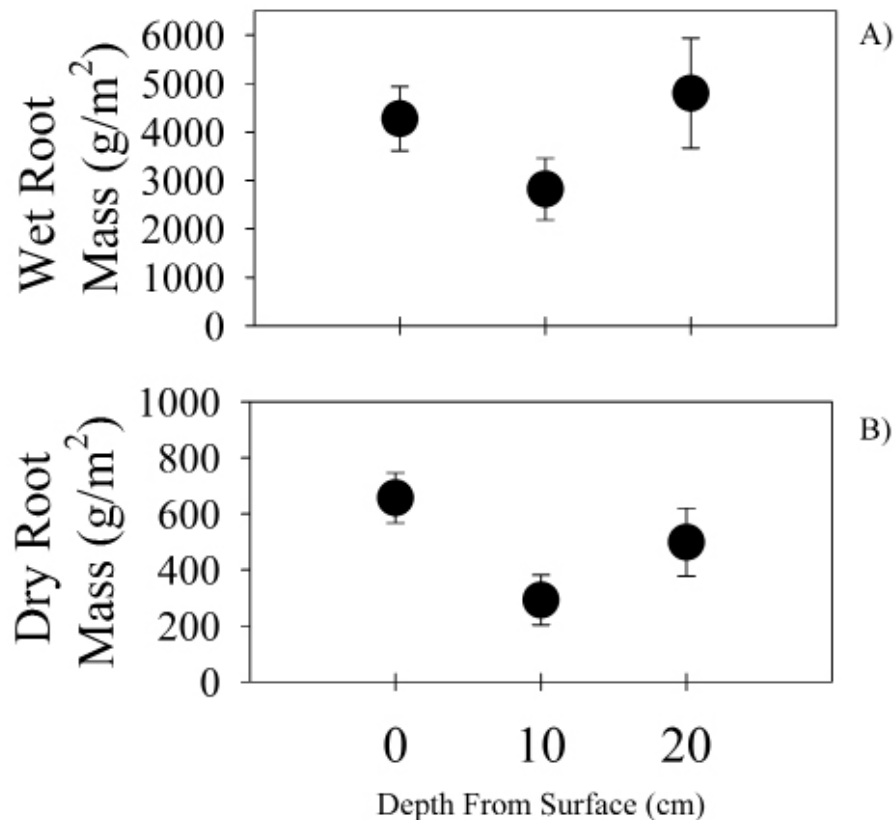


FIGURE 10. Mean a) wet root mass and b) dry root mass (g/m²) and depth from the surface.

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