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C Sequestration by Vetiver Grass in Nakhon Ratchasima, Northeast Thailand

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Abstract: Vetiver grass (*Vetiveria* spp.) was grown in the Thai Tapioca Development Institute, Nakhon Ratchasima, northeast Thailand, along contours for soil erosion reduction in tapioca fields. Soils after continually cultivating vetiver grass for 1, 2, 3, 5 and 7 years were measured for soil organic C (SOC) and organic N using a sequential fumigation incubation procedure for microbial biomass C ($C_{microbial}$), labile organic C (C_{labile}) and potential turnover rate (k). This study found SOC and organic N levels increased significantly due to continuous vetiver grass cover. The mean C storages in soil at 120 cm depth were 23.63, 28.62, 66.30, 28.68 and 228.90 ton C ha^{-1} for the 1, 2, 3, 5, to 7 yr site, respectively. The $C_{microbial}$ content enriched maximally at 0-10 cm (0.15 mg g^{-1}soil) and for the C_{labile} content the maximum was at 60-120 cm (0.744 mg g^{-1}soil). The potential C turnover was the fastest at 18 days from 0-10 cm layer of the 3 yr site and for the slowest was 108 days at 60-120 cm layer of the 7 yr site.

Key words: Vetiver · Soil microbial biomass C · Soil labile organic C · C turnover rate

INTRODUCTION

Soil organic C is one type of C pools in the pedosphere, which the current SOC pool in the world soils is estimated at 1500 Pg [1]. The SOC pool is about 2.1 times that of the atmosphere pool and about 2.7 times that of the biotic pool comprising land plants. Processes that enhance SOC content are plant biomass production, humification, aggregation and sediment deposition, while processes that degrade SOC content are soil erosion, leaching and soil organic matter (SOM) decomposition.

SOC is important for improving soil quality and regulating global C cycling. SOC contains fractions with a rapid turnover rate as well as fractions with a slower turnover rate [2]. Microbial biomass C and labile organic C, the rapidly responding fractions in C turnover, suggested as an early indicator of the effects of land use on SOC pools [3] and as an important indicator of soil quality. The majority of studies on SOC and its different labile fractions have used only topsoil samples; however, few studied have focused on a connection with soil profile and plant species.

Vetiver grass is a tropical plant which grows naturally. In Thailand, vetiver grass can be found growing in a wide range of areas from highlands to lowlands in various soil conditions. Two ecotypes commonly found in Thailand are *Vetiveria zizanioides* Nash and *Vetiveria nemoralis* A. Camus [4]. The grass has a deep thick root system which spreads vertically rather than horizontally. The roots densely bind together like an underground curtain or wall enabling it to store water and moisture. His Majesty King Bhumibhol Adulyadej of Thailand has long expressed his ideas about vetiver, the wonder grass with proven potential in preventing erosion and conserving soil moisture and its multifold applications such as prevention and treatment of polluted water and contaminated land.

The objective of this study was to evaluate C sequestration efficiency of vetiver grass by investing the distribution of SOC and its active fractions through soil profile.

MATERIALS AND METHODS

Site Description: The research was conducted at the Thai Tapioca Development Institute (TTDI), Nakhon Ratchasima Province, northeast Thailand (15°16' N, 101°51' E; 312 m above sea level). This land,

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approximately 320 ha, mainly cultivates tapioca since last 10 years ago. Vetiver grass was planted along contours across the slope to protect soil erosion. The organic C input into soil was through manure, plant root system, stubble and crop residues remaining on the field. The soils in the study area are sandy loam and sandy clay loam. During 1977-2007, the mean annual temperature ranged between 21.9 and 32.8°C with average monthly precipitation of about 89 mm (Nakhon Ratchasima Meteorology Station).

Soil Sampling: Soil samples were collected on January 2008, from the contours of continuous vetiver grass for 1, 2, 3, 5 and 7 years. Soil was bulked into a composite sample with three depth layers at 0-10, 10-60 and 60-120 cm. Soil samples were separated into two parts: first, fresh-moist soils were kept at 4°C for investigating for $C_{microbial}$ and C_{labile}; and second, soils were air-dried for physical and chemical analysis.

