

Article

Production of *Phaseolus vulgaris* L. Genotypes with *Tithonia diversifolia* (Hemsl.) Gray and *Cajanus cajan* (L.) Millsp.

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Abstract: Adding mulch biomass prior to crop seeding may improve production of tropical soil. We evaluated the response of four bean (*Phaseolus vulgaris* L.) genotypes to the addition of mulch biomass from *Tithonia diversifolia* (Hemsl.) Gray and *Cajanus cajan* L. Millsp. The addition of mulch did not result in significant differences ($p < 0.05$) in soil characteristics when compared to a control (no mulch addition) except for soil potassium (K), which was significantly greater ($p < 0.05$) in the *T. diversifolia* mulch biomass treatment. Bean yield and shoot biomass were significantly greater ($p < 0.05$) in the mulch biomass treatments compared to the control (no biomass added). In these treatments, Phosphorus (P)-efficient bean genotypes had a significantly greater ($p < 0.05$) yield and shoot biomass. Bean shoot nutrient concentrations were significantly different ($p < 0.05$) between mulch biomass treatments and between bean genotypes (P, K and magnesium (Mg) only). Phosphorus utilization and uptake efficiencies were significantly different ($p < 0.05$) between mulch biomass treatments and between bean genotypes. Bean root biomass was not significantly different ($p < 0.05$) between mulch biomass treatments, but was significantly different ($p < 0.05$) between bean genotypes. The number of root nodules was significantly greater ($p < 0.05$) in the *T. diversifolia* mulch biomass treatment and was significantly different between bean genotypes.

Keywords: bean genotypes; mulch; bean yield; shoot biomass; phosphorus uptake efficiency; phosphorus utilization efficiency

1. Introduction

Phosphorus (P) is a limiting factor for plant production on steep slopes in the humid tropics, where many weathered volcanic ash soils (Andosols) are located [1]. Low P availability can often be crop yield-limiting; and it is difficult to mitigate, because of P fixation by iron- and aluminum-oxides [2]. In addition, many parts of the world have inadequate access to fertilizers, because sources of high-grade P for fertilizer processing are limited and/or fertilizers are not economically feasible for agricultural producers [3].

Management strategies, such as the development of P-efficient genotypes, may help improve crop production. For example, the common bean (*Phaseolus vulgaris* L.) is the most important dietary legume in Latin America, because it contains up to 25% protein, in addition to its large fiber and complex carbohydrate content [4]. However, bean yields continue to be low in Central America, due to poor soil P availability [5]. Bean genotypes that can efficiently use soil P reserves have played a significant role in improving food security in this region [6]. Lynch [7] found that efficient bean genotypes have greater root mass, root length and root/shoot ratio, and their roots demonstrated an extraordinary ability to sense and respond to localized changes in P availability. To date, the bean breeding program has focused on improving this crop's root architecture for enhancing P uptake [8]. Henry *et al.* [6] suggested that traits that increase P acquisition may also result in an increase in bean shoot biomass and yield, in addition to a greater concentration of P in shoot tissue.

The addition of manure, compost or plant biomass (mulch) in a cut-and-carry system is an agroecosystem management strategy commonly used in tropical regions to help maintain soil fertility and improve P availability for crop growth [9]. In a cut-and-carry system, defined as the transfer of biomass from external sources to the area of crop production, mulch is strategically located on the soil surface. The mulch biomass acts as a soil protector by minimizing erosion and as a soil amendment by enhancing soil fertility and crop yield [10]. Biomass for cut-and-carry systems is typically obtained from perennial trees or legumes that are capable of acquiring a large fraction of P from relatively less available forms of soil P and are capable of accumulating a greater P concentration in leaves [11]. Palm *et al.* [12] suggested that some biomass materials with a greater P ($>2.5 \text{ mg P g}^{-1}$) concentration have the potential to increase P availability in soils. The Mexican sunflower [*Tithonia diversifolia* (Hemsl.) Gray] may be a suitable shrub species for cut-and-carry systems, because of its greater foliar nutrient content [13]. In Ghana, Partey *et al.* [14] observed that *T. diversifolia* leaves had the greatest rate of decomposition and nutrient release rates when compared to four other leguminous species [*Senna spectabilis* (DC) H. Irwin & Barnaby, *Gliricidia sepium* (Jacq.) Walp., *Laucaena leucacephala* (Lam.) De Wit and *Acacia auriculiformis* Benth.] used as mulch biomass in agroforestry systems.

For the long-term sustainability and assurance of food and soil security, the addition of mulch biomass in combination with mineral P fertilizer or manure may be the most optimal fertilization strategy [12]. To date, most studies have evaluated the response of *T. diversifolia* mulch biomass

addition on soil fertility and crop production [15–17]. Some studies have also evaluated the influence of *T. diversifolia* mulch biomass on bean genotype production on a P-limited soil [18]. However, no study has evaluated the response of bean genotypes to the application of leguminous and non-leguminous mulch biomass in a P-limited soil. The objectives of this study were to quantify biomass production, nutrient concentrations and yield of four different bean genotypes in response to the addition of mulch biomass from *T. diversifolia* and *Cajanus cajan* (L.) Millsp. in a cut-and-carry system to a P-deficient Andosol.

