Effects of vinasse on rhizospheric bacteria and microbial C-biomass and CO_2-activity of a Pachic Haplustoll soil

Efectos de vinazas sobre bacterias rizosféricas y en la actividad-CO_2 y biomasa-C microbiana de un suelo Pachic Haplustoll

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Abstract

The effect of the application of vinasse, a byproduct of the fuel alcohol industry on growth promoting rhizospheric bacteria (*Pseudomonas fluorescens* and *Bacillus subtilis*), in CO_2-activity and microbial C-biomass and metabolic quotient-qCO_2 on a Pachic Haplustoll, and its relationship to the yield of green bean (*Phaseolus vulgaris* L.). We used a completely randomized design with seven treatments and five repetitions. The treatments were designed based on the requirements of K+ by the crop (150 kg/haK_2O, Vallejo and Estrada 2004) using as sources KCl and vinasse, alone and combined. The results show significant differences in activity and microbial biomass in the second and third sampling respectively. The highest values of microbial activity are presented in T5 and T7 minors; Microbial biomass presented the highest values in T3 and T7 minors. Rhizospheric bacteria differs significantly; *Pseudomonas fluorescens* in the second sampling, showing the highest values T5 and T6 minors. *Bacillus subtilis* differs in the third sample and the highest values reported in T4 and T1 minor. The negative correlation between metabolic ratio and bean yield, in this way, higher dry matter values are shown in the T6 and T5 minor.

Key words: Metabolic quotient, microbial biomass, *Phaseolus vulgaris*, rhizospheric bacteria; vinasse.

Resumen

En condiciones de casa de malla de la Universidad Nacional de Colombia sede Palmira se estudió los efectos de la aplicación de vinaza, un subproducto de la industria de alcohol carburante, sobre las bacterias rizosféricas *Pseudomonas fluorescens* y *Bacillus subtilis* promotoras de crecimiento, la actividad-CO_2, biomasa microbiana-C y el cociente metabólico-qCO_2 en un suelo Pachic Haplustoll y su relación con el rendimiento de habichuela (*Phaseolus vulgaris* L.). Se utilizó un diseño completa-mente al azar con siete tratamientos y cinco repeticiones. Los tratamientos se seleccionaron con base en los requerimientos de K del cultivo (150 kg/ha K_2O) utilizando como fuentes KCl y vinaza solos y en mezclas. Los tratamientos evaluados y la época de muestreo influyeron ($P < 0.05$) en la actividad y biomasa microbiana. Los menores valores de estas variables se presentaron en la época de floración del cultivo cuando la demanda de nutrientes es alta. La mezcla en partes iguales de vinaza y KCl favorece la mayor producción de habichuela sin afectar la actividad microbiana; el cociente metabólico indicó estabilidad del sistema en el tiempo y las bacterias rizosféricas presentaron el mejor crecimiento en la mezcla 75% de potasio como vinaza y 25% como KCl.

Palabras clave: Bacterias rizosféricas, biomasa microbiana, cociente metabólico, *Phaseolus vulgaris,* vinaza.

Introduction

The microbiological component of soil contributes to the improvement of physical, chemical and biological properties require keeping soil fertility (Sánchez and Gómez, 2001). The increase in production of agroindustrial residues requires the searching of new alternatives for their management. In the department of Valle del Cauca, Colombia, there is high volume of production of vinasse, and organic residue from sugarcane ethanol production. Because of its high organic matter content, vinasse is a highly contaminant material, especially for water sources since its COD and BOD values for a solid content of 10% w/w are 116,000 and 41,200 ppm, respectively, thus, it needs a suitable treatment before being used (García *et al.*, 2002).

Vinasse from agroindustrial processes has a variable pH between 3.5 and 4.5 and, a wide variety of organic compounds, among them, alcohol, aldehydes, ketones, esters, acids and sugars (Morales and Larrahondo, 1998) that when applied to the soil affects the benefic microorganisms in it.

