RESOURCE PARTITIONING OF SOIL ORGANIC PHOSPHORUS: INVESTIGATIONS FROM A TROPICAL MOUNTAIN FOREST

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Biology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

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Abstract

One of the major limitations of resource-niche theory to explain plant species diversity and distribution is the paucity of recognized resources. Recent investigations in grassland and tundra ecosystems indicate that plant species can specialize to exploit different forms of soil nitrogen. I hypothesized that a similar phenomena occurs in the tropics with soil phosphorus (P). I grew seedlings of arbuscular mycorrhizal (AM) *Mollinedia darensi* and *Podocarpus olieofolius*, ectomycorrhizal (EM) *Oreomunnia mexicana*, and nonmycorrhizal (NM) *Roupala montana* tree species in a hydroponic growth medium containing exclusively either inorganic, monoester, diester, inositol-P, or a no-P control. In addition, I assayed the production of P-mono and diesterase enzymes activity of each species to determine their capacity to remineralize P from organic sources. My results support the potential for resource partitioning to promote coexistence between mycorrhizal and nonmycorrhizal species. The mycorrhizal tree species exhibited similar growth, nutritional, and allocational responses across treatments, with growth and total P content high in inorganic-P and monoester-P and low in the inositol and diester-P treatments. When limited to inositol P, *R. montana* (NM) exhibited high growth, significantly greater total and specific leaf area, and significantly greater P use efficiency when compared to the other experimental treatments. *R. montana* (NM) also had 3 fold greater total P in the inositol P treatment than in the no-P control ($p=0.064$), and had significantly greater P-mono and diesterase activity than both AM and EM species. Together these results indicate that the potential exists for partitioning of soil P between mycorrhizal and NM plants, but not between AM and EM plants.

Acknowledgements

I would like to thank Jim Dalling and Ben Turner for the intellectual, institutional and financial assistance without which this project would not have been possible. Carlos Espinoza, Alberto Gonzales, and Arturro Morris provided invaluable assistance in collecting and identifying plants, constructing the grow house, and maintaining the experiment at the Fortuna Field Station. Claire Baldeck provided valuable editing for both this thesis work, presentations based off of it, and grant and fellowship applications to support its completion. Mike O' Mara assisted in the harvest, transportation, and weighing of plant material. McKenna Kelly helped collect seedlings from the field and transfer them to a hydroponic growth medium, edited every scrap of text and presentation, and kept me sane throughout this masters degree. Financially, I am indebted to the Smithsonian Tropical Research Institute, Clark, Tinker and Masters Research Support Grants, and the Govindjee and Rajni Award for Excellence in Biological Science.

Table of Contents

Introduction

The partitioning of limiting resources promotes species coexistence by reducing inter-species competition (Hutchinson 1959, Tilman 1982). In a classic example, different species of Galapagos ground finch (*Geospiza* spp.) occupying the same island have evolved beak morphologies to feed on seeds of different sizes, reducing interspecies competition for food (Grant 1986). While numerous other case studies corroborate the success of resource partitioning in animal communities (Shoener 1974, Pyke 1982, Grant and Grant 2006), there is less evidence to suggest its importance in plant communities (Connell 1978, Huston 1979). This bias reflects both the difficulty of resolving the mechanisms of below ground competition (Casper and Jackson 1997, Silvertown *et al.* 1999), and the paucity of defined resources limiting plant productivity across the globe (sometimes referred to as the "paradox of diversity"). While the competitive exclusion principle predicts that the number of species cannot exceed the number of limiting resources (Stewart and Levin 1973, Armstrong and McGhee 1983), there are approximately 300,000 terrestrial plant species, and only 20 recognized resources (including light, water, and mineral nutrients) (Harrison 1987). Further, global analyses reveal that most terrestrial plant productivity is limited by either soil nitrogen (N), phosphorus (P), or co-limited by both (Aerts and Chapin 2000, Elser *et al.* 2007). Resolving the paradox of plant species diversity involves redefining soil N and P as composite resources, present in a host of different chemical compounds. If plant species differ in their ability to exploit different chemical forms of a limiting soil resource, then the potential exists for resource partitioning to promote plant species coexistence.

In fact, several studies suggest that coexisting plants species are adapted to acquire N from different chemical sources. In a landmark study, McKane *et al.* (2002) exposed plant species from a N-limited arctic tundra to ^{15}N-labelled ammonium, nitrate, or glycine (a simple amino acid). They observed marked differences in which form of N different species exploit. Further, they found that dominant plant species exploit the most abundant form of soil N available. This suggests that plant species turnover can be determined by the distribution of different chemical forms of soil N. Resource partitioning of soil N has also been demonstrated in temperate grasslands (Weigelt *et al.* 2005, Kahmen *et al.* 2006). However, the ability of plants to partition soil P remains less clear, despite the diversity of chemical forms of soil P, the unique physiological challenges they pose for plants, and their heterogeneous distribution (Turner

2008).

Total soil P is present in a variety of different chemical forms, including labile inorganic and organic fractions. Inorganic P (P_i) can be taken up directly, without the aid of specialized cell transporters or enzymes (Silverbush and Barber 1983). Organic P (P_o), which can constitute anywhere from 30-90% of the total soil P (Harrison 1987), is present in compounds that differ markedly in their biological availability (Condron et al. 2005). These include P monoesters (glucose phosphate, mononucleotides), diesters (RNA, DNA), and monoester inositols (phytic acid) (Turner 2008). In order for plants to exploit P_o, they must first re-mineralize it into P_i (Trafadar and Classen 1988, Adams and Pate 1992). This process can be achieved through the exudation of specialized phosphatase enzymes, which cleave ester bonds and liberate phosphate from P_o (Antibus *et al*. 1992). The different forms of soil P can be placed along a gradient of increasing investment required for exploitation, depending on the metabolic cost of producing enzymes capable of liberating P_i from organic substrates (Turner 2008). Thus, monoester P requires hydolysis by the enzyme monoesterase, diester P by both mono and diesterase, and inositol P by monoesterase and phytase (Fig. 1).

Changes in relative abundance of inorganic, monoester, diester, and inositol P along gradients of decreasing soil P roughly mirror their bioavailability to plants (Turner *et al.* 2007). Soils high in total P are made up primarily of P_i. As soils age and total soil P declines, there is an increase in relative proportion of P_o (Syers and Walker 1969, Parfitt *et al.* 2005). Initially, this P_o is composed primarily of P monoesters and inositols. As total P decreases still further, monoester and inositol P decline rapidly, and diester P increases in abundance (Turner *et al.* 2007) (Fig. 2). Finally, the most P poor soils on earth contain low amounts of P_o, with most P_i sorbed to soil cations (Al and Fe in acid soils, Ca in alkaline soils) (Lambers *et al.* 2008). Concomitantly, there is a turnover of plant species that employ different P-acquisition strategies along gradients of total P availability. These include association with different functional groups of mycorrhiza (arbuscular and ectomycorrhiza) and/or formation of proteiod cluster roots (Lambers *et al.* 2008, Turner 2008). Soils high in inorganic P tend to host arbuscular mycorrhizal (AM) plant species, while more weathered soils host ectomycorrhizal (EM) plants, and the most P-deficient soils tend to host nonmycorrhizal (NM) plants that form root clusters (Richardson *et al.* 2004, Lambers *et al.* 2006, Crews *et al.* 1995) (Fig. 3).

Of particular interest is whether plants with different P-acquisition strategies specialize to take up different chemical forms of P. Mycorrhizas provide plants with mycelia that expand the volume of soil where P can be obtained, providing as much as 80% of plants' total P uptake (Douds *et al.* 2000). In addition, several studies have demonstrated that AM and EM differ in their production of phosphatase enzymes. AM species are generally considered P scavengers, which use mycelia to extensively search the soil for inorganic P (Smith and Read 1993). To contrast, EM species are well known to re-mineralize P from organic esters through the production of mono and diesterase, and phytase enzymes (Antibus *et al.* 1992). However, this paradigm may be shifting, as more recent studies heave demonstrated the ability of some AM species to complete their life cycle when limited to organic P (Joner *et al.* 2000, Koide and Kabir 2000). Plant species that form proteiod cluster roots, particularly in the family Proteaceae, exist on some of the most P-deficient soils on earth and are often described as P miners. While these species are almost exclusively non-mycorrhizal (Lambers *et al.* 2008), their roots exude both phosphatase enzymes and abundant organic acids capable of re-mineralizing organic P and releasing P_i sorbed to soil cations, respectively (Adams and Pate 1992, Watts and Evans 1999).

P-limited soils are distributed primarily at tropical and subtropical latitudes, where the lack of recent glaciations has allowed both physical weathering and biological sequestration of soil P to proceed uninterrupted over long periods of time (10^3-10^5 years) (Vitousek 2004). These latitudes also contain the most species rich plant assemblages on the planet, with tropical forests alone containing more than half of all plant species on less than 5% of the earth's surface (Prance 1982). Moreover, anthropogenic increases in other limiting resources, via atmospheric N deposition and increased CO2 emissions, threaten to drive tropical plants towards more marked P-limitation (Norisada *et al.* 2006, Pheonix *et al.* 2006). If these environmental inputs affect a similar increase in interspecies competition, they may lead to the extinction of plant species that are poor competitors for soil P. Alternatively, resource partitioning of soil P could buffer interspecies competition against increased P-limitation, facilitating coexistence in the face of global climate change.

Mechanistically, plant species that are adapted to acquire P from different sources should also differ in their production of phosphatase enzymes. Assays of P-monoesterase are used as proxies of both the capacity to remineralize simple P-monoesters (e.g. glucose-phosphate) as well as

monoester inositol P (phytic acid) (Antibus *et al.* 1992). Additionally, if plants specialize to exploit different forms of P, they should elicit several growth, allocational, functional, and nutritional reactions when limited preferred P forms, relative to less preferred forms. These include:

(1) increased growth

(2) increased allocation in photosynthetic structures (leaves) relative to nutrient acquisition structures (roots) (Shipley and Mezaine 2002).

(3) increased specific leaf area (SLA), a functional leaf trait describing the ratio of leaf area to leaf mass that correlates positively with plant nutrition and light limitation (Hirose *et al.* 1988, Witkowski *et al.* 1992, van Arendonk *et al.* 1997, Meziane and Shipley 1999) .

(4) increased P-concentration ([P]), which should correspond inversely to P limitation, but is also subject to increases due to luxury consumption of excess P and translocation from abscised leaf and root tissues.

(5) increased total P, which indicates the uptake of P.

(6) increased P use efficiency (PUE), which measures the efficiency with which plant biomass is accumulated per unit of P uptake.

