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SUBSTRATE-INDUCED RESPIRATION IN PUERTO RICAN SOILS: MINIMUM GLUCOSE AMENDMENT

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ABSTRACT

Soil microbiota –usually quantified as microbial biomass –is a key component of terrestrial ecosystems, regulating nutrient cycling and organic matter turnover. Among the several methods developed for estimating soil microbial biomass, Substrate-Induced Respiration (SIR) is considered reliable and easy to implement; once the maximum respiratory response is determined for a particular substrate amendment and study area. We determined the minimum glucose amendment of three forest types in Puerto Rico: dry, moist, and wet subtropical forests. The minimum glucose concentration was 3 mg C-glu/g dry soil for these forests. Additionally, we discuss soil properties to be considered for the implementation of SIR in tropical soils.

RESUMEN

La microbiota del suelo –generalmente cuantificada como biomasa microbiana –es un componente clave de los ecosistemas terrestres; regulando el ciclo de nutrientes y la rotación de materia orgánica. Entre los varios métodos desarrollados para estimar la biomasa microbiana del suelo, la Respiración de Sustrato-Inducida (SIR, por sus siglas en inglés) es considerada una técnica segura y fácil de aplicar; una vez la respuesta respiratoria máxima es determinada para un sustrato y área de estudio en particular. Se determinó la cantidad mínima de glucosa (sustrato) necesaria para estimar la respiración inducida en tres tipos de bosques en Puerto Rico: seco, húmedo y mojado subtropical. La concentración mínima de la glucosa fue de 3 mg C-glu / g de suelo seco para estos bosques. Adicionalmente, discutimos las propiedades de suelo que deben ser consideradas para la implementación de SIR en suelos tropicales.

Microbial biomass (MB) is an important component of most terrestrial ecosystems because it reflects the abundance of microbial populations, which in turn can regulate nutrient cycling and soil organic matter turnover. Microbial biomass represents a highly labile source of nutrients for plants (Ritz *et al*. 1994). Moreover, the non-labile fraction (primary microbial cell wall constituents) is an important component of soil organic matter (Zou *et al*. 2005). Several methods have been developed for the estimation of MB; chloroform fumigation and incubation (CFI), chloroform fumigation and extraction (CFE), and substrate-induced respiration (SIR) are among the more widely used (Paul *et al.* 1999). Substrate-induced respiration is a technique in which an easily assimilated substrate (usually glucose) is added to the sample and the respiration response (mainly CO_2 evolution rates) is measured during an incubation period (Anderson and Domsch 1978; Lin and Brookes 1999). In soil amendments, it is assumed that the initial maximum respiration rate is proportional to the microbial biomass present. To apply this method, it is necessary to know the minimum glucose concentration required for achieving a maximal initial respiration response. This concentration varies greatly between soils and must be determined for each location (Horwarth and Paul 1994). The main goal of this study was to determine the amount of glucose needed for maximum microbial stimulation (i.e., maximum initial respiration rate) for Puerto Rican soils. We determined this concentration for forest soils in three bioclimatic zones: subtropical moist, subtropical wet, and subtropical dry forests (Ewel and Whitmore 1973). The results obtained provide guidelines for the use of the SIR technique in Puerto Rican and potentially tropical soils in general. Microbial biomass is not only an important source of labile C and nutrients, but also is involved in key processes such as nitrification and decomposition of recalcitrant soil organic matter. Therefore, the standardization of reliable methods for the estimations of microbial biomass is an important contribution to the study of tropical soil ecology. Moreover, SIR is a widely used technique for monitoring soil quality (Machulla 2003), assessing the effect of fertilizers and pesticides (Jones and Ananyeva 2001; Cederlund and Stenström 2004) and evaluating the affect of management strategies in arable soil (Dilly *et al*. 2003).

