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Synergic Effect of *Mucuna pruriens* var. *Utilis* (Fabaceae) and *Pontoscolex corethrurus* (Oligochaeta, Glossoscolecidae) on the Growth of *Quercus insignis* (Fagaceae) Seedlings, a Native Species of the Mexican Cloud Forest

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Propagation of native species in local nurseries is an important activity in reforestation and forest restoration programs. A requisite for successful plantation is that nursery produced plants are of a size and quality that allows optimal establishment under field conditions. Manipulation of edaphic processes through the combined use of the earthworm *Pontoscolex corethrurus*, *Mucuna pruriens* and inorganic fertilizers may promote faster biomass gain. This study assessed the activity of *P. corethrurus*, its association with *M. pruriens* (green manure) and inorganic fertilizers, on the growth of *Quercus insignis* seedlings under greenhouse conditions. Measured variables were basal diameter, height, biomass and foliar nitrogen content. Growth rates of basal diameter ($F = 5.33$; $P < 0.0001$) and height ($F = 2.84$; $P < 0.0087$) were significantly greater in the treatment of *P. corethrurus*-*M. pruriens*-inorganic fertilizer, relative to the control. Also, leaf biomass and total biomass of the seedlings were greater in the treatment of *P. corethrurus*-fertilizer ($F = 2.32$; $P < 0.0290$, $F = 3.71$; $P < 0.0011$, respectively) compared to the control treatment. Foliar nitrogen content was significantly higher ($F = 2.54$; $P < 0.01742$) in the treatment of *P. corethrurus*-inorganic fertilizer. Incorporating biological soil management techniques in propagation of native species is a good choice to assist reforestation and forest restoration.

Keywords: Nursery; Oak Seedlings; Earthworms; Green Manure; Inorganic Fertilizers; Plant Propagation

Introduction

Cloud forest hosts around 6790 plant species (Villaseñor, 2010). Regrettably, this diversity is in decline due to several factors of disturbance, most of which are anthropogenic (Ramírez-Marcial et al., 2001; Lamb et al., 2005). This situation affects the population dynamics of plant species that are endangered, threatened or susceptible to forest fragmentation (Saunders et al., 1991; Cayuela et al., 2006). This is the case with *Quercus insignis*, a species native to this ecosystem. Despite a broad geographic range that spreads from eastern-central Mexico to Costa Rica, this species is a narrow habitat-specialist and is highly susceptible to disturbance (Valencia, 2004). It was included in the Red List of Oaks under the endangered category (Oldfield & Eastwood, 2007). More recently, and due to the accelerated destruction of its habitat, this species was re-classified to the Critically Endangered category in the Red List of Mexican cloud forest trees (González-Espinosa et al., 2011).

A potential mechanism to reverse this situation and restore degraded areas is the establishment of forest plantations, or the reintroduction of locally extinct species (Vázquez-Yanes & Cervantes, 1993; Meli, 2003; Pedraza & Williams-Linera, 2003; Lamb et al., 2005). This process must be complemented by the production of native species in nurseries (Benítez et al., 2002). At present, there is nil to very low availability of native species in local nurseries in Mexico, and the few plants that are produced are lacking in both quality and size (Arriaga et al., 1994; Meza-Sánchez et al., 2009). Moreover, the use of black plastic bags with repeated watering causes the soil compaction and poor root development in the seedlings. Novel propagation techniques are therefore required in order to improve seedling quality in nurseries (Benítez et al., 2002), as well as complementary strategies with which to facilitate the future field establishment in the field.

In nurseries that are devoted to the massive production of native forest species, common use is made of organic remains,

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sand, fertilizers and pesticides. However, the use of green manure could become an important source of nutrients (mainly nitrogen) to increase soil fertility (Smyth et al., 1991; Blanchart et al., 2006). The legume *Mucuna pruriens* var. *utilis* has been used as a cover crop for these purposes (Hulugalle et al., 1986; Smyth et al., 1991; Buckles, 1995; Ortiz-Ceballos et al., 2012) and its use has also enabled the biological control of weeds, pests and diseases, while it acts to influence the composition and activity of the soil biota, particularly the earthworms. All of these effects may indirectly favor plant growth (Ortiz-Ceballos & Fragoso, 2004; Blanchart et al., 2006).

The soil biota is known to regulate the availability of nutrients necessary for plant growth and development (Wardle et al., 2004). There is also evidence of the important role of earthworms in the functioning of natural- and agro-ecosystems through the conservation of soil fertility (Wardle, 2002; Bhadauria, 2010) and enhancement of plant growth (Lee, 1985; Haimi & Einbork, 1992; Pashanasi et al., 1992; Edwards & Bohlen, 1996; Doube et al., 1997; Scheu, 2003; Ortiz-Ceballos et al., 2007). However, most information published to date on plant-earthworm interactions is derived from studies based on domesticated plants (Edwards & Bater, 1992; Brown et al., 1999; Scheu, 2003; Ortiz-Ceballos & Fragoso, 2004). It is therefore important to examine the influence of earthworms on plants from natural environments (Scheu, 2003), such as cloud forest. The earthworm *Pontoscolex corethrurus* (Müller, 1857) is a peregrine species of the Glossoscolecidae family and native to the Neotropics. It is an endogeic earthworm with a broad environmental tolerance that occurs in various habitats and soil types (Lavelle et al., 1987; Lee, 1985; Fragoso et al., 1999; Buch et al., 2011), including cloud forest (Fragoso et al., 1999). This species plays a crucial role in organic matter decomposition as well as the mineralization of nitrogen and phosphorous (Barois et al., 1999; Bhadauria & Saxena, 2010) and has been successfully used in bio-fertilization and bio-stimulation techniques, producing significant positive effects on crop yields (Lavelle & Pashanasi, 1989; López-Hernández et al., 1993; Senapati et al., 1999).