2. Materials and Methods

2.1. Study Site and Experimental Design

The research was conducted at the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) Experimental Station located in San Juan Sur (9°53'N, 83°38'W), Costa Rica. The average annual precipitation was 2679 mm, the mean annual temperature was 21.8 °C and the site was located 950 m above sea level. The soil was classified as Andosol (FAO), with a clay loam to clay texture and a reddish-brown color. The soil structure and bulk density (0.68 Mg m^{-3}) allowed for good internal drainage in the upper 20 cm of the soil profile. Prior to initiating this study, the soil was characterized to a 20 cm depth with a pH (water) of 4.9, an exchangeable acidity of $1.35 \text{ cmol}_c^+ \text{ L}^{-1}$ and an exchangeable aluminum of 1.27% [18]. Values of exchangeable cations ($\text{cmol}_c^+ \text{ L}^{-1}$) ranged from 0.19 [calcium (Ca)], 0.13 [magnesium (Mg)] to 0.07 (K), with a P retention of 96.5% and an availability of 3.5 mg L^{-1} [18]. The study site was surrounded by a very humid pre-montane native tropical forest [19] and extensive agricultural production with *P. vulgaris*, maize (*Zea mays* L.) and coffee (*Coffea arabica* L.).

The site was previously dominated by natural fallow vegetation and was not fertilized for two years. Three months before the study was initiated, the fallow vegetation was cut and removed, and the site was ploughed and amended with 900 g m^{-2} poultry manure and 260 g m^{-2} calcium carbonate (CaCO_3). Analysis of the poultry manure showed a nutrient concentration of 27.2 g N kg^{-1} , 24.8 g P kg^{-1} , 28.1 g K kg^{-1} , $9.5 \text{ Mg mg kg}^{-1}$ and 107 g Ca kg^{-1} .

The study was a split plot design. The experimental units were randomly assigned treatments of fresh mulch biomass input from *T. diversifolia* (8000 g m^{-2}), *C. cajan* (900 g m^{-2}) and an un-mulched control. Each experimental unit was replicated three times ($n = 3$), with a whole plot size of $6 \text{ m} \times 5.5 \text{ m}$. *Tithonia diversifolia* and *C. cajan* biomass was cut above the soil surface in an adjacent site [17] and were chosen because of their potential to increase soil P or soil P availability.

Immediately after adding the mulch biomass, the whole plots of the experimental units were divided into four subplots, each with a size of $3 \text{ m} \times 2.75 \text{ m}$. Each subplot was randomly assigned to four different bean genotypes, including Chirripo Rojo, Negro Huasteco, Dor-364 and CIAT G-1937. Bean genotypes were seeded, using two seeds per planting hole, at a spacing of 0.3 m within row and 0.4 m between rows. After bean emergence, plants were thinned to one plant per planting hole, and missing plants were replanted using thinned plants. The bean genotypes were inoculated with *Rhizobium*, which was added as a thin layer to the beans at sowing time using a sugar solution as an adherent. The crops were managed according to local practices, including a planting density of $166,666 \text{ plants ha}^{-1}$,

manual weeding, manual harvest of pods and retaining crop residues in the field. This study was conducted during the bean growing season, which occurred from March through June in 2002. Three of the four bean genotypes belonged to the Mesoamerican gene pool. Chirripo Rojo, a newly developed P-efficient genotype, also referred to as Bribri or UCR-55, is one of the most productive Costa Rican genotypes used by local small-scale growers [20]. Negro Huasteco is an older Costa Rican genotype, which has been planted successfully for many years. Dor-364 is a high yielding commercial genotype developed in Central America [21]. CIAT G-1937 was developed by the International Centre for Tropical Agriculture (CIAT) in Cali, Colombia, and is characterized to be high yielding under P-limited conditions [22].

2.2. Soil, Shoot and Root Characteristics of *P. vulgaris* Genotypes

At the bean flowering phase (43 days after seeding), four random soil samples (0–12 cm) were extracted from each subplot using a soil corer with a 2.5 cm inner diameter. The extracted soil was air-dried and sieved (2 mm) and evaluated for soil chemical characteristics, including pH (1 soil:2.5 water suspension), exchangeable acidity and exchangeable cations K, Ca and Mg ($\text{cmol}_c^+ \text{L}^{-1}$; modified Olsen, pH 8.5 and 1 N KCl extractants) and available P (mg kg^{-1} ; Mehlich No. 3).

Within each subplot, beans were harvested from a randomly selected area of 1.2 m² at the flowering phase (43 days after seeding). The beans were harvested using a wide spade to excavate each individual plant with its root system. Bean shoots were dried at 60 °C until a constant dry weight was obtained. The dried samples were weighed, ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) to 1 mm and analyzed for P, K, Ca and Mg (mg g^{-1}) using wet digestion [23] in a mixture of 5:1 nitric-perchloric acid by atomic absorption (Perkin Elmer 100, Norwalk, CT, USA).

Soil and decomposed debris was removed from bean roots in the field, and the number of nodules was counted. Roots were hand washed over a 0.2 mm sieve to remove any remaining soil and placed in a petri dish for further removal of non-root derived debris. The washed roots were scanned using WinRhizo (v. 4.1; Regent Instruments, Quebec, Canada). Specific root length (SRL) was determined by dividing root length into root dry mass. Once root length was determined, the roots were dried at 60 °C until a constant dry weight was obtained to determine their biomass. After 90 days of bean growth, beans were harvested from a randomly selected area 1.2 m² within each subplot to determine yield. Harvested bean pods were air-dried to separate beans from pods and subsequently weighed to estimate water content in order to express yield on a 5% water content. Phosphorus uptake efficiency ($\text{mg P m g}^{-1} \text{SRL}$) was quantified by dividing P content in dry weight by the specific root length, whereas P utilization efficiency ($\text{g DW mg}^{-1} \text{P}$) is the inverse of P concentration of bean shoot dry weight [24].