Main features of the forests studied are as follows: The first site was a lowland moist mature secondary forest located in the Botanical Gardens of the town of Río Piedras in Puerto Rico (18º22'59.0"N, 66º03'12.7"W). The site has a mean annual temperature of 26.2° C, and an annual rainfall of 1712 mm (Gould *et al*. 2006). Vegetation in this site is dominated by *Manilkara bidentata* (A. DC.) A. Chev. and *Ocotea leucoxylon* (Sw) Lanessan, with S*yzygium jambos* (L.) Alst*., Spathodea campanulata* Beauv*., Faramea occidentalis* (L.) A. Rich.*, Chrysophyllum argenteum* Jaquin*,* and *Miconia prasina* (Swartz) DC. as other well represented species (Gould *et al*. 2006). Soils are well drained, derived from volcanic rocks, with a water holding capacity (WHC) of 68 percent (G. González, unpublished data; Percentage based on g water/g dry soil). The second site was a subtropical wet forest, located in the Luquillo Mountains, near El Verde Field Station (18º19'10.3"N, 65º49'02.6"W). Mean annual temperature is 24.5 ° C and annual rainfall is about 3456 mm (García-Martinó *et al*. 1996), soils are a complex of well and poorly drained ultisols and oxisols (Ruan *et al*. 2004), with high clay content and a 71 percent WHC (G. González unpublished data). Vegetation is composed by a *Dacroydes excelsa* Vahl-*Tetragastris balsamifera* (Sw) Kuntzee community with *Prestoea montana* (R. Graham) Nichols*, Miconia tetrandra* (Swartz) D. Don*, Manilkara bidentata* (A.DC.) A.Chev.*, Ormosia krugii* Urban (Gould *et al*. 2006). The third site was a subtropical dry forest located in the eastern coast of the Island (18°13'57.8"N, 65°36'01.6"W). Mean annual temperature is 27.5° C and annual rainfall is 1262 mm (Gould *et al*. 2006). Soils are well drained, silty clay loams, derived from volcanic uplands (USDA 1977) and 88 percent WHC (G. González, unpublished data). Vegetation is composed by *Guapira fragrans* (Dum.-Cours.) Little-*Bucida buceras* L. community with *Erythoxylon brevipes* DC., *Eugenia biflora* (L.) DC., *Bursera simaruba* (L.) Sarg, *Bourreria succulenta* Jacq. (Gould *et al*. 2006).

Soil samples (from 0 to 10 cm depth) were collected and analyzed during April-July 2004 and April-May 2005 for each forest. Soil was sieved through 3.36 mm mesh. Rocks, roots and other organic debris were extracted by hand. The soil was stored at 4° C (for no more than 2 weeks) until analyzed. Respiratory response (as CO_2 evolution) to the addition of glucose was measured with an automated respirometer (Oxymax ER-10 –Columbus instruments) equipped with 10 independent chambers. Incubations were done over a minimum of three hours and a maximum of 20 hours at 25 °C and 800.2 mm Hg. Moisture content was adjusted to 60 percent of WHC according to each soil type. Glucose concentrations used ranged from 2 to 9 mg C-glucose g^{-1} dry soil. Glucose solutions were prepared using deionized and 0.2 μm-filtered water. Soil subsamples equivalent to 10 grams dry wt were incubated in one of the 10 chambers of the respirometer. Eight randomly selected chambers received each one of the glucose amendments, while the two additional chambers were used as controls adding either deionized or filtered water or no amendment (basal respiration), respectively. The procedure was done for 2-6 successive days for each forest and the averages per treatment were then used for calculations and analysis. From the complete curve of CO_2 evolution, we selected the interval of time in which respiration was more stable. This period was between 6 and 15 h, since the first segment of CO_2 evolution curve was characterized by a stabilization phase and the final stages showed a decline due to substrate depletion (usually near the 20 h of incubation). We used the average amount of CO_2 evolved during the stable period to then determine microbial biomass. Calculation of microbial biomass from CO_2 evolved where made from a wide range of conversion factors derived from literature and summarized by Sparling (1995). For the three forest types studied, we obtained the maximal respiratory response at glucose concentrations equal or greater than 2 mg C-glu g dry soil^{-1} (Figure 1). Soils from the moist and wet forests achieved the maximal respiratory response at 2 mg C-glu g dry soil^{-1} (Figure 1b and c), while for the dry forest, maximal respiratory response was achieved with 3 mg of C-glucose on June 2004 (Figure 1a). In general, the respiratory response did not significantly increase after glucose concentrations greater than 3 mg. Even though there were significant seasonal variations in the amount of soil respiration for the different forest types (mainly due to transient changes in the soil microclimate), the maximum respiration response was achieved at about the same glucose amendment (Figures 1a-c).

Two soil characteristics important to be considered when applying this technique for comparative purposes of MB in different soils are: WHC percent and texture. The WHC of the soils should be kept around 55-60 percent because high water contents may inhibit respiration and obscure the stimulation driven by the addition of glucose (Ilstedt *et al*. 2000). However in Puerto Rico, water addition trials of wet forest soils have not shown a significant decrease in respiration at 100-150 percent WHC (M. Zalamea, personal observation). Thus, it is possible that the optimum WHC percent for SIR varies according to the soil type. Potentially, this might imply that comparisons between forests with different water regimes should be done at the optimum moisture for each soil type, instead of a constant WHC for all. It has also been postulated that the texture of soils can determine the magnitude of the glucose amendment. Soils with high clay content may require higher glucose concentrations because the complex spatial structure created by micro-aggregates in clayed soils makes it more difficult for the microorganisms to obtain carbon sources (van Gestel *et al*. 1991). However, given the results of this study, we can not support that contention of soil texture effects on SIR glucose amendments. In this study, we did not observed large differences in soil respiration given large differences in glucose amendments in the wet forest soils which have a clayey texture. In fact, the highest glucose amendment needed to stimulate soil respiration was obtained in the dry forest, a soil with loamy texture.