Vinasses produced in the department of Valle del Cauca, Colombia, are characterized by an acidic pH and high contents of organic carbon, potassium, calcium, magnesium, sulfur and electrolyte concentration (Narváez *et al.*, 2010). They include between 2.1 and 3.4 kg/m^3 of K_2O (López and Da Silvieira, 2004) and are used, mainly, to supply potassium requirements in sugarcane and grape crops. Because of its high potassium content are an alternative to correct soil salinity problems; however, when applied special attention is needed on the changes to electrical conductivity (EC) (Subiros and Molina, 1992). Rojas *et al.* (2008) found that the hydraulic conductivity of saturated soils is reduced with increments in application of vinasse. On the other hand, Montenegro *et al.* (2009) estimated that the microbial biomass and the metabolic efficiency in the soil are not recovered in time when vinasse has been applied. Nárvaez *et al.* (2010) consider that the negative effect in the dehydrogenase activity through the time is due to the reduction of the soil porous space (bulk density > 1.4 g/cm^3) by vinasse application.*et al.*

Sánchez *et al.* (2010) observed in Mollisols soils that the inorganic and organic fractions of available and moderately available P are favored by vinasse application, increasing the continuous availability of this element through the time. According to Gasca *et al.* (2011) vinasse application favors some chemical and biological properties of the soil, contributing with the reduction of Exchangeable Sodium Percentage (ESP) and the sodium Adsorption Ratio (SAR), with the increase in microbial C-biomass and higher biological activity. The studies on the use of vinasse for the control of plant pathogen fungi are scarce, in this sense, Santos *et al.* (2008) did not find any effect of vinasse on growth promoting rhizospheric bacteria.

This research had as objective to evaluate, under greenhouse conditions, the effect of potassium application as vinasse and KCl on a Pachic Haplustoll soil cultivated with green beans, on the activity of growth promoting rhizospheric bacteria (*P. fluorescens* and *B. subtilis*), CO_2 activity, microbial C-biomass and soil metabolic ratio qCO_2.

Materials and methods

This research was performed in the Universidad Nacional de Colombia - Palmira, department of Valle del Cauca, between 3° 30' 45" N and 76° 18' 29" O, at 965 MASL and average temperature of 24 °C. A Pachic Haplustoll soil (Velásquez and Sánchez de Prager, 2011) was used, it had low potassium (K) content and was sterilized on autoclave at 120 °C at 20 psi for 2 h, except for treatments T5 and T6. An initial analysis of the physical and chemical properties of the soil was performed and the vinasse used was characterized by Velásquez and Sánchez de Prager (2011) by determining pH, organic matter (OM), major and minor elements and electrical conductivity (EC).

Treatments and experimental design

The experimental design was completely randomized with seven treatments (Table 1) and five replicates, for a total of 35 experimental units that consisted on 5 kg capacity pots filled with soil in which two green bean plants of the Unapal Milenio variety (Vallejo and Estrada, 2004) were planted. The K doses were chosen according to the crop requirements (150 kg/ha of K_2O) to produce 20 t/ha (Vallejo and Estrada, 2004). KCl and vinasse were used as sources, having the latest one 19% of total solids (TS) and 1.43% (w/v) of K.

To inoculate the benefic microorganisms the concentration used was 1 x 10^9 cfu/ml of the bacteria strain *P. fluorescens* (PN1), native from the valley zone of Valle del Cauca and, commercial *B. subtilis* (BC2). Samplings were done four times in the crop.

Measurements

Measurements were done 8, 16, 35 and 45 days after sowing (das) which agree with the key phases of physiological development of the crop: germination, adaptation, pre-flowering and grain filling.

The microbial CO_2-activity (µg of C/g of soil per day) was measured in 50 g of soil using the respirometry method according to the Center of Agrobiology of Brazil described by Cadena and Madriñan (1998). The microbial biomass (µg of C/g of soil) was measured in 20 g of dry soil according to the fumigation extraction method (Vance *et al.*, 1987). CO_2 was estimated by the methods of Vance *et al.* (1987) and the Center of Agrobiology of Brazil. The microbial biomass was measured for each experimental unit and the metabolic ratio (qCO_2) was calculated by the following equation:

$$q\,(CO_2) = \frac{Microbial\ activity\ (\frac{\mu C-CO_2}{g\ of\ soil\ per\ day})}{Microbial\ biomass\ (\frac{\mu gC}{g\ of\ soil})}$$

For the quantification of soil microbial communities was used the methodology proposed by the Laboratory of Microbiology of the Universidad Nacional de Colombia - Palmira; for that effect soil samples from each experimental unit were taken, air dried and sieved on 2 mm mesh before taking 200 g subsamples to estimate by triplicate the colony forming units (cfu). To calculate these units, soil dilutions in distilled water were used from 10^{-6} till 10^{-9} and sowed on Potato Dextrose Agar (PDA) media in the case of *B. subtilis* and in KB (King B) for *P. fluorescens* and incubated at28 °C. Counting of developed colonies in each dilution was done after 24 h of incubation (Benjumea, 1998) before calculating the colony forming units. Biomass accumulation

Table 1. Description of the treatments used.