While previous studies have established differences in root enzyme activities in plant species (Adams and Pate 1992, Antibus *et al.* 1992, Watts and Evans 1999), these studies have not contrasted the enzyme production, growth, allocation, nutrition, and functional characteristics of coexisting plant species representative of multiple P-acquisition strategies. This depth is required in order to bridge the gap between pot studies and ecology due to the myriad influences affecting competitive success, such as growth/P uptake trade-offs. These characteristics are important as plants with comparable ability to re-mineralize the same P source may also differ in their P-requirements and uptake efficiency—both factors that would affect fitness and, therefore, capacity to coexist on a shared soil substrate. Moreover, previous studies have focused exclusively on temperate plant species, while a majority of terrestrial plants growing on P-deficient soils are found at tropical latitudes. Here I provide the first growth experiment

comparing tropical AM, EM, and NM (with cluster roots) plant species ability to grow and acquire P from inorganic, monoester, diester, and inositol P.

Methods

Plant species exhibiting AM, EM, and NM root systems were collected from the Fortuna Forester Reserve and either grown in soilless media with P provided exclusively in different chemical forms or assayed for the production of P-mono and diesterase enzymes.

Study Site

Fieldwork was conducted within the Fortuna Forest Reserve (19,500 ha), Chiriqui Province, along the Central Cordillera of Panama (Fig. 4). Fortuna consists of lower montane forests between 1000 and 1500 m a.s.l., with mean annual rainfall from 1500-6800 mm and mean annual temperature ranging from 19 to 22 °C (Cavelier *et al.* 1996). Seedlings were collected from outside 1-hectare plots at Quebrada Chorro, Honda "B", and Hornito.

Both Quebrada Chorro and Quebrada Honda "B" have soil formed on rhyolite tuff with very low concentrations of N and P (Table. 1). The Chorro plot is dominated primarily by palm species, but also contains the AM focal species *Podocarpus oleifolius*. Honda "B" is dominated by the EM emergent canopy tree *Oreomunnea mexicanum*, Junglandaceae (29% of the trees > 10 cm DBH; 42% of the basal area). Hornito is characterized by relatively nutrient rich dacitic soils (Anderson *et al.* 2010, Dalling *unpublished data*).

Study Species

***Mollinedia darienensis* (AM). Order: Laurales, Family: Monimiaceae.** *M. dariensis* is a shade-tolerant canopy tree with a wide distribution across sites at the Fortuna Forest Reserve. It occurs from Costa Rica to southern Colombia (GBIF 2011). It is classified as AM on the basis of the documented association of other genera within Monimiaceae (Duousso *et al.* 2008, Ruiz and Davey 2005). It was collected outside Honda B.

***Podocarpus oleifolius* (AM with root nodules). Order: Pinales, Family : Podocarpaceae.** *P. oleifolius* is a shade-tolerant conifer found on rhyoltic tuff soils at the Honda and Chorro sites within the Fortuna Forest Reserve, with a clustering of emergent canopy trees at the Chorro site. It occurs from southern Mexico, throughout Central America, and along western South America into Boliva (GBIF 2011). Plants in the Podocarpaceae form spherical root nodules, which can become infected with AM mycorrhiza. Podocarps are known to associate on weathered soils high

in inositol and diester P (Turner *et al.* 2007). *P. oleoifolius* is classified as AM due to the consistent documentation of AM infection both within Podocarpaceae and the genus *Podocarpus* (Wang and Qui 2006). It was collected outside Chorro.

***Oreomunnea mexicana* (EM) Order: Fagales, Family: Juglandaceae.** *O. mexicana* is a shade tolerant canopy tree found with a wide distribution across sites at the Fortuna Forest Reserve, but with a marked clustering around the rhyolytic soils of Honda A and B. It achieves monodominance only within the Honda B site, where it forms thick swaths of seedlings. It occurs from southern Mexico to central Panama (GBIF 2011). *O. mexicana* was previously characterized as EM by Quist *et al.* (2000). Surface observations of infected root tips of *O. mexicana* at Fortuna support this characterization. It was collected outside Honda B.

***Roupala montana* (NM). Order: Proteales, Family: Proteaceae.** *R. montana* is a shade-tolerant canopy tree with a wide distribution across the sites at the Fortuna Forest Reserve. It occurs from southern Mexico, throughout Central America, and into Southern Brazil (GBIF 2011). It forms clusters of fine roots, which is a common trait amongst the Proteaceae. It is characterized as NM on the basis of the common lack of mycorrhizal infection documented within the Proteaceae (Brundrett 2008, Lambers *et al.* 2008). However, some evidence suggests that even cluster root forming species can host AM fungi, though they are considered redundant in terms of augmenting host-plant nutrition (Boulette & Lambers 2006). It was collected outside of Hornito.

Nutrient Stock Solutions

Experimental and control plants were fertilized every other day with 30 mL of solution containing all essential nutrients. Nutrient stock solutions were prepared by first preparing a "master" no-P control solution with a Scott's 15-0-15 (mass ratio of N:P:K) Dark Weather Feed® fertilizer, supplemented with $MgSO_4$. For the P treatment solutions, the nutrient solution also included the addition of sodium phosphate monobasic (PO4, Sigma S0761), D-glucose 6-phosphate disodium salt hydride (G6P, Sigma G7250), phytic acid sodium salt hydride (INS, Sigma P0109), or RNA from torula yeast (Sigma R6625) (Table. 2).

Plant Collections

Forty-six bare-root seedlings of each focal species were collected from field soil at the Fortuna Forest Reserve between February 22-March 23, 2010. Six seedlings from each species were harvested before the experiment began to estimate species' initial values for all dependent variables. The roots of the remaining 40 seedlings were gently washed with water and the plants were transplanted into 30×5 cm (length × diameter) containers filled with acid washed marine sand (4.3 μg/cm^3 resin P, 1.9μg/cm^3 microbial P, Dalling *unpublished data*). Seedlings were divided into 5 treatment groups with 8 replicates each. Replicates were organized to minimize the difference between average stem height and leaf number between treatments. These treatments correspond to plants provided with all essential nutrients with P as either (1) inorganic phosphate (PO4), (2) organic monoester phosphate (G6P), (3) inositol phosphate (INS), (4) diester phosphate (RNA), and (5) a P-free control. Initial stem height and leaf number were recorded for each replicate for use as potential covariates.

Conetainers were placed in a growing house with a transparent plastic roof and 75% shade-cloth. Species were grouped together on the same bench with the placement of treatments randomized. Approximately every 2 weeks the position of each plant was changed on the bench to correct for any heterogeneity in light and temperature. After the first four weeks, the shade cloth was reduced to 50%. After 4-months plants were removed from the sand media. Leaves were scanned on a flatbed scanner to determine leaf area, and plants were dried for three days at 70°C and weighed to determine total dry mass, and leaf, stem, and root dry mass independently. All of the leaf, stem, and root tissue were ground into a fine powder. The P concentration of a random sample taken of the composite powder was digested in acid, measured using inductively coupled plasma spectroscopy, and expressed on a mg P g^{-1} basis.

Analysis

Plant dry mass was used to calculate relative growth rate (RGR),

$$RGR=[\ln(M_f)-\ln(M_i)]/(\Delta t)$$

where M_f is final mass, M_i is initial mass estimated as the average dry mass of the pre-experimental harvest, and Δt is the duration of the experiment in days. Specific leaf area, a measure of leaf thinness, was calculated as the sum of leaf area over leaf dry mass. Mass ratios were calculated as the ratio of leaf, stem, and root dry mass over total plant dry mass. Total P

measurements were calculated as the product of P-concentration and total dry mass. P use efficiency (PUE) was calculated as

$PUE= (M_f-M_i)/(P_f \cdot M_f)$

where P_f is the final P concentration.

To assess the significance of species and species*treatment interaction on the dependent variables, composite ANOVAs with the four species were run with species, treatment, species*treatment interaction, and initial leaf number (when significant) as model statements. For each species separately, differences in treatment means for each dependent variable were analyzed using ANOVA, with initial leaf number used as a covariate when it explained a significant amount of variation ($p<0.05$). When within-species ANOVA returned p-values $\leq$ 0.05, differences in treatment means were analyzed using least significant difference (LSD).

Enzyme Assays

10-14 individuals from each of the focal species used in the growth experiment were potted in field soil for approximately 10 months and then harvested to assay their production of P-mono and diesterase using a protocol modified from Antibus *et al.* (1992).

Assay buffer was prepared by taking a 20mM solution of sodium acetate (Sigma), adjusted to pH 5.0 by addition of acetic acid. Stock solutions of 25mM p-nitrophenyl phosphate (Fisher Scientific, NPP), and bis(p-nitrophenyl)phosphate (Sigma, BNPP) were prepared in assay buffer and kept frozen until the day assays were run. Plant roots were rinsed gently in deionized water. Forceps were used to remove soil particles that remained adhered to fine roots. For each plant, 3 to 5 root tips per vial were removed and placed in one of five 7.5 mL glass vials filled with either 4.9 mL (experimental treatments) or 5.0 mL (control) of assay buffer. Glass vials were kept in an incubator (Boekel Scientific) for approximately 30 minutes to raise the temperature to 26°C. The assays began with the addition of 100 uL of enzyme substrate, for final substrate concentration of 0.5mM in 5.0mL. The glass vials were then transferred to a shaker (Eberbach 6010) operating at 3 Hz, where they remained for 45 minutes.

To terminate the reactions, 500 uL of the reaction mixture of NPP, BNPP, and a no-substrate control were added to 4.5 mL of 0.125 M NaOH (pH 10). The release of product was measured

by reading the absorbance of samples at 405 nm in a spectrophotomoter (Hach DR500). Absorbance values were converted to mM using standard curves prepared with *p*-nitrophenyl (Sigma). Roots were removed from glass vials, placed in labeled aluminum foil envelopes, and dried at 105 °C for 24 hrs. Enzyme activity was expressed as μM product • mg^{-1} dry root mass • $hour^{-1}$, with control values for each plant replicate subtracted from the experimental values. For each assay, species were analyzed using ANOVA, with significant differences determined at ($p<0.05$). Differences in species means were analyzed using least significant difference (LSD).

Results

Nearly all dependent variables varied significantly with treatment, with the exceptions of root mass fraction (RMF) and phosphorus use efficiency (PUE). The most significant treatment effects were observed in P concentration, total P, RGR, and total leaf area (TLA). Most dependent variables varied more significantly between species, with the exceptions of SLA and P concentration. The most significant of these include the growth and biomass allocation variables TLA, root mass fraction (RMF), and leaf mass fraction (LMF). Further, significant species x treatment interactions were found in all variables with the exception of LMF and total P, with the most significant occurring in TLA, P concentration, SLA and RGR. When *R. montana* (NM) was removed from the model, the species x treatment interaction disappeared for all variables. This did not occur for any of the other species. The leaf covariate had a significant effect on RGR and P-concentration only. There was no significant interaction between initial leaf number and species or treatment for all dependent variables (Table 3).