Obtaining information on the interaction between *P. corethrurus* and native cloud forest plant species in general and *Q. insignis* in particular may be of great relevance, as this can allow us to develop new practices aimed at supplementing and reinforcing the management and propagation of native plant species within conservation and restoration programs for this mountain ecosystem. The goal of this study was to evaluate integrated soil fertility management, *i.e.*, how the combination of biomass (*Mucuna pruriens* var. *utilis*), inorganic fertilizer, and earthworms could impact the soil fertility as well as the growth and leaf nitrogen content of *Quercus insignis* seedlings under nursery conditions.

Methods

Study Site and Soil

The study was conducted in the greenhouse and laboratory of the Instituto de Biotecnología y Ecología Aplicada (INBIOTECA) in the center of Veracruz State, a region of Mexico that features cloud forest. A total of 200 kg of soil was collected to a depth of 40 cm from the Plan de San Antonio cloud forest (19˚26'N, 96˚59'W; 1430 m elevation), located 20 km from the town of Coatepec city. Soil samples were sealed in plastic bags and transported to the INBIOTECA greenhouse where they were air-dried at ambient temperature. The physical and chemical soil properties of the collected soils were; pH 5.7, organic matter content 16.6%, total nitrogen 0.68%, 0.32 $cmol \cdot kg^{-1}$ K, 0.4 $cmol \cdot kg^{-1}$ Ca, 1.7 $cmol \cdot kg^{-1}$ Mg, 26.9 $cmol \cdot kg^{-1}$ cation exchange capacity, 54% moisture content (soil collected), 17% clay, 32% silt, 49% sand.

Earthworms, Green Manure and Oak Seedlings

Juvenile individuals of *P. corethrurus* used in this study corresponded to the first generation obtained under laboratory conditions from earthworms collected in a secondary cloud forest. *Pontoscolex corethrurus* is an exotic species that has been reported in Mexican cloud forests and is also commonly found in areas that have been transformed from cloud forest to pastureland or crops, such as maize or beans, among others (Fragoso et al., 1999; Fragoso, 2001).

Juvenile *P. corethrurus* earthworms were raised following the protocol of Ortiz-Ceballos et al. (2005): (1) two adult earthworms, each with a conspicuous clitellum, were placed in plastic boxes (12 × 12 × 8 cm) filled with 300 g of soil, mixed with 3% *Mucuna* and wetted to field capacity (42%). The boxes were incubated at 26˚C ± 2˚C, and the soil replaced and cocoons collected fortnightly. These cocoons were incubated in Petri dishes at 27˚C. In the greenhouse, plants of *M. pruriens* were grown for a period of three months (September - November 2009). The foliage was then collected, dried (63˚C ± 3˚C, 48 h), ground (to 2 mm), and placed in paper bags; total nitrogen content of the *M. pruriens* was determined (2.23%) using the Kjeldahl digestion method following the Mexican Official Norm NOM-021 (SEMARNAT, 2002). Seeds of the studied oak species, *Quercus insignis* were collected in November 2009 from cloud forest fragments located at 19˚12'N, 96˚59'W, at an elevation of 1460 m . This species bears large acorns (5 cm diameter) and its seeds may be classed as recalcitrant. Seeds were placed in nursery beds (1 × 8 m), where they germinated between 15 - 20 days after sowing, presenting a germination rate > 60%.

Experimental Setup

The experiment was conducted in the greenhouse of INBIOTECA from May to November 2010. We used eight treatments: 1) *Pontoscolex* (P); 2) *Pontoscolex-Mucuna* (PM); 3) *Pontoscolex*-fertilizer (PF); 4) *Pontoscolex-Mucuna*-fertilizer (PMF); 5) *Mucuna* (M); 6) fertilizer (F); 7) *Mucuna*-fertilizer (MF); 8) Control (C) [only soil]. There were 20 replicates (15 × 25 cm plastic containers) by treatment. The containers were filled with 540 g of dry soil that was then wetted to field capacity (43%), and placed on a metallic table. One day later, an 8 - 12 day old *Q. insignis* seedling of approximate height 15 cm was planted in each container. One week later, two juvenile *P. corethrurus* earthworms (106 ± 12 mg) were added to the appropriate treatments, and *Mucuna* foliage was added to the soil surface (8 g; 2.23 N%) (nitrogen content of *Mucuna* is equivalent to that of the applied inorganic fertilizer), as well as the slow-release fertilizer (ammonium nitrate [0.7 g; 26 N 13 S%]) according to the requirements of each treatment. Seedlings were grown for a period of 180 days and watered weekly to maintain the soil moisture at field capacity (43%). Mean temperature in the greenhouse was 25.6˚C ± 4.3˚C, and weeds were manually removed from the soil. During the experiment, aphids were found on the back of some leaves, these were controlled manually and no pesticide was applied.