Treatments	Composition	Vinasse amount and KCl/ pot
T1	100% of K as vinasse + *P. fluorescens* + *B. subtilis*	500 ml
T2	100% of K as KCl + *P. fluorescens* + *B. subtilis*	9.25 g
T3	50% of K as vinasse and 50% KCl + *P. fluorescens* + *B. subtilis*	250 ml + 4.63 g
T4	75% of K as vinasse and 25% KCl + *P. fluorescens* + *B. subtilis*	375 ml + 2.31 g
T5	100% of K as vinasse and without bacteria	500 ml
T6	100% de K as KCl and without bacteria	9.26 g
T7	Control without K sources + *P. fluorescens* + *B. subtilis*	No application

(DM, %) was determined from the dry weight of the leaves, stem and roots harvested in each experimental unit. The data was subjected to analysis of variance and mean comparison test, additionally correlations and regressions were performed using the SPSS 20 software (IBM 2011).

Results and discussion

Vinasse and soil characterization

The vinasse used was highly acidic and had high OM content, high electrical conductivity (EC) and good K_2O content (1.43%). Soil is silt loam with slightly acidic pH (6.23), high content of phosphorus (197.35 mg. kg), OM (4.78%), calcium (17.34 Cmol. kg), magnesium (5.88 Cmol. kg), boron (1.41 mg. kg), manganese (47.85 mg. kg-1), sulfur (55.56 mg. kg), and low contents of potassium (0.2 Cmol.kg), copper, iron and zinc (1.42, 7.19, 3.55 mg. kg respectively) (Velásquez and Sánchez de Prager, 2011).

Microbial activity.

The CO_2-microbial activity showed significant values ($P < 0.05$) in the second time of sampling (Table 2). In Figure 1 is observed that the highest values of microbial activity were shown in the first sampling in all the treatments, followed by the second and forth sampling. In this first sampling the highest values were observed in treatment T6 (100% K as KCl, without bacteria) with 343.35 μgC-$C0_2$/g, and the lowest in the treatment T2 (100% of K as KCl + *P. fluorescens* + *B. subtilis*) with 236.62 μgC-$C0_2$/g.

In the third sampling the lowest values were found again in treatment T2 (10.06 μgC-$C0_2$/g), whereas in treatment T1 (100% of K as vinasse + *P. fluorescens* + *B. subtilis*) had the highest (20.84 μgC-$C0_2$/g).

The microbial activity values observed in the different samplings had a descendent order according to the treatments: T5 > T1 > T3 > T6 > T4 > T2 > T7. The highest values in the first sampling and the reduction in the others

Table 2. Analysis of variance for the microbial activity and biomass per sampling.

Variable	Sampling 1	Sampling 2	Sampling 3	Sampling 4
Microbial activity	0.68 NS	0.001**	0.88 NS	0.32 NS
Microbial biomass	0.72 NS	0.70 NS	0.003**	0.096 NS

**P<0.05.

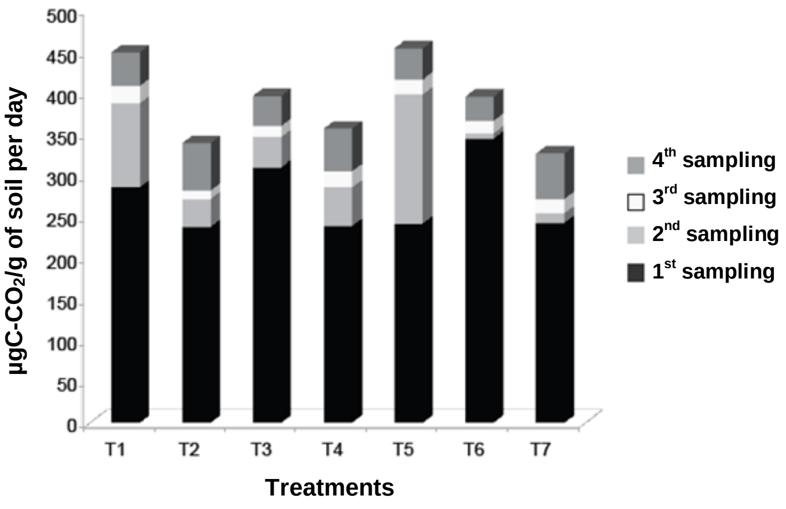

Figure 1. Behavior of the microbial activity by treatment in the sampling times.