Physical and Chemical Properties of Soils: Dried soils were sieved to 2 mm and analyzed for soil particle size distribution by the hydrometer method [5], bulk density [6], $pH_{(H2O)}$ (1:5 soil water ratio), EC in 1:5 soil water suspension, cation, exchangeable cations, SOC by the Walkley-Black wet oxidation method [7] and total N by the Kjeldahl method [8]. The mean C storage in soil at 120 cm depth was calculated as following Eq. (1):

$$C\ Storage\ (gC\ m^{-2}) = Bulk\ density \times SOC \times Soil\ depth \quad (1)$$

Soil Microbial Biomass C: $C_{microbial}$ was measured with a chloroform fumigation incubation technique [9]. Two 30 g subsamples of field-moist soils were placed in 100 mL containers. One subsample was fumigated with free alcohol $CHCl_3$ in the dark for 24 h, while the other subsample was kept in the dark at 4°C for 24 h. Both fumigated and nonfumigated soils were incubated at 25°C for 10 days. The CO_2 evolved from the soils was absorbed in 1 M NaOH and determined titrimetrically with 1 M HCl using phenolphthalein as an indicator. Microbial biomass C was calculated from Eq. (2).

$$B = \frac{F}{K} \quad (2)$$

B is soil biomass C in mgC g^{-1} soil; F is the CO_2-C evolved during the 10 days' incubation from the fumigated, minus the nonfumigated soil; and K is 0.45.

Soil Labile Organic C: C_{labile} was determined by a sequential fumigation-incubation procedure [10]. The fumigated soil was incubated 50-80 days (5-8 cycles). The CO_2-C evolution was measured every 10 days, after adding 0.5 mL supernatant of 1 g inoculated soil. The amount of CO_2-C (C_t) was calculated from Eq. (3) [11].

$$C_t = \frac{\left[\left(A_t - V_t\right)N_t E - Q_t\right]}{w} \quad (3)$$

A_t is the volume (mL) of acid used to titrate the blank; V_t is the volume (mL) of acid used to titrate the treatment; N_t is the normality of titrating acid; E = 6 is equivalent weight; w is the initial weight of soil. Q_t is a correction factor for the inoculated soil after each fumigation as shown in Eq. (4).

$$Q_t = \frac{C'}{(r+1)} + \sum\left[\frac{C_{t-1}}{(r+1)}\right], t = 1,...,n, \quad (4)$$

C' is the amount of CO_2-C from the nonfumigated soil during the first 10 days incubation; r is the weight ratio of fumigated soil to inoculation soil. The accumulated CO_2-C (M_t, t = 1,...,n) from the fumigated soil is calculated following to Eq. (5) [12].

$$M_t = C_{labile}\left(1 - e^{-kt}\right) \quad (5)$$

C_{labile} is estimated from pool size of soil labile organic C and k is the potential turnover rate. Eq. (5) can be transformed to linear regression in Eq. (6).

$$Ln(C_t) = Ln(k\ C_{labile}) - kt, \quad (t = 1, 2,..., n) \quad (6)$$

k is the slope, $Ln(kC_{labile})$ is the intercept (a) and C_{labile} equals to e^a/k.

Statistical Analysis: Statistical analysis of the data was carried out on the replicates by one-way ANOVA. If the main effects were significant at $p < 0.05$, a post hoc separation of means was done by univariate least significant difference (LSD) test. Statistical analysis was conducted with SPSS and Microsoft Excel for Window 2000.

RESULTS AND DISCUSSION

Soil Organic C and Nitrogen: The physical and chemical soil properties for each site and soil layer are shown in Table 1. The SOC and N concentrations at 0-10 and 10-60 cm layer were greater than that of 60-120 cm,

Table 1: Selected characteristic of the study soils from the sites at TTDI, Nakhon Ratchasima, Thailand.