Within each species, treatment had a significant effect on most dependent variables. Exceptions include *M. dariensis* TLA and SLA, *P. oleifolius* SLA, and *O. mexicana* RMF and SLA (Table 4). The leaf covariate had a significant effect on *M. dariensis* TLA and LMF, *O. mexicana* total P, and *R. montana* RGR and TLA. There was no significant interaction between initial leaf number and treatment for all combinations of species and dependent variables.

Relative Growth Rate– AM *P. oleifolius* and *M. dariensis* had similar patterns of growth between treatments, with relatively high growth in PO4 and G6P, and lower growth in INS and RNA. However, in both species, only growth in the PO4 treatment varied considerably from the NO/P control. *O. Mexicana* (EM) had a maximum growth rate of nearly one-third the other species, and experienced negative growth in the RNA treatment (consistent with leaf and/or root abscission). *R. montana* (NM) was the fastest growing of the four species, with a unique pattern of growth between treatments. Its maximum RGR was the in INS treatment. Further, *R. montana* was the only species with significantly different RGR between the monoesters G6P and INS. However, growth in both the PO4 and INS treatments did not differ significantly from the NO/P control (Fig. 5).

Total Leaf Area– AM *M. dariensis* and *P. oleifolius* had nearly identical patterns of TLA between treatments, which mirror the patterns of RGR between treatments. *O. mexicana* (EM) had the highest TLA amongst species, but with the PO4 treatment not differing significantly from the NO/P control. Further, *O. mexicana* TLA was significantly lower in the RNA treatment than in the NO/P control. *R. montana* (NM) TLA was significantly greater in INS than in all other treatments. This treatment effect is also strong in magnitude—the TLA of the INS treatment is between 2 to 3 fold higher than in the PO4 and NO/P treatments (Fig. 6).

Specific Leaf Area– Mycorrhizal *M. dariensis*, *P. oleifolius*, and *O. mexicana* had no significant differences in SLA between treatments. *R. montana* (NM) had significantly greater SLA in INS than in all other treatments (Fig. 7).

Leaf Mass Fraction– *M. dariensis* (AM) and *O. mexicana* (EM) had no differences in LMF between treatments. AM *P. oleifolius* LMF in PO4 and G6P was significantly greater than in NO/P control. *R. montana* (NM) LMF was significantly greater in INS than in the G6P and RNA treatments. However, LMF of INS and PO4 did not differ significantly from the NO/P control. Both *O. mexicana* and *R. montana* exhibit significantly higher LMF than the other species (Fig. 8).

Stem Mass Fraction– In general, there was an inverse relationship between SMF and RGR. Both *M. dariensis* (AM) and *O. mexicana* (EM) had significantly greater SMF in RNA than all the other treatments. *P. oleifolius* (AM) had similarly high SMF in RNA, though it did not differ significantly from the INS treatment. *R. montana* (NM) had the greatest and least SMF in the two monoester treatments—G6P and INS, respectively. In addition, *R. montana* SMF was significantly greater in the NO/P than it is in the INS treatment (Fig. 8).

Root Mass Fraction– Neither *M. dariensis* (AM) nor *O. mexicana* (EM) exhibit significant differences in RMF between treatments. *P. oleifolius* (AM) had significantly greater RMF in NO/P than in all other treatments. *R. montana* (NM) RMF was significantly higher in NO/P and RNA treatments than in the INS treatment, which exhibits the lowest RMF (Fig. 8). *R. montana* roots in the NO/P treatment also differed qualitatively from the other treatments. Whereas at the beginning of the experiment none of the *R. montana* replicates contained cluster roots, at harvest

6 of 8 *R. montana* had developed simple root clusters. Cluster root formation was not observed in any other treatment.

Phosphorus Concentration ([P])– AM *M. dariensis*, *P. oleifolius*, and NM *R. montana* had similar patterns of [P] between treatments, with PO4, G6P, and RNA significantly greater than NO/P [P]. *O. mexicana* (EM) [P] was greatest in the RNA treatment, with a nearly 2.5 fold difference between [P] in the RNA and NO/P treatments. *R. montana* had a wide range of [P] values between treatments with similar RGR, including a nearly 8-fold difference in [P] between PO4 and NO/P treatments. There was no significant difference in *R. montana* [P] between the INS and NO/P treatments (Fig. 9).

Total Phosphorus– Of the mycorrhizal species, only *M. dariensis* (AM) and *O. mexicana* (EM) had significantly higher total P in G6P than in the other treatments. *R. montana* (NM) total P was significantly greater in PO4 and RNA than in the other treatments, whereas G6P did not differ from the NO/P control (Fig. 10*)*. The total P in the INS treatment was nearly 3-fold greater than in the NO/P control, with a near-significant p-value of 0.064.

Phosphorus Use Efficiency (PUE)– The mycorrhizal species all had significantly less PUE in the RNA compared to the other treatments. *R. montana* (NM) PUE was markedly greater in the NO/P control, with roughly 6-fold greater PUE than the PO4 treatment. In addition, *R. montana* PUE was significantly greater in the INS treatment than in any of the other experimental treatments, with roughly 3-fold the magnitude of the PO4 value (Fig. 11).

P mono and diesterase– R. montana (NM) produced significantly more P-monoesterase than the other focal species. *R. montana* also produced significantly more P-monoesterase in root clusters than in fine roots. *R. montana* also produced significantly more P-diesterase than the other species, though root clusters and fine roots did not differ significantly (Fig. 12).

Discussion

My results support the potential for mycorrhizal and nonmycorrhizal species to partition soil organic P. The disappearance of the significant species x treatment interaction for RGR, total leaf area, SLA, RMF, P concentration, and PUE (Table 3) when *R. montana* (NM), but not the other species, were removed from the model indicates that the AM and EM species did not differ significantly in their response to different chemical forms of P. Relative the mycorrhizal plant species, *R. montana* (NM) exhibits a number of unique reactions to inositol and diester P. While *R. montana* growth did not differ significantly between the inositol-P treatment and no-P control (Fig. 5d), resource allocation and functional characteristics indicate that the inositol-P treatment were primarily light limited, while plants in the no-P control were nutrient limited. Thus, *R. montana* in the inositol-P treatment invested significantly more resources in photosynthetic structures (greater leaf area and thinner leaves) than in any other treatment (Figs. 6d, 7d), and allocated significantly less biomass to roots than in the no-P control (Fig. 8d). Moreover, *R. montana* produced root clusters only in the no-P control, indicating that it was not stressed for additional P in the experimental treatments.

The nutritional data further corroborates *R. montana*'s significant and unique exploitation of inositol P. *R. montana* produced significantly greater levels of P-monoesterase than the other species (Fig. 12), which has been demonstrated to act as an effective proxy of P-uptake from inosoitol-P (Antibus *et al*. 1992). Further, *R. montana* total P was more than 3 fold greater in the inositol-P treatment than in the no-P control, and PUE in the inositol-P treatment was significantly greater than any of the other experimental groups—a unique result amongst species (Fig. 11). The marked differences in PUE between treatments reflect *R. montana*'s ability to sustain high growth under a wide range of different P concentrations. While plants in the inorganic-P and no-P control did not differ significantly in RGR, they varied more than 8-fold in P-concentration, and 6-fold in total P (Figs. 9d, `10d). Thus, the apparently uniform growth of *R. montana* in inorganic-P, inositol-P, and the no-P control belies a host of allocational, functional, and nutritional differences indicating (1) luxury consumption of P in inorganic-P, (2) efficient and biologically significant exploitation of P in inositol-P, and (3) efficient mobilization of P reserves and the onset of P-stress in the no-P control. This result suggests NM Proteaceae

possess a hereto unappreciated versatility of adaptations to growth on soils with different forms of soil P.

While the mycorrhizal species exhibit enhanced growth and nutrition when limited to glucose relative to inositol-P, the NM *R. montana* had an opposite reaction. Thus, *R. montana* had significantly greater RGR and PUE in the inositol than in the glucose-P treatment (Fig. 5d,11d). Further, while both *M. dariensis* (AM) and *O. mexicana* (EM) had significantly greater total P in the glucose-P treatment than the no-P control, *R. montana* did not (Fig. 10). Both inositol P and glucose-phosphate are monoesters, and thus susceptible to hydrolysis by the high levels of *R. montana* P-monoesterase (Fig. 12d). As mycorrhiza can take up and metabolize simple sugars applied externally to infected root tips (Bücking and Shachar-Hill 2005), it is possible that glucose addition increased the fitness of C-limited mutualistic AM and EM, while the effect on *R. montana* (NM) was only to increase the fitness of free living microbes with which it competed for available N and P. If so, future experimentation should reveal that mycorrhizal plant species, but not uninnoculated controls, benefit more from unphosphorylated glucose addition than NM plant species.

P. oleifolius (AM with nodules) appears to have no enhanced ability to exploit organic P relative either *M. dariensis* (AM) or *O. mexicana* (EM), despite its documented affinity for low P soils with high proportions of diester and inositol P (Turner *et al.* 2007, Turner 2008). It seems likely that *P. oleifolius* are adapted to respond to gradients of inorganic P availability, rather than for the acquisition of organic P. Generally, podocarps respond to mixed-fertilizer addition with decreased growth and survivability (Carswell *et al.* 2003, Pareliussen *et al.* 2006). It is also possible for podocarps to promote low P conditions by creating high C:P leaf litter, which resists decomposition (Wardle *et al.* 2008). The role of the root nodules as adaptations to low nutrient soils remains unknown; though they are currently implicated in (1) hosting facultative N-fixing bacteria and (2) increasing the efficiency of AM mutualists (McGee *et al.* 1999). Of these two possibilities, the first is probably spurious, and based on the confounding of N-fixation of free-living bacteria in soil around podocarp roots for symbiotic N-fixation via podocarp nodules (Khan and Valder 1972, Silvester and Bennett 1973). The second possibility warrants further investigation, perhaps by assessing the magnitude that AM inoculum enhances podocarp growth and nutrition relative to other sympatric plant species.

Although EM species are documented to both produce greater amounts of phosphatase enzymes and to outcompete AM species on soils high in organic P, the P-mono and diesterase of *O. mexicana* did not differ significantly from the AM species, while the RGR of AM species was greater than *O. mexicana* in all treatments. In other tropical ecto-monodominant forests the establishment of common hyphal networks is thought to augment both the shade tolerance and nutrition of EM seedlings (Nara 2006, McGuire 2007). However, the RGR of *O. mexicana* growing in field soils is not known, so it is not possible at this point to assess the effect of lost biological interaction under experimental conditions. Further, in forest soils EM hypha expand horizontally to increase the rhizosphere volume within thin surface layers of organic matter. This spatially expansive "scavenger" strategy may not be amenable to growth in relatively narrow diameter conetainers.