We recorded seedling height and basal diameter monthly. A destructive harvest of all plants from all treatments was carried out 180 days after the start of the experiment. For each plant, the biomass weight was calculated (dried 65˚C ± 3˚C; 72 h) in roots, stem and leaves. To determine leaf nitrogen content a sample of the leaves was taken for each treatment and nitrogen content was determined with the Kjeldahl digestion method, following the Mexican Official Norm NOM-021 (SEMARNAT, 2002). At the end of the study, the number of earthworms and cocoons in each treatment was recorded.

Statistical Analysis

Analysis of variance (ANOVA) was performed to compare between treatments for each of the following variables: a) growth rate in height, b) growth rate in basal diameter, c) total and component-specific dry biomass, and d) total foliar nitrogen content. When the ANOVA yielded a significant result, means were compared with a Tukey multiple-comparison test. Prior to the ANOVA, numeric values were transformed to natural logarithm, the normality and homogeneity of variance were fulfilled. Growth rate (T_C) was estimated with the following equation:

$$T_C = \left(\ln C_2 - \ln C_1\right)/t$$

where C_2 is final seedling height or diameter, C_1 is initial seedling height (cm) or diameter (mm), and t is elapsed time (months). In addition, biomass allocation to leaves (L) was assessed relative to root biomass (R), and expressed as the L/R ratio between treatments. Percentage values of total nitrogen content in the leaves were also compared between treatments with a Tukey test (GLM Proc, SAS 9.2; SAS Institute Inc., 2009).

Results

Growth of *Quercus insignis* Seedlings

Basal diameter growth rate of the *Q. insignis* was significantly higher in the treatment PMF ($F = 5.33$; $P < 0.0001$) than in all the other treatments (**Figure 1**).

Regarding plant height, significant differences were found ($F = 2.84$; $P < 0.0087$) among the treatments PMF, fertilizer (F) and the control treatment (C) (**Figure 2**). However this trait did not differ significantly between the treatments *P. corethrurus-M. Pruriens-fertilizer* (PMF), *P. corethrurus*-fertilizer (PF) and *M. pruriens*-fertilizer (MF).

Total Leaf Nitrogen

Total foliar nitrogen content in *Q. insignis* seedlings was significantly different between treatments ($F = 2.54$; $P < 0.01742$). Values of this trait were significantly higher in the treatment PF compared to the other treatments. It is noteworthy that the *Q. insignis* plants that grew in the control treatment (soil only) presented the lowest values of foliar nitrogen, as is shown in **Figure 3**.

Biomass in *Q. insignis* Seedlings

Significant differences between treatments ($F = 2.32$; $P < 0.0290$) were recorded for leaf biomass values in *Q. insignis*. This was particularly noteworthy in the contrast between the *P.*

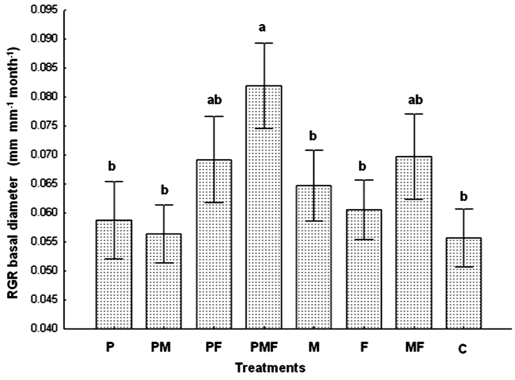

Figure 1.
Effects of eight treatments on growth of *Quercus insignis* seedlings. Abbreviations: *P. corethrurus* (P), *P. corethrurus-M. pruriens* (PM), *P. corethrurus*-fertilizer (PF), *P. corethrurus-M. pruriens*-fertilizer (PMF), *M. pruriens* (M), fertilizer (F), *M. pruriens*-fertilizer (MF), Control (C). Mean values of seedling basal diameter are shown. Vertical lines represent standard errors. Different letters indicate significant differences between treatments.

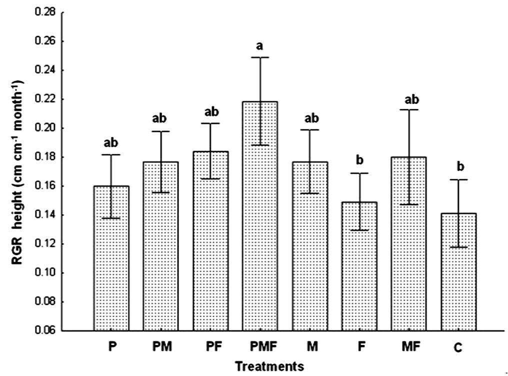

Figure 2.
Effects of eight treatments on growth of *Quercus insignis* seedlings. Mean values of seedling height are shown. Vertical lines represent standard errors. Different letters indicate significant differences between treatments.