was due, possibly, to the largest application of OM in the first case and to the decomposition of the substrates by the soil microorganisms in the second case, which agrees with findings by Silva *et al.,* (2005). Tenorio *et al.* (2000) associate this behavior of the microbial activity with changes in soil pH. The values found in this study are higher than the ones found by Montenegro *et al.* (2009) and lower to the ones reported in field conditions by Gasca *et al.* (2011).

Microbial biomass

This characteristic had lower values ($P < 0.05$) in the third sampling (Table 2). In Figure 2 is observed that the highest values of this parameter were presented in the treatment T3 (50% of K as vinasse and 50% of K as KCl + *P. fluorescens* + *B. subtilis*) followed by the treatments T5, T4, T2, T6 and T1. In the second sampling the highest values for microbial biomass were presented in the treatment T3 (50% of K as vinasse and 50% of K as KCl + *P. fluorescens* + *B. subtilis*) with 603.79 µgC/g of soil and the lowest values in the treatment T4 (75% of K as vinasse and 25% as KCl + *P. fluorescens* + *B. subtilis*), with 302.15 de µgC/g of soil.

The lowest values for microbial biomass were presented in the third sampling in the treatment T7 (Control) with 34.03 µgC/g of soil, whereas in the treatment T5 (100% of K as vinasse, without bacteria) had the highest values in this sampling (292.87 µgC/g of soil). According to the results found by Fauci and Dick (1994) the biomass values in this study are considered as normal for the first and fourth sampling, low for the third and high for the second.

The microbial biomass showed a similar behavior to the microbial activity since both parameters showed the lowest values in the third sampling at the beginning of flowering when the plant has higher activity, besides that in this time all the mineralization processes had happened and the substrate for microorganisms is reduced, which agrees with

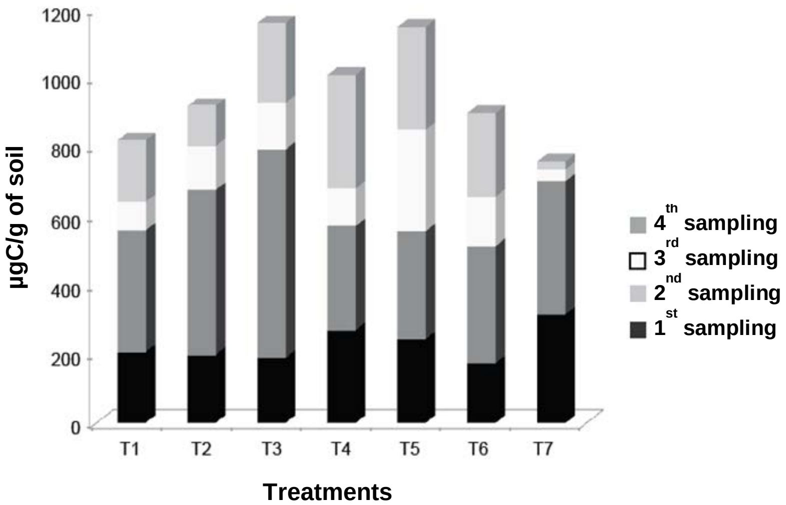

Figure 2. Behavior of the microbial biomass by treatment in the sampling times.

Table 3. Metabolic ratio by treatment effect.

Ratio	Treatment						
	T1	T2	T3	T4	T5	T6	T7
qCO_2	0.56	0.72	0.85	0.43	0.65	0.85	0.50

the findings of Tate (2000) cited by Montenegro *et al.* (2009).

Metabolic rate

The analysis of variance did not showed significant differences (P > 0.05) by effect of the treatments. The highest values of the metabolic ratio are presented in the treatment T6 and, the lowest in treatment T4 (Table 3). In descendent order T6 > T3 > T2 > T5 > T1 > T7 > T4. This ratio tended to descend through the time in all the treatments, showing processes associated with OM decomposition, since the values are < 1 and, of system stability. These results agree with the ones of Doran *et al.* (1994) who found high qCO_2 values in young (immature) ecosystems and low in mature ecosystems, i.e. the relation between the total respiration and the total biomass of an ecosystem should decrease progressively as it reaches the equilibrium or stability.