The low growth response amongst all species when limited to diester-P group (Fig. 1) does not appear to be caused by P-deficiency. Each species produces significant levels of P-diesterase, had significantly greater P-concentration in the diester-P treatment than in the no-P control. Most species total P did not differ significantly between the diester and inorganic-P treatments (with the exception of *O. mexicana*, which had a significantly *greater* P-concentration in the diester-P treatment) (Fig. 5). It appears that either diester-P posed a formidable metabolic cost for plants to acquire or produced a toxic effect retarding plant growth. Some support exists for the increased metabolic cost of remineralizing diester-P, which occurred at around one half the rate of monoester-P amongst the focal species in this experiment (Figure 12c). However, differences in RGR and total P between treatments indicate that *R. montana* is the only species to make a trade-off between growth and P-uptake from diesters. *R. montana*, which had the greater P-diesterase activity than the mycorrhizal plant species (Fig. 12b), was the only species with significantly higher total P in the diester-P group relative the no-P control, despite growing significantly less (Figs. 5d, 10d). To contrast, the mycorrhizal species had decreased growth and similar total P between the diester-P and no-P control group (Fig5a-c, Fig 10a-c). An interesting possibility is that the uptake of incomplete products of RNA re-mineralization, such as short-chain RNA molecules and mononucleotides, negatively affects plant growth. Paungfoo-Lonhienne (2010) has established plants can take up intact nucleic acids, which can act both as a P source and a signaling molecule. I suggest a metabolomic approach aimed at establishing the relationship of

plant tissue RNA concentrations, growth, and nutrition could shed light on the potentially toxic effect of nucleic acid P.

Results from this study lead to some working predictions on the relative fitness of the focal species growing in soils with varying P availability. Thus, *R. montana* (AM) should be successful on both young soils rich in inorganic P, and on older weathered soils with high proportions of inositol and diester P. *M. dariensis* (AM) and *O. mexicana* (EM) may outcompete *R. montana* on soils containing P-monoesters bound to simple sugars. *P. oleifolius* (AM with root nodules) should not compete with plants over organic P, but rely on a host of other adaptations to persist on low P soils. Future studies should utilize both descriptive methods aimed at relating the distribution of plant taxa to the magnitude and proportions of soil P present in different chemical forms, as well as experimental manipulations of (1) common gardens of focal species growing on different soil substrates and (2) tracking P^{32} and P^{32}/C^{14} tracers attached to organic substrates as they are taken up by plants.

NM plant species, such as those of Proteaceae, may have the ability to form an ecological niche based upon the exploitation of inositol and diester-P. However, given that a majority of terrestrial plant species are mycorrhizal (Brundrett 2009), resource partitioning of soil organic P may not play a primary role in promoting their coexistence. Resource partitioning should be considered separately from adaptations that allow plants to grow on soil with low P-availability, such as low growth rate, long lived leaves, and high foliar C:P. This is particularly relevant given increases in N-deposition and atmospheric CO_2, which may favor NM species that invest in exploiting recalcitrant inositol and diester-P pools to maintain high growth rates on soils with low P availability. Thus, a comprehensive understanding of plant species coexistence should include resource partitioning of soil organic P.

Tables and Figures

Table 1. Adapted from Anderson *et al.* (2010). Site location, tree species collected, and environmental characteristics of three 1-ha plots in lower montane forests in western Panama. Temperature is estimated using adiabatic lapse rates in Cavelier *et al.* (1996). All soil chemistry values are means of 15 samples (0-10 cm depth) ± standard errors. Values with different letters indicate significantly different means after Bonferroni correction (P<0.05).

Environmental Variables	**Chorro**	**Honda B**	**Hornito**
Substrate	Rhyolitic tuff	Rhyolitic tuff	Dactitic
Topsoil	Organic	Organic	Organic
Tress species collected	*P. oliofolius* (AM with root nodules)	*O. mexicana* (EM), *M. darienensis* (AM)	*R. montana* (NM)
Latitude (N)	8°44'58"	8°45'26"	8°40'26"
Longitude (W)	82°13'46"	82°14'37"	82°12'51"
Elevation (m)	1100	1241	1379
Temperature (°C)	20.5	19.7	19.2
Annual rainfall (mm $year^{-1}$)	4290	6153	5083
Fisher's α	1.71	3.89	2.40
Soil properties			
Bulk density (g cm^{-3})	0.08 ± 0.06^{b}	0.13 ± 0.03^{a}	0.39 ± 0.09^{a}
pH	3.91 ± 0.07	3.63 ± 0.03	5.76 ± 0.12
N:P	0.25 ± 0.03	0.36 ± 0.12	0.5 ± 0.1
Inorganic N (μg cm^{-3})	0.63 ± 0.06^{c}	0.80 ± 0.13^{b}	4.52 ± 0.99^{a}
P (μg cm^{-3})	2.74 ± 0.23^{b}	4.10 ± 0.63^{b}	10.92 ± 1.94^{a}
Al (μg cm^{-3})	316 ± 48^{b}	356 ± 58^{b}	913 ± 102^{a}
Ca (μg cm^{-3})	97 ± 22^{b}	40 ± 12^{c}	1358 ± 229^{a}
Fe (μg cm^{-3})	55 ± 11	108 ± 23	212 ± 26
K (μg cm^{-3})	18.8 ± 2.5^{b}	30 ± 5.2^{b}	96.1 ± 7.9^{a}
Mg (μg cm^{-3})	18.9 ± 3.1^{b}	26.4 ± 7.1^{b}	254.0 ± 44.7^{a}
CEC (%)	53.3 ± 6.2	37.5 ± 5.1	52.0 ± 3.1
Base saturation (%)	19.4 ± 4.3^{b}	18.4 ± 5.6^{b}	43.2 ± 5.9^{a}

Table 2. The mM concentration and chemical form of all macro and micronutrients in the hydroponic feed solution. Note that experimental treatments received one of the four possible forms of P, while the different forms of N were provided simultaneously (percentages indicate the relative mass of each form).

Nutrient	Chemical Form	mM in feed solution
Phosphorus (P)	Sodium phosphate Glucose Phosphate Phytic acid Ribonucleic acid	0.833
Nitrogen (N)	82.6 % nitrate 13.9% urea 3.5% ammonia	24.988
Potassium (K)	Potassium nitrate	8.952
Magnesium (Mg)	Magnesium sulfate	16.39
Sulfur (S)	Magnesium sulfate	16.39
Calcium (Ca)	Calcium nitrate	6.404
Iron (Fe)	Fe-EDTA	0.021
Manganese (Mn)	Mn-EDTA	0.011
Copper (Cu)	Cu-EDTA	0.001
Boron (B)	Boric acid	0.015
Zinc (Zn)	Zn-EDTA	0.001
Molybdenum (Mo)	Ammonium molybdate	.0218

Table 3. F-table of composite ANOVA for all dependent variables of the growth experiment, with leaf rows shaded when they do not explain a significant amount of variation.

Attribute	species	treatment	species × treatment	leaf
Relative growth rate	31.03***	12.78***	2.43**	4.32*
Total leaf area	95.83***	10.44***	6.25***	
Leaf mass fraction	55.058***	6.46***	1.55	
Stem mass fraction	10.41***	10.21***	2.39**	
Root mass fraction	61.16***	0.95	2.63**	
Specific leaf area	2.39	3.13*	2.55**	
P-Concentration	21.74***	39.10***	6.17***	3.95*
Total P	34.22***	14.92***	1.58	
PUE	6.25**	2.32	2.75**	

* P<0.05, **P<0.01, ***P<0.001

Table 4. F-table for within species ANOVA for all dependent variables of the growth experiment, with leaf rows omitted when they do not explain a significant amount of variation.

Attribute	Statement (degrees of freedom)	*Mollinedia* (AM)	*Podocarpus* (AM w/ nodules)	*Oreomunnia* (EM)	*Roupala* (NM)
Relative growth rate	*treatment* (4)	5.05**	5.31**	4.78**	3.90*
	leaf (1)				5.54*
Total leaf area	*treatment* (4)	4.48**	8.36**	7.18**	9.74***
	leaf (1)	4.32*			4.71*
Leaf mass fraction	*treatment* (4)	0.62	4.49**	5.33**	3.97*
	leaf (1)	5.23*			
Stem mass fraction	*treatment* (4)	4.32**	4.90**	4.48**	3.18*
	leaf (1)				
Root mass fraction	*treatment* (4)	0.63	4.54**	1.80	3.39*
	leaf (1)				
Specific leaf area	*treatment* (4)	0.21	0.53	1.50	4.87**
	leaf (1)				
P-Concentration	*treatment* (4)	5.95**	7.02**	9.51***	18.31***
	leaf (1)				
Total P	*treatment* (4)	4.66**	10.01***	2.94*	7.70**
	leaf (1)			4.36*	
PUE	*treatment* (4)	4.27**	3.17*	0.89	24.90***
	leaf (1)				

* P<0.05, **P<0.01, ***P<0.001

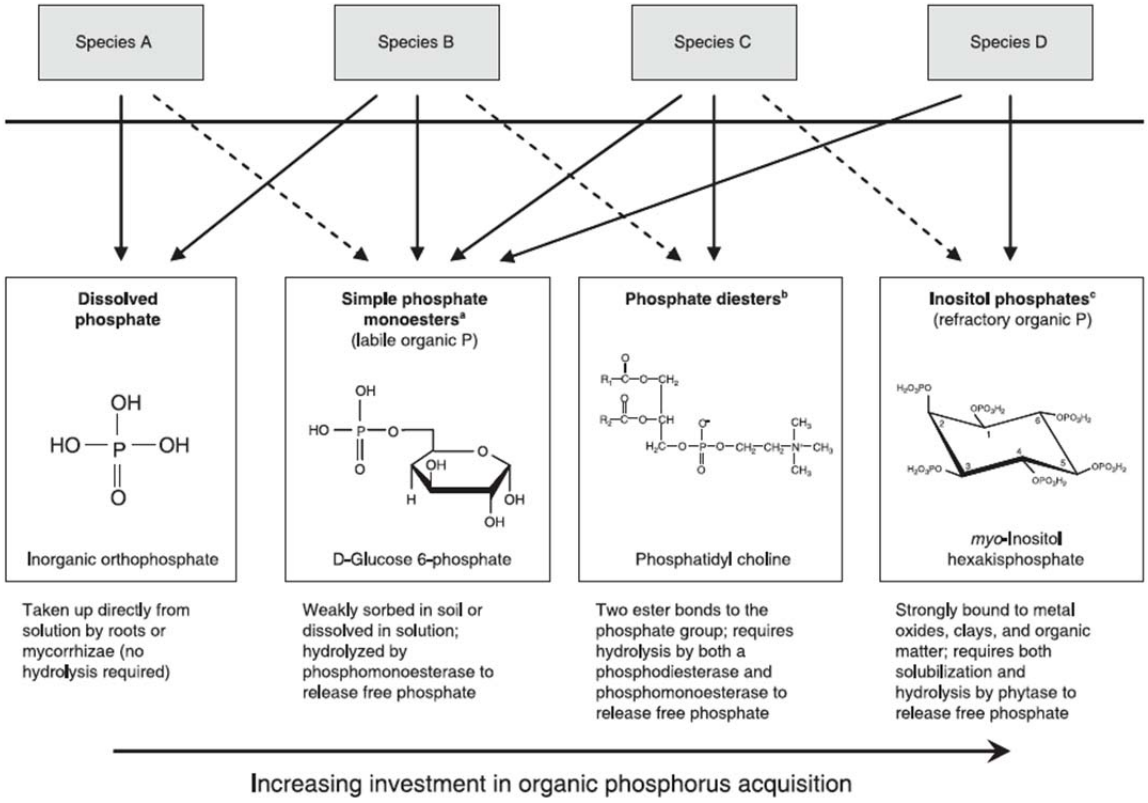

Figure 1. Adapted from Turner (2008). A conceptual diagram of different plant species (A-D) either completely (solid line) or partially (dotted line) exploiting monoesters (a), diester (b), and inositol (c) P along a gradient of increasing investment.

