Biomass production, nutrients and root characteristics of fallow species and the utilization of its biomass as a phosphorus source for the common bean (*Phaseolus vulgaris* L.)

By

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Dedication

To my family for all the memorable times we have shared.

To CATIE, my friends all over the world.

To my friend Alexander Solano.

To my new friends Dr. Maren Oelbermann and Ing. Luis Crouch Bogaert
and those people in Turrialba,
who kept me alive and happy during my stay.

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Biography

Pedro Salvador Jorge Mustonen is from the Dominican Republic, born in September 1964. His elementary and high school education was conducted in La Salle School in Santo Domingo. He obtained a B.S. in agricultural engineering, in soil science at the Universidad Autónoma de Santo Domingo (UASD) in 1989. He has been managing the agronomy area at Jorge Mustonen CxA, a family consulting firm, since 1989. Most of the company projects are carried out in consortium with international consulting firms for environmental investments related to hydroelectric and agroforestry projects. From 1990 – 1992, he obtained his masters degree in agroforestry and silviculture from CATIE. Since 1994, he has taken part in the development of a construction company, CHH constructions CxA, for hydraulic projects using channel and dam technology. In May 1999, he initiated his doctoral studies at CATIE with an emphasis in agroforestry systems. He completed his PhD in 2005 under the Joint Doctoral Program of CATIE and the University of Wales (UWB). His career goals are to optimize plant growth and farm production, to establish and succeed in agroforestry farming projects, to promote the development of consulting activities in agriculture and agroforestry in association with the construction of hydraulic and hydroelectric projects.

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Jorge Mustonen, P.S. (2005). Biomass production, nutrients and root characteristics of fallow species and the utilization of its biomass as a phosphorus source for the common bean (*Phaseolus vulgaris* L.). Ph.D. Thesis, CATIE-UWB Joint Doctoral Program, Turrialba, Costa Rica, 227 p.

Key words: agroforestry, *Tithonia diversifolia* genotypes, *Cajanus*, soil phosphorus fractions, slash biomass, bean cultivars, bean yield.

Summary

Potential utility of a six-month long fallow period and the application of slash biomass from densely planted fallow species to increase soil phosphorus availability and bean yield was evaluated on a phosphorus-limited Andisol in Costa Rica. The Mexican *Tithonia diversifolia* genotype in monocultural fallow performed best for nutrient and biomass accumulation, even though the nutrient concentrations in biomass were similar among fallow species and for natural regeneration. In fact, phosphorus concentration in *Tithonia* genotypes did not exceed 6 milligram of phosphorus per gram of biomass even at high phosphorus availability (1 mM of phosphorus). Maximum biomass accumulation occurs without excessive P concentration in the plant. In *Tithonia* genotypes, biomass accumulation was positively correlated with root length density but not with leaf phosphorus concentration. Higher root length density increases plant capacity to maximize phosphorus acquisition, but root feedback mechanisms maintain an optimal level of phosphorus within the plant. The six-month fallow period and the applied slash biomass of fallow species had no consistent and significant effect toward conversion of less available soil phosphorus fractions into more readily available phosphorus fractions. However, bean cultivar yield was higher after the fallow period with the Costa Rican *Tithonia* genotype in monoculture and the application of slash biomass from fallow species; but nevertheless the application of slash biomass only increased potassium concentration in bean tissue. Additionally, the application of slash biomass did not increase the number of adventitious roots, but increased the number of root nodules in bean cultivars.

Jorge Mustonen, P.S. (2005). Producción de biomasa, nutrientes y características de las raíces de especies de barbecho y la utilización de su biomasa como fuente de fósforo para el fríjol común (*Phaseolus vulgaris* L.). Tesis de Ph.D., CATIE-UWB Programa de Doctorado Conjunto, Turrialba, Costa Rica, 227 p.

Palabras claves: agroforestería, genotipos de *Tithonia diversifolia*, *Cajanus*, fracciones de fósforo en el suelo, abonos verdes, variedades de fríjol, rendimiento de fríjol.

Resumen

La contribución de seis meses de barbecho y la aplicación de la biomasa de especies de barbecho densamente plantadas para incrementar la disponibilidad de fósforo en el suelo y el rendimiento de variedades de fríjol fue evaluada en un Andisol con limitada disponibilidad de fósforo en Costa Rica. El barbecho con el genotipo de *Tithonia diversifolia* de México en monocultivo se comportó mejor con respecto a la acumulación de biomasa y de nutrientes, pero las concentraciones de los nutrientes en la biomasa fueron similares entre las especies de barbecho. De hecho, las concentraciones de fósforo en los genotipos de *Tithonia* no fueron superiores a los 6 miligramos por gramo de biomasa aun bajo una alta disponibilidad de fósforo (1 mM de fósforo). La acumulación máxima de biomasa ocurre sin un excesivo contenido de fósforo. En los genotipos de *Tithonia*, la acumulación de biomasa estuvo correlacionada positivamente con la densidad de raíces pero no con la concentración de fósforo en las hojas. Una alta densidad de raíces incrementa la capacidad de la planta para la adquisición de fósforo, pero los mecanismos de regulación en las raíces mantienen el nivel óptimo de fósforo dentro de la planta. Los resultados indican que los seis meses de barbecho y la biomasa aplicada de las especies de barbecho no tuvieron un efecto consistente y significativo en la conversión de las fracciones de fósforo menos disponibles hacia las fracciones de fósforo más disponibles en el suelo. Sin embargo, el barbecho con el genotipo de *Tithonia* de Costa Rica en monocultivo y la aplicación de abonos verdes permitieron el mayor rendimiento de las variedades de fríjol, aunque la aplicación de abonos verdes solamente incrementó la concentración de potasio en los tejidos del fríjol. Adicionalmente, la aplicación de abonos verdes tampoco incrementó el número de raíces adventicias, pero si incrementó el número de nódulos en las raíces de las variedades de fríjol.

List of abbreviations and acronyms

AM	Arbuscular mycorrhizal
ANOVA	Analysis of variance
CATIE	Tropical Agricultural Research and Higher Education Centre
CEDAF	Centro para el Desarrollo Agropecuario y Forestal
CIAT	International Center for Tropical Agriculture
DIAS	The Danish Institute of Agricultural Sciences
DM	Dry matter
ECEC	Effective cation exchange capacity ($cmol_c\ kg^{-1}$)
ECM	Ectomycorrhizal
FAO	Food and Agriculture Organization
GLM	General linear model
HPLC	High-Performance Liquid Chromatography
ICRAF	International Council for Research in Agroforestry
ISRIC	International Soil Reference and Information Centre
LOADS	Low-molecular weight organic acids
MAG	Ministerio de Agricultura, Costa Rica
MANOVA	Multivariate analysis
NaF	Sodium floureno
NPK	Nitrogen, Phosphorus and Potassium
P	Phosphorus
RLD	Root length density ($cm\ cm^{-3}$ or $m\ mm^{-3}$)
SAS	Statistical analysis system
SRL	Specific root length ($m\ g^{-1}$)
UCR	University of Costa Rica
UK	United Kingdom
UNA	Universidad Nacional de Costa Rica
UWB	University of Wales, Bangor

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1. Introduction

Phosphorus (P) is an essential element for plant growth whose input into agricultural soils is often necessary for profitable crop production (Zhongqi *et al*., 2004), but P availability in native soils is seldom adequate for optimal plant growth (Epstein, 1972). Due to its chemical properties, P is a relatively immobile soil nutrient (Haynes *et al.,* 1991). In addition, plants need to deal with heterogeneous P distribution in soils. Hence, co-localization of root foraging and resource distribution become important factors in resource capture (Rubio *et al*., 2001). P is the second most limiting nutrient (Nwoke *et al*., 2003). It is estimated that at least 60% of the bean production areas in Latin America and 50% in Africa are severely P deficient (CIAT, 1987; Wortmann and Allen, 1994) due to the large proportion of total P unavailable to plants (Sánchez and Cochrane, 1980). Low P availability is a primary constraint for agricultural productivity in many low-input systems and represents a significant production cost in high-input systems.

This is especially true in high-input systems where soil chemical conditions "fix" applied fertilizer into less available forms over time so that continual high inputs are needed (Lynch and Beebe, 1995). However, in low-input systems in tropical latitudes, maintenance or restoration of soil fertility traditionally occurs through fallow periods (Kass and Somarriba, 1999) such as agroforestry systems that can be promising alternatives to short-fallow shifting cultivation or other mono-cropping systems (Hoang Fagerström *et al.,* 2002). Through improved management, by combining hedgerow intercropping and planted fallow, it should be possible to increase crop yields in shifting cultivation systems and, at the same time, decrease the adverse environmental impacts (Hoang Fagerström, 1998). One of the promising options for stabilizing subsistence agriculture systems could be short-duration, managed or improved fallows, in which natural vegetation is intentionally replaced by planted and managed, fast-growing N-fixing leguminous and non-leguminous species (Buckels *et al.,* 1998; Noordwijk van, 1999; Kolawole *et al.,* 2004).

The local slash/mulch system, known, as “fríjol tapado”, is the most widely low-input bean system used in Costa Rica (Araya and González, 1994; Meléndez, 2004). This system is commonly established in P-limited environments and represents 47% of the national production and covers 63% of the area sown to beans (Alfaro, 1984) but, in the last several years, the system has lost some of its former importance and presently only accounts for 24% of national bean production (Meléndez, 2004). Sepsa (2005) estimated that in Costa Rica, the national bean area is 20,267 hectares with a production of 15,088 metric tons in 2003, of which approximately 3,290 hectares are “Fríjol Tapado” (Cedeco, 2003).

Organic production has been gaining more attention by scientists, agriculturalists, and farmers as an environmentally clean production technology and especially smallholder farmers have been utilizing more organic production methods because it implies lower input costs, lower health risks, lower environment problems and higher market prices for the production. There is increased interest in making effective use of the soil phosphate reserves by seeking crop genotypes and management systems that result in more efficient soil-P uptake and utilization (Smith, 2002). Within this context, this research linked the *Tithonia* Project (University of Wales-CATIE-CIAT-ICRAF) and the Bean and Phosphorus Project (Danish Institute of Agricultural Sciences-CATIE) research objectives.

The objectives of the *Tithonia* Project (Kass, 1999) were the determination of plant mechanisms for P uptake and accumulation in *Tithonia diversifolia* (Hemsley), Gray genotypes in relation to its natural range, soil conditions and mycorrhizal infection. It also contributed to define a technical domain for *Tithonia* use as green manure. The Bean and Phosphorus Project (DIAS/CATIE, 1999), included root morphology of bean cultivars with high and low acclimation to P-limited soils and the suitability of *T. diversifolia* and pigeon pea [*Cajanus cajan* (L.) Millsp.] as green manures for the Costa Rican and exotic CIAT bean cultivars.

This study looked at biomass accumulation, nutrient concentrations and root characteristics of *T. diversifolia* genotypes and *C. cajan* in low fertility soils as potential

agroforestry species for improved fallow systems. The potential of *Tithonia* genotypes was tested for biomass utilization (mainly as a P source), P cycling and for estimating the increments in bean yields that can be expected from different green manure species. Particular emphasis was given to bean cultivar root responses to slash biomass applications and to the evaluation of the potential of the Costa Rican and exotic CIAT bean cultivars to grow in P-limited soil.

1.1 Justification

P availability is a limiting factor for plant production in many agricultural soils (Fairhurst *et al.,* 1999), especially in steep slopes in the humid tropics where many weathered volcanic ash soils (Andisols) are found (Sánchez and Logan, 1992). Management strategies are needed to maintain adequate P for plant growth while minimizing adverse environmental impacts of P (Zhongqi *et al*., 2004), such as more efficient genotypes and green manure applications. Low P availability can often be yield-limiting (Kwabiah *et al.,* 2003b) and is, in general, difficult to correct due to the fixation of P by Fe and Al oxides or allophane and recalcitrant organic matter (Sánchez and Uehara, 1980). In addition, agricultural systems in many parts of the world have limited access to fertilizers, sources of high-grade P ore for fertilizer processing are limited, non-renewable (Cathcart, 1980) and fertilizers are often too expensive (Kwabiah *et al.,* 2003a; Saïdow *et al.,* 2003) for farmers. Plant materials that can contribute to replace or minimize costly inorganic fertilizers as P sources are needed for all farming systems.

It is known that plants have a variety of mechanisms for enhancing P uptake from soils (Marschner, 1995) and understanding the nature of plant acclimation to limited P availability has relevance for both low-input agriculture and for high-input systems, where more P efficient crops and cropping systems would require less fertilizer, thus causing less environmental pollution (Lynch and Beebe, 1995). Plant mechanisms to reduce P deficiency can come from the selection of genotypes with longer and denser root hairs (Bates and Lynch, 2001), greater root density for fine roots (Haynes *et al.,* 1991; Eissenstat, 1992; Nielsen, 1997; Miller, 1998), better association with mycorrhiza

fungi (George, 2000; Godbold and Sharrock, 2003) and organic acid release (Hoffland *et al.,* 1989; Ae *et al.,* 1990; Jones and Darrah, 1994; Ström, 1998).

An alternative to reducing the severity of low P availability for crops can be the use of plant materials (as slash biomass or "green manure") of deliberately planted species for carbon and nutrient accumulation during the fallow period. Fallowing is one of the few means available for maintaining satisfactory physical and chemical soil fertility (Boffa, 1999). Small-farmers are in urgent need of technologies that enhance and accelerate fallow functions, thus providing a similar level of ecological benefits such as restoration of soil fertility or maintenance of soil productivity over a shorter time period than that with natural bush fallow (Cairns *et al.,* 1998).

T. diversifolia is native to Central America (Nash, 1976) but is now widespread in the tropics (Buresh, 1999a). It has the potential to improve agroforestry systems because it grows quickly (Kass, 1999) and is easily propagated (Rios, 2002). Additionally, *Tithonia* grows well in soils with low P availability and can potentially complement chemical fertilizers as green manure due to above normal nutrient concentrations for P, N and cations in its above-ground biomass (Buresh, 1999b; Jama *et al.,* 2000; Thor *et al.,* 2002; Sharrock *et al.,* 2004). *Tithonia* may have the potential to release adequate amount of P to replenish P in soil for crop uptake in tropical farming systems.

T. diversifolia usage in the cut-and-carry system is well known among farmers from Malang, Indonesia, but there is no information available on how much and how long *Tithonia* biomass influences soil P availability or the main mechanisms for P mobilization (Hairiah and Noordwijk van, 2000). This suggests that research should be conducted on *T. diversifolia* genotypes or other fallow species that utilize part of the soil P reserves corresponding to the less available soil P fractions or if their slash biomass can facilitate P uptake for subsequent crops. In addition, research must make special efforts to obtain more knowledge of root characterization, mycorrhizal infection and external fungal mycelium of fallow species and *Tithonia* genotypes that might contribute to explain the differences in biomass accumulation and nutrient concentrations.

An understanding of the processes of nutrient acquisition would be valuable in assessing the magnitude of variations in nutrient uptake and tissue concentrations of *Tithonia* provenances as well as developing management practices to enhance retrieval and cycling of soil nutrients in agroforestry systems (Jama *et al.,* 2000). Current research at the University of Wales, ICRAF, CIPAV and CATIE is being conducted on P accumulation in tissues of *T. diversifolia* genotype to evaluate their potential as a species for improved fallow systems (Sinclair *et al.,* 1999).

The use of organic materials as P sources is of considerable interest in smallholder farming systems in tropical and subtropical environments, mainly because of its potential as alternatives to inorganic fertilizers. For many cropping systems in the tropics, applications of P from organic and inorganic sources are essential to maximize and sustain high crop yield potential in continuous cultivation systems (Kwabiah *et al.,* 2003b). Additionally, yield of the common bean (*Phaseolus vulgaris* L.) in tropical soils is often limited by low P availability, and P deficiency is a major nutritional limiting factor to N_2 fixation in the crop (Araújo and Teixeira, 2000). Cultivars of the common bean capable of yielding well at low levels of native or added P are highly desirable for many tropical production systems (Beebe *et al.,* 1997; Smith, 2002).

Considerably higher yields, biological N_2 fixation and P solubilization would probably occur if part of the effort for improving crops and legumes for intensive agriculture systems leads to improved fallow and crop species used in the traditional fallows in Latin America which have been proven sustainable over wide areas for millennia (Kass and Somarriba, 1999). The common bean crop was chosen for this research because even though thousands of legume species exist, the common bean is consumed more than any other (Broughton *et al.,* 2003). It represents the species of choice for the study of grain legumes.

In Latin America and Africa, the improvement of grain legume productivity, such as the common bean, remains a major priority for research due to its importance as a staple food crop with a short growing cycle and its prevalence in local cropping systems for human consumption (Giller and Wilson, 1991; Beebe *et al.,* 1997; Graham and

Ranalli, 1997). Many of the concepts and processes of the common bean systems may also be relevant to other crops and wild plants (Lynch and Brown, 2001). In addition, linking nitrogen-fixing symbiosis in beans with cultivars that possess greater P acquisition efficiency would be an important contribution to food security, where many regions have low fertility soils and inadequate fertilizer inputs (Lynch, 1998).

1.2 Objectives

The general objectives are to:

- Describe the relationships of biomass accumulation, nutrient concentrations and root characteristics of fallow species in P-limited environment.
- Determine the effects of fallow species and natural regeneration on soil P fractions in weathered volcanic tropical soil.
- Compare bean cultivars in yield, growth and root characteristics where green manure from fallow species were removed, retained or transferred.

The specific objectives are to:

- Quantify biomass accumulation and nutrient concentrations for natural regeneration and *T. diversifolia* genotypes grown in monoculture and in association with *C. cajan* on an Andisol with low P availability.
- Estimate *T. diversifolia* genotype, *C. cajan* and natural regeneration nutrient uptake mechanisms such as root length density, specific root length, root hair length, internal organic acid concentrations in the root tips, fungal structures in fine roots (mycorrhizal infection) and external fungal mycelium that are potentially important for biomass accumulation and nutrient concentrations.
- Determine the effects of natural regeneration, *T. diversifolia* genotype and *C. cajan* fallows on soil P fractions and bean cultivar yields.
- Determine P concentration in *T. diversifolia* genotypes under controlled environment conditions at different P availability levels in sand culture.

- Quantify plant growth response and root growth in *T. diversifolia* genotypes under controlled environment conditions at different P availability levels.
- Quantify biomass production, nutrient concentrations and yield of the Costa Rican bean cultivars grown on an Andisol with low P availability in which fallow species biomass were removed or retained.
- Estimate the Costa Rican and exotic CIAT bean cultivar nutrient uptake mechanisms such as root dry weight partitioning (mainly adventitious and basal root dry weights), root system components (number of root nodules and root types) and root extension (root-stem length and specific root length), and fungal structures in fine roots (mycorrhizal infection) that are potentially important for yield, plant growth, P uptake and utilization efficiencies.
- Quantify biomass production, nutrient concentrations and yield of the Costa Rican and exotic CIAT bean cultivars to *T. diversifolia* and *C. cajan* biomass applications in the cut-and-carry system on an Andisol with previous calcium carbonate and poultry manure applications.

1.3 Research hypotheses

It can be expected that *T. diversifolia* genotypes, *C. cajan*, natural regeneration and bean cultivar differences in biomass accumulation, nutrient concentration or yield depend on the contributions of root system characteristics, fungal structures or intracellular composition. Some of these can be used as reliable criteria to distinguish superior fallow species and bean cultivars for biomass production, nutrient accumulation or yield in improved fallow systems.

The research hypotheses are:

1. Biomass accumulations of fallow species differ when they are grown on a weathered tropical Andisol with low P availability.
2. Nutrient concentrations of fallow species differ when they are grown on a weathered tropical Andisol with low P availability.

3. Leaf P concentration of *T. diversifolia* genotypes can exceed 10 mg P g^{-1} when P availability is increased from 10 to 1,000 μM of P in sand culture.
4. Densely planted fallows have higher efficiency rates for foraging nutrients than natural regeneration.
5. Root characteristics and the abundance of fungal structures of fallow species play a significant role for higher biomass accumulation and nutrient concentrations.
6. The ranking of organic acid concentrations in the root tips of *T. diversifolia* genotypes corresponds to the ranking of biomass accumulation.
7. Less available soil P fractions in San Juan Sur Andisol can be converted into more readily available soil P fractions during six-months of fallow period and after the application of fallow species biomass.
8. Following densely planted fallows bean cultivar yields are higher than natural regeneration.
9. Bean cultivars which received fallow species slash biomass have higher yield than the control.
10. Nutrient concentrations of the Costa Rican and exotic CIAT bean cultivars are higher under slash biomass applications on an Andisol with low P availability.
11. Fallow species slash biomass increase the number of adventitious roots and the abundance of fungal structures in the fine roots of Costa Rican and exotic CIAT bean cultivars.

2. Literature review

2.1 Nutrient supply for plants

Gruhn *et al.* (2000) pointed out that as long as agriculture remains as a soil-based industry, major increases in productivity are unlikely to be attained without ensuring plants receive an adequate and balanced nutrient supply. An appropriate environment must exist for nutrients to be available to crops in the right form, in the correct absolute and relative amounts and at the right time for high yields to be realized in the short and long terms (Gruhn *et al.,* 2000). Agricultural crops require the additions of P, nitrogen (N), potassium (K), sulphur (S) and trace elements (Cu, Zn, Mn, Mo) to the soils for higher yields. However, increasing plant adaptation for a better performance under nutrient-limited environments (Raghothama, 1999) may help to reduce the actual necessity of mineral nutrient supply (Nielsen, 1997) without affecting significantly crop yields.

Forest soils in the tropics encompass a large range of mineral nutrient availabilities, and above-ground primary productivity may be limited by atmospherically derived nutrients such as N or rock-derived nutrients such as P (Chadwick *et al.,* 1999) and cations (Marschner, 1995). By definition, mineral nutrients have specific and essential functions in plant metabolism (Marschner, 1995). The main functions of mineral nutrients such as N, S, and P are to serve as constituents of proteins and nucleic acids. However, the relative importance of different soil minerals influencing plant growth depends on specific soil conditions and plant physiological adaptations in a given site (Chapin and Cleve*,* 1989).

2.2 Nutrient uptake

Nutrient uptake is defined as the plant ability to absorb nutrients from the soil solution through roots and/or mycorrhizae. Nutrient uptake often depends on plant anatomical and physiological complexity. Physiological responses usually occur before the morphological (Drew and Saker, 1978), thus increased ion uptake may act as a signal

of locally improved soil nutrient status and trigger new root investment in the patch zone (Hodge, 2004).

The movement of inorganic ions adsorbed by the soil colloids, the root surfaces or hyphal surfaces, occurs by diffusion and mass flow (Reid, 1990). Nitrate moves via mass flow, but P mobility in soil is governed by diffusion, which makes its acquisition very dependent on the temporal and spatial exploration of the soil by the plant root system (Barber, 1995). Two processes account for increased soil exploration during vegetative growth: first, root elongation, and second, enlargement of the radius of the depletion zone around existing roots (Ge *et al*., 2000). Due to anatomical and physiological characteristics, there can be large differences in optimal and critical nutrient values for different species or biotypes, even under the same growing conditions (Drechsel and Zech, 1993).

Furthermore, the absorption of nutrients into the roots and their transport upwards to the shoots are not merely passive consequences from the transpiration-induced water flow (mass flow) but are metabolically active processes (Epstein, 1977). At chemical equilibrium, no further net movements of solute can occur without the application of a driving force. One way of accomplishing this task is to couple transport to the hydrolysis of adenosine triphosphate (ATP) (Taiz and Zeiger, 1998). Each membrane in every organism apparently has at least one kind of ATPase, whose major function in membranes is to release energy in ATP to transport ions and other solutes across the membrane against a free-energy gradient (Salisbury and Ross, 1992).

2.3 Root system functions

The root system is designed to perform a number of essential functions, including absorption, conveyance and storage of water and nutrients from the soil, anchorage of the plant in the soil (Hillel, 1998), establishment of biotic interactions at the rhizosphere (López *et al*., 2003) as well as the synthesis of plant hormones (Schiefelbein and Benfey, 1991). However, root functions change with root ontogeny (an inherently acropetal -top down- developmental process) (Zobel, 2003). Age and position in the branched hierarchy of the root system determine root function. The function of young “pioneer” or

"framework" roots changes from water and nutrient absorption (Taylor and Terrel, 1982; Smith, 2002) to transport, anchorage and production of lower order absorptive roots (Wells and Eissenstat, 2003). Therefore, a whole root system comprises root segments produced during different growth periods (Fitter, 1991) and the life expectancy of a individual root is related to its position on the branching root system, because average root diameters continuously decrease from the proximal to the distal end of any branching structure in a wide variety of plants (Pregitzer, 2002).

Higher plant structure is limited because vital functions require maximizing surface exposure rather than minimizing it, both above and below ground, since nutrient resources are normally diffused rather than concentrated (Hillel, 1998). The need for such exposure becomes apparent since the primary function of roots is to gather water and nutrients continuously from a medium that often holds only a meagre water supply per unit volume and generally contains only dilute concentrations of soluble nutrients (Hillel, 1998).

Experimental evidence supports the possibilities that nutrients are absorbed either over the entire root surface or only at the apical regions of the root axes or branches (Taiz and Zeiger, 1998). According to Clarkson and Hanson (1980) K, nitrate, ammonium, and phosphate can be absorbed freely at all locations of the root surface. However, nutrient uptake activity varies along a root axis (Russell and Anderson, 1967; Wells and Eissenstat, 2003). The young region immediately behind the meristem has greater nutrient uptake activity than other segments of the root axis, although older segments maintain some nutrient uptake activity (Gao *et al*., 1998).

Early on, fine absorptive roots exhibit high capacities for water and nutrient uptake and benefit from symbiotic associations with mycorrhizal fungi (Wells and Eissenstat, 2003). Gao *et al*. (1998) suggested that in young root systems nutrient uptake activity declined rapidly over time, while older root systems maintained a relatively low and gradually declining uptake activity as roots aged. Also as they aged, most fine absorptive roots eventually undergo pigmentation, cortical cell death, loss of mycorrhizal associations, and substantial reductions in the capacity for water and nutrient absorption (Wells and Eissenstat, 2003). However, fine root demographic studies suggest that young

white roots make up only a small fraction of total fine root length in plants during much of the favorable growing season. Older roots may contribute significantly to water and nutrient uptake. Although the water and nutrient uptake capacities of those older roots are reduced, they are not negligible, and they represent by far the largest potential surface area for uptake (Wells and Eissenstat, 2003) only beaten by the temporally emission of root hairs or white fine roots during "flushes" of favorable environmental conditions. In particular, the ratio of young roots to old roots changes with time, and root systems with a larger proportion of young root segments should generally have higher nutrient uptake activities (Gao *et al*., 1998). Thus, the evolutionary determinants governing whether or not a plant is more fit by investing in a highly suberized and lignified exodermis probably includes trade-offs between short-lived, highly absorptive roots and long-lived roots with lower absorptive capacity (Eissenstat and Achor, 1998).

2.4 P nutrition, acquisition and utilization

P is an important nutrient in crop production because it is a component of key molecules such as nucleic acids, phospholipids, and ATP, and consequently, plants cannot grow without a reliable supply of this nutrient (Schachtman *et al.,* 1998). P is used in much lower quantities than N. There seems to be a general consensus that N and P, in that order, are usually the most deficient plant nutrients in the tropics. If only legumes are considered, the emphasis shifts to P (Fox, 1978). According to Sánchez and Logan (1992) low soil P availability is a primary limitation to agricultural production in the humid tropics and subtropics.

Large applications of P as a fertilizer are required to maintain high productivity of plants (Clark and Duncan, 1991) since many soils in their native state do not contain sufficient available P to maximize crop yield (Barber, 1995). Only a small fraction of the organic P pool, about 1% per year, is mineralized, supplying inorganic P for plant uptake (Tiessen *et al.,* 1984). This creates a tight, conservative system in which P is recycled very slowly and in very small amounts (Kwahiah e*t al.,* 2003a).

In most agricultural soils, between 30-70% of the total soil P is present in its organic form (Ozanne, 1980; Marschner, 1995), which is not readily available for plants,

and under this condition P deficiency occurs. Generally, P deficiency and aluminium toxicity are two inseparable limiting factors for crop productivity on acid soils, which may account for about 40% of world arable land (Kochian, 1995). In addition, P losses from topsoil of cultivated lands by erosion can be particularly detrimental to plant growth since many tropical soils are inherently low in plant available P and the applied P is easily fixed into unavailable forms.

There is no biological process, comparable to N_2 fixation, by which P may be added to the soil (Sinclair *et al.,* 1999). However, mycorrhizae can help plants to utilize part of the fixed P. Furthermore, due to its chemical properties, P is a relatively immobile soil nutrient, making it very dependent on soil exploration by roots or their symbionts (Haynes *et al.,* 1991).

Under P-deficiency conditions, the typical plant symptoms are dark green leaves and selective growth inhibitions, affecting shoots much more than roots (Marschner, 1995). Additionally, root hairs become longer and denser (López *et al.,* 2003). In the common bean, for example, these symptoms are related to reduced cell expansion, reduced leaf initiation (Lynch *et al*., 1991) and an increased number of epidermal cells that differentiate into trichoblasts (López *et al.,* 2003).

Definitions of nutrient efficiency vary depending on the element, kind of study, and the investigator (Clark and Duncan, 1991). The term “efficiency” is used frequently to the genotypic and species differences for nutrient uptake and utilization. More efficient P utilization is an important factor in the development of more sustainable agricultural systems (Nielsen, 1997). Nutrient acquisition and/or utilization efficiencies may also be important components of plant fitness and species competition (Nielsen, 1997; Dong *et al.,* 2004). Thus, P efficiency comprises two categories: (i) efficiency in P acquisition (unit of P per specific root length) and (ii) efficiency in acquired P utilization (dry-mater produced per unit of P) (Dong *et al.,* 2004). The first category depends on root length, root length density and architecture (Nielsen, 1997; Miller, 1998), root hair length, secretion of phosphatases, organic acids and protons, alteration of root gravitropism, stimulation of adventitious roots, alteration of lateral root branching (Bates and Lynch, 2001), mycorrhizal colonization, production of aerenchyma and root respiration rate in

predominantly wet or flooded and P-limited soils (Fan *et al.,* 2003). The second category is most commonly defined as the amount of biomass produced per unit of nutrient (or simply the inverse of nutrient concentration). Therefore, high nutrient utilization efficiency indicates that more biomass has been produced per nutrient unit. This is a common plant response to low nutrient availability (Chapin and Cleve*,* 1989) and depends on P partitioning among plant parts, P reallocation within the plant from vegetative to reproductive plant organs and to yield (Rao *et al.,* 1999), P remobilization from senescent or non-productive tissues to growing or productive tissues, low P content in tissue and P reserves in seeds which mainly depend on seed size (Lynch, 1990). Smaller seed size in cultivated bean is related to lower seed P reserves, which have a significant effect on early vigour of bean seedlings under P stress (Yan *et al.,* 1995).

For a given genotype to exhibit efficient topsoil foraging, one or more individual strategies may be employed. For example, some genotypes may preferentially develop adventitious roots, while others may have more plastic gravitropism in their basal roots for a similar effect. In species other than the common bean, various root types could develop preferentially in the upper soil layers to permit more extensive topsoil exploration (Lynch and Brown, 2001). Optimum nutrient-utilization efficiency usually occurs when the tissue nutrient concentration approaches critical concentration. This is assumed to be because theoretical maximum yields should occur without excessive nutrient concentration in the plant (Clark and Duncan, 1991).

Beans show that P utilization efficiency varies among specific crosses according to parents (Whiteaker *et al*., 1976). P efficiency in chickpea (*Cicer arientinum* L.) and pigeon pea (*C. cajan*) was greater at low soil P in comparison to maize or soybean. This was explained as the ability of chickpea and pigeon pea to absorb P less rapidly, to have more extensive root-hair development, greater innate ability to solubilize soil P, and to enhance root associations with mycorrhiza (Itoh, 1987), and P re-mobilization from senescent or non-productive tissues to growing or productive tissues. On the other hand, P-deficiency tolerance may have a different genetic basis than P-utilization efficiency in some plants, since the former could be related to the ability of root exudates to solubilize P fixed in soil particle complexes (Clark and Duncan, 1991).

2.5 Root morphology, architecture and nutrient uptake mechanisms

Root morphology refers to the surface features of a single root axis as an organ, including characteristics of the epidermis such as root hairs, root diameter, the root cap, the pattern of appearance of daughter roots, undulations of the root axis and cortical senescence (Lynch, 1995). Most of the characteristics of root systems, including primary root growth and the formation of root hairs, are controlled by plant growth regulators (López *et al.,* 2003). The overall shapes of root systems are controlled mainly by genetic rather than environmental mechanisms (Hurd, 1974; Salisbury and Ross, 1992). However, the ultimate configuration of a root system under natural conditions is largely determined by environmental factors (Schiefelbein and Benfey, 1991).

Root systems extend radially from subterranean stems and grow with certain orientation with respect to gravity (gravitropism) (Evans, 1991). However, when roots develop in the soil, their architecture and demography vary because their development is plastic and responds to the exceptionally heterogeneous environment that prevails in soils, both temporally and spatially (Eissenstat and Yanai, 1997; Fitter, 1998; Grabov *et al.*, 2005) such as gravity, soil strength, mechanical impedance of substrate, microorganisms both beneficial and detrimental to root growth, interactions with other roots, availability of water, mineral nutrient availability and oxygen status. These environmental signals are translated to the differential growth of root cells through the activity of a variety of genes controlling plant morphological phenotype (Grabov *et al.*, 2005), which can vary according to plant species.

Root architecture, defined as the explicit spatial configuration of the root system (Lynch, 1995), determines soil exploration in time and space and therefore should influence the P acquisition efficiency of the root system (Nielsen, 1997). Variation in the common bean root growth and architecture has been reported with P-efficient genotypes characterized as those having vigorous, highly branched root systems with many growing root tips (Lynch and Beem van, 1993).

Root growth trajectories respond to P deficiency by decreasing gravitropism sensitivity of both basal and tap roots which led a more through exploration of the

shallow soil profile where P is more available (Miller, 1998). Root systems can only respond to spatially and temporally heterogeneous nutrient supplies because of their dynamic nature (Fitter, 1998). Therefore, root architecture generally becomes quite complex and is constantly shifting (Lynch, 1995) and also depends upon ions being immobile (such as phosphate) or mobile (such as inorganic N forms) (Hodge, 2004). Co-localization of root foraging and resource distribution becomes an important factor in resource capture in environments where limiting resources are heterogeneously distributed in the soil (Rubio *et al*., 2001), such as the typical situation in P-limited environments. Hence the "benefit" of root proliferation in phosphate-rich patches is relatively easy to interpret. As NO_3^- is highly mobile in soil it should not be necessary for roots to proliferate such as with immobile ions (Hodge, 2004). Root proliferation response to K is perhaps less commonly reported because plants are better able to redistribute K internally (de Jager, 1982).

During favorable environmental conditions for plant growth, the production of fine roots occurs at different levels throughout the growing season and is punctuated by one or more seasonal "flushes" of very high root proliferation. There is now a growing body of evidence that suggests the form of branching of fine root systems in trees is directly related to its function (Pregitzer, 2002). Some observations suggest that root growth increases in patches rich in inorganic nutrients (St. John *et al.,* 1983). In contrast, fungal hyphae growth increases in local patches of organic matter.

Plants respond to limited soil nutrients by increasing biomass allocation to fine roots, altering root morphology or increasing fine root lifespan (Bloom *et al.,* 1985; Eissenstat and Yanai, 1997), and by aerenchyma formation that reduces root respiration (Fan *et al.,* 2003). The effect of P deprivation in root systems is therefore local rather than systemic. One of the most conspicuous changes in root architecture that results from nutrient deficiency is the induction of some epidermal cells to form root hairs (López *et al.,* 2003).

These subcellular extensions from the root epidermis facilitate the acquisition of immobile nutrients by increasing the absorptive surface area of the root and allowing the root to explore a greater soil volume (Bates and Lynch, 2001; Ma *et al*., 2001). The

length and density of root hairs are extremely plastic and highly local in response to soil P availability (Bates and Lynch, 2001). Many plant species exhibit increased length of root hairs in response to P deficiency (Bates and Lynch, 1996). Gahoonia and Nielsen (1997) provided direct evidence on the substantial participation of root hairs in P uptake from the soil. For several species, root hairs are the most active of the entire root surface in phosphate absorption (Fohse *et al.*, 1991). The surface of root hairs can represent up to 70% of the total root surface of primary and lateral roots (López *et al.,* 2003). In short-lived annuals, root hairs may still confer an advantage in mycorrhizal roots since root hairs appear at the onset of radicle emergence, whereas the mycorrhizal symbiosis takes some time to develop and to become beneficial to the host plant (Smith and Read, 1997).

Under low P soil conditions a lesser branched root architecture is more efficient (Nielsen 1997, Nielsen *et al.*, 1994) because greater volumes of soil could be explored at low cost until resources are found (Lynch and Brown, 2001). Roots prefer to grow into new soil regions, avoiding the formation of additional branch or feeder roots, which only occur in localized regions with more available nutrients. No lateral branches of high developmental orders are formed within the depletion zone, leading to low root density and relatively low inter-root competition (Nielsen, 1997).

2.5.1 Bean root system

In crops such as beans, the root system is typical of annual dicots (Lynch and Brown, 2001). It is composed of four root types: adventitious roots, basal roots, taproot, and lateral roots arising from the first three types. The taproot has strong positive gravitropism and usually goes straight downwards. Adventitious roots emerge from the subterranean portion of the hypocotyls and grow horizontally to explore soil volumes closer to the soil surface. Basal roots arise from the basal part of the root system (Nielsen, 1997; Miller, 1998; Rubio *et al.,* 2003) and account for a large portion of it (Liao *et al*., 2001). They form the skeleton or scaffold upon which much of the mature root system develops as a set of lateral roots (Zobel, 1975; Stoffella *et al.*, 1979). Adventitious roots have less lateral branching and more horizontal growing planes than basal roots which serve to disperse root foraging over larger soil volumes for a given metabolic investment.

However, all parts of the bean root system are involved in topsoil foraging, and all are subject to regulation by P availability (Lynch and Brown, 2001). Basal roots can be plastic in terms of growth rate, branching and growth angle in response to low P availability (Lynch and Beem van, 1993). The impact of basal root gravitropism on plant performance in the field is more complex, because of tradeoffs between topsoil resources, such as P with deeper resources such as water, as well as competitive interactions with neighbouring plants (Rubio *et al*., 2003). Comparisons of contrasting bean genotypes in controlled environments and in the field show that plants with better topsoil foraging have superior P acquisition and growth in low P soils (Lynch and Brown, 2001).

Efficient genotypes have either a higher number of adventitious and basal roots or the roots have a higher specific root length (SRL) and therefore possibly a lower construction cost (Eissenstat, 1992). Adventitious roots have greater SRL defined as root length per unit dry weight and lower construction cost than other root types (Miller, 1998). A high SRL represents an effective use of resources to maximize soil contact. In general, SRL seems lower in dicotyledonous species than in monocotyledonous species (Atkinson, 2000). However, trees develop more extensive root systems than annual crops that allow them to have higher ability to obtain both water and mineral nutrients from the soil.

In species other than the common bean, various root types can develop preferentially in the upper soil layers to permit more extensive topsoil exploration (Lynch and Brown, 2001). Because the common situation is that P is concentrated in the topsoil, shallow rooted genotypes have a growth advantage (Rubio *et al*., 2003). If P is homogeneously distributed down soil profile, a shallow root system does not confer any growth advantage.

Shallower root systems explore more soil per unit of root biomass than deeper systems, because shallower systems have more dispersed basal roots and therefore less inter-root competition, which occurs when neighbouring roots have overlapping P depletion zones (Ge *et al*., 2000). Some genotypes may preferentially develop adventitious roots, while others can utilize plastic gravitropic responses of their basal root

systems for a similar effect. In the field, shallow rooted recombinant inbred lines are more productive than deep-rooted recombinant inbred lines (Rubio *et al*. 2003).

2.5.2 Plant growth rate and nutrient accumulation

The nutrient accumulation rate of the plant is a measurement of the physiological potential of the plant to absorb nutrients from the environment (Chapin and Cleve, 1989). The rate at which roots remove nutrient ions from the rhizosphere depends on the plant growth rate and on its previous nutritional history (Drew, 1990), and on its ability to maximize nutrient uptake though fine roots and mycorrhizas. Trees accumulate nutrients as their biomass increases, because nutrients are incorporated to the new organs. On a dry weight basis, the highest accumulations of nutrients are in the foliage during the plant's first growing phases (Drechsel and Zech, 1993).

Genotypes that grow well on soils of low nutrient availability not only may have a higher rate of uptake and translocation of a particular mineral nutrient, but may also show higher nutrient utilization efficiency at the cellular level (compartmentation, binding stage, etc.), including high rates of re-mobilization from older to younger leaves, seeds, and storage organs (Marschner, 1995). Evidence is accumulating that inherently fast growing species from nutrient-rich habitats display a higher degree of root morphological plasticity in response to nutrient enrichment than inherently slow growing species from nutrient-poor habitats (Cadwell *et al.,* 1992), but slow growing species are assumed to maintain large, long-lived root systems which remain viable under prolonged periods of nutrient stress and enable them to instantaneously increase the nutrient pulses (Grime *et al.,* 1986).

2.5.3 Plant plasticity

Plant plasticity is the mechanism whereby plant responses vary to any stress as the need arises (Liao *et al*., 2001). Variability of root system development enables plants to cope with a wide range of soil factors and heterogeneity and patchiness of the soil (de Kroon and Hutchings, 1995). As field soils are heterogeneous, their biological, chemical and physical properties vary in both time and space. Plant root systems are able to adjust

to such variation in soil conditions through their physiological and morphological plasticity (Hodge, 2004). However, because it is an inducible or facultative response, it can be reversed in anatomical (e.g. the turnover of root hairs and fine roots during less favorable soil conditions) and physiological aspects for better acclimatation to the available resources when the stress has been abated. Plant characteristics that influence performance have different opportunities for expression in different years (Ludlow and Muchow, 1989) according to the variability in soil conditions. Plasticity is an important response characteristic for intermittent environment stress.

Eissenstat and Caldwell (1988) and Campbell and Grime (1989) found preferential root proliferation in favorable (fertilizer) micro sites, but root reactions also depended on element mobility in the soil (Fitter, 1987). Physiological plasticity is generally assumed to be more important in enhancing capture of mobile ions, such as NO_3^-, as NO_3^- uptake is not limited by diffusion in the soil but by its uptake at root surface (Hodge, 2004). By contrast, immobile ions such as phosphate (Haynes *et al.,* 1991) are more limited by diffusion (Barber, 1995; Marschner, 1995) to the root surface, so enhancing ion uptake at root surface will not greatly enhance phosphate capture. Plants may need to respond rapidly to increase capture of mobile ions before they diffuse to other roots, while a rapid response is less crucial for immobile ions, allowing time for new root construction (Hodge, 2004). Both physiological and morphological plasticity come at the price of ATP expenditure to maintain high ion-uptake capacities, construction of new roots or increased extension rates of existing roots. However, the cost of maintaining uptake systems of higher affinity and ability to move ions across cell membranes is likely to be small relative to those of constructing and maintaining roots (Eissenstat and Yanai, 1997). To date, the most extensively studied morphological plasticity is the response of roots to localized patches of mineral nutrients (Bingham and Bengough, 2003).

Differential root hair growth in response to different environmental conditions is a possible example of root plasticity for the purpose of acquiring essential resources. Since root hairs are outgrowths of single root epidermal cells, the plasticity response of root hairs is relatively faster than root growth and root branching. Therefore, root hair growth

may represent the most immediately beneficial morphological response that roots have for changing environmental conditions (Bates and Lynch, 2000).

On this point dicotyledons (including hyperaccumulators) exhibit a higher plasticity than grasses (Eissenstat 1992; Taub and Goldberg, 1996). However, roots experiencing nutrient-rich patches can also enhance their physiological ion-uptake capacities compared with roots of the same plant outside the patch zone (Hodge, 2004). These plastic responses by root systems have been proposed as the major mechanism by which plants cope with the naturally occurring different supplies of nutrients in the soil. This exploitation is aided by the modular structure of the root system, which enables exceptional flexibility in architectural patterns and allows root development in nutrient-rich zones (Hodge, 2004).

2.5.4 Rhizodeposition

The release of carbon compounds from living plant roots (rhizodeposition) and the consequent proliferation of microorganisms in the surrounding soil, coupled with the physical presence of a root and processes associated with nutrient uptake, gives rise to a unique zone of soil called rhizosphere (Jones *et al.,* 2004) having different chemical, physical and biological characteristics to bulk soil (Barber, 1995). Theoretically, almost any soluble component present in the root can be lost to the rhizosphere. However, current evidence suggests that exudation is dominated by low molecular weight solutes such as sugar, amino acids and organic acids that are present in the cytoplasm at high concentration (Farrar *et al.,* 2003). Apart from sugars and amino acids, organic acids typically represent the next largest exudates group (Jones *et al.,* 2003a). The most common low-molecular weight organic acids (LOAs) identified in soils are oxalic, succinic, tartaric, fumaric, malic, citric, and salicylic.

There are two exudate classes: exudates which are lost simply as a result of passive diffusion and over which the plant exerts little control (basal exudation); and exudates which are released for a specific purpose and over which the plant exerts a close degree of control (Jones *et al.,* 2004). Based upon a range of studies, it is likely that basal root exudation constitutes approx. 3-5% of carbon fixed in photosynthesis (Pinton *et al.,*

2001). Small amounts of organic acids enter the soil from atmospheric deposition and through fall from canopy. Most organic acids in soils arise from root exudation and lyses and by release from soil microorganisms (Millet *et al.,* 1997; Ryan *et al.,* 2001). LOAs are naturally produced in soils as microbial metabolites or plant exudates from living or dead cells (Kpomblekou and Tabatabai, 2003).

The major factors that may influence the kind and quality of organic acids are plant species, plant age and light intensity (Hale and Moore, 1979). Root exudates are generally released in greater quantities from plants experiencing P deficiency and are caused by an increase in membrane leakiness. Replenishment of the soil organic acid pool depends almost entirely upon continued release from either plant or animal cells and can therefore be expected to be more temporally variable (Jones *et al.,* 2003b). Stevenson (1967) found that LOAs reach their highest equilibrium level during active plant residue decomposition. The concentration of organic acids may also be very spatially localized due to the very low diffusion coefficients of most organic acids in soil, the size of these hot spots may be only a few μm in diameter if release occurs from the tip of a root hair, fungal hyphae or bacterial cell (Jones *et al.,* 2003b). In addition, a possible explanation for the low organic acid concentrations detected in soil solutions is their continual removal by the soil microbial community (Jones *et al.,* 2003b) because organic acids are a readily utilizable source of carbon. Unlike long-chain fatty acids that may persist in soils for long periods of time, LOAs have a transitory existence; the amount present in the soil at any time is a balance between synthesis and destruction processes controlled by microorganisms. A number of studies have shown that the rate of organic acid turnover in soil is extremely rapid with half-lives ranging from 1 to 5 hours in organic topsoils, to 5 to 12 hours in subsoils (Jones, 1998).

Organic acids have been hypothesized to perform many functions in soils, including root nutrient acquisition, mineral weathering, microbial chemotaxis and metal detoxification (Jones *et al.,* 2003b). High root exudation rates of LOAs from plants could be an adaptation to limiting nutrient conditions in the soil (Ström, 1998). Although, Merckx *et al.* (1987), studying rhizodeposition in soils with different nutrient availability levels, showed that soils with low available nutrients had lower organic rhizodeposition

incorporated into microbial biomass compared with high nutrient availability soils. Despite that, rhizodeposition may still improve phosphate nutrition.

The mechanisms to access poorly available inorganic P, mainly in low nutrient soils, focus on the release of organic acids (citrate, malate) and H^+ ions into the rhizosphere to dissolve P containing minerals and to remove P from the soil exchange phase. This enhances the amount of P in the soil solution, thus facilitating root access (Sinclair *et al.,* 2000). This excretion may or may not be associated with dramatic changes in root morphology and plant carbon partitioning (Dinkelaker *et al.,* 1989). However, the role of organic acids in P acquisition is often viewed in isolation from other potential mechanisms for increasing plant P uptake such as mycorrhizas, root hairs, P transporter up-regulation, phosphatases, and alterations in H^+-ATPase activity (Jones *et al.,* 2004). Root exudates can also stimulate hyphal growth and branching rather than spore germination *per se* (Jones *et al.,* 2004).

Fitter (1998) found that root exudates, such as citrate, have altered ion solubility in the rhizosphere. Malate, citrate and oxalate may play a role in many operating processes in the rhizosphere, including nutrient acquisition, metal detoxification, anaerobic stress alleviation in roots, mineral weathering and pathogen attraction (Jones and Farrar, 1999). It has been shown that under P deficiency, roots of a variety of plants release large quantities of organic acids such as malic, citric or piscidic acids (Hoffland *et al.,* 1989; Ae *et al.,* 1990). After release, these organic acids can directly affect the behaviour of inorganic P in soils in a variety of ways: they can block P sorption sites in soil thereby enhancing the rate of P diffusion; they can directly displace P from sorption sites; they can complex metal cations on the sorption surface, causing a loss of sorption site; and they can complex cations on mineral surfaces, releasing P into solution (Jones 1998). In addition, organic acids can bring organic P into solution, which then becomes available (Gerke, 1993).

Organic anions formed by the decomposition of organic additions can compete with P for the same adsorption sites and thereby increase P availability in soil (Nziguheba *et al.,* 1998). The anions might block exchange sites and serve as substrates for microbial activity. The reduction in P adsorption apparently resulted from competition for

adsorption sites by organic anions produced during the high quality *T. diversifolia* green manure decomposition (Nziguheba *et al.,* 1998). Geelhoed (1998) found that at pH of 6, citrate clearly reduced P sorption and increased the P concentration in the soil solution. For a pH greater than 6, the impact was small or absent.

In species such as lupines (Fabaceae) and some sedges (Cyperaceae), citrate secretion may be a key element in their phosphate acquisition strategy, notably when it is combined with the morphological development of cluster roots (Lamont, 1993; Dinkelaker *et al.,* 1995). The ability of pigeon pea (*C. cajan*) and soybean (*Glicine max* [L.] Merr.) to efficiently utilize ironbound P was attributed to the root exudates, mainly piscidic acid and its derivatives (Ae *et al.,* 1990). P deficiency induced mainly oxalate and malate whereas aluminium toxicity induced solely citrate in two contrasting soybean genotypes (Dong *et al.,* 2004). Baxi 10, a known P-efficient soybean tended to excrete much more oxalate than P-inefficient Bendi 2 under P starvation (Dong *et al.,* 2004).

Some of the exudates have no capacity to mobilize soil P (e.g. sugar) but can activate microorganisms. Other organic acids can potentially mobilize significant quantities of inorganic soil ions (Jones and Farrar, 1999). Among them, oxalic acid is the most effective in releasing P from low reactive phosphate rocks (Kpomblekou and Tabatabai, 2003). Comparison of the amounts of P released from the low reactive phosphate rocks using various organic acids indicated that the tri-carboxylic acids (cis-aconitic and citric acids) and the di-carboxylic acids (oxalic, malonic, fumaric, and tartaric acids) were more effective in complexing metal ions in low reactive phosphate rocks and releasing more P than the mono-carboxylic acids (glycolic, pyruvic, and salicylic acids) (Kpomblekou and Tabatabai, 2003). However, under greenhouse conditions, organic acids did not have a positive effect on dry-matter yield when mixed with high reactive phosphate rocks. When mixed with low and medium reactive phosphate rocks, maize dry-matter yield significantly increased but neither organic acid was consistently better than other (Kpomblekou and Tabatabai, 2003).

Investigations on the role of synthetic organic acids in the mobilization of plant nutrients in seven contrasting soils indicated that malate was poor at mobilizing micronutrients, whilst citrate was capable of mobilizing significant quantities (Jones and

Darrah, 1994). The cations Na, K and Mn were not mobilized to any extent in any of the soils either by malate or citrate. The amounts of Ca and Mg mobilized were similar for both malate and citrate. On average, for every μmol of citrate added to the soil, 0.8 μmol of cations were mobilized (Jones and Darrah, 1994). The highest soil specific reaction for P mobilization was only about 0.03 μmol of P released for every μmol of citrate added. The release of organic acids in response to P deficiency can only be expected to be significant in soils which possess a large acid soluble Ca-P component (Jones and Darrah, 1994). Laboratory studies showed that the organic acids are effective in releasing P from low and medium reactive phosphate rocks, but very ineffective in releasing P from high reactive phosphate rocks (Kpomblekou and Tabatabai, 2003).

2.5.5 Mycorrhizae

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs which live symbiotically in the roots of host plants (Giovannetti, 2000) and often are considered as a plant adaptation to low soil P conditions (George, 2000). AM are the most widespread symbiotic association, occurring in two-thirds of all land plants. Moyersoen *et al.* (1998) pointed out that the distribution of AM fungi colonization in different plant species in a tropical rainforest was not related to specific edaphic parameters. Ectomycorrhizal (ECM) and AM can occur, with wide variations of colonization, throughout the litter, soil organic and mineral horizons.

Kling and Jakobsen (1998) indicated that AM fungi constitute a living bridge for nutrient transport from the soil to the plant root. The AM group of soil microorganisms is of most direct importance for nutrient uptake in herbaceous plants. Allowing colonization by AM fungi is not a plant response to a specific environmental stress, it is part of the normal life cycle of most plant species (George, 2000) and AM fungi have evolved and survived for 400 million years (Giovannetti, 2000). Read (1984) suggested that mycorrhizal fungi may be the largest microbial biomass component of many tropical soils. Allen (1991) stated that mycorrhizal fungi might be the single largest consumer group of net primary production in many, if not in most terrestrial biomes. So far, strong evidence has been found that plants evolved with mycorrhizae in natural ecosystems and

are highly dependent on them for their contributions to growth, health and sustainability (Varma, 1998).

The role of mycorrhizae in the rhizosphere should be conceptualized in the context of the importance of soil nutrient availability to the success of plants in their terrestrial habitats (Reid, 1990). The benefits of mycorrhizae for improving plant nutrient status are largely related to the hyphal net extramatrical mycelia in the soil, providing a large surface for nutrient uptake (Rousseau *et al.,* 1994). Mycorrhizae should: (i) be less expensive to construct than fine roots; (ii) be able to detect and respond to short-lived nutrient patches or pulses that roots cannot detect; and (iii) be able to penetrate to the sites of patch decomposition and therefore be able to compete directly with other soil microorganisms for the nutrient released (Hodge, 2004). For example, because of a very effective association with mycorrhizae, cassava (*Manihot sp*.) can grow well in very low P soils.

AM fungal hyphae grow at least 12 cm from the root surface (Li *et al.,* 1991) and make up over 80% of the total hyphal length in the soil (Kabir *et al.,* 1996). AM hyphal length densities in soil are often between 2 to 25 m g^{-1} (Li *et al.,* 1991; Ravnskov *et al.,* 1999; Schweiger *et al.,* 1999) with a rapid turnover of external hyphae, measured to be 5-6 days (although AM runner hyphae may persist for up to 30 days) and a large input of carbon into the soil environment (Staddon *et al.,* 2003). ECM trees have a more competitive advantage than AM trees in the absorption of nutrients, such as P (Moyersoen *et al*., 1998) because some ECM have rhizomorphs and greater hyphal development than AM (Bending and Read, 1995). This is particularly true when the nutrients are poorly soluble and in low concentrations.

Nutrient uptake efficiency due to the increased adsorbing surface area is likely to be one of the most important features of mycorrhizae (Reid, 1990). Through mycorrhizae, plants have developed strategies that increase the absorbing surface area of the root with the least amount of energy expenditure (Reid, 1990). Some organic P may be directly accessed by the roots or the associated mycorrhizal partners through phosphatase enzyme excretion into the soil (Tarafdar and Claassen, 1988). In this context, subapical zones of branched hyphae may be of particular importance for P

uptake (Bago *et al.,* 1998). Mycorrhizal fungal symbionts have transported the ion to the root surface from beyond the depletion zone surrounding the root via its hyphae (Fitter, 1998). Formation of polyphosphates in the hyphae allows for high P transport rates (Saito, 2000). More than 70% of the total plant P content may be taken up by hyphae in compartment boxes with separate growing zones for roots and hyphae (George *et al.,* 1994). However, the rate of phosphate absorption by fungal hyphae is controlled by the fungal P status (Chapin and Cleve*,* 1989).

Plant root colonization by AM fungi can greatly increase plant nutrient uptake as compared with non-mycorrhizal ones (Reid, 1990) probably because root surface and plant metabolism is enhanced in mycorrhizal roots (George, 2000). This is expected due to higher metabolic activity in mycorrhizal versus nonmycorrhizal roots (Saito, 2000). Nutrient metabolic compounds such as ATPase and nitrate may be regulated by fungal colonization (George, 2000). The symbiotic association between plants and mycorrhizae changes the physiology and morphology of roots and plants in general (Linderman, 1988) and leads to altered root exudation and affects microbial communities around roots.