Rhizospheric bacteria.

The effect of the application of the different treatments on the growth promoting rhizospheric bacteria showed differences (P < 0.5) in *P. fluorescens* for the second sampling time and for *B. subtilis* in the third sampling (Table 4).

The highest values for colony forming units of *P. fluorescens* (cfu/g of dry soil) are presented in the fourth sampling (Figure 3), being the highest in treatment T5 (100% of K as vinasse, without bacteria) with 330.83 x 10^6 ufc/g and the lowest (67.01 x 10^6 cfu/g) in the treatment T6 (100% of K as KCl, without bacteria). The lowest values of rhizospheric bacteria (5.47 x 106 cfu) were shown in the third sampling of treatment T1 and the highest (14.06 x 10^6 cfu/g) in the treatment T3 (50% of K as vinasse and 50% as KCl + *P. fluorescens* + *B. subtilis*).

The highest number of cfu was in treatment T5 and in descendent order were the treatments T1 > T4 > T3 > T7 > T2 > T6 (Figure 3). *Pseudomonas fluorescens* presented the highest values (330.22 x 10^6 cfu/g) in the

Table 4. Analysis of variance of the rhizospheric bacteria by sampling.

Bacteria	Sampling 1	Sampling 2	Sampling 3	Sampling 4
P. fluorescens	0.61NS	0.03**	0.80 NS	0.68 NS
B. subtilis	0.49 NS	0.41 NS	0.002**	0.18 NS

**P<0.05.

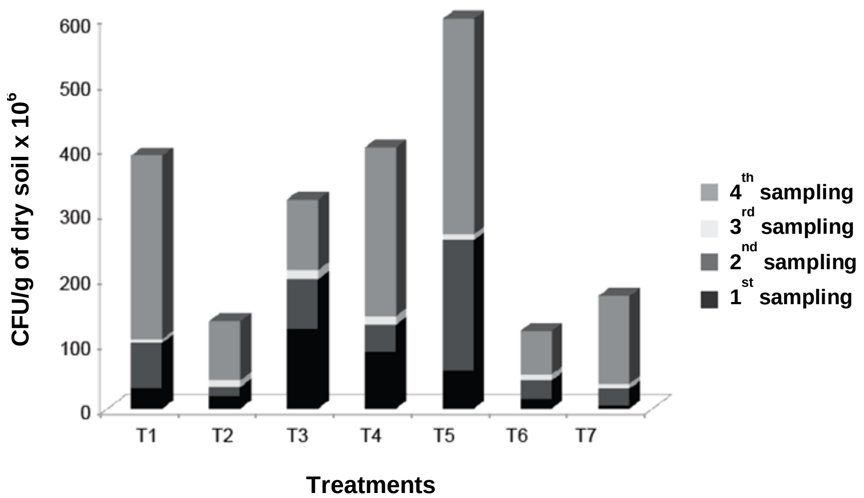

Figure 3. Behavior of *P. fluorescens* rhizospheric bacteria by treatments

fourth sampling of treatment T5 (100% vinasse without bacteria inoculation), whereas the lowest values (8.86 x 10^6 cfu/g) were presented in the third sampling, matching the crop flowering time.

The low values of the third sampling (8.90 x 10^6 cfu/g) can be considered as normal, since in this moment the nutrients´ demand changes the rhizosphere and can generate some changes in their exudates that affect the bacteria as well as the K+ ion of the KCl.

The population of *B. subtilis* was the lowest ($P < 0.05$) in the third sampling (Table 4). In the Figure 4 can be observed that the highest values for the bacteria were found in treatment T4 followed in descendent order by the treatments T6 > T7 > T2 > T3 > T5 > T1.

The sampling time affected the cfu of *B. subtilis* (Figure 4), being their number higher (78.57 x 10^6 cfu/g) in the first sampling and in treatment T4 (75% of K as vinasse and 25% as KCl + *P. fluorescens* + *B. subtilis*). The lowest values (1.38 x 10^6 cfu/g) were shown in the third sampling in treatment T1 (100% of K as vinasse + *P. fluorescens* + *B. subtilis*) and the highest (19.09 x 10^6 cfu/g) were shown in the first sampling. The low cfu values in this treatment could be due to a pH effect that characterizes the vinasse. The best results for these bacteria are presented in the treatments with the lowest vinasse doses.