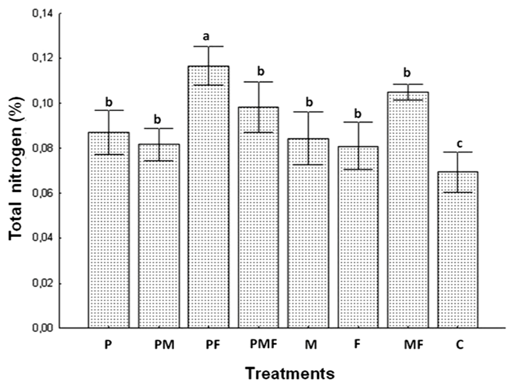

Figure 3.
Effects of treatments with *P. corethrurus* (P), *M. pruriens* (M) and fertilizer (F), and combinations of these, on total leaf nitrogen content in seedlings of *Quercus insignis*. Values are means for plants grown in each treatment. Vertical lines represent standard errors. Different letters indicate significant differences between treatments.

corethrurus-fertilizer (PF) treatment and the control. However the root and shoot biomass *Q*. *insignis* seedlings did not differ significantly between treatments (see **Table 1**).

Total biomass was similar between treatments, and significant differences ($F = 3.71$; $P < 0.0011$) were only found among the treatments PF, F and the control (**Table 1**). The leaf/root biomass ratio was >1 in all treatments, indicating a larger resource allocation to photosynthetic tissues in *Q*. *insignis* seedlings, regardless of treatment. No significant differences were observed between treatments in this trait.

Discussion

It is widely accepted that the presence of earthworms is generally beneficial to plant growth (Edwards & Bohlen, 1996; Bohlen et al., 2002, 2004; Scheu, 2003; Eisenhauer et al., 2009). Similarly, the addition of organic matter to the soil, mediated by leguminous plants such as *M*. *pruriens*, is known to provide nutrients such as nitrogen, and to improve soil fertility and thus increase plant productivity (Becker et al., 1995; Konboon et al., 2000). In our study, the treatments with *P*. *corethrurus*, *M*. *pruriens*, inorganic fertilizer and the interaction between these, presented similar values in terms of growth variables. However, significantly higher values in all plant growth variables were found between *P*. *corethrurus*-*M*. *pruriens*-fertilizer (PMF) treatment versus the control treatment. Brown et al. (2004) and Scheu (2003) reported increases in the growth of several cultivated plant species, mostly cereals and grasses, in the presence of earthworms. Likewise, other studies have led to the conclusion that the use of *Mucuna pruriens* favors the growth and productivity of *Zea mays* (Ile et al., 1996; Buckles & Triomphe, 1999; Eilitta et al., 2003).

Our results indicate that the combination of *P*. *corethrurus*-*M*. *pruriens*-fertilizer (PMF) positively affects the growth in height of *Q*. *insignis* plants. However, the combination *P*. *corethrurus*-*M*. *pruriens* (PM) and *P*. *corethrurus*-fertilizer (PF) can be equally effective in producing increased height, an aspect which is of vital importance to tree species that are propagated in greenhouses. The use of inorganic fertilizers may potentially induce variability in the effects of earthworms on plants, which may largely depend on soil nutrient status (Laossi et al., 2010a).

According to our findings, inorganic fertilizer appears to interact synergistically with *P*. *corethrurus* and *M*. *pruriens*, or with *M*. *pruriens* only, promoting the growth of *Q*. *insignis* during early stages of its life cycle. This condition is likely to be the result of the relatively rapid liberation of nitrogen provided by the synthetic fertilizer. If true, this would partly explain the improved plant growth in the treatments with *P*. *corethrurus*, *M*. *pruriens* and synthetic fertilizer, and in combinations of these. The fact that the inorganic fertilizer alone cannot produce a growth increase in these seedlings must not be overlooked.

Increases in stem and root biomass in plants that have been exposed to earthworm activity have been repeatedly documented (Wurst & Jones, 2003); however, other studies have reported contrasting results (Scheu, 2003). Ortiz-Ceballos et al., (2007) observed increased root biomass in plants of *Zea mays* exposed to the combined effects of the activity of the earthworm *Balanteodrilus pearsei* with the leguminous *M*. *pruriens*. Conversely, the presence of *P*. *corethrurus*, and its combination with *M*. *pruriens*, did not significantly affect root and stem biomass in *Q*. *insignis* seedlings. It must be stressed that most studies reporting increases in root and stem biomass have focused on species with short life cycles (annual or biennial) that are often herbaceous, while the effect of this interaction with long-lived trees is less well understood. Moreover, the effect of earthworms on plant growth may also vary depending on the functional traits of the different plant and earthworm species (Brown et al., 2004; Eisenhauer et al., 2009; Laossi et al., 2010b). As a long-lived tree species, *Q*. *insignis* could respond differently in terms of resource allocation to different tissues during a very early phase of its juvenile development. Ultimately, this possibility could obscure the synergistic effect of *P*. *corethrurus* and *M*. *pruriens* on root and shoot biomass allocation in *Q*. *insignis* seedlings at the early stages of their development in a nursery.

Leaf biomass is seldom assessed as a response in studies of plant-earthworm interactions. In our analysis, the comparison of leaf biomass of *Q*. *insignis* revealed a homogeneous increase between the various treatments, with the exception of the control. The uniform increase of leaf biomass among treatments

Table 1.
Effects of treatments with *P*. *corethrurus* (P), *M*. *pruriens* (M) and fertilizer (F), and combinations of these, on the biomass of aboveground and underground components and leaf/root ratio of *Q*. *insignis* seedlings.