The P contained in plants differs according to the AM species. For example, Drew *et al.* (2003) found that plants grown with *Glomus intraradices* obtained almost three times more P^{33} than those grown with *G. mosseae. G. intraradices*, which obtained a greater proportion of P at distance from the host, was more affected by sand pore size than *G. mosseae.* Most studies indicate that mycorrhizal plants do not use particularly different soil P forms than nonmycorrhizal plants (Bolan, 1991). This does not exclude that a distinct soil P fraction be more depleted which appears to depend more on its relative availability in the soil than on the mobilization properties of the hyphae or mycorrhizal roots (Nurlaeny *et al.,* 1996).

2.6 Agricultural strategies for improving P acquisition and use

P inputs from the atmosphere are usually small, commonly ranging from 0.1 to 0.5 kg P ha^{-1} annually. Inputs from weathering vary greatly among ecosystems, but are often of the same order of magnitude. Annual plant uptake is commonly 2-25 kg P ha^{-1} (Binkley and Vitousek, 1989).

Agricultural strategies to increase P availability for crops are: fertilizers, genotype selection, rhizosphere microbial technology, shrub-based accelerated fallow, tree mulching, manure and compost. To reclaim lost organic matter and nutrients for agricultural use, chemical inputs are recommended, especially lime and P fertilizers (Cramb, 2005). Organic amendments, such as farmyard manure, cover crops, green manures, and compost, are also advocated to help ameliorate acid soil conditions and build up depleted soil organic matter (Lefroy *et al.,* 1995; Whitbread and Blair, 1999).

2.6.1 Fertilizers

Fertilizers play an important role in increasing crop production, either through increased fertilizer application or improved efficiency (Bhalla *et al.,* 1999). However, unless fertilizer prices decrease and food prices rise dramatically, the economically optimal quantity of fertilizer will not likely approach an agronomic optimum (Bhalla *et al.,* 1999). Studies suggest that the profitable use of fertilizer in many unirrigated areas will generally require greater fertilizer efficiency through a balanced dose, timely planting, improved application timing and improved placement (Bhalla *et al.,* 1999).

In agricultural soils, fertilization and cultivation increase P bioavailability in the topsoil but in most cases with only very slow movement of P into the subsoil. Patches of localized soil P availability may retain their boundaries within millimetres or centimetres over some years (Lynch, 1995). As a result, P availability usually declines substantially with soil depth. P application is required in soils with less than 4–5 ppm P availability (Howeler, 1996), and the key to P fertility is the maintenance of at least the equivalent to 4 kg P ha^{-1} of available P fraction in the soil solution (Fairhurst *et al.,* 1999). Fox (1978) reported that external P requirements for numerous crops are from ~0.01to 0.4 ppm in the soil solution.

Analyzing nutrient availability in fertilizers to meet plant demand, the term “available” remains confusing. “Availability” infers plant availability, but no one knows how to actually measure true plant availability. In the case of fertilizer, P availability has to be expressed on terms of weak acid solubility. Even completely water-soluble P fertilizer (completely plant available) when manufactured, it does not remain this way

very long after it is applied to the soil. The process of available P being made unavailable to plants is called "P fixation" (Penas and Sander, 1993). The reverse of plant-available fertilizer to plant-unavailable P is a process that cannot be avoided, but fertilizer P efficiency can be increased by proper management: (i) acid soils can be limed to increase soil pH to between 6.5 and 7.0; (ii) P fertilizer can be applied in small amounts and frequently rather than in large amounts at one time; (iii) trying to reduce soil fertilizer contact; (iv) placing P fertilizers in soil areas where roots are most active.

2.6.2 Genotype selection

The application of phosphate fertilizers is not an adequate solution to P-deficient soils because of rural poverty, poor access to appropriate fertilizers and limited fertilizer efficiency in highly weathered soils (Liao *et al*., 2001). An alternative or complementary approach is the development of cultivars with superior growth and yield in soils with low P availability or with "P efficiency" (Rubio *et al*., 2003).

Koyama *et al.* (1973) concluded that the tolerance of the rice (*Oriza sativa*) variety Drawk Mali to P deficiency was not due to a lower P requirement of the variety but rather a greater ability to absorb P from the soil. P acquisition efficient genotypes may have a higher number of adventitious and basal roots, or the roots may have a higher SRL and, therefore, possibly a lower construction cost per root surface area unit (Eissenstat, 1992).

P efficiency in bean appears to be associated primarily with enhanced P acquisition from the soil through superior root growth and architecture rather than through microbial associations and chemical modification of the rhizosphere or leaf acid phosphatase activity (Lynch and Beebe, 1995; Yan *et al.*, 2001; Rubio *et al*., 2003). The response of a set of contrasting bean genotypes to P stress (Yan *et al.,* 1995) suggests that the genotype ranking was independent of mycorrhizal infection. Therefore, the adaptation of a genotype to low P, relative to other genotypes, is determined by inherent root traits rather than by symbiotic efficiency.

Intraspecific variability in P-efficient has been observed in the common bean (Lynch and Beebe, 1995). P-efficient genotypes may be superior due to a greater ability to recover P from fertilizer and/or native fractions of soil P under P-limiting conditions, or due to a greater productivity per unit of P absorbed, irrespective of the mechanism involved (Beebe *et al.,* 1997). Wild beans in general performed relatively poorly to P stress; it appears that P-efficiency traits in *P. vulgaris* have been acquired during or after domestication (Beebe *et al.,* 1997). In contrast, wild oat (*Avena fatua*) has a higher root to shoot ratio and is less likely to experience P deficiency than cultivated oats (*Avena sativa*) in a given soil (Koide, 2000).

In the common bean, the more efficient genotypes have greater root mass, root length, root: shoot ratio (Yan *et al*., 1995) and their roots also demonstrate a high ability to sense and respond to localized changes in P availability (Lynch, 1995). A possible indicator for P uptake efficiency and root architecture in the common bean was found using fractal dimension to estimate the three-dimensional complexity of root systems (Nielsen, 1997). Efficient genotypes were found to have a higher planar fractal dimension (measured by tracing the root intercepts with horizontal planes) than less efficient genotypes (Nielsen, 1997). Root systems that have a small root diameter or a root architecture that reduces overlap between P depletion zones could be beneficial under low P soil conditions. However, much lower root length densities are sufficient for the depletion of nitrate than for phosphate depletion from the soil solution due to a higher soil diffusion coefficient for nitrate than for phosphate (George, 2000).

2.6.3 Rhizosphere microbial technology

Microbial interactions in the rhizosphere are perhaps more complex in soils than in any other microbiological habitat, especially in the presence of growing plants (Meeting, 1993). New microbial habitats are produced continually through root growth. In contrast to the rhizosphere soil, the bulk of nonrhizosphere soil is oligotrophic, in essence a nutritional desert (Meeting, 1985). Because of the different spatial scales of root processes (e.g. root exudation and ion uptake) and the different diffusion rates of solutes in soil, each position in the rhizosphere is chemically unique (Jones *et al.,* 2004).

The rhizosphere effect is a stimulation for microbial growth surrounding the roots because of the release of organic compounds (Elliott *et al.,* 1994). Cultivars for sustainable systems will probably need to maximize microbial interactions (Parke and Kaeppler, 2000).

Plants modify their environment at many spatial scales: the global, the ecosystem, the soil horizon, and the rhizosphere (Jones *et al.,* 2004). In all ecosystems, plants transform the surrounding soil, making and maintaining a habitat more favorable for growth (Marschner, 1995). Root mediated changes to soils are mainly associated with ways to increase their potential nutrient and water acquisition (Jones *et al.,* 2004).

Rhizosphere manipulation is a common technique to alter either the composition or the activities of soil microorganisms. Simultaneous dual inoculation of plants with AM fungi and phosphate solubilizing bacteria in phosphate deficient soils has been shown to be more effective in growth promotion than single inoculation (Barea *et al.,* 1975). Improved P uptake by soil fungi and/or bacteria may be due to the production of acidic compounds that increase the calcium phosphate solubility (Gyaneshwar *et al.,* 2002) and the production of extracellular phosphatase, which releases P from organic compounds (Gryndler, 2000).

2.6.4 Shrub-based accelerated fallow

Forest-fallow systems evolve into bush-fallow, then short-fallow systems or shrub-based accelerated fallow, and finally annual or even multiple cropping (Cairns *et al.,* 1998; Cramb, 2005). The transition from one stage to the next occurs when the growth of population makes it necessary to increase total food output from a given area of land (Cramb, 2005). Whereas forest and bush-fallow systems rely on the ash from the burned fallow vegetation to fertilize the soil, short-fallow cultivators depend almost exclusively on manure from their draught and other domestic animals, while in annual and multiple cropping systems, additional sources of fertilization are needed, including green manuring, marling (applications of lime and clay), and composts (Cramb, 2005).

Fallow systems are rotation systems in which crops are grown with short, intervening fallow periods during which the land is left to revegetate, but they tend to give way to continuous cultivation as population pressure increases (Ruthenberg, 1980); this fallow system, mainly corresponds to natural fallow. However, sometimes natural fallow is replaced by planted fallow -cultivation of selected species during the fallow phase of shifting cultivation system- (Nair, 1993; Snapp *et al.,* 1998) to guide or accelerate the process of soil restoration. As the fallow periods are not long enough to fully restore the fertility of the soil, the level of production decreases with the intensity of soil use on all but the most fertile soils, unless nutrients are imported to replace those leached or removed in the crops (Giller and Wilson, 1991).

Before the advent of N fertilizers, it was typical to maintain 25-50% of a farm in a legume-rich pasture or cover crop which produced relatively few commodities, but regenerated soil fertility through biological fixation of atmospheric dinitrogen (N_2) by legume-rhizobial symbiosis in plant biomass (Smil, 2001; Crews, 1993). Legumes also remain important components of farming systems in resource-poor nations or where pulses form part of a staple diet. The introduction of N fertilizers presents an alternative to legume rotations that were expensive in both labour and land (Crews and Peoples, 2004). However, fallows of leguminous trees do not only add N to the system but also improve the physical and hydrological status of the soil, which is backed up by the finding that infiltration rates are doubled under improved fallows (Raussen *et al.,* 1999).

However, the amount of available nutrients that are accumulated during the fallow period depends on the duration of the fallow, the concentration of nutrients in the different layers of the soil profile, and the quality of fallow vegetation (Saïdou *et al.,* 2003). Plant bodies contain many elements, some in relatively large amounts, some in trace quantities, and others in between (Boyd, 2004). Unfortunately, the most important macronutrients (N, P and K) that can be used as plant nutrient sources are only available in low concentrations in terms of the plant dry mass basis. No hyperaccumulation of macronutrients is reported in the literature, but there are significant differences in N, P and K between different plant species and some plants have “higher” nutrient concentrations than others. Hyperaccumulation occurs principally for metal ions, such as

Cu, Fe, Zn, Mn, Ni, Co and Se (Cobbett, 2003), which are essential micronutrients (Gutiérrez, 1998) and the other nonessential metals, such as Cd, Cr, Pb, Al and As, that can cause toxicities when present in excess (Marschner, 1995; Cobbett, 2003; Reeves, 2003). Some species are termed hyperaccumulators because of their extraordinary level of accumulation of certain elements on a dry mass basis. The threshold that defines hyperaccumulator is –100-1000-folds the levels normally accumulated in most species.

Cover crop mulching are improved fallows and, by reducing the time needed for fallowing, can aid in making such systems more sedentary (Erenstein, 2003). Careful management has shown weeds to be an effective cover crop (Cairns *et al.,* 1998). Fast development of a dense Asteraceae canopy, for example could further assist nutrient retention by filling the gap left after harvest and shielding the soil from erosion and leaching losses (Cairns *et al.,* 1998). As the stand matures, copious quantities of litterfall decompose quickly, releasing nutrients that are recaptured by Asteraceaes´s extensive lateral roots, thus mimicking the tight nutrient cycling of natural forest ecosystems (Cairns *et al.,* 1998). These attributes appear to speed up the nutrient accumulation process and contribute to a steeper soil fertility restoration curve (Cairns *et al.,* 1998). South East Asian examples regarding "shrub-based accelerated fallow" are primarily based on nutrient-scavenging members of Asteraceae family and N-fixing *Mimosa* species (Cairns *et al.,* 1998). However, it should be kept in mind that different plant species might have an array of possible mechanisms for the use of so-called "unavailable" nutrient sources, and they may mobilize nutrients from recalcitrant sources (Marschner, 1995).

Enrichment of native vegetation by species that accumulate and/or circulate a "greater" amount of nutrients is generally referred to as a biologically enriched fallow as opposed to an economically enriched fallow in which the favoured species increases the economic value of the fallow (Raintree and Warner, 1986; Kass and Somarriba, 1999). Many agroforestry systems accumulate P in their biomass and return it to the soil when litters decompose (Sánchez and Palm*,* 1996). Like upland primary forests, agroforestry systems have a dense, deep and permanent network of roots, contributing to more efficient nutrient cycling and preventing losses due to leaching of mobile nutrients, which

is often the main cause of soil fertility loss in annual crop land use systems. Tree canopies and leaf litter also protect the soil against erosion (losses of immobile nutrients) and high temperatures (Alfaia *et al.,* 2004).

A great deal of work remains to test and optimize different crop combinations appropriate to particular regions (Crews and Peoples, 2004), especially looking at competition for nutrients when plants are grown in mixed cropping systems. On this sense, for example, Siriri and Raussen (2003) found that maize after two years fallow increased significantly from 1.6 Mg ha^{-1} continuous cropping to 3.5, 4.1, 5.9 and 6.2 Mg ha^{-1} in the *Tephrosia*, *Alnus*, *Calliandra* and *Sesbania* fallow systems, respectively.

2.6.5 Mulching (green manure), manure and compost

Farmers occasionally use animal manures and crop residues for soil fertility replenishment. Crop residues are often used as livestock fodder. Hence, the amount of organic inputs and their quality are often insufficient to meet crop demands. Furthermore, distances to the fields make transport of the bulky organic inputs difficult (Siriri and Raussen, 2003).

Crop response to organic and biological nutrient carriers is not as spectacular as responses to fertilizers, but the supplementary and complementary use of these sources enhances the nutrient utilization efficiency of applied fertilizers when the organic material has high to moderate quality. Additionally, organic and biological nutrient carriers also improve physical and chemical soil properties, prevent micronutrient deficiencies (Yadav *et al.,* 2000), stabilize and enhance crop yield (Erenstein, 2003). Experiences so far have highlighted positive, neutral, and negative impacts on crop yields in the short-term. Overtime, the yield effects tend to be neutral to positive (Erenstein, 2003). The potential of cover crop mulching seems to be restricted to the sub-humid zones and to instances where the opportunity cost of using land for grown cover crops is limited (Erenstein, 2003).

Agroforestry systems tend to combine elements of an *ex situ* and live mulches (Erenstein, 2003). Mulch is strategically located at the soil-atmosphere interface and acts

both as soil protector and as a soil amendment. It thereby affects: soil conservation, soil ecology, crop yield, and environment (Erenstein, 2003). An ideal perennial tree for biomass production is one that transfers nutrients to P deficient soils by acquiring a large fraction of its P from relatively less available soil P forms and accumulates a "high" P concentration in leaves (Buresh *et al.,* 1997). Some organic materials with a high P (>2.5 mg P g^{-1}) concentration have the potential to increase P availability in the soils (Palm *et al.,* 1997). Hairiah and Noordwijk van (2000) indicated that the application of *T. diversifolia* shoot biomass improved microbial biomass and increased P soil availability associated with a reduced P-adsorption. The effect on reduced P-adsorption could be much stronger than expected from the increased P supply (Hairiah and Noordwijk van, 2000).

Green manure application timing also influences yields in a way that seems to vary according to rainfall distribution (Boffa, 1999) and the manure composition. Higher yields of rice (*Oryza sativa*) are obtained by incorporating green manure up to ten days before transplanting compared with longer periods before transplanting (Meelu, 1994). In Cameroon, Roy *et al.* (1988) suggested that *Sesbania* and sun hemp (*Crotolaria juncea*) green manures should be incorporated about two weeks before transplanting rice.

Green manures, through providing nutrients, encourage plant growth and promote the activity of desirable microorganisms which play a key role in transforming and liberating plant nutrients in the soil and also by discouraging some undesirable microorganism growth (Meelu, 1994). Green biomass of *Tithonia* has been recognized as an effective source of nutrients for lowland rice in Asia and more recently for maize (*Zea mays*) and vegetables in eastern and southern Africa (Jama *et al.,* 2000). Kwabiah *et al*. (2003b) found that the effects of organic material from *T. diversifolia* and *Croton megalocarpus* on maize yield were similar to 50 kg P ha^{-1} + 120 kg N ha^{-1} as inorganic fertilizer when leaves and small twigs of these species were applied at 5 Mg ha^{-1} in a Ultisol of Western Kenya. Under laboratory incubation conditions, *T. diversifolia* and *Croton megalocarpus* were found to contain >3.0 g kg^{-1} of total P in dry weight and were identified as having the potential to release adequate P to replenish solution P for crop uptake (Kwabiah *et al.,* 2003a).

Yadav *et al.* (2000) working in Punjab, India, found that *Sesbania* green manuring was more promising than other legume crops. Diekmann and De Datta (1990) found that in a 45-day growth period, *Sesbania rostrata,* accumulated 114 kg N ha^{-1} when planted at a rate of 40 kg of seeds per hectare. This was higher than the 100 kg N ha^{-1} that accumulated when planted at 50 kg of seeds per hectare. Becker *et al.* (1988), in a trial of vegetative propagation, observed that 30-cm stem cutting (100 cuttings per m^2) of 8-week old *S. rostrata* produced more biomass and N than 80 kg seed ha^{-1} or 20-cm cuttings. However, leguminosae have problems growing in acidic soils with deficient levels of K, P, Ca, Mg, Mn and Mo. In addition, acidic soils are not suitable for most *Rhizobium* microorganisms (FAO, 1986).

Alley cropping can increase production potential (Kass, 1985) in fragile environments to meet food and fuel needs as well as improve soil fertility (Dalzell *et al.,* 1987; Yadav *et al.,* 2000). The sustainability of alley cropping systems depends in part on the rate of resprouting and annual biomass output (nutrient cycling) of pruned shrubs (Chesney, 2000). However, competition of plants occurs both above and below-ground. Whereas above-ground competition involves one principal resource (light), below ground competition encompasses a broader spectrum of resources, including water and all the essential mineral nutrients.

For long-term sustainability, mulching in combination with mineral P fertilizer would be a more optimal fertilization strategy (Palm *et al.,* 1997). Sole application of mulch from agroforestry trees in Cotonou, Benin, had no significant effects on maize yields, while combined application of pruning and NPK fertilizers or sole NPK increased yields significantly (Saïdou *et al*., 2003). The application of an integrated economic-environmental accounting framework to evaluate the sustainability of agricultural production systems revealed that the gross margins of steady-state in organic farming systems were found to be higher than the corresponding conventional farming system gross margins and also organic farming systems performed better than integrated and conventional farming systems with respect to N losses, pesticide risk, herbaceous plant biodiversity and most of the other environmental indicators (Pacini *et al*., 2003).

3. Materials and Methods

3.1 Experimental site

This study was conducted from June 2001 and September 2002 at the CATIE Experimental Station, San Juan Sur, Turrialba, Costa Rica. The experimental area was located at 09° 53´ N and 83° 38´ W, approximately 8 km SW of Turrialba and consists of hills of 15 to 50% slopes facing the morning sun (east to south) at 950 meters above sea level, where coffee and pasture with dispersed orchards are common. The annual rainfall is 2,679.3 mm (Appendix 1), average annual temperature is 21.8 °C and the corresponding Holdridge life zone is very humid Pre-montane Forest (bvh-P) (Holdridge, 1987) (CATIE, 2002). The native vegetation is tropical rainforest.

San Juan Sur soil is classified as an Andisol Acrudoxic Melanudand by ISRIC (1994) and is quite extensively used for agriculture in the townships of Cervantes and Paraíso. San Juan Sur Andisol is loamy-clay to clay, reddish brown with soil structure and apparent density favorable for good internal drainage in the upper twenty centimetres of the soil profile. Mean soil bulk density is 0.68 Mg m^{-3} (Garzón, 1991). It is characterized in the upper 0.60 m by: pH≅5.1; low base status (0.39 $cmol^{+}$ kg^{-1}); high exchangeable aluminium (1.35 $cmol^{+}$ kg^{-1}) in which aluminium represents >60% in CEC; high total P, but low P availability (3.2 ppm); high P retention (96.8%) and Fe oxide/clay ratio >0.2.

In the experimental area, soil pH was lower than 5.5 (acid) and the sum of cations (Ca, K and Mg) was below of 4 $cmol_c$ kg^{-1} of effective cation exchange capacity (ECEC) indicating low cation exchange capacity in the topsoil and limited ability to retain nutrient cations against leaching. The pH values from Experiment 1 and 2 sites were similar even though in Experiment 2, calcium carbonate and poultry manure were previously applied to bean plots used to propagate bean cultivar seeds. Calcium carbonate increased the pH by 0.16 units in the 0-12 cm layer. Available P and Ca concentrations were also higher in Experiment 2. However, available P was below the critical level of 12 mg P L^{-1} (Bertsch, 1995) in both experiments. Soil chemical properties in the initial and

after fallow species treatments are shown in Appendix 2. These soil fertility parameters suggested the presence of low soil fertility conditions.

Phosphate sorption isotherms were carried out to estimate P buffering capacity in Experiment 1 (Appendix 3). The topsoil (0-12 cm) P-isotherm patterns were similar between the plots in the experimental area. However, contrasting curves were obtained at 12.5-25 cm soil depth. P-isotherm response curves at 12.5-25 cm soil depth showed higher sorption strength indices (less remaining P) for higher levels of P added in solution. As a reference, plants attained near maximum growth when P was found at 0.2 ppm of P in the soil solution (Beckwith, 1964). However, this P value was not reached when P was added during P-isotherm tests. In addition, pH values in sodium floureno (NaF) were >8.5 at the 0-12.5 cm and 12.5-25 cm soil depths, indicating the presence of considerable amounts of amorphous material formed by weathering of volcanic ash. Alvarado and Buol (1985) reported pH NaF=10.1 for the Costa Rican Andisols in similar ranges of altitude, but with ustic soil moisture regimens (soil remains dry for >90 days per year, but <60 consecutive days). The Al_o+½Fe_o values were around 2.1%, indicating significant amorphous clay mineral content (Shoji *et al.,* 1993) and that P availability was limited in this soil. In addition, P retention values were high (>85.0%). According to above P parameters, the experimental area possesses as a P-limited environment.

3.2 Experimental materials of field experiments

T. diversifolia genotypes were colleted in Central America and Mexico in 1999, and established in a germplasm collection at San Juan Sur, Costa Rica. The Asian *Tithonia* genotypes sent by ICRAF were also included in the collection. In Experiment 1, three of these genotypes were used: Heredia, Costa Rica; Tapachula, Mexico and Malaga, Indonesia, as well as a woody tropical legume pigeon pea (*C. cajan*). These plant species were identified as having the quality to increase P in soils or to increase P availability in laboratory incubation studies (Kwabiah *et al.,* 1995, 1996; Aguiar, 2001) and litterbag decomposition experiments under field conditions (Kwabiah *et al.,* 1999, 2001; Cobo *et al.,* 2002). Experiment 1 also used two Costa Rican bean cultivars belonging to the

Mesoamerican gene pool, the Chirripo Rojo (the newly developed cultivar also called Bri-bri or UCR-55) and the common black bean cultivar (Negro Huasteco).

In Experiment 2, four bean cultivars were used: the two Costa Rican cultivars mentioned above and two other exotic CIAT cultivars: Dor-364, a high yielding bred line developed in Central America (Liao *et al*., 2001); and CIAT G-1937 a landrace adapted to P-limited conditions and relatively high yielding. For both experiments (1 and 2), bean cultivar seeds were obtained from Fabio Baudrit Experimental Station, University of Costa Rica (UCR).

3.3 Description of field experiments

3.3.1 Experiment 1: Retaining or removing slash fallow species biomass

Experiment 1 was conducted at the CATIE experimental station to evaluate biomass and nutrient accumulation and root characteristics of fallow species, and their effects on bean yield, biomass production, nutrient concentrations and root responses on an Andisol with low P availability. The experimental design consisted of a 4 x 2 factorial, in a magic 8 x 8 Latin square design with split-plots and comprised 64 plots (divided into four subplots labelled S_1b_1, S_1b_2, S_2b_1 and S_2b_2) of 6.0 x 5.0 m and sixty plants each (Figure 1). This trial included eight main treatments (main plots) sown with three *Tithonia* genotypes in monoculture and in association with *Cajanus* and natural regeneration with and without *Cajanus* for *in situ* produced mulch (Photo 1). Natural regeneration represented the existing pastures in short-fallow shifting cultivation and was composed of a mixture of locally grown fallow species that included non-legume and N-fixing species. Un-mulched plots are referred to as controls.

The "improved" practices were represented by the treatments in mono and in association with non-legume and legume plant species: *Tithonia*, a non-legume that produces many flowers principally for honeybees and *Cajanus,* a N-fixing species that can produce edible beans if cropping periods are longer than six-months (but depending on the planting date and cultivars, the occurrence of the flowering phase varies). After six-months with fallow species treatments, each main plot was divided into four split-

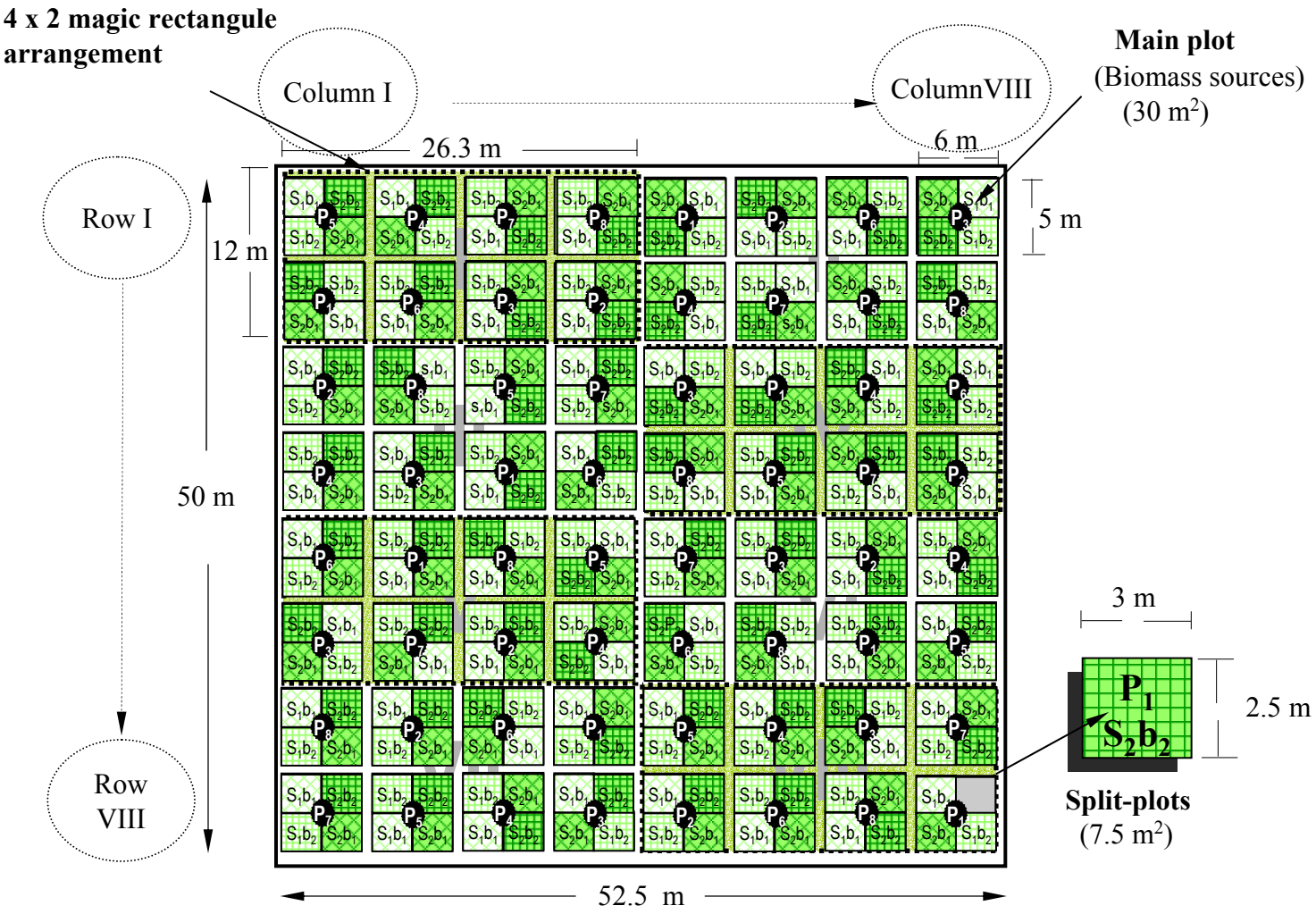

Figure 1. 4 x 2 factorial experiment arranged in magic 8 x 8 latin square design with split-plots. P=biomass sources (8 treatments; main plots), S=biomass application treatments and b=bean cultivars (4 split-plots).

Photo 1. *T. diversifolia* and *C. cajan* slash biomass application treatments in split-plots harvested six-months after planting in Experiment 1, San Juan Sur, Turrialba, Costa Rica.

plots, which were subjected to two different biomass management treatments (with and without removal of slash biomass) and two Costa Rican bean cultivars (the Chirripo Rojo and Negro Huasteco) were sown to evaluate their responses in yield, biomass production, nutrient concentrations and root characteristics.

3.3.2 Experiment 2: Mulch transference from outside plots (Cut-and-carry system)

Experiment 2 evaluated bean cultivar yield, growth and root responses to *Tithonia* and *Cajanus* mulches in a cut-and-carry system. The experimental design consisted of a randomized block design with two ex-site organic mulches, four bean cultivars and three replications on an Andisol with previous applications of poultry manure (≈9.0 Mg ha^{-1})

and calcium carbonate (≈2.6 Mg ha^{-1}). This experiment combined the most appropriate elements of organic technology to maximize bean yield in acid soils.

Experiment 2 comprised 9 main plots (divided into four subplots labelled: B_1, B_2, B_3 and B_4. Each subplot dimension was 3.0 x 2.75 m to allocate bean cultivars. Pruning in Experiment 1 for mulching in Experiment 2 was carried out as follows: All biomass of fallow species pruned and removed from nearby Experiment 1 were transferred to the corresponding mulch treatments (*ex-situ* mulch). Therefore, application rates of slash biomass were equivalent to 80 and 9.0 Mg ha^{-1} for *Tithonia* and *Cajanus* treatments, respectively. The biomass applications for one crop parcel were equivalent to two and eight areas of *Cajanus* and *Tithonia* fallows, respectively. Important considerations in assessing suitability of the fallow species for study were availability, high quality (i.e., high total P and N concentrations) and sufficiency of supply (Kwabiah *et al.,* 2003b).

3.4 Field experiment management

Before the installation of the field experiments, only pastures in natural fallow occupied the experimental area. The area was safely kept from cattle and no fertilizers were used for the previous two years. The field area was ploughed three times prior to planting fallow species to control weeds, accelerate the decomposition of natural vegetation and minimize the presence of plant debris when sampling roots.

Twenty-centimetre long *Tithonia* stakes were pre-rooted and inoculated with mycorrhizal fungi by planting the stakes in the inter-row space of the *Tithonia* germplasm collection to ensure sufficient mycorrhizal inoculation. AM infected roots of standing *Tithonia* plants inoculated the stakes while new roots developed. Only bare-rooted stakes were transplanted to the experimental plots (Experiment 1) while *Cajanus* was sown directly using two seeds per planting hole soon after transplanting all *Tithonia* genotype stakes into their corresponding plots.

Plot demarcation was done along the contour lines. Each fallow species treatment was sown manually in a dense planting arrangement to avoid biomass lignification of *Tithonia* and *Cajanus*. The planting density was 60,000 plants ha^{-1} (0.30 m between

plants and 0.4 m between rows). All around the experimental area, a double and dense row of *Canavalia ensiformis* was planted to prevent an attack of leaf-cutter ants (*Atta cephalotes*) (Donald Kass, personal communication).

Tithonia and *Cajanus* were left to grow for six-months, until pruning. However, every two months, *Tithonia* and *Cajanus* roots were cut with a spade along each plot perimeter to prevent roots growing outside the planted area. Roots were cut at 20-25 cm depth to limit the majority of root development during the fallow period, based on root samples taken in the two-year old *Tithonia* germplasm collection in San Juan Sur, adjacent to the Experiment 1. Weed control was done manually, periodically scouting the plots and uprooting the weeds during the first four months of fallow and immediately before slashing biomass. Natural regeneration treatment was not weeded.

During the six-months (24 weeks) of the fallow period, biomass samples and root measurements were taken at 6, 17, 23 and 24 weeks after planting fallow species. On the first three sampling times, non-destructive measurements for height, stem length and the number of branches were recorded. The stem length in *Tithonia* genotypes and *Cajanus* was measured from the plant stock. In each treatment, two destructive plant samples were taken during and at the end of fallow period. Plants from the central rows in each plot were taken randomly for destructive and non-destructive samples. Soil samples were taken during the fallow period and at fallow clearance to determine the effect of fallow systems on soil P fractions. Similar sampling was done for above-ground biomass which was separated into lignified and non-lignified stems, leaves and flowers.

Six-months after planting Experiment 1, all plots of fallow species treatments were harvested and stumps left. The root systems of fallow species were left intact. Biomass was weighed, broadcasted uniformly onto randomly selected slash and mulch plots (for *in situ* mulch). In the retained biomass treatments, biomass was left on the soil (not incorporated) and not chopped into small pieces that could be blown by wind. In control plots, biomass was cut and removed before planting bean cultivars. Bean cultivars were sown immediately after slashing biomass for a crop cycle of three months (90 days), in both experiments. The removed slash biomass in Experiment 1 was transferred to the corresponding mulch treatments in Experiment 2 for *ex situ* mulch applications. In

Experiment 2, the control and mulch treatment plots were randomly split and four bean cultivars were sown.

Fallow species were not left for re-growth during the bean growing cycle and had to be pruned three times, in accordance with the sprouting vigour of the fastest growing fallow species, only to avoid light competition with the bean crop. Biomass production after the first pruning was very low and it was cut and left in each plot, but not included in the total biomass production of each fallow species. No litter was collected in the fallow species treatments.

3.5 Crop management in field experiments

In Experiment 1 and 2, bean cultivars were sown using two seeds per planting hole soon after the treatments were applied. In Experiment 1, individual parcels (split-plots) were 2.5 x 3.0 m (7.5 m^2), thus the plot width accommodated six rows of beans at a spacing of 0.3 x 0.40 m. In Experiment 2, individual parcels (split-plots) were 3.0 x 3.0 m (9.0 m^2), thus the plot width accommodated seven rows of beans at a spacing of 0.3 x 0.40 m.

Rhizobium bean inoculant produced at the University of Costa Rica was used for Experiments 1 and 2. At sowing time, *Rhizobium* inoculant was added to the bean seeds using a sugar solution as an adherent (Donald Kass, personal communication). After bean plant emergence, the missing plants were immediately replanted to maintain a population around 166,666 plants ha^{-1}.

Bean cultivar plots were managed (planting, weeding, application of insect repellent and harvesting) according to local practices, but entirely as organic agriculture (Donald Kass, Reinhold Muschler, Adolfo Solano and Francisco Nunez, personal communications) to facilitate experimental interpretation and to assure the use of clean production technology for the environment.

Bean cultivars were sprayed with a blend of hot pepper “Chile Panameño” (*Capsicum chinense*) and garlic (*Allium sativum* L.) (8-10 g of hot pepper and garlic per litre) on a weekly basis (Donald Kass, personal communication) from the time the plants

developed their fourth leaf to the bean maturity phase to avoid insect damages (*Cerotoma ruficornis* and *Diabrotica balteata*). From time to time, white bags and dogs were used to keep wild rabbits and "cuzucos " away from bean plots (Donald Kass and Adolfo Solano, personal communications). There were no serious incidences of weeds, wild rabbits, cuzucos, insects or diseases. Weeding was done whenever appropriate.

Cattle and rabbits damaged some bean plants on several plots during the growing phase. The damage occurred principally on young leaves and only a few plants were lost. And finally, at physiological maturity of 12 weeks (for the Chirripo Rojo) and 13 weeks (for the Negro Huasteco, Dor-364 and CIAT G-1937) bean cultivars were harvested on each entire split-plot.

3.6 Supporting experiments

The supporting experiments consisted of two independent studies in Costa Rica, one with potted plants under greenhouse conditions and another with girdling stems in the *Tithonia* germplasm collection. In addition, three potted experiments in a controlled environment were held in Denmark. All experiments were carried out using completely randomized designs with 5-6 replications (plants) per treatment.

3.6.1 External mycelium, root hair and organic acid experiments

The objectives of these two experiments were to determine the contribution of external fungal mycelia and root hairs in soil exploitation, and the concentration of internal organic acid in the root tip and leaf tissue as potential root exudates for improving nutrient uptake. In both experiments, the same three *Tithonia* genotypes were used: the Costa Rican (Heredia), Indonesian (Malaga) and Mexican (Tapachula). *Tithonia* genotype was compared with each other for variables mentioned above.

External fungal mycelium was determined on one-year old *Tithonia* potted plants grown under greenhouse conditions at the Cabiria, CATIE experimental station. *Tithonia* genotype plants were sown in pots of 10.5 cm diameter and 8 cm height containing two substrate types. The mycorrhizal inoculant (from topsoil, upper layer, 0 to 12 cm.) was

obtained from the area of the *Tithonia* germplasm collection at San Juan Sur. The soil containing mycorrhizal inoculum was mixed with washed sand in even proportions or used alone. In mixed substrates, sand was sterilized in an autoclave prior to mixing with soil to avoid external sources of contamination. Black plastic film was placed over the substrate to avoid algae, weed growth and reduce direct water evaporation. Potted plants were left to grow for one year and no nutrients were added to the irrigation water until harvesting. Potted plants were also used for root system characterization and as an additional source of root samples for counting fungal structures in *Tithonia* genotypes.

Internal organic acid concentrations in the root tip and leaf tissue were measured in six-week old girdled stems randomly chosen from the *Tithonia* germplasm collection at San Juan Sur. Succinic, malic, fumaric and oxalic acids as well as citric acid were determined at the Universidad Nacional (UNA, Heredia, Costa Rica) using four repetitions and three clones per *Tithonia* genotype. The type of organic acids were previously identified in *Tithonia* root tips and leaves in exploratory experiments carried out in Bangor, United Kingdom. Root tips were obtained from girdled stems once emerging roots reached a few centimetres. It took six to eight weeks until the root tips were ready to be sampled in all *Tithonia* genotypes. Immediately after, leaf tissue samples from the same girdled stems were taken. Internal organic acid composition in the leaf tissue was also determined to evaluate the possibility of predicting organic acid composition in the root tip by only sampling leaf tissue, which is easy to obtain. In addition, root samples from girdled stem and from the topsoil layer of the *Tithonia* germplasm collection were sampled for root hair length determination. Root segments with root hairs were obtained from girdled stems or fine roots, which had been covered by aluminium foil for several weeks to hold moisture and promote root proliferation.

3.6.2 *Tithonia* response to different mineral P levels in sand culture

Three controlled environment experiments (S-A, S-B and S-C) were conducted using two *Tithonia* genotypes grown at different mineral P levels in a re-circulating aerated hydroponic system. A solid-phase alumina buffer (Al_2O_3P) technique as described in Lynch *et al.* (1990) was used. Three P activated alumina buffers

(MARTINSWERK, 2000) with different desorbing P concentrations (10, 160 and 1,000 μM) were used in potted experiments (Ottosen, 2000). P concentrations were considered to be low-P, medium-P and high-P, respectively. Alumina buffers helped to maintain stable P concentrations in the medium (Hansen, 1997). Loaded alumina was hand mixed with sand at a 1% mass/volume basis (1 g alumina in 100 mL sand) and poured into 10-cm containers. Each substrate enriched with P was prepared separately using 10 kg of sand and 200 g of the different activated alumina P buffers. In Experiment S-C, a plastic separator was used in each pot to allocate different phosphate enriched substrates. The sand-alumina mix was prevented from running out of the drainage holes in the bottom of the containers by 0.5 mm plastic mesh placed prior filling with the mix. The composition of P-free nutrient solution was (in μM): 420 K_2SO_4, 1,720 KNO_3, 1,350 $Ca(NO_3)_2$, 320 $MgSO_4$, 830 NH_4NO_3, 500 $Mg(NO_3)_2$, 110 NaCl, 40 Fe-EDTA, 5 $MnSO_4$, 2 $ZnSO_4$, 0.6 $CuSO_4$, 8 H_3BO_3 and 0.008 $NH_4Mo_7O_{24}$. The temperature within the greenhouse was 25±4 ºC during the day and 19±2 ºC during the night.

Vegetative cuttings of *Tithonia* genotypes of similar length (around 10 cm) were used. Cuttings were collected from adult plants of each genotype, cloned and rooted directly in containers containing different P treatments (Carl-Otto Ottosen and Kai Nielsen, personal communications). During the first 8 to 10 days, cuttings were covered with a mesh and plastic until the roots were able to keep the plants turgid through the day. Plants were automatically irrigated for one to three minutes per day into saturation with P-free nutrient solution. The nutrient solution was replaced every week to maintain nutrient concentrations, principally N and the pH around 6.5. Plants were irrigated once per day, in the morning, during the first three weeks and twice a day from the fourth week until harvest. Treatments were assigned to different trays to ensure that each received the corresponding P treatments. Additional space between plants was provided when needed.

The objectives of these experiments were to determine growth, P concentration and accumulation in *Tithonia* genotype biomass in response to different P levels. Growth rate and P concentration in plant tissue were possible through weekly sampling of plant growth and P concentration that might be potentially important for P uptake and

utilization efficiencies, indicating how *Tithonia* grows and how much P can be accumulated in plant tissue under different P conditions (Ottosen, 2000). Only the *Tithonia* genotypes available at DIAS were used: the Costa Rican and Colombian genotypes. Two clones were used for the Costa Rican genotype and only one for the Colombian genotype.

In Experiment S-A, three levels of P availability were employed (10, 160 and 1,000 μM of P). Plants were irrigated for six weeks with P-free nutrient solution. This experiment tested for leaf, stem and root P concentrations and growth rate of two *Tithonia* genotypes (the Costa Rican and Colombian). Potted plant measurements were performed at four different stages of growth: 23, 28, 34 and 39 days after planting.

Experiment S-B was a replicate of Experiment S-A; however, a control treatment with no P added to the sand substrate was included. Treatments were harvested every 5 to 6 days to measure the rate of root growth and P concentration in plant tissue. No root samples from this experiment were scanned. P uptake and utilization efficiencies, P content (plant dry weight x P concentration) were calculated for each treatment. Efficiency in P uptake is equal to P content in dry weight divided by root dry weight (Zhu *et al.,* 2001) or by specific root length. In Experiment S-A, efficiency in P uptake was also expressed per unit of root length. P utilization efficiency corresponds to the inverse of P concentration in above-ground dry weight.

In Experiment S-C, the same P treatments as in Experiment S-A were used but three more P treatments with split P levels in the same container were added. Each container with split P treatments was loaded with three different combinations of P treatments (160/10, 10/1,000 and 160/1,000 μM of P) to evaluate the root to above-ground biomass ratio at different P availability levels. However, only one clone from the Costa Rican genotype was used and plants were irrigated with a nutrient solution containing 10 μM of P until harvest.

Tithonia plants were harvested four to six weeks after planting. However, in Experiment S-A, where leaf, stem and root samples were obtained eight weeks after planting. Using small scissors, above-ground biomass was separated into senescent leaves

(with brown or yellow color), functional leaves (with green color) and stems. Once the roots were separated from sand, they were stored for one week in water in a cool room and then in 20 and 40% ethanol. After that, roots were cut with a pair of scissors into segments up to three centimetres and vigorously mixed in water. Random sub samples of 100-300 segments were collected and spread in a flat bed scanner in 2-3 millimetres of water and scanned (Kai Nielsen, personal communication). In Experiment S-A, root samples were stained with Neutral Red solution (50 g per litre) for 24 hours before scanning. However, in Experiment S-C, roots were not stained before scanning because roots showed sufficient pigmentation for proper scanning. Scanned root images were analyzed using the software Winrhizo program version 4.1 to obtain root length and root length diameter distribution. Plant parts were put in paper bags, dried (70-°C, 24 h) and dry weights were recorded. Finally, dry weight samples were ground and burned at 500 °C and P analysis of plant tissues were conducted using a colorimetric method (Murphy and Riley, 1962).

3.7 Plant growth and nutrient analysis

In Experiment 1, plant growth was determined using above-ground biomass and stem length. Samples were taken randomly at the eighteenth and twenty-third week after planting using three repetitions per fallow species treatment. Above-ground biomass was also determined 24 weeks after planting, when the whole Experiment 1 was harvested. In natural regeneration treatment, fresh biomass samples were randomly taken using a frame of 0.25 x 0.4 m (0.1 m^{-2}). Fresh biomass samples were weighed and divided into lignified stems (diameter >0.6 cm) and the remaining non-lignified stems (diameter ≤0.6 cm) as well as leafy and flower parts. For bean cultivars, above and below-ground biomass were determined at the growing, flowering and maturity phases using at least 16 plants per bean cultivar in both experiments (1 and 2).

Biomass samples were put in paper bags and brought to the laboratory on the same day for dry weight determinations (oven-drying at 60 ºC for about 60 h of 300-500 g sub-samples) in order to obtain the converting factors of each biomass material to express dry weigh in mega-gram per hectare. Biomass samples were chopped with

laboratory mill to pass a 1 mm screen. Later each dry biomass was used for nutrient analysis. However, harvested bean pods were air-dried to separate beans from pods and subsequently weighed to estimate water content in order to express yield on 5% water content.

In Experiment 1, nutrient concentrations in dry weight were determined at the seventeenth and twenty-third week for fallow species treatments, using three plants per treatment. For bean cultivars, biomass samples were taken at the fourth, sixth and eighth week after planting, which correspond to the end of the bean growing, flowering and maturity phases, respectively in Experiment 1; but in Experiment 2, biomass samples were only taken at the bean flowering phase. Plant biomass samples were dried at 45-60 °C for 48 hours to determine dry weight. The dried samples were ground and analyzed in the CATIE soil laboratory. N tissue concentration was determined by the semi-micro Kjeldahl method. Nutrient concentrations (P, Ca, Mg and K) in dry weight were determined by wet digestion in a mixture of 5:1 nitric-percloric acid (Diaz Romeu and Hunter, 1978; Mills and Jones, 1996) by atomic absorption (PERKIN ELMER, Model ANALYSIS 100, Serial No.040N8051505, Norwalk, CT, U.S.A.) and for P by UV-V spectrophotometer (THERMOSPECTRONIC, Model HELIOS ALFHA, Serial No. 092210, Cambridge, United Kingdom). Nutrient concentrations were expressed in milligrams per gram of dry weight. Total C was estimated using an average conversion factor of 44.78% in dry weight for different organic materials. This factor was taken from Cobo *et al.* (2002) for the calculation of C:N and C:P ratios.

3.8 Root system characterization

In Experiment 1, samples for root characterization were taken at the twelfth, seventeenth and twenty-third week for fallow species treatments using three sampling sites per treatment. The samples were obtained by an impact-type, vertical soil core sampler (auger cylinder with a diameter of 8 cm) at 25 cm equi-distant from the planting arrangement (30 x 40 cm) for the eight fallow species treatments: *Tithonia* genotypes (the Costa Rican, Mexican and Indonesian), *Cajanus,* natural regeneration and the three *Tithonia* genotypes in association with *Cajanus*. Each core sample (0-24 cm depth) was

split into three different depths (0-8, 8-16 and 16-24 cm), which included a variable but large fraction of total fine roots of the fallow species. For the three associated fallow species treatments (*Tithonia* genotypes with *Cajanus*), no root separation by fallow species was carried out and root parameter determinations corresponded to the mixture of the fallow species as in natural regeneration (untreated check). Root system characterization analysis was also determined in potted plants at the Cabiria greenhouse.

For *P. vulgaris*, the freshly excavated root samples were taken during the bean growing, flowering and maturity phases. A wide spade was carefully used to excavate around each bean plant for the extraction of the root system. Soils and decomposed debris were gently removed from roots in the field, then the number of nodules per plant (diameter ± 0.5 mm) was counted while roots were still fresh. The roots were separated from the shoot and stored in safe plastic containers in a refrigerator. In the root laboratory, the numbers of both adventitious and basal roots were counted and recorded separately and each type of root was removed from hypocotyls (the part of stem beneath the cotyledons). A relatively easy distinction can be made between adventitious and basal roots when examining extracted hypocotyls of mature plants (Miller, 1998). Adventitious roots arise at a 90° angle along the length of the hypocotyls (Miller, 1998). Basal roots arise from one verticil with an angle of less than 90° at the base of the hypocotyls, a zone approximately 0.5 cm long (Zobel, 1986). The part of hypocotyl where adventitious roots appear was considered as upper root-stem and the part of hypocotyl with basal roots was considered as lower root-stem. Both root-stem parts were measured to the nearest mm. Finally bean roots were submitted to a second cleaning at the root laboratory. A new variable was created (called root-stem surface) to analyze the interaction between the root-stem diameter and upper root-stem length. To describe the bean root system, root variables were grouped into three categories: root dry weight partitioning (adventitious root, basal root, upper root-stem and lower root-stem dry weights), root components (number of nodules, adventitious and basal roots) and root extension (specific root length of adventitious and basal roots, upper and lower root-stem lengths) in order to compare them using multivariate analysis.

For all roots samples (retrieved from the auger cylinder, pots and bean plants), the soil or sand was removed from the roots by hand washing using a 0.2 mm sieve. After that, root samples were placed in petri dish to manually remove the largest plant debris other than roots, using tweezers. According to the amount of debris the cleaning of the root samples varied (around 1 hour for samples taken at 0-10 cm soil depth and 30 minutes for samples taken at 8-16 and 16-24 cm soil depths). Roots were kept frozen in 30% ethanol until root length measurements were taken. No staining procedure was employed and finally roots were cut with small scissors into segments up to 3 cm long before scanning (Kai Nielsen, personal communication). After vigorously mixing the root segments, random sub-samples of 100-300 segments were collected and spread in a flat bed scanner in 2-3 mm of water for scanning using a WhiRhizo program version 4.0 B (Regent Instruments, 1997). Root image analyses were done to obtain root length measurements. Once scanned roots were dried and weighed, root measurements were expressed on a whole root system basis for each bean cultivar and on soil sample volume basis of auger cylinder for each *Tithonia* genotype*, Cajanus* and the other associated fallow species treatments. Root length for each sample was divided by soil sample volume (cm^3) to express as root length density (RLD) in ($cm\ cm^{-3}$) and divided by root dry weight to express specific root length (SRL) in (m of root g^{-1} root DW).

For root hair length determination, a visual morpho-metrical method aided by a light microscope at 5X and 10X magnifications was used. Root segments with root hairs were stored in 30% ethanol with glycerol and formalin until microscope measurements were taken. Only the relatively straight root hairs were measured. An eyepiece micrometer was employed for scale conversion. Root segments were randomly selected for measurement from the central part of each root segment. Average root hair length was measured from a representative root hair, which remained in focus throughout its length. This process was repeated five times for each root segment.

3.9 Mycorrhizal studies

In root samples collected by the author in Central America and Mexico for the *Tithonia* Project, the degree of mycorrhizal colonization was examined at the University

of Wales, Bangor, UK, as well as the families of arbuscular mycorrhizas associated with roots of *Tithonia* genotypes using family specific PRC primers (Sharrock *et al.,* 2004). Therefore, this study was limited to the counting of fungal structures on roots for *Tithonia* genotypes and bean cultivars by root image examination and the determination of external fungal mycelium length for three *Tithonia* genotypes.

3.9.1 Fungal structures in roots

For the fungal structures in roots, *Tithonia* genotype samples were obtained from Experiment 1 (harvested twenty-three weeks after planting), *Tithonia* germplasm collection (two-year old plantation) and one-year old potted plants at Cabiria greenhouse. However, for bean cultivars, one sampling time was taken at the bean flowering phase in Experiment 1. Bean roots were obtained in the proximity of each plant using metal cylinders with a diameter of 2.5 cm and three random sampling plants per repetition.

Samples of approximately 25-50 root segments were collected per repetition and stored in 30% ethanol with glycerol. The root segments were 1-3 cm in length and around 0.5 mm diameter. Root segments were left overnight in 10% KOH, then rinsed with water followed by a 5% HCl. Samples were further rinsed with water and stained for 16 hours with 0.05% trypan blue solution in glycerol (Godbold and Sharrock, 2003). This clearing and staining procedure was used to make root segments easily visible under microscopic examination. The root segments were mounted on glass slides for microscopic examination (entire root image analysis) (Varma, 1998). Fungal entry points, vesicles and arbuscules were counted on a percent-length basis (Douglas Godbold, personal communication) using 100X and 160X magnification with a NIKON phase contrast –2 1.25 to determine the abundance of fungal structures.

For the calculation of the proportion of fungal structures in roots, at least one fungal structure in root segment was enough to consider the presence of AM fungi. Chi-square tests were employed for the frequency of fungal structure occurrence analysis in root samples.

3.9.2 External fungal mycelium length

Two grams of fresh soil, in which "arbuscular mycorrhizal" inoculated plant genotypes, were suspended in 90 cm^3 of deionised water and vigorously stirred with a magnetic device for five minutes. The suspension was passed through a 250 μm screen. Fifteen cm^3 of the suspension were passed through a cellulose membrane (0.45 μm pore size) and submerged in an acid glycerol trypan blue solution for 1 hour, rinsed with deionised water and vacuum-filtered (Photo 2). The membranes were allowed to dry and a 17 x 17 mm piece was placed on a microscope slide wetted with acidic glycerol, and covered with a cover glass (Godbold *et al.,* 1997).

Photo 2. External fungal mycelium in cellulose membrane after being submerged in an acid glycerol trypan blue solution.

Mycelia were measured by means of an eyepiece micrometer per optic field grid. Mycelia in nine optic fields per sub-sample were used to determine the number of mycelia per mm^2 and mycelium length in microns per mm^2. Mycelium density in pots with and without *Tithonia* genotype plants was determined by the technique described in Sylvia (1992). However, the difference between external mycelia in pots with *Tithonia* genotypes and pots with bare soils was not determined to avoid negative values when free mycelium length was greater in bare soil than in pots with *Tithonia* plants. Instead,

mycelium density values for *Tithonia* genotypes and unplanted pots (control) were separately presented.

3.10 Internal concentration of organic acids in the root tip and leaf tissue

For internal organic acid concentration in the root tip and leaf tissue, pieces of root tips of 1-2 cm. long. and leaf samples (1 to 3 g of fresh weight) were taken and placed inside 10 mL syringes, which were previously weighted. Samples were kept closed to avoid desiccation. The fresh weights were taken and the samples were submerged in liquid nitrogen for a few seconds to break cell membranes. In the syringes, 1.5 mL Eppendorf micro-test tubes were placed to collect the root or leaf extracts during centrifuging. The fresh samples were centrifuged at speeds of 2,000 to 3,000 rotations per minute for 5 to 10 minutes. Once the extracts were obtained, they were stored in 1.5 mL safe-lock Eppendorf and frozen immediately in liquid nitrogen to preserve the organic acids and then stored in a deep freezer (Douglas Godbold and Patrick A.W. van Hees, personal communications). Concentrations of internal organic acids were measured using High-Performance Liquid Chromatography (HPLC) against concentrations of organic acid standard solutions. Organic acid standards (in specific concentrations) were run first to identify the peak position of each acid. The extract samples were centrifuged at 4,500 rpm before HPLC analysis to separate dirt. Then, the samples were diluted and filtered in a 0.45 μm nylon membrane. Direct injection in a column Supelcogel G-610H-H (25 cm x 4.6 mm) was employed (Gerardo Rodriguez, personal communication). The mobile phase description was 0.1% H_3PO_4, 0.5 mL minute^{-1}, temperature 30 °C, detection UV, 210 nm (SUPELCO, 2000).

3.11 Soil P fraction analysis

Soil samples for P fraction determination were taken at the first, seventeenth, twenty third and fifty third week after starting Experiment 1 (from July 2001 to August 2002) using metal cylinders with a diameter of 2.5 cm at four random sampling positions per fallow species treatments (natural regeneration, *Cajanus* fallow, *Tithonia* in monoculture and in association with *Cajanus*) in the same plots. Soil P fractionation

procedure was used to examine the P cycling and biochemistry of San Juan Sur Andisol in response to the different fallow species and mulch treatments.

The sequential Hedley fractionation procedure for soil P was employed in the organic and inorganic fractions of P in which soils were quantified by means of several P extractants of different strengths (Hedley *et al.,* 1982; Lajtha *et al.,* 1999; Szott and Meléndez, 2001). The procedure starts from P easily extracted fraction in water (that can be directly exchangeable with the soil solution) and alkaline bicarbonate extract that provides a measure of relatively labile and plant-available P sorbed onto soil surfaces, as bicarbonate mimic the respiration activity of plant roots and will depress the activity of Ca^{2+} in high Ca soils. The bicarbonate step is more effective in relatively unweathered soils than in tropical soils; however, the NaOH extracts amorphous and some crystalline Fe and Al phosphates, as well as P strongly bound by chemisorption to Fe and Al compounds (Lajtha *et al.,* 1999). Seven P fractions were determined and grouped in four categories, employing functional concepts to qualify them as: P in the soil solution (Resin-Pi), labile P ($NaHCO_3$-Pi + $NaHCO_3$-Po), potentially labile P (NaOH-Pi + NaOH-Po) and occluded P (HCl-P + Residual-P) (Lajtha *et al*., 1999; Hoang Fagerström *et al.,* 2002; Nwoke *et al*., 2003).