In this study, microbial biomass ranged between 0.46 -1.43 mg/g of soil (Table 1). These values are within the range reported for these forests using other methods such as Chloroform Fumigation and Incubation (Ruan *et al*. 2004). Microbial biomass increases from wet to dry forest (Figure 2), showing an inverse relation with rainfall. The three forests types selected also are part of an elevation gradient, along which microbial biomass increases as elevation decrease (Zalamea and González, unpublished data).

In summary, results from our trials show that a range between 2 to 3 mg glucose-C/g dry soil is appropriate for these Puerto Rican soils. Given the differences among the three forest types considered, this range is rather narrow, and we consider that a general concentration of 3 mg glucose-C/g dry soil can be used for estimating microbial biomass in these tropical soils.

FIGURE 1. CO_2 evolution (µg . g dry soil^{-1} . hr^{-1}) after addition of different concentrations of glucose (mg C-glu. g dry soil^{-1}) for the three forest types studied: a. Dry, b. Moist, and c. Wet. Error bars = Standard error (n = 9).

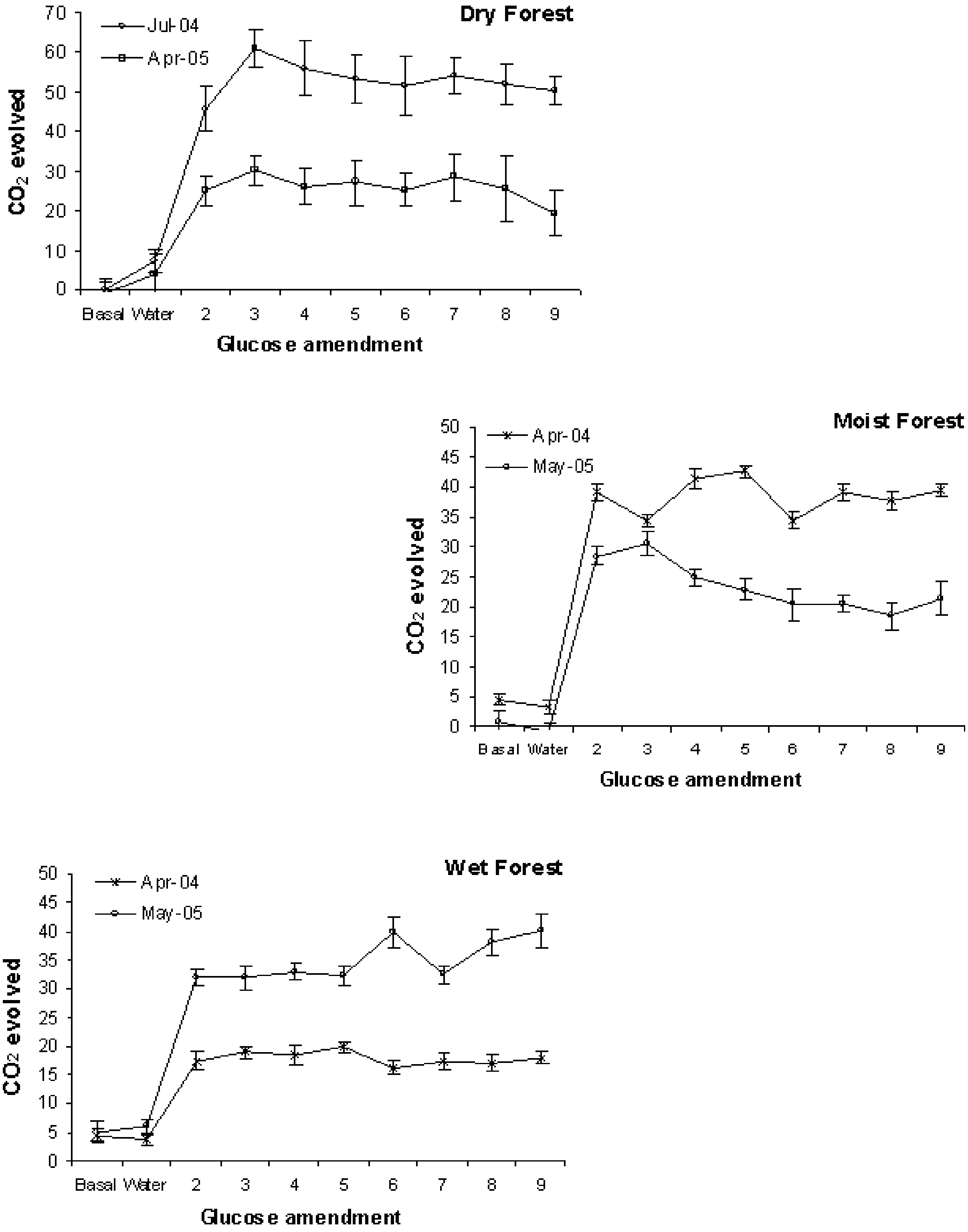

Figure 2. Microbial biomass for the three forest types studied. p values for comparisons between sites were: Dry vs. Moist and Dry vs. Wet: $p < 0.015$, Moist vs. Wet: $p = 0.054$).

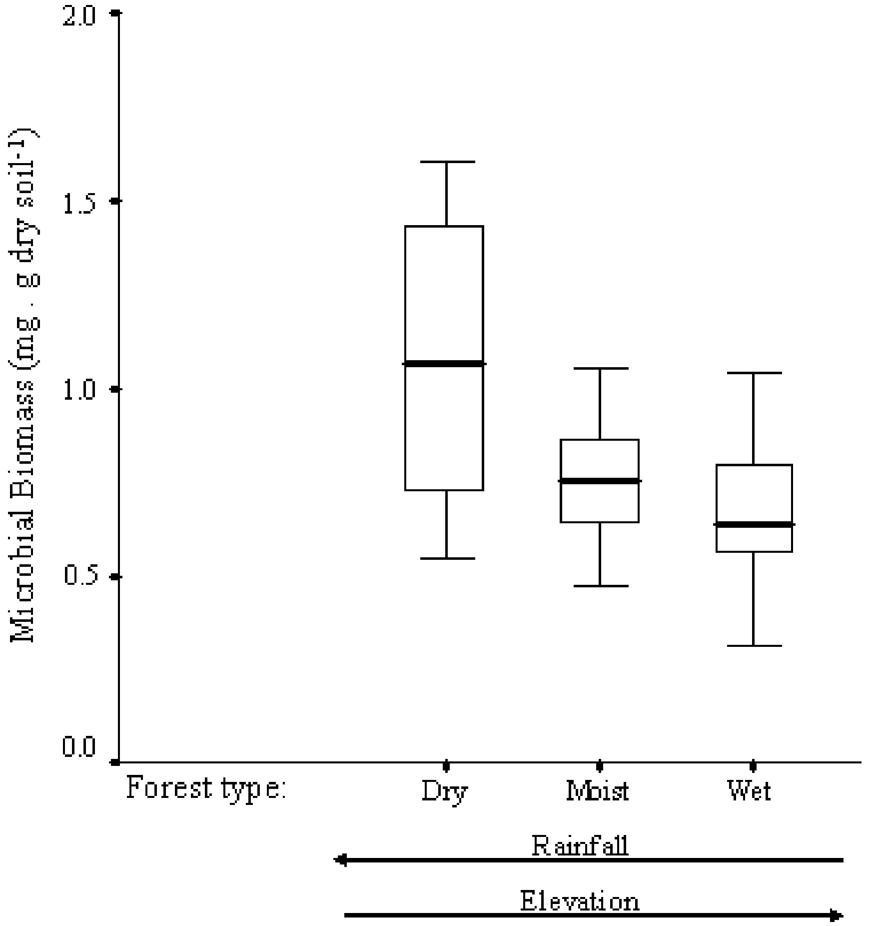

Table 1. Mean ± SE (n = 9) of microbial biomass usign conversion factors from Sparling (1995).

	Microbial Biomass (mg/g soil)			
	Forest type	**Date**	**Mean + SE**	**Minimum**
Dry	Jul-04	1.43 ±0.04	1.62	1.29
	Apr-05	0.72 ±0.03	0.81	0.64
Moist	Apr-04	0.92 ±0.04	1.04	0.83
	Apr-05	0.66 ±0.04	0.75	0.59
	Apr-04	0.46 ±0.03	0.51	0.41
Wet	May-05	0.94 ±0.05	1.07	0.85

We recommend that future development of the SIR technique in tropical soils include the determination of the optimal concentration of selective inhibitors to estimate fungal and bacterial contributions to soil biomass through their respective respiration responses.

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