Treatments (n)	Root biomass (g dry weight)	Stem biomass	Leaf biomass	Total biomass	Leaf/root ratio
P (17)	2.2 ± 0.9a	2.3 ± 1.2a	3.7 ± 1.7ab	8.3 ± 2.2ab	2.1 ± 1.2a
PM (16)	2.1 ± 0.8a	1.9 ± 0.7a	3.2 ± 1.3ab	7.1 ± 1.5ab	2 ± 1.1a
PF (18)	2 ± 0.9a	2.6 ± 1a	**4.1 ± 1.3a***	**8.8 ± 2.3a****	2.4 ± 1.6a
PMF (16)	1.9 ± 0.9a	1.8 ± 0.8a	3.4 ± 0.7ab	7.0 ± 2.1ab	2.2 ± 1.3a
M (16)	1.8 ± 0.8a	1.8 ± 1a	2.9 ± 1.5ab	6.5 ± 2.2ab	2 ± 1.2a
F (17)	1.9 ± 0.6a	1.7 ± 0.9a	2.7 ± 1.4ab	6.3 ± 2.1b	1.6 ± 0.9a
MF (17)	2 ± 0.6a	1.9 ± 0.8a	2.9 ± 1.2ab	6.7 ± 2.5ab	1.7 ± 1.1a
C (17)	1.7 ± 0.4a	1.8 ± 1a	2.6 ± 1.4b	6.1 ± 1.6b	1.6 ± 1a

Note: Values are means ± SE. Values in parentheses (n) indicate the number of plants per treatment at the end of the experiment. Different letters within the same column indicate significant differences between treatments. *$p < 0.0290$; **$p < 0.0011$.

involving the addition of *P. corethrurus*, *M. pruriens*, the inorganic fertilizer, or combinations of these, suggests that plants tend to accumulate photosynthetic tissue when they have unlimited access to sufficient nutrients provided through biological and/or inorganic fertilization. Moreover, the comparison of total biomass in *Q. insignis* showed similar patterns among those treatments that combined biological fertilization with inorganic fertilizer, the only significant differences being in the treatment that only included fertilizer, and in the control. Laossi et al. (2010a) reported that the interaction of *Lumbricus terrestris* with a fertilizer had significant effects on the total biomass of two herb species, *Poa annua* and *Veronica persica*. Similarly, the increase in total biomass of *Q. insignis* suggests a positive effect of this endogeic earthworm, provided its activity is supplemented by an external source of nitrogen that can be assimilated by the plant in the short term. Thus, the activity of *P. corethrurus*, combined with the use of *M. pruriens* and/or chemical fertilizer positively affects both leaf and total biomass in *Q. insignis* seedling.

Total foliar nitrogen content was significantly higher in the treatment *P. corethrurus*-fertilizer (PF). This result is to be expected given that an increase of leaf nitrogen is related to greater photosynthetic activity, which in theory makes it easier for a plant to access higher quantities of useful resources vital to growth and biomass accumulation in different tissues (Garnier, 1991; Gleeson, 1993; Hikosaka, 2004). Although leaf nitrogen concentration often decreases as plants grow (Gastal & Lemaire, 2002), this does not seem to be the case in *Q. insignis*. This may change, however, later in the life of the plant after its stage transition from sapling to young adult. Plants tend to optimize resources, and they therefore require a balance in the allocation of nutrients, such as nitrogen, in order to maximize growth (Hilbert, 1990; Gleeson, 1993; Göran & Franklin, 2003). However, the effect of earthworms on this plant resource allocation has been poorly documented and understood (Scheu, 2003). In this context, we observed that leaf biomass is generally greater than root biomass in seedlings of *Q. insignis*, regardless of treatment. This situation suggests that the priority for seedlings of this oak species is to produce more photosynthetic tissues, which is logical considering that this is a late successional species that establishes under limited light conditions in its natural habitat.

Conclusion

The integration of a biological component such as the earthworm *P. corethrurus* into soil management, along with use of *M. Pruriens*, could favor the growth of *Q. insignis.* This could be further optimized by the addition of inorganic fertilizer. This combination represents a good option for soil enrichment, diversification of the practices of plant propagation and reduced use of inorganic fertilizers. Day-to-day practices in nurseries include the application of inorganic fertilizers; however, as this study shows, their use does not necessarily translate into improved plant growth. The ultimate goal of biological soil management, including the use of earthworms and green manure, is to promote the integration of edaphic processes such as natural nutrient cycling and degradation to forms that plants can readily assimilate. These processes play decisive roles in the soil dynamics of an ecosystem and promote robust plant development. Full consideration should therefore be given to the integration of soil biological management into the propagation techniques of native species aimed at conserving and restoring fragmented forest ecosystems.

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REFERENCES

Arriaga, V., Cervantes, V., & Vargas-Mena, A. (1994). *Manual de reforestación con especies nativas*. México: Instituto Nacional de Ecología, Secretaría de Desarrollo Social and Universidad Nacional Autónoma de México.

Barois, I., Lavelle, P., Brossard, M., Tondoh, J., Mártinez, M. A., Rossi, J. P., Senapati, B. K., Angeles, A., Fragoso, C., Jiménez, J. J., Decaëns, T., Lattaud, C., Kanyonyo, J., Blanchart, E., Chapuis, L., Brown, G. G., & Moreno, A. (1999). Ecology of earthworm species with large environmental tolerance and/or extended distributions. In: P. Lavelle, L. Brussard, & P. Hendrix (Eds.), *Earthworm management in tropical agroecosystems* (pp. 57-85). Wallingford, UK: CABI.