3.12 Soil chemical properties, P-isotherms and the pH in NaF

In Experiment 1, soils were sampled at the surface (0-12 cm) and subsurface (12-24 cm) at the first and twenty-third week (before fallow and immediately before the application of slash and mulch treatments) using metal cylinders with a diameter of 2.5 cm at three random sampling positions per site. Four sampling sites from an area of approximately 1 hectare were chosen randomly. Two soil samples were taken in Experiment 1 and only one at the Experiment 2, six-weeks after bean planting in control and mulch treatments. Each soil sample was brought to the laboratory for partial air drying at room temperature, sieved to less than 2 mm to remove the coarse materials, stones and plant debris, and finally homogenized to obtain a representative sample of surface and subsurface soil layers. The topsoil samples were analyzed for soil texture by the hydrometer method, pH in 1:2.5 soil: water suspension, exchangeable cations and

exchangeable acidity by modified Olsen (pH 8.5) and 1 N KCl extractants. Soil samples were run in triplicate to assess analysis precision and representative.

For soil P fixation characterization, P-isotherms and NaF pH were determined at the University of Idaho for the samples collected at the beginning of the fallow period. In the P-isotherm method, soil was equilibrated with different solutions containing increasing P concentrations and adsorption isotherms were determined from the relation between adsorbed and dissolved P (Fox and Kamprath, 1970). The procedure used three grams of soil weighted in eight 50 ml plastic tubes. Duplicate 30 ml P solutions of 5, 25, 50 and 75-μg mL^{-1} were added to the tubes. Samples were shaken slowly for 3 consecutive days. They were then placed in a centrifuge at 1,200 rpm for 15 minutes and 4 mL of clear liquid were pipetted into spectrophotometer cuvette tubes containing 6 mL of triple distilled water (TDW). To each sample or standard, 2 mL of combined reagent B (reagent A - ammonium molybdate and potassium antimony tartrate plus ascorbic acid) was added and mixed well. After 30 minutes, absorbance was read at 660 nm on the spectrophotometer. The μg mL^{-1} of P (as phosphate) left in solution was calculated by preparing a standard curve and determining the linear regression equation. Data from the regression equation was multiplied by 2.5 (4 to 10 dilution) to calculate actual μg mL^{-1} P remaining in the solution. The μg P sorbed per gram of soil was determined by subtracting the μg mL^{-1} P remaining in solution from each of the P additions (5, 25, 50, or 75) and multiplied by 10 (30 ml solution added per three grams of soil). P-isotherms were prepared by plotting μg mL^{-1} P remaining in solution on a logarithmic scale (X-axis) and μg P sorbed per gram of soil on the Y-axis. Plots with straight-line curves indicated most of the P was sorbed by the soil (Paul A. McDaniel and Anita L. Falen, personal communications).

NaF pH was determined by adding 50 mL 1-M NaF (sodium floureno) solution to 1 gram of soil in a 50 mL beaker. The mixture was stirred for one minute, allowed to stand for one minute and read using a standardized pH meter. High NaF pH numbers are a general indicator of amorphous material presence such as those that would form from volcanic ash weathering.

3.13 Analytical methods

The variables measured in fallow species and bean cultivars in the Experiment 1, Experiment 2 and the supporting experiments were used to answer the research hypotheses.

In Experiment 1 and 2:

Fallow species:

1. Biomass production and nutrient accumulation of *Tithonia* genotypes in monoculture and in association with *Cajanus*;
2. *Tithonia* genotypes and *Cajanus*: Above-ground biomass, dry weight partitioning (leaf, non-lignified and lignified stems and flower), stem length and the number of branches;
3. Nutrient concentrations (N, P, K, Ca and Mg) in the dry weight of fallow species;
4. Root extension characteristics (specific root length and root length density) in the different fallow species treatments and at different soil depths;
5. Specific root length per root diameter class and root hair length, and
6. Fungal structures in *Tithonia* roots: Entry points, vesicles and arbuscules.

Bean cultivars:

1. Above-ground biomass, dry weight partitioning (leaf, stem and pod) and yield under different fallow species treatments, slash biomass application, and mulch treatments;
2. Nutrient concentrations (N, P, K, Ca and Mg) in dry weight under different fallow species treatments, slash biomass application and mulch treatments;
3. P uptake and utilization efficiencies;
4. Root dry weight partitioning (adventitious root, basal root, upper root-stem and lower root-stem dry weights) under different slash biomass application and mulch treatments;
5. Root component (number of nodules, adventitious and basal roots) under different slash biomass application and mulch treatments;

6. Root extension (specific root length of adventitious and basal roots, upper and lower root-stem lengths) under different slash biomass application and mulch treatments;

7. Root architecture (number of nodules, adventitious and basal roots, upper and lower root-stem lengths) under different slash biomass application and mulch treatments;

8. Root to shoot biomass ratio, diameter of root-stem at the plant base part and at the transition zone between adventitious and basal roots, and

9. Fungal structures on bean roots (entry points, vesicles and arbuscules) under different slash biomass application treatments.

Soil variables:

1. Soil P fractionation: P in the soil solution (Resin-Pi), $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pi, NaOH-Po, HCl-P and residual-P under different fallow species treatments (Experiment 1);

2. Soil chemical properties (pH, exchangeable acidity, exchangeable cations, total P, N, minor nutrients, Al and Fe) in Experiments 1 and 2 for field site characterization, and

3. P-isotherms in Experiment 1.

In supporting experiments:

Potted plants under greenhouse and girdled stems with *Tithonia* genotypes:

1. External fungal mycelium: number of mycelia, mycelium length and mycelium length density;

2. Organic acids in the root tip and leaf tissue on girdled stems: oxalic, malic, succinic, fumaric and citric acids;

3. Above and below-ground dry weights, root to above-ground biomass ratio and root hair;

4. Number of roots and stems, root length, specific root length, root length density and root length diameter distribution, and

5. Fungal structures in roots: entry points, vesicles and arbuscules.

In controlled environment experiments with *Tithonia* genotypes:

1. P concentration in leaf, stem and root of *Tithonia* genotypes at different P availability levels;

2. P uptake and utilization efficiencies at different P availability levels;
3. Above and below-ground biomass at different P availability levels, and
4. Root length, root length diameter distribution and root to above-ground biomass ratio at different P availability levels.

For the randomized block design with split-plot design (Experiment 2), supporting experiments in randomized design and random sampling in the *Tithonia* germplasm collection, the linear additive models are not explained in detail. However, for Experiment 1 in a magic latin square with split-plot design, the following linear additive model was used to explain the response of experimental observations for the different factor effects and error:

$$Y_{ijk(l)mn} = \mu + a_i + n_j + s_k + r_{(l)} + \varepsilon_{ijk} + b_m + q_n + (bq)_{mn} + (rbq)_{(l)mn} + \varepsilon_{ijk(l)mn}$$

where Y_{ijklmn} represents the observation of the i^{th} row, j^{th} column, k^{th} magic square, l^{th} treatments, m^{th} subfactors and n^{th} subfactors;

μ = population mean;

a_i = effect of the i^{th} latin square row;

n_j = effect of the j^{th} latin square column;

s_k = effect of the k^{th} magic square;

r_l = effect of the l^{th} treatment in the main-plot (sources of biomass);

ε_{ijk} = experimental error associated to the main plots;

b_m = effect of the m^{th} subfactor (biomass application treatments);

q_n = effect of n^{th} subfactor (bean cultivars);

$(bq)_{mn}$ = effect of the $(bq)mn^{th}$ two interaction subfactors;

$(brq)_{(l)mn}$ = effect of the $(rbq)(l)mn^{th}$ three interaction factors and

$\varepsilon_{ijk(l)mn}$ = experimental error associated to subplots.

Analysis of variance (ANOVA) evaluated the main effect of factors (source of biomass in fallow species treatments, biomass applications, bean yield, genotypes and cultivars), and their interactions using general linear model procedures (GLMs) of SAS, statistical program, version 8 (SAS, 1999). The estimated probability in SAS was always presented, and for statistical interpretation, the significant levels of α=0.05 and α=0.01 were used in ANOVA tests. Either orthogonal contrast or Duncan's multiple range test (DMRT) was used to compare means. Mean and standard errors were present for treatment comparisons. Orthogonal contrasts were employed to find the differences occurring in specific groups of treatments (Gilberto Páez, personal communication). The most important orthogonal contrasts for the response variables in Experiment 1 were:

- *Tithonia* in monoculture versus *Tithonia* and *Cajanus* association;
- *Tithonia* and *Cajanus* association versus control (natural regeneration);
- Bean response in absence of slash biomass versus bean response in presence of slash biomass, and
- Costa Rican bean (the Chirripo Rojo and Negro Huasteco) versus exotic CIAT bean cultivars (the Dor-364 and CIAT G-1937).

Since data included simultaneous measurements on several nutrients, fungal structures in root and plant characteristic variables, multivariate analysis (MANOVA) using Wilks' Lambda test (Dallas, 2000) was applied to understand the relationship between variables such as growth response, root system characteristics and their association with nutrient acquisition mechanisms and biomass accumulation. Significant differences in MANOVA test means that the vector dimension fell into a subspace other than zero. The dimensional space of the mean vector can be ascertained inspecting the eigenvalues: if they show a dimension m=1 with a significant F value (p<0.05) for each group of variables then sample means (the canonical variables) are spread along a straight line (one-dimensional space). To determine the dimensional representation for each mean, the canonical values are calculated and plotted on (X, Y) scatter graphs. Canonical variables (new derived variables that compress in less number of variables the effects of groups of the related original variables) were used to analyze the relationship between the

plant attributes that they represent (e.g. root extension, root components, above and below-ground dry weight partitioning).

Multi-linear regression was calculated using SAS command STEPWISE to detect which root system characteristics were strongly and positively associated with pod production and above-ground dry weight (valid for the bean cultivars). Cluster analysis was used to reduce the source of multi collinearity between root characteristic variables in bean cultivars before the multi-linear regression analysis. The standard distance (1-abs (Pearson)) and 0.55 as the cut criteria point (Johnson and Wichern, 1998) were used to identify groups of root variables. One root variable per cluster was chosen. The variable selected per cluster included the ones from the three groups (dry weight partitioning, root component and root extension measurements), but priority was given to those variables that were simple to measure. Linear correlation coefficients were determined using the PROC CORR procedure of SAS and simple regression models (with PROC REG statements) between different root characteristics, P concentration in dry weight and above-ground biomass accumulation.

Covariable analysis was applied to adjust bean yield in plants corresponding to some treatments that were more damaged than others by cattle and rabbits. For the case of bean seed germination at harvest time, covariable adjustment was not necessary because the partial seed germination was mainly uniform between plot treatments. However, adjustments for the loss in bean yields due to seed germination were conducted by correction factors for bean yields. Over 30% of the harvested bean plots were randomly sampled to estimate the correction factors with enough precision for each treatment and bean cultivar.

4. Results

4.1 Fallow species on an Andisol with low P availability

4.1.1 Above-ground biomass accumulation

Above-ground biomass accumulation, measured 24 weeks after planting, indicated that fallow species treatments differed (p<0.0001) according to ANOVA test (Table 1). The highest above-ground biomass was for the Mexican *Tithonia* genotype in monoculture (10.5 Mg ha^{-1}) (Photo 3), followed by the Mexican *Tithonia* genotype in association with *Cajanus* (7.4 Mg ha^{-1}) (Figure 2). The lowest biomass accumulation were for *Cajanus* and the Indonesian *Tithonia* fallow. *Cajanus* fallow was statistically similar to the Indonesian *Tithonia* fallow, according to Duncan's test.

An important source of variation within fallow species treatments was captured by the non-biased group of comparisons (orthogonal contrasts), which were contemplated in the experimental design, allowing for the detection where the differences were occurring in above-ground biomass accumulation (Table 1). *Cajanus* fallow differed from *Tithonia* fallows in monoculture and *Tithonia* in association with *Cajanus* fallows (p<0.0001). The Mexican *Tithonia* genotype in monoculture was higher than the Indonesian *Tithonia* genotype in monoculture (p<0.0001). The Mexican *Tithonia* genotype in association with *Cajanus* was higher than the Indonesian *Tithonia* genotype in association with *Cajanus* (p<0.0001) and *Tithonia* fallows in monoculture were higher than *Tithonia* in association with *Cajanus* fallows (p=0.0255).

4.1.2 Above-ground biomass allocation and branching patterns

Above-ground biomass allocation significantly differed between *Tithonia* genotypes in lignified and non-lignified stems, leaves and flowers (p=0.0178, p=0.0231, p=0.0070 and p<0.0001), respectively (Figure 3). In *Tithonia* genotypes, the above-ground biomass allocations were significantly different (p<0.0001) by the Wilks' Lambda multivariate test. The Mexican *Tithonia* genotype had more biomass allocation into the flower part than the Costa Rican and Indonesian genotypes six-months after

Table 1. Orthogonal breakdown of treatment effects on biomass accumulation six-months after planting in Experiment 1 at San Juan Sur, Turrialba, Costa Rica, (n=64).

Source of variation	df	MS	F value	Pr > F	Interpretation
Rows	7	6.48	1.17	0.3420	n.s.
Columns	7	27.11	4.90	0.0005	**
SuperRow*Supercolumn	3	8.16	1.47	0.2364	n.s.
Fallow species treatments (A)	7	150.97	27.27	<0.0001	**
Natural regeneration VS. All species	1	12.15	2.20	0.1465	n.s.
Cajanus VS. *Tithonia* and *Tithonia* /*Cajanus*	1	103.51	18.69	0.0001	**
Tithonia VS. *Tithonia* /*Cajanus*	1	29.86	5.39	0.0255	**
CR *Tithonia* VS. MEX and IND *Tithonia*	1	87.40	15.78	0.0003	**
CR *Tithonia* /*Cajanus* VS. MEX and IND *Tithonia* /*Cajanus*	1	22.14	4.00	0.0526	n.s.
MEX *Tithonia* VS. IND *Tithonia*	1	689.48	124.52	<0.0001	**
MEX *Tithonia* /*Cajanus* VS. IND *Tithonia* /*Cajanus*	1	112.27	20.28	<0.0001	**
E. Error (a)	39	5.54	5.12	<0.0001	-
Subtreatments: (B) and (C)	3	1.02	0.95	0.4195	n.s.
Interaction (AxBxC)	21	1.30	1.21	0.2518	n.s.
E. Error (b)	168	1.08			
Total	255				

* Significant differences at 5% level; ** Highly significant differences at 1% level; n.s. Non significant differences

Note: The relative efficiency of the magic latin square design was 52.8%, 31.5% and 3.2% with respect to completely random design, randomized complete block design and latin square design.

Photo 3. The Mexican (left) and Costa Rican (right) *Tithonia diversifolia* genotypes harvested six-months after planting in Experiment 1 at San Juan Sur, Turrialba, Costa Rica. *Canavalia* shown in front of *Tithonia* genotypes was used to protect from leaf-cutter ants (Zompopo) all around Experiment 1.

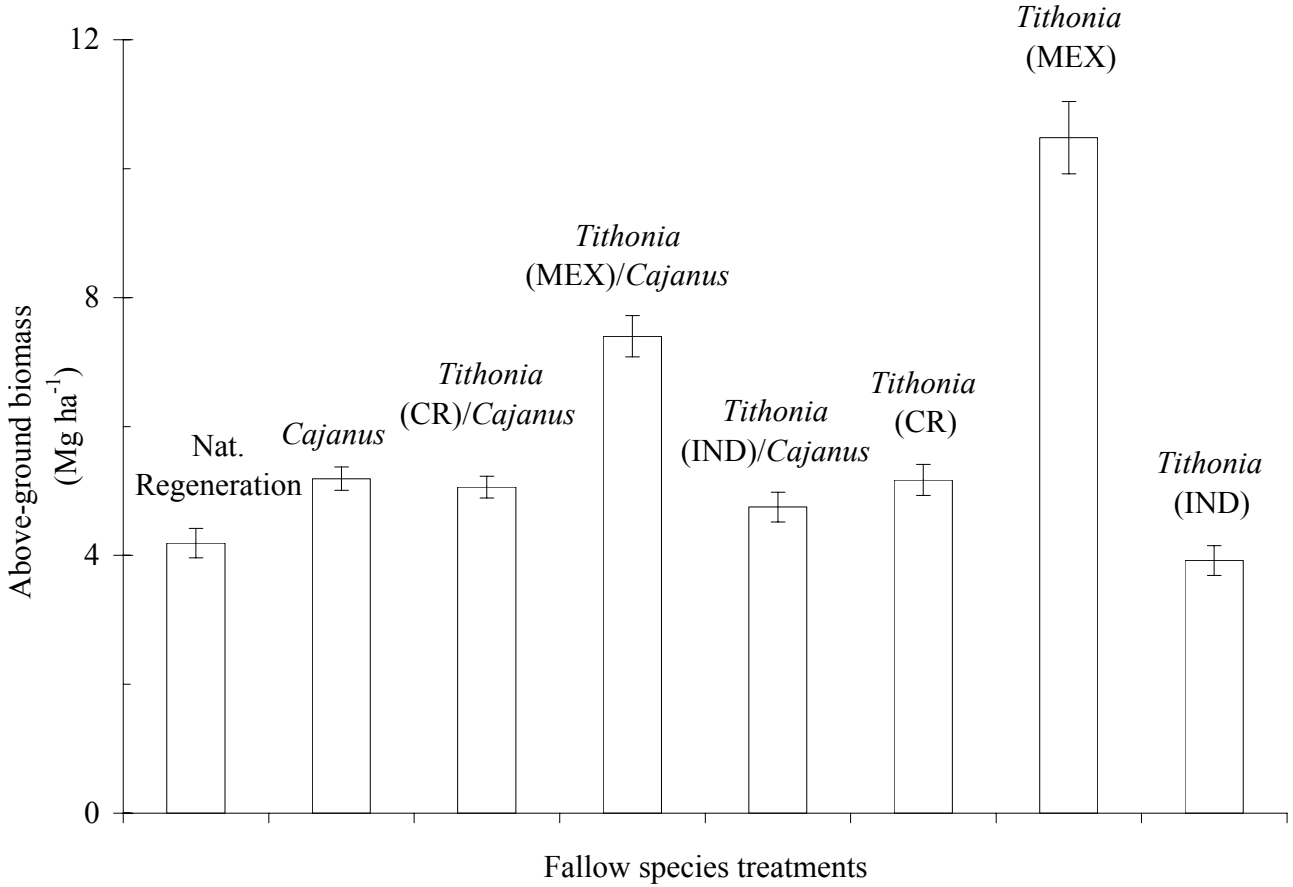

Figure 2. Above-ground biomass of natural regeneration, *Cajanus* and *Tithonia* genotypes with and without *Cajanus* harvested six-months after planting on an Andisol with low P availability, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=32).

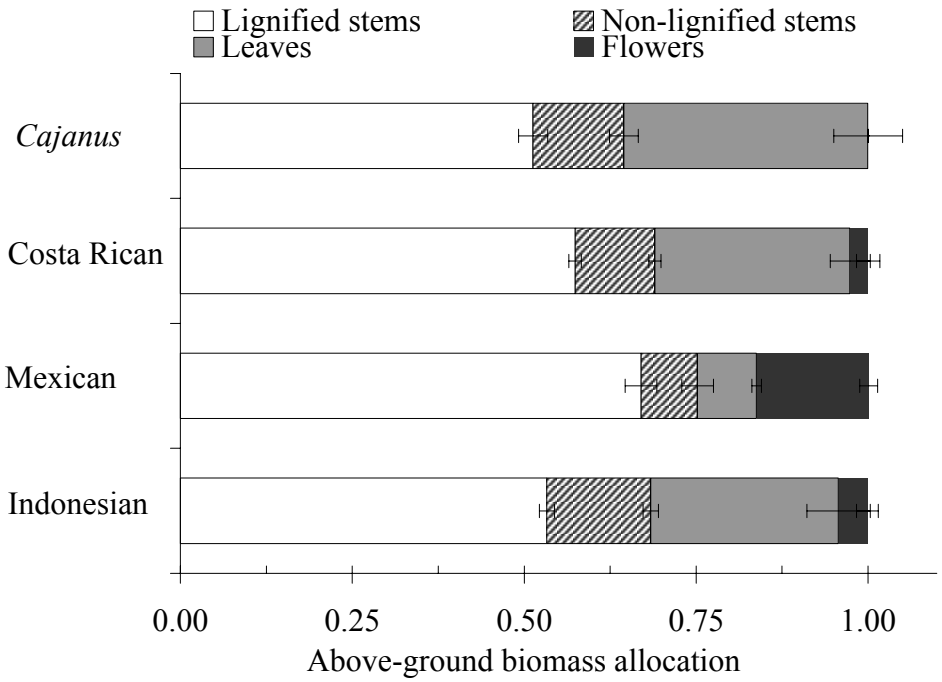

Figure 3. Above-ground biomass allocation of the Costa Rican, Mexican and Indonesian *Tithonia* genotypes and *Cajanus* harvested six-months after planting on an Andisol with low P availability at San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=6). Wilks' Lambda multivariate test (p <0.0001) showed fallow species were significantly different for above-ground biomass allocation. Variability in flowering time was detected in the different *Tithonia* genotypes. The Indonesian was the earliest and the Costa Rican the lasted. Sampling time (February) coincided with the flowering phase of the Mexican *Tithonia* genotype.

planting in Duncan's test. In orthogonal contrast comparisons, the Indonesian *Tithonia* genotype had the most biomass allocated into non-lignified stem parts (15%, p=0.0069), followed by the Costa Rican *Tithonia* genotype. The Costa Rican *Tithonia* genotype had more biomass allocated into leaves than the Mexican and Indonesian genotypes (p=0.0174). The Mexican *Tithonia* genotype had more biomass allocated into lignified stem part (75%) which was not different than the Costa Rican genotype, but different from the Indonesian genotype (p=0.0062).

The Mexican *Tithonia* genotype was larger and had fewer stems (2) than the other genotypes. The Indonesian and Costa Rican genotypes resemble small shrubs with many stems (3 to 5). When planted in monoculture, the Mexican genotype showed greater biomass accumulation over the short-term (Figure 4-A), and showed greater variation in above-ground biomass accumulation per plant and greater stem length in comparison to *Cajanus* and the other *Tithonia* genotypes (Figures 4-A and 4-B). Branching of the Costa Rican and Indonesian genotypes tended to appear below 0.75 m height and have a greater number of stems than the Mexican genotype. The Costa Rican and Indonesian genotype stem lengths did not continue increasing 12 weeks after planting because of the development of secondary/tertiary branching, but not the case for the Mexican genotype. The Mexican genotype also had a greater primary stem diameter than the Costa Rican and Indonesian genotypes. In the Indonesian genotype, secondary and tertiary branching increased rapidly 17 weeks after planting, which partially explains the gain in biomass at final harvesting (Figure 4-A) and the decrease in the mean stem length (Figure 4-B).

4.2 Nutrient concentration and accumulation in the biomass of fallow species

Nutrient concentrations of N, P, K and the other macronutrients of the fallow species treatments as nutrient sources are presented in Appendix 4. In ANOVA test, no significant nutrient concentration differences were found between fallow species treatments six-months after planting. The only exception was the difference in Mg and K concentrations between *Cajanus* and the Costa Rican *Tithonia* genotype in Duncan's comparisons (Appendix 4). Figure 5 shows the range of P and Mg concentration means for the *Tithonia* genotypes, *Cajanus* and natural regeneration 17 and 24 weeks after

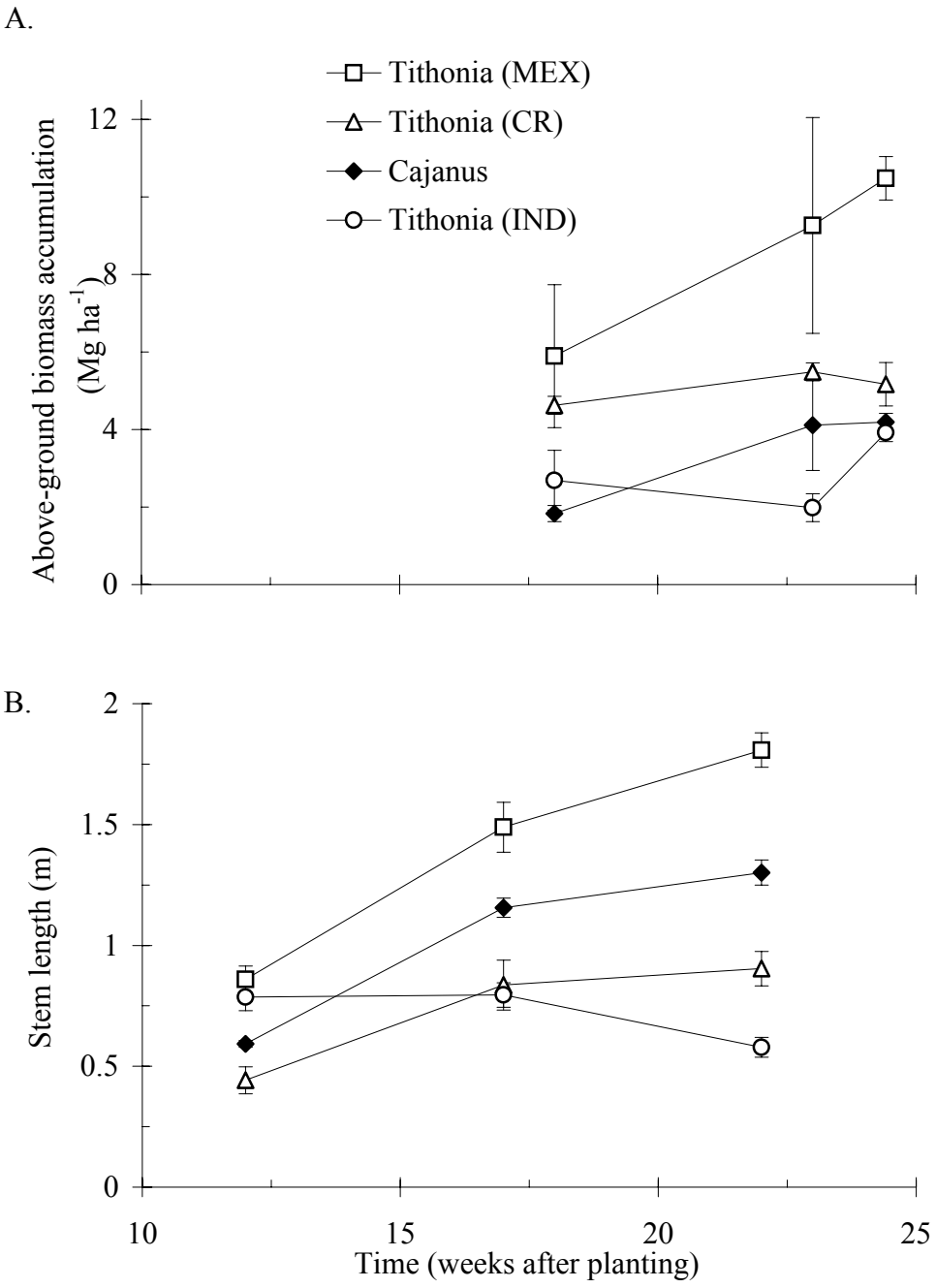

Figure 4. Above-ground biomass accumulation (A) and stem length (B) of *T. diversifolia* genotypes and *Cajanus* in monocultural plots at three different harvest times. Standard error bars are displayed (n=8).

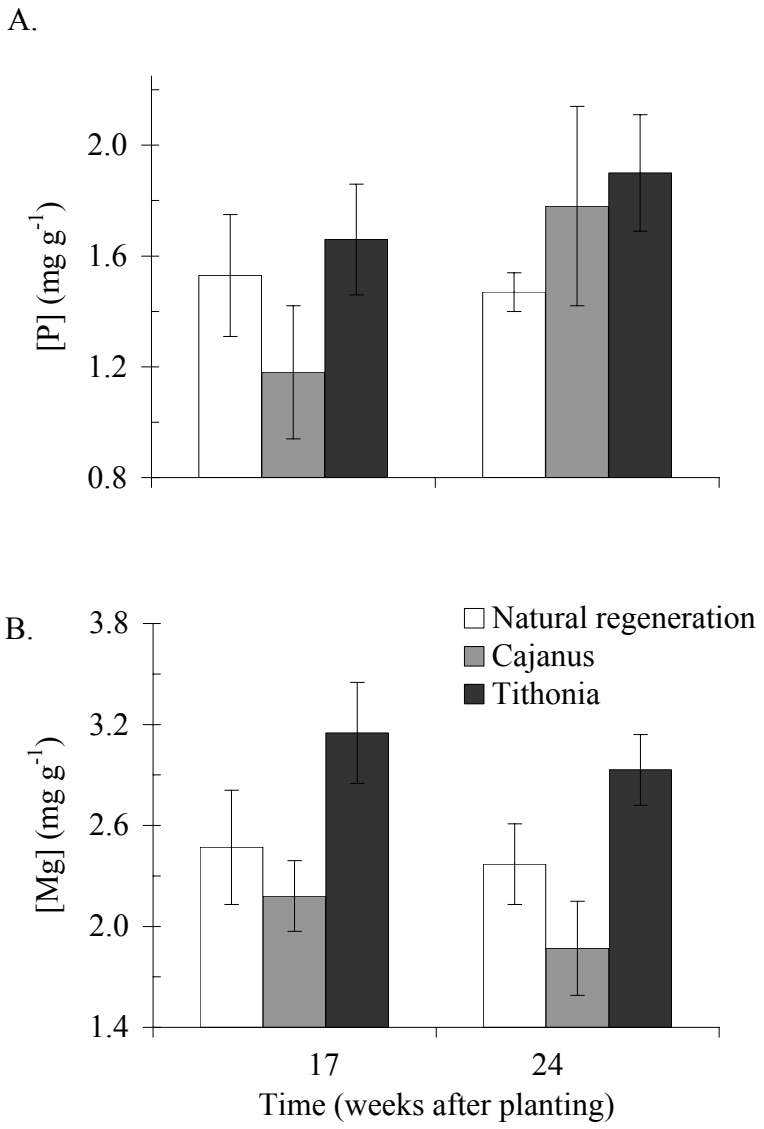

Figure 5. Phosphorus (A) and magnesium (B) concentrations in the biomass of *Tithonia* , *Cajanus* and natural regeneration at two different harvest times in San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=3).

planting. These nutrient concentrations for fallow treatments 24 weeks after planting corresponded to the time when accumulated biomass was cut and applied to the corresponding slash application treatments. In multivariate analysis using Wilks' Lambda test for nutrient concentrations, no significant differences were found between natural regeneration, *Cajanus*, *Tithonia* in monoculture and *Tithonia/Cajanus* association treatment groups (p=0.0869, Figure 6). However, the nutrient inputs (concentrations x biomass) in cut and retained biomass with the Mexican *Tithonia* genotype (*in situ* mulch) were higher than the other fallow species for the six-month fallow period. On the other hand, *Cajanus* was left far behind, mostly by a much lower biomass accumulation.

Therefore, most of the differences in nutrient accumulation corresponded only to the differences in biomass accumulation by the fallow species because nutrient concentrations were similar between fallow species (Appendix 4). The Mexican *Tithonia* genotype grown in monoculture and in association with *Cajanus* accumulated the highest amount of nutrients in plant tissue during the six-month fallow period (Appendix 5). The total N inputs ranged from 115 kg N ha^{-1} for *Cajanus* to 208 kg N ha^{-1} for the *Tithonia* genotype coming from 4.19 and 10.5 Mg ha^{-1} above-ground biomass applied with *Cajanus* and the Mexican genotype, respectively. P accumulation range was 8-18 kg P ha^{-1} between fallow species treatments. At least, the nutrient amounts presented in Appendix 5 returned to the soil for *in-situ* mulch treatments.

The nutrients accumulated mainly in above-ground biomass in most of the fallow species biomass, but *Cajanus*, in which considerable litter (mostly leaves) fell at the beginning of bud-flower emergence (six-months after planting, just at the pruning time). The contribution of litter and below-ground biomass as a source of nutrients was not included in total nutrient accumulation mentioned above.

4.3 Development of fallow species root systems

When the three different root sampling times were considered, root length density (RLD) did not show significant differences between fallow species treatments (p=0.2303), but showed significant differences between sampling times (p<0.0001),

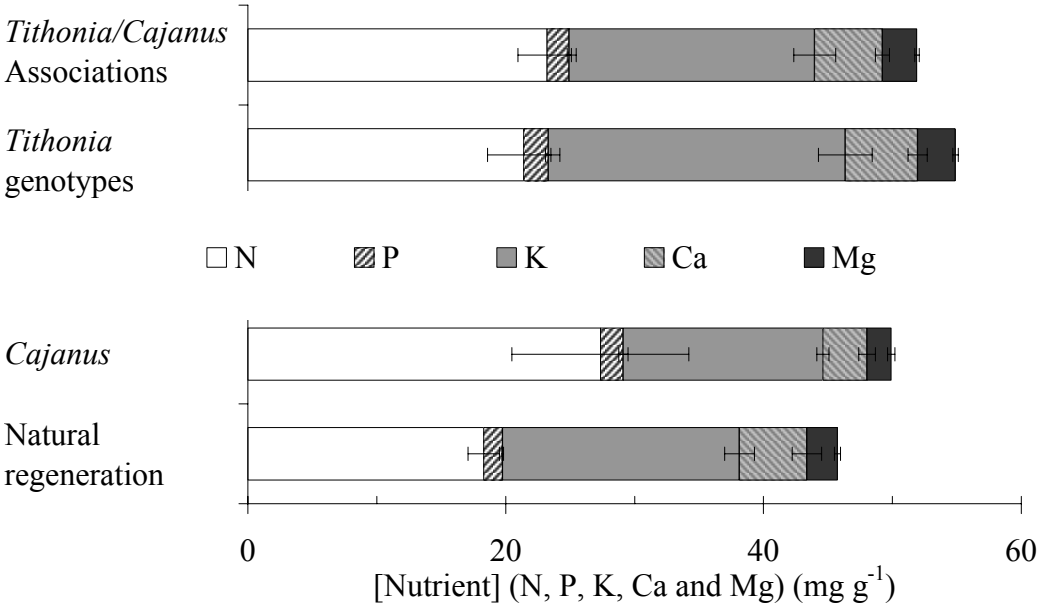

Figure 6. Nutrient concentrations based in above-ground biomass of *Tithonia*/*Cajanus* associations, natural regeneration, *Tithonia* genotypes and *Cajanus* in monocultural plots harvested six-months after planting on an Andisol with low P availability at San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=6). Wilks' Lambda multivariate test (p =0.0869) showed fallow species were not significantly different for nutrient concentrations.

depths (p<0.0001) and the interaction sampling times and depths (p<0.0001), but not for the interaction fallow species and sampling depths. No differentiation in RLD between fallow species was found in the first root sampling times (12 and 17 weeks after planting). As examples, RLD for natural regeneration and Mexican *Tithonia* genotype are shown in Figure 7. RLD of the Mexican genotype continued increasing in the 0-8 cm and 8-16 cm soil sampling depths during fallow period (Figure 7-B), but showed a gradual decrease at 16-24 cm soil sampling depth 17 weeks after planting.

However, 23 weeks after planting, analysis of variance for RLD detected significant differences between fallow species treatments (p=0.0254) and between soil sampling depths (p<0.0001) (Appendix 6). In *Tithonia* genotypes, above-ground biomass was significantly correlated with RLD in the upper layers (Figure 8-A). However, P concentration in *Tithonia* genotype biomass was poorly and negatively correlated with RLD (Figure 8-B).

The interaction of fallow species treatments and soil sampling depths was not significantly different for RLD. In comparison to the densely planted fallow species treatments, RLD in control (natural regeneration) for 0-8 cm soil sampling depth decreased markedly in the deeper layers (Figure 9-A). The upper soil layer had higher soil fertility, which was implied by the balance between the different soil P fractions down soil profile (Appendix 7) and the high RLD in this layer. Higher RLD down soil profile (0-25 cm) were obtained in the Mexican *Tithonia* genotype in association with *Cajanus,* reaching 10.97 cm of roots cm^{-3} of soil (Table 2).

No significant differences were detected between fallow species and root sampling depths for specific root length (SRL) (Table 3 and Appendix 8). However, according to SRL means, three different patterns can be recognized down soil profile between fallow species treatments (Figure 9-B). Down soil depths, *Cajanus* showed an inverse pattern in SRL in relation to natural regeneration and the other *Tithonia* genotypes. Natural regeneration had an even pattern in SRL down soil profile and *Tithonia* genotypes showed higher SRL at 8-16 cm soil depth in relation to the other soil sampling depths. The most abundant species in natural regeneration were: *Paspalum*

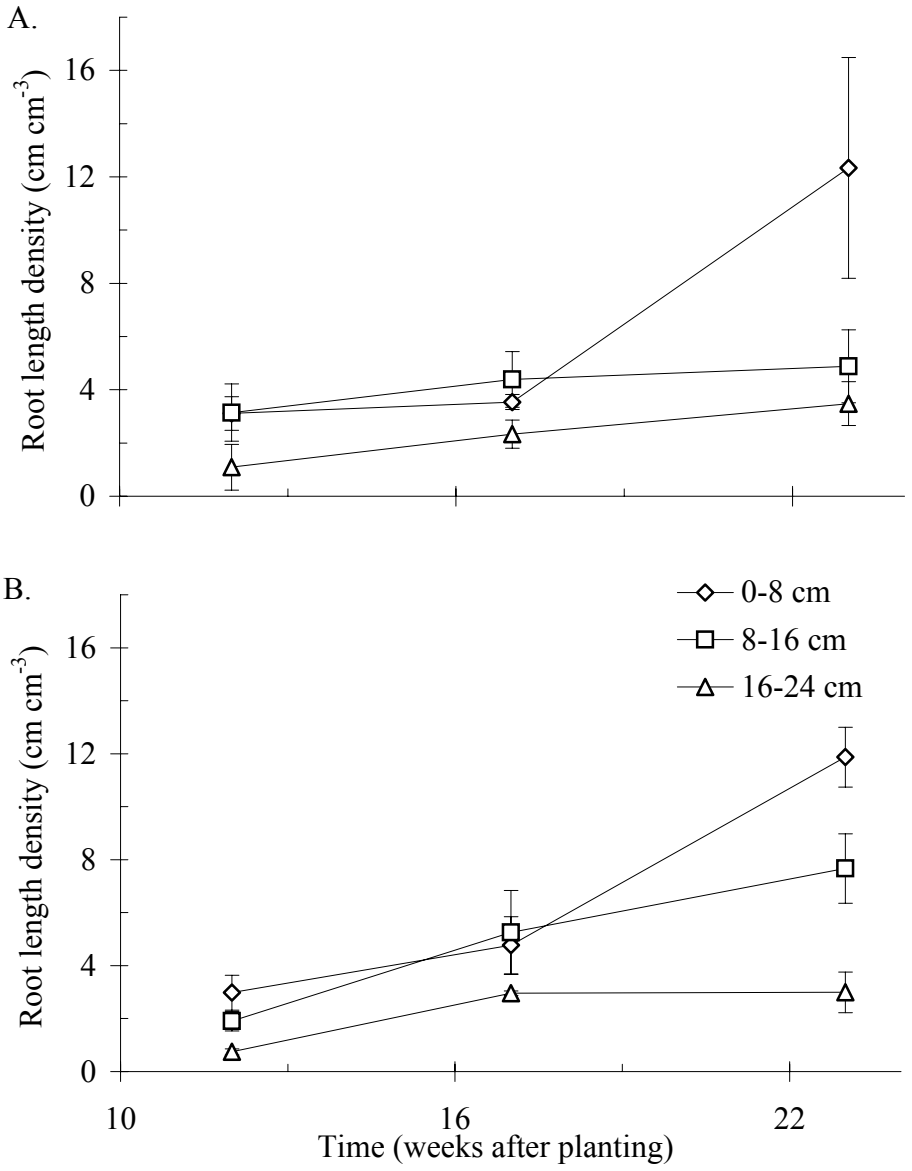

Figure 7. Root length density of natural regeneration (A) and the Mexican *Tithonia* genotype fallow (B) harvested 12, 17 and 23 weeks after planting at three soil sampling depths on an Andisol with low P availability, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=3).

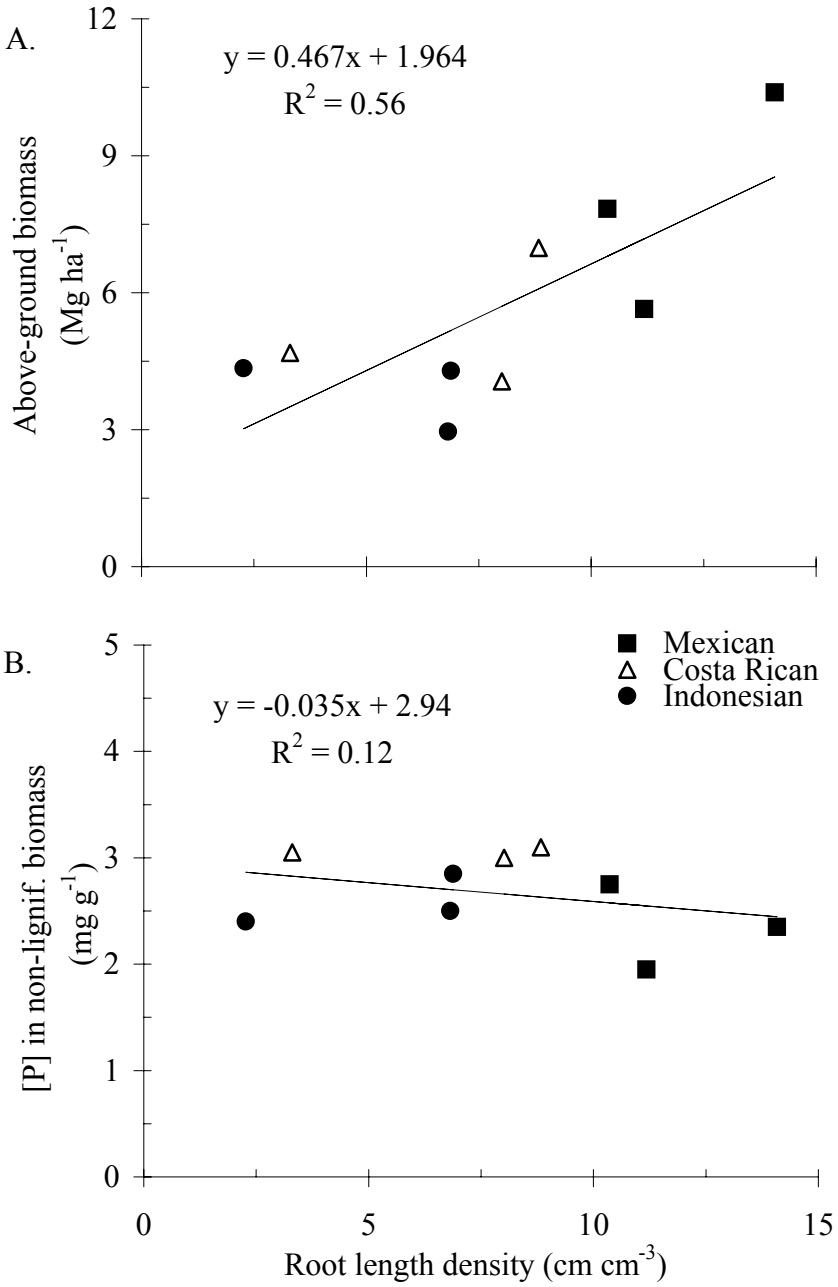

Figure 8. Relationships between above-ground biomass (A), P concentration in non-lignified biomass (B) and root length density for three *T. diversifolia* genotypes harvested 23 weeks after planting in Experiment 1 at San Juan Sur, Turrialba, Costa Rica, (n=3). Pearson correlation coef. (0.748; p=0.0206 and -0.34; p=0.3696) for above-ground biomass and P concentration, respectively. Non-lignified biomass includes non-lignified stems and leaves.

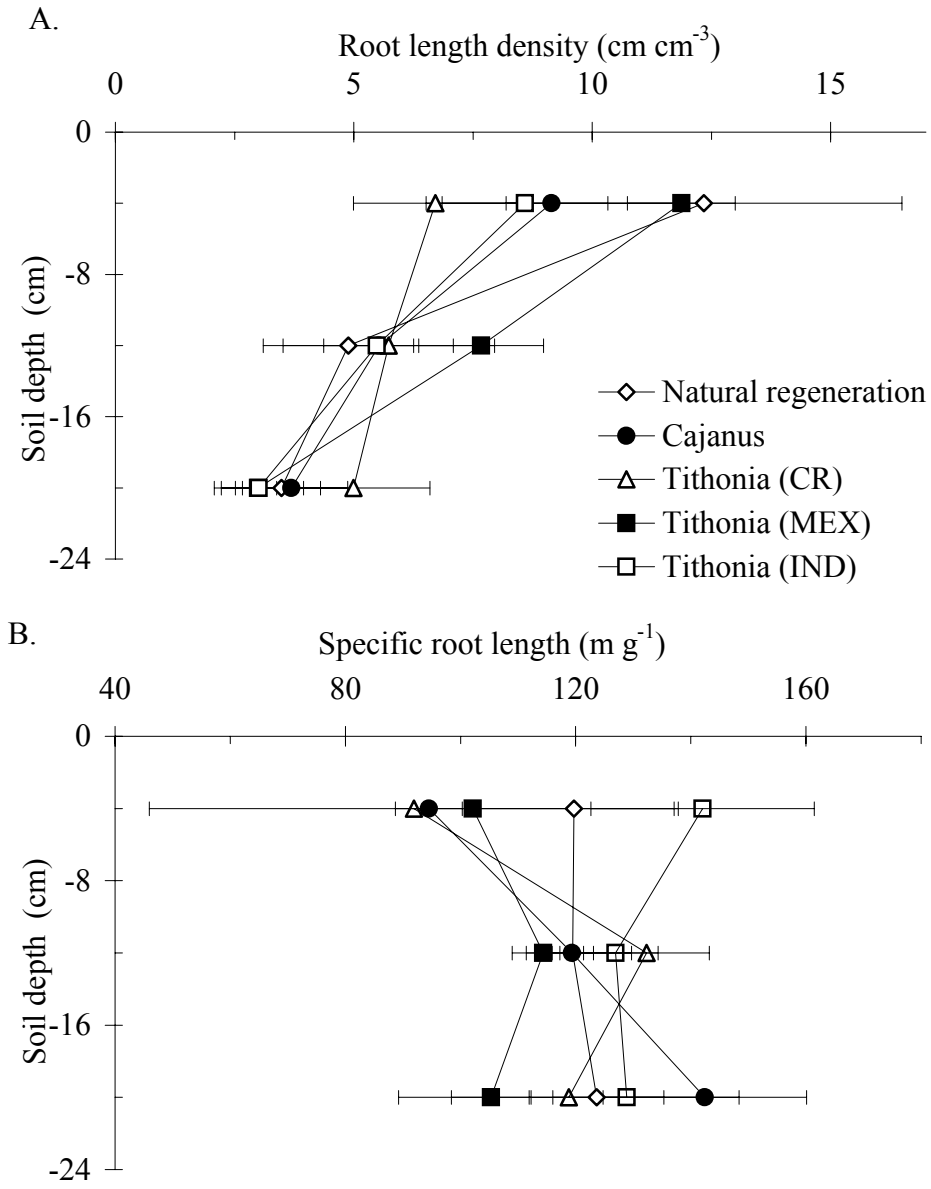

Figure 9. Root length density (A) and specific root length (B) down soil profile under natural regeneration, *Cajanus* and *T. diversifolia* genotype fallows harvested six-months after planting on an Andisol with low P availability, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=3).

Table 2. Root system parameters estimated by sampling (n=9) in the upper 25 cm of soil depth for fallow species treatments harvested 23 weeks after planting in Experiment 1.

Fallow species treatments	**Root length density** ($cm\ cm^{-3}$)	**Specific root length** ($m\ g^{-1}$)
Without *Cajanus cajan*		
Natural regeneration	6.90±1.88 (B)	120.94±6.18 (A)
Tithonia from Costa Rica	5.81±0.82 (B)	114.33±17.14 (A)
Tithonia from Mexico	7.51±1.39 (B)	107.27±4.15 (A)
Tithonia from Indonesia	5.69±0.96 (B)	132.61±7.43 (A)
With *Cajanus cajan*		
Tithonia (CR) + *Cajanus*	4.44±1.02 (B)	121.45±12.13 (A)
Tithonia (MEX) + *Cajanus*	10.97±2.19 (A)	124.83±9.51 (A)
Tithonia (IND) *Cajanus*	6.83±1.44 (B)	117.24±2.27 (A)
Cajanus	6.12±1.35 (B)	118.76±9.25 (A)

Means with the same letters along the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. Values are means ± standard errors.

Table 3. Analysis of variance, Duncan's and multivariate test outputs for root system characteristics of *T. diversifolia* genotypes on one-year old potted plants, Cabiria, Turrialba, Costa Rica.

Source of variation	ANOVA output interpretation (p values)			Wilks' Lambda multivariate test (p values)
	Root length	Specific root length	Root length density	
Genotypes (A)	0.0106	<0.0001	0.0106	<0.0001
Substrates (B)	<0.0001	<0.0001	<0.0001	<0.0001
Interaction (AxB)	n.s.	n.s.	n.s.	n.s.
	Means and the corresponding Duncan's test interpretation			
	Root length (m plant^{-1})	Specific root length (m of root g^{-1} of root DW)	Root length density (m mm^{-3})	
Genotypes (A)				
Mexican	205.6±35.8 (A)	69.4±5.6 (B)	0.39±0.07 (A)	
Costa Rican	131.0±20.4 (B)	56.1±4.0 (C)	0.25±0.04 (B)	
Indonesian	136.8±14.6 (B)	84.1±4.5 (A)	0.26±0.03 (B)	
Substrates (B)				
Soil	203.0±19.5 (A)	78.5±4.0 (A)	0.39±0.04 (A)	
Soil/Sand	93.3±9.0 (B)	58.7±4.1 (B)	0.18±0.02 (B)	

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.
n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=10.

paniculatum, *Digitaria ciliaris*, *Homolepis auterensis*, *Ageratum conyzoides*, *Cassia leiophylla*, *Calopogonium mucunoides*, *Sida rhombidolia*, *Hyptis pectinata*, *Mimosa pudica*, *Cuphea carthagenensis*, *Ageratum conyzoides, Castilleja arvensis*, *Clibadium sp*. and *Solanum americanum*.

SRL diameter distributions were similar between fallow species. The lower root diameter classes (<1.0 mm) captured the highest SRL even though part of the roots in these classes were unavoidably lost during the cleaning process because of attachment to small organic matter particles. More fine roots were found in 0-0.25 and 0.25-0.50 mm root diameter classes than any other root diameter classes (Appendix 9). However, no significant differences for SRL in the root diameter classes (0-0.5, 0.5-1, 1-1.5 and 1.5-2.0 mm) were found between fallow species in ANOVA test (Appendix 10). In multivariate analysis using the Wilks' Lambda test for root diameter classes, no significant differences were found between fallow species, but significant differences were found between root sampling depths (p=0.0038).

In one-year old potted plants, the Mexican genotype had the highest root length mean for most of the root diameter classes between 0.5 to 1.0 mm (Figure 10). Root length per diameter classes between 1.25 to 2.75 mm showed also significant differences between *Tithonia* genotypes, but these root diameter classes had very low root length. The Indonesian genotype had the greatest SRL (Table 3). However, when all diameter classes in the root system are considered, the Indonesian *Tithonia* genotype showed higher SRL than the Costa Rican and Mexican genotypes. In addition, the Indonesian genotype had the greatest number of roots coming from the root-stem (Table 4) between *Tithonia* genotypes (p=0.0005). The root to above-ground biomass ratio of the Costa Rican *Tithonia* genotype was higher than the Indonesian genotype. The Costa Rican and Mexican *Tithonia* genotypes presented similar root to above-ground biomass ratios.

Root hair length (RHL) showed significant differences between *Tithonia* genotypes in the first and second sampling times (p=0.0013 and p=0.0461), respectively. However, the *Tithonia* genotype with the highest RHL mean was different for each

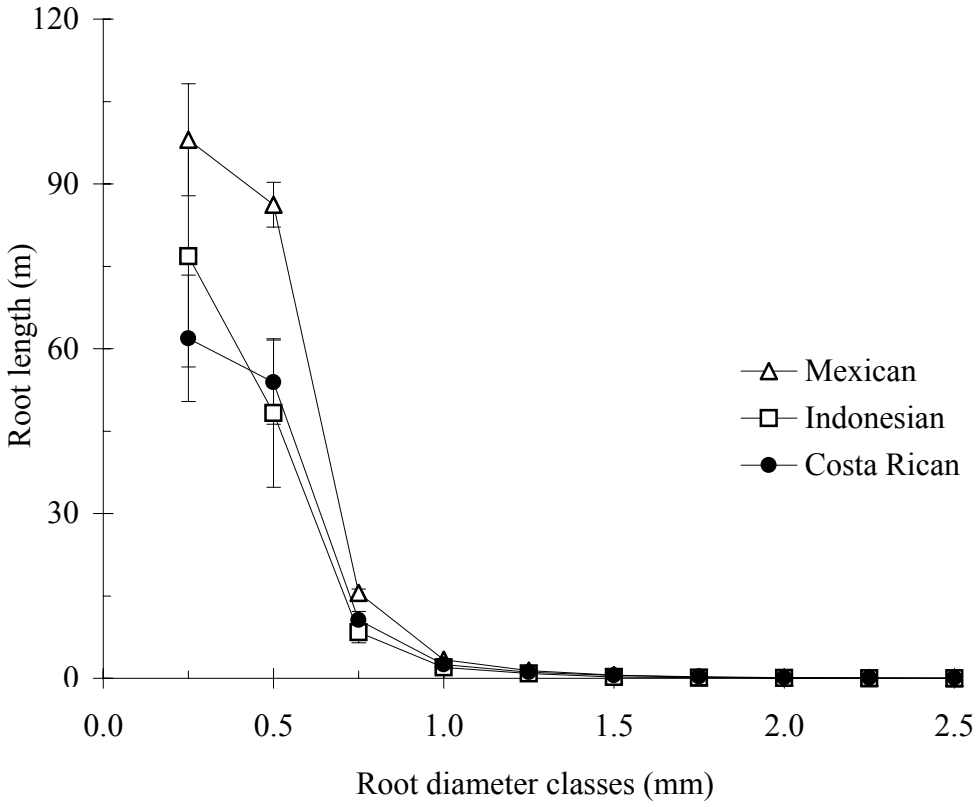

Figure 10. Root length diameter distribution for one-year old potted plants of three *T. diversifolia* genotypes at the Cabiria greenhouse, Turrialba, Costa Rica. Standard error bars are displayed (n=10). Wilks' Lambda multivariate test (p =0.0004) showed *Tithonia* genotypes were significantly different for root length. Wilks' Lambda contrasts showed significant differences for Indonesian versus Mexican and Costa Rican (p =0.0378), and Mexican versus Costa Rican genotype (p =0.0008).

Table 4. Above-ground biomass, number of roots and stems, and root to above-ground biomass ratio of *T. diversifolia* genotypes on one-year old potted plants, Cabiria, Turrialba, Costa Rica.

***Tithonia* genotypes**	**Above-ground biomass** (g)	**Number of roots**	**Number of stems**	**Root to above-ground biomass ratio**
Costa Rican	1.8±0.2 (B)	11±2 (C)	3±1 (B)	1.3±0.1 (A)
Mexican	2.8±0.3 (A)	22±4 (B)	2±0 (B)	1.0±0.1 (A)
Indonesian	3.2±0.2 (A)	35±5 (A)	5±1 (A)	0.5±0.0 (B)

Same letters between the same column indicate no significant differences (p<0.05) by the Duncan's test. Values are means ± standard errors.

sampling time. The first sampling time had a small sample size (n=9) because it was obtained from fine roots in the topsoil (Figure 11). To increase the sample size, the second and the third sampling times were obtained from girdled stems in the *Tithonia* germplasm collection. In the second sampling time, the Costa Rican genotype had higher RHL than the Indonesian genotype by orthogonal contrast comparison (p=0.0330), but in the last and larger sample taken, no significant differences in RHL were found between *Tithonia* genotypes.

4.4 External fungal mycelium and the abundance of fungal structures in *Tithonia* genotype roots

For the number of external fungal mycelia per square millimetre, external fungal mycelium length and fungal mycelium length density, no significant differences were detected between *Tithonia* genotype potted plants (Table 5). However, they showed significant differences between substrates. In *Tithonia* genotypes, external fungal mycelia did not provide additional soil exploration area. However, the Mexican genotype showed larger fungal mycelium length density than the Costa Rican genotype by Duncan's test.