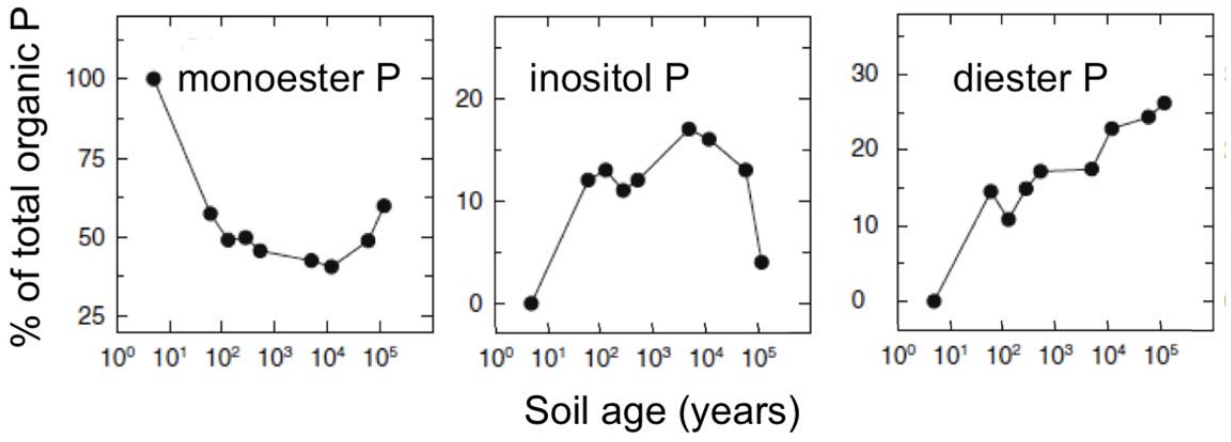

Figure 2. Adapted from Turner et al. 2007. The % of total organic P (y axis, note difference in scale) found in monoester P, inositol P, and diester P along a 10,000 year gradient of soil age (x-axis) in the Franz Joseph chronosequence, NZ.

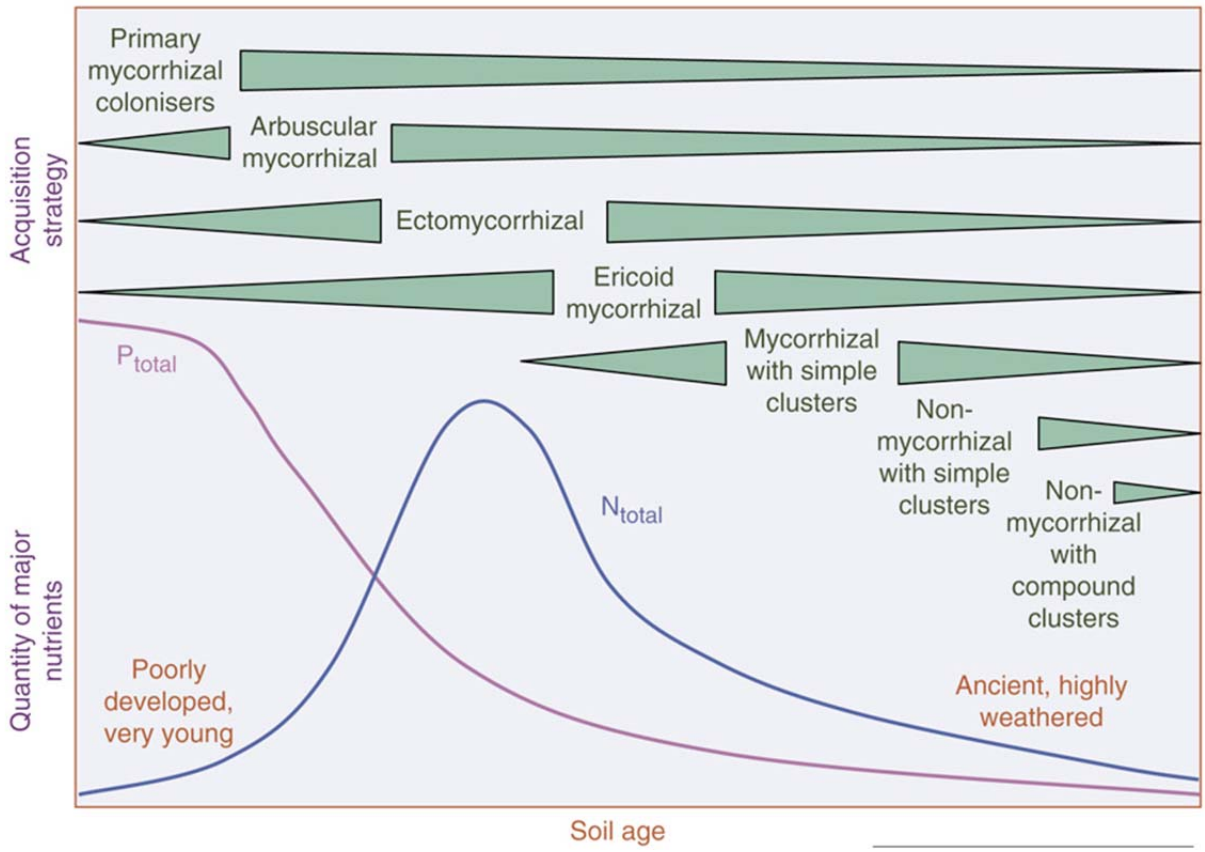

Figure 3. Adapted from Lambers *et al.* 2008. A conceptual diagram showing changes in total soil P (purple) and N (blue) along gradients of soil age. The relative abundance of plants with different P acquisition strategies is indicated by the width of the corresponding triangles.

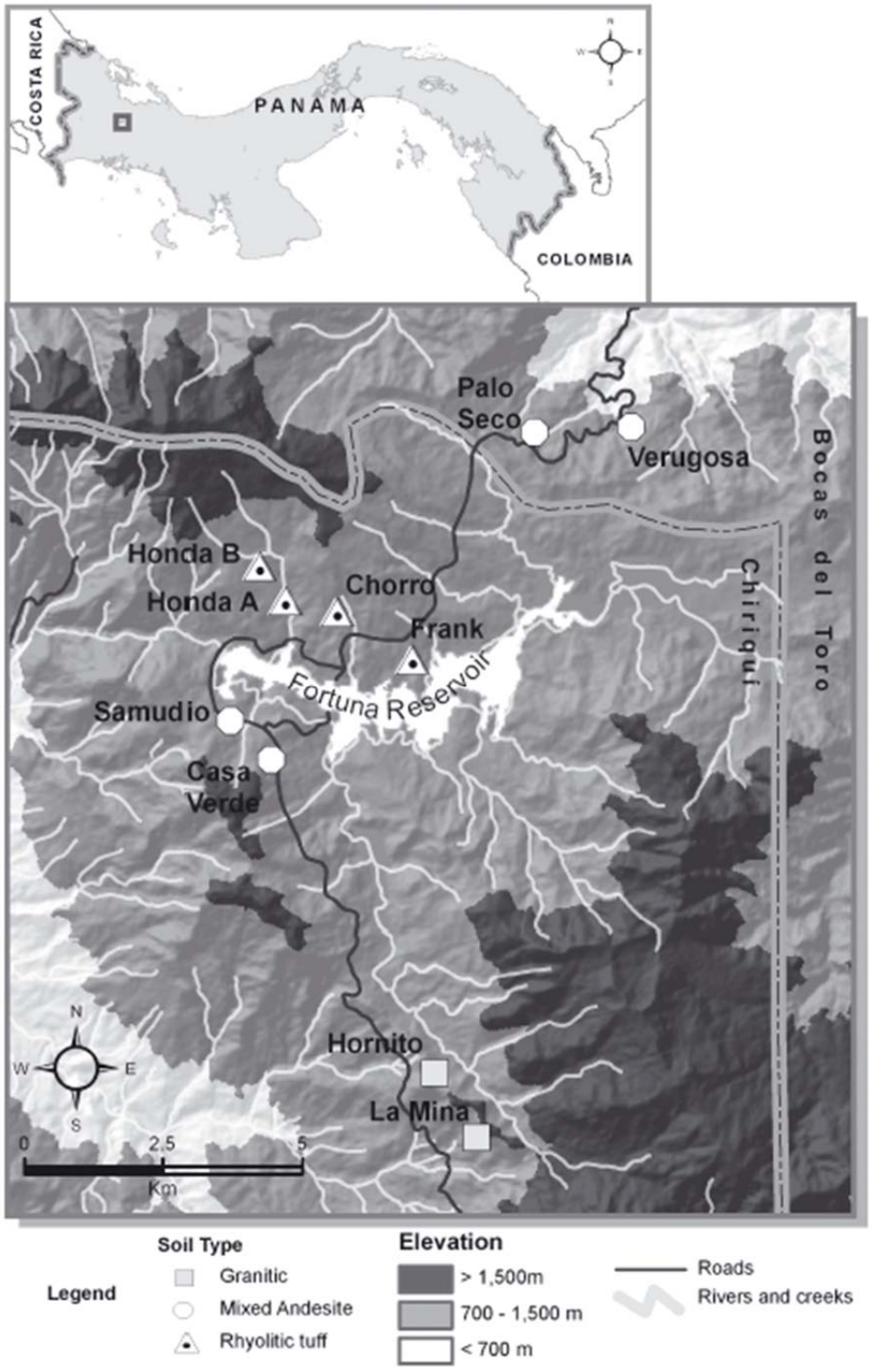

Figure 4. Adapted from Anderson *et al.* (2010). A map showing the location, elevation, and soil type of ten 1-ha plots in the Fortuna (Chiriqui) and Palo Seco (Bocas del Toro) Forest Reserves. Plant collections were made at (from top to bottom) Honda B, Chorro, and Hornito.