Becker, M., Ladha, J. K., & Ali, M. (1995). Green manure technology: Potential, usage and limitations. A case study for lowland rice. *Plant Soil, 174,* 181-194. http://dx.doi.org/10.1007/BF00032246

Benítez, G., Equihua, M., & Pulido-Salas, M. T. (2002). Diagnóstico de la situación de los viveros oficiales de Veracruz y su papel para apoyar programas de reforestación y restauración. *Revista Chapingo. Serie Ciencias Forestales y del Ambiente, 8,* 5-12.

Bhadauria, T., & Saxena, K. G. (2010). Role of earthworms in soil fertility maintenance through the production of biogenic structures. *Applied and Environmental Soil Science, 2010,* Article ID: 816073. http://dx.doi.org/10.1155/2010/816073

Blanchart, E., Villenave, C., Viallatoux, A., Barthes, B., Girardin, C., Azontonde, A., & Feller, C. (2006). Long-term effect of a legume cover crop (*Mucuna pruriens* var. *utilis*) on the communities of soil macrofauna and nematofauna, under maize cultivation, in southern Benin. *European Journal of Soil Biology, 42,* 136-144. http://dx.doi.org/10.1016/j.ejsobi.2006.07.018

Bohlen, P. J., Edwards, C.A., Zhang, Q., Parmelee, R. W., & Allen, M. (2002). Indirect effects of earthworms on microbial assimilation of labile carbon. *Applied Soil Ecology, 20,* 255-261. http://dx.doi.org/10.1016/S0929-1393(02)00027-6

Bohlen, P. J., Parmelee, R. W., & Blair, J. M. (2004). Integrating the effects of earthworms on nutrient cycling across spatial and temporal scales. In: C. A. Edwards (Ed.), *Earthworm ecology* (pp. 161-180). Boca Raton, FL: CRC Press. http://dx.doi.org/10.1201/9781420039719.ch9

Brown, G. G., Pashanasi, B., Villenave, C., Patron, J. C., Senapati, B. K., Giri, S,, Barois, I., Lavelle, P., Blanchart, E., Blakemore, R. J., Spain, A. V., & Boyer, J. (1999). Effects of earthworms on plant production in the tropics. In: P. Lavelle, L. Brussard, & P. Hendrix (Eds.), *Earthworm management in tropical agroecosystems* (pp. 87-137). Wallingford, UK: CABI.

Brown, G. G., Edwards, C. A., & Brussaard, L. (2004). How earthworms affect plant growth: burrowing into the mechanisms. In: C. A. Edwards (Ed.), *Earthworm ecology* (pp. 13-49). Boca Raton, FL: CRC Press.

Buch, A. C., Brown, G. G., Niva, C. C., Sautterc, K. D., & Lourenc, L. F. (2011). Life cycle of *Pontoscolex corethrurus* (Müller, 1857) in tropical artificial soil. *Pedobiologia, 54,* S19-S25. http://dx.doi.org/10.1016/j.pedobi.2011.07.007

Buckles, D. (1995). Velvetbean: A new plant with a history. *Economic*

Botany, 49, 151-162. http://dx.doi.org/10.1007/BF02862271

Buckles, D., & Triomphe, B. (1999). Adoption of mucuna in the farming systems of northern Honduras. *Agroforestry Systems, 47,* 67-91. http://dx.doi.org/10.1023/A:1006205702691

Cayuela, L., Golicher, D. J., Benayas, J. M. R., González-Espinosa, M., & Ramírez-Marcial, N. (2006). Fragmentation, disturbance and tree diversity conservation in tropical montane forests. *Journal of Applied Ecology, 43,* 1172-1181. http://dx.doi.org/10.1111/j.1365-2664.2006.01217.x

Doube, B. M., Williams, P. M. L., & Willmott, P. J. (1997). The influence of two species of earthworm (*Aporrectodea trapezoids* and *Aporrectoedea rosea*) on the growth of wheat, barley and faba beans in three soil types in the greenhouse. *Soil Biology and Biochemistry, 29,* 503-509. http://dx.doi.org/10.1016/S0038-0717(96)00037-5

Edwards, C. A., & Bater, J. E. (1992). The use of earthworms in environmental management. *Soil Biology and Biochemistry, 24,* 1683-1689. http://dx.doi.org/10.1016/0038-0717(92)90170-3

Edwards, C. A., & Bohlen, P. (1996). *Biology and ecology of earthworms*. New York: Chapman and Hall.

Eisenhauer, N., Milcu, A., Sabais, A. C. W., & Scheu, S. (2009). Earthworms enhance plant regrowth in a grassland plant diversity gradient. *European Journal of Soil Biology, 45,* 455-458. http://dx.doi.org/10.1016/j.ejsobi.2009.06.001

Eilitta, M., Sollenberger, L. E., Littell, R. C., & Harrington, L. W. (2003). On-farm experiments with maize-mucuna systems in the Los Tuxtlas region of Veracruz, Mexico. I. *Mucuna* biomass and maize grain yield. *Experimental Agriculture, 39,* 5-17. http://dx.doi.org/10.1017/S0014479702001126

Fragoso, C., Kanyonyo, J., Moreno, A., Senapati, B., Blanchart, E., & Rodríguez, C. (1999). A survey of tropical earthworms: Taxonomy, biogeography and environmental plasticity. In: P. Lavelle, L. Brussard, & P. Hendrix (Eds.), *Earthworm management in tropical agroecosystems* (pp. 1-26). Wallingford, UK: CABI.