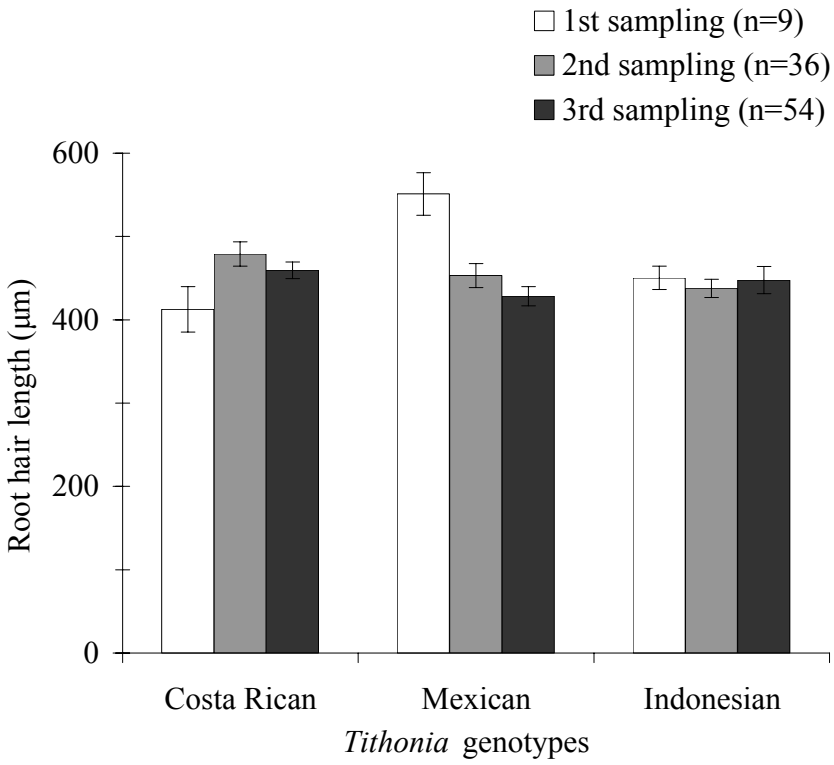

Figure 11. Root hair length measured using a light microscope at 5X and 10X magnifications for *T. diversifolia* genotype roots harvested from topsoil (1st sampling) and in six-week old girdled stems (2nd and 3rd sampling) at the *Tithonia* germplasm collection, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed.

Table 5. Analysis of variance, Duncan's and multivariate test outputs for the external mycelium in *T. diversifolia* genotype roots on one-year old potted plants, Cabiria, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)			**Wilks' Lambda multivariate test** (*p* values)
	Number of mycelia	Mycelium length	Mycelium length density	
Tithonia genotypes (A)	n.s.	n.s.	n.s.	<u><0.0001</u>
Substrates (B)	<u>0.0077</u>	<u>0.014</u>	<u>0.0145</u>	<u>0.0223</u>
Interaction (AxB)	n.s.	n.s.	n.s.	n.s.
	Means and the corresponding Duncan's test interpretation			
	Number of mycelia (units mm^{-2})	Mycelium length (micras mm^{-2})	Mycelium length density (m g^{-1} of soil)	
***Tithonia* genotypes (A)**				
Mexican	4±1 (A)	40.7±7.9 (A)	0.11±0.02 (A)	
Indonesian	4±1 (A)	30.1±5.5 (A)	0.08±0.02 (A) (B)	
Costa Rican	5±1 (A)	21.6±5.6 (A)	0.06±0.02 (B)	
No plant (Control)	4±1 (A)	29.8±7.3 (A)	0.07±0.02 (A) (B)	
Substrates (B)				
Soil	5±0 (A)	37.5±4.6 (A)	0.10±0.01 (A)	
Soil/Sand	4±0 (B)	21.1±4.0 (B)	0.05±0.01 (B)	

Same letters in the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$. Values are means ± standard errors, n=10.

For fungal structure proportion, a significant difference was found in the occurrence of fungal entry points in root segments in the different *Tithonia* genotypes in Experiment 1 by Chi-square test (Table 6). The Indonesian genotype had the lowest proportion of roots with absence of fungal entry points. The Mexican and Indonesian genotypes differed in the proportion of entry points in root segments (p=0.0123). ANOVA test did not detect significant differences in the abundance of arbuscules between *Tithonia* genotypes (p=0.0744) in Experiment 1 (Appendix 11). However, the Duncan's test found differences between the Costa Rican and Indonesian *Tithonia* genotypes (Table 7) and orthogonal contrast comparison between the Costa Rican genotype versus the Mexican and Indonesian genotypes detected also significant differences in the abundance of arbuscules (p=0.0386) (Appendix 11).

Table 6. Proportion of fungal entry points on six-month old *T. diversifolia* genotype roots in Experiment 1, San Juan Sur, Turrialba, Costa Rica, (n=64). In each square, the top value corresponds to the frequency of entry point occurrence and the value in the parenthesis corresponds to the percentage of occurrence in the sample. Chi-Square (p=0.0326) showed the proportion of fungal entry points was significantly different between *Tithonia* genotypes.

	Categories for fungal entry points in roots		
***Tithonia* genotypes**	**Absence** 0	**Presence** 1	**Total**
Costa Rican	10 (6.17)	44 (27.16)	54 (33.33)
Mexican	13 (8.02)	47 (29.01)	60 (37.04)
Indonesian	2 (1.23)	46 (28.40)	48 (29.63)
Total	25 (15.43)	137 (84.57)	162 (100.00)

Table 7. Proportion of occurrence and abundance of fungal structures in *T. diversifolia* genotype roots harvested six-months after planting in Experiment 1, San Juan Sur, Turrialba, Costa Rica.

***Tithonia* genotypes**	**Means and the corresponding Duncan's test for the proportion of fungal structure occurrence**		
	Entry points	Vesicles	Arbuscules
Costa Rican	0.81±0.05 (B)	0.30±0.06 (A)	0.39±0.07 (A)
Mexican	0.78±0.05 (B)	0.28±0.06 (A)	0.35±0.06 (A)
Indonesian	0.96±0.03 (A)	0.35±0.07 (A)	0.40±0.07 (A)
***Tithonia* genotypes**	**Means and the corresponding Duncan's test for the abundance of fungal structures** (units cm^{-1})		
	Number of entry points	Number of vesicles	Number of arbuscules
Costa Rican	11±1 (A)	3±1 (A)	4±1 (A)
Mexican	11±1 (A)	2±1 (A)	3±1 (A)(B)
Indonesian	10±1 (A)	1±0 (A)	2±0 (B)

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. Values are means ± standard errors, n=54.

In potted plants at Cabiria, only the Mexican *Tithonia* genotype had significantly more abundance of entry points than others (p=0.008) (Appendix 12). In the germplasm collection no significant differences were found for the abundance of entry points and vesicles between *Tithonia* genotypes (Appendix 13), but significant differences were found for the abundance of arbuscules (p<0.0001). The Indonesian *Tithonia* genotype had higher abundance of arbuscules than the Costa Rican and Mexican *Tithonia* genotypes.

Wilks' Lambda multivariate test did not detect significant differences between the three *Tithonia* genotypes for the abundance of fungal structures (entry points, vesicles and arbuscules) (Photo 4) in Experiment 1, *Tithonia* germplasm collection and Cabiria greenhouse (Appendices 11, 12, 13 and Figure 12).

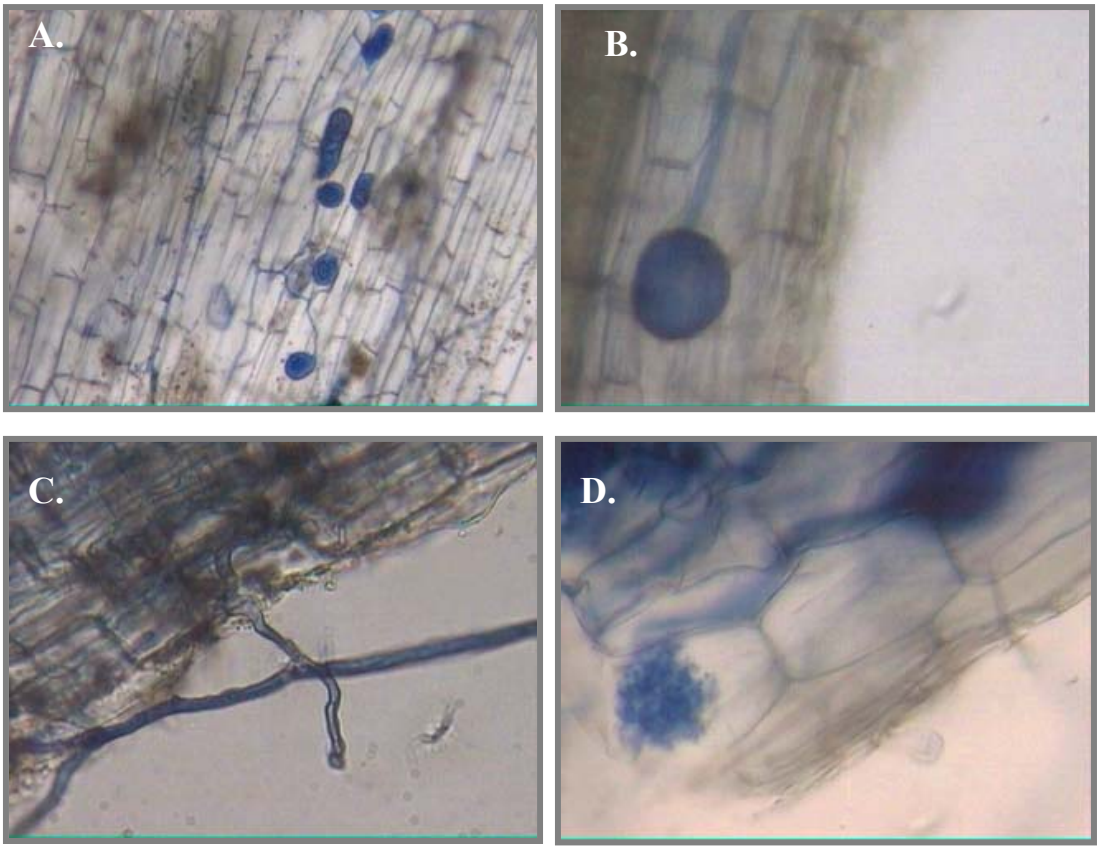

Photo 4. Fungal structures in *Tithonia* and bean roots: vesicles and spores (A) vesicle (B) entry points of hyphae (C) arbuscule and vesicle (D).

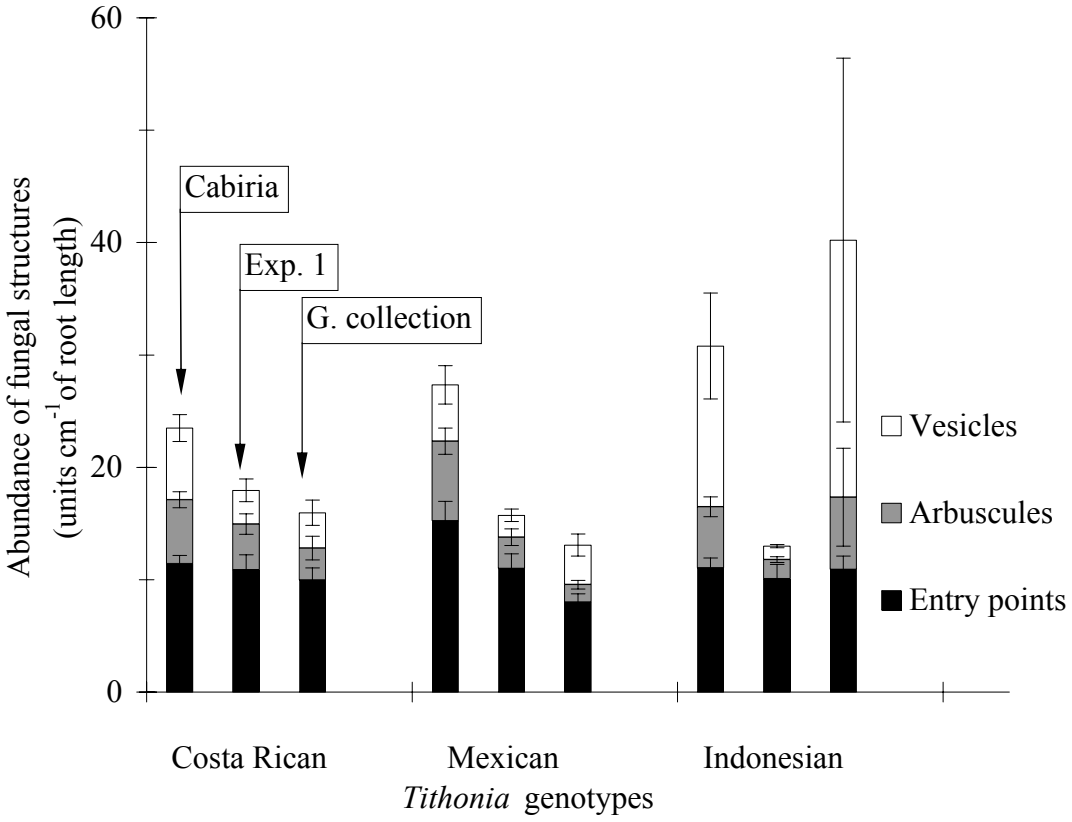

Figure 12. Abundance of fungal structures in the roots of *T. diversifolia* genotype potted plants at the Cabiria greenhouse, field experiment (Exp. 1) and *Tithonia* germplasm collection. Within each genotype, the first column corresponds to the potted plants (Cabiria) (n=88), the second one to the field experiment (Exp. 1) (n=54) and the last of each group to the *Tithonia* germplasm collection (G. collection) (n=100). Standard error bars are displayed.

4.5 Organic acids in the root tip and leaf tissue of *Tithonia* genotypes

In a multivariate test for the internal organic acid concentrations in the root tip and leaf tissue of girdled stems, significant differences were found between *Tithonia* genotypes, clones and the interaction (genotypes x clones) as shown in Table 8 and Appendix 14. In ANOVA test, the differences for internal organic acid concentrations in the root tips between *Tithonia* genotypes were found only for succinic acid; the other organic acids (citric, malic and oxalic) had no significant differences in the root tips (Figure 13). Between *Tithonia* clones within each genotype and the interaction genotypes x clones, there were significant differences for the internal organic acid concentrations in the root tips (succinic and citric acids) (Table 8) and leaf tissues (oxalic, citric and malic acids) (Appendix 14).

When testing differences between specific genotypes or combination of genotype groups, by *a priori* stated orthogonal contrasts, the Mexican genotype in comparison to the Indonesian genotype showed significant difference for succinic acid in the root tips (p=0.002) and grouping *Tithonia* genotypes, the Costa Rican versus the Mexican and Indonesian genotypes differed for malic acid. However, in the leaf tissue, the differences between *Tithonia* genotypes were due principally to citric and malic acids. The Mexican *Tithonia* genotype differed significantly from the Indonesian genotype for malic acid (p=0.004) and citric acid (p=0.015) in the leaf tissue.

The first pair of canonical variables for the internal organic acid concentrations in the leaves and root tips was not completely independent (p=0.0287) and the correlation coefficient was equal to 0.799, thus detecting the main source of association. The organic acid concentrations for the leaf tissue showed a weakly positive correlation with internal organic acid concentrations in the root tip (Figure 14).

Table 8. Analysis of variance, orthogonal contrast, multivariate and Duncan's test outputs for internal organic acid concentrations in six-week old root tips of girdled stems of *T. diversifolia* clones and genotypes (n=12) at the *Tithonia* germplasm collection, San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation for organic acids** (*p* values)					**Wilks' Lambda multivariate test** (*p* values)
	Oxalic	Citric	Malic	Succinic	Fumaric	
Genotypes (A)	n.s.	n.s.	n.s.	0.007	n.s.	<0.0001
Clones (B)	n.s.	0.046	n.s.	0.003	n.s.	0.003
Interaction (AxB)	n.s.	0.031	n.s.	0.012	n.s.	0.001
	Means and the corresponding Duncan's test interpretation					
	In the root tips (μM g^{-1} of fresh weight)					
	Oxalic	Citric	Malic	Succinic	Fumaric	
Genotypes (A)						
Costa Rican	2.406 (A)	3.972 (A)	14.853 (A)	9.577 (A)(B)	0.762 (A)	
Mexican	0.908 (A)	2.343 (A)	7.199 (A)	12.599 (A)	0.812 (A)	
Indonesian	3.105 (A)	1.559 (A)	6.897 (A)	5.412 (B)	0.899 (A)	
Comparisons	**Orthogonal contrast**					
C.R VS. MEX and IND	n.s.	0.087	0.044	n.s.	n.s.	
MEX VS. IND	n.s.	n.s.	n.s.	0.002	n.s.	

Same letters in the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$.

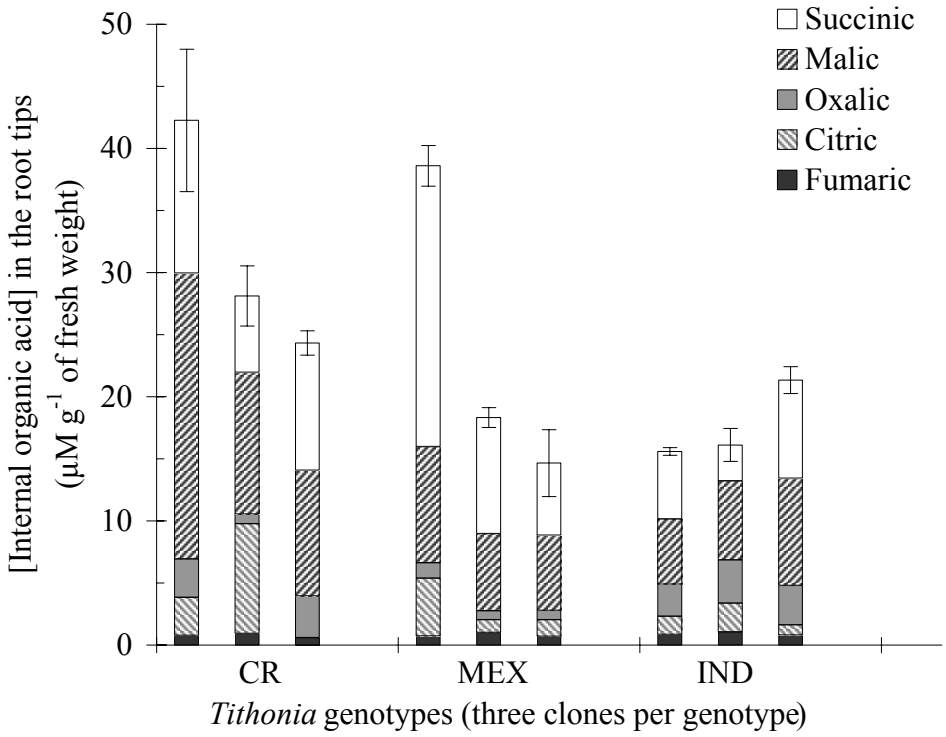

Figure 13. Internal organic acid concentrations in the root tips measured using High-Perfomance Liquid Chromatography (HPLC) in six-week old girdled stems of *T. diversifolia* genotypes and clones at the *Tithonia* germplasm collection, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=12). Wilks' Lambda multivariate test (p <0.0001) showed organic acids were significantly different among *Tithonia* clones, genotypes and genotypes x clones. The differences were due principally to succinic acid.

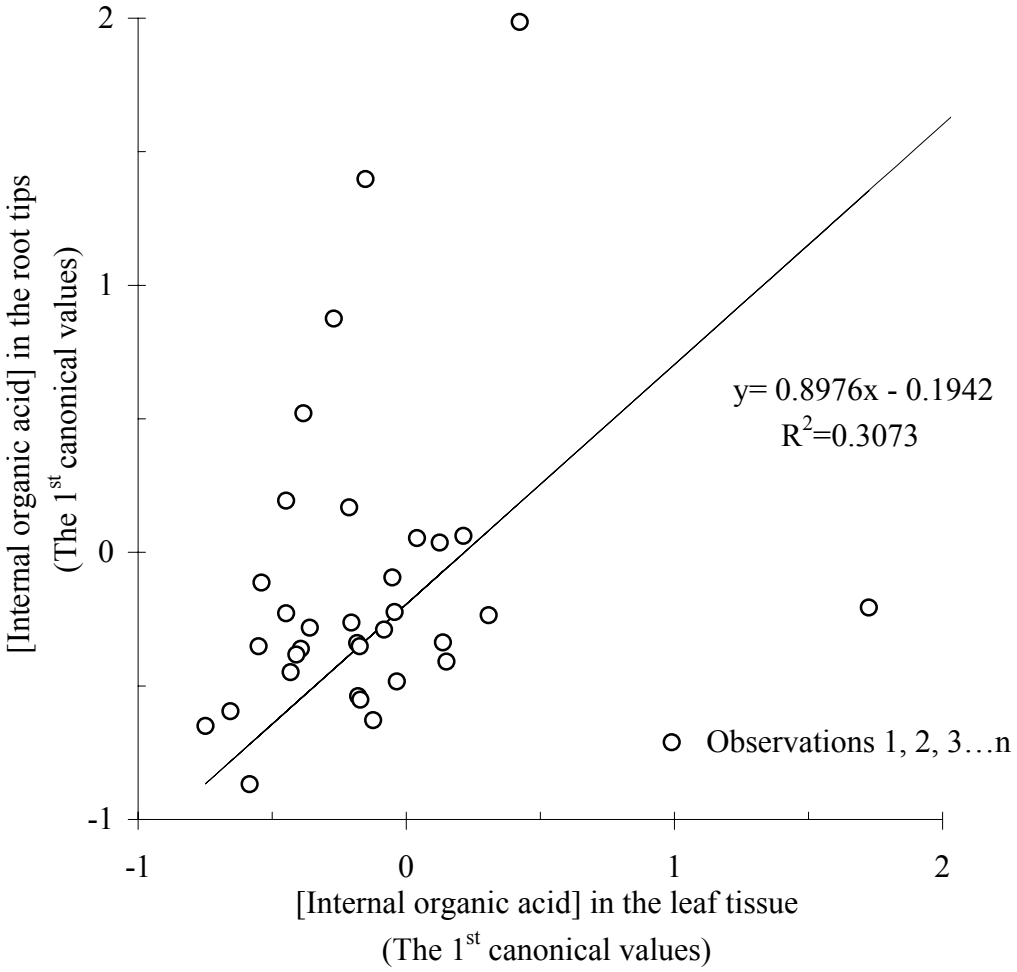

Figure 14. Relationship between internal organic acid concentrations in the leaf tissue and root tips in six-week old girdled stems of *T. diversifolia* genotypes using the first pairs of canonical values, (n=36). Pearson correlation coef.=0.554 and p=0.0006. Canonical variables captured in statistically significant variables, the effects of the original variables for organic acid concentrations in the root tips and leaf tissue.

4.6 Effects of fallow species on soil P fractions

The highest soil P fraction concentrations prevailed in potentially labile P (NaOH-Pi and NaOH-Po) and residual-P at 0-12 and 12-24 cm soil sampling depths (Appendices 15 and 16), respectively. At both soil sampling depths (0-12 and 12-24 cm) around 10% of P in soil corresponded to labile P (Figure 15). However, potentially labile P was higher than 50% (Figure 16 and Appendix 7). The inorganic P concentration in the soil solution (Pi) for San Juan Sur soil is about 1 mg P kg^{-1} (1 ppm) at the 0-12 cm soil sampling depth and 0.2 mg P kg^{-1} at the 12-24 cm soil depth. However, total P reserves were over 900 mg P kg^{-1} at the 0-12 cm soil sampling depth and over 700 mg P kg^{-1} at the 12–24 cm soil depth (Figure 16). During the experimental period (July 2001 to August 2002), soil P fractions were higher in the 0-12 than 12-24 cm soil sampling depth where changes in soil P fractions were mainly registered 17 weeks after planting fallow species, just before slashing above-ground biomass (Figures 15 and 16).

At the 0-12 cm soil sampling depth, the soil P fractions that registered significant changes between groups of fallow species treatments were: P in soil solution, $NaHCO_3$-Pi, $NaHCO_3$-Po, HCl-Pi and residual-P (Appendix 15). *Tithonia* in monoculture had the highest Pi in the soil solution. However, between natural regeneration and the planted fallow species treatments, Pi in the soil solution and $NaHCO_3$-Pi fractions did not show significant differences (Appendix 15). Labile P ($NaHCO_3$-Pi and $NaHCO_3$-Po) showed a decline at both soil depths over the short-term for the fallow species treatments but it always remained above 35 mg P kg^{-1} (Figure 15). In addition, at the 12-24 cm soil sampling depth, only residual-P showed significant differences between fallow species treatments (Appendix 16). Labile P ($NaHCO_3$-Pi and $NaHCO_3$-Po) and occluded P (HCl-Pi and residual P) declined over the three different sampling times at the 12-24 cm soil sampling depth (Figures 15 and 16).

In the topsoil (0-24 cm soil depth), there were significant differences in Wilks' Lambda test for the different groups of fallow treatments for soil P fractions ($p<0.0001$), as well as soil depth ($p<0.0001$) (Table 9), which showed evidence that some fallow species treatments produced more changes in soil P fraction concentrations than others

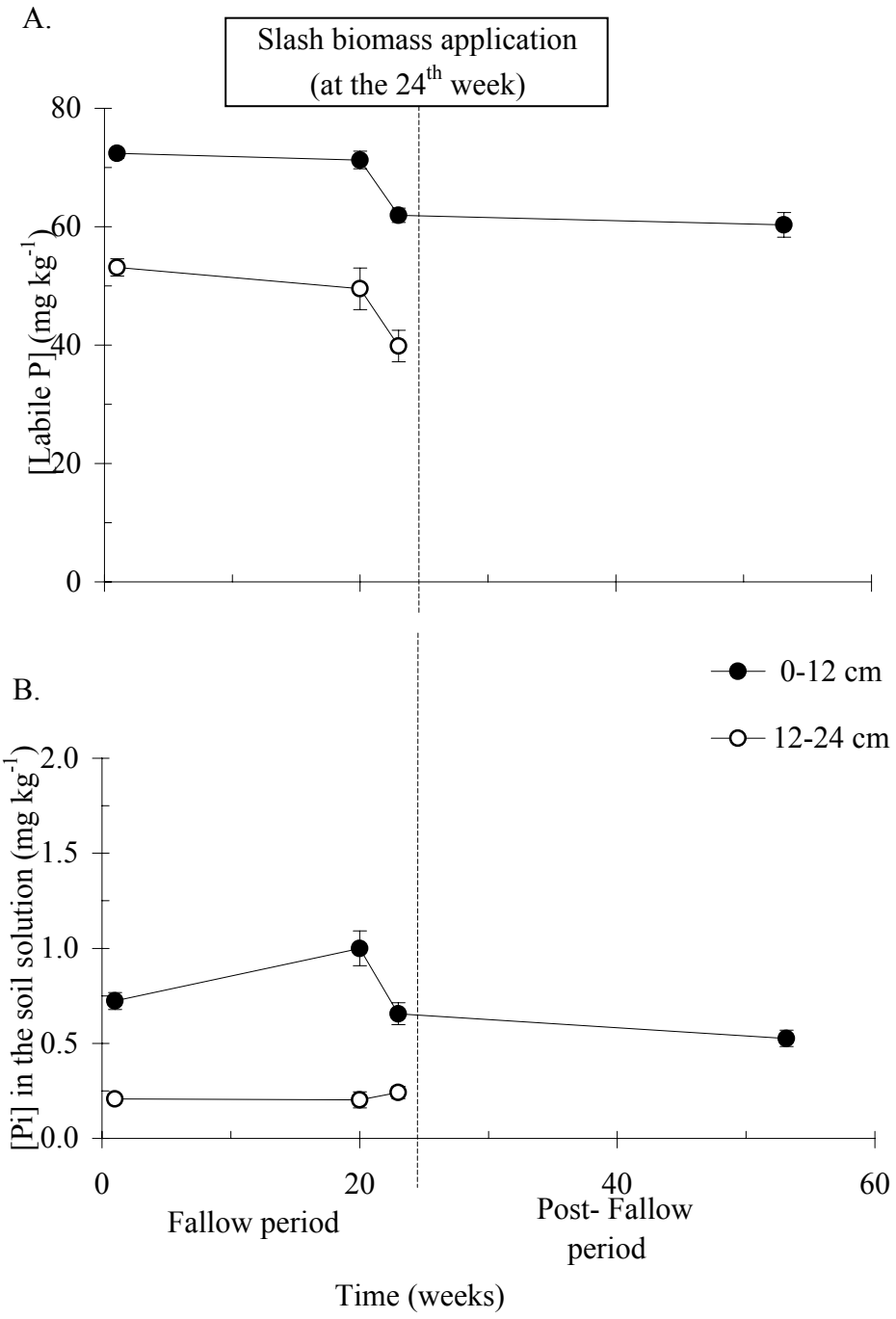

Figure 15. Labile P (A) and Pi in the soil solution (B) fractions at three and four different sampling times on an Andisol with low P availability using two soil sampling depths at San Juan Sur, Turrialba, Costa Rica. Pi=inorganic P and Po=organic P. Standard error bars are displayed. Sampling sizes were (n=36) and (n=12) for 0-12 and 12-24 cm soil sampling depths, respectively.

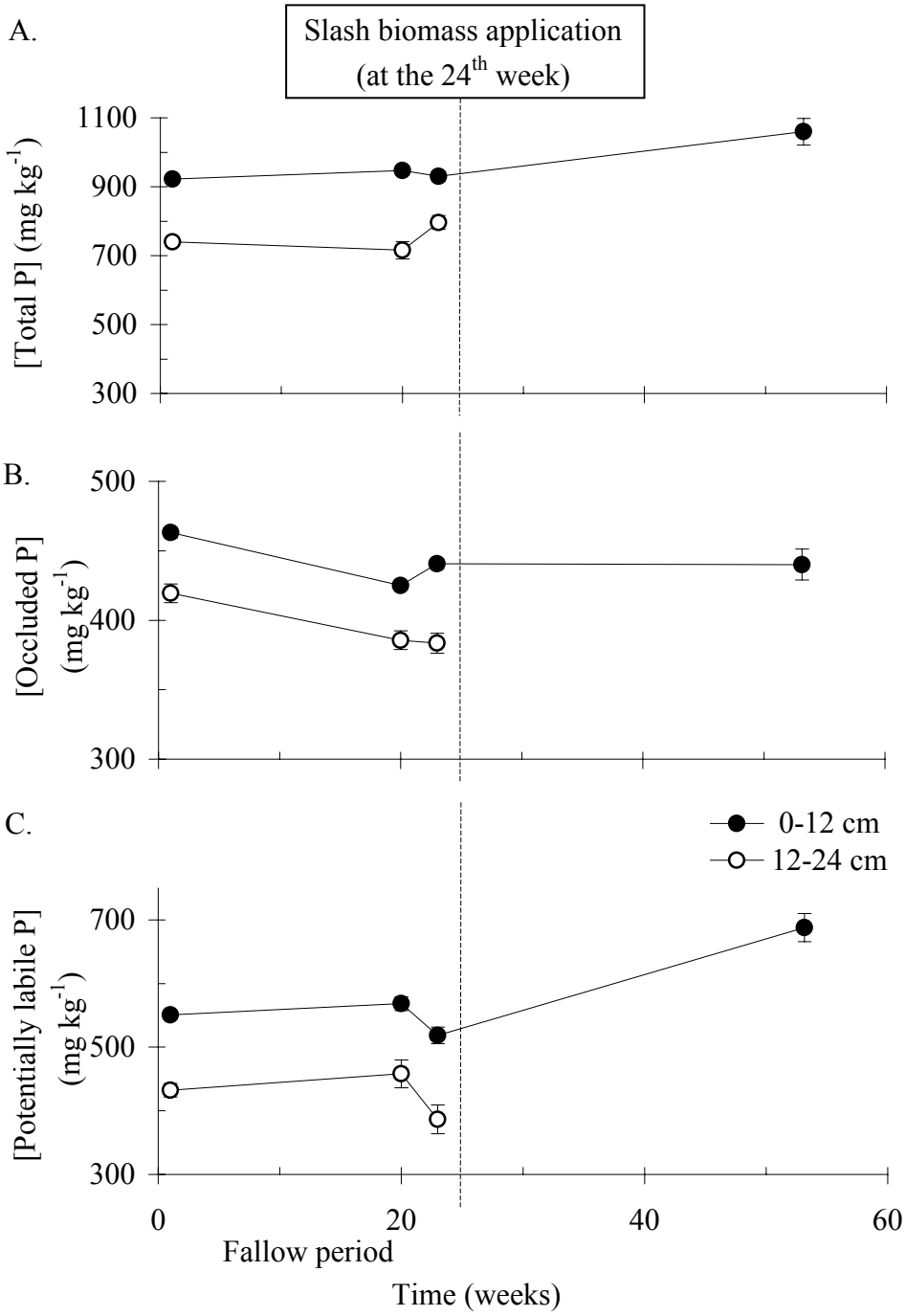

Figure 16. Total P (A), occluded P (B) and potentially labile P (C) concentrations at three and four different sampling times on an Andisol with low P availability using two soil sampling depths at San Juan Sur, Turrialba, Costa Rica. Pi=inorganic P and Po=organic P. Standard error bars are displayed. Sampling sizes were (n=36) and (n=12) for 0-12 and 12-24 cm soil sampling depths, respectively.

Table 9. Analysis of variance, orthogonal contrast, Duncan's and multivariate test outputs for the soil P fractions at 0-24 cm soil sampling depth 1, 20, 23 and 55 weeks after planting fallow species at San Juan Sur, Turrialba, Costa Rica, (n=12).

Source of variation	**ANOVA output interpretation for soil P fractions** (*p* values)							**Wilks' Lambda multivariate test**
	Pi	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-Pi	Residual-P	(*p* values)
Group of fallow species treatments	≤0.0001	0.0692	0.0340	0.0746	n.s.	≤0.0001	≤0.0001	≤0.0001
Time	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001
Depth	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001
Groups of fallow species treatments	**Means** (mg kg^{-1}) **and the corresponding Duncan's test interpretation**							
	Pi	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-Pi	Residual-P	
Natural regeneration	0.59 (B)	9.23 (A)	56.51 (AB)	153.15 (C)	370.18 (A)(B)	1.92 (B)	445.61 (A)	
Cajanus fallow	0.51 (B)	9.77 (A)	51.91 (C)	163.24 (B)	356.30 (B)	1.99 (B)	417.89 (B)	
Tithonia in monoculture fallow	0.86 (A)	9.86 (A)	53.52 (BC)	162.58 (B)	374.04 (A)(B)	2.02 (B)	427.50 (B)	
Tithonia and *Cajanus* fallow	0.65 (B)	9.93 (A)	59.62 (A)	171.32 (A)	388.60 (A)	2.68 (A)	447.24 (A)	
Fallow species comparisons	**Orthogonal contrast comparisons** (*p values*)							
Nat. Reg. VS. Planted fallows	n.s.	n.s.	0.0395	0.0279	n.s.	0.0100	≤0.0001	
Cajanus VS. *Tith.* and *Tith.* /*Caj.*	0.0270	0.0096	0.0942	n.s.	n.s.	0.0031	0.0153	
Tithonia VS. *Tith.* /*Caj.*	0.0001	n.s.	n.s.	n.s.	0.0520	0.0001	0.0117	

Means with the same letters along the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.

n.s. = non significant differences at p <0.05.

(Appendix 17). However, soil P fractions did not show clear trends during the six-month fallow period (Appendix 7) even though time showed significant differences (p<0.0001) (Table 9).

Tithonia genotypes and *Cajanus* depleted more labile P than the other fallow species treatments at 0-12 cm soil sampling depth 23 weeks after planting (Figure 17). The association of *Tithonia* and *Cajanus* showed a uniform decrease of soil labile P during the first 23 weeks of the fallow period. As shown in Figure 17-B, there were no clear time trends of how potentially labile P was changing under different fallow species treatments.

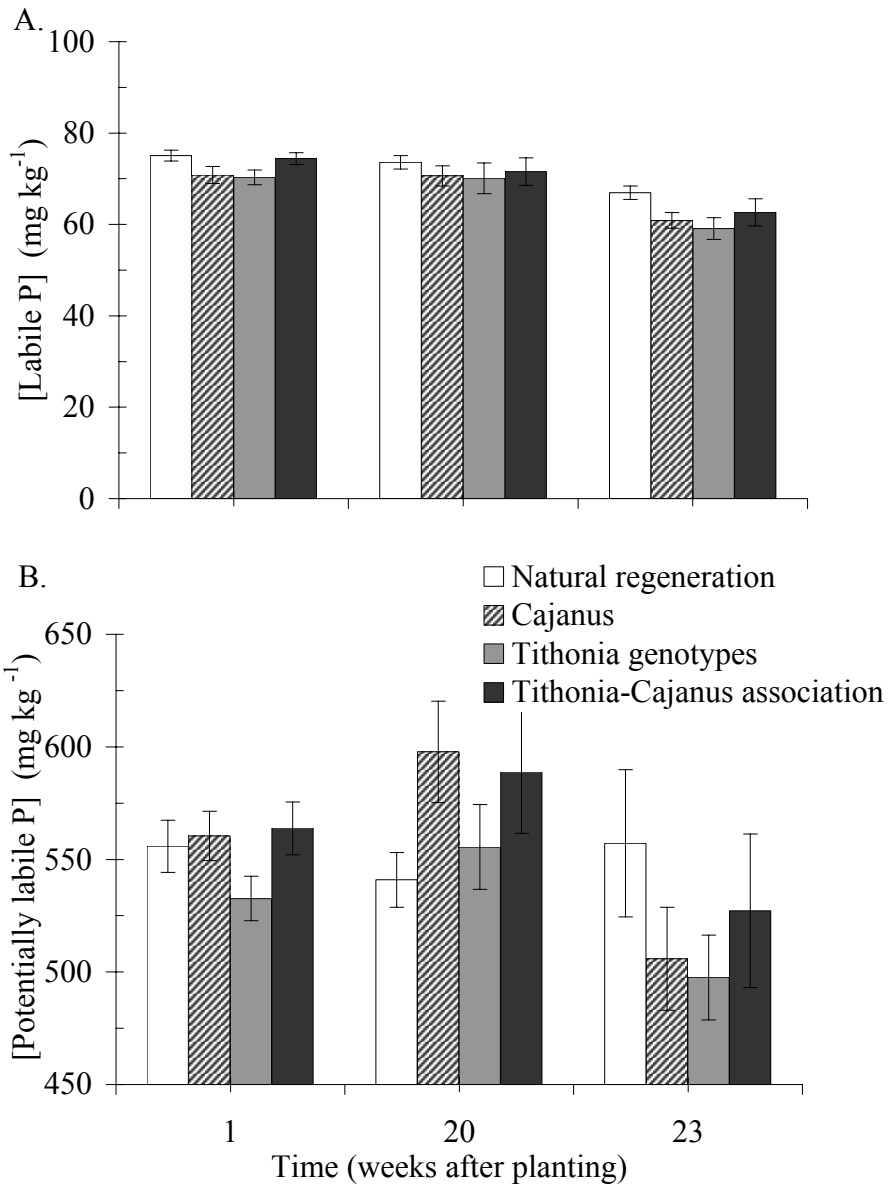

Figure 17. Labile P (A) and potentially labile P (B) concentrations 1, 20 and 23 weeks after planting fallow species at 0-12 cm soil sampling depth on an Andisol with low P availability, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=12).

4.7 Bean growth response experiments

4.7.1 Experiment 1: Retaining or removing slash fallow species biomass

4.7.1.1 Bean yield

Bean yield was significantly different between fallow species treatments (p=0.004) and higher with the Costa Rican *Tithonia* fallow treatment in comparison to other *Tithonia* monoculture fallows in orthogonal contrast comparisons (p=0.0002) (Table 10 and Appendix 18). However, bean yield increased at a higher rate with the slash biomass of natural regeneration in comparison to the biomass of the planted fallow species (Figure 18). The removal of slash biomass negatively affected bean yield (p=0.0004) in the Chirripo Rojo more than the Negro Huasteco (p=0.0463). No significant contributions were detected for the interaction of bean cultivars and slash biomass application effects (p=0.7963) (Appendix 18). The global mean gain in bean yield represented around 0.15 Mg ha^{-1} (18.1%) and 0.05 Mg ha^{-1} (6.03%) for the presence of slash biomass of fallow species and bean cultivar selection, respectively. Bean yield with the Costa Rican *Tithonia* fallow treatment was 0.28 Mg ha^{-1} higher than the other fallow treatments.

4.7.1.2 Bean biomass production

Multivariate analysis detected significant differences for above-ground biomass allocation regarding bean cultivar effects (B) at the end of the flowering and bean maturity phases (p=0.047 and p<0.0001), respectively (Table 11 and Appendix 19). For slash biomass management treatment effects (C) and the interaction (CxB), no significant differences were detected for above-ground biomass allocation at the end of the flowering phase (Photo 5). However, at the bean maturity phase, the slash biomass management treatment effects became significant different (p=0.0145) (Table 11).

ANOVA did not detect significant differences in above-ground biomass between bean cultivars at the end of the flowering and bean maturity phases; however, it was significantly different before flowering (p=0.0417) as shown in Figure 19. Pod dry

Table 10. Bean cultivar yield after fallow species treatments with and without slash biomass removal harvested at the bean maturity phase on an Andisol with low P availability, San Juan Sur, Turrialba, Costa Rica, (n=64).

Fallow species treatments	With slash biomass removal		Without slash biomass removal		Mean
	Chirripo Rojo	Negro Huasteco	Chirripo Rojo	Negro Huasteco	
	Yield (Mg ha^{-1})				
Without *C. cajan*					
Natural regeneration	0.81±0.14	0.85±0.12	0.85±0.17	0.95±0.15	0.87±0.07 (B)
Tithonia (CR)	1.02±0.15	0.90±0.16	1.43±0.15	1.17±0.08	1.13±0.08 (A)
Tithonia (MEX)	0.71±0.16	0.66±0.12	1.01±0.16	0.98±0.19	0.84±0.08 (B)
Tithonia (IND)	0.73±0.09	0.91±0.18	0.86±0.07	0.73±0.11	0.80±0.06 (B)
	---	---	---	---	---
	0.83	0.82	1.00	0.94	0.90
With *C. cajan*					
Tith. (CR)/*Cajanus*	0.97±0.15	0.65±0.10	0.82±0.13	0.96±0.15	0.85±0.07 (B)
Tith. (MEX)/*Cajanus*	0.79±0.08	0.76±0.16	0.96±0.15	0.95±0.09	0.86±0.06 (B)
Tith. (IND)/*Cajanus*	0.77±0.08	0.74±0.05	1.22±0.29	0.80±0.11	0.89±0.09 (B)
Cajanus	0.89±0.10	0.81±0.12	0.70±0.08	0.87±0.80	0.81±0.05 (B)
	---	---	---	---	---
	0.84	0.75	0.96	0.92	0.92
Mean	0.83 (b)	0.78 (b)	0.98 (a)	0.93 (b)	

Same capital letters in the same column indicate no significant differences (p<0.05) by the Duncan's test. Same small letters in the same row indicate no significant differences (p<0.05) by the Duncan's test. Values are means ± standard errors.

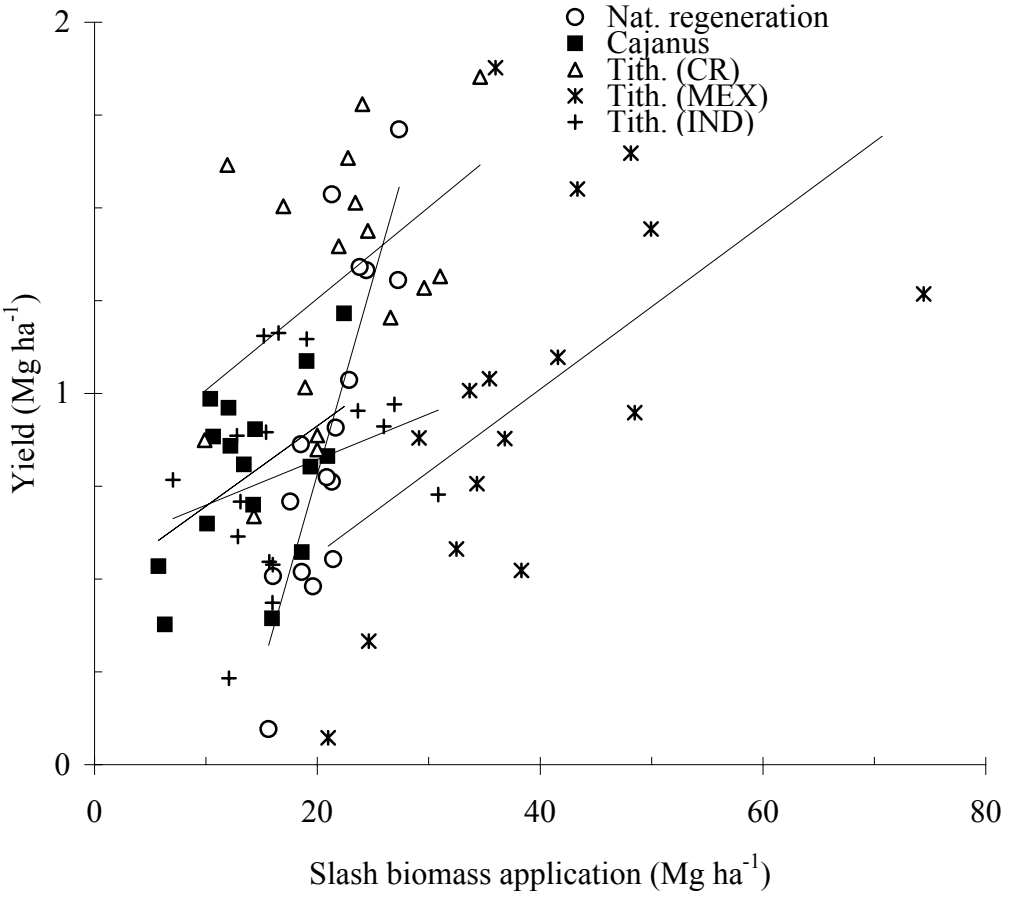

Figure 18. Relationship between bean yield and slash biomass application (*in situ* mulch) of the different fallow species in Experiment 1, (n=16). Bean yield increased at a higher rate with the slash biomass of natural regeneration, but the Costa Rican *Tithonia* biomass produced higher yield than the other fallow species biomass at lower slash biomass application.

Table 11. Analysis of variance and multivariate test outputs for above-ground biomass allocation of bean cultivars between fallow species and slash biomass application treatments harvested at the bean maturity, before and at the end of the flowering phases, San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)			**Wilks' Lambda multivariate test** (*p* values)
	At the bean maturity phase (3 variables; n=16)			
	Leaf	Stem	Pod	
Fallow treatments (A)	0.0052	0.0049	0.099	
Bean cultivars (B)	0.0002	<0.0001	n.s.	<0.0001
Slash biomass treatments (C)	0.0937	n.s.	n.s.	0.0145
Interaction (BxC)	0.0815	n.s.	n.s.	n.s.
	At the end of the flowering phase (3 variables; n=8)			
	Leaf	Stem	Pod	
Fallow treatments (A)	n.s.	n.s.	0.0456	
Bean cultivars (B)	n.s.	n.s.	0.0255	0.0047
Slash biomass treatments (C)	n.s.	0.0752	n.s.	n.s.
Interaction (BxC)	n.s.	n.s.	n.s.	n.s.
	Before the flowering phase (2 variables; n=8)			
	Leaf	Stem	Pod	
Fallow treatments (A)	0.0057	0.0007	-	
Bean cultivars (B)	0.0524	0.0222	-	n.d.
Slash biomass treatments (C)	n.s.	0.0825	-	n.d.
Interaction (BxC)	n.s.	n.s.	-	n.d.

The underlined *p* values indicate significant differences at $p < 0.05$.

n.s. = non significant differences at $p < 0.05$.

n.d. = non determined.

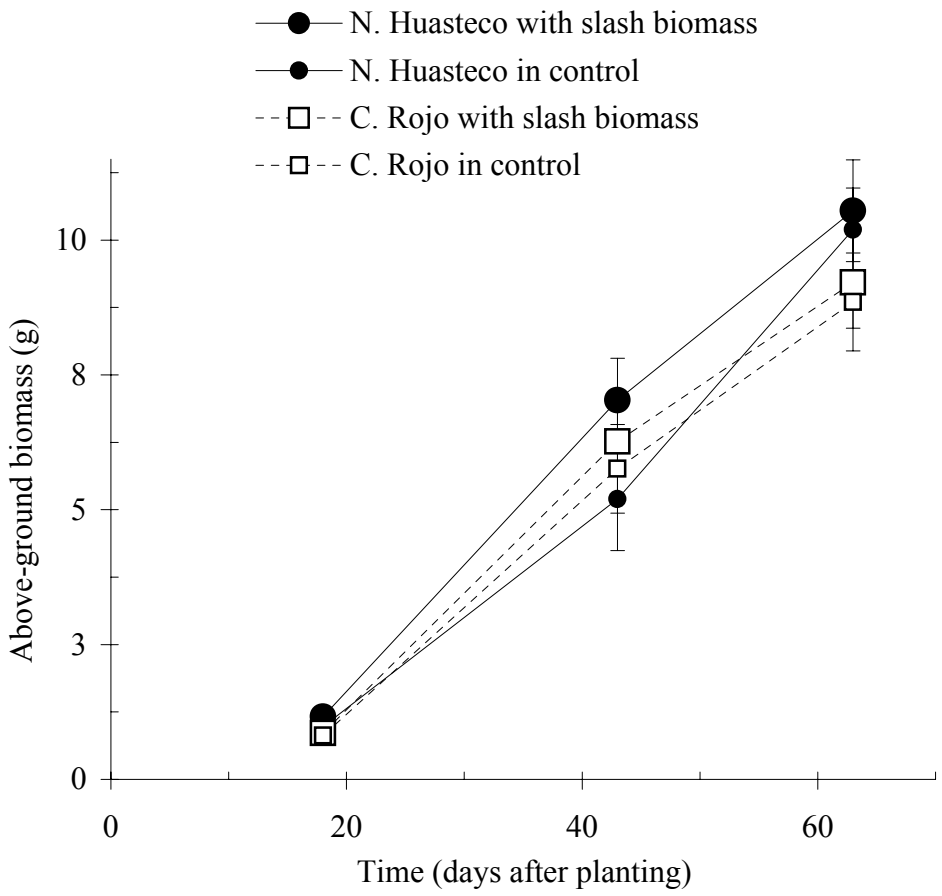

Figure 19. Above-ground biomass of bean cultivars in control and in the presence of slash biomass application treatments 18, 43 and 63 days after planting at San Juan Sur, Turrialba, Costa Rica. Closed circle, Negro Huasteco; open square, Chirripo Rojo. Standard error bars are displayed. Sampling sizes were (n=8) for the first two sampling times and (n=16) for the last sampling time. ANOVA (p =0.042) showed bean cultivars were significantly different for above-ground biomass harvested 18 days after planting.

weight was significantly different for fallow species treatments (p=0.0456) and between bean cultivars (p=0.0255) at the end of the flowering phase (Table 11 and Figure 20) but not before the flowering and bean maturity phases.

Photo 5. Bean spit-plots at the growing phase in Experiment 1, San Juan Sur, Turrialba, Costa Rica.

Bean cultivar growth showed positive association with soil exchangeable bases (Ca and Mg) and Zn concentrations. The corresponding Pearson coefficients and the associated probability values were (0.7099, p=0.0001; 0.7051, p=0.0001 and 0.7014, p=0.0007) for Ca, Mg and Zn, respectively. More bean plants with low vegetative growth were found at lower concentrations of Ca, Mg and Zn. The number of plants with low vegetative plant growth was higher for the Chirripo Rojo than for the Negro Huasteco bean cultivar. Soil P concentration was not associated with plant growth in bean cultivars (Pearson coefficient equal to 0.2730 and p=0.1969).

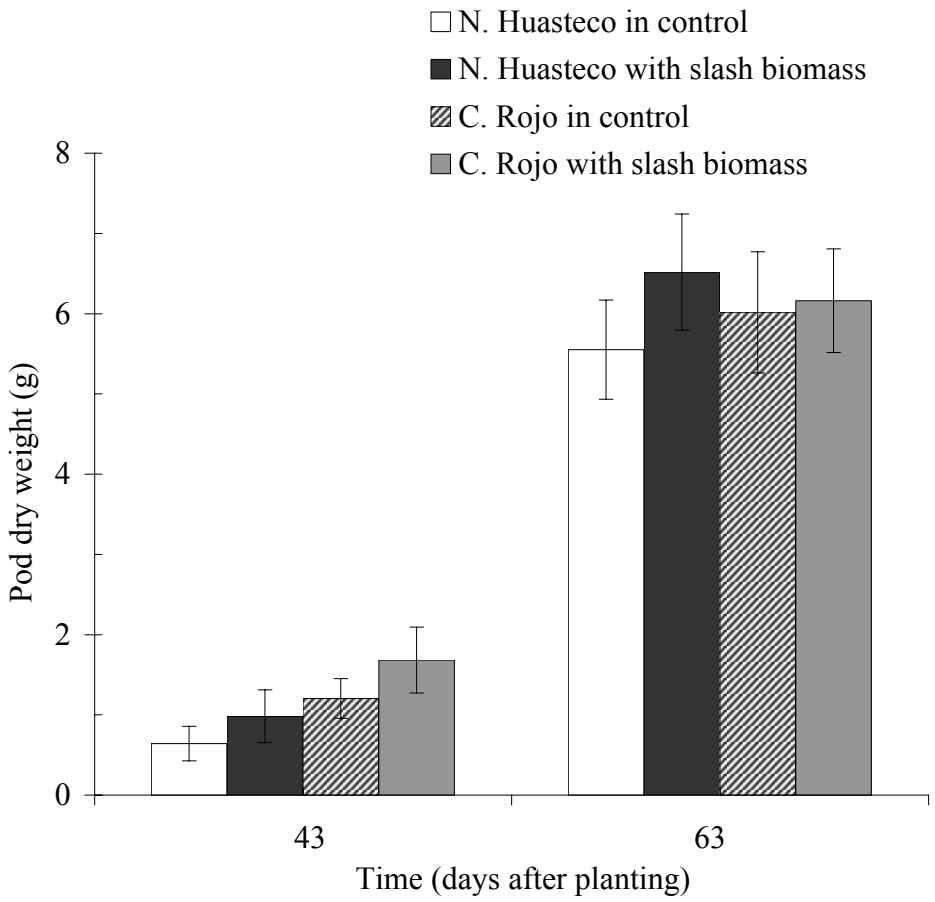

Figure 20. Pod dry weight of bean cultivars in control and in the presence of slash biomass application treatments 43 and 63 days after planting at San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed. Sampling sizes were (n=8) and (n=16) for 43 and 63 days after planting, respectively. ANOVA (p =0.026) showed bean cultivars were significantly different for pod dry weight harvested 43 days after planting.

4.7.1.3 Bean nutrient concentrations

Using Wilks' Lambda multivariate analysis, nutrient concentrations in above-ground dry weight had significant differences for bean cultivar effects (A) (p=0.0391), but not for slash biomass treatments (B) (p=0.0636) before the flowering phase (Appendix 20). This was also true at the end of the flowering phase for bean cultivar effects (p=0.0001) (Table 12). The interaction between bean cultivars and slash biomass application treatments did not show significant differences in any of the two mentioned sampling times. However, significant differences were detected between fallow species treatments for Ca and K concentrations in bean cultivars before the flowering phase (p=0.0002 and p=0.0085), respectively (Appendix 20) as well as at the end of the flowering phase for Ca, K and P (p=0.0002, p=0.0025 and p=0.0143), respectively in the ANOVA test (Table 12).

The Mexican and Costa Rican *Tithonia* genotype fallows induced higher P concentration in bean cultivars than the Indonesian *Tithonia* and *Cajanus* fallows at the end of the flowering phase in Duncan's test. Significant differences were found between the slash biomass application treatment effects (without slash biomass removal versus with slash biomass removal) (p=0.0036) for K concentration before the flowering phase (Appendix 20) and at the end of the flowering phase (p=0.0132) (Table 12). K concentration significantly increased when slash biomass was retained before and at the end of the flowering phase (Appendix 20 and Table 12). However, no significant differences were found between bean cultivars for K concentrations at the mentioned sampling times. In orthogonal contrast comparisons, the Chirripo Rojo bean cultivar had significantly higher nutrient concentrations for P, Ca and Mg than the Negro Huasteco bean cultivar at the end of the flowering phase (Table 12 and Figure 21). Before the flowering phase, the Chirripo Rojo also had higher a P concentration than the Negro Huasteco bean cultivar (p=0.0035) (Appendix 20 and Figure 21-A). At the end of the flowering phase, the Negro Huasteco bean cultivar had a higher P utilization efficiency than the Chirripo Rojo (p<0.0001) (Appendix 21), but, P uptake efficiency did not show significant differences between bean cultivars in the ANOVA test. Slash biomass

Table 12. Analysis of variance, orthogonal contrast, Duncan's and multivariate test outputs for bean cultivar nutrient concentrations between fallow species and slash biomass application treatments harvested at the end of the flowering phase, San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)				**Wilks' Lambda multivariate test** (*p* values)
	P	Ca	Mg	K	
Fallow species treatments (A)	0.0143	0.0002	n.s.	0.0025	
Bean cultivars (B)	<0.0001	0.0461	0.0006	n.s.	0.0001
Slash biomass applications (C)	n.s.	n.s.	n.s.	0.0132	n.s.
Interaction (BxC)	n.s.	n.s.	n.s.	n.s.	n.s.
Comparisons	**Orthogonal contrast outputs** (*p* values)				
Without Sl. biom. VS. With Sl. biom.	n.s.	n.s.	n.s.	0.0117	
Chirripo Rojo VS. Negro Huasteco	<0.0001	0.0379	0.0005	n.s.	
Interaction (B. cultivars x S. biomass)	n.s.	n.s.	n.s.	n.s.	
Biomass application treatment comparisons	**Means** (mg g^{-1}) **and the corresponding Duncan's test interpretation**				
	P	Ca	Mg	K	
Control (with slash biomass removal)					
Chirripo Rojo	2.1±0.1 (A)	7.9±0.7 (A)	4.7±0.5 (A)	21.5±2.6 (B)	
Negro Huasteco	1.8±0.1 (B)	6.3±0.6 (A)	3.3±0.2 (B)	21.7±2.3 (B)	
Without slash biomass removal					
Chirripo Rojo	2.2±0.1 (A)	6.9±0.6 (A)	4.0±0.2 (A)(B)	28.2±2.1 (A)	
Negro Huasteco	1.7±0.1 (B)	6.5±0.5 (A)	3.1±0.2 (B)	24.0±1.3 (A)(B)	

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.

n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=8.