2.3. Statistical Analysis

All data were examined for homogeneity of variance and found to have normal distributions using SPSS (SPSS Science Inc., Armonk, NY, USA, 2009). To quantify differences between mulch biomass treatments with respect to soil chemistry, bean shoot and root characteristics and between genotypes, data were analyzed using ANOVA in SPSS (SPSS Science Inc. 2009). Significantly different main effects were further tested using the least significant differences multiple comparison test [25].

Significant simple effects were tested using the estimated marginal mean function in SPSS. For all statistical analyses, the threshold of probability level for determining significant differences was $p < 0.05$.

3. Results

Bean genotypes did not influence soil characteristics significantly. As such, values presented in Table 1 are mean values of soil chemical characteristics from all bean subplots within each treatment. Only K was significantly different between mulch treatments, showing a greater concentration in the *T. diversifolia* mulch biomass treatment.

Table 1. Soil chemical characteristics (0–12 cm) of an Andosol at the bean flowering phase in treatments with no mulch or mulch biomass from *T. diversifolia* and *C. cajan* in a Costa Rican Andosol. Standard errors are given in parentheses.

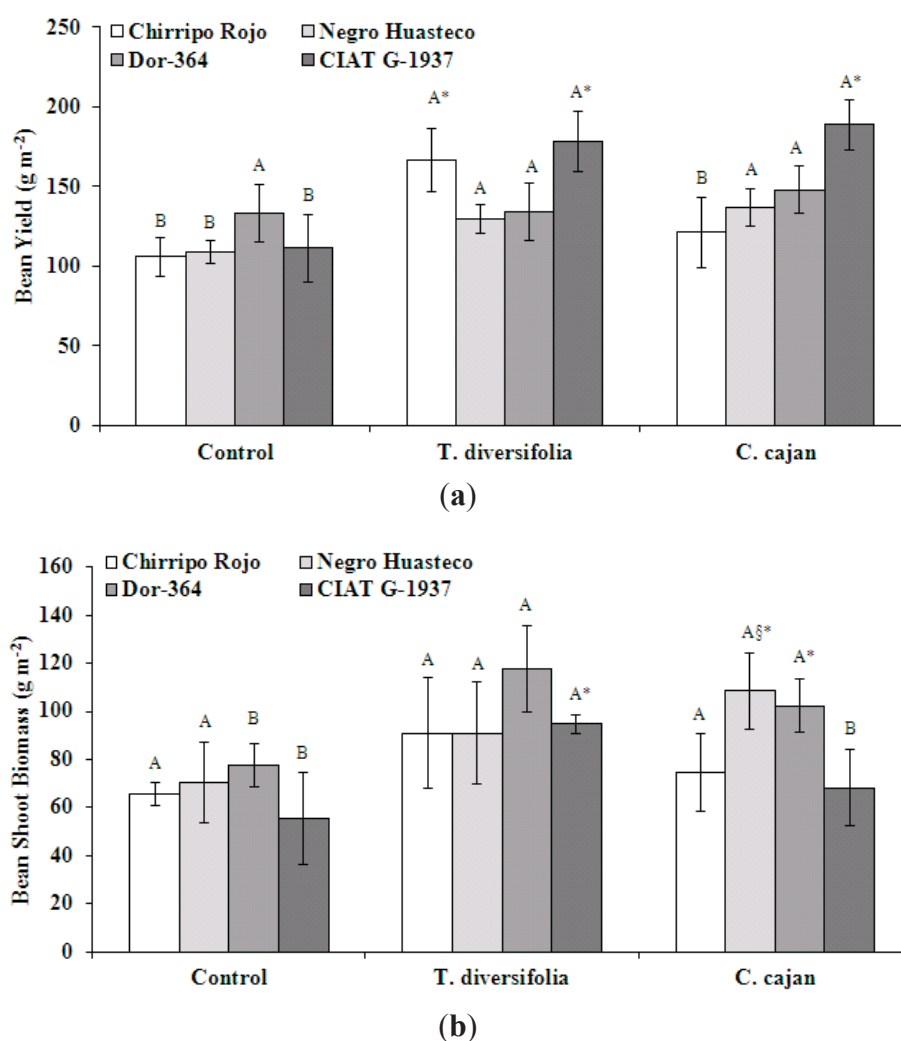
	Control	<i>T. diversifolia</i>	<i>C. cajan</i>
pH	4.9 (0.1) ^A	4.7 (0.1) ^A	4.9 (0.1) ^A
Exchangeable acidity (cmol _c ⁺ L ⁻¹)	1.3 (0.2) ^A	1.2 (0.1) ^A	1.1 (0.2) ^A
Available P (mg kg ⁻¹)	6.6 (1.0) ^A	7.0 (0.6) ^A	6.6 (0.5) ^A
Exchangeable K (cmol _c ⁺ L ⁻¹)	0.2 (0.1) ^B	0.4 (0.1) ^A	0.2 (0.1) ^B
Exchangeable Ca (cmol _c ⁺ L ⁻¹)	1.9 (0.5) ^A	1.7 (0.4) ^A	1.8 (0.4) ^A
Exchangeable Mg (cmol _c ⁺ L ⁻¹)	0.3 (0.1) ^A	0.3 (0.1) ^A	0.3 (0.1) ^A

Values followed by different upper case letter, comparing differences between the control and mulch treatments, are significantly different at $p < 0.05$.