Fragoso, C. (2001). Las lombrices de tierra de México (Annelida, Oligochaeta): Diversidad, ecología y manejo. *Acta Zoológica Mexicana, 1,* 131-171.

Garnier, E. (1991). Resource capture, biomass allocation and growth in herbaceous plants. *Trends in Ecology & Evolution, 6,* 126-131. http://dx.doi.org/10.1016/0169-5347(91)90091-B

Gastal, F., & Lemaire, G. (2002). N uptake and distribution in crops: An agronomical and ecophysiological perspective. *Journal of Experimental Botany, 53,* 789-799. http://dx.doi.org/10.1093/jexbot/53.370.789

Gleeson, S. K. (1993). Optimization of tissue nitrogen and root-shoot allocation. *Annals of Botany, 71,* 23-31. http://dx.doi.org/10.1006/anbo.1993.1003

González-Espinosa, M., Meave, J. A., Lorea-Hernández, F. G., Ibarra-Manríquez, G., & Newton, A. C. (2011). *The red list of Mexican cloud forest trees*. Cambridge: Fauna and Flora International (FFI).

Göran, I. A., & Franklin, O. (2003). Root: Shoot ratios, optimization and nitrogen productivity. *Annals of Botany, 92,* 795-800. http://dx.doi.org/10.1093/aob/mcg203

Haimi, J., & Einbork, M. (1992). Effects of endogeic earthworms on soil processes and plant growth in coniferous forest soil. *Biology and Fertility of Soils, 13,* 6-10. http://dx.doi.org/10.1007/BF00337230

Hilbert, D. W. (1990). Optimization of plant root: Shoot ratios and internal nitrogen concentration. *Annals of Botany, 66,* 91-99.

Hikosaka, K. (2004). Interspecific difference in the photosynthesis-nitrogen relationship: Patterns, physiological causes, and ecological importance. *Journal of Plant Research, 6,* 481-494. http://dx.doi.org/10.1007/s10265-004-0174-2

Hulugalle, N. R., Lail, R., & Kuile, C. H. H. (1986). Amelioration of soil physical properties by Mucuna after mechanized land clearing of tropical rain forest. *Soil Science, 14,* 219-224. http://dx.doi.org/10.1097/00010694-198603000-00007

Ile, E., Hamadina, M. K., Zufa, K., & Henrot, J. (1996). Note on effects of a *Mucuna pruriens* var. *utilis* crop on the growth of maize (*Zea mays*) on an acid ultisol in southeastern Nigeria. *Field Crops Research, 48,* 135-140. http://dx.doi.org/10.1016/S0378-4290(96)01028-3

Konboon, Y., Blair, G. J., Lefroy, R. D. B., & Whitbread, A. M. (2000). Tracing the nitrogen, sulfur and carbon released from plant residues in a soil/plant system. *Australian Journal of Soil Research, 38,* 699-710. http://dx.doi.org/10.1071/SR99075

Lamb, D., Erskine, P. D., & Parrota, J. A. (2005). Restoration of degraded tropical forests landscapes. *Science, 310,* 1628-1632. http://dx.doi.org/10.1126/science.1111773

Laossi, K. P., Ginot, A., Noguera, D. C., Blouin, M., & Barot, S. (2010a). Earthworm effects on plant growth do not necessarily decrease with soil fertility. *Plant and Soil, 328,* 109-118. http://dx.doi.org/10.1007/s11104-009-0086-y

Laossi, K. P., Noguera, D. C., & Barot, S. (2010b). Earthworm mediated maternal effects on seed germination and seedling growth in three annual plants. *Soil Biology and Biochemistry, 42,* 319-323. http://dx.doi.org/10.1016/j.soilbio.2009.11.010

Lavelle, P., Barois, I., Cruz, I., Fragoso, C., Hernández, A., Pineda, A., & Rangel, P. (1987). Adaptative strategies of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. *Biology and Fertility of Soils, 5,* 188-194. http://dx.doi.org/10.1007/BF00256899

Lavelle, P., & Pashanasi, B. (1989). Soil macrofauna and land management in Peruvian Amazonia (Yurimaguas, Loreto). *Pedobiologia, 33,* 283-291.

Lee, K. E. (1985). *Earthworms: Their ecology and relationships with soils and land use*. Sydney: Academic Press.

López-Hernández, D., Lavelle, P., Fardeau, J. C., & Niño, M. (1993). Phosphorus transformations in two P-sorption contrasting tropical soils during transit through *Pontoscolex corethrurus* (Glossoscolecidae: Oligochaeta). *Soil Biology and Biochemistry, 25,* 789-792. http://dx.doi.org/10.1016/0038-0717(93)90124-T

Meli, P. (2003). Restauración ecológica de bosques tropicales: Veinte años de investigación académica. *Interciencia, 28,* 581-589.

Meza-Sánchez, R., Ruiz-Espinoza, F. H., & Navejas-Jiménez, J. (2009). *Guía para la producción de planta y plantación con especies nativas*. Baja California Sur: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP).