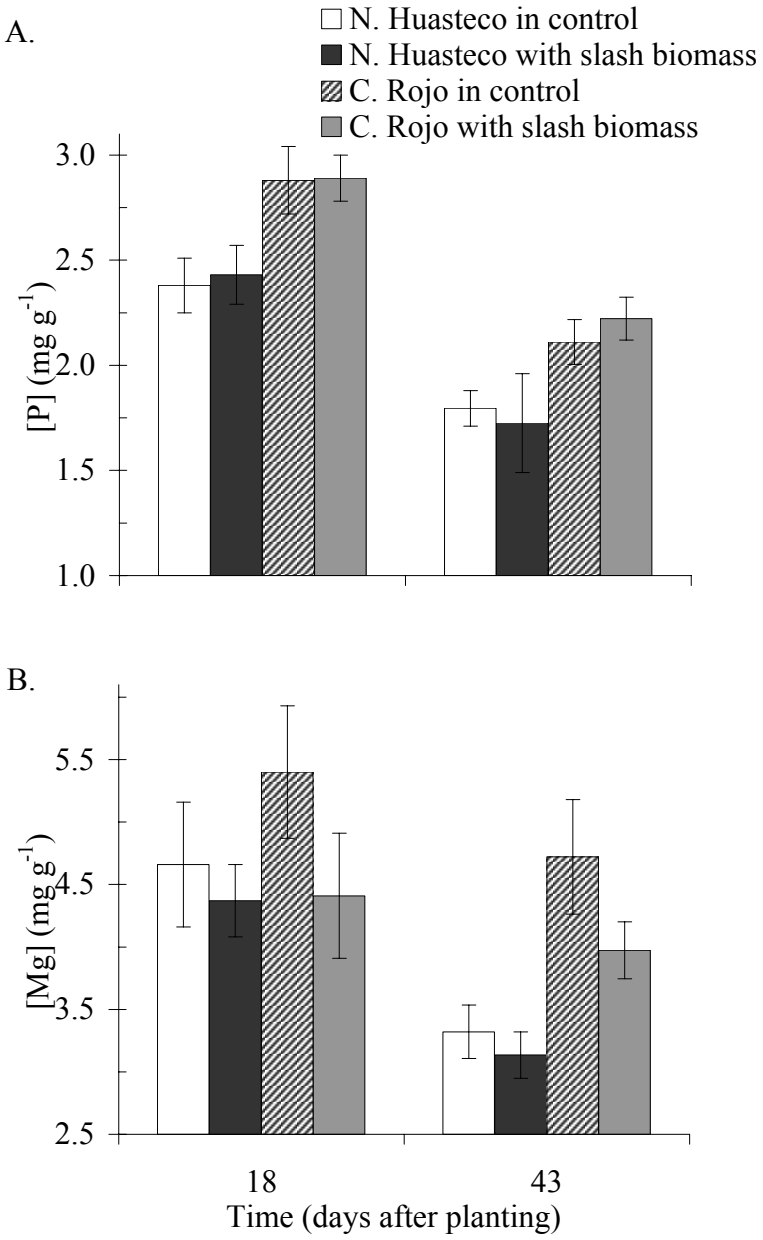

Figure 21. Phosphorus (P) (A) and magnesium (Mg) (B) concentrations for bean cultivars at two different harvest times under slash biomass applications in Experiment 1. Standard error bars are displayed (n=8). In A, ANOVA (p =0.0001) showed bean cultivars were significantly different for P concentration at both harvest times. In B, ANOVA (p =0.0006) showed bean cultivars were significantly different for Mg concentration 43 days after planting.

treatments and the interaction (bean cultivars x slash biomass treatments) did not show significant differences for P utilization and uptake efficiencies.

4.7.1.4 Bean root system characteristics

Figures 22 and 23 illustrate root system characteristics and above-ground biomass allocation for the two bean cultivars in control plots (with slash biomass removal) at the end of the flowering phase. For both bean cultivars, it can be seen that upper root-stem and basal root dry weights represented the principal parts of the root system dry weight; however, adventitious roots were in higher quantity and had higher SRL than basal roots (Appendix 22).

In Wilks' Lambda multivariate test, significant differences between bean cultivars were detected for root system components (p=0.0025), root extension (p=0.0311), but not for root dry weight partitioning (p=0.081) at the end of the flowering phase (Appendix 23). The effects of slash biomass management treatments did not show significant differences for root system components (p=0.0527) (Appendix 23). Similarly, the interaction of slash biomass treatments and bean cultivars did not show statistical differences in the groups of root system characteristics. Statistical significance was found for two variables within the root system components: the number of nodules and adventitious roots (p=0.0148 and p=0.017), respectively (Figure 24). Within root dry weight partitioning, only adventitious root dry weight and lower root-stem dry weight were significant (p=0.0086 and p=0.0321), respectively (Appendix 23). Contrast comparisons for adventitious root dry weight, lower root-stem, and the number of nodules and adventitious roots detected significant differences between the Chirripo Rojo and Negro Huasteco bean cultivars (Figures 24 and 25). Slash biomass treatments produced only significant differences for the number of adventitious roots in which the number of adventitious roots was lower when the slash biomass was left than the control (with slash biomass removal) (p=0.002). However, the number of nodules did not significantly increase when slash biomass was left on the site at the end of flowering phase (Appendix 23 and Figure 24). Within the root system extension variables, only lower root-stem length was statistically different for bean cultivars (p=0.0340) (Appendix

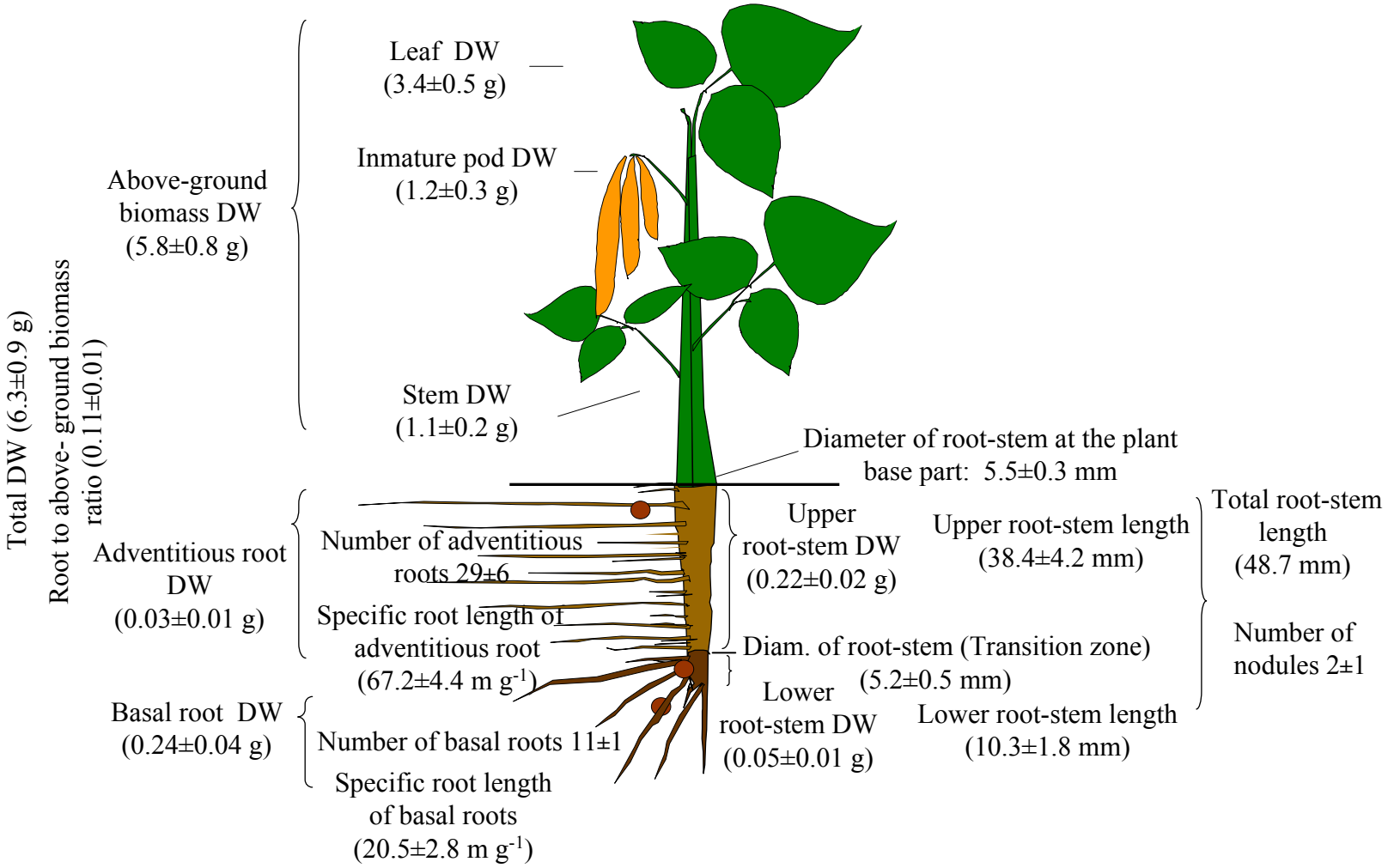

Figure 22. Above-ground biomass allocation and root system characteristics of the Chirripo Rojo bean cultivar without slash biomass treatment at the end of the flowering phase, (n=8). Values are means ± standard errors.

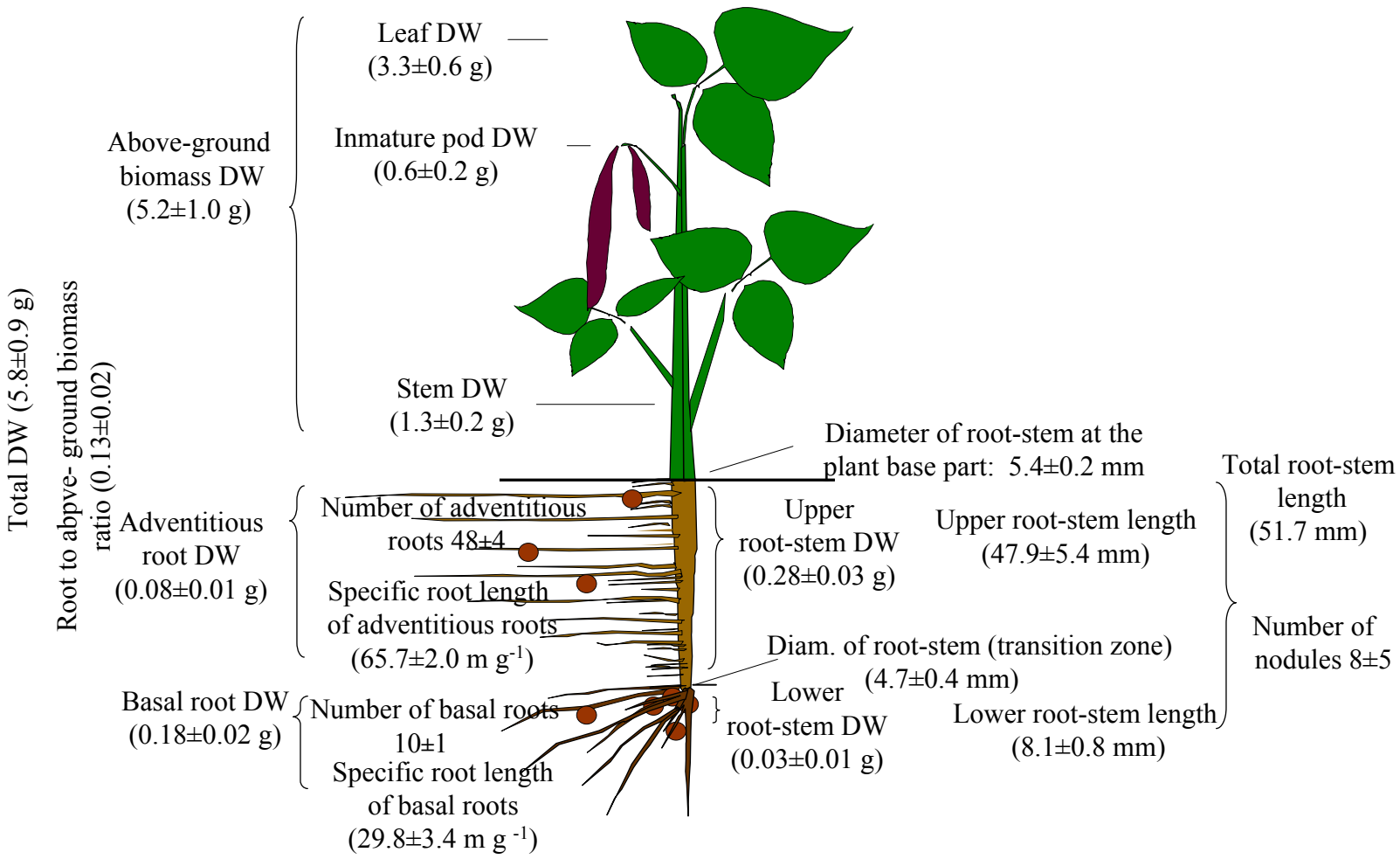

Figure 23. Above-ground biomass allocation and root system characteristics of the Negro Huasteco bean cultivar without slash biomass treatment at the end of the flowering phase, (n=8). Values are means ± standard errors.

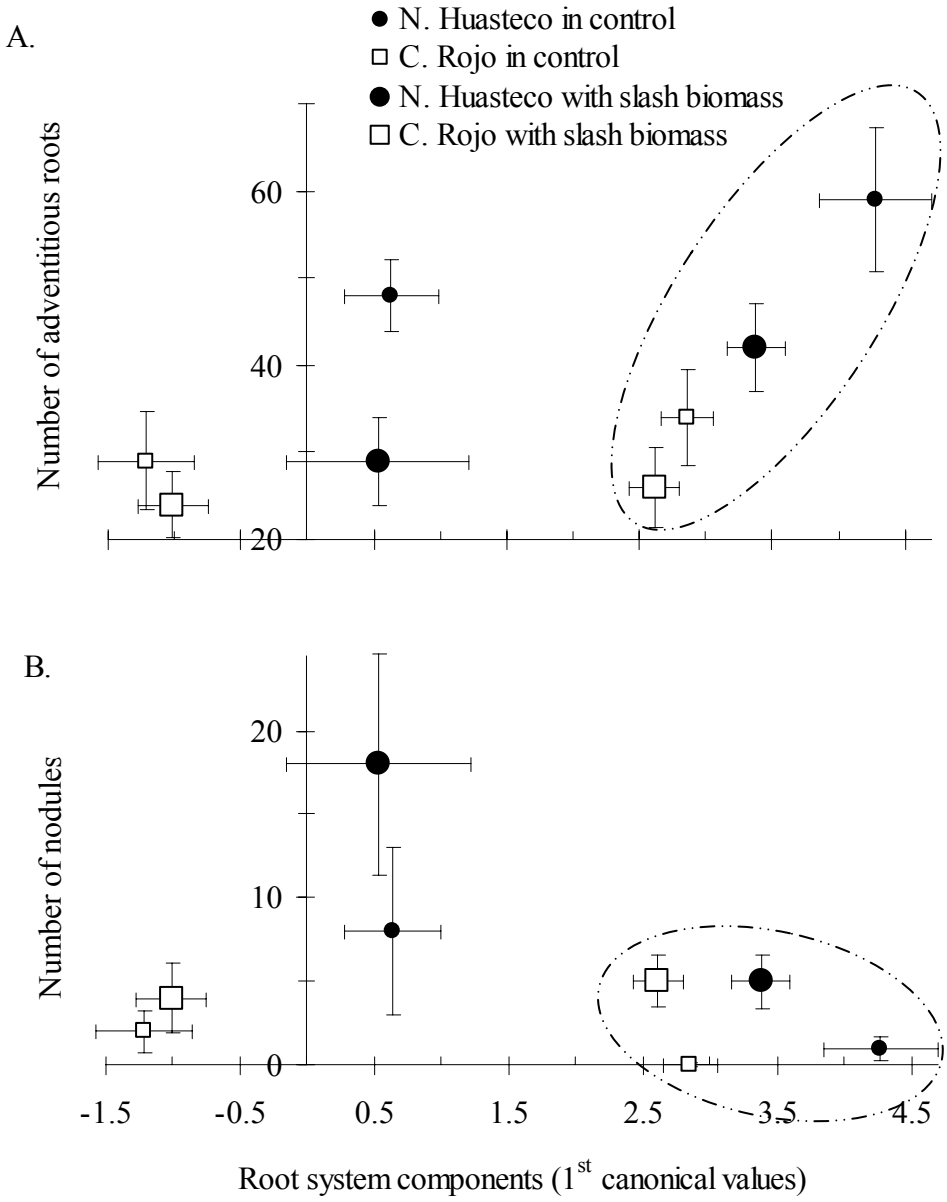

Figure 24. Number of adventitious roots (A) and nodules (B) for bean cultivars under slash biomass treatments at two different harvest times, (Data set, n=8). Closed circle, Negro Huasteco; open square, Chirripo Rojo. In A and B, the samples encircled by the ovals correspond to the pod filling phase (Data set, n=16). Not-encircled data refer to the end of the flowering phase. The 1st canonical values of root system components were used to represent different data sets of the same groups of variables. In A, contrast comparisons detected the following significant differences at the end of the flowering phase: control versus slash biomass treatments (p=0.02), Chirripo Rojo versus Negro Huasteco (p=0.02) and Negro Huasteco in control versus Negro Huasteco with slash biomass treatments (p=0.01). In B, contrast comparisons detected significant differences between the Chirripo Rojo and| Negro Huasteco (p=0.01) at the end of the flowering phase.

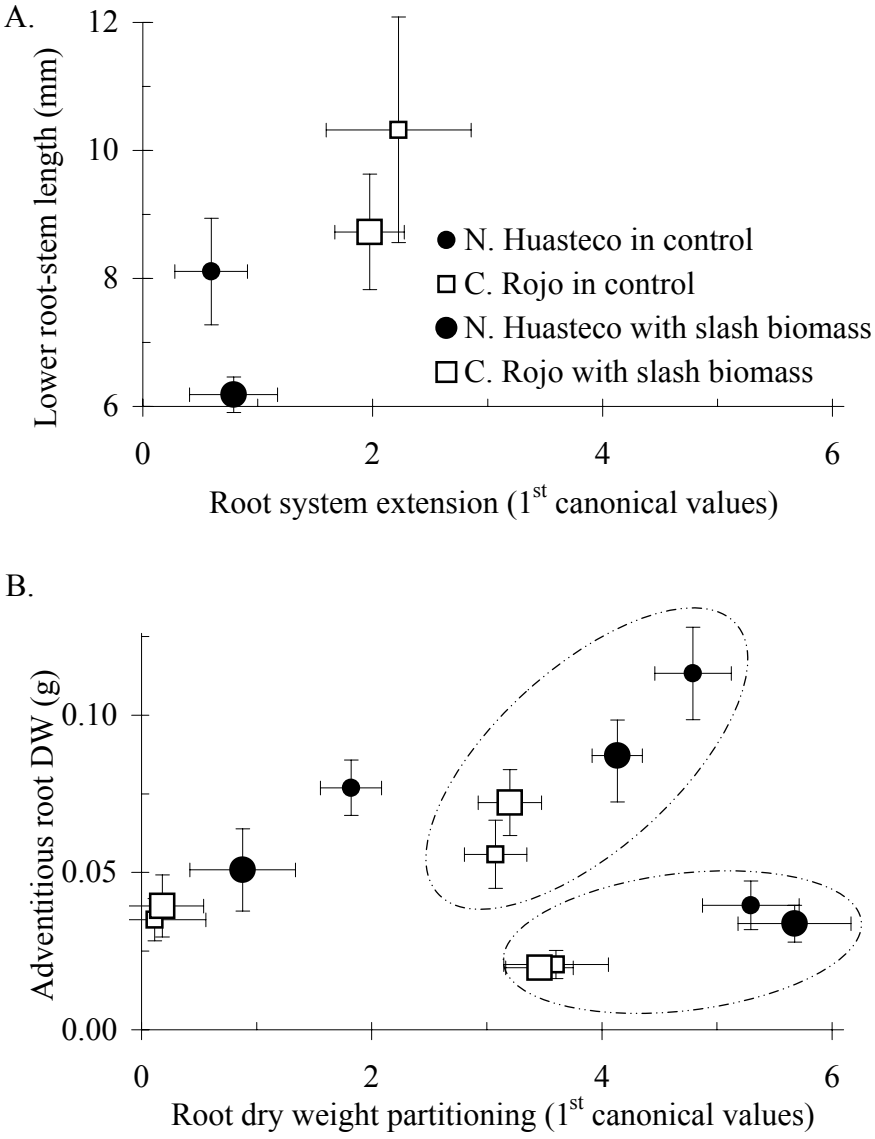

Figure 25. Lower root-stem length (A) and adventitious root dry weight (B) of bean cultivars under slash biomass treatments at three different harvest times. Closed circle, Negro Huasteco; open square, Chirripo Rojo. In A and B, not-encircled data refer to the end of the flowering phase (Data set n=8). In B, the samples encircled by the ovals correspond to the flower initiation (the ones closer to X axes, data set, n=8) and to the pod filling phases, (Data set, n=16), respectively. The 1st canonical values were used to represent different data sets of the same groups of variables. In A and B, contrast comparisons detected significant differences between the Chirripo Rojo and Negro Huasteco (p =0.03) and (p =0.01), respectively at the end of the flowering phase.

23), but it did not show significant differences for slash biomass treatments (p=0.1081) (Figure 25-A). For the surface of root-stem, orthogonal contrast detected differences between bean cultivars (p=0.0405) (Appendix 21). The Chirripo Rojo bean cultivar had a smaller root-stem surface (42.27 mm^2) than the Negro Huasteco (66.25 mm^2) (p=0.0405). There were no differences due to the presence or absence of slash biomass or to the interaction of bean cultivars with the presence or absence of slash biomass (AxB).

In an earlier sample from a separate data set collected at the beginning of the flowering phase, significant differences were detected for the root dry weight partitioning between bean cultivars (p=0.002) using Wilks' Lambda test (Appendix 24). However, root system components did not show significant differences between the Chirripo Rojo and Negro Huasteco bean cultivars. As bean plants reached maturity, significant differences were detected between bean cultivars for root dry weight partitioning (p=0.0002) and root components (p=0.0012) using Wilks' Lambda test (Appendix 25). Likewise, differences were found between slash biomass treatments for root system components and root extension (p=0.0006 and p=0.0002), respectively. No interaction effects were significant between bean cultivars (A) and slash biomass treatments (B) for root dry weight partitioning, root components and root extension (Appendix 25). Most of the root dry weight variables were also significantly different between bean cultivars. The number of adventitious roots and the adventitious root dry weight continued to show significant differences between bean cultivars (p=0.011 and p=0.0006), respectively. The number of nodules was not significantly different between bean cultivars; however, it was significantly increased when slash biomass was left on the site (p=0.0007). Figures 24 and 25 integrate the most significant differences in root system characteristics before and at the end of the flowering and the bean maturity phases.

Root variables with less source of collinearity between each other were identified using cluster analysis (Figure 26). On these root variables, multi-regression analysis permitted the choice of the best predictors for pod production and above-ground dry weight at the end of the flowering phase. The regression coefficient R indicated that SRL of basal roots, basal root dry weight, upper root-stem dry weight and lower root-stem length were the best predictors for pod production. The corresponding partial R-squares

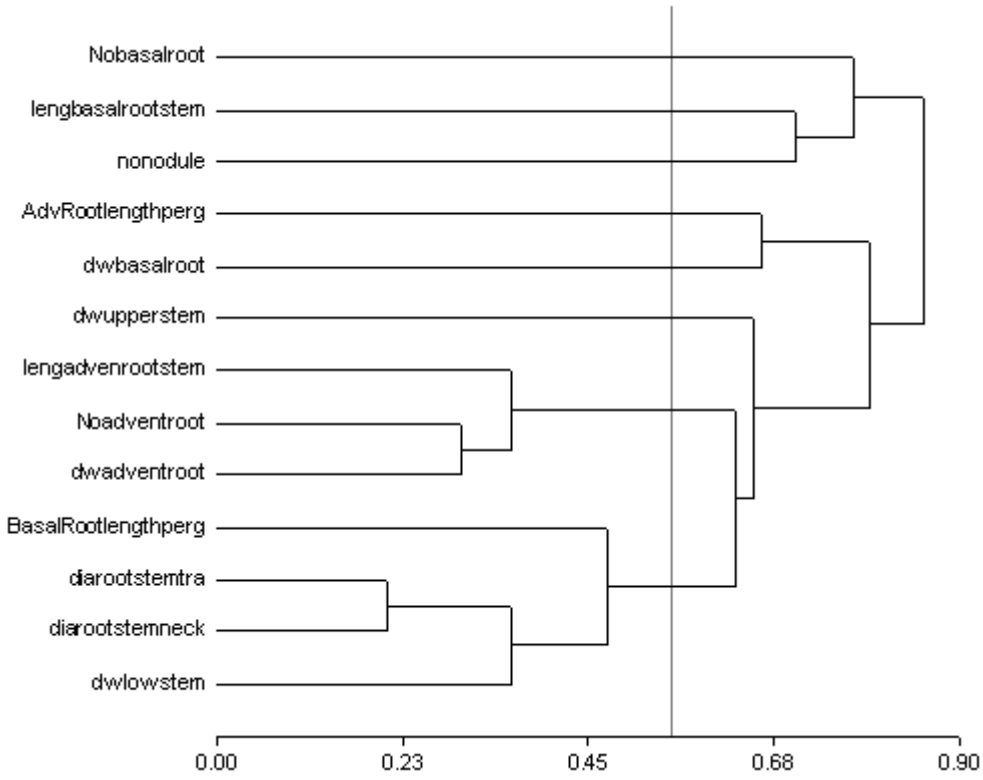

Figure 26. Clusters of root variables for bean cultivars using the standard distance (1-abs (Pearson)) and 0.55 as the cut criteria point.

and the significance levels for each one were R^2=34.37, p=0.004; R^2=14.16, p=0.0073; R^2=7.86, p=0.0318 and R^2= 5.87, p=0.0489, respectively. The linear regression model for these four variables was responsible for 62.76% of the variation in pod production and was significant (p<0.0001). As bean plants reached maturity, SRL of basal roots and upper root-stem dry weight were no longer part of the best predictor variables for pod production. Instead, basal root dry weight, lower root-stem length and the number of basal roots were the best predictors for pod production. However, they only reached a R^2 equal to 30.85. Most of the variables that were identified as the best predictors for pod production were not the same that contributed to detect differences in root characteristics for bean cultivars, except for lower root-stem length. The best predictors for above-ground dry weight were upper root-stem dry weight, SRL of basal roots and the number

of adventitious roots and nodules, which reached an R^2 equal to 72.14%, (p<0.0001) for the two Costa Rican bean cultivars (the Chirripo Rojo and Negro Huasteco).

4.7.1.5 Fungal structures in bean cultivar roots

Chi-Square test for the fungal structures in bean roots showed significant differences for the proportion of arbuscules at the end of the flowering phase (p=0.0111) (Table 13). In bean cultivar roots, the largest arbuscule percentage (7.32%) was found using the Indonesian *Tithonia* genotype in association with *Cajanus* and with slash biomass application treatments. The associated Mexican *Tithonia* genotype with *Cajanus* allowed the highest proportion of bean roots without arbuscules (10.73%). Another significant difference found in ANOVA test was the interaction between bean cultivars and slash biomass applications within the fallow species treatments (p=0.0087) for the proportion of vesicles. However, Chi-Square only detected significant differences in the Chirripo Rojo bean cultivar with and without slash biomass treatments for the proportion of vesicles (p=0.0171).

The abundance of fungal structures (entry points, vesicles and arbuscules) per root length in bean root systems showed significant differences between bean cultivars (p=0.0282) using multivariate analysis (MANOVA) in Wilks' Lambda test. However, no significant differences were detected for slash biomass treatment effects and the interaction of bean cultivar and slash biomass treatment effects (Appendix 26). The abundance of entry points and arbuscules showed significant differences between fallow species treatments (p<0.0001 and p=0.001), respectively (Appendix 26). However, differences were not found between bean cultivars for slash biomass treatment effects. The associated Mexican *Tithonia* genotype with *Cajanus* had the highest abundance of arbuscules in Duncan's test (Appendix 26). The abundance of vesicles was significantly different between the Chirripo Rojo and Negro Huasteco bean cultivar, but not for the abundance of entry points and arbuscules in ANOVA. The Negro Huasteco bean cultivar showed superiority regarding the abundance of vesicles with and without slash biomass treatments (p=0.0117) in orthogonal contrast comparisons (Figure 27).

Table 13. Proportion of arbuscules in bean cultivar roots of fallow species treatments harvested at the flowering phase in Experiment 1, San Juan Sur, Turrialba, Costa Rica, (n=50). In each square, the top value corresponds to the frequency of the occurrence of arbuscules and the value in the parenthesis corresponds to the percentage of occurrence in the sample. Chi-Square (p=0.0111) showed that the proportion of arbuscules in bean cultivar roots was significantly different between fallow species treatments.

Fallow species treatments	**Categories for arbuscule percentage in roots**		
	Absence	**Presence**	**Total**
Natural regeneration	24 (5.85)	24 (5.85)	48 (11.71)
Cajanus fallow	28 (6.83)	20 (4.88)	48 (11.71)
Tithonia (CR) fallow	24 (5.85)	26 (6.34)	50 (12.20)
Tithonia (CR) and *Cajanus* fallow	34 (8.29)	14 (3.41)	48 (11.71)
Tithonia (MEX) fallow	30 (7.32)	23 (5.61)	53 (12.93)
Tithonia (MEX) and *Cajanus* fallow	44 (10.73)	19 (4.63)	63 (15.37)
Tithonia (IND) fallow	31 (7.56)	21 (5.12)	52 (12.68)
Tithonia (IND) and *Cajanus* fallow	18 (4.39)	30 (7.32)	48 (11.71)
Total	233 (56.83)	177 (43.17)	410 (100.00)

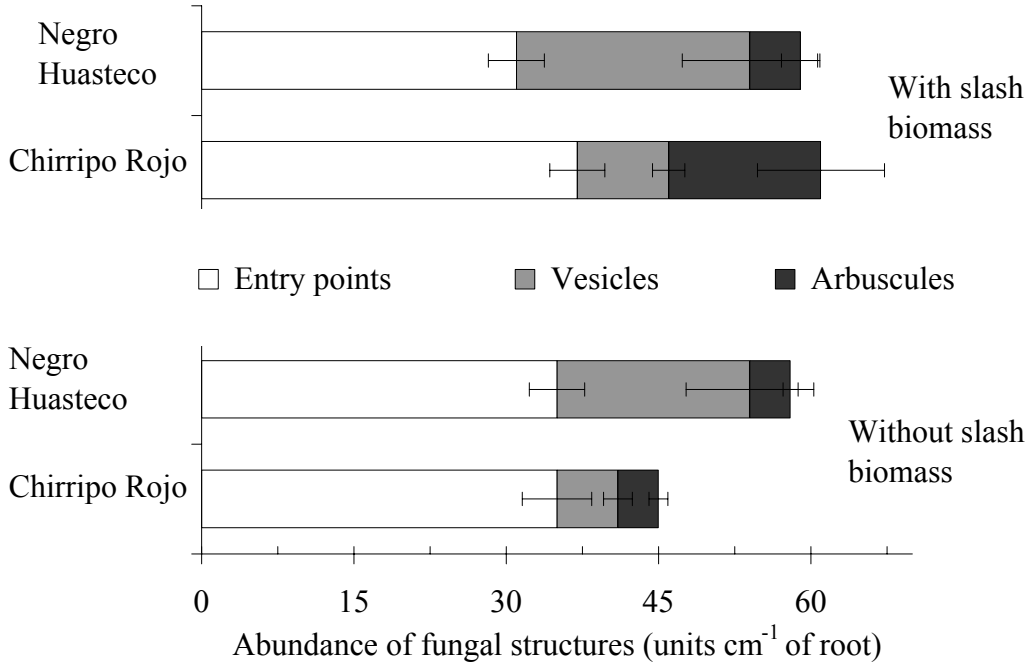

Figure 27. Abundance of fungal structures in bean cultivar roots grown with and without slash biomass applications harvested at the end of the flowering phase. Standard error bars are displayed (n=100). ANOVA (p =0.012) showed bean cultivars were significantly different for abundance of vesicles. Wilks' Lambda multivariate test showed bean cultivars were significantly different for the abundance of fungal structures in roots (p =0.03).

4.8.1 Experiment 2: Mulch transference from outside plots (Cut-and-carry system)

4.8.1.1 Bean yield

With a previous application of calcium carbonate and poultry manure (three months before planting), bean yield was significantly different between mulch application treatments (p=0.0217) in ANOVA test and was higher with mulch application treatments than the control (p=0.0063) in orthogonal contrast comparisons. However, there were no significant differences between *Cajanus* and *Tithonia* mulches. In addition, no contributions came from bean cultivars and the interaction of bean cultivars and mulch application treatment effects. The orthogonal contrasts did not detect additional significant differences between the exotic (the DOR-364 and CIAT G-1937) and the Costa Rican bean cultivars (the Chirripo Rojo and Negro Huasteco) (p=0.0688). Only the Negro Huasteco bean differed from the CIAT G-1937 in bean yield (p=0.0397). In control, the best performance was obtained by the DOR-364 (Appendix 27).

When slash biomass was transferred from outside plots in *Cajanus* and *Tithonia* mulch application treatments (on soils with previous applications of calcium carbonate and poultry manure), the global mean gain in bean yield represented 0.35 Mg ha^{-1} (30.8%) with respect to the control and 0.15 Mg ha^{-1} (11.7%) for bean cultivar selection (between the exotic and the Costa Rican bean cultivars) (Appendix 28).

There were no significant differences in ANOVA test for the above-ground biomass between the four bean cultivars and also for the comparisons of the control, *Tithonia* and *Cajanus* mulch application treatments (Appendix 29). Orthogonal contrasts did not detect significant differences between the exotic and the Costa Rican bean cultivars (p=0.0997) neither within the exotic bean cultivars (DOR-364 versus CIAT G-1937) for above-ground biomass at the flowering phase (p=0.0973).

4.8.1.2 Bean nutrient concentrations

When Ca, Mg, K and P concentrations were grouped as a set of nutrient variables, no statistical differences were detected in nutrient concentrations for bean cultivar effects (p=0.0956) using Wilks' Lambda multivariate analysis (MANOVA). However, for mulch application treatments there were significant differences (p=0.0014) (Appendix 30). There were significant differences between the control and mulch application treatments for Ca, Mg and K concentrations in above-ground biomass (Appendix 31). The control showed higher Ca and Mg concentrations than the mulch application treatments, but lower for K concentration.

Only Mg concentration in the above-ground dry weight had significant differences between the four bean cultivars (p=0.0291) (Figure 28-B). The Negro Huasteco bean cultivar had lower Mg concentration than the Chirripo Rojo bean cultivars. For Mg concentration, the Negro Huasteco bean cultivar showed significant differences with the Chirripo Rojo in contrast comparisons (p=0.0071) and with the CIAT G-1937 bean cultivar in Duncan's test (Appendix 30).

The *Cajanus* mulch was significantly better than *Tithonia* mulch (p=0.0102) (Appendix 31) at enhancing P concentration in bean tissue. Calcium carbonate and poultry manure applications were more effective in increasing P concentration in bean cultivar tissue than the slash biomass treatments (Appendix 32). Significant differences were found only between mulch application treatments for P utilization efficiency as shown in Appendix 29. P uptake efficiency was not statistically different for mulch application and bean cultivar treatments (Figure 29-B). Neither P uptake efficiency nor P utilization efficiency were effective parameters to discriminate and rank bean cultivars. However, the Chirripo Rojo showed higher P uptake efficiency than the CIAT G-1937 in Duncan's test.

4.8.1.3 Bean cultivar root system characteristics

For root dry weight partitioning variables, only the basal root dry weight showed significant differences for bean cultivars (p=0.0123). This was not the case for mulch

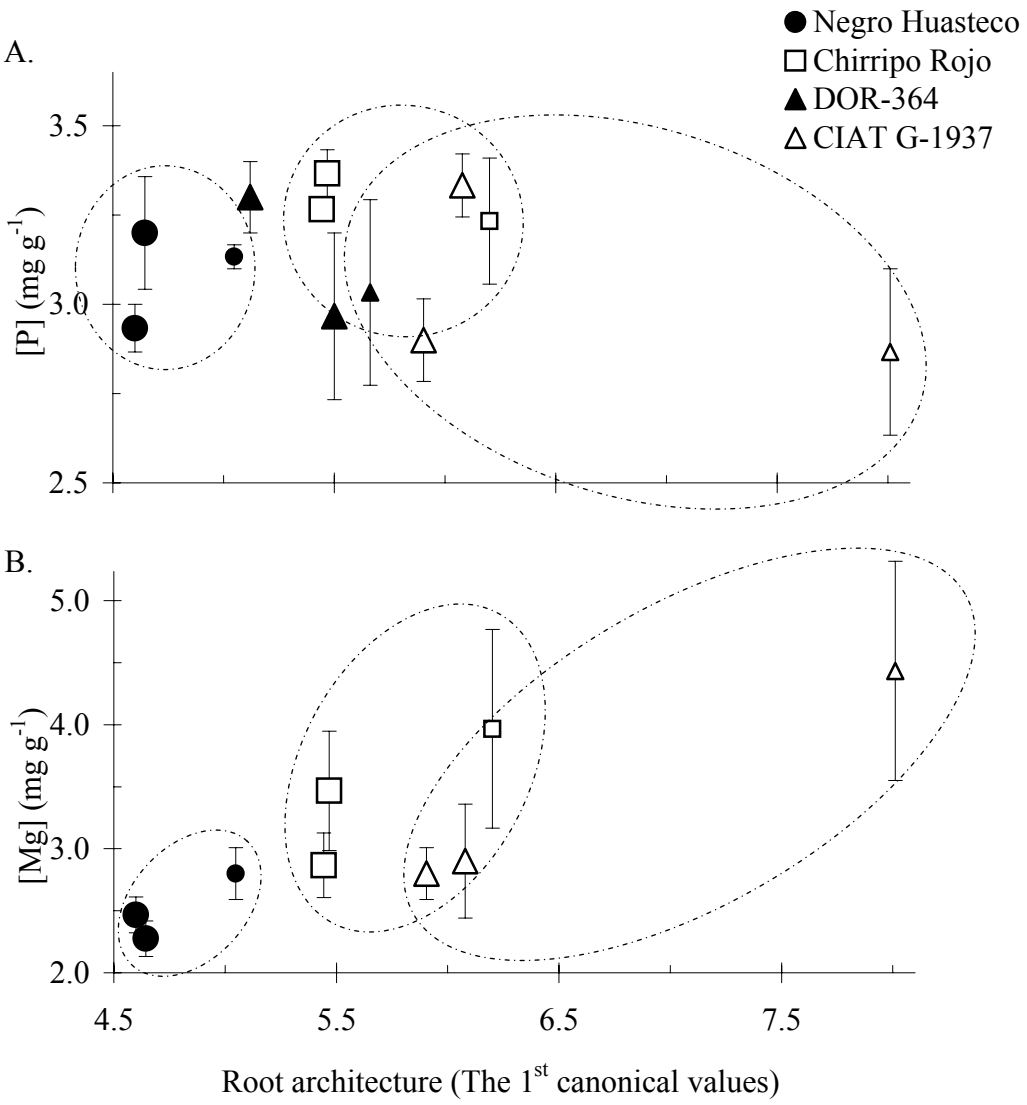

Figure 28. Relationships between P (A), Mg (B) concentrations and root architecture for four bean cultivars grown under different mulch application treatments harvested at the flowering phase in Experiment 2. The 1st canonical values were used to capture, in statistical significant variables, the effects of the original root variables. Standard error bars are displayed (n=3). Close circle, Negro Huasteco; open square, Chirripo Rojo; close triangle, Dor-364; open triangle, CIAT G-1937. Group of cultivars encircled by ovals that do not overlap indicate significant differences for root architecture. The enlarged (x,y) points correspond to *Tithonia* and *Cajanus* mulch treatments. In A, contrast comparisons detected significant differences between *Cajanus* and *Tithonia* mulches (p =0.0102). In B, contrast comparisons detected significant differences between control and mulch treatments (p =0.0001) and between the Chirripo Rojo and Negro Huasteco (p =0.007). The (x,y) points of the DOR-364 bean cultivar were hidden for a better representation of statistical differences detected.

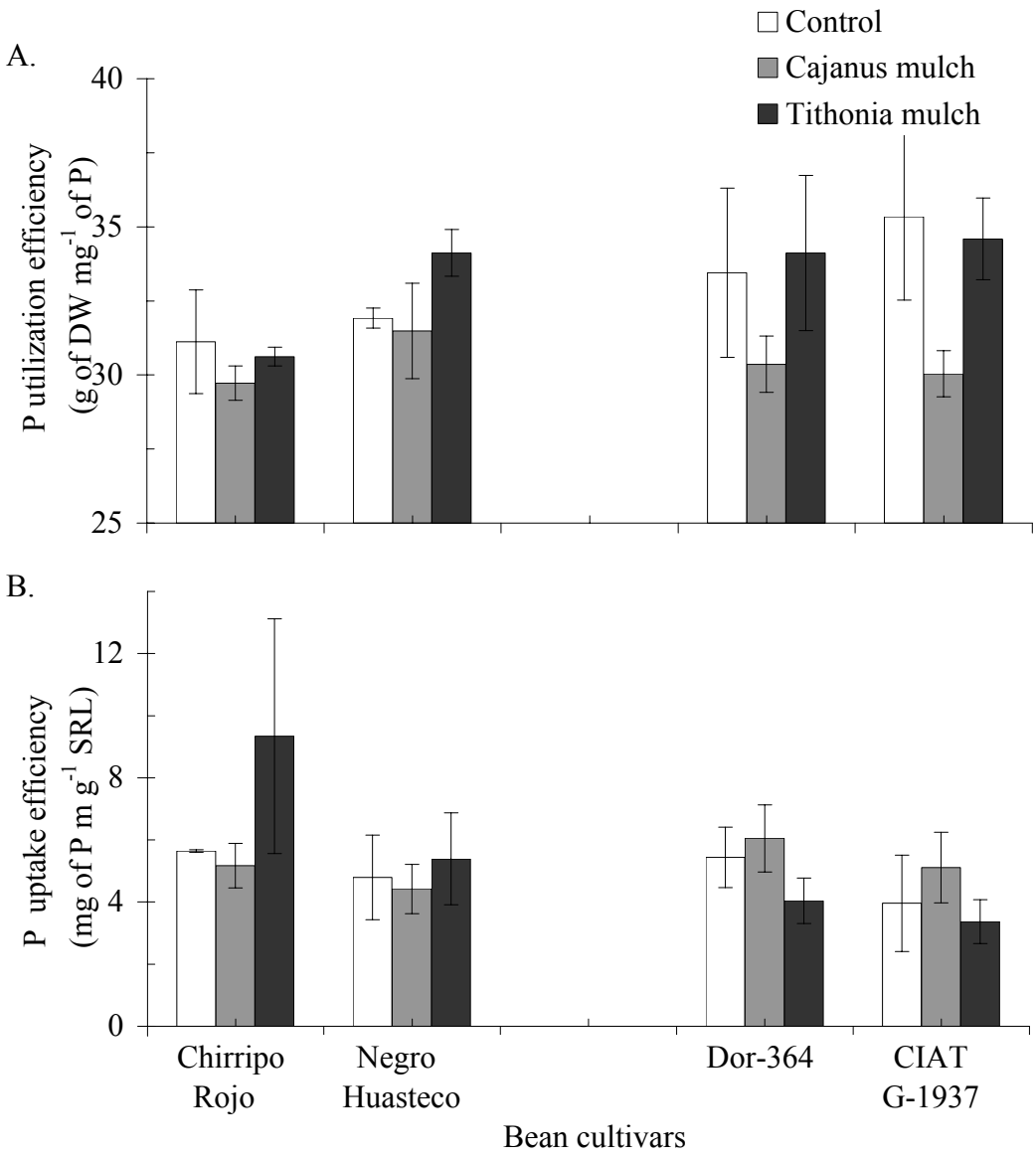

Figure 29. Comparison between the Costa Rican (Chirripo Rojo and Negro Huasteco) and exotic CIAT bean cultivars (Dor-364 and CIAT G-1937) for P utilization (A) and uptake (B) efficiencies under different mulch application treatments harvested at the flowering phase in Experiment 2. Standard error bars are displayed (n=3). In A, ANOVA (p =0.03) showed mulch treatments were significantly different and contrast comparisons detected significant differences between *Cajanus* and *Tithonia* mulches.

application treatments in ANOVA test (Appendix 33). The CIAT G-1937 and the Negro Huasteco bean cultivars had higher basal root dry weight than the Dor-364 and the Chirripo Rojo. No significant differences were detected in a multivariate analysis for root dry weight partitioning variables. For root architecture, a multivariate analysis using Wilks' Lambda test indicated significant differences for bean cultivar effects (B) (p=0.0002) and for mulch application treatments (A) (p=0.0017) (Appendix 34), but the interaction between bean cultivars and mulch application treatments (AxB) did not show significant differences. Bean cultivars significantly differed in upper root-stem length (p=0.0498) and the number of nodules (p=0.0002) (Figure 30). However, upper root-stem length was not significantly affected by the presence of mulch on the soil surface. Bean cultivars developed a higher number of nodules in *Tithonia* mulch than *Cajanus* mulch treatment (Appendix 34 and Figure 30). However, the comparison between the control and mulch treatments did not show significant differences for the number of nodules.

As shown in Figure 31, the first canonical variable correlation analysis detected evidence that root dry weight partitioning variables and root architecture variables are not completely independent (since p=0.0001). In addition, more than two canonical variables were statistically significant. The first and second canonical variables had correlation coefficients equal to 0.7428 and 0.5981, respectively.

When above-ground biomass was used as a response variable in a multi-linear regression model with root dry weight partitioning and root architecture variables for the four bean cultivars (the Costa Rican and exotics) at the flowering phase, the best predictors were the lower root-stem dry weight, the number of nodules and the lower root-stem length (R^2=26.02; p<0.0001; R^2=14.26; p=0.0001 and R^2=5.39; p=0.0115), respectively. The linear regression model had an R^2 equal to 45.68.

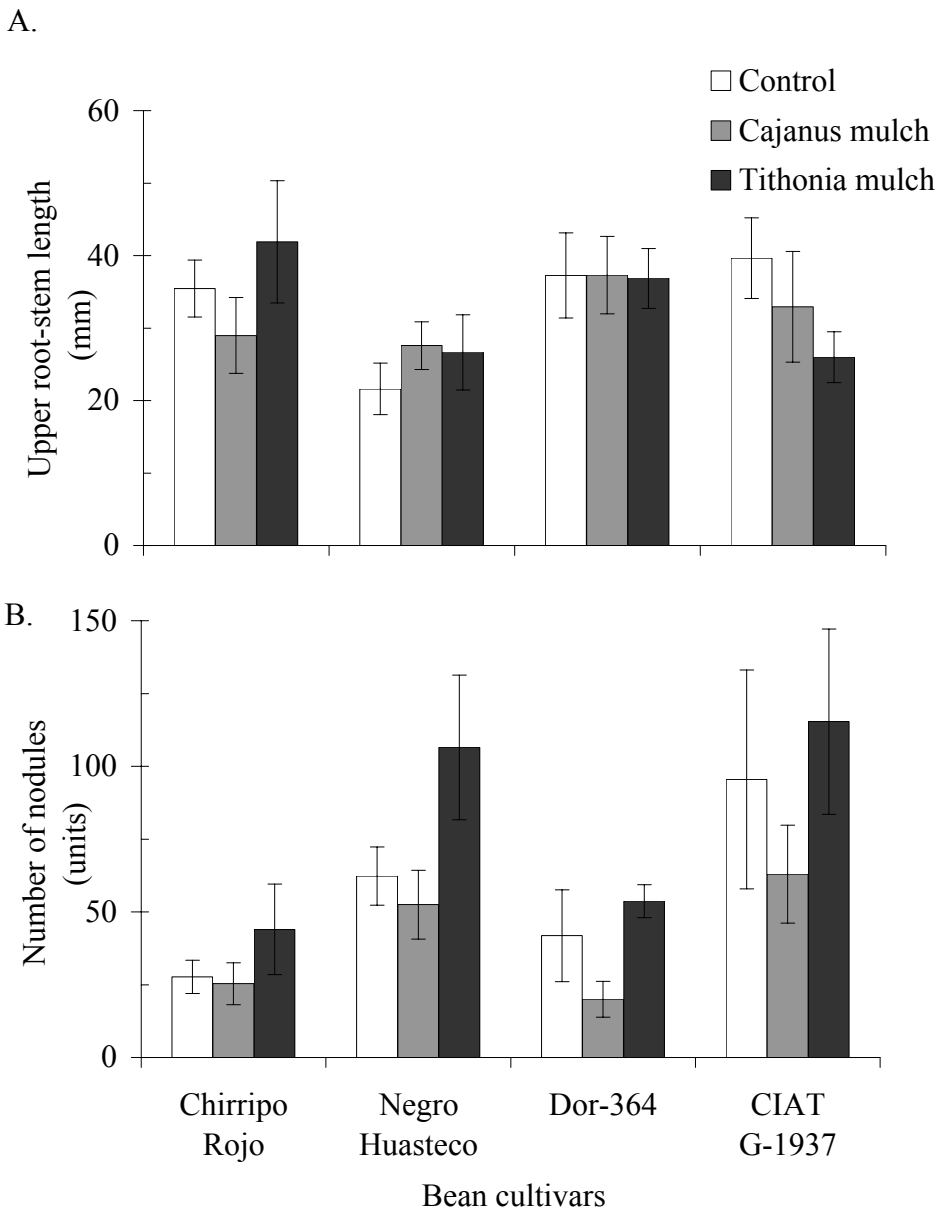

Figure 30. Upper root-stem length (A) and the number of nodules (B) in control, *Cajanus* and *Tithonia* mulch application treatments harvested at the flowering phase in Experiment 2. Standard error bars are displayed (n=12). In A, ANOVA detected significant differences among bean cultivars (p =0.05). In B, ANOVA detected significant differences for mulch application treatments and bean cultivars (p =0.01 and p =0.002), respectively. Contrast comparisons detected significant differences between *Cajanus* and *Tithonia* mulches (p =0.0026).

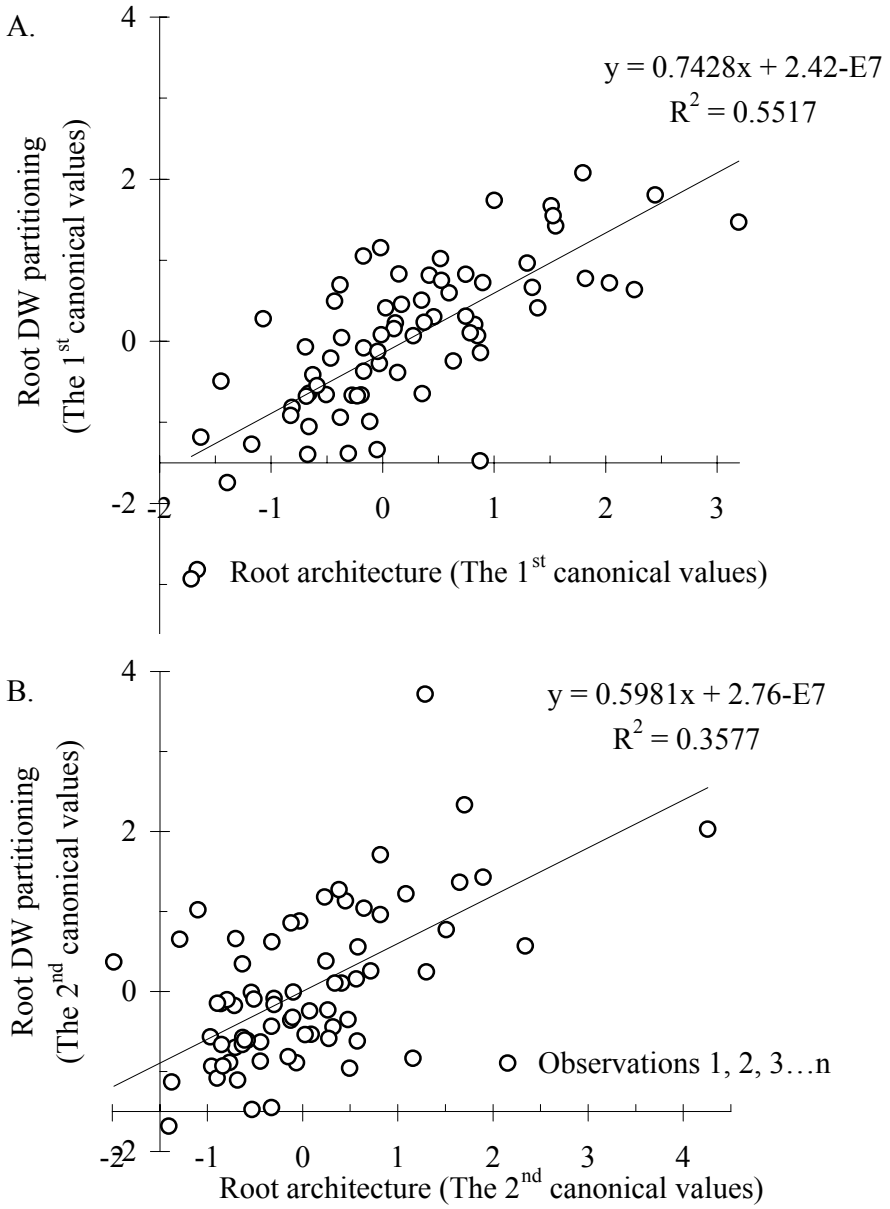

Figure 31. Relationships between the 1st (A) and 2nd (B) canonical variables for root dry weight partitioning and root architecture between four bean cultivars harvested at the flowering phase in Experiment 2, (n=72). Pearson correlation coef. and probability: (0.743, p <0.0001 and 0.598, p <0.0001) for A and B, respectively. Canonical variables captured, in statistical significant variables, the effects of the original variables for root architecture and root dry weight partitioning. Only two pairs of canonical variables are needed to adequately represent the association between the two sets of original variables. Both pairs of canonical variables have about an equal and positive amount of influence in the characterization of the bean root system. Therefore, it is necessary to measure both groups of original variables for a better characterization of the bean root system.

4.9. *Tithonia* response to different mineral P availability levels

4.9.1 Growth parameters of *Tithonia* genotypes at different P availability levels

Above-ground dry weight for the Colombian and Costa Rican *Tithonia* genotypes increased as P availability levels increased (Figure 32 and Photo 6). Those plants grown under medium P availability conditions (160 μM of P) or under high P conditions (1 mM of P) performed significantly better than their low P conditions (10 μM of P) counterparts when exposed to different P levels overtime. For example, under medium and high P levels, above-ground dry weight was two to four times higher than that under lower P conditions. At the lowest P treatments, *Tithonia* genotype plants were very small and had dark green leaves and more senescent leaves.