Bean yield (g m⁻²) was significantly different between mulch biomass treatments; with a significantly greater yield in both mulch biomass treatments compared to the control (Figure 1a). When yield is expressed as a mean value of bean genotypes, yield for *T. diversifolia* was 152 g m⁻², 149 g m⁻² for *C. cajan* and 115 g m⁻² for the control treatment. Chirripo Rojo and CIAT G-1937 had a significantly greater yield in the *T. diversifolia* mulch biomass treatment. In the *C. cajan* mulch biomass treatment, only CIAT G-1937 had a significantly greater yield. Expressing yield as a mean value of mulch biomass treatments, CIAT G-137 (159 g m⁻²) had the greatest yield followed by Dor-364 (138 g m⁻²), Chirripo Rojo (131 g m⁻²) and Negro Huasteco (125 g m⁻²). The interaction effects of mulch treatment-by-bean genotype were not significant for bean yield [$F(6, 25) = 0.619$, $p = 0.713$].

Bean shoot biomass (g m⁻²) was significantly different between mulch biomass treatments (Figure 1b), with the greatest biomass in the *T. diversifolia* mulch treatment for the Dor-364 and CIAT G-1937 genotypes. When shoot biomass is expressed as a mean value of bean genotypes, shoot biomass production for *T. diversifolia* was 99 g m⁻², 88 g m⁻² for *C. cajan* and 67 g m⁻² for the control treatment. Only Negro Huasteco and Dor-364 were significantly greater compared to the other genotypes in the *C. cajan* mulch biomass treatment. Expressing shoot biomass as a mean value of mulch treatments, Dor-364 (99 g m⁻²) had the greatest yield followed by Negro Huasteco (90 g m⁻²), Chirripo Rojo (77 g m⁻²) and CIAT G-1937 (73 g m⁻²). The interaction effects of mulch treatment-by-bean genotype were not significant bean shoot biomass [$F(6, 25) = 0.514$, $p = 0.792$].

Figure 1. (a) Bean yield (g m^{-2}) at harvest of four different bean genotypes in treatments with no mulch or mulch from *T. diversifolia* or *C. cajan* of four different genotypes in treatments with no mulch or mulch from *T. diversifolia* or *C. cajan* on a Costa Rican Andosol. Different upper case letters above standard error bars indicate significant differences at $p < 0.05$ in the comparison of mulch treatments within the same genotype. Values followed by *, comparing differences between bean genotypes and within mulch treatments, are significantly different at $p < 0.05$; **(b)** Bean shoot biomass (g m^{-2}) at the flowering phase of four different genotypes in treatments with no mulch or mulch from *T. diversifolia* or *C. cajan* on a Costa Rican Andosol. Different upper case letters above standard error bars indicate significant differences at $p < 0.05$ in the comparison of mulch treatments within the same genotype (§ *C. cajan* was significantly different from the control only). Values followed by *, comparing differences between bean genotypes and within mulch treatments, are significantly different at $p < 0.05$.



Bean shoot nutrient concentrations (mg g^{-1}) were significantly different between mulch biomass treatments (Table 2). Bean shoot P concentration was significantly greater in the *T. diversifolia* mulch biomass treatment compared to *C. Cajun* (CIAT G-1937 genotype only) and the control (Chirripo Rojo and CIAT G-1937 genotypes only). Potassium concentrations followed a similar pattern, showing a

significantly greater concentration of this nutrient in the *T. diversifolia* mulch biomass treatment compared to that with *C. cajan* (Negro Huasteco genotype only) and the control (Negro Huasteco, Dor-364 and CIAT G-1937 bean genotypes only). The control treatment had a significantly greater Ca concentration compared to the treatment with *T. diversifolia* (Chirripo Rojo, Negro Huasteco and CIAT G-1937 genotypes only) and *C. Cajun* (Negro Huasteco and CIAT G-1937 genotypes only). The control treatment also had a significantly greater Mg concentration compared to the treatment with *T. diversifolia* (Chirripo Rojo, Negro Huasteco and CIAT G-1937 genotypes only) and *C. Cajun* (Negro Huasteco and CIAT G-1937 genotypes only). When comparing differences between bean genotypes, only Chirripo Rojo had a significantly lower P concentration in the control treatment, whereas K concentration was significantly greater for CIAT G-1937 in the *T. diversifolia* mulch biomass treatment. For Mg, Negro Huasteco had a significantly lower concentration of this nutrient in the control treatment. The interaction effects of mulch treatment-by-bean genotype were not significant shoot P [$F(6, 24) = 0.413, p = 0.863$], K [$F(6, 24) = 0.377, p = 0.886$], Ca [$F(6, 24) = 0.477, p = 0.819$] and Mg [$F(6, 24) = 0.675, p = 0.671$] concentrations.

Table 2. Shoot nutrient concentrations (mg g^{-1}) of four bean genotypes at the flowering phase in treatments with biomass addition from *T. diversifolia* or *C. cajan* or without biomass addition (control) on a Costa Rican Andosol. Standard errors are given in parentheses.