Site	Soil depth (cm)	Bulk Density (mg m^{-3})	Particle size distribution: Sand (g kg^{-1} soil)	Silt	Clay	pH$_{(H2O)}$	EC (μS cm^{-1})	Exchangeable Cation: Ca (cmol kg^{-1} soil)	K	Mg	Na	CEC (cmol kg^{-1} soil)	SOC (g kg^{-1} soil)	Total N
1 yr	0-10	1.37	82.48	12	5.52	8.68^{B}	5.10^{Da}	0.108^{Da}	0.035^{Da}	0.023^{Cb}	0.033^{Ba}	0.43^{Cb}	1.37^{Cb}	0.27^{Ca}
						(0.18)	(0.82)	(0.001)	(0.001)	(0.001)	(0.006)	(0.08)	(0.13)	(0.02)
	10-60	1.15	79.98	9.5	10.52	8.33^{B}	10.90^{Db}	0.094^{Eb}	0.027^{Cb}	0.024^{Eb}	0.027^{Aa}	0.56^{Dbc}	1.95^{Da}	0.28^{Ca}
						(0.21)	(1.04)	(0.001)	(0.006)	(0.001)	(0.006)	(0.16)	(0.25)	(0.03)
	60-120	1.19	76.48	6	17.52	8.64^{C}	8.45^{Da}	0.034^{Ea}	0.025^{Db}	0.038^{Da}	0.013^{Cb}	1.42^{Da}	1.49^{Db}	0.01^{Cb}
						(0.38)	(0.25)	(0.001)	(0.001)	(0.001)	(0.006)	(0.10)	(0.12)	(0.01)
2 yrs	0-10	1.37	86.48	8	5.52	9.59^{A}	7.53^{Eb}	0.088^{Ea}	0.013^{Eb}	0.015^{Db}	0.005^{Ea}	0.46^{Cb}	1.97^{C}	0.01^{Db}
						(0.43)	(0.68)	(0.001)	(0.006)	(0.001)	(0.001)	(0.03)	(0.21)	(0.01)
	10-60	1.08	81.98	6	12.02	9.47^{A}	6.96^{Eb}	0.133^{Da}	0.010^{Eb}	0.076^{Aa}	0.015^{Bb}	0.73^{Da}	1.85^{D}	0.28^{Ca}
						(0.23)	(0.64)	(0.006)	(0.000)	(0.001)	(0.001)	(0.13)	(0.36)	(0.02)
	60-120	1.54	70.98	11.5	17.52	9.96^{A}	9.68^{Da}	0.122^{Db}	0.013^{Eb}	0.076^{Ba}	0.023^{Ba}	0.07^{Ea}	1.72^{D}	0.27^{Ba}
						(0.25)	(0.58)	(0.001)	(0.006)	(0.001)	(0.006)	(0.12)	(0.15)	(0.01)
3 yrs	0-10	1.04	75.88	8.57	15.55	8.47^{Ba}	114.89^{Ca}	0.413^{Ca}	0.313^{Aa}	0.064^{Ab}	0.023^{Cb}	2.77^{A}	8.37^{Ba}	0.74^{Ba}
						(0.35)	(1.04)	(0.001)	(0.015)	(0.001)	(0.006)	(0.75)	(0.70)	(0.08)
	10-60	1.00	66.88	3.57	29.55	7.69^{Cb}	48.72^{Cb}	0.553^{Ca}	0.226^{Ab}	0.053^{Ca}	0.015^{Bb}	2.70^{A}	4.60^{Bb}	0.84^{Ba}
						(0.43)	(1.13)	(0.001)	(0.001)	(0.001)	(0.001)	(0.35)	(0.56)	(0.07)
	60-120	1.09	50.45	36.00	13.55	7.56^{Db}	25.26^{Ca}	0.498^{Cb}	0.135^{Aa}	0.083^{Aa}	0.033^{Aa}	3.47^{B}	5.29^{Bb}	0.44^{Ab}
						(0.15)	(0.63)	(0.001)	(0.001)	(0.006)	(0.006)	(0.19)	(0.10)	(0.07)
5 yrs	0-10	0.92	60.76	19.07	20.17	9.18^{Aa}	145.98^{Aa}	0.987^{Bb}	0.195^{Ca}	0.045^{Ba}	0.043^{Aa}	1.92^{B}	8.70^{Ba}	1.41^{Aa}
						(0.16)	(1.78)	(0.001)	(0.001)	(0.001)	(0.006)	(0.34)	(0.45)	(0.09)
	10-60	0.64	51.76	17.00	31.24	7.19^{Da}	72.21^{Bb}	0.945^{Ba}	0.195^{Ba}	0.063^{Bb}	0.025^{Ab}	1.80^{C}	3.72^{Cb}	1.26^{Aa}
						(0.08)	(1.06)	(0.001)	(0.001)	(0.001)	(0.001)	(0.05)	(0.48)	(0.05)
	60-120	0.40	49.76	17.00	33.24	7.68^{Db}	58.91^{Ba}	1.777^{Ba}	0.077^{Cb}	0.076^{Ba}	0.025^{Bb}	2.28^{C}	3.64^{Cb}	0.48^{Ab}
						(0.18)	(0.64)	(0.001)	(0.006)	(0.001)	(0.001)	(0.25)	(0.08)	(0.09)
7 yrs	0-10	1.07	50.31	25.14	24.55	8.71^{Bb}	118.61^{Ba}	1.632^{Ab}	0.213^{Ba}	0.045^{Bb}	0.014^{D}	2.95^{Ab}	10.74^{Ab}	0.85^{Bb}
						(0.21)	(1.88)	(0.001)	(0.006)	(0.001)	(0.001)	(0.13)	(0.39)	(0.10)
	10-60	0.97	51.98	20.50	27.52	6.77^{Da}	83.40^{Aa}	1.615^{Aa}	0.015^{Da}	0.045^{Db}	0.014^{B}	2.33^{Ba}	7.87^{Aa}	1.33^{Aa}
						(0.24)	(1.55)	(0.001)	(0.001)	(0.001)	(0.002)	(0.08)	(0.55)	(0.07)
	60-120	0.55	48.98	15.50	35.52	9.30^{Ba}	92.85^{Ab}	2.219^{Aa}	0.127^{Bb}	0.064^{Ca}	0.015^{C}	3.90^{Aa}	53.96^{Aa}	0.44^{Aa}
						(0.27)	(1.20)	(0.001)	(0.006)	(0.001)	(0.001)	(0.05)	(1.44)	(0.14)