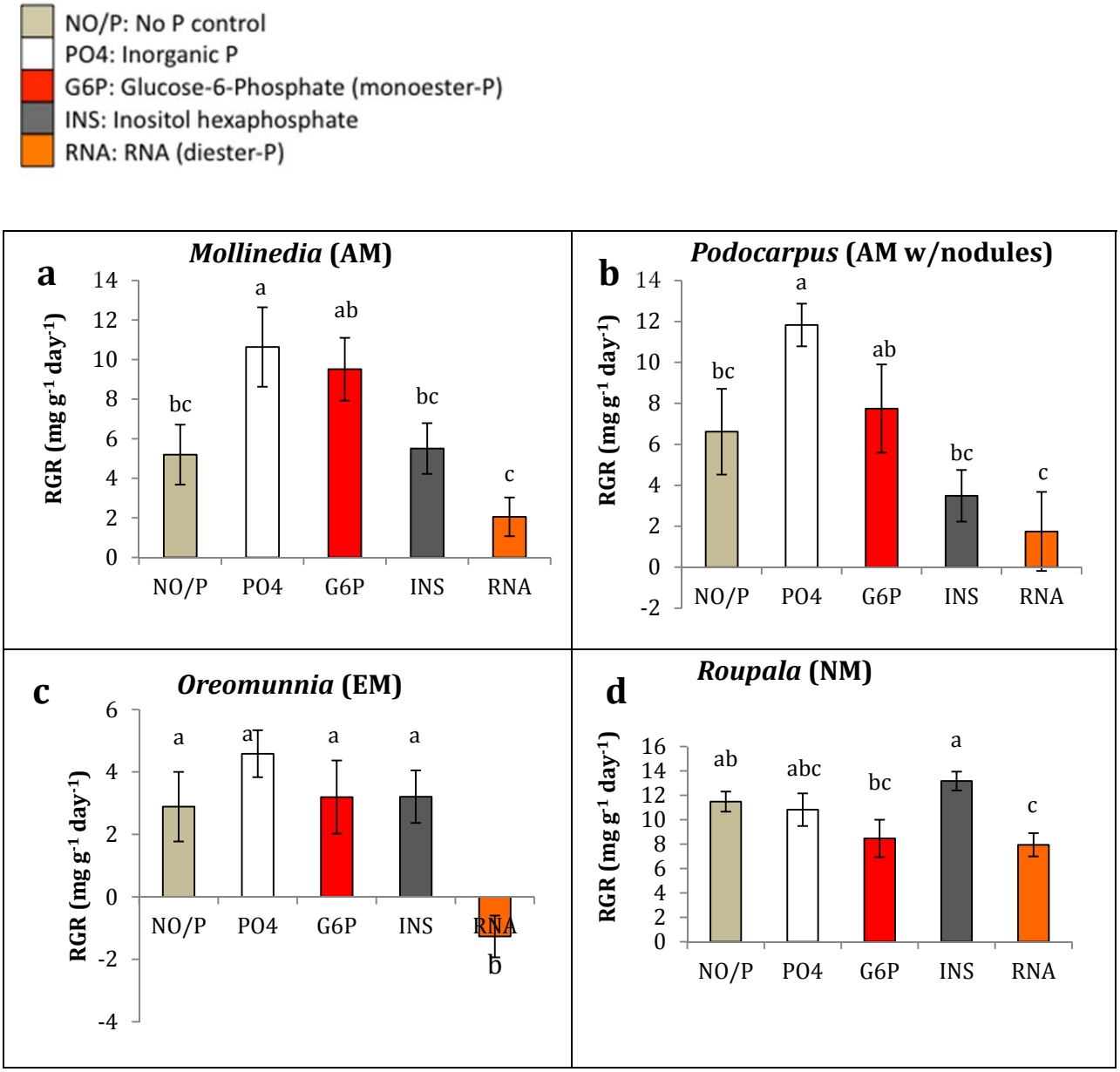

Figure 5. Mean relative growth rate +/- standard error for each treatment of (a) *Mollinedia darienensis*, (b) *Podocarpus olieofolius*, (d) *Oreomunnia mexicana*, and (d) *Roupala montana*. Within each species, bars with different letters are significantly different. Note the difference in scale for *O. mexicana*.

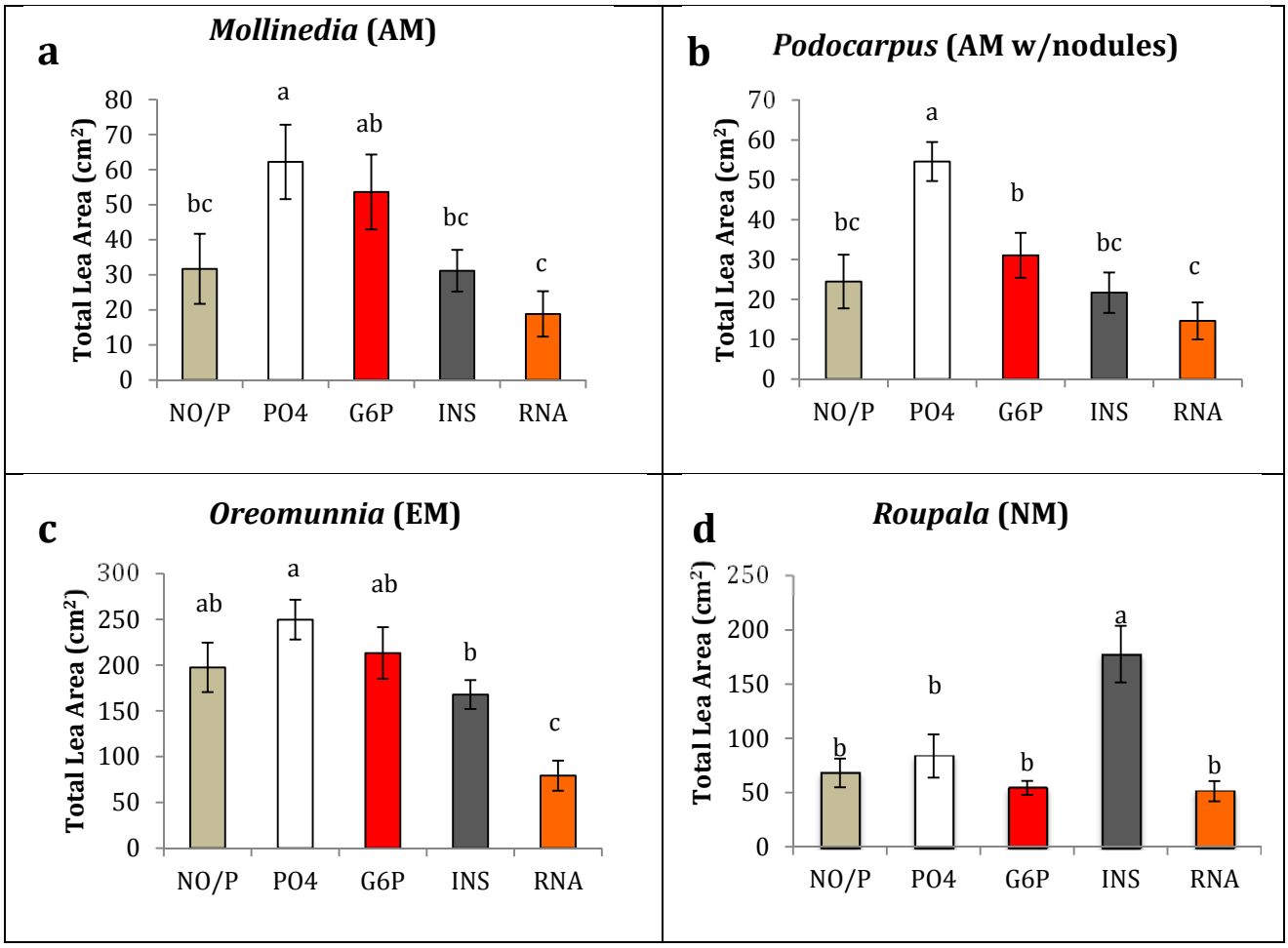

Figure 6. Mean total leaf area +/- standard error for each treatment of (a) *M. darienensis*, (b) *P. olieofolius*, (c) *O. mexicana*, and (d) *R. montana.* Within each species, bars with different letters are significantly different. Note the differences in scale.

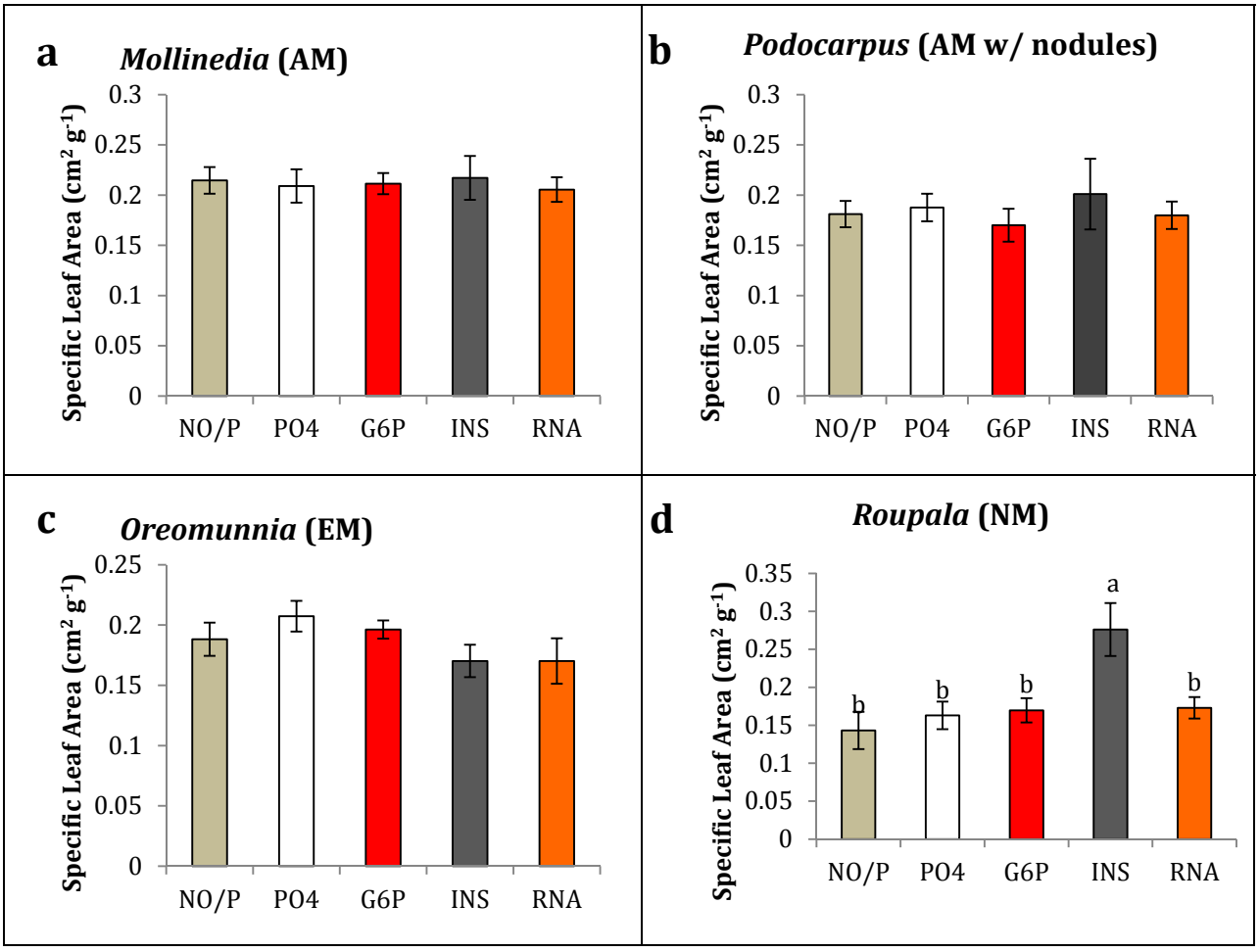

Figure 7. Mean specific leaf area +/- standard error for each treatment of (a) *M. darienensis*, (b) *P. olieofolius*, (c) *O. mexicana*, and (d) *R. montana*. Within each species, bars with different letters are significantly different. Note the differences in scale.