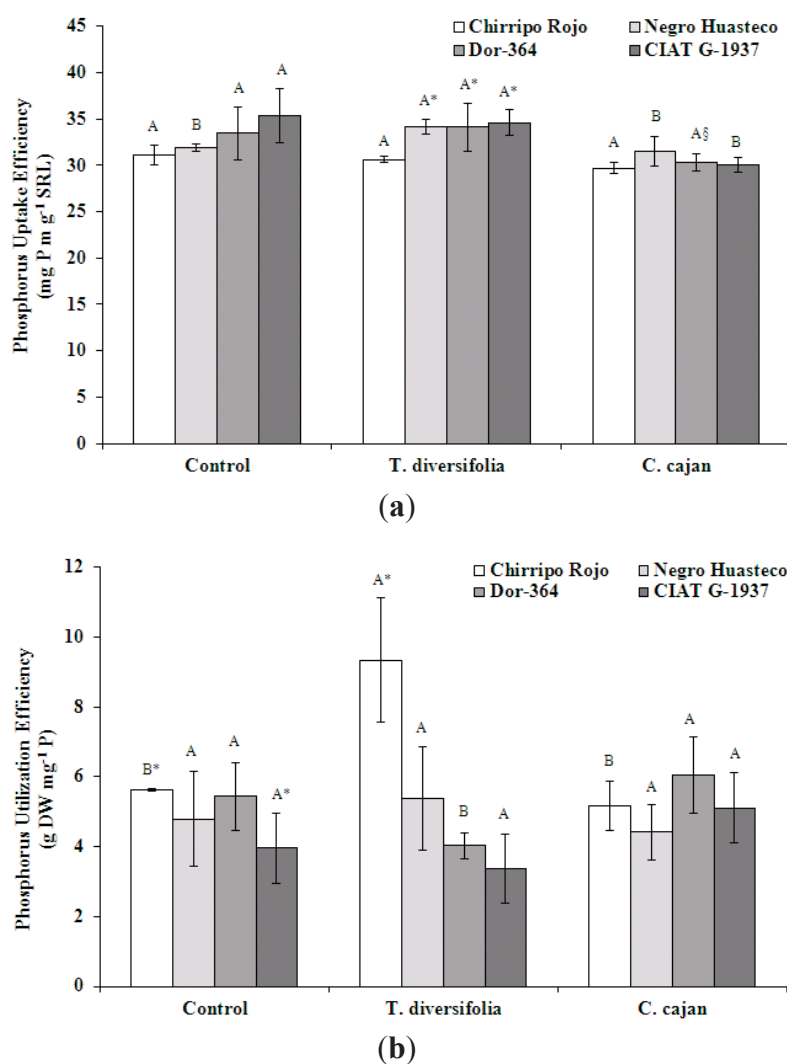
		P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})
Control	Chirripo Rojo	2.23 (0.18) ^B *	28.3 (5.8) ^A	19.9 (2.1) ^A	3.97 (0.80) ^A
	Negro Huasteco	3.13 (0.33) ^A	31.3 (5.5) ^B	22.3 (1.2) ^A	2.80 (0.21) ^A *
	Dor-364	3.03 (0.26) ^A	28.3 (7.2) ^B	21.1 (1.4) ^A	3.33 (0.18) ^A
	CIAT G-1937	2.87 (0.23) ^B	29.6 (8.5) ^B	20.5 (0.9) ^A	4.43 (0.88) ^A
<i>Tithonia diversifolia</i>	Chirripo Rojo	3.37 (0.07) ^A	35.6 (3.4) ^A	16.0 (1.1) ^B	3.47 (0.48) ^A
	Negro Huasteco	3.20 (0.18) ^A	40.5 (3.2) ^A	18.3 (2.1) ^B	2.28 (0.17) ^B
	Dor-364	3.30 (0.10) ^A	41.5 (1.6) ^A	17.8 (2.5) ^A	2.63 (0.34) ^B
	CIAT G-1937	3.35 (0.07) ^A	46.9 (2.1) ^A *	17.5 (0.4) ^B	3.05 (0.61) ^B
<i>Cajanus cajan</i>	Chirripo Rojo	3.27 (0.33) ^A	39.1 (3.8) ^A	19.0 (0.8) ^A	2.87 (0.26) ^B
	Negro Huasteco	2.93 (0.17) ^A	34.6 (1.2) ^B	16.7 (2.2) ^B	2.47 (0.15) ^A
	Dor-364	2.97 (0.23) ^A	36.9 (5.0) ^A	17.9 (1.5) ^A	2.77 (0.18) ^B
	CIAT G-1937	3.03 (0.18) ^B	41.8 (3.6) ^A	18.7 (2.0) ^B	2.63 (0.15) ^B

Values followed by different upper case letters indicate significant differences at $p < 0.05$ in the comparison of mulch treatments within the same bean genotype. Values followed by *, comparing differences between bean genotypes and within mulch treatments, are significantly different at $p < 0.05$.

Phosphorus uptake efficiency (mg P m g^{-1} SRL) was significantly different between mulch biomass treatments (Figure 2a). Negro Huasteco had a significantly greater P uptake efficiency in the *T. diversifolia* mulch biomass treatment compared to the control. Phosphorus uptake efficiency was also significantly greater for Negro Huasteco, Dor-364 and CIAT G-1937 in the *T. diversifolia* mulch biomass treatment compared to *C. cajan*. When P uptake efficiency is expressed as a mean value of bean genotypes, P uptake efficiency was 33 mg P m g^{-1} SRL for the *T. diversifolia* and control treatments and 30 mg P m g^{-1} SRL for the *C. cajan* mulch biomass treatment. Negro Huasteco, Dor-364 and CIAT G-1937 were significantly greater compared to Chirripo Rojo only in the

T. diversifolia mulch biomass treatment. Expressing P uptake efficiency as a mean value of mulch treatments, P uptake efficiency was 33 mg P m g⁻¹ SRL (*T. diversifolia*) and 30 mg P m g⁻¹ SRL (*C. cajan*) for Chirripo Rojo. The interaction effects of mulch treatment-by-bean genotype were not significant for P uptake efficiency [$F(6, 25) = 0.498, p = 0.804$].