The highest green bean yields were found in the treatment T6 (100% KCl without bacteria inoculation) and the lowest in treatment T5 (100% vinasse without bacteria inoculation) (Table 5). When yield was analyzed in function of the metabolic ratio (Figure 5) the regression analysis by lineal modeling was highly significant ($P < 0.01$). The analysis associates the microbial activity and biomass and demonstrates that as the metabolic ratio is lower than one (< 1), the yield is high, possibly be

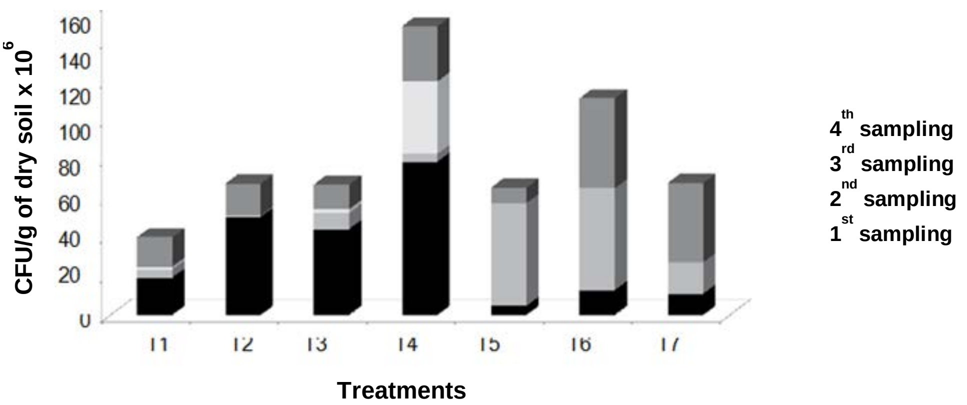

Figure 4. Behavior of *B. subtilis* rhizospheric bacteria by treatments

Table 5. Treatment effect on green bean yield.

Treatment	Yield in dry matter percentage		
	1	2	3
T5	23.77	–	–
T4	23.89	–	–
T1	25.07	25.07	–
T3	30.40	30.40	30.40
T7	31.75	31.75	31.75
T2	–	33.85	33.85
T6	–	–	36.54

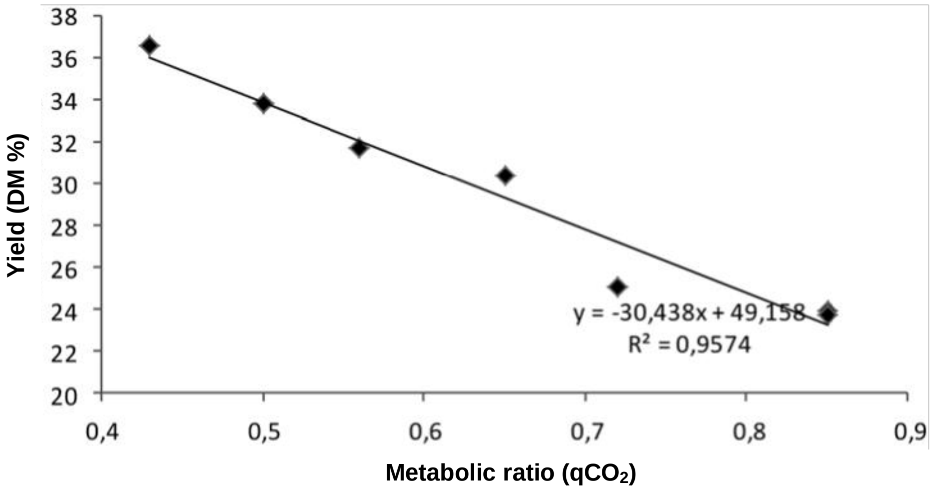

Figure 5. Relation between green bean yield and the metabolic ratio.

cause of a higher nutrient availability due to OM decomposition and mineralization processes.

Conclusions

- The treatments and sampling times, which match the physiological stages of green bean, affected significantly the microbial activity and biomass. The lowest values for both parameters were shown when the plant was at flowering and demanded more nutrients.
- The mix in equal parts of K and vinasse benefited the microbial biomass in the experimental soil. The metabolic ratio showed stability through the time in this system.
- Rhizospheric bacteria showed their best growth in the mix of 75% of K as vinasses plus 25% as KCl.
- The best dry matter yields of green bean, *P. vulgaris* L., were found in the treatments that include KCl as K source mixed with vinasses.

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