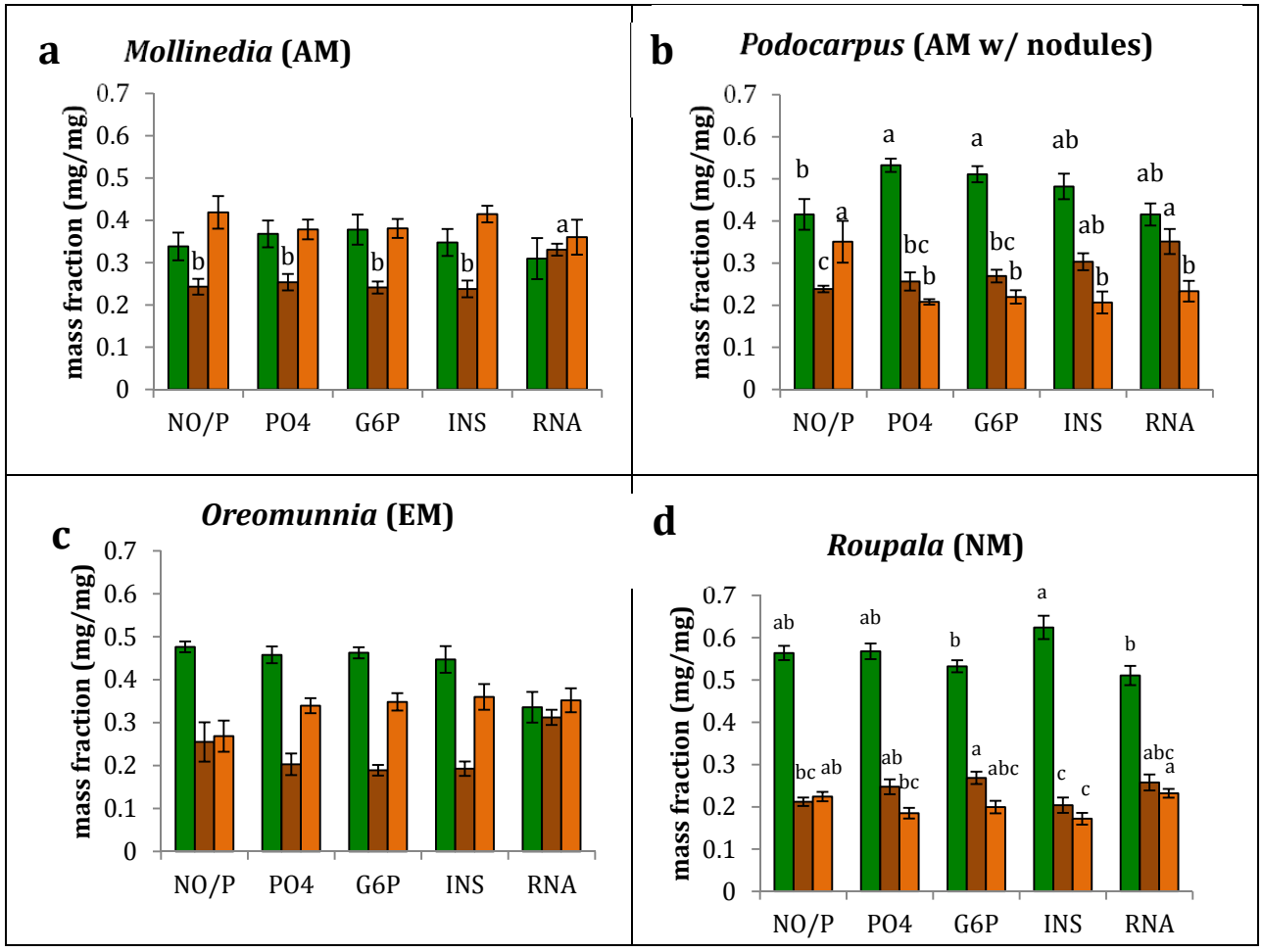

Figure 8. Mean fraction of total mass +/- standard error for the leaves, stem, and roots for each treatment of (a) *M. darienensis*, (b) *P. olieofolius*, (c) *O. mexicana*, and (d) *R. montana.* Within each species and plant part, bars with different letters are significantly different.

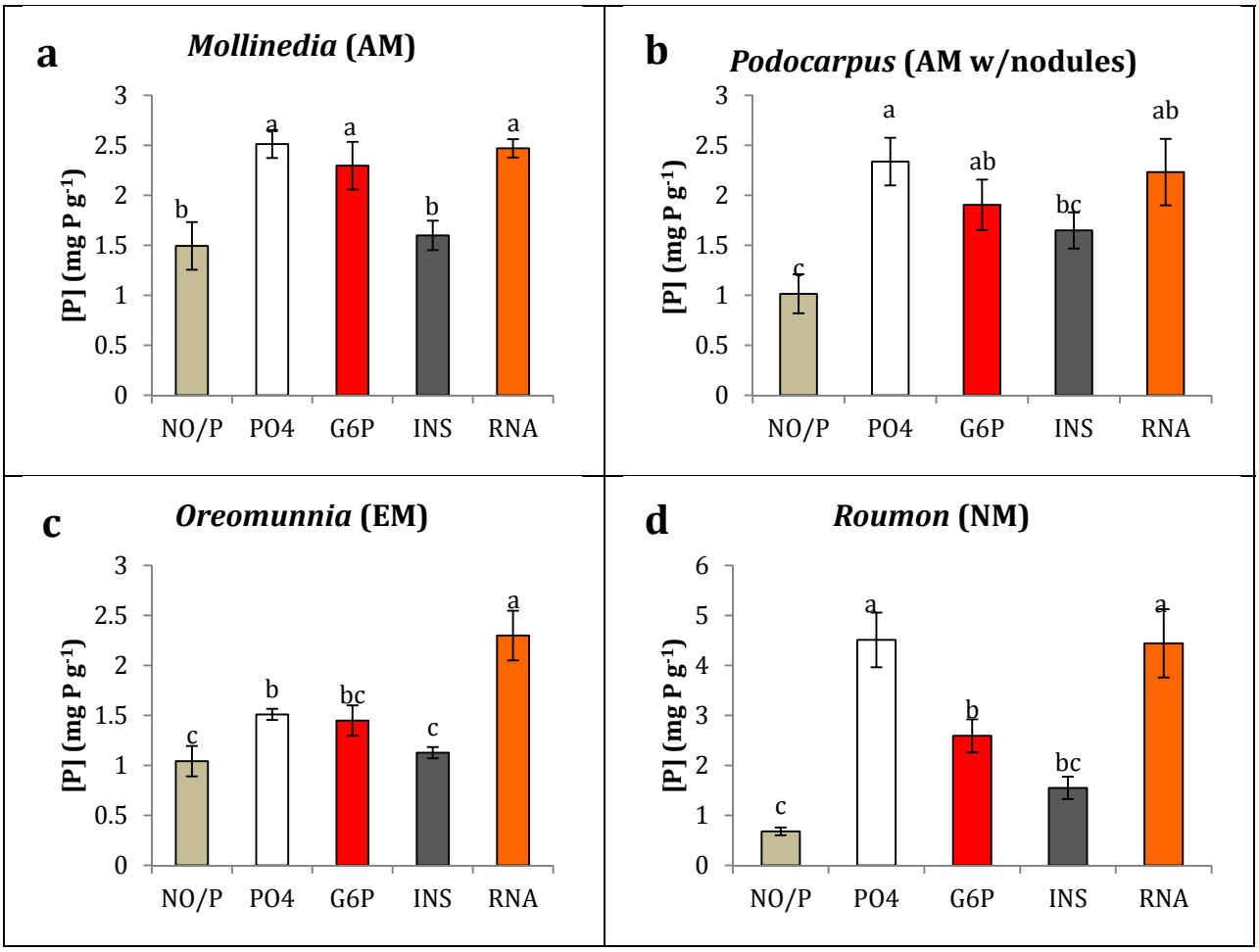

Figure 9. Mean [P] of composite samples containing leaves, stem, and roots +/- standard error for each treatment of (a) *M. darienensis*, (b) *P. olieofolius*, (c) *O. mexicana*, and (d) *R. montana.* Within each species, bars with different letters are significantly different. Note the difference in scale in *R. montana*.