Figure 2. (a) Bean genotype phosphorus uptake efficiency (mg P m g⁻¹ SRL), where SRL is specific root length at the flowering phase in treatments with no mulch or mulch from *T. diversifolia* or *C. cajan* on a Costa Rican Andosol. Different upper case letters above standard error bars indicate significant differences at $p < 0.05$ in the comparison of mulch treatments within the same genotype (*C. cajan* were significantly different from *T. diversifolia* only). Values followed by *, comparing differences between bean genotypes and within mulch treatments, are significantly different at $p < 0.05$; (b) Bean genotype phosphorus utilization efficiency (g DW mg⁻¹ P) at the flowering phase in treatments with no mulch or mulch from *T. diversifolia* or *C. cajan* on a Costa Rican Andosol. Different upper case letters above standard error bars indicate significant differences at $p < 0.05$ in the comparison of mulch treatments within the same genotype. Values followed by *, comparing differences between bean genotypes and within mulch treatments, are significantly different at $p < 0.05$.

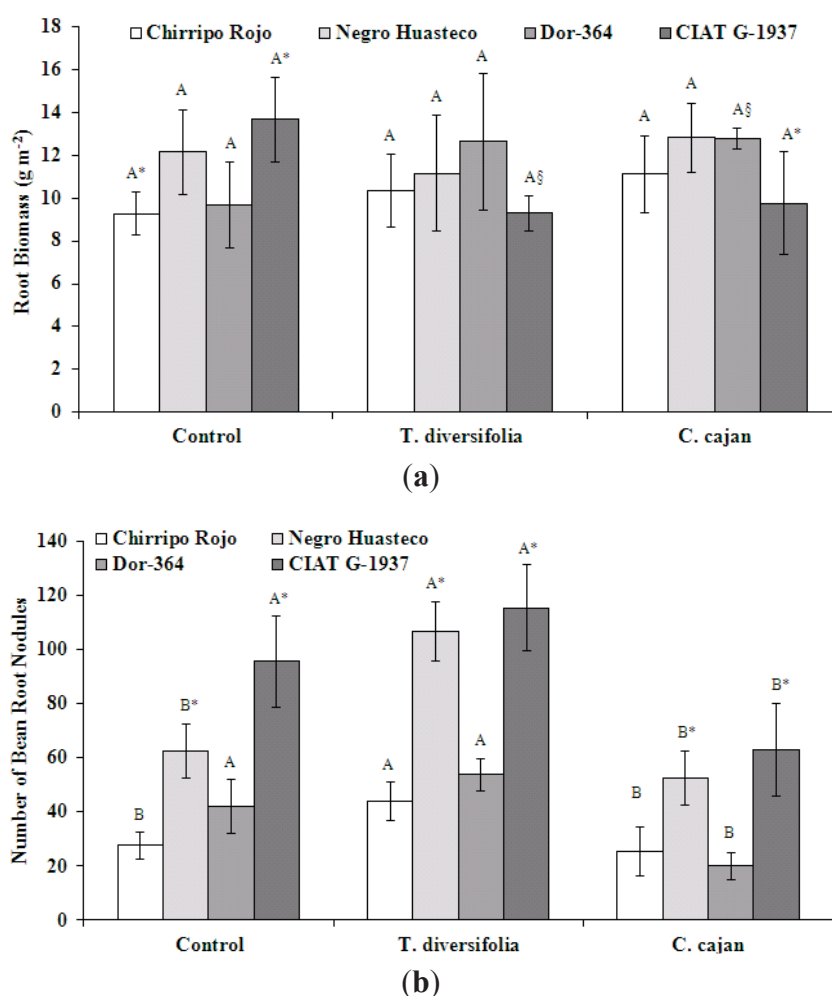


Phosphorus utilization efficiency ($\text{g DW mg}^{-1} \text{P}$) was significantly different between mulch biomass treatments, with the greatest utilization efficiency occurring in Chirripo Rojo in the *T. diversifolia* treatment (Figure 2b). When P utilization efficiency is expressed as a mean value of bean genotypes; P utilization efficiency was $5 \text{ g DW mg}^{-1} \text{P}$ for the *C. cajan* and control treatments and $6 \text{ g DW mg}^{-1} \text{P}$ for the *T. diversifolia* mulch biomass treatment. Chirripo Rojo had a significantly greater P utilization efficiency compared to CIAT G-1937 in the control treatment only. Expressing P uptake efficiency as a mean value of mulch treatments, P uptake efficiency was $7 \text{ g DW mg}^{-1} \text{P}$ for Chirripo Rojo, $5 \text{ g DW mg}^{-1} \text{P}$ for Negro Huasteco and Dor-364 and $4 \text{ g DW mg}^{-1} \text{P}$ for CIAT G-1937. The interaction effects of mulch treatment-by-bean genotype were not a significant P utilization efficiency [$F(6; 25) = 0.888; p = 0.519$].