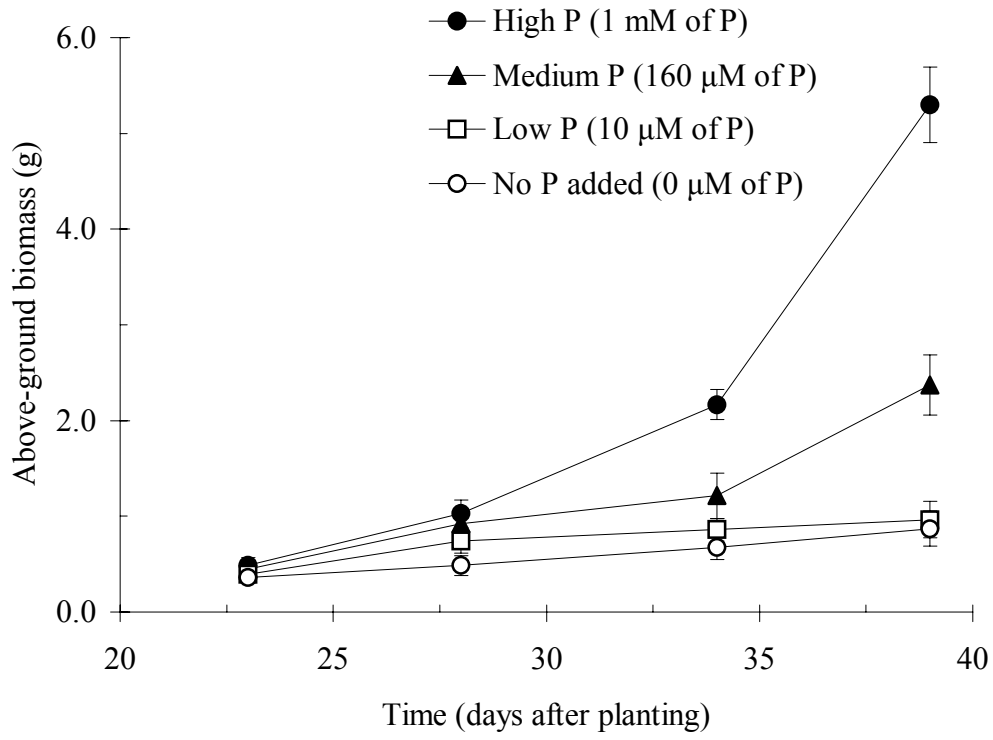

Figure 32. Above-ground biomass for *T. diversifolia* genotype potted plants at four different P availability levels in sand culture and four different harvest times. Standard error bars are displayed (n=12).

Photo 6. The Costa Rican and Colombian *T. diversifolia* genotypes growing at 0, 10, 160 and 1,000 μM of P in sand culture, Årslev, Denmark. The first two groups of plants (starting from the left) are from Costa Rica and the last one from Colombia.

Significant differences were detected between P availability levels for above and below-ground dry weight allocation ($p<0.0001$ and $p<0.0001$), respectively. However, no significant differences were detected between the Colombian and Costa Rican genotypes for root and stem dry weights, except for green and senescent leaf dry weights ($p=0.0395$ and $p<0.0001$), respectively. The potted plant heights and stem diameters were significantly higher in the Colombian than the Costa Rican genotype ($p=0.0259$ and $p=0.0351$), respectively. However, both genotypes were similar in leaf area ($p=0.1643$).

4.9.2 P concentration and accumulation in *Tithonia* genotypes

There were significant differences for leaf, stem and root P concentrations ($p<0.0001$, $p<0.0001$ and $p=0.0003$), respectively at different P availability levels 39

days after planting, but not between the Costa Rican and Colombian genotypes. A total of 192 samples were examined for leaf P concentration in potted plants that were grown under different P availability levels harvested 39 days after planting. The overall P concentration rank was higher for the leaves than for the stems and roots (Table 14). More P was allocated to the leaves than to any other organ. Leaf, stem and root P concentrations were superior to 2.6 mg P g^{-1} for both *Tithonia* genotypes when potted plants were grown at 1 mM of P. Leaf P concentrations were constant over exposure time at low (10 μM of P) and medium (160 μM of P) levels of P. P concentrations in green leaves were around 2 mg P g^{-1} (Figure 33).

Table 14. Leaf, stem and root P concentrations for two *T. diversifolia* genotype potted plants at four different P availability levels harvested 39 days after planting in sand culture.

P availability levels (μM of P)	**P concentrations** (mg g^{-1})					
	***Tithonia* genotypes**					
	Costa Rican			**Colombian**		
	Root	Stem	Leaf	Root	Stem	Leaf
0	0.7±0	0.7±0.1	2.1±0.2	1.3±0.4	1.1±.1	2.0±0.1
10	0.9±0	0.9±0	1.8±0.1	1.1±0.1	1.1±0.2	1.8±0.1
160	1.4±0.1	1.2±0.1	2.5±0.1	1.4±0.2	1.4±0.2	2.1±0
1,000	6.2±0.9	3.1±1.0	5.3±0.2	3.8±0.3	2.6±0.1	4.8±0.2

Sampling sizes were n=16 and n=8 for the Costa Rican and Colombian genotypes, respectively. Values are means ± standard errors.

P utilization efficiency declined with increasing P availability levels, but P uptake efficiency increased with P availability levels (Figure 34). Significant differences were found for P uptake and utilization efficiencies at different P availability levels 39 days after planting, but significant differences between the Colombian and Costa Rican

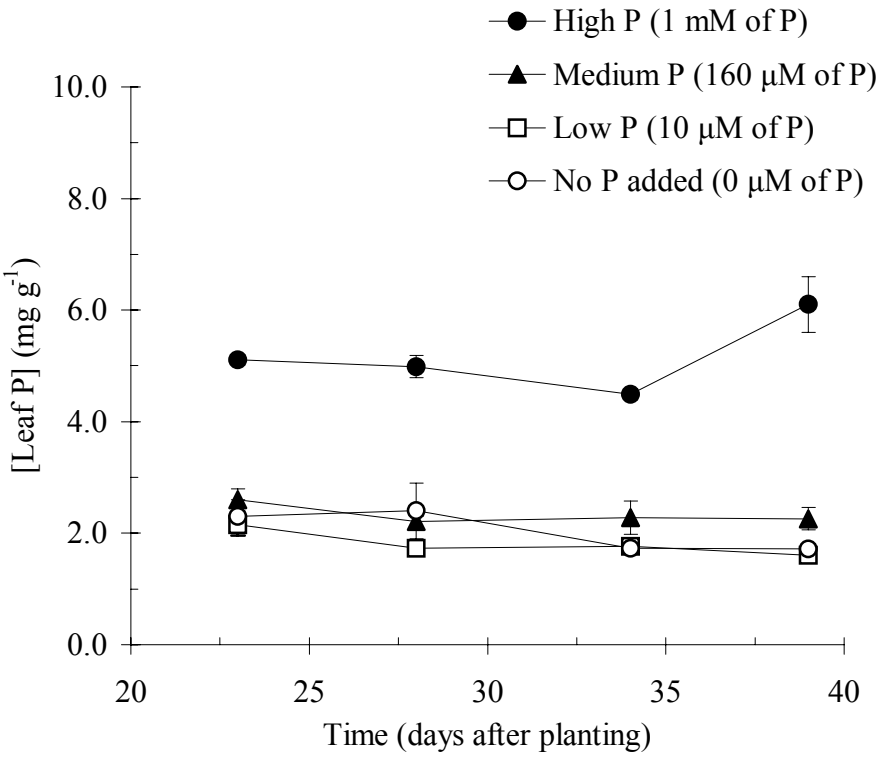

Figure 33. Leaf P concentration for *T. diversifolia* genotype potted plants grown at four different P availability levels in sand culture and four different harvest times. Standard error bars are displayed (n=16).

genotypes occurred only in P utilization efficiency (p=0.005). Performance ratings for P utilization efficiency in above-ground biomass dry weight indicated that the Costa Rican genotype (Clones 1-CR and 4-CR) could have a higher biomass production per P uptake at low P availability levels than the Colombian genotype (Clone 6). These differences were not found at medium and high P availability levels, as shown in Figure 34-A.

4.9.3 Root length of *Tithonia* genotypes under different P availability levels

P fertilization significantly increased root length (p<0.0001) in *Tithonia* genotypes. The Colombian genotype developed a higher root length than the Costa Rican (p<0.0001) at 10, 160 and 1,000 µM of P (Figure 35). Greater differences in root length were found at the highest P availability level. There were significant differences between

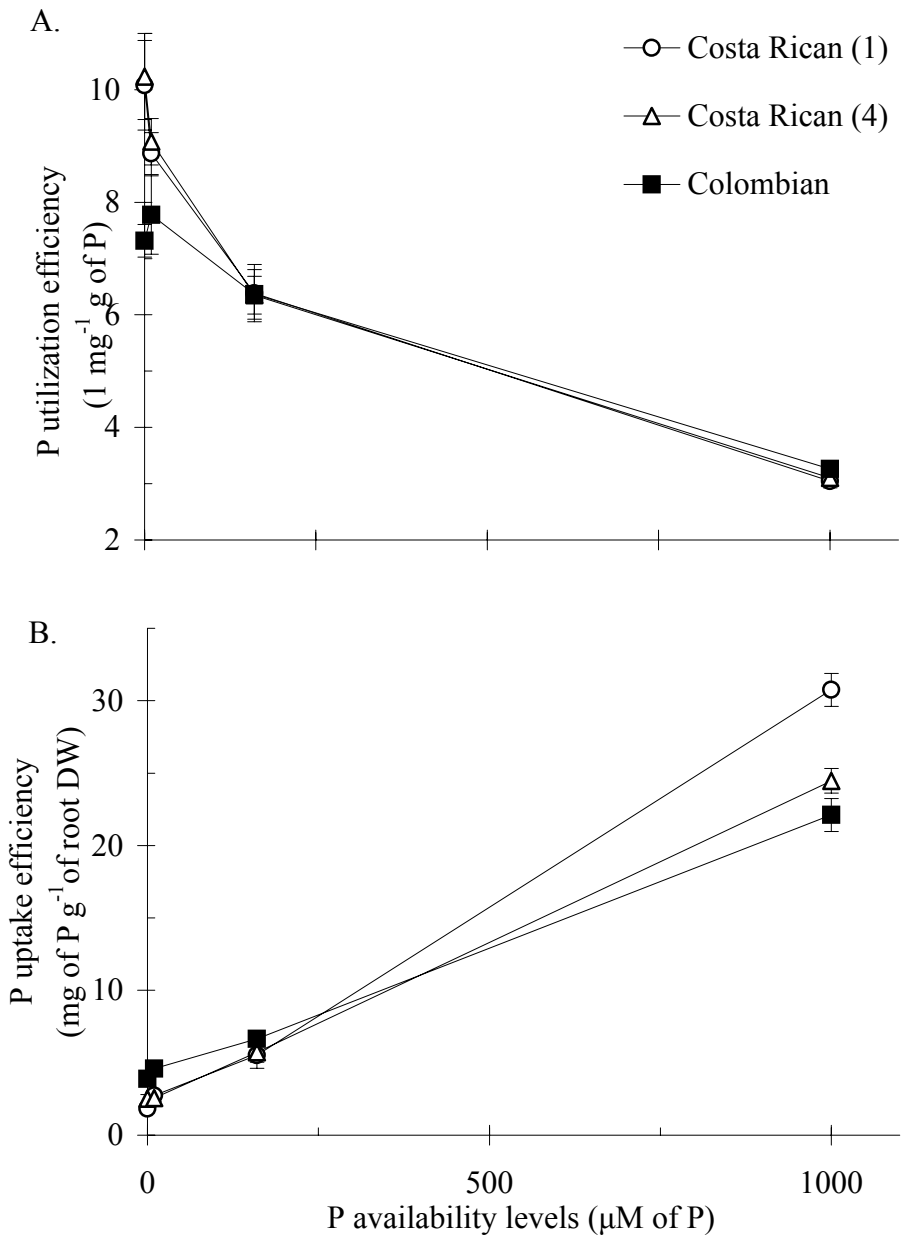

Figure 34. P utilization (A) and P uptake (B) efficiencies of *T. diversifolia* genotypes at four different P availability levels in sand culture harvested 39 days after planting. Standard error bars are displayed (n=6). Two clones were used for the Costa Rican (CR-1 and CR-4) and one for the Colombian genotype.

P levels for SRL (p<0.0001), but not between the Costa Rican and Colombian genotypes. Duncan's test detected significant differences between the highest P level and the lowest P level for SRL. Mean SRL were 45.8, 44.2 and 72.3 m of root g^{-1} of root dry weight for low, medium and high P levels, respectively.

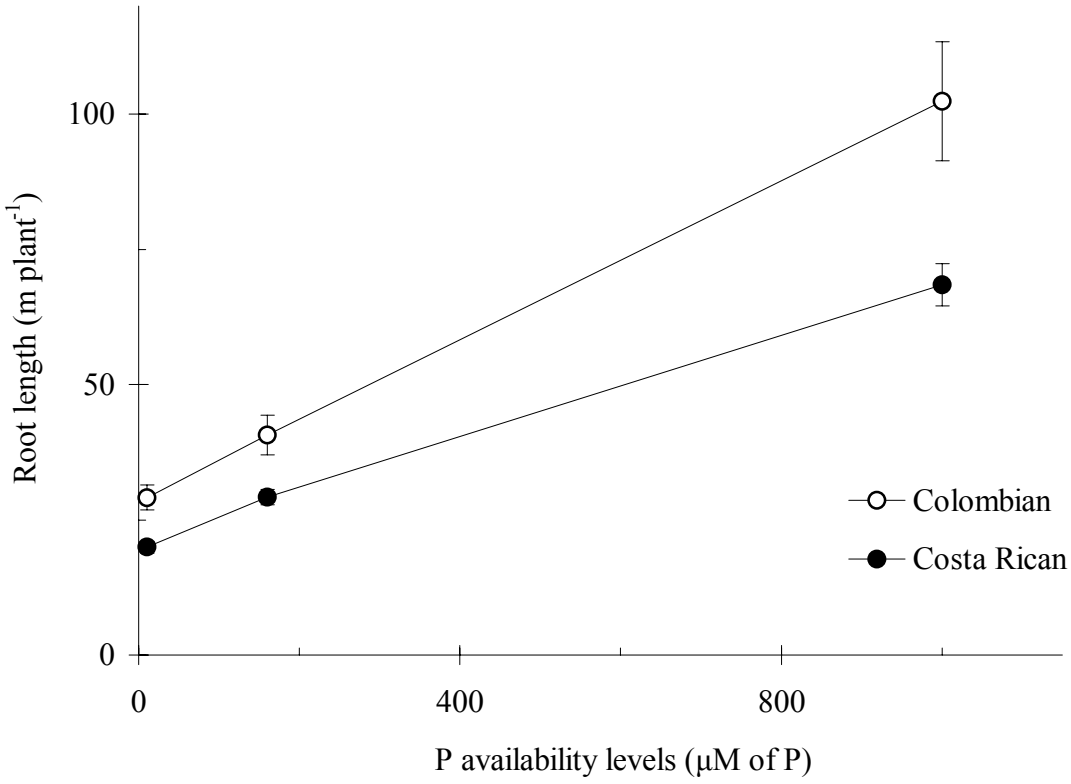

Figure 35. Total root length for the Colombian and Costa Rican *T. diversifolia* genotypes in six-week old potted plants at three different P availability levels in sand culture. Standard error bars are displayed. Sampling sizes were n=5 and n=25 for the Colombian and Costa Rican genotypes, respectively.

Figure 36 shows the root length diameter distribution for the Costa Rican and Colombian *Tithonia* root systems at three P availability levels on six-week old potted plants (42 days after planting). Root length increased with P availability for the root diameter classes below 1.75 mm. However, significant differences between the Costa Rican and Colombian genotypes were mainly found for the root diameter classes below 0.75 mm.

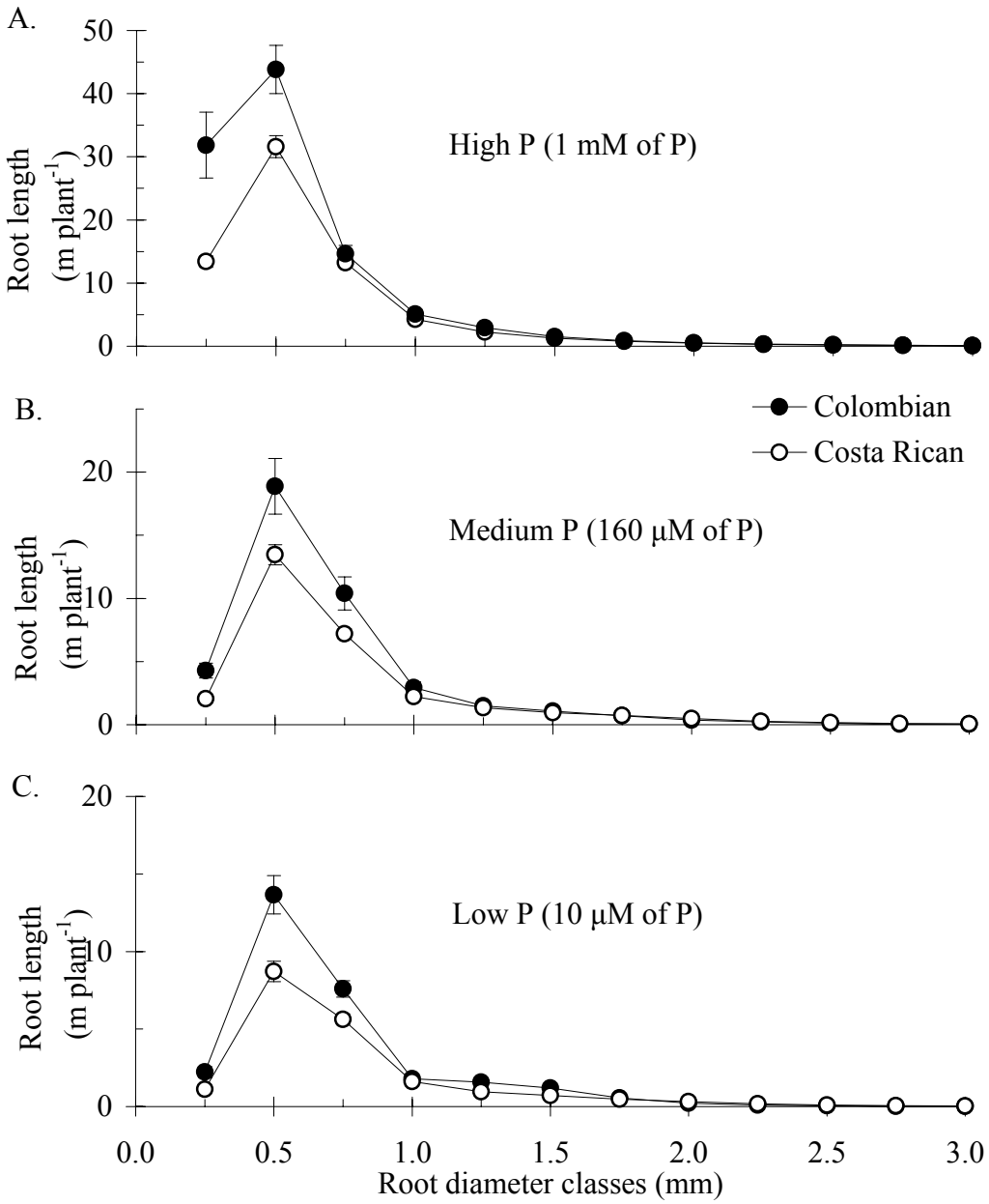

Figure 36. Root length diameter distribution of the Costa Rican and Colombian *T. diversifolia* genotypes in six-week old potted plants at three different P availability levels in sand culture. Open circle, Costa Rican; closed circle, Colombian. Sampling sizes were n=25 and n=5 for the Costa Rican and Colombian genotypes, respectively. Standard error bars are displayed. ANOVA (p <0.0001) showed *Tithonia* genotypes were significantly different for root length diameter distribution at 1 mM of P.

4.9.4 Root to above-ground biomass ratio of *Tithonia* genotypes

The effects of increasing P fertilization decreased the root to above-ground biomass ratio of the Costa Rican and Colombian genotypes (Figure 37). An important aspect is that the root to above-ground biomass ratio decreased less in the Colombian genotype with P fertilization. In fact, dry weight allocation in the Colombian genotype varies less than the Costa Rican genotype in P fertilization (p<0.0001). When different P availability levels were used in the same pot (split P fertilization) with the Costa Rican *Tithonia* genotype, root to above-ground biomass ratios in split pots were similar to the highest P levels of the respective P level treatments in non-split P fertilization treatments (Appendix 35).

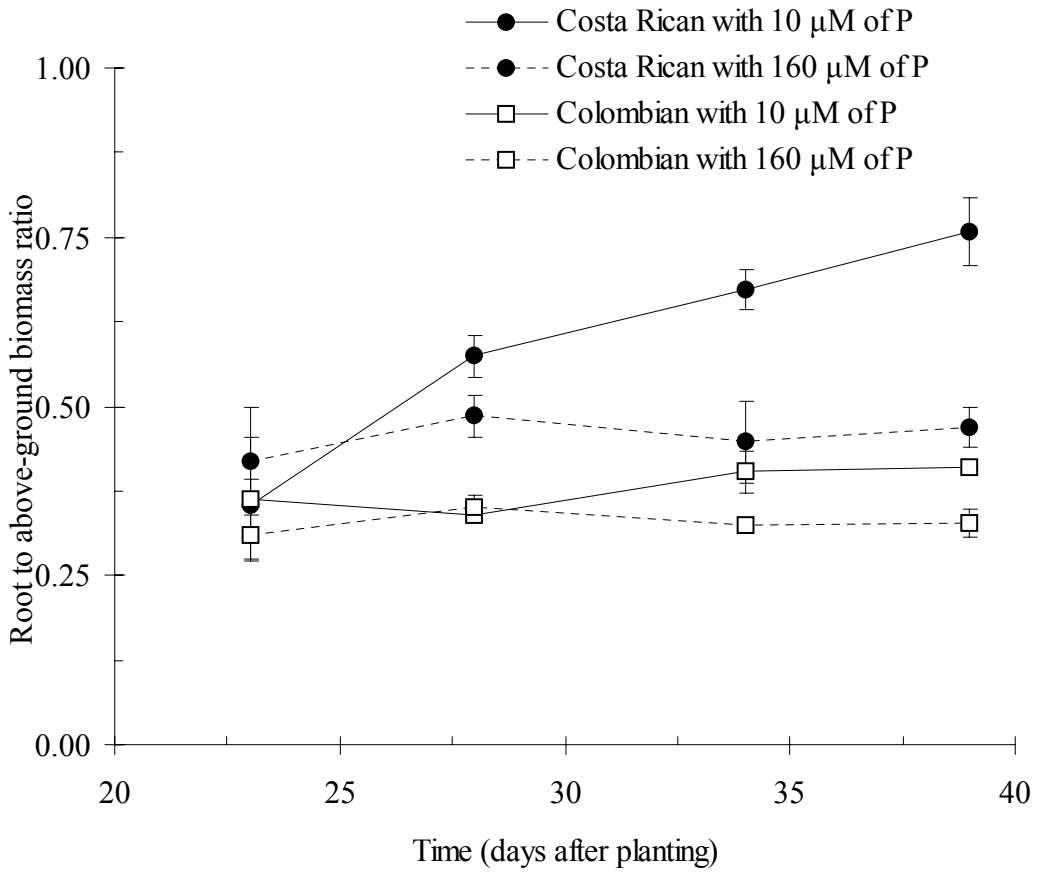

Figure 37. Root to above-ground biomass ratio for the Costa Rican and Colombian *T. diversifolia* genotype potted plants at two different P availability levels in sand culture and four different harvest times. Standard error bars are displayed. Sampling sizes were n=5 and n=25 for the Colombian and Costa Rican *Tithonia* genotypes, respectively.

5. Discussion

5.1 Biomass and nutrient accumulation of fallow species

5.1.1 Biomass accumulation of fallow species

Tithonia is an attractive species to accelerate the rehabilitation of the original ecosystem when biomass and nutrient accumulation are considered. At least five densely planted fallow species accumulated more biomass than natural regeneration. Considering that adequate productivity for many ecosystems is about 5.0 Mg ha^{-1} (Bradshaw, 1984), the Mexican *Tithonia* genotype was found to be the fastest growing species on an Andisol with low P availability because it accumulated up to 10.5 Mg ha^{-1} in densely planted fallows six-months after planting. It seems that *Tithonia*, in densely planted fallows, better utilizes the environmental resources found in San Juan Sur Andisol to maximize above-ground biomass accumulation. However, densely planted fallows cannot reach the biodiversity and complexity of ecosystem structure registered in natural fallow.

Reduction in above-ground biomass accumulation for *Tithonia* genotypes in Experiment 1 field site did not occur at limited soil P availability (4.9 mg P kg^{-1}) (Figure 2). Different nutrient acquisition mechanisms may be operating in the field to maintain the observed fast growth of *Tithonia* in San Juan Sur Andisol because of *Tithonia* potted plants did not growth in sand culture at 10 μM of P (Figure 32). As a reference, in soils well supplied with phosphate, P concentrations in the soil solution are likely to be less than 2 μM of P (Smith, 2002; Raghothama, 1999).

In this type of soil, Lebeuf (1993) observed that biomass accumulation was less than 4.0 Mg ha^{-1} for *Erythrina fusca,* in hedgerow intercropping. López (1995) reported 1.7 Mg ha^{-1} for velvet bean (*Mucuna deeringiana*) and *Erythrina berteroana* when *Mucuna* was planted at 80,000 plants ha^{-1} and *Erythrina* planted at 4.0 m between hedgerow and 0.5 m between plants and pruned twice a year. Arriaza (1995) recorded 1.4 Mg ha^{-1} for *Erythrina* in alley farming. Comparatively, Gichuru and Kang (1989), working with a fast-growing legume (*Calliandra calothyrsus*), reported only 6.0 Mg ha^{-1} of biomass accumulation per year for an alley cropping system on an Oxic Paleustalf in

southwestern Nigeria. In comparison to these systems with the above mentioned species, the Mexican *Tithonia* genotype in short fallow period at 60,000 plants ha^{-1} attained a higher biomass accumulation. This indicates a higher acclimation of the Mexican genotype to this soil because of its higher root length density (Table 3) or other root advantages that increase plant capacity to maximize nutrient acquisition and utilization when *Tithonia* is grown in densely planted fallow.

Even though the densely planted fallows showed higher biomass production in comparison to alley cropping, they are limited because agricultural crops cannot be established at the same time biomass accumulation of fallow species is taking place. Therefore, to maintain a sustainable food supply for human consumption, additional areas are needed for cultivation outside of fallow areas during densely planted fallows. In addition, green manure species re-grow vigorously after pruning (such as *Tithonia* genotypes in Experiment 1) during the cropping phase, but compete with desired crops to some extent (Giller and Wilson, 1991). Two pruning were needed to control the re-growth of *Tithonia* during the cultivation of a bean crop cycle.

In *Tithonia* genotype fallow treatments, significant differences were found in above-ground biomass accumulation and stem height between the different genotypes. Lower biomass accumulation (around 3.9 Mg ha^{-1}) and stem height were found for the Indonesian *Tithonia* genotype due to early flower development on most of the branches in detriment of new stem and leaf production. Additionally, this genotype also exhibited lower biomass accumulation and stem height in the *Tithonia* germplasm collection at San Juan Sur, Turrialba, Costa Rica. *Tithonia* genotype stem height showed the same ranking as biomass accumulation (Figure 4). Three stem height classes were found to exist between different genotypes at the *Tithonia* germplasm collection. This also corresponded to the three different stem height classes observed in Experiment 1, where the Mexican *Tithonia* genotype was tallest, the Costa Rican (Central American) intermediate and the Indonesian (Asian) shortest.

As a biomass accumulator, the Mexican *Tithonia* genotype concentrated its biomass in fewer and larger branches which is inconvenient for mulching and fodder, but

it proved to be an important species for the cut-and-carry agroforestry system because it can accumulate more non-lignified biomass per unit of area (>3.2 Mg ha^{-1}) than the other *Tithonia* genotypes and *Cajanus* (around 2.4 Mg ha^{-1}). Natural regeneration accumulated more green mulch (5.19 Mg ha^{-1}) if all its slash biomass is considered as non-lignified (Figures 2 and 3). Slaasts *et al.* (1996) and Herrera (1997) indicated that *Tithonia,* like other *Asteraceae* plants, typically attains maximum biomass and nutrient accumulation in three to five years. This agroforestry species produced mainly leaf biomass in the first six-months but stem growth dominated thereafter (George *et al.,* 2002a).

Tithonia might be a better non-lignified biomass producer in longer fallows if the same biomass allocations are maintained after six-months of fallow. However, the amount of non-lignified biomass for *Tithonia* genotypes did not increase continuously in the two-year old *Tithonia* plants at the *Tithonia* germplasm collection because there was a significant reduction in non-lignified biomass immediately after its main flowering season (November-February). *Tithonia* re-growth started profusely at the end of February and attained maximum biomass accumulation from September to October. Higher green mulch occurs for *Calliandr*a (4.9 Mg ha^{-1}) and *Alnus* (5.4 Mg ha^{-1}) in longer fallows (2-year) (Siriri and Raussen 2003). However, *Calliandra* is usually considered a poor fallow species because of its slow rate of biomass decomposition and therefore slower nutrient release (Handayato *et al.,* 1994). On the contrary, *Tithonia* biomass decomposes very quickly and releases most of its nutrients in the first few weeks after application (Cobo *et al.,* 2002). As such, even though *Tithonia* produces lower green mulch, its nutrient release into the system is quicker mainly when grown in short term densely planted fallow because of its lower contents of lignin and polyphenols.

5.1.2 Nutrient concentration and accumulation

A major focus of this study was to address the potential of fallow species biomass as nutrient sources, especially for P. However, it was found that nutrient composition (N, P, K, Mg and Ca) in the biomass of the different fallow species is similar when nutrient concentrations are considered together in a multivariate test (Figure 6). This can be explained by the similar feedback regulation mechanisms of nutrients among the fallow

species used. Glass and Siddiqi (1984) also found that plants exhibit a strong tendency to maintain constant cytoplasmic concentrations of ions such as N, P, K and Cl in despite of the large fluctuations in the external concentrations of those ions such occur when nutrients are supplied through fertilizers.

Differences in nutrient additions to the system by each fallow species corresponded mainly to differences in biomass accumulation because nutrient concentrations were similar (Appendix 5). Nutrient accumulation was likely to be more beneficial when rapid biomass producing fallow species were used and low average N, P, and K tissue concentrations such as in Experiment 1. The other possible option may be to use slow growing fallow species, compensating with very high N, P and K concentrations in the biomass. However, this qualification of very high nutrient concentrations refers only to slightly above normal concentrations in higher plants because the most important macronutrients (N, P and K) are only available in low concentrations in plant dry matter (Boyd, 2004). Therefore, slow growing species are more limited to the accumulation of nutrients than rapid biomass producers. Slow growing species cannot meet the most important criterion as biomass sources for maintaining sufficient and available supplies (Kwabiah *et al.,* 2003b).

Nutrient concentrations reported in Appendix 4 for *Tithonia* genotypes and natural regeneration were below the mean N, P and K concentrations indicated by Nagarajah and Nizar (1982) for leaves and non-lignified stems in Sri Lanka. *Tithonia* genotypes in monoculture and natural regeneration biomass had an average N-concentration of 20.7 mg N kg^{-1} DW (Appendix 4) which is slightly lower than the 25 mg N kg^{-1} recommended for organic material to be used as green manure (Hoang Fagerström *et al.,* 2002). However, the biomass used in Experiments 1 and 2 included leaves, flowers, non-lignified and lignified stems in which lignified stems represented more than 50% of the biomass produced during the six-month fallow with *Tithonia* genotypes and *Cajanus* as fallow species. Early pruning might improve biomass quality, but could reduce biomass production. According to Palm *et al.* (1997), plant tissue concentrations ranging from 18 to 22 mg N kg^{-1} represent the critical threshold in the transition from N immobilization to net N mineralization. All of the fallow species treatments and natural

regeneration in short fallows were found to meet the N quality parameter, making them good sources for green mulch.

P concentrations in dry matter for fallow species were found to be slightly below the optimal growth range of 3-5 mg P g^{-1} (0.3–0.5%) during the vegetative growth stage (Marschner, 1995) and were 5 times lower than N. However, visible signs of P deficiency, such as purple leaves, did not appear in any of the fallow species during the six-month fallow period (Figure 5-A), even under the prevailing P-limited conditions in San Juan Sur Andisol. In addition, it was observed that leaf P concentration in *Tithonia* genotypes did not exceed 6 mg P g^{-1} even under high P availability (1 mM of P) in controlled environmental conditions (Figure 33). Maximum biomass accumulation occurs without excessive P concentration in the plant. It seems that root feedback mechanisms detect an increase in cellular concentrations of inorganic P, due both to its own uptake and to fungal supply. Correspondingly, they reduce the expression of their own P transporter genes to maintain an optimal level of nutrition within the plant. The probability of P toxicity increases at P concentrations higher than 10 mg P g^{-1} (Marschner, 1995; Shane *et al.,* 2004). Jama *et al.* (2000) indicated that the highest known P concentrations in *Tithonia* were found in the green leaves of plants growing on the Sukula Hills phosphate deposit (7 mg P g^{-1}) and in the Busumbu phosphate deposit (7.3 mg P g^{-1}) in eastern Uganda. Therefore, the utilization of *Tithonia* as a P source would be limited to a few milligrams per gram of biomass when grown on an Andisol with low P availability (<4.9 mg P kg^{-1}) and/or under high P availability level (1 mM of P) in controlled environmental conditions.

Among the fallow species, nutrient accumulation was higher for N and K, but lower for P, because P in plant tissue is only used for energy storage while N and K have much more extended biochemical functions in higher plants such as constituent of amino acids, coenzymes, cofactor for enzymes and in establishing cell turgor and maintaining cell electroneutrality. The Mexican *Tithonia* genotype in monoculture accumulated the equivalent of 208 kg N ha^{-1} in six-month fallow period (Appendix 4). Similar results were reported by Gichuru and Kang (1989) in an alley cropping system with *C. calothrysus*. Barrios and Cobo (2004) in longer term *Tithonia* planted fallow (27 month

fallow period) reported 417 kg N ha^{-1}, 85. 3 kg P ha^{-1}, 928 kg K ha^{-1}, 299 kg Ca ha^{-1} and 127 kg Mg ha^{-1} in 37 Mg ha^{-1} of above-ground biomass.

In field experiments, the Mexican *Tithonia* genotype in monoculture or in association with *Cajanus* also supplied more P in comparison to the other fallow species because of its higher biomass accumulation. This could enrich the soil for subsequent crop growth. P supplied over a six-month fallow period (18 kg P ha^{-1}) was very low, but it was higher than P supplied by velvet bean (*M. deeringiana*) and *E. berteroana* when these were applied every six-months (3.38 and 3.41 kg P ha^{-1}), respectively (López, 1995). Kwabiah *et al.* (2003b) also noted that *Tithonia*, applied at 5 Mg ha^{-1} DW, supplied a maximum of 9-15 kg P ha^{-1} 35 days after incorporation.

The organic materials of *Cajanus*, the Costa Rican *Tithonia* genotype in monoculture and the associated Indonesian *Tithonia* genotype with *Cajanus* had mean C:N ratio less than 20:1 (Appendix 5), suggesting that the equilibrium between mineralization and immobilization processes during decomposition would likely shift in favor of net mineralization of N (Constantinides and Fownes, 1994). Commonly, organic materials produced by fast growing species in short term fallow have low C:N ratio because of their higher proportions of non-lignified biomass that have more N per gram of dry weight. *Cajanus* registered the lowest C:N ratio (16.4), followed by the Costa Rican *Tithonia* genotype in monoculture with 19.3 ratio. The other fallow species biomass (from the Mexican *Tithonia* genotype in monoculture and in association with *Cajanus*) might induce a temporary immobilization of N if they are used immediately as mulch for crops since they have a higher C:N ratio. Considering C:P and N:P ratios, the best quality organic material sources were from the Costa Rican and Indonesian *Tithonia* genotypes in monoculture fallows. The other fallow species materials fall outside the critical quality levels and ranged from 2.0 to 2.7 mg P g^{-1} for total P, 156:1 to 252:1 for C:P ratio and 7.1 to 14.1 for the N:P ratio (Kwabiah *et al.,* 2003a). However, these ratios (C:N, C:P and N:P) for plant materials are time-dependent, implying that the application management or crop planting time is critical if crops are to benefit from the additional nutrient inputs provided by fallow species biomass.

The most suitable source of organic materials for improving soil fertility might be the fallow system that accumulates biomass the quickest, such as the Mexican *Tithonia* genotype in order to accelerate nutrient cycling in shorter time period. *Tithonia* was not shown to contain significant quantities of lignin and phenolic compounds that might negatively impact biomass decomposition (Cobo *et al.,* 2002). Based on the results that indicated the highest nutrient accumulation was registered by the Mexican genotype when grown in monoculture fallow, its biomass may be the best source for improving N, P and K balances for subsequent crops *in situ* or *ex-situ* mulch agroforestry systems. However, nutrient releases between *Tithonia* genotypes and *Cajanus* biomass need to be determined because there were significant differences in above-ground biomass allocation (Figure 3). Differences in the proportion of lignified plant parts might cause different decomposition rates and nutrient release patterns between the organic materials of fallow species.

Not all plant parts release nutrients immediately when applied to crops as mulch. The less lignified biomass in *Cajanus* and the Indonesian and Costa Rican *Tithonia* genotypes, such as leaves and green succulent stems, may be quicker to decompose and possibly release nutrients quicker to crops than the Mexican genotype, which contained more lignified parts. In addition, some fallow species biomass may lose more nutrients than others when the mulch is longer exposed at the soil surface. For example, it was estimated that 17% of N was lost as ammonia within 30 days when alfalfa mulch was placed on the soil (Larsson *et al.,* 1998). However, P in mulches can only be lost in important quantities by soil erosion, through small cracks in topsoil and soil P fixation or being taken up by neighboring crops or forests. Incorporation of fallow species biomass rather than surface mulching may reduce N loss and promote quick nutrient acquisition.

In the San Juan Andisol, the average available P (4.9 mg P L^{-1}) was equivalent to 18.32 kg ha^{-1} of P_2O_5 in the upper 12 cm of the soil profile (Appendix 2), assuming a soil bulk density of 0.68 Mg m^{-3} (Garzón, 1991). If beans require between 98 and 195 kg of P_2O_5 ha^{-1} to reach maximum yields on an Andisol in San Vito, Costa Rica (Rosemeyer, 1994), then a deficit of between 80 and 177 kg ha^{-1} P_2O_5 was present at the Experiment 1 field site. To meet P demand through the use of slash biomass from the Mexican *Tithonia*

genotype fallow (18 kg P ha^{-1} equivalent to 82.5 kg ha^{-1} of P_2O_5), the producing area has to be one to two times greater than the bean production area for a six-month fallow period. This means that one to two hectares of the Mexican *Tithonia* genotype in densely planted fallow are needed to satisfy the needs of one hectare of beans.

Although *Tithonia* combined the best qualities as a green manure source in a densely planted fallow, the probability of growing *Tithonia* on land reserved for crop production is very low due to limited land availability and to limited labor as well. In San Juan Sur, soils are generally used for bean and vegetable production. However, there are areas of less favorable topography that probably are available for biomass production of improving plants such as *Tithonia* or *Cajanus*. Nziguheba *et al,* (2002) indicated that in small scale farming systems, the more likely scenarios would be *Tithonia* on marginal areas and or field boundaries.

5.2 Root characteristics of fallow species

5.2.1 Root length density (RLD), specific root length (SRL) and root hair length (RHL)

The Mexican *Tithonia* genotype in monoculture had a RLD similar to natural regeneration in the upper 8 cm of soil depth due to a high proliferation of roots in combination with a dense planting arrangement, maximizing soil colonization by roots (Figure 9-A). The RLD in almost all of the fallow species was similar, but with different nutrient and biomass accumulation 23-weeks after planting in Experiment 1 (Figure 2 and Table 2). The way to explain the differences in above-ground biomass accumulation might be that some fallow species root systems located nutrient-rich patches faster and/or exploited soils with different intensities utilizing more active root systems per root length to accumulate higher biomass. Therefore, for short term fallow, at least one specific *Tithonia* fallow was more efficient than natural regeneration in terms of nutrient and biomass accumulation. In fact, the Mexican *Tithonia* genotype was the most efficient planted fallow for foraging nutrients.

In *Tithonia* genotypes, above-ground biomass was significantly correlated with RLD in the topsoil (Figure 8-A). It seems that RLD in the upper soil layer is an important root characteristic of the Mexican *Tithonia* genotype in densely planted fallows and explains the greater accumulation of above-ground biomass than the Costa Rican and Indonesian genotypes. *Tithonia* genotypes RLD reached a mean of 6.3-cm root per cm^{-3} of soil. Commonly, crop plant RLD in the plow layer is 5-cm root cm^{-3} of soil (Hendriks *et al.,* 1981; Jungk*,* 1996). According to Gallagher *et al.* (1999), *Tithonia* is characterized by having a strong 'nutrient-scavenging' ability. During the cropping phase, this aggressive nutrient scavenger is a major weed (McFadyen, 1996), but as a fallow species in rotation with annual crops, it has the ability to significantly improve soil productivity (Gallagher *et al.,* 1999).

All fallow species showed a higher root length density (RLD) in the upper 8 cm of the soil profile (Figure 9-A), indicating shallow root systems, which are better for topsoil foraging. Nutrient concentrations (such as N, P and K) are normally highest in the topsoil. Shallow root systems also allow the plant species to avoid low P layers and Al toxicity. An increase in RLD is more important for the enhanced capture of immobile ions rather than for mobiles, giving better acclimatation in P-limited environments (Hodge, 2004). However, P concentration in *Tithonia* genotype biomass was negatively correlated with RLD in the upper soil horizon (Figure 8-B). The mere extension of the root systems does not always explain differences in P uptake efficiency between plant species or genotypes of the same species (Baligar *et al.,* 1990).

Root length per diameter class among fallow species showed similar root patterns; higher root length corresponded to the root diameter classes below 1.0 mm (Appendix 8). The lowest root diameter classes are the most important for a larger extension of the root system. Keller *et al.* (2003) presented similar root length diameter distributions for a hyperaccumulator (*Thalpsi caerulescens*), an annual (*Zea mays*) and a perennial plant (*Salix viminalis*). For the *Tithonia* genotype potted plants (Figure 10), the Mexican genotype had the highest root length for 0.25 to 1.0 mm root diameter classes; i.e., the largest proportion of root length was found in the smallest diameter classes which are more active for nutrient uptake, presumably allowing intense soil exploitation (Appendix

9). This could be another reason why the Mexican genotype grows faster than the other genotypes. The Costa Rican genotype had larger proportion of root length for 2.0 to 2.5 mm diameter class but, at this range root lengths were shorter and consequently a lower soil volume was exploited by roots, reducing their ability to seek for essential nutrients.

Atkinson (2000) indicated that root length is a parameter that expresses the potential of the root systems for absorption of nutrients or water, assuming that every segment of the root system has the same function and capacity to uptake nutrients and water (Pregitzer, 2002). However, there is evidence that even within fine root diameter classes nutrient and water uptake capacities vary according to age and position in the roots (Wells and Eissenstat, 2003). In fact, several authors have considered that root length in fine roots can give only a partial explanation of the root system capacity of fallow species and that it is not an absolutely convenient parameter to assess the efficiency of root systems. Root length is not necessarily a good indicator of the length of xylem that is open for water and nutrient conductions (McCully, 1999). In spite of, a large scaffold of roots occupying the richest soil volume during its lifespan and may use it as a base for the production of more new roots. The Mexican *Tithonia* genotype is able to keep more fine roots in the upper soil layer. Therefore, more rapid nutrient acquisition responses may occur during favorable environmental conditions (principally moisture) when most nutrients become more available for plant growth. However, a larger scaffold of roots does not necessarily imply a more active root system per root length for nutrient acquisition, but can contribute to locate nutrient-rich patches faster.

In a slash/mulch system on nutrient-poor soils, fallow species that are likely to have a high RLD (large and potentially competitive root systems), such as the Mexican *Tithonia* genotype (Figure 10), may not be the best for planting in sequence with beans. After pruning fallow species, many roots may remain active, developing below-ground competition for available nutrients. However, some competition is probably inevitable for efficient use of the available soil resources by the tree-crop association as a whole (Schroth and Sinclair, 2003). A significant reduction in maize grain yield during the establishment phase of *Tithonia* and *Crotolaria grahamiana* fallows was recorded, possibly as a result of competition between the inter-planted fallow and the maize (Thor

et al., 2002). Apparently, the Costa Rican and Indonesian *Tithonia* genotypes produce less below-ground competition due to their slightly lower RLD than the Mexican genotype. However, these genotypes also produced less above-ground biomass for mulching than the Mexican genotype.

Specific root length (SRL) was not useful for the detection of differences between fallow species due to the wide range of variation for SRL in the upper 25 cm soil depth 23-weeks after planting (107 to 133 m of root g^{-1} of root dry weight). According to other studies for SRL, apple trees (*Malus sp.*) had values as low as 5 m g^{-1} and *Lolium perenne* were as high as 750 m g^{-1} (Atkinson, 2000). When compared to these species, fallow species were closer to the lower than the higher SRL values, which implies that fine roots in these fallow systems might not be considered as the most important root characteristic in enhancing nutrient uptake and soil exploration nor in explaining the differences in biomass accumulation.

Fallow species showed similar SRL down the soil profile, but *Cajanus* exhibited a trend to produce more fine roots at increased soil depth, presumably allowing nutrient uptake from lower soil horizons. It is possible that the *Cajanus* root system is unable to modify its inherited architecture when its roots are growing in moderate to high water availability environment. Natural regeneration showed an even pattern in SRL down the soil profile (Figure 9-B) because most of the roots were from different herbaceous weed and shrub species, which have mainly adventitious roots.

Similar to SRL, root hair length (RHL) showed a range of variation that makes it difficult to rank *Tithonia* genotypes. The different *Tithonia* genotypes in this study had nearly equal RHL. Barber (1995) reported that root hairs are relatively uniform within a species. Therefore, no *Tithonia* genotype had an advantage over the others with respect to the radius of the soil exploitation volume by root hairs. According to the range of RHL in higher plants reported by Dittmer (1949), Caradus (1979) and Hofer (1996), RHL can vary in length from 80 to 1,500 μm (0.08 to 1.5 mm). *Tithonia* genotypes fell into a low-intermediate value (Figure 11). If the surface of root hairs can represent up to 70% of the total root surface of primary and lateral roots (López *et al.,* 2003) and the contribution via

longer root hairs is not occurring between *Tithonia* genotypes, then the enhanced absorptive area may be a result of densely packed root hairs. However, when the root hair density is high, the nutrient uptake rate levels off due to overlapping of the depletion zones of individual roots, reflecting an inter-root competition for nutrients (Marschner, 1995). Because of this, RHL cannot be used to explain the differences in biomass accumulation; it is possible that differences are due to different root hair metabolic activity and/or larger fine root surface with root hairs among fallow species. Further research need to be carried out to clarify the root hair role in nutrient and biomass accumulation.

In conclusion, no one criterion has proven definitive in predicting how fallow species will respond to nutrient patches based on SRL, root demography and biomass allocation within the patch zone (Hodge, 2004). Plant species that exhibit smaller root systems may exploit soil more intensely by more active root systems per unit of root length that allow them to survive under poor–nutrient soil conditions. However, perennial species from nutrient-rich habitats indeed seem dependent on high root biomass production or better root architecture to sustain high nutrient capture rates, as is suggested by several authors (Grime *et al.,* 1986; Sibly and Grime, 1986; Crick and Grime, 1987; Nielsen, 1997). Nevertheless, all root characteristics play a direct or indirect role in nutrient uptake (Itoh, 1987; Hillel, 1998; Miller, 1998; Bates and Lynch, 2001), irrespective of the mechanisms by which they contribute in plant performance. However, care should be taken in interpreting the relationships between the modifications in root system characteristics and plant growth responses to biotic and abiotic factors because of the complexity for understanding plant behavior.

5.2.2 Fungal structures and external mycelia in roots

Statistical differences were not found for the abundance of fungal structures in *Tithonia* genotype roots in Experiment 1. However, when the proportion of roots with entry points was considered, the Indonesian *Tithonia* genotype had the highest number of roots with fungal entry points, an indication of a better source of arbuscular mycorrhizae (AM) inoculant for subsequent crops. In addition, fungal mycelia may enhance soil

exploration volume in comparison with the other *Tithonia* genotypes but its contribution to biomass and nutrient accumulation did not show it to be a more effective mechanism than those used by the Mexican and Costa Rican *Tithonia* genotypes; indeed the Indonesian genotype was the lowest above-ground biomass accumulator. Consequently, fungal structure determinations did not help to explain the differences in biomass accumulation between *Tithonia* genotypes.

External fungal mycelium density was lower than 0.11 m g^{-1} and similar between *Tithonia* genotype potted plants. AM hyphal length densities in soil are often between 2 to 25 m g^{-1} (Li *et al.,* 1991; Ravnskov *et al.,* 1999; Schweiger *et al.,* 1999) and under greenhouse conditions about one or more meters of hyphae per gram of soil (Smith and Read, 1997). In lowland, neotropical rainforest, Powers *et al.* (2004) reported very low mycelium length density (0-10 cm soil depth), ranging from 0.16±0.06, 0.15±0.07 and 0.15±0.07 m g^{-1} at the La Selva Biological Station (Costa Rica), Barro Colorado Island (Panama) and Cashu Biological Station in Manu National Park (Peru), respectively. Probably, the mycelia of AM fungi that colonized tropical species are fixed to the host root surfaces rather than in a profuse network of mycelium beyond the soil exploited by root hairs.

In the different *Tithonia* genotype potted plants, external mycelium length differences were not found. External hyphae length did not significantly increase the soil exploitation volume in any particular *Tithonia* genotype because *Tithonia* quickly develops a high root density when grown in pots. It is possible that when root density is high, such as in the upper soil horizons, most hyphae become firmly fixed to fine roots and root hairs and then external hyphae lengths are not completely taken into account. In fact, many truncated hyphae persisted in *Tithonia* fine roots after stirring soil suspension during external hyphae length determination.

Additionally, hyphae growth in pots without plants (bare soil) and in pots with *Tithonia* plants had similar external mycelium parameters, indicating that free mycelia were the main source of the external mycelia observed. Therefore, correlation analyses between above-ground biomass accumulation, P concentration in dry weight and the

external hyphae length could not be carried out. According to Smith *et al.* (2000) the correlation between acquisition of P and hyphae lengths in soil does not always exist, indicating that several other factors must be involved in regulating P uptake and transfer to the host plant. Even though, the development of a network of fine extraradical hyphae always increases the absorption rate of slow-diffusing nutrients, mainly phosphate, from the soil to the plant (Jackobsen, 1999).

5.2.3 Internal concentration of organic acids in the root tip and leaf tissue

In *Tithonia* genotypes, succinic and malic dominated the organic acids found in the root tip and leaf tissue. Several studies have shown that plant roots exude a variety of organic acids, but generally one dominates the spectrum (Hocking, 2001). The highest concentrations of the other organic acids (citric, oxalic and fumaric acids) were dispersed in the *Tithonia* genotypes and clones, making it complicated to associate organic acid concentrations for any specific *Tithonia* genotype in order to account for the differences in biomass accumulation. However, these organic acids may still function as root exudates (Ström 1998; Jones and Farrar, 1999), which potentially facilitate nutrient uptake. P-deficient plants, that have higher organic acid concentrations in the roots, can also exude more organic acids (Hoffland *et al.,* 1989; Ryan *et al.,* 2001). However, in most cases, the correlation does not hold over time (Ryan *et al.,* 2001). This lack of correlation might be due to changes in the stage of development of the roots and/or as a consequence of the development of microbial communities in the immediate vicinity of roots.

Malic and succinic acid concentrations found in the root tips make limited contributions to differences observed in biomass accumulation in *Tithonia* genotypes (Table 8 and Figure 13). Only the observed ranking for succinic acid concentrations in *Tithonia* genotypes corresponded to the same ranking for biomass and nutrient accumulation. Additionally, while succinic acid is least effective in mobilizing P, malate is moderately effective (Nagarajah *et al.,* 1970). To determine whether or not succinic or malic acid functions in enhancing P uptake or in developing Al tolerance, additional

research need to be conducted to detect which *Tithonia* genotypes release more of these acids in the rhizosphere.

The interaction of genotypes and clones indicated that the internal concentrations of succinic acid within each genotype were not similar for, at least, one clone per genotype. In natural *Tithonia* populations, other genotypes may exhibit higher or lower internal organic concentrations as a potential mechanism for mobilizing nutrients. Even though there is little information on intraspecific variation in organic exudation from plants in relation to P acquisition, there is evidence that species which exude less organic acids differ from other species that exude more in the ability to access the various pools (fractions) of soil P (Hocking, 2001).

Tithonia genotypes showed similar organic acid concentrations in the root tips collected from girdled stems. Probably, these root tip samples could not fully evaluate the effects of soil conditions on organic acid concentrations because root tips were not in direct contact with the rhizosphere. Additionally, internal organic acid concentrations in the root tips may not have a relationship with the organic acid efflux of roots, based on the currently available data, as well as on consideration of intracellular organic acid compartmentalization and transmembrane thermodynamics (Ryan *et al.,* 2001). The difficulty of measuring organic acid efflux from root tips may be avoided by using intracellular organic acid concentrations in root tips and/or leaf tissues which as such, provides a limited indication of the capacity of the organic acid efflux involved in mobilizing soil-bound nutrients but, at the same time, only the organic acids that are found in cells can be released. Further research need to be carried when roots are growing directly in the rhizosphere.

5.3 Effects of fallow species and slash biomass addition on soil P fractions

The balance in soil P fractions in the upper 8 cm of soil depth was poorly influenced by a short-fallow period with different fallow species and slash biomass application. Densely planted fallow species treatments and natural regeneration in short fallow period could not optimize P cycling by re-allocating P from potentially labile P

(NaOH-Pi and NaOH-Po), occluded P (HCl-Pi and residual-P) to the soil labile P and P in the soil solution. In the subsurface, a relative steady state of soil P fractions was also detected where the less labile soil P fractions continued in higher proportions but there was evidence of P desorption from the soil P fixed site occurring at the expense of potentially labile P and occluded P in natural regeneration and *Cajanus*, but that did not increase the labile P ($NaHCO_3$-Pi and $NaHCO_3$-Po) (Appendix 16).

Under the influence of fallow species, fallow period and the organic additions in Experiment 1, the inter-conversion mechanisms between non-labile soil P and labile fractions occurred in diverse directions, not necessarily changing the same soil P fractions and following the inverse strength sequence of the Hedley P fractionation procedure. It is reasonable to assume that the soil P fraction balance may be differentially addressed, even within the traditional agricultural strategies for maintaining satisfactory physical and chemical soil fertility. The effects of the different agricultural strategies used may counteract and enhance at the same time the different soil P fractions even if all are promoters for soil fertility amelioration. Similar findings are reported by Kolawole *et al.* (2004) where natural and *Pueraria* fallow systems and residue management options (burning, incorporation and mulching) had no consistent and significant effects on soil P fractions. Derry *et al.* (2005) indicated that long-term trend under fríjol tapado was not toward redistribution of P among fractions. However, Phiri *et al.* (2001) found that a *Tithonia* fallow of one year could significantly increase soil P fractions related to plant available P. In addition, Matta-Machado and Jordan (1995) indicated that *Albizia julibrissin*, in a three year period in an alley cropping system, was more efficient in tapping unavailable forms of soil P and achieved a higher P stock than the annual legume-based cropping system. It is possible that a longer fallow period than the six-months used in this study allows enough time to produce more defined trends of the inter-conversion of soil P fractions.

Even significant changes in the soil P fractions were detected in the different fallow treatments by the Wilks' Lambda multivariate test (Table 9); only the soil labile P ($NaHCO_3$-Pi and $NaHCO_3$-Po) declined at 0-12 and 12-24 cm soil depths during the different sampling times (Figure 15), contrary to the expectancy of an increment in P

availability. In fallow species treatments, changes in the labile P fractions were more detectable than P in the soil solution (Figure 15) because whenever the roots deplete P in the soil solution, P is replenished by the solid phase where it is released, mainly by desorption (Jungk *et al.,* 1993). There is a rapid movement from labile P to P in the soil solution. However, when the labile P is being depleted, the movement from the non-labile soil P fractions to the labile P is slow (Whitelaw, 2000).

P in the soil solution remained very low (<1 mg P kg^{-1}) from the first to the last sample taken, indicating that fallow species treatments did not reduce or increase P in the soil solution, even after slashing fallow species biomass. In soils like this, the phosphates that are released to the soil solution during organic matter mineralization become quickly converted into more stable, but less labile soil P fractions by Fe and Al oxides, allophone and recalcitrant organic matter. They create a tight, conservative system in which P recycled very slowly and in very small amounts. Sorption reactions maintain low concentrations of phosphate in the soil solution while buffering the amount of phosphate in the soil solution (Smith 2002). Similar P concentrations in the soil were found in the savannas of West Africa where P varied from 1 to 14 mg P kg^{-1} (Nwoke *et al*., 2003).

The other soil P fractions (potentially labile P, occluded P and residual-P) appear to have fluctuated, probably due to the inherent variability of soil properties rather than the effects of fallow species treatments and slash biomass applications. Kass *et al.* (1999) indicated that correlations of soil P fractions with plant response have been poor and several publications have shown that other factors, such as soil type, soil texture and nutrient status, can affect the amounts of the different soil P fractions as much as management practices. Soil chemical properties play a major role in controlling P dynamics and the mechanism of inter-conversion between soil P fractions could be complicated (Zhongqi *et al*., 2004).