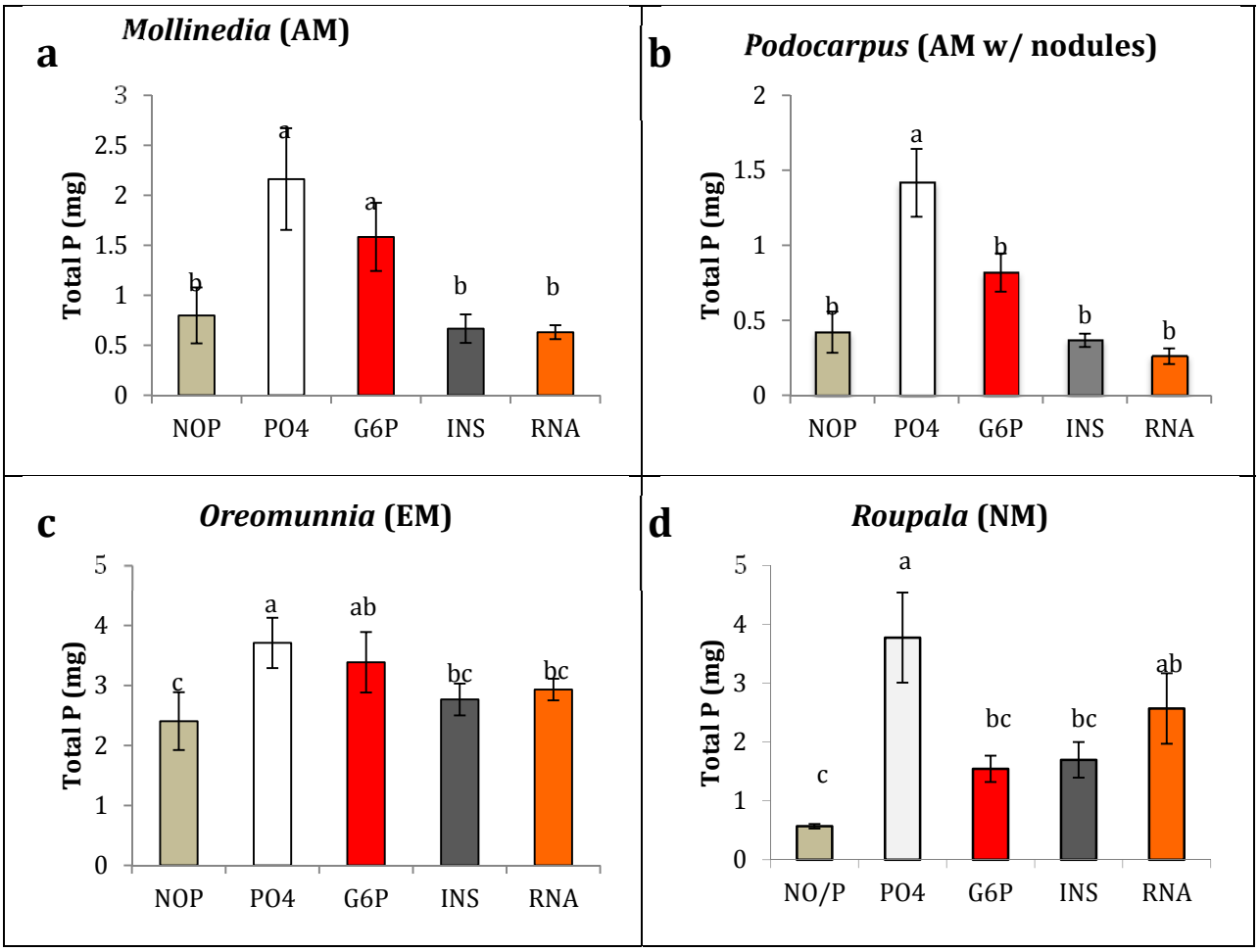

Figure 10. Mean total P +/- standard error for each treatment of (a) *M. darienensis*, (b) *P. olieofolius*, (c) *O. mexicana*, and (d) *R. montana.* Within each species, bars with different letters are significantly different. Note the differences in scale.

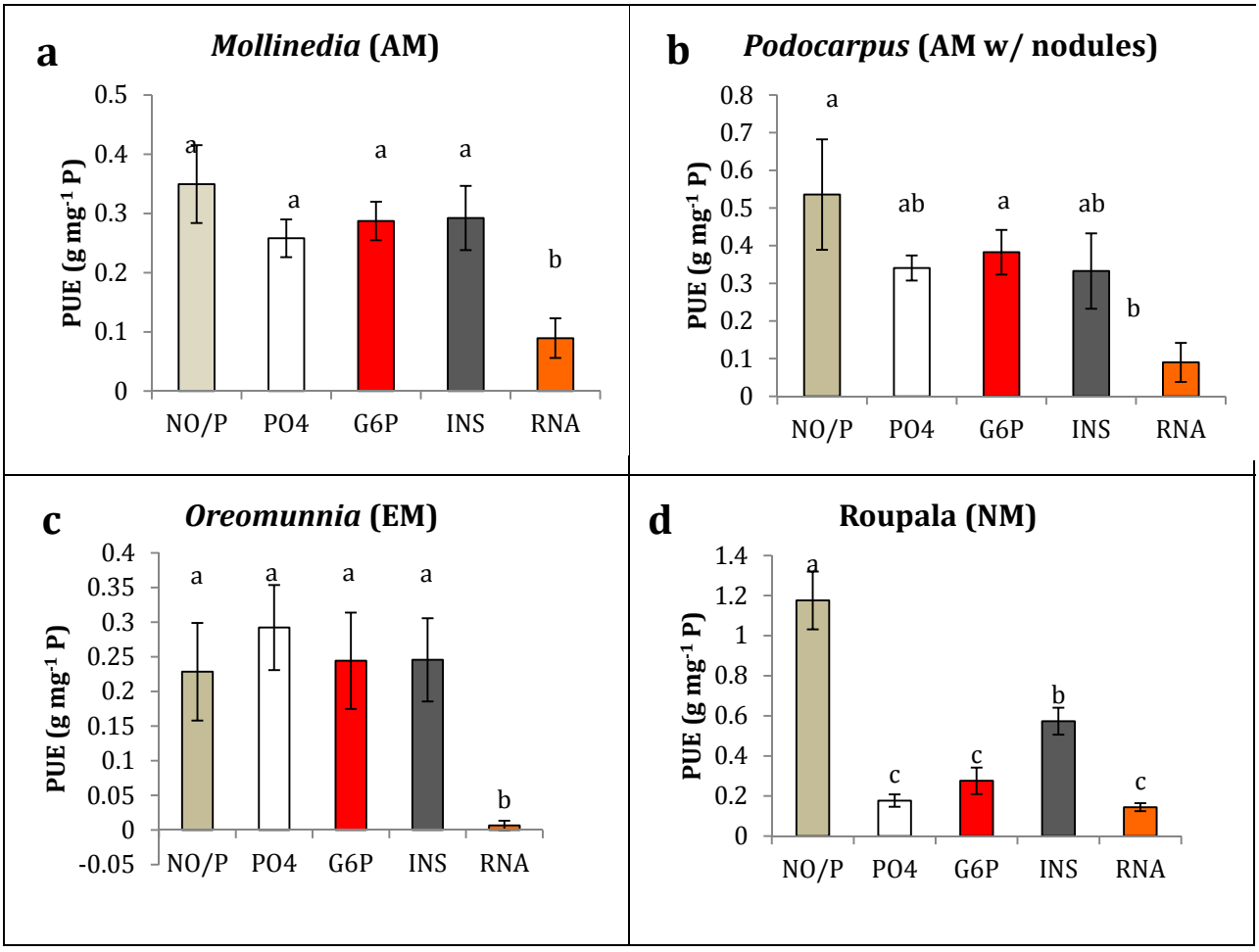

Figure 11. Mean total PUE +/- standard error for each treatment of (a) *M. darienensis*, (b) *P. olieofolius*, (c) *O. mexicana*, and (d) *R. montana.* Within each species, bars with different letters are significantly different. Note the differences in scale.

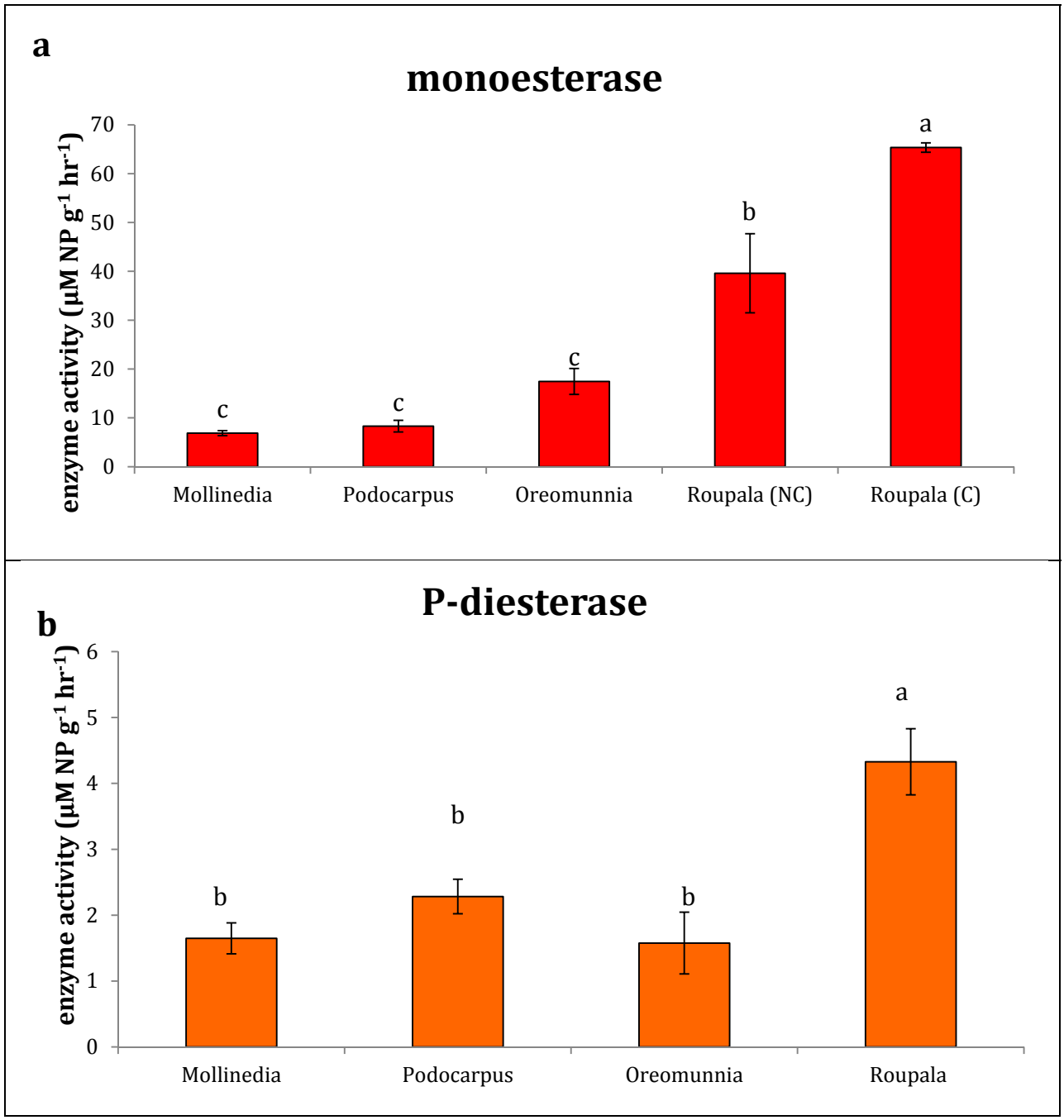

Figure 12. Mean (a) P-monoesterase and (b) P-diesterase activity +/- standard error for *M. darienensis, P. oliefolius, O. mexicana, and R. montana* fine roots (NC) and root clusters (C).

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