Bean root biomass (g m^{-2}) was not significantly different between mulch treatments (Figure 3a). When root biomass is expressed as a mean value of bean genotypes, root biomass was $\sim 11 \text{ g m}^{-2}$ for all treatments. Differences between bean genotypes showed that Chirripo Rojo had a significantly lower root biomass compared to CIAT G-1937 in the control treatment only. Expressing root biomass as a mean value of mulch treatments, root biomass was 12 g m^{-2} for Negro Huasteco and Dor-364, 11 g m^{-2} CIAT G-1937 and 10 g m^{-2} for Chirripo Rojo. The interaction effects of mulch treatment-by-bean genotype were not significant for root biomass [$F(6, 25) = 0.833, p = 0.556$].

The number of root nodules was significantly different between mulch biomass treatments (Figure 3b). The number of root nodules was significantly greater for Chirripo Rojo and Negro Huasteco in the *T. diversifolia* mulch biomass treatment compared to the control. All genotypes had a significantly greater number of root nodules in the *T. diversifolia* mulch biomass treatment compared to the *C. cajan* treatment. When the number of root nodules is expressed as a mean value of bean genotypes, the number of nodules was 80 in the *T. diversifolia* mulch treatment, 57 in the control and 40 in the *C. cajan* mulch biomass treatment. In all three treatments, CIAT G-1937 had a significantly greater number of root nodules. In the *T. diversifolia* and *C. cajan* treatment, Negro Huasteco also had a significantly greater number of root nodules. Expressing the number of root nodules as a mean value of mulch treatments, the root nodule number was 91 for CIAT G-1937, 71 for Negro Huasteco, 39 for Dor-364 and 32 for Chirripo Rojo. The interaction effects of mulch treatment-by-bean genotype were not significant for the number of root nodules [$F(6, 25) = 0.637, p = 0.699$].

Figure 3. (a) Root biomass (g m^{-2}) of four different bean genotypes at the flowering phase grown in treatments with no mulch or mulch from *T. diversifolia* or *C. cajan* on a Costa Rican Andosol. Different upper case letters above standard error bars indicate significant differences at $p < 0.05$ in the comparison of mulch treatments within the same genotype [*C. cajan* (within Dor-364) and *T. diversifolia* (within CIAT G-1937) were significantly different from the control only]. Values followed by *, comparing differences between bean genotypes and within mulch treatments, are significantly different at $p < 0.05$; **(b)** Number of root nodules of four different bean genotypes at the flowering phase grown in treatments with no mulch or mulch from *T. diversifolia* or *C. cajan* on a Costa Rican Andosol. Different upper case letters above standard error bars indicate significant differences at $p < 0.05$ in the comparison of mulch treatments within the same genotype. Values followed by *, comparing differences between bean genotypes and within mulch treatments, are significantly different at $p < 0.05$.



4. Discussion

Results from this study showed that the addition of mulch from *T. diversifolia* or *C. cajan* did not substantially affect soil chemistry, including soil pH. Similarly, Amusan *et al.* [26] and Das *et al.* [27] did not observe changes in soil pH when either manure or biomass or a combination of manure and biomass were added to an acidic soil in southwestern Nigeria and India. The lack of a significant

increase in soil pH may also be due to the smaller amount of mulch biomass added from *T. diversifolia* or *C. cajan* compared to other studies [28]. However, the significantly greater soil K concentration was likely due to a larger quantity of this nutrient in *T. diversifolia* biomass compared to *C. cajan*. Mustonen *et al.* [17] observed that the concentration of K in aboveground *T. diversifolia* biomass was 20.1 mg g^{-1} compared to 15.5 mg g^{-1} in *C. cajan*.

A lack of change in most soil chemical properties evaluated in this study, despite the addition of various amendments, may also be due to the short-term nature of this study. Miller and Miller [29] suggested that the application of organic materials to crop land could affect soil properties, but this may not be apparent over the short-term (<5 years). For example, The *et al.* [30] found that the long-term application of organic amendments resulted in increased soil P, Ca, Mg and organic matter; and the slow release of these nutrients increased soil fertility and crop yield in subsequent years [31].

Bean yield was within range of that reported by Mustonen *et al.* [17], Lopez [32] and Gosh *et al.* [33] in the *T. diversifolia* and *C. cajan* mulch biomass treatments. Results from our study were similar to that of Mukuralinda *et al.* [34], who found that maize grain yield in Rwanda increased three-fold when biomass from *T. diversifolia* was added to the growing maize crop. The increased bean yield observed in our study likely occurred due to the additional nutrients contributed from the input of *T. diversifolia* or *C. cajan* mulch biomass [32,33]. Additionally, previous application of poultry manure on this site in combination with the mulch biomass input from *T. diversifolia* or *C. cajan* may have resulted in a synergistic effect that positively influenced bean yield, especially in P efficient genotypes, such as CIAT G-1937 [26]. This is because organic resources from mulch biomass treatments may have the capacity to enhance the availability of P through a variety of mechanisms, including the blocking of P-sorption sites, preventing P-fixation and stimulating microbial P-uptake [1].

Bean shoot biomass fell within range of that reported from other studies in Central America [35]. For example, in Costa Rica, Henry *et al.* [36] quantified a shoot biomass of 98 g m^{-2} for Chirripo Rojo, which was similar to that in the *T. diversifolia* mulch biomass treatment in our study. Aboveground biomass production of all bean genotypes in our study was greater compared to that reported by Roy *et al.* [37] in control and mulch treatments. This may be due to the prior input of poultry manure in our study in addition to a better quality of the mulch biomass from *T. diversifolia* or *C. cajan* compared to that of rice straw (*Oryza sativa* L.) and tropical whiteweed (*Ageratum conyzoides* L.) in the study by Roy *et al.* [37].