Müller, F. (1857). II. Description of a new species of earthworm (*Lumbricus corethrurus*). *Journal of Natural History Series 2, 20,* 13-15. http://dx.doi.org/10.1080/00222935709487865

Oldfield, S., & Eastwood, A. (2007). *The red list of oaks*. Cambridge: Fauna and Flora International (FFI).

Ortiz-Ceballos, A. I., & Fragoso, C. (2004). Earthworm populations under tropical maize cultivation: The effect of mulching with velvetbean. *Biology and Fertility of Soils, 39,* 438-445. http://dx.doi.org/10.1007/s00374-004-0732-8

Ortiz-Ceballos, A. I., Fragoso, C., Equihua, M., & Brown, G. G. (2005). Influence of food quality, soil moisture and the earthworm *Pontoscolex corethrurus* on the growth, reproduction and activity of a tropical earthworm *Balanteodrilus pearsei*. *Pedobiologia, 49,* 89-98. http://dx.doi.org/10.1016/j.pedobi.2004.08.006

Ortiz-Ceballos, A. I., Fragoso, C., & Brown, G. G. (2007). Synergistic effect of a tropical earthworm *Balanteodrilus pearsei* and velvetbean *Mucuna pruriens* var. *utilis* on maize growth and crop production. *Applied Soil Ecology, 35,* 356-362. http://dx.doi.org/10.1016/j.apsoil.2006.07.009

Ortiz-Ceballos, A. I., Aguirre-Rivera, J. R., Osorio-Arce, M. M., & Peña-Valdivia, C. (2012). Velvet Bean (*Mucuna pruriens* var. *utilis*) a cover crop as Bioherbicide to preserve the environmental services of soil. In: R. Alvarez-Fernandez (Ed.), *Herbicides-environmental impact studies and management approaches* (pp. 167-184). Cambridge: University of Cambridge.

Pashanasi, B., Meléndez, G., Szott, L., & Lavelle, P. (1992). Effect of inoculation with the endogeic earthworm *Pontoscolex corethrurus* (Glossoscolecidae) on availability, soil microbial biomass and the growth of three tropical fruit tree seedlings in a pot experiment. *Soil Biology and Biochemistry, 24,* 1655-1659. http://dx.doi.org/10.1016/0038-0717(92)90165-T

Pedraza, R. A., & Williams-Linera, G. (2003). Evaluation of native tree species for the rehabilitation of deforested areas in a Mexican cloud forest. *New Forests, 26,* 83-99.

http://dx.doi.org/10.1023/A:1024423511760

Ramírez-Marcial, N., González-Espinosa, M., & Williams-Linera, G. (2001). Anthropogenic disturbance and tree diversity in montane rain forests in Chiapas, Mexico. *Forest Ecology and Management, 154,* 311-326. http://dx.doi.org/10.1016/S0378-1127(00)00639-3

Saunders, D. A., Hobbs, R. J., & Margules, C. R. (1991). Biological consequences of ecosystem fragmentation: A review. *Conservation Biology, 5,* 18-32. http://dx.doi.org/10.1111/j.1523-1739.1991.tb00384.x

Senapati, B. K., Lavelle, P., Giri, S., Pashanasi, B., Alegre, J., Decaëns, T., Jimenez, J. J., Albrecht, A., Blanchart, E., Mahieux, M., Rousseaux, L., Thomas, R., Panigrahi, P. K., & Venkatachalam, M. (1999). In-soil earthworm technologies for tropical agroecosystems. In: P. Lavelle, L. Brussaard, & P. Hendrix (Eds.), *Earthworm management in tropical agroecosystems* (pp. 199-237). Wallingford, UK: CABI.

SAS Institute Inc. (2009). SAS system for windows version 9.2. http://www.sas.com/

SEMARNAT (2002). *Nom -021-SEMARNAT-2000 Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos, estudio, muestreo y análisis, 2nd Sect*. México: Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT).

Scheu, S. (2003). Effects of earthworms on plant growth: Patterns and perspectives. *Pedobiologia, 47,* 846-856. http://dx.doi.org/10.1078/0031-4056-00270

Smyth, T. J., Cravo, M. S., & Melgar, R. J. (1991). Nitrogen supplied to corn by legumes in a Central Amazon Oxisol. *Tropical Agriculture (Trinidad), 68,* 366-372.

Valencia, A. S. (2004). Diversidad del género *Quercus* (Fagaceae) en México. *Boletin de la Sociedad Botanica de México, 75,* 33-53.

Vázquez-Yanes, C., & Cervantes, V. (1993). Reforestación con árboles nativos de México. *Ciencia y Desarrollo, 19,* 52-58.

Villaseñor, J. L. (2010). *El bosque húmedo de montaña en México y sus plantas vasculares: Catálogo florístico-taxonómico*. México: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO)—Universidad Nacional Autónoma de México (UNAM).

Wardle, D. A. (2002). *Communities and ecosystems: Linking aboveground and belowground components*. Princeton, NJ: Princeton University Press.

Wardle, D. A., Bardgett, R. D., Kironomos, J. N., Setälä, H., Van der Putten, W. H., & Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science, 304,* 1629-1633. http://dx.doi.org/10.1126/science.1094875

Wurst, S., & Jones, T. H. (2003). Indirect effects of earthworms (*Aporrectodea caliginosa*) on an above-ground tritrophic interaction. *Pedobiologia, 47,* 91-97. http://dx.doi.org/10.1078/0031-4056-00173