While it is clear that agricultural plants differ in the amounts of P they obtain from the same soil (McLachlan, 1976; Randall, 1995), it is difficult to know if they simply access the same soil P fractions at different rates or whether they access different soil P fractions (Hocking, 2001). However, there is little evidence that plants take up organic

forms of soil P, including colloidal P, from the soil solution. Soil organic P must first be mineralized to inorganic P before it can be taken up by plants (Bieleski, 1973; Marschner, 1995). P becomes available as result of the biochemical action of plants, mycorrhizas or other soil microbes. Therefore, soil P fractions that plants can directly access are usually limited to P in the soil solution and $NaHCO_3$-Pi. However, for these soil P fractions only the labile P is reduced in the topsoil (0-12 cm) after a six-month fallow (Figure 15). However, in reference to sharper decline recorded in the soil P fractions at 12-24 cm, there is no evidence showing that P, from non-labile soil P fractions in the subsoil, can be taken more easily than P, from the same non-labile P in the topsoil. In fact, there are more satisfactory physical and chemical conditions in the topsoil than in the subsoil (Appendix 7) for P uptake by fallow species.

The reduction in the labile P fraction began to occur at the end of the fallow period when root systems of the fallow species were most developed and consequently greater soil exploitation was taking place (between 17 and 23-weeks under fallow) (Figure 15). To detect trends in the labile P fractions ($NaHCO_3$-Pi $NaHCO_3$-Po) in field experiments under fallow as an agricultural strategy for ameliorating soil fertility, the fallow period may need to be longer than half a year, allowing more P to accumulate in above-ground biomass. Harmand (1999), working in the Sub-Saharan Africa savannas, pointed out that there is a lag time, varying from species to species, between planting and the changes in chemical soil properties. Probably, P re-allocation for higher P availability may not occur in longer fallow under standing vegetation, because any labile P coming from more stable soil P fractions will be re-taken by the roots or become fixed. However, George *et al.* (2002b) found that *Tithonia* reduced P in the soil solution (resin-P) but *Tithonia* and *Tephosia* reduced NaOH-Po (organic) in a rhizopot experiment. *Tephosia* potentially enhanced P solubility by increasing rhizosphere pH, whereas *Tithonia* appeared to increase P solubility despite acidification of the rhizosphere, suggesting the production of organic anions (George *et al.,* 2002b).

Another turning period in soil P fractions may occur immediately after slashing fallow species biomass (between 23 and 53-weeks after starting Experiment 1) considering that *Tithonia* biomass decomposition is nearly complete 12-weeks after

application (Cobo *et al.,* 2002). Higher net P release in legume and non-legume species occurs during the first eight weeks under laboratory incubation conditions (Kwabiah *et al.,* 2003a). However, Aguiar (2001) did not find significant effects of tropical green manure species residues in soil P fractions after a 60-day incubation period, which indicated a transfer of P from unavailable to more available soil P fractions.

A reduction in P-desorption was not induced by biomass decomposition 29-weeks after biomass application. Biomass additions from fallow species did not enhance the labile soil P fractions, such as causing P desorption from the soil P fixed sites and residual P, but increased NaOH-Po (Figure 16). It appears that the increment in the potentially labile P fractions (NaOH-Pi and NaOH-Po) were mainly due to the organic P fraction (NaOH-Po) (around 161 mg P kg^{-1}) in the post-fallow at a soil depth of 0-12 cm. However, the increment in this organic P fraction did not take place at the expense of other soil P fractions, only occluded P registered a minimal decrease (22.97 mg P kg^{-1}) and the other soil P fractions did not register changes of that quantity 53 weeks after initiating Experiment 1. The P organic sources that increased NaOH-Po were the same that enhanced total P at 0-12 cm soil sampling depth (Figure 16).

P coming from organic additions is more likely to become readily available for uptake by higher plants than P coming from non-labile soil P fractions. Organic P sources will also contribute to the maintenance of the native soil P fraction balance because organic P fractions are the major components in tropical soils. According to Jama *et al.* (1998), inputs of organic resources are essential for building the organic P pool and sustaining high crop yield in both continuous cultivation and rotation systems. Nevertheless, inorganic P inputs from plant material (*in situ* or *ex situ* mulches) appeared insufficient to change the equilibrium of the stable soil P fractions though it did appear that an adequate quantity of high quality plant biomass could lead to a significant increase in water holding capacity, nutrient supply, and micro and macroorganism activity. These soil conditions may have affected the mineralization rates in the upper centimeters of the soil profile. Therefore, the potential of organic P inputs to improve the quality of tropical soils is not limited to P-reallocation. Further detailed examination of

the interchange patterns between soil P fractions may provide more an accurate interpretation of what is occurring during the fallow period and after organic additions.

5.4 Effects of the utilization of fallow species on beans

5.4.1 Bean yield response

Significantly higher bean yields were recorded after the Costa Rican *Tithonia* fallow in comparison to the other fallows but, bean yield increased at a higher rate with the slash biomass of natural regeneration in comparison to the biomass of the planted fallow species in Experiment 1 (Figure 18). Densely planted fallow produced higher bean yield than natural regeneration (Table 10 and Figure 18) and fríjol tapado system (0.49 Mg ha^{-1}) (Meléndez, 2004) because they produced mainly more slash biomass per unit of area. In addition, the Costa Rican and Indonesian *Tithonia* genotypes supplied less lignified biomass and their biomass had lower N:P ratio (Appendix 5) than the Mexican genotype (Figure 3). These may affect the nutrient release rates and patterns that are partially controlled by the resource quality of the materials (Gachengo *et al.,* 1999). Nutrient releases from *Tithonia* and *Cajanus* biomass and other interaction factors (fallow species x crop x organic addition) may have higher importance for maximum bean yield than the quantity of nutrients supplied by the applied slash biomass of fallow species at the doses used (4.0 to 12.0 Mg ha^{-1}).

Even though the Mexican *Tithonia* genotype supplied a higher quantity of nutrients thus improving soil fertility, a higher RLD (Table 2) may have resulted in greater below-ground competition for nutrients in subsequent crops. It is possible that some roots remained active after pruning. However, below-ground competition may lessen gradually as above-ground biomass is slashed since some of the roots will die as a result of pruning and root turnover (Chesney, 2000). The Mexican genotype may be more suitable for biomass production when grown outside cultivated areas and when its biomass is added to the cultivated areas in a cut-and-carry system so as to reduce below-ground competition.

Higher bean yields were obtained when slash biomass was added (Table 10, Figure 18 and Appendix 18). The gain in bean yield was twice that gained from bean cultivar selection (the Chirripo Rojo or Negro Huasteco). This increment might be superior when fallow species biomass is incorporated than slashing biomass (surface mulching) as it was found by Kolawole *et al.* (2004) under natural regrowth and *Pueraria* cover crop fallows. The promotion of biomass decomposition by its incorporation in the soil guarantees a quick nutrient cycling that works better for crops with a short growing cycle such as beans.

In Experiment 2, soil amendments (such as calcium carbonate and poultry manure) and the multiple effects of mulch cover, including nutrients supplied by the slashed biomass played a more important role in bean yield enhancement than bean cultivar selection. It seems that soil amendments removed the most limiting soil environmental factors that affect nutrient availability in San Juan Sur Andisol by reducing the solubility and sorption potential of Al and Fe oxides and allophone. However, in a cut-and-carry system (*ex situ* mulch) with previous applications of calcium carbonate and poultry manure, mulch application over 9.0 Mg ha^{-1} did not show a significant increase in bean yield. Bean yield difference in control plots represents an increase of about 0.32 Mg ha^{-1} (38%) between Experiment 1 (without previous applications of calcium carbonate and poultry manure) and Experiment 2 (with previous applications of calcium carbonate and poultry manure) (Appendix 28). Similarly, Lebeuf (1993) recorded bean yields of 2.2 and 2.0 Mg ha^{-1} with *Inga edulis* and *E. fusca* mulches, respectively, but applying 2.6 Mg ha^{-1} of calcium carbonate to San Juan Sur Andisol. López (1995) working with velvet bean (*M. deeringiana*) as a cover crop and *E. berteroana* mulch, reported 0.87 and 0.96 Mg ha^{-1} bean yields, respectively. The bean yields reported by López (1995) were similar to the bean yields obtained with *Tithonia* and *Cajanus* fallows in the present study, but in both cited studies smaller applications of mulch were used. However, when P was applied at 50 kg ha^{-1} of P_2O_5, bean yield reached 1.54 Mg ha^{-1} for *Mucuna* and 1.69 Mg ha^{-1} for *E. berteroana* mulches (Arriaza, 1995).

A high variability in bean cultivar responses was recorded in experimental plots (Experiment 2), probably due to differences in soil fertility along the hillside. Lebeuf

(1993) observed a variability in soil characteristics along slopes in San Juan Sur. In fact, depth to B horizon intrusions was observed to be highly variable, which indicates movement of soil within the field. Intense soil erosion may have occurred before the establishment of Experiment 2, when the upland area had been under cultivation in the past.

Yield differences between bean cultivars were minimal in Experiment 1 where the Chirripo Rojo had a higher yield than the Negro Huasteco (Table 10). However, the exotic CIAT bean cultivars (the Dor-364 and CIAT G-1937) had similar yields as the Costa Rican (the Chirripo Rojo and Negro Huasteco) in Experiment 2. In comparison with the Negro Huasteco, the Chirripo Rojo may have a more efficient root system for taking up nutrients (at least for N and K), genetic variation in the distribution of basal roots and/or more convenient biomass allocations resulting in a higher yield (Tables 10 and Appendix 19). In fact, the Negro Huasteco allocated more dry weight to leaf and stem at the maturity phase (Table 11). In addition, other morphological and/or physiological plant characteristics may exist in the Chirripo Rojo bean cultivar as to able it to obtain higher yields. Chirripo Rojo might have better acclimation to the San Juan Sur Andisol environmental and edaphic conditions. In fact, the Chirripo Rojo is one of the last bean cultivars developed by the University of Costa Rica (UCR) for high-yielding that do better on highly weathered acid soils.

5.4.2 Bean nutritional status

Slash biomass additions only increased K nutritional status of bean cultivars before and at the end of the flowering phases (Table 12 and Appendix 20) even though N as well as K were in similar concentrations in the applied biomass of fallow species (Appendix 4). It seems that N nutritional status of beans is subordinated to N-fixation rather than N coming from the slash biomass added. The other nutrients (P, Ca and Mg) coming from the slash biomass addition did not increase bean nutritional status. Cobo *et al.* (2002) reported that during the first two weeks of decomposition, the leaves of *Tithonia* released 45, 60, 70 and 95% of P, Ca, Mg and K, respectively. The peak in P, Ca, Mg and K released by the slash biomass (mostly leaves) might have occurred before

the bean flowering phase and nutrients were utilized in plant growth. This would be in agreement with the highest nutrient concentration values recorded in above-ground biomass before the flowering phase (Figure 21 and Appendix 20). However, no significant differences among fallow species treatments and slash biomass applications were found for Mg and P in bean tissue before the flowering phase.

In the case of P, it is reasonable to accept that slash biomass additions at the bean planting time did not increase P concentration in bean cultivars because P inputs coming from the biomass of fallow species were very low (<18.0 kg P ha^{-1}) (Appendix 5) and an important part of the P added may have become fixed by the sorption reactions at the San Juan Sur Andisol as a tropical weathered volcanic-ash soil. Additionally, P is an immobile nutrient in soil (retaining its boundary within millimeters from the applied site) and is released at a low rate during biomass decomposition (Cobo *et al.,* 2002). López (1995) also found that P uptake by beans was similar between alley farming and organic amendments in the San Juan Sur Andisol. However, when poultry manure and calcium carbonate soil amendments were added to the soil, P concentration in bean tissues increased substantially (Appendix 32), mainly because these amendments increased the pH of acid soils (Appendix 2). Raising soil pH consequently increases P availability (Giller and Wilson, 1991) and reduces toxic concentrations of Al and Mn (Sánchez and Logan, 1992).

Between the Negro Huasteco and Chirripo Rojo bean cultivars, there were significant differences in the P concentrations (Figure 21-A). However, Araújo and Teixeira (2000) did not find significant differences for P concentration between bean cultivars. Gabelman and Gerloff (1983) reported that bean cultivar yield grown under a low P supply was associated with P utilization efficiency. In addition, P uptake efficiency was not a suitable parameter to be used in ranking bean cultivars according to their capacity to uptake P. Consequently, better utilization of acquired P may be occurring in the Negro Huasteco bean cultivar rather than a higher P uptake, according to the recorded differences in root system characteristics between these bean cultivars.

Higher yields of the Chirripo Rojo were positively associated with P concentrations, but negatively associated with P utilization efficiency (Appendix 21) when the bean cultivars were grown on a low P Andisol (Appendix 7). An explanation of the higher P concentrations in the Chirripo Rojo might be that a higher P uptake rate has occurred at early stages of development in the Chirripo Rojo. This hypothesis deserves comprobation.

Similar to P, Ca and Mg inputs were too low to induce changes in the bean nutritional status for either bean cultivar (Appendix 20). However, in the case of K, it is not surprising that slash biomass of fallow species increased K concentrations in the beans because high K concentrations were found in the slash biomass. K is released more quickly than other nutrients (P, Ca and Mg) and is better distributed internally to satisfy plant demand. In addition, root morphology and architecture differences between bean cultivars may play secondary roles in the case of mobile nutrients such as N and K.

5.4.3 Bean root system response to organic and soil amendments

The application of organic amendments from fallow species to the soil surface was thought to facilitate bean root system development by enhancing nutrient and moisture availabilities in the upper soil layers. However, neither slash biomass of fallow species (*in situ* mulch) in Experiment 1, nor mulch treatments in Experiment 2 increased the number of adventitious roots (Figure 24-A and Appendix 34), or the upper root-stem length when determined before and at the flowering and maturity phases of bean cultivars (Appendices 23, 24, 25, 34 and Figure 30). Nevertheless, they increased the number of nodules at the bean maturity phase (Figure 24-B). Under the effects of minor environmental stress in the upper soil layers due to mulch applications, bean cultivar root systems produced minor morphological expressions in root characteristics because bean plants were able to exploit soil resources in a similar or more efficient manner with less investment in the development of bigger root systems. However, this was not the same for nodulation which increased under better environmental conditions (Figures 24 and 25), considering that a higher population of N-fixing bacteria can develop when the topsoil is not dried out.

Both Costa Rican bean cultivars (Negro Huasteco and Chirripo Rojo) reduced the number of adventitious roots in response to the "new nutrient-rich patches" and/or better environmental conditions created by slash biomass additions on the topsoil (Figure 24-A). Adventitious roots, rather than any other kind of root system mechanism for nutrient acquisition, are more likely to occupy and proliferate within the nutrient-rich zones located in the first centimeters of soil (Nielsen, 1997; Miller, 1998; Lynch and Brown, 2001; Rubio *et al*., 2003). However, the behavior of adventitious roots in bean cultivars was opposite to the expected root performance for the acquisition of an immobile nutrient such as P, unless the effects of slash biomass application on P availability were overshadowed by the improvement of other environmental conditions.

Miller (1998) indicated that P efficient bean genotypes responded to low P with an increased number of adventitious roots throughout development which increased in length and dry weight. However, a greater number of adventitious roots, presumably associated with better nutrient uptake, was not observed in the bean cultivar with the highest yield (Chirripo Rojo). The Chirripo Rojo did better for P concentrations in tissues and yields even though the Negro Huasteco had a greater number of adventitious roots. This happened because Chirripo Rojo had other advantages for growing in this environment.

Previous applications of poultry manure and calcium carbonate in Experiment 2 showed a greater number of root nodules (Appendix 34) than Experiment 1 (without these soil amendments) (Figure 24-B and Appendix 22) for the Costa Rican bean cultivars at their flowering phases. When soil amendments are used, P availability, cations and pH in the topsoil are increased, aluminium saturation is reduced and other micronutrient deficiencies are corrected. Under these soil conditions, better initial establishment of N-fixing bacteria occurs (Giller and Wilson, 1991) which results in a higher nodulation. It is possibly that N-fixation efficiency in bean cultivars was also enhanced by these soil amendments. In addition, the number of nodules was lower at the bean maturity phase than at the end of the flowering phase in Experiment 1. Araújo and Teixeira (2000) indicated that the development of nodulation depends on the P supply and differs between cultivars; some cultivars show an intense decline in the number of

nodules after flowering. Time of sampling strongly altered the rank of bean cultivars for nodulation as was found in the Experiment 1 (Figure 24-B).

Another important point is that even though the Negro Huasteco had a higher number of nodules than the Chirripo Rojo during the flowering phase in Experiments 1 and 2, it did not correlate positively with bean yield. It seems that N inputs from slash biomass of the fallow species could balance the N demand for both bean cultivars, and that the lower nodulation of the Chirripo Rojo did not affect its yield. Therefore, the highest yield of the Chirripo Rojo may not come from the greater dimension and the number of root system characteristics that showed significant differences. The Chirripo Rojo may have a more active root system per unit of root length for nutrient acquisition and/or other plant characteristics such as above-ground biomass allocation that caused the higher yield in this bean cultivar. In fact, the Chirripo Rojo had more dry weight allocated to pod production until the end of flowering phase (Table 11). However, a root system which is more active per unit of root length is not necessarily more active for all essential nutrients. For example, the Chirripo Rojo had a similar P uptake efficiency as the Negro Huasteco (Appendix 21). It seems that P uptake plays a secondary role in the explanation of the differences in bean yield.

In comparison to the Chirripo Rojo, the Negro Huasteco showed greater plasticity in its root system morphology and architecture to environmental soil changes introduced by the presence of slash biomass cover. The Chirripo Rojo had smaller root modifications in root dry weight partitioning, root system components and root extension measurements between the control and plots with slash biomass additions (Figures 24 and 25). It seems that the Negro Huasteco is more "programmed" to be able to acclimate to varied conditions but not to higher yield. In control plots (with slash biomass removal), the Negro Huasteco maximized soil colonization in search of nutritional resources through a higher adventitious root dry weight and a greater number of adventitious roots as the Chirripo Rojo bean cultivar (Appendix 22). Kuruvadi and Aguilera (1990), in a greenhouse experiment with 20 genotypes of the common bean, found that the Negro Huasteco showed one of the best and deepest developed root systems (81-100 cm). It is possible, there are bean cultivar differences in the tendency of roots to grown with certain

orientation to gravity that enhance topsoil foraging (Ge *et al.,* 2000; Lynch and Brown 2001; Rubio *et al.,* 2001; López *et al.,* 2003). Further research should put more attention in observing and analyzing the architecture of the bean cultivar roots growing in soil.

The integration of simultaneous measurements of root characteristic variables, such as root dry weight partitioning, root components and root extension, captured all the partial modifications in root system morphology and architecture in response to organic amendment treatments and also facilitated differentiation bean cultivars, principally at the bean maturity phase. Root dry weight partitioning was not altered by organic amendments. But, for root components, at least one root characteristic was modified in a detectable way (the number of adventitious roots was reduced and the number of nodules was increased) (Figure 24 and Appendix 25). In addition, root extension (SRL of basal roots was increased) was modified by organic amendments (Appendix 25). When bean cultivars are compared, the Chirripo Rojo and Negro Huasteco assigned differently root dry weight allocation (upper root-stem, adventitious and basal roots) and root components (number of basal roots and nodules) (Appendix 25).

The interactions between the canonical variables for root dry weight allocation and root architecture canonical variables were important sources of explanation for the observed root system characteristics (Appendices 33, 34 and Figure 31). By the combination of both groups of variables bean cultivar differences helped to detect root system characteristics and responses to improved soil conditions. The relationships of these groups of variables had some effects in root system characterization. Both pairs of canonical variables have about an equal and positive amount of influence in the characterization of bean root system. Therefore, it is necessary to measure both groups of original root variables for a better characterization of bean root system.

Another important point is that root reactions also depend on element mobility in the soil (Fitter, 1987). Physiological plasticity is generally assumed to be more important in enhancing the capture of mobile ions (NO_3^-) before they diffuse to other roots down the soil profile. In contrast, immobile ions, such as in the case of phosphates, a rapid response is less crucial, allowing time for new root construction (Hodge, 2004). Root

systems need to deal with this duality and have to balance root morphology and architecture for maximizing macronutrient captures. Therefore, the contribution of root morphological and architectural characteristics to take advantage of nutrient supplies by mulch treatments, does not always imply significant modifications in the bean root system characteristics, even under P-limited conditions.

It was not possible to detect which bean cultivar was superior in maximizing nutrient and water capture according to root characteristics because the desired dimensions of root characteristics were not found in any one specific bean cultivar. However, Ketring *et al.* (1982) found that the shoot dry weight was highly correlated with the number of roots and taproot length in peanuts. Each bean cultivar compensated for its weakness in specific root characteristics with modifications in other characteristics. The most efficient bean cultivar would be one that combined properties of both cultivars: longer lower root-stem in the Chirripo Rojo and higher adventitious root dry weight and higher number of adventitious and nodules in the Negro Huasteco.

Hybridization may be helpful to obtain a better bean cultivar for high yield in acid soils. Lynch and Beebe (1995) reported that the interaction between genotype, season and P level implied inconsistent behaviours of some genotypes over the growing seasons in their response to low P. This complicates the identification and selection of P-tolerance genotypes in the field. Potted experiments under greenhouse might help a better identification of advantages of root system morphologies and architectures that contribute to maximization of bean yields. So, additional series of field experiments are needed to overcome inconsistent behaviours. These findings underscore the difficulty of relying on yield trials as a criterion for selection. Molecular tracers would be especially useful for selecting genes for P efficiency, considering the difficulties cited previously in yield trials to identify P-efficient genotypes (Lynch and Beebe 1995). However, morphological and physiological phenotypic plasticity of bean cultivars have to be taken into account for a complete explanation when yield differences are found between bean cultivars.

The hypothesis that slash biomass additions from fallow species have a positive influence on the number of fungal structures in bean roots was rejected (Appendix 26).

However, most of the fallow species treatments improved the colonization of the Costa Rican bean cultivar roots by VAM mycorrhizae. It seems that natural regeneration as well as the densely planted fallow treatments maintained high levels of VAM inoculum, which could be important for enhancing P uptake in a six-month fallow period. For better plant nutrition, it is ideal to plant in rotation so as to maintain high levels of VAM inoculum and extend the hyphae networks, allowing the crops to interact with well established VAM colonies as early as possible. This is very important for legumes as they have both short roots and short root hairs. In such root systems, mycorrhizal responsiveness is high (Marschner, 1995).

The Negro Huasteco bean cultivar had the highest proportion of arbuscules, but the Chirripo Rojo had the highest abundance of vesicles per centimeter of root (Appendix 26). However, the advantage of having a higher proportion of arbuscules was not enough to make the Negro Huasteco bean a better cultivar for obtaining a higher yield than the Chirripo Rojo. It is possible that the Negro Huasteco allowed a longer lifespan for arbuscules than the Chirripo Rojo. In addition, the number of vesicles needs to be managed carefully because many, but not all, endomycorrhizal fungi form vesicles as a lipid-rich storage organ (Marschner, 1995). Also, Nielsen *et al.* (1998) suggested that mycorrhizal infections are less important as specific P acquisition mechanisms for the development of more P efficient cultivars because mycorrhizal infections are high in bean plants under most field conditions. The ranking of the genotypes with respect to biomass production is independent of mycorrhizal infection, and the construction cost seemed not significantly affected by mycorrhizal infection (Nielsen *et al.,* 1998).

These findings imply that higher efficiency in nutrient uptake may rely on other factors such as the existence of more effective associations with N-fixing bacteria and arbuscular mycorhizas, better biomass allocation and/or better acclimation to the San Juan Sur Andisol environmental and edaphic conditions. The combination of these factors may have made the Chirripo Rojo, a high-yielding bean cultivar that does better on highly weathered acid soils. Further research in the topics mentioned above might find the plant mechanisms that are operating in the Chirripo Rojo bean cultivar for better performance and yield in San Juan Sur Andisol.

6. Conclusions

The Mexican *Tithonia* genotype in densely planted fallow can maximize biomass and nutrient additions in slash and mulch agroforestry systems when grown on a weathered Andisol with low P availability. However, the Costa Rican *Tithonia* genotype in monoculture showed more favorable biological interactions with bean cultivars for maximizing yield after six-month fallow period under slash and mulch agroforestry systems. In addition, densely planted fallows cannot reach the biodiversity and complexity of ecosystem structure registered in natural fallow, neither, for higher bean yield per Mg of slash biomass applied. Therefore, the fallow treatment with higher biomass and nutrient accumulation, such as the Mexican *Tithonia* genotype, is not necessarily the most appropriate for improving nutrient cycling and bean yield in low-input agriculture.

Tithonia is not a P hyperaccumulating plant species in any given soil P availability level. Therefore, to meet bean P demand, large applications of *Tithonia* slash biomass are required. Over a six-month fallow period with biomass from the Mexican *Tithonia* genotype in densely planted fallow, the biomass producing area has to be one to two times greater than the bean production area.

The biomass accumulation in *Tithonia* genotypes was positively correlated with root length density in the upper 25 cm of soil depth. The Mexican *Tithonia* genotype is able to keep more roots occupying the richest soil volume during its lifespan and may use it as a base for the production of more new roots when favorable environmental conditions take place, such as sufficient moisture. A larger number of fine roots in the topsoil can help to locate nutrient-rich patches faster and develop more rapid nutrient acquisition responses, but these do not necessarily imply a more active root system per root length for nutrient acquisition.

Contrary to expectations, root hair length, external hyphae length and fungal entry points in fine roots did not significantly increase the soil exploitation volume, in some *Tithonia* genotypes. In addition, organic acid concentrations in the root tips did not

explain the differences observed in biomass accumulation between *Tithonia* genotypes because only the ranking for succinic acid concentration corresponded to the ranking for biomass accumulation, but succinic acid is least effective in mobilizing P. None of them has proven definitive in predicting why some *Tithonia* genotypes grow faster than the other genotypes in a P-limited Andisol. The explanation of the differences in biomass accumulation between *Tithonia* genotypes is more complex to evaluate than just measuring root system characteristics of fallow species. To determine whether or not these root characteristics function in enhancing plant nutritional status or in developing genotypes with better acclimation to P-limited soils, additional research need to be conducted to examine root performance, architecture and colonization in controlled environments.

The six-month fallow period and the applied slash biomass of densely planted fallow species had no consistent and significant effect toward conversion of less available soil P fractions into more readily available P fractions. To produce a significant inter-conversion of soil P fractions from non-labile P to labile P, if possible in the short-term or long-term fallow, alternatives other than a densely planted fallow and fallow species biomass additions are needed to deal with the higher P-sorption capacity of the San Juan Sur Andisol.

Higher yield for the Costa Rican bean cultivars occurred when slash biomass of fallow species was added as *in situ* mulch systems at 4.0 to 12.0 Mg of biomass ha^{-1}, but it could not be associated with soil P-reallocation. However, when soil amendments including poultry manure and calcium carbonate were used, the yield and P nutritional status in bean cultivars increased as well as soil P availability, cations and pH. These soil amendments may be able to ameliorate the balance of soil P fractions which will result in higher P nutritional status in bean cultivars. When soil amendments, slash biomass of fallow species or fallow period are not possible, the other alternative or complementary approach for sustaining bean production in soils with high P-sorption capacity is the development of bean cultivar with superior growth and yield in soils with low P availability such as the Chirripo Rojo.

Slash biomass additions on soil surface did not significantly increase the P nutritional status of bean cultivars. This is possibly due to the low P concentration in the slashed biomass and the high soil P-sorption capacity in San Juan Sur Andisol. In addition, bean cultivars did not develop more adventitious roots in response to slash biomass additions, which are more likely to occupy and proliferate in the nutrient-rich soil horizons. In fact, P uptake efficiency in bean cultivars was not increased by slash biomass additions. Improving the contact of *Tithonia* slashed biomass with bean roots by mulch incorporation might promote higher plant nutritional status and nutrient availabilities for ions with limited mobility in the soil such as P.

The applications of poultry manure and calcium carbonate were more effective in enhancing bean yields and P, K, Ca and Mg nutritional status of the Costa Rican and exotic CIAT bean cultivars than the slash biomass addition in the cut-and-carry system (ex *situ* mulch). It seems that poultry manure and calcium carbonate soil amendments removed the most limiting soil chemical factors for bean production in the San Juan Sur Andisol. Therefore, to maximize crop yield in weathered Andisols, soil amendments are required, but at higher cost for farmers. It should be noted that additional input of external nutrients and soil amendments may not compensate the higher cost of using them with respect to a gain in crop yield.

Contrary to expectations, the number of adventitious roots and the upper root-stem length of the Costa Rican and exotic CIAT bean cultivars did not increase in response to the "new nutrient-rich patches" and/or better environmental conditions created by slash biomass additions on the topsoil. Bean cultivars were able to exploit soil resources in similar or more efficient ways with less investment in developing a bigger root system. This was not the case for the number of nodules, which was higher in response to slash biomass and soil amendment additions at bean maturity.

In response to the environmental soil changes introduced by slash biomass additions, the Negro Huasteco bean cultivar showed greater modifications in the adventitious root dry weight, root-stem length, number of adventitious root and nodules than the Chirripo Rojo cultivar, but not a higher yield. Therefore, greater plasticity in its

root system morphology and architecture to environmental soil changes introduced by the presence of slash biomass cover does not necessarily have to be detectable in the root system to induce changes in bean yields. Root architecture and physiological plasticity in the root system might increase nutrient uptake per unit of root length without producing important expressions in root characteristics.

Even though the Negro Huasteco bean cultivar had a greater number of adventitious roots and nodules and a greater adventitious root dry weight, which presumably allow a better nutrient uptake, the Chirripo Rojo had higher yield and P concentration in bean tissue. Larger root system characteristics were not necessarily associated with higher P concentration in bean tissue and yield. A more efficient root system per unit of root length (at least for N and K) and/or more effective genetic variation in the distribution of basal roots for taking up nutrients may have a more determinant role in bean cultivar yields than the root characteristic differences observed in bean cultivars. However, a more active root system per unit of root length for nutrient acquisition does not necessarily occur for all essential nutrients because of their different mobility rates within the soil matrix that may request different nutrient acquisition mechanisms.

In addition, the abundance of fungal structures in fine roots was similar among bean cultivars with and without slash biomass removal and could not be associated with superior yield and nutritional status for the Costa Rican bean cultivars. These findings imply that higher efficiency in nutrient uptake may rely on other factors such as the existence of more effective associations with N-fixing bacteria and arbuscular mycorhizas, better biomass allocation and/or better acclimation to the San Juan Sur Andisol environmental and edaphic conditions. The combination of these factors may have made the Chirripo Rojo, a high-yielding bean cultivar that does better on a highly weathered acid Andisol.

7. Recommendations

New research efforts should focus on fine tuning the interaction of fallow species, crop, soil and management factors in densely planted fallow systems in order to maximize crop yield and the carry over effects in restoring soil fertility of agricultural systems for smallholder farmers. The appropriate combination of the above mentioned management factors for the local environment conditions may help to define an improved fallow system with *Tithonia diversifolia*, *Cajanus* and other fallow species in tropical environments.

Screening fallow species for green manure production should not only include the determination of nutrient and biomass accumulation, but also nutrient release rates from the applied biomass to define the optimal time and doses for biomass application according to each fallow species. In the case of *Tithonia*, it is necessary to ascertain the availability of non-lignified biomass in the different genotypes during the year and determine agricultural crop yield and above and below-ground competitions for *in situ* and *ex situ* mulch systems with *Tithonia*.

The carry over effects of fallow species on agroecosystems should be studied in fallow systems shorter than one year and when agroforestry management practices be applied upon fields. Fallows longer than one year may not be considered as improved fallow systems if they are conceived as sustainable alternatives to accelerate the regeneration of soil fertility, overcome the problems of the traditional land use system and increase agricultural crop production and income.

Even though, low bean yield responses to short term fallows and green manure additions were found in this research, these agricultural practices are the best alternatives to recycle nutrients in low input systems. They guarantee a sustainable use of renewable resources, minimizing the dependency on external inputs and lowering the risks to the environment. Other alternatives to recycle nutrients different from plant biomass and fallow will probably not be found in the near future. However, in the meantime the use of organic materials from agroforestry systems can be optimized for weathered volcanic ash

soils; the utilization of fertilizer, calcium carbonate, poultry manure and compost in an integrated nutrient management system remain as the best alternatives in the short-term for maximizing food production for high-input systems.

Future studies of plant species as P sources should include the effects of organic compounds released during biomass decomposition of different fallow species on soil P availability rather than searching for P hyperaccumulator plant species, if they really exist. Some of the research questions that still need to be addressed include: Are the organic compounds that are released during biomass decomposition different from the ones detected when internal organic acids are determined in the fresh plant tissue of fallow species? Can they bring low available nutrients into solution, which then become available?

Further work should also evaluate the effects of organic material and calcium carbonate additions on soil P fractions using laboratory incubation methods. Mixing soil with organic materials and calcium carbonate at increasing doses should be periodically sampled at 1, 2, 3, 4, 8 and 16 weeks to permit the complete examination of the occurrence of inter-conversion process from non-labile to labile P, if it really occurs. Here, the questions to be asked are: What are the effects of these amendments on soil P fractions? Are the doses to ameliorate soil P availability higher than the doses to maximize crop yield?

For a detailed characterization of bean cultivar root systems in response to organic material additions in the greenhouse, it is necessary to ascertain if roots are increasing in activity per unit of root length, and how root system dimensions and number of root components are modified in response to presumably minor environmental stresses induced by organic material additions. Similarly, it might be pertinent to determine both the above-ground biomass allocation and root to above-ground biomass ratio of bean cultivars in response to organic material additions.

The classification of each root system characteristic as: root dry weight partitioning, root extension, root components and root architecture as integrated root

system features before using multivariate analysis will help to facilitate the differentiation of bean cultivars. Similarly, the interpretation of root system should be based on multiple correlations between the integrated root system characteristics, yield, biomass and nutrient accumulation for better understanding of plant responses to biotic or abiotic factors.

The procedure for measuring external mycelia in roots needs some fine tuning because many truncated hyphae persisted in the fine roots after stirring the soil suspension. As such, the determination of external hyphae length was inaccurate. A correction factor must be included in the estimation of external hyphae length to take in account the external hyphae that remain attached to fine roots and root hairs.

Finally, the issue that bean yield increased at a higher rate with the slash biomass of natural regeneration in comparison to the biomass of the planted fallow species deserves further research. Natural regeneration may have other advantages for improving soil fertility and consequently crop yields. The identification of these advantages must be considered in designing improved fallow systems with agroforestry species. Probably, the best way to study relies on crop yield evaluation where biodiversity of natural regeneration is altered. For example different categories of plants such as legume, non-legume, grass, shrub and forest can be removed or retained to evaluate system behavior under new environmental conditions.

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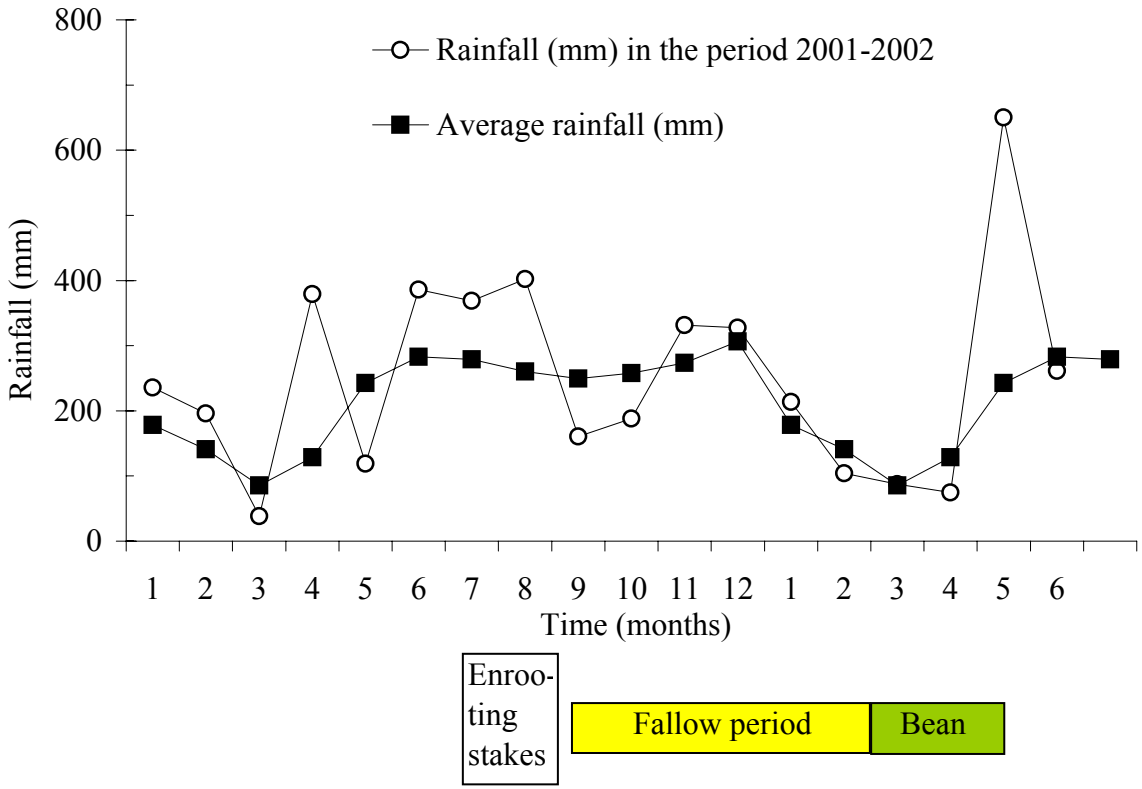


Appendix 1. Monthly rainfall during the field experiment (June 2001 to June 2002), Turrialba, Costa Rica. Source: Catie, (2002).

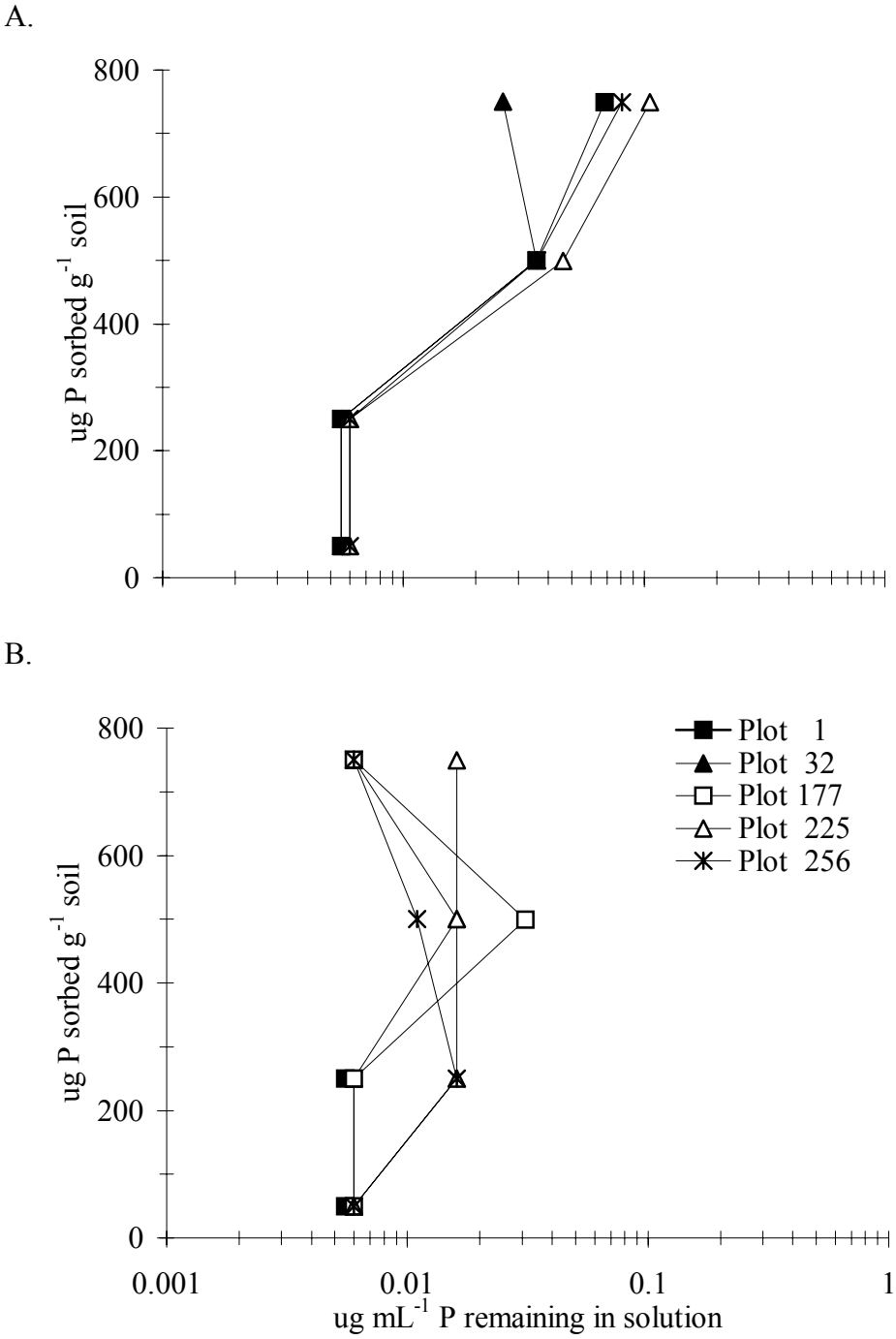
Appendix 2. Chemical soil properties at experimental site, San Juan Sur, Turrialba, Costa Rica.

	Experimental soil site characterization	Depth	pH (Water)	Exchangeable acidity[1] ($cmol^+$) L^{-1}	Exchangeable cations[1] ($cmol^+$) L^{-1}			Available P[1] (mg L^{-1})	Organic matter[2] (%)	Nitrogen[3] (%)
					Ca	Mg	K			
Reference	Catie soil report NR01-079-ORIG	0-20 cm	4.9	1.35	0.19	0.13	0.07	3.5		
Experiment 1	Without previous applications of $CaCO_3$ + Poultry manure									
	Before planting beans	0-12 cm	5.14±0.06	0.9±0.1	1.5±0.1	1.0±0.1	0.4±0.0	4.9±0.2	n.d.	n.d.
	With removal of slash biomass (Control)	0-12 cm	4.71±0.06	1.7±0.1	0.7±0.1	0.5±0.1	0.2±0.1	5.0±0.6	n.d.	n.d.
	Without removal of slash biomass	0-12 cm	4.73±0.04	1.3±0.1	0.6±0.1	0.5±0.1	0.3±0.0	4.4±0.3	n.d.	n.d.
Experiment 2	With previous applications of $CaCO_3$ + Poultry manure									
	With *Cajanus* mulch	0-12 cm	4.93±0.09	1.1±0.2	1.8±0.4	0.3±0.1	0.2±0.0	6.6±0.5	n.d.	n.d.
	With *Tithonia* mulch	0-12 cm	4.73±0.12	1.2±0.1	1.7±0.4	0.3±0.1	0.4±0.1	7.0±0.6	n.d.	n.d.
	Control	0-12 cm	4.86±0.13	1.3±0.2	1.9±0.5	0.3±0.1	0.2±0.0	6.6±1.0	n.d.	n.d.
Potted Experiment	San Juan Sur Soil + Sand	n.d	6.63±0.12	0.04±0.0	2.6±0.1	1.7±0.1	0.4±0.0	2.4±0.2	2.1±0.4	0.1±0.0
	San Juan Sur Soil	n.d	4.73±0.03	0.7±0.1	2.5±0.2	1.7±0.2	0.3±0.0	3.6±0.5	11.7±0.2	0.6±0.0

Appendix 2. Chemical soil properties at experimental site, San Juan Sur, Turrialba, Costa Rica. (Continuation)

	Experimental soil silte characterization	Minor nutrients (mg L^{-1})				P retention[4] (%)	Fe[5] (%)	Al[5] (%)	Texture[6]
		Cu	Zn	Mn	Fe				
Reference	Catie soil report NR01-079-ORIGN	n.d.	n.d.	n.d.	n.d.	96.51	n.d.	n.d.	n.d.
Experiment 1	Without previous applications of $CaCO_3$ + Poultry manure								
	Before planting beans	n.d.	n.d.	n.d.	n.d.	85.4±0.7	1.6±0.0	1.27±0.1	n.d.
	With removal of slash biomass (Control)	13.0±0.9	0.9±0.1	9.7±1.1	346.8±36.7	n.d.	n.d.	n.d.	n.d.
	Without removal of slash biomass	14.3±1.0	0.9±0.1	9.3±0.6	329.3±26.4	n.d.	n.d.	n.d.	n.d.
Experiment 2	With previous applications of $CaCO_3$ + Poultry manure								
	With *Cajanus* mulch	16.2±0.2	1.1±0.0	9.9±1.0	445.3±5.5	n.d.	n.d.	n.d.	n.d.
	With *Tithonia* mulch	16.3±1.5	1.2±0.1	17.0±3.1	437.8±56.8	n.d.	n.d.	n.d.	n.d.
	Control	16.0±0.8	1.1±0.2	11.4±1.2	442.8±52.0	n.d.	n.d.	n.d.	n.d.
Potted Experiment	San Juan Sur Soil + Sand	n.d.	n.d.	n.d.	n.d.	n.d.	0.7±0.1	0.1±0.0	LS
	San Juan Sur Soil	n.d.	n.d.	n.d.	n.d.	n.d.	1.5±0.0	1.0±0.0	LS

1/ Modified Olsen pH=8.5 and KCl 1N; 2/ Walkey-Black method; 3/ Semi-micro Kjeldahl; 4/ New Zeland method; 5/ Ammonium oxalate extraction method; 6/ Bouyucos method.
n.d. = no determined. Values are means ± standard errors, n=12.



Appendix 3. P-isotherms at 0-12.5 (A) and 12.5-25 (B) cm soil sampling depths before starting Experiment 1, San Juan Sur Andisol, Costa Rica, (n=5). Means pH in sodium fluoreno (NaF) were 9.92±0.22 and 10.43±0.26 at 0-12.5 and 12.5-25 cm soil depths, respectively.

Appendix 4. Nutrient concentrations in above-ground biomass of fallow species harvested six-months after planting in Experiment 1 at San Juan Sur, Turrialba, Costa Rica.

Fallow species treatments	**Nutrient concentrations and Duncan's test interpretation**				
	(mg g^{-1})				
	N	P	K	Mg	Ca
Without *Cajanus cajan*					
Natural regeneration	18.3±1.2 (A)	1.5±0.1 (A)	18.4±1.2 (A)(B)	2.4±0.2 (A)(B)	5.2±1.2 (A)
Tithonia from Costa Rica	23.2±5.8 (A)	2.1±0.4 (A)	28.6±4.3 (A)	3.2± 0.4 (A)	5.6±1.4 (A)
Tithonia from Mexico	19.8±4.3 (A)	1.7±0.3 (A)	20.1±3.0 (A)(B)	2.7±0.4 (A)(B)	5.0±1.2 (A)
Tithonia from Indonesia	21.6±5.3 (A)	1.9±0.4 (A)	21.4±3.4 (A)(B)	3.0±0.4 (A)(B)	6.3±1.5 (A)
With *Cajanus cajan*					
Tithonia (CR) + *Cajanus*	26.2±3.9 (A)	1.8±0.3 (A)	18.7±2.9 (A)(B)	2.4±0.2 (A)(B)	4.8±0.9 (A)
Tithonia (MEX) + *Cajanus*	20.0±3.6 (A)	1.6±0.3 (A)	18.7±2.4 (A)(B)	3.0±0.4 (A)(B)	6.1±1.1 (A)
Tithonia (IND) + *Cajanus*	23.4±4.4 (A)	1.8±0.3 (A)	19.6±3.3 (A)(B)	2.6±0.2 (A)(B)	4.8±0.8 (A)
Cajanus	27.4±6.9 (A)	1.8±0.4 (A)	15.5±0.5 (B)	1.9±0.3 (B)	3.4±0.6 (A)

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test.
Values are means ± standard errors, n=8.

Appendix 5. Total nutrient and carbon accumulation, C:N, C:P, and N:P in the different fallow species treatments harvested six-months after planting on an Andisol with low P availability in Experiment 1 at San Juan Sur, Turrialba, Costa Rica.

Fallow species treatments	**Total nutrient accumulation in slash biomass (N –P- K) + Ca + Mg** (kg ha^{-1})	**Total carbon**[/1] (Mg ha^{-1})	**C:N**	**C:P**	**N:P**
Without *Cajanus cajan*					
Natural regeneration	(95 – 8 – 96) + 27 + 12	2.37±0.14 (C)	24.5	304.6	12.5
Tithonia from Costa Rica	(120 – 11 – 148) + 29 + 16	2.61±0.19 (D)	19.3	209.3	10.8
Tithonia from Mexico	(208 – 18 – 211) + 53 + 29	5.94±0.18 (C)	22.6	258.8	11.5
Tithonia from Indonesia	(85 – 8 – 84) + 25 + 12	1.79±0.21 (C)	20.7	238.2	11.5
With *Cajanus cajan*					
Tithonia (CR) + *Cajanus*	(132 – 9 – 95) + 25 + 12	2.38±0.10 (A)	17.1	250.2	14.6
Tithonia (MEX) + *Cajanus*	(148 –12 – 139) + 45 + 22	3.54±0.17 (B)	22.4	274.7	12.3
Tithonia (IND) + *Cajanus*	(111 – 8 – 94) + 23 + 12	2.45±0.13 (D)	19.1	255.9	13.4
Cajanus	(115 – 8 – 65) + 14 + 8	1.91±0.13 (C)	16.4	251.6	15.4
			20.3±2.8	255.4±27.4	12.8±1.6

Same letters in the same column indicate no significant differences (p<0.05) by the Duncan's test.

1/ Estimated using a convertion factor for total carbon in dry weight equal to 0.4488. Values are means ± standard errors, n=32.

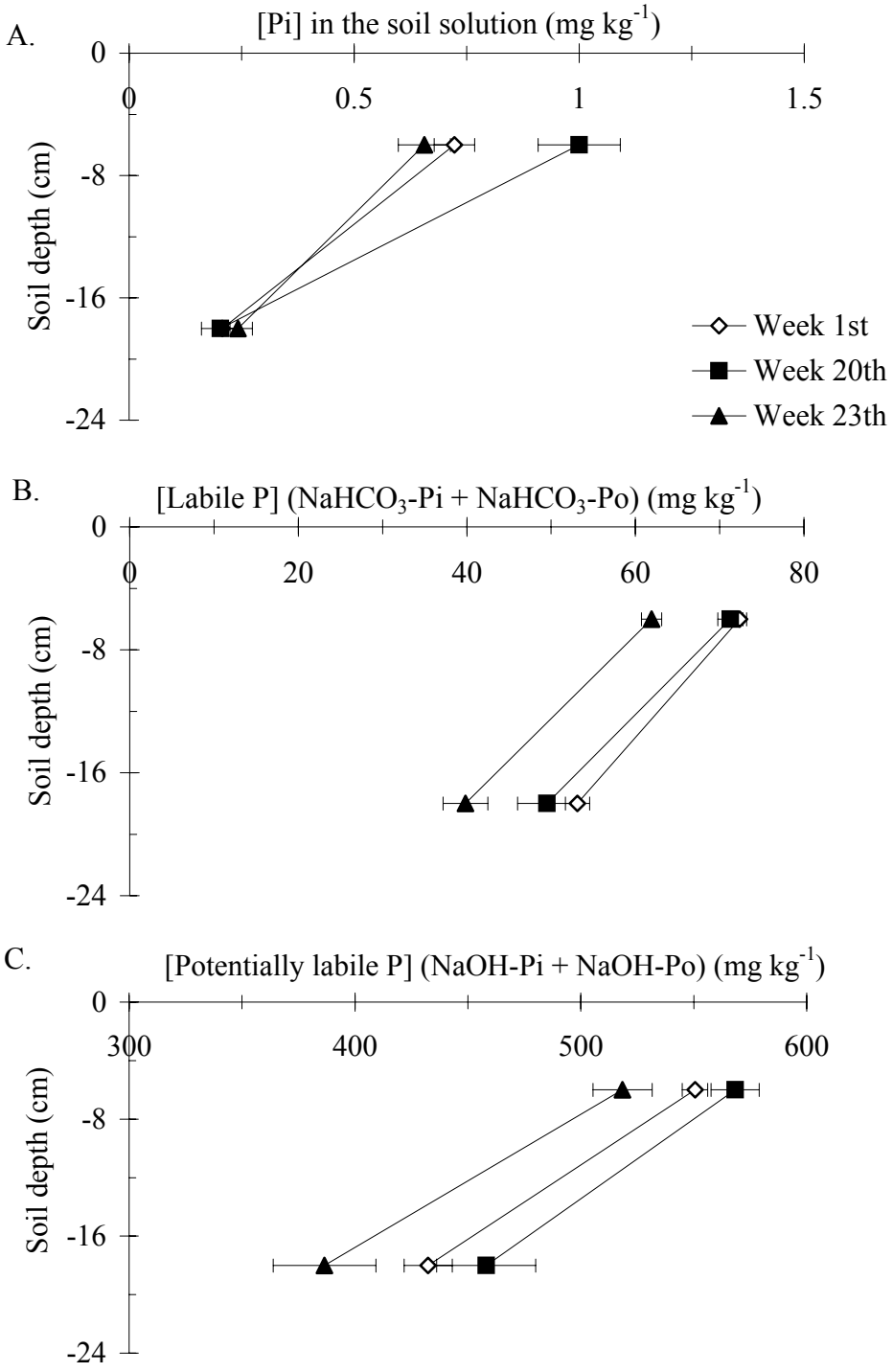
Appendix 6. Analysis of variance and Duncan's test outputs for root length density of fallow species harvested 23 weeks after planting in Experiment 1, San Juan Sur, Turrialba, Costa Rica.

Source of variation	df	MS	F value	Pr > F	Interpretation
Fallow species treatments (A)	7	33.57	2.56	0.0254	*
Sampling depths (B)	2	250.66	19.09	<0.0001	**
Interaction (AxB)	14	5.63	0.43	0.9571	n.s.
Error	48	13.13			
Total	71				

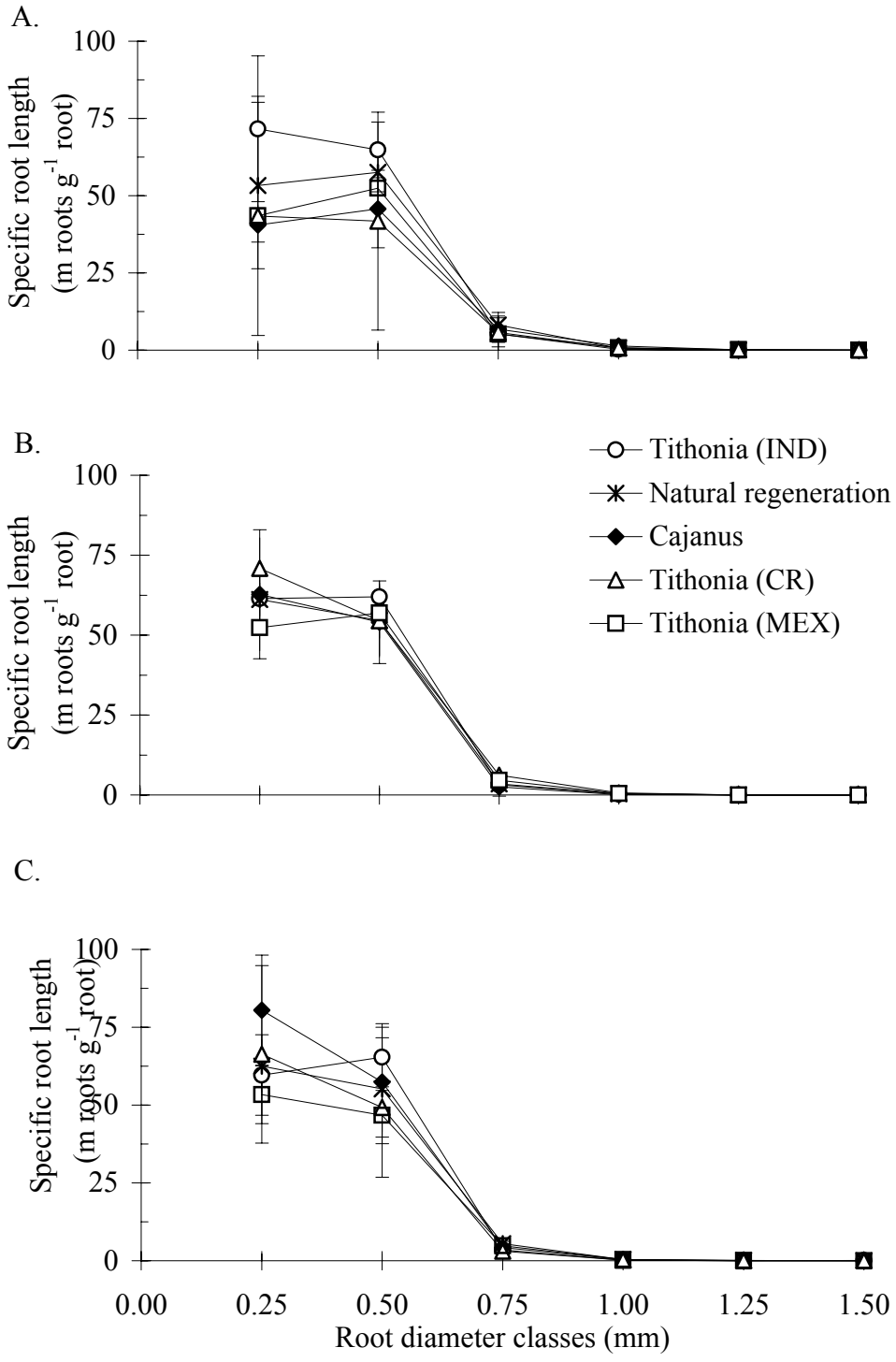
Means and the corresponding Duncan's test interpretation

Sampling depths (cm)	Root length density (cm cm^{-3})	
0-8	10.22±1.08	(A)
8-16	6.34±0.60	(B)
16-24	3.80±0.43	(C)

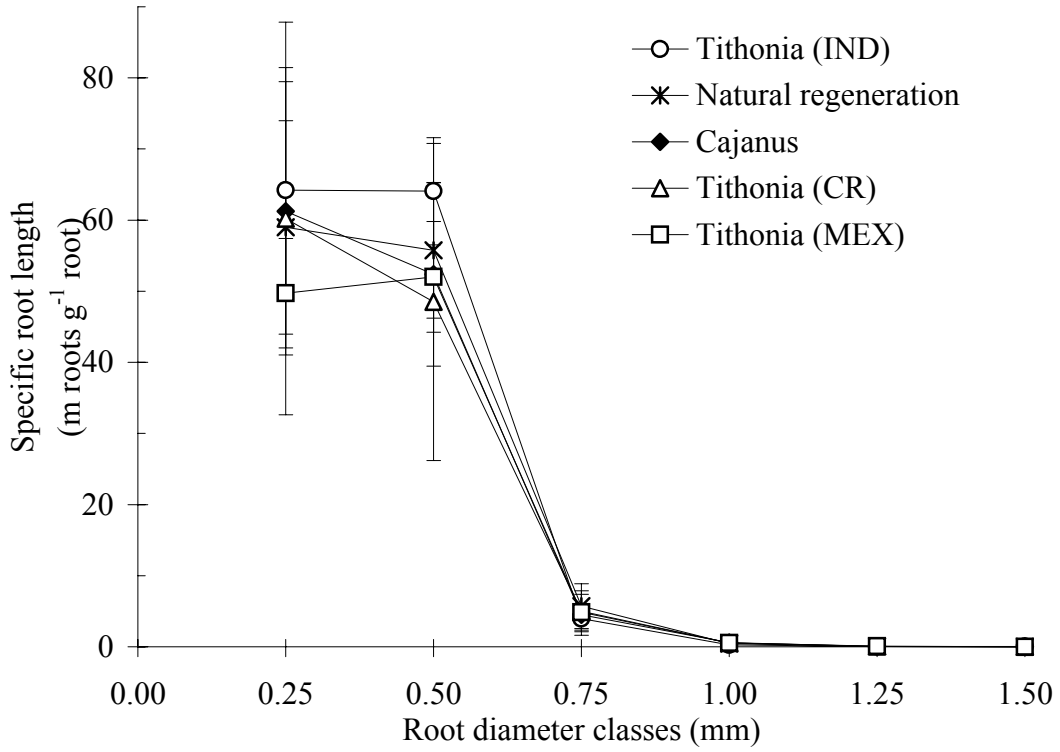
* Significant differences at 5% level; ** Highly significant differences at 1% level; n.s. Non significant differences.
Same letters along the same column indicate no significant differences (p <0.05) by the Duncan's test.
The underlined p values indicate significant differences at p <0.05. Values are means ± standard errors, n=9.