Miller *et al.* [8] and Fageria *et al.* [38] also observed a greater variability among bean genotypes with respect to bean yield and biomass production. Although shoot biomass production is closely related to yield, this relationship may be difficult to establish in legumes [38]. This was evident in the P-efficient CIAT G-1937 genotype, which had a greater yield compared to shoot biomass in the mulch treatments. This suggested that the efficient genotypes may allocate a larger amount of resources to the production of pods rather than shoot biomass. In a field experiment in Brazil, evaluating the effect of soil chemistry on bean shoot nutrient concentration, Fageria *et al.* [5] reported results similar to that of our study and found that K had the greatest concentration and P the lowest concentration in bean shoot biomass. This suggested that bean genotypes responded differently to the addition of mulch biomass depending on the nutrient under evaluation [5]. Ho *et al.* [39] pointed out that such differences may be due to a variation in root architecture among bean genotypes and, therefore, their ability to take-up and accumulate nutrients in aboveground biomass.

Mukuralinda *et al.* [34] and Niang *et al.* [40] reported a 3.5-fold increase in P uptake in treatments with *T. diversifolia* mulch biomass compared to other commonly used agroforestry species in West Africa. This may be due to the larger nutrient quality of *T. diversifolia* biomass compared to that of *C. cajan* [17] and its greater mineralization potential [14]. Nielsen *et al.* [22] also found differences among bean genotypes, where the P-efficient CIAT G-1937 had a greater P uptake and utilization efficiency when grown under low, medium and high P availability compared to Dor-364. Although the common bean is a diverse species that includes genotypes with contrasting P uptake and utilization efficiencies, results from our study implied that differences in P utilization efficiency were minimal. Nielsen *et al.* [22] suggested that this is because, at the cellular level, efficient utilization of a commonly limiting nutrient may have been subject to natural selection, and utilization efficiency at the whole plant level may likely be related to fundamental differences in form, size and phenology.

Results from our study showed that the addition of mulch biomass from *T. diversifolia* or *C. Cajun* did not affect bean root biomass production. Similarly, Roy *et al.* [37] did not observe differences in bean root biomass in treatments with mulch, compost or vermicompost compared to a control. This suggested that the additional nutrients added from *T. diversifolia* and *C. cajan* mulch biomass were likely allocated to aboveground components, which was reflective of a larger bean yield. Miller *et al.* [8] found that variation among bean genotypes with respect to root biomass production is common even when levels of soil P were low or adequate. Results from our study support that of Nielsen *et al.* [22], who found that P efficient bean genotypes have a larger root biomass under low P availability. Our study showed that in the control treatment, the CIAT G-1937 genotype was more efficient in P uptake ($35 \text{ mg P m}^{-1} \text{ SRL}$) and P utilization ($4 \text{ g DW mg}^{-1} \text{ P}$) and had a larger root biomass (14 g m^{-2}) compared to Chirripo Rojo, which had a P uptake and utilization of $31 \text{ mg P m}^{-1} \text{ SRL}$ and $6 \text{ g DW mg}^{-1} \text{ P}$ and a root biomass of 9 g m^{-2} .

Vargas and Graham [41] found a large variation in the number of nodules on bean roots and noted that this was mostly dependent on the genotype. They observed that the number of nodules ranged from 0 to 190 per plant [41]. Increased number of root nodules, especially in the *T. diversifolia* mulch treatment, suggested that when soil amendments are used, the establishment of N_2 -fixing bacteria is facilitated, resulting in the formation of a larger number of nodules [42]. Teixeira *et al.* [43] indicated that nodulation depends on the P supply and differs between bean cultivars; some cultivars show an intense decline in the number of nodules after flowering. Time of sampling, therefore, may strongly affect the results in studies evaluating the effect of mulch biomass addition to P-limited soils. Additionally, the input of external amendments, such as manure, inorganic fertilizers or mulch biomass increased the number of nodules [33]. Gosh *et al.* [33] attributed this to the repeated application of organic matter from manure and/or mulch biomass, which led to an improved soil physical and chemical environment for nitrogenase activity [33].

5. Conclusions

A major challenge facing agricultural producers under current climate change scenarios is the long-term assurance of food and soil security. As such, sustainable agroecosystem management practices capable of maintaining or increasing crop yields, while at the same time conserving soil resources, are imperative. Results from the present study showed that the addition of mulch biomass

from non-leguminous (*T. diversifolia*) and leguminous (*C. cajan*) species in a cut-and-carry system did not lead to changes in soil chemistry, which was likely due to the short-term nature of this study. However, the addition of mulch biomass contributed to a greater bean yield, shoot biomass, nutrient concentration and number of root nodules compared to the control. This may be due to the additional input of nutrients from the mulch biomass. The added mulch biomass likely led to a nutrient synchronization between its mineralization and subsequent uptake of nutrients by the growing bean crop. This was also reflected in greater P uptake and utilization efficiencies in the mulch biomass treatments. Depending on the evaluated variable, results from this study indicated the influence of mulch biomass on bean production was greater than differences between bean genotypes. This suggested the potential for sustainable crop production solely through the addition of mulch biomass, which may also enhance soil fertility over the long-term. Future studies should focus on the potential of mulch biomass to enhance soil chemistry and ensure the long-term fertility of soil and P availability to the growing crops. Additionally, further information on treatment effect on bean genotype nodulation should also be taken into consideration in order to understand the response of bean genotypes to soil N. Future studies should also evaluate the long-term effects of manure and CaCO₃ input and their role in removing the most limiting soil chemical factors in weathered Andosols.

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