Appendix 7. Inorganic P (Pi) in the soil solution (A), labile P (B) and potentially labile P (C) concentrations at two soil sampling depths 1, 20 and 23 weeks after planting fallow species at San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed. Sampling sizes were (n=12) and (n=4) for 0-12 and 12-24 cm soil sampling depths, respectively. Pi=inorganic P and Po=organic P.



Appendix 8. Specific root length across root diameter classes at 0-8 (A), 8-16 (B) and 16-24 (C) cm soil sampling depths for different fallow species harvested 23 weeks after planting in Experiment 1, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=3).



Appendix 9. Specific root length across root diameter classes in the upper 25 cm of soil sampling depth for different fallow species harvested 23 weeks after planting in Experiment 1, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=9).

Appendix 10. Analysis of variance, orthogonal contrast and multivariate test outputs for specific root length per root diameter class of fallow species harvested 23 weeks after planting in Experiment 1, San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)						**Wilks' Lambda multivariate test** (*p* values)
	Specific root length (m g^{-1}) **at different root diameter classes** (6 variables)						
	0-0.5 mm	0.5-1.0 mm	1.0-1.5 mm	1.5-2.0 mm	2.0-2.5 mm	>2.5 mm	
Fallow treatments (A)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Depths (B)	0.0878	<u>≤0.0001</u>	n.s.	n.s.	n.s.	n.s.	<u>0.0094</u>
Interaction (AxB)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Comparisons	**Orthogonal contrasts** (*p* values)						
Natural. Regener. VS. All fallow species	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Caj. VS. *Tith.* and *Tith.* /*Caj.*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Tith. VS. *Tith.* /*Caj.*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
C.R *Tith.* VS. Mex and Ind *Tith.*	n.s.	n.s.	n.s.	n.s.	<u>0.0086</u>	0.0654	
C.R *Tith.* /*Caj.* VS. Mex. and Ind. *Tith.* /*Caj.*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Mex. *Tith.* VS. Ind. *Tith.*	0.0565	n.s.	n.s.	n.s.	n.s.	n.s.	
Mex. *Tith.* /*Caj.* VS. Ind. *Tith.* /*Caj.*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Fallow species treatments	0-0.5 mm	0.5-1.0 mm	1.0-1.5 mm	1.5-2.0 mm	2.0-2.5 mm	>2.5 mm	
Natural regeneration	114.75±6.82 (A)	6.17±1.09 (A)	0.04±0.02 (A)	0.0000±0.00 (A)	0.0000±0.00 (B)	0.0000±0.00 (A)	
Cajanus cajan	113.62±9.86 (A)	5.09±1.23 (A)	0.05±0.03 (A)	0.0000±0.00 (A)	0.0000±0.00 (B)	0.0011±0.00 (A)	
Tithonia (CR)	108.72±16.41 (A)	5.51±1.11 (A)	0.06±0.03 (A)	0.0144±0.01 (A)	0.0122±0.01 (A)	0.0022±0.00 (A)	
Tithonia from (CR) and *Cajanus*	115.15±12.17 (A)	6.12±0.73 (A)	0.16±0.06 (A)	0.0133±0.01 (A)	0.0011±0.00 (B)	0.0011±0.00 (A)	
Tithonia (MEX)	101.74±4.36 (A)	5.42±0.50 (A)	0.09±0.04 (A)	0.0778±0.01 (A)	0.0000±0.00 (B)	0.0000±0.00 (A)	
Tithonia from (MEX) and *Cajanus*	120.21±10.03 (A)	4.50±1.09 (A)	0.11±0.06 (A)	0.0000±0.00 (A)	0.0000±0.00 (B)	0.0000±0.00 (A)	
Tithonia (IND)	128.30±7.26 (A)	4.24±0.55 (A)	0.05±0.03 (A)	0.0111±0.01 (A)	0.0000±0.00 (B)	0.0000±0.00 (A)	
Tithonia from (IND) and *Cajanus*	111.58±2.63 (A)	5.56±0.55 (A)	0.07±0.05 (A)	0.0144±0.01 (A)	0.0000±0.00 (B)	0.0000±0.00 (A)	

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.

n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=9.

Appendix 11. Analysis of variance, orthogonal contrast and multivariate test outputs for fungal structures in *T. diversifolia* genotype roots harvested six-months after planting in Experiment 1, San Juan Sur,Turrialba, Costa Rica, (n=54).

Source of variation	**ANOVA output interpretation** (*p* values)			**Wilks' Lambda multivariate test** (*p* values)
	Proportion of fungal structure occurrence			
	Entry points	Vesicles	Arbuscules	
Tithonia genotypes (A)	<u>0.0323</u>	n.s.	n.s.	n.d.
Comparisons	**Orthogonal contrast outputs** (*p* values)			
Costa Rican VS. Mexican and Indonesian	n.s.	n.s.	n.s.	
Mexican VS. Indonesian	<u>0.0123</u>	n.s.	n.s.	
Source of variation	**ANOVA output interpretation** (*p* values)			**Wilks' Lambda multivariate test** (*p* values)
	Number of fungal structures cm^{-1} of root length			
	Entry points	Vesicles	Arbuscules	
Tithonia genotypes (A)	n.s.	n.s.	0.0744	n.s.
Comparisons	**Orthogonal contrast outputs** (*p* values)			
Costa Rican VS. Mexican and Indonesian	n.s.	n.s.	<u>0.0386</u>	
Mexican VS. Indonesian	n.s.	n.s.	n.s.	

The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$.
n.d. = non determined.

Appendix 12. Analysis of variance and multivariate test outputs for the abundance of fungal structures in *T. diversifolia* genotype roots on one-year old potted plants, Cabiria,Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)			**Wilks' Lambda multivariate test** (*p* values)
	Abundance of fungal structures cm^{-1} of root length			
	Number of entry points	Number of vesicles	Number of arbuscules	
Tithonia genotypes (A)	0.008	n.s.	n.s.	n.s.
Clones (B)	0.0425	n.s.	n.s.	n.s.
Interaction (AxB)	<0.001	0.0372	n.s.	n.s.
	Means and the corresponding Duncan's test interpretation			
	Number of entry points (units cm^{-1} of roots)	Number of vesicles (units cm^{-1} of roots)	Number of arbuscules (units cm^{-1} of roots)	
***Tithonia* genotypes (A)**				
Costa Rican	11±1 (B)	6±1 (A)	6±1 (A	
Mexican	15±2 (A)	5±2 (A)	7±1 (A)	
Indonesian	11±1 (B)	14±5 (A)	5±1 (A)	

The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$.
Values are means ± standard errors, n=88.

Appendix 13. Analysis of variance, Duncan's test, orthogonal contrast and multivariate test outputs for the abundance of fungal structures in *T. diversifolia* genotype roots harvested on two-year old plants at the *Tithonia* germplasm collection, San Juan Sur,Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)			**Wilks' Lambda multivariate test** (*p* values)
	Number of entry points	Number of vesicles	Number of arbuscules	
Tithonia genotypes (A)	n.s.	n.s.	<u>≤0.0001</u>	n.s.
Clones (B)	n.s.	n.s.	<u>≤0.0001</u>	n.s.
Interaction (AxB)	n.s.	n.s.	<u>≤0.0001</u>	
	Means and the corresponding Duncan's test interpretation			
	Number of entry points (units cm^{-1} of roots)	Number of vesicles (units cm^{-1} of roots)	Number of arbuscules (units cm^{-1} of roots)	
***Tithonia* genotypes (A)**				
Costa Rican	10±1 (A)	3±1 (A)	3±1 (B)	
Mexican	8±1 (A)	4±1 (A)	2±0 (B)	
Indonesian	11±1 (A)	23±16 (A)	6±4 (A)	
Comparisons	**Orthogonal contrast outputs** (*p* values)			
CR VS. MEX and IND	0.8997	0.2343	n.d.	
MEX VS. IND	<u>0.0293</u>	0.0539	n.d.	

Same letters in the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$.
n.d. = non determined.
Values are means ± standard errors, n=100.

Appendix 14. Analysis of variance, orthogonal contrast, multivariate and Duncan's test outputs for internal organic acid concentrations in the leaf tissue of six-week old girdled stem of *T. diversifolia* clones and genotypes (n=12) at the *Tithonia* germplasm collection, San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation for organic acids** (*p* values)					**Wilks' Lambda multivariate test** (*p* values)
	Oxalic	**Citric**	**Malic**	**Succinic**	**Fumaric**	
Genotypes (A)	0.083	<u>0.027</u>	<u>0.013</u>	n.s.	n.s.	<u>0.045</u>
Clones (B)	<u>0.042</u>	<u>0.010</u>	0.080	n.s.	n.s.	<u>0.014</u>
Interaction (AxB)	<u>0.017</u>	<u>0.023</u>	<u>0.002</u>	n.s.	n.s.	<u>0.032</u>
Genotypes (A)	**Means and the corresponding Duncan's test interpretation** In the leaf tissue (μM g^{-1} of fresh weight)					
	Oxalic	**Citric**	**Malic**	**Succinic**	**Fumaric**	
Costa Rican	0.455 (A)	2.504 (A)	4.173 (A)	68.780 (A)	0.042 (A)	
Mexican	0.272 (A)(B)	2.684 (A)	5.546 (A)	97.902 (A)	0.025 (A)(B)	
Indonesian	0.117 (B)	0.871 (B)	0.935 (B)	63.198 (A)	0.009 (B)	
Comparisons	**Orthogonal contrast**					
C.R. VS. MEX and IND	<u>0.047</u>	n.s.	n.s.	n.s.	0.064	
MEX VS. IND	n.s.	<u>0.015</u>	<u>0.004</u>	n.s.	n.s.	

Same letters in the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$.

Appendix 15. Analysis of variance, Duncan's test, orthogonal contrast and multivariate test outputs for the soil P fractions at 0-12 cm soil sampling depth of fallow species treatments 1, 20, 23 and 55 weeks after starting Experiment 1 at San Juan Sur, Turrialba, Costa Rica, (n=36).C25

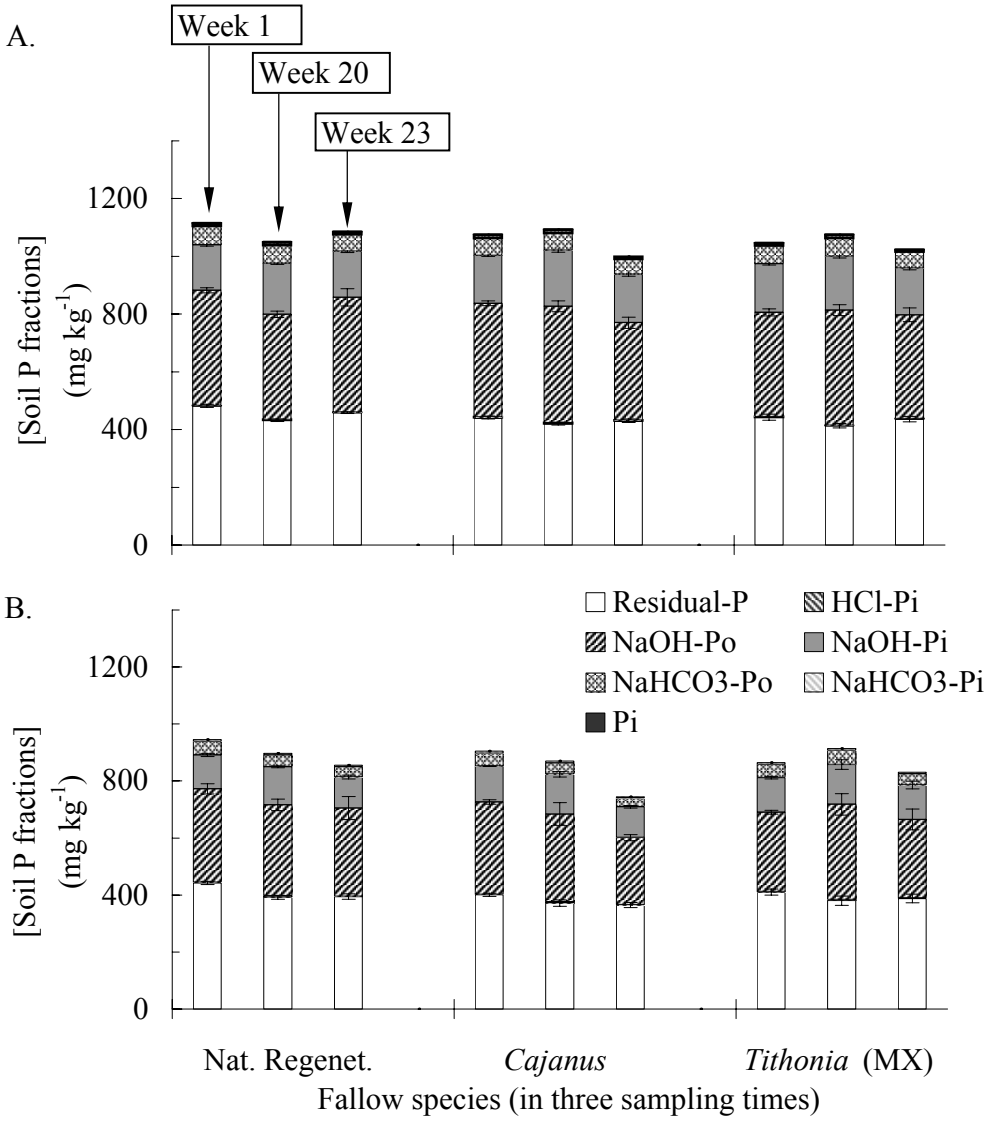
Source of variation	**ANOVA output interpretation for soil P fractions** (p values)							**Wilks' Lambda multivariate test** (p values)
	Pi	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-Pi	Residual-P	
Group of fallow species treatments	<u>≤0.0001</u>	<u>0.0382</u>	<u>0.0183</u>	n.s.	n.s.	<u>≤0.0001</u>	<u>≤0.0001</u>	<u>≤0.0001</u>
Time	<u>≤0.0001</u>	<u>≤0.0001</u>	<u>≤0.0001</u>	<u>≤0.0001</u>	<u>≤0.0001</u>	<u>≤0.0001</u>	<u>≤0.0001</u>	<u>≤0.0001</u>
Group of fallow species treatments	**Means** (mg kg^{-1}) **and the corresponding Duncan's test interpretation**							
	Pi	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-Pi	Residual-P	
Natural regeneration	0.72 (B)	10.33 (A)(B)	61.54 (A)	163.99 (B)	387.34 (A)	1.61 (C)	457.66 (A)	
Cajanus fallow	0.60 (B)	11.05 (A)	56.39 (B)(C)	176.11 (A)	378.58 (A)	1.88 (B)(C)	430.89 (B)	
Tithonia in monoculture fallows	0.96 (A)	10.53 (A)(B)	54.95 (C)	168.46 (A)(B)	387.00 (A)	1.98 (B)	433.31 (B)	
Tithonia and *Cajanus* fallows	0.65 (B)	9.93 (B)	59.62 (A)(B)	171.32 (A)(B)	388.60 (A)	2.68 (A)	447.24 (A)	
Fallow species comparisons	**Orthogonal contrast comparisons**							
Natural regener. VS. Planted fallows	n.s.	n.s.	<u>0.0136</u>	<u>0.0369</u>	n.s.	<u>≤0.0001</u>	<u>≤0.0001</u>	
Cajanus VS. *Tith.* and *Tith.*/*Caj.*	<u>0.0089</u>	<u>0.0047</u>	n.s.	n.s.	n.s.	<u>0.0006</u>	0.0747	
Tithonia VS. *Tith.*/*Caj.*	<u>0.0001</u>	n.s.	n.s.	n.s.	0.0525	<u>≤0.0001</u>	<u>0.0100</u>	

Means with the same letters along the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.
n.s. = non significant differences at p <0.05.

Appendix 16. Analysis of variance, Duncan's and multivariate test outputs for the soil P fractions at 12-24 cm soil sampling depth of fallow species treatments 1, 20 and 23 weeks after starting Experiment 1 at San Juan Sur, Turrialba, Costa Rica, (n=12).

Source of variation	**ANOVA output interpretation for soil P fractions** (p values)							**Wilks' Lambda multivariate test** (p values)
	Pi	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-Pi	Residual-P	
Fallow species treatments	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0039	0.0046
Time	n.s.	0.0522	0.0029	0.0033	n.s.	<0.0001	0.0001	<0.0001
Fallow species treatments	**Means** (mg kg^{-1}) **and the corresponding Duncan's test interpretation**							
	Pi	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-Pi	Residual-P	
Natural regeneration	0.19 (A)	5.90 (A)	41.42 (A)	120.62 (A)	318.73 (A)	2.89 (A)	409.47 (A)	
Cajanus fallow	0.22 (A)	5.91 (A)	38.45 (A)	124.63 (A)	289.47 (A)	2.33 (A)	378.92 (B)	
Mexican *Tithonia* fallow	0.24 (A)	5.83 (A)	44.97 (A)	127.27 (A)	296.30 (A)	2.34 (A)	392.63 (A) (B)	

Means with the same letters along the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.
n.s. = non significant differences at p <0.05.



Appendix 17. Soil P fraction composition at 0-12 (A) and 12-24 cm (B) soil sampling depths in three fallow species treatments at Experiment 1, San Juan Sur, Turrialba, Costa Rica. Within each fallow treatment, the first, second and third columns of each group correspond to the 1st, 20th and 23th week after planting fallow species, respectively. Sampling sizes: (n=12) and (n=4) for 0-12 and 12-24 cm soil sampling depths, respectively. Pi=inorganic P and Po=organic P.

Appendix 18. Orthogonal breakdown of fallow species treatment and slash biomass application effects on bean yield in Experiment 1, San Juan Sur,Turrialba, Costa Rica, (n=64).

Source of variation	df	MS	F value	Pr > F	Interpretation
Rows	7	413,100.99	4.32	0.0013	**
Columns	7	1,556,578.41	16.26	<0.0001	**
SuperRow*Supercolumn (Magic quadrates)	3	395,957.48	4.14	0.0122	**
Species treatments (A)	7	349,963.34	3.66	0.004	**
Natural regeneration VS. All species	1	21,052.88	0.22	0.6417	n.s.
Cajanus VS. *Tithonia* and *Tithonia* /*Cajanus*	1	229,140.10	2.39	0.1299	n.s.
Tithonia VS. *Tithonia* /*Cajanus*	1	139,697.63	1.46	0.2343	n.s.
C.R. *Tithonia* VS. Mex. and Ind. *Tithonia*	1	1,560,044.36	16.30	0.0002	**
C.R. *Tithonia* /*Cajanus* VS. Mex. and Ind. *Tithonia* /*Cajanus*	1	3,028.07	0.03	0.8597	n.s.
Mex. *Tithonia* VS. Ind. *Tithonia*	1	107,649.02	1.12	0.2954	n.s.
Mex. *Tithonia* /*Cajanus* VS. Ind. *Tithonia* /*Cajanus*	1	826.91	0.01	0.9264	n.s.
E. Error (a)	39	95,704.01	1.10	0.3267	
Slash biomass applications and bean cultivars	3	514,178.36	5.93	0.0007	**
Slash biomass treatments (B)	1	1,114,017.20	12.85	0.0004	**
Bean cultivars (C)	1	349,330.80	4.03	0.0463	*
Interaction Bean cultivars x S. biomass treatments (BxC)	1	5,794.20	0.07	0.7963	n.s.
Interaction (AxBxC)	21	141,536.20	1.63	0.0472	*
Covariable (Number of damaged plants per plot)	1	549,391.65	6.34	0.0128	*
E. Error (b)	167	86,676.17			
Total	255				

* Significant differences; ** Highly significant differences; n.s. Non significant differences.

Appendix 19. Biomass allocation and root dry weight of bean cultivars with and without slash biomass removal harvested at the bean maturity phase on an Andisol with low P availability, San Juan Sur, Turrialba, Costa Rica, (n=16).

	With slash biomass removal		**Without slash biomass removal**	
	Chirripo Rojo	Negro Huasteco	Chirripo Rojo	Negro Huasteco
	Biomass allocation (g plant $^{-1}$)			
Leaf	1.8±0.2 (B)	2.7±0.2 (A)	1.8±0.2 (B)	2.2±0.2 (B)
Stem	1.0±0.1 (B)	1.9±0.2 (A)	1.2±0.1 (B)	1.9±0.1 (A)
Pod	6.0±0.8 (A)	5.6±0.6 (A)	6.2±0.6 (A)	6.5±0.7 (A)
Above-ground biomass	8.9±0.9 (A)	10.2±0.8 (A)	9.2±0.8 (A)	10.5±0.9 (A)
Root	0.6±0.0 (C)	0.8±0.1 (A)	0.6±0.1 (B)(C)	0.7±0.0 (A)(B)
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Total	9.4±0.9 (A)	11.0±0.8 (A)	9.8±0.9 (A)	11.3±1.0 (A)

Same letters along the same row indicate no significant differences (p<0.05) by the Duncan's test. Values are means ± standard errors.

Appendix 20. Analysis of variance, orthogonal contrast, Duncan's and multivariate test outputs for bean cultivar nutrient concentrations in above-ground biomass between fallow species, slash biomass application treatments and bean cultivars harvested before the flowering phase at San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)				**Wilks' Lambda multivariate test** (*p* values)
	P	Ca	Mg	K	
Fallow treatments (A)	n.s.	0.0002	n.s.	0.0085	
Bean cultivars (B)	0.0035	n.s.	n.s.	n.s.	0.0391
Slash biomass applications (C)	n.s.	n.s.	n.s.	0.0036	0.0636
Interaction (BxC)	n.s.	n.s.	n.s.	n.s.	n.s.
Comparisons	**Orthogonal contrast outputs** (*p* values)				
Control VS. Slash biomass	n.s.	n.s.	n.s.	0.0036	
Chirripo Rojo VS. Negro Huasteco	0.0035	n.s.	n.s.	n.s.	
Interaction (B. cultivars x Slash biomass)	n.s.	n.s.	n.s.	n.s.	
Bean cultivar comparisons	**Means** (mg g^{-1}) **and the corresponding Duncan's test interpretation**				
	P	Ca	Mg	K	
Control (with slash biomass removal)					
Chirripo Rojo	2.9±0.4 (A)	9.4±2.7 (A)	5.4±1.5 (A)	30.1±6.7 (A)(B)	
Negro Huasteco	2.4±0.4 (B)	8.9±3.0 (A)	4.7±1.4 (A)	27.2±8.7 (B)	
Without slash biomass removal					
Chirripo Rojo	2.9±0.3 (A)	8.1±2.7 (A)	4.4±1.4 (A)	34.4±4.4 (A)	
Negro Huasteco	2.4±0.4 (B)	9.2±2.5 (A)	4.4±0.8 (A)	34.6±5.6 (A)	

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.
n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=8.

Appendix 21. Analysis of variance and Duncan's test outputs for P uptake and utilization efficiencies of bean cultivars between fallow species and slash biomass application treatments harvested at the end of the flowering phase at San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)		
	P uptake efficiency	P utilization efficiency	Surface of upper root-stem
Fallow treatments (A)	n.s.	0.013	n.s.
Bean cultivars (B)	0.0574	<0.0001	0.0405
Slash biomass applications (C)	n.s.	n.s.	n.s.
Interaction (BxC)	n.s.	n.s.	n.s.
Bean cultivar comparisons	**Means and the corresponding Duncan's test interpretation**		
	P uptake efficiency (mg of P m g^{-1} SRL)	P utilization efficiency (Total g of DW mg^{-1} of P)	Surface of upper root-stem (mm^{-3})
Control (with Sl. biomass removal)			
Chirripo Rojo	4.2±0.4 (A)	48.1±2.5 (B)	38.7±9.4 (B)
Negro Huasteco	3.4±0.3 (A)	56.5±2.5 (A)	78.9±10.1 (A)
Without Sl. biomass removal			
Chirripo Rojo	3.5±0.6 (A)	45.7±2.1 (B)	45.9±11.3 (A)(B)
Negro Huasteco	2.9±0.3 (A)	58.9±2.9 (A)	53.7±14.4 (A)(B)

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.

n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=8.

Appendix 22. Root characteristics and dry weight allocation of bean cultivars with and without slash biomass removal harvested at the end of the flowering phase in Experiment 1, San Juan Sur, Turrialba, Costa Rica, (n=8).

Root system characteristics	With slash biomass removal		Without slash biomass removal	
	Chirripo Rojo	Negro Huasteco	Chirripo Rojo	Negro Huasteco
Root dry weight partitioning (g)				
By root-stem parts				
Upper part	0.22±0.02 (A)	0.28±0.03 (A)	0.28±0.05 (A)	0.27±0.02 (A)
Lower part	0.05±0.01 (A)(B)	0.03±0.01 (B)	0.06±0.01 (A)	0.04±0.01 (A)(B)
By types of roots				
Adventitious	0.04±0.01 (B)	0.08±0.01 (A)	0.04±0.01 (B)	0.05±0.01 (A)(B)
Basal	0.24±0.04 (A)(B)	0.18±0.02 (B)	0.38±0.11 (A)	0.25±0.02 (A)(B)
Root system components (units)				
Nodules	2±1 (B)	8±5 (A)(B)	4±2 (B)	18±7 (A)
Adventitious roots	29±6 (B)	48±4 (A)	24±4 (B)	29±5 (B)
Basal roots	11±1 (A)	10±1 (A)	10±0 (A)	9±1 (A)
Root extension				
Root-stem length (mm)				
Upper part	38.4±4.2 (A)	47.9±5.4 (A)	38.1±2.9 (A)	38.8±4.7 (A)
Lower part	10.3±1.8 (A)	8.1±0.8 (A)(B)	8.7±0.9 (A)(B)	6.2±0.3 (B)
SRL (m g^{-1})				
Adventitious roots	67.2±4.4 (A)	65.7±2.0 (A)	77.3±7.8 (A)	61.9±6.9 (A)
Basal roots	20.5±2.8 (A)	29.8±3.4 (A)	25.8±4.6 (A)	24.8±2.2 (A)
Root-stem perimeter (mm)				
In the top of root-stem	17.2±0.9 (A)	16.9±0.6 (A)	17.8±1.1 (A)	17.7±0.8 (A)
In the bottom of root-stem	16.4±0.6 (A)	14.8±1.2 (A)	16.8±1.3 (A)	17.2±1.0 (A)
Dry weight allocation (g)				
Leaf	3.4±0.5 (A)	3.3±0.6 (A)	3.2±0.5 (A)	4.3±0.4 (A)
Stem	1.1±0.2 (A)	1.3±0.2 (A)	1.3±0.2 (A)	1.8±0.2 (A)
Pod	1.2±0.3 (A)(B)	0.6±0.2 (B)	1.7±0.4 (A)	1.0±0.3 (A)(B)
Above-ground biomass	5.8±0.8 (A)	5.2±1.0 (A)	6.3±1.0 (A)	7.0±0.8 (A)
Total root dry weight	0.5±0.0 (A)	0.6±0.1 (A)	0.8±0.1 (A)	0.6±0.0 (A)
Total plant	6.3±0.9 (A)	5.8±1.0 (A)	7.0±1.1 (A)	7.6±0.8 (A)

Means with the same letters along the same row indicate no significant differences ($p<0.05$) by the Duncan's test. Values are means ± standard errors.

Appendix 23. Analysis of variance and multivariate test outputs for root characteristics of bean cultivars between fallow species and slash biomass application treatments harvested at the end of the flowering phase, San Juan Sur,Turrialba, Costa Rica, (n=8).

Source of variation	**ANOVA output interpretation** (p values)				**Wilks' Lambda multivariate test** (p values)
	Root dry weight partitioning (4 variables)				
	Lower root-stem DW	Upper root-stem DW	Adventitious root DW	Basal root DW	
Fallow treatments (A)	n.s.	0.0634	n.s.	n.s.	
Bean cultivars (B)	<u>0.0321</u>	n.s.	<u>0.0086</u>	n.s.	0.081
Sl. biomass treatments (C)	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction (BxC)	n.s.	n.s.	n.s.	n.s.	n.s.
	Root system components (3 variables)				
	Number of basal roots	Number of adventitious roots	Number of nodules		
Fallow treatments (A)	n.s.	n.s.	n.s.		
Bean cultivars (B)	n.s.	<u>0.017</u>	<u>0.0148</u>		<u>0.0025</u>
Sl. biomass treatments (C)	n.s.	<u>0.0202</u>	n.s.		0.0527
Interaction (BxC)	n.s.	n.s.	n.s.		n.s.
	Root extension (4 variables)				
	SRL of adventitious roots	SRL of basal roots	Lower root-stem length	Upper root-stem length	
Fallow treatments (A)	<u>0.0306</u>	<u>0.0022</u>	n.s.	n.s.	
Bean cultivars (B)	0.0923	0.0945	<u>0.034</u>	n.s.	<u>0.0311</u>
Sl. biomass treatments (C)	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction (BxC)	n.s.	<u>0.043</u>	n.s.	n.s.	n.s.

The underlined p values indicate significant differences at $p<0.05$.
n.s. = non significant differences at $p<0.05$.

Appendix 24. Analysis of variance and multivariate test outputs for root characteristics of bean cultivars between fallow species and slash biomass application treatments harvested before the flowering phase at San Juan Sur, Turrialba, Costa Rica, (n=8).

Source of variation	**ANOVA output interpretation** (p values)				**Wilks' Lambda multivariate test** (p values)
	Root dry weight partitioning (4 variables)				
	Lower root-stem DW	Upper root-stem DW	Adventitious root DW	Basal root DW	
Fallow treatments (A)	0.0188	0.0072	n.s.	n.s.	
Bean cultivars (B)	n.s.	<0.0001	0.0063	0.0112	0.002
Sl. biomass treatments (C)	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction (BxC)	n.s.	n.s.	n.s.	n.s.	n.s.
	Root system components (3 variables)				
	Number of basal roots	Number of adventitious roots	Number of nodules		
Fallow treatments (A)	n.s.	0.0024	n.s.		
Bean cultivars (B)	n.s.	n.s.	n.s.		n.s.
Sl. biomass treatments (C)	n.s.	n.s.	n.s.		n.s.
Interaction (BxC)	n.s.	n.s.	n.s.		n.s.
	Root extension (2 variables)				
		Lower root-stem length	Upper root-stem length		
Fallow treatments (A)		0.0555	n.s.		
Bean cultivars (B)		n.s.	n.s.		
Sl. biomass treatments (C)		n.s.	n.s.		
Interaction (BxC)		n.s.	n.s.		

The underlined p values indicate significant differences at $p<0.05$.
n.s. = non significant differences at $p<0.05$.

Appendix 25. Analysis of variance and multivariate test outputs for root characteristics of bean cultivars between fallow species and slash biomass application treatments harvested at the bean maturity phase, San Juan Sur, Turrialba, Costa Rica, (n=16).

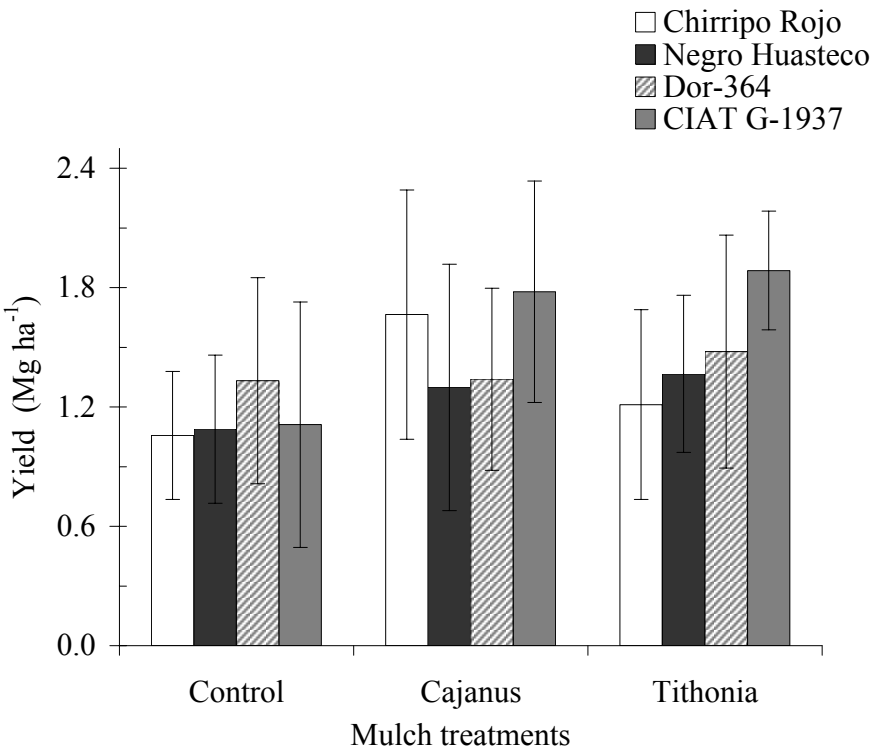
Source of variation	**ANOVA output interpretation** (p values)				**Wilks' Lambda multivariate test** (p values)
	Root dry weight partitioning (4 variables)				
	Lower root-stem DW	Upper root-stem DW	Adventitious root DW	Basal root DW	
Fallow treatments (A)	0.0214	0.0562	0.0002	0.0465	
Bean cultivars (B)	n.s.	0.0006	0.0006	0.0131	0.0002
Sl. biomass treatments (C)	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction (BxC)	n.s.	n.s.	0.0353	n.s.	n.s.
	Root system components (3 variables)				
	Number of basal roots	Number of adventitious roots	Number of nodules		
Fallow treatments (A)	n.s.	n.s.	n.s.		
Bean cultivars (B)	0.0117	0.0011	n.s.		0.0012
Sl. biomass treatments (C)	n.s.	0.0353	0.0007		0.0006
Interaction (BxC)	n.s.	n.s.	n.s.		n.s.
	Root extension (4 variables)				
	SRL of adventitious roots	SRL of basal roots	Lower root-stem length	Upper root-stem length	
Fallow treatments (A)	0.0006	n.s.	n.s.	n.s.	
Bean cultivars (B)	n.s.	n.s.	n.s.	n.s.	n.s.
Sl. biomass treatments (C)	0.0768	<0.0001	n.s.	n.s.	0.0002
Interaction (BxC)	n.s.	n.s.	n.s.	n.s.	n.s.

The underlined p values indicate significant differences at $p<0.05$.
n.s. = non significant differences at $p<0.05$.

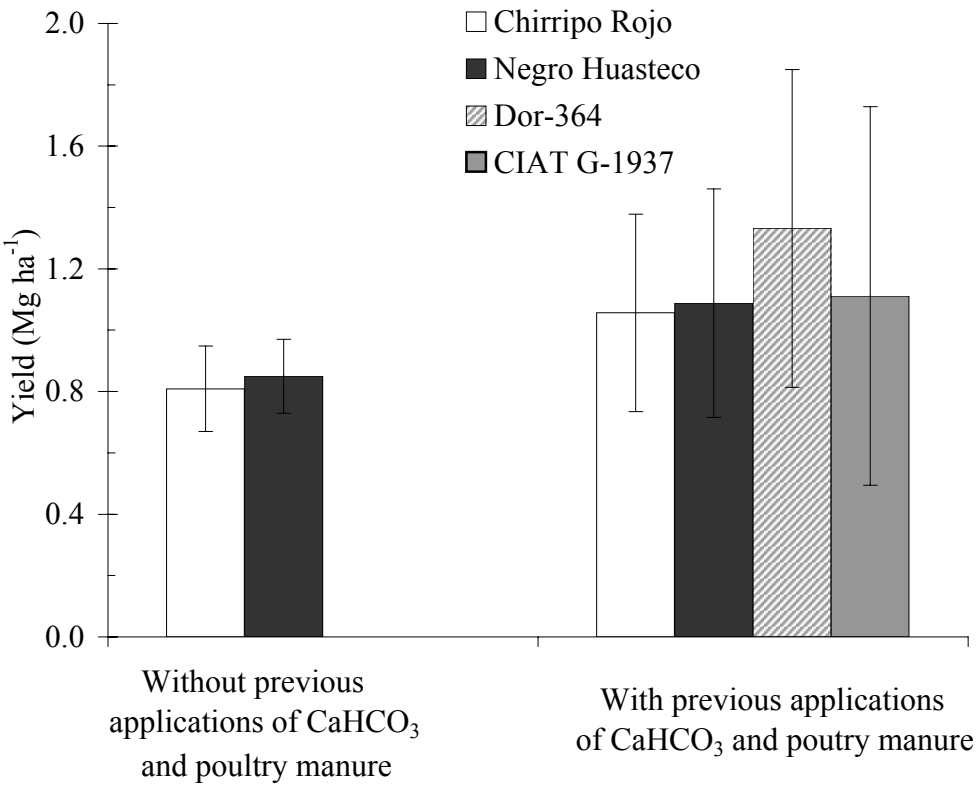
Appendix 26. Analysis of variance, Duncan's test, orthogonal contrast and multivariate test outputs for the abundance of fungal structures in bean cultivar roots between fallow species and slash biomass application treatments harvested at the end of the flowering phase, San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (p values)			**Wilks' Lambda multivariate test** (p values)
	Number of entry points	Number of vesicles	Number of arbuscules	
Fallow species treatments (A)	<u>≤0.0001</u>	0.0553	<u>0.001</u>	
Bean cultivars (B)	n.s.	<u>0.0120</u>	n.s.	<u>0.0282</u>
Slash biomass applications (C)	n.s.	n.s.	n.s.	n.s.
Interaction (BxC)	n.s.	n.s.	n.s.	n.s.
Fallow species treatments (A)	**Means and the corresponding Duncan's test interpretation**			
	Number of entry points (units cm^{-1} of roots)	Number of vesicles (units cm^{-1} of roots)	Number of arbuscules (units cm^{-1} of roots)	
Natural regeneration	43±5 (A)(B)	26±13 (A)(B)	5±2 (B)	
Cajanus fallow	44±6 (A)	11±2 (A)(B)	5±1 (B)	
Tithonia from Costa Rica	38±4 (A)(B)	5±1 (B)	4±1 (B)	
Tithonia from Costa Rica and *Cajanus*	20±2 (D)	7±3 (B)	3±0 (B)	
Tithonia from Mexico	35±4 (A)(B)	31±8 (A)	3±1 (B)	
Tithonia from Mexico and *Cajanus*	42±3 (A)(B)	8±3 (B)	34±17 (A)	
Tithonia from Indonesia	22±3 (C)(D)	8±2 (B)	4±1 (B)	
Tithonia from Indonesia and *Cajanus*	31±4 (B)(C)	16±9 (A)(B)	5±2 (B)	
Comparisons	**Orthogonal contrast outputs** (p values)			
Without Sl. biomass VS. With Sl. biomass	n.s.	n.s.	n.s.	
Chirripo Rojo VS. Negro Huasteco	n.s.	<u>0.0117</u>	n.s.	
Interaction (Bean cultivars x Sl. biomass)	n.s.	n.s.	n.s.	

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.
n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=50.



Appendix 27. Yield of bean cultivars in response to three different mulch treatments on an Andisol with carry over effects of calcium carbonate and poultry manure in Experiment 2 at San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed (n=3).



Appendix 28. Bean cultivar yield in control treatments without and with carry over effects of soil amendments at San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed. Sampling sizes were (n=64) and (n=9) for the groups without and with soil amendments, respectively. Mean yield increment represents ±0.32 Mg ha^{-1} between without and with carry over effects of soil amendments.

Appendix 29. Analysis of variance, Duncan's test and contrast comparison outputs for above-ground biomass, P utilization and uptake efficiencies under different mulch treatments harvested at the flowering phase in Experiment 2, San Juan Sur, Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (p values)		
	P utilization efficiency	P uptake efficiency	Above-ground biomass
Mulch treatments (A)	0.033	n.s.	n.s.
Bean cultivars (B)	n.s.	n.s.	n.s.
Blocks	n.s.	n.s.	<0.0001
Mulch treatments (A)	**Means and the corresponding Duncan's test**		
	P utilization efficiency (g of DW mg^{-1} of P)	P uptake efficiency (mg of P m g^{-1} SRL)	Above-ground biomass (g)
Control	32.96±1.05 (A)	4.96±0.52 (A)	4.62±0.52 (A)
Cajanus mulch	30.47±0.56 (B)	5.13±0.44 (A)	5.50±0.71 (A)
Tithonia mulch	33.36±0.82 (A)	5.53±1.13 (A)	5.27±0.50 (A)
Bean cultivars (B)	**Means and the corresponding Duncan's test**		
	P utilization efficiency (g of DW mg^{-1} of P)	P uptake efficiency (mg of P m g^{-1} SRL)	Above-ground biomass (g)
Chirripo Rojo	30.49±0.58 (A)	6.71±1.29 (A)	4.42±0.56 (B)
Negro Huasteco	32.41±0.74 (A)	4.82±0.61 (A)(B)	4.89±0.66 (A)(B)
Dor-364	32.64±1.29 (A)	5.18±0.55 (A)(B)	4.93±0.51 (A)(B)
CIAT G-1937	33.32±1.25 (A)	4.15±0.64 (B)	6.30±0.86 (A)
Comparisons	**Contrast outputs** (p values)		
Control VS. Mulch treat.	n.s.	n.s.	n.s.
Caj. mulch VS. *Tith.* mulch	0.0146	n.s.	n.s.

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.
n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=9.

Appendix 30. Analysis of variance, contrast comparison, Duncan's and multivariate test outputs for nutrient concentrations in above-ground biomass between mulch treatments harvested at the flowering phase in Experiment 2, San Juan Sur,Turrialba, Costa Rica.

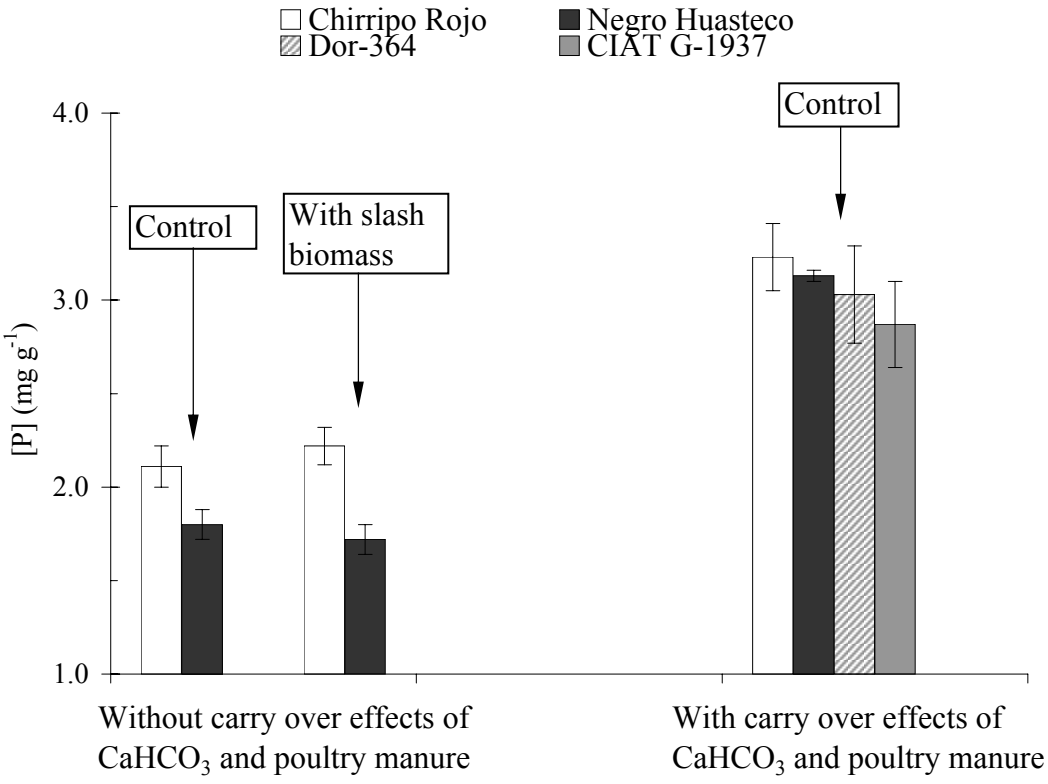
Source of variation	**ANOVA output interpretation** (*p* values)				**Wilks' Lambda multivariate test** (*p* values)
	P	Ca	Mg	K	
Mulch treatments (A)	0.0344	0.0040	0.0061	0.0014	0.0014
Bean cultivars (B)	n.s.	n.s.	0.0291	n.s.	0.0956
Blocks	n.s.	0.0433	0.0572	0.0065	
Interaction (AxB)	n.s.	n.s.	n.s.	n.s.	n.s.
Bean cultivar comparisons	**Contrast outputs** (*p* values)				
Costa Rican VS. Exotic	n.s.	n.s.	n.s.	n.s.	
C. Rojo VS. N. Huasteco	0.0997	n.s.	0.0071	n.s.	
Dor-364 VS. CIAT G-1937	n.s.	n.s.	n.s.	n.s.	
Bean cultivars	**Means** (mg g^{-1}) **and the corresponding Duncan's test interpretation**				
	P	Ca	Mg	K	
Chirripo Rojo	3.3±0.1 (A)	18.3±0.9 (A)	3.4±0.3 (A)	34.3±2.7 (A)	
Negro Huasteco	3.1±0.1 (A)	19.0±1.2 (A)	2.5±0.1 (B)	36.0±2.2 (A)	
Dor-364	3.1±0.1 (A)	18.9±1.1 (A)	2.9±0.2 (A)(B)	35.6±3.2 (A)	
CIAT G-1937	3.0±0.1 (A)	19.5±0.8 (A)	3.4±0.4 (A)	37.3±3.8 (A)	

Same letters in the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$. Values are means ± standard errors, n=9.

Appendix 31. Duncan's test and contrast comparison outputs for nutrient concentrations in above-ground biomass between mulch treatments harvested at the flowering phase in Experiment 2, San Juan Sur, Turrialba, Costa Rica.

Mulch treatments	**Means** (mg g^{-1}) **and the corresponding Duncan's test interpretation**			
	P	Ca	Mg	K
Control	3.1±0.1 (B)	21.0±0.7 (A)	3.6±0.3 (A)	29.4±3.0 (B)
Cajanus mulch	3.3±0.1 (A)	17.3±0.8 (B)	2.9±0.2 (B)	41.1±1.6 (A)
Tithonia mulch	3.0±0.1 (B)	18.7±0.8 (B)	2.7±0.1 (B)	36.5±1.7 (A)
Mulch treatment comparisons	**Contrast comparison outputs** (*p* values)			
Control VS. Mulch treatments	n.s.	<u>0.0025</u>	<u>0.0010</u>	<u>0.0005</u>
Cajanus VS. *Tithonia*	<u>0.0102</u>	n.s.	n.s.	n.s.

Same letters in the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$. Values are means ± standard errors, n=8.



Appendix 32. P concentrations without and with carry over effects of soil amendments in control and slash biomass treatments for four bean cultivars harvested at the flowering phase, San Juan Sur, Turrialba, Costa Rica. Standard error bars are displayed. Sampling sizes were (n=8) and (n=3) for the groups without and with soil amendments, respectively. Mean P concentration increment represents ±64% between without and with carry over effects of soil amendments and ±0.01% between the control and slash biomass treatments without previous applications of soil amendments.

Appendix 33. Analysis of variance, Duncan's and multivariate test outputs for root dry weight partitioning of bean cultivars under different mulch application treatments harvested at the flowering phase in Experiment 2, San Juan Sur, Turrialba, Costa Rica.

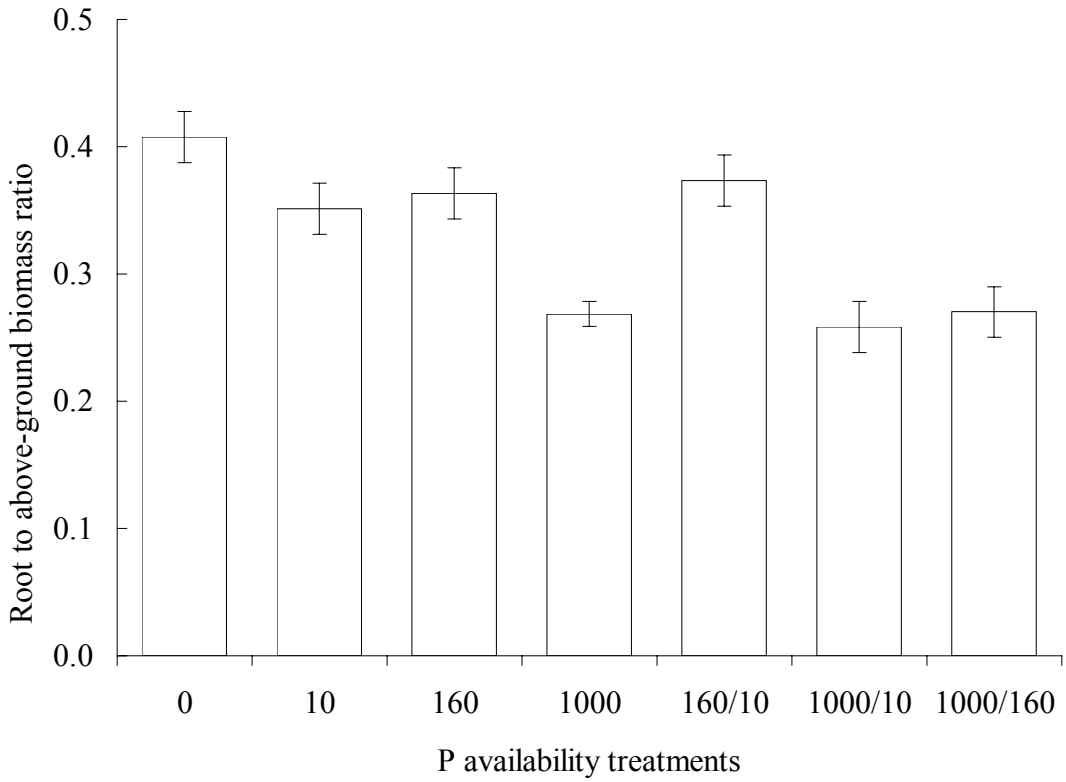
Source of variation	**ANOVA output interpretation** (p values)				**Wilks' Lambda multivariate test** (p values)
	Root dry weight partitioning (g) (4 variables)				
	Lower root-stem DW	Upper root-stem DW	Adventitious root DW	Basal root DW	
Mulch treatments (A)	n.s.	n.s.	n.s.	n.s.	n.s.
Bean cultivars (B)	n.s.	n.s.	n.s.	<u>0.0123</u>	0.1179
Blocks	<u>0.0012</u>	<u>0.0026</u>	n.s.	<u>0.0285</u>	
Interaction (AxB)					n.s.
Bean cultivars (B)	**Duncan's test interpretation**				
	Lower root-stem DW	Upper root-stem DW	Adventitious root DW	Basal root DW	
Chirripo Rojo	0.04±0.00 (A)	0.18±0.02 (A)	0.08±0.02 (A)	0.28±0.03 (B)	
Negro Huasteco	0.04±0.01 (A)	0.17±0.02 (A)	0.11±0.03 (A)	0.37±0.05 (A)(B)	
Dor-364	0.03±0.00 (A)	0.19±0.02 (A)	0.08±0.01 (A)	0.33±0.03 (B)	
CIAT G-1937	0.04±0.01 (A)	0.19±0.03 (A)	0.09±0.02 (A)	0.46±0.04 (A)	

Same letters in the same column indicate no significant differences (p <0.05) by the Duncan's test. The underlined p values indicate significant differences at p <0.05.
n.s. = non significant differences at p <0.05. Values are means ± standard errors, n=9.

Appendix 34. Analysis of variance, orthogonal contrast, Duncan's and multivariate test outputs for root architecture of bean cultivars under different mulch application treatments harvested at the flowering phase in Experiment 2, San Juan Sur,Turrialba, Costa Rica.

Source of variation	**ANOVA output interpretation** (*p* values)					**Wilks' Lambda multivariate test** (*p* values)
	Root architecture (5 variables)					
	Number of basal roots	Number of adventitious roots	Number of nodules	Lower root-stem length	Upper root-stem length	
Mulch treatments (A)	n.s.	0.0033	0.0101	n.s.	n.s.	0.0017
Bean cultivars (B)	0.0707	n.s.	0.0002	n.s.	0.0498	0.0002
Blocks	n.s.	n.s.	n.s.	0.0405	n.s.	
Interaction (AxB)						n.s.
Mulch comparisons	**Orthogonal contrast outputs**					
Control VS. Mulches	0.0737	0.0008	n.s.	n.s.	n.s.	
Cajanus VS. *Tithonia*	n.s.	n.s.	0.0026	n.s.	n.s.	
Bean cultivars	**Duncan's test interpretation**					
	(units)	(units)	(units)	(mm)	(mm)	
Chirripo Rojo	8±0 (B)	15±1 (A)	32±6 (B)	6.39±0.54 (A)	35.4±3.6 (A)	
Negro Huasteco	10±1 (A)	16±2 (A)	74±11 (A)	7.04±0.73 (A)	25.3±2.3 (B)	
Dor-364	10±1 (A)	19±2 (A)	39±7 (B)	8.16±1.07 (A)	37.1±2.8 (A)	
CIAT G-1937	9±0 (A)(B)	15±2 (A)	91±17 (A)	6.95±0.36 (A)	32.9±3.4 (A)(B)	

Same letters in the same column indicate no significant differences ($p < 0.05$) by the Duncan's test. The underlined *p* values indicate significant differences at $p < 0.05$.
n.s. = non significant differences at $p < 0.05$. Values are means ± standard errors, n=9.



Appendix 35. Root to above-ground biomass ratio for the Costa Rican *T. diversifolia* genotype potted plants at different and combined P availability treatments in sand culture harvested six-weeks after planting. Standard error bars are displayed (n=18). The last three P availability treatments (160/10, 100/10 and 1,000/160) correspond to different combinations of P availability levels in the same pots. P treatment names indicate the P availability levels in µM of P.