Data are means with the standard deviation in parentheses (n = 3). Different higher and lower case letters represent significant differences ($p < 0.05$) of the treatment among sites and among treatment within the same site, respectively

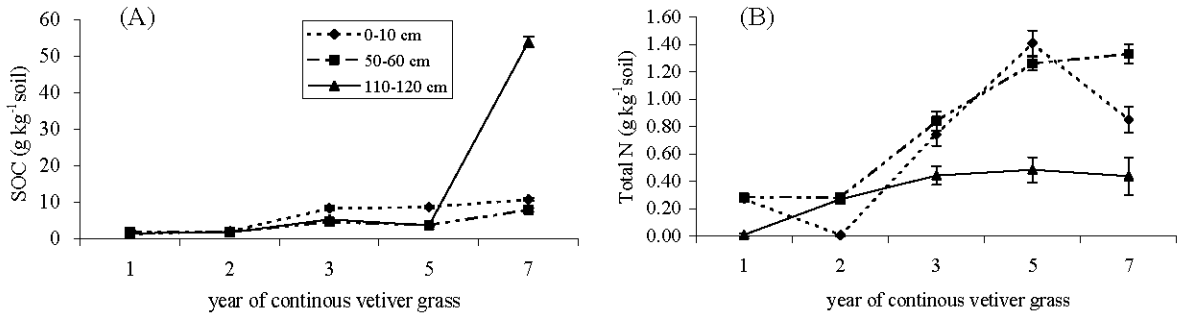

Fig. 1: Levels of SOC (A) and organic N (B) in a chronosequence of soils under continuous vetiver grass. Error bars indicate standard deviation (n = 3).

except at the 7 yr site which SOC at 60-120 cm layer increased steeply to 53.96 g kg^{-1} soil (Fig. 1). The SOC and N concentrations varied significantly with year of continuous vetiver grass (p<0.05) by the SOC increase being maximum in the 7 yr site and the N content being maximum in the 5 yr site. Both SOC and N contents increased distinctly after continuous vetiver grass for 3 years.

The mean C storages in soil at 120 cm depth were 23.63, 28.62, 66.30, 28.68 and 228.90 ton C ha^{-1} for the 1, 2, 3, 5 and 7 yr site, respectively (Fig. 2). C sequestration under a field experiment was significantly relative with the year of continuous vetiver grass as shown in Eq. (7). The mean C storage at 120 cm increased with a decreasing rate, which the storage was 9.69 times larger in the 7 yr site compared to the 1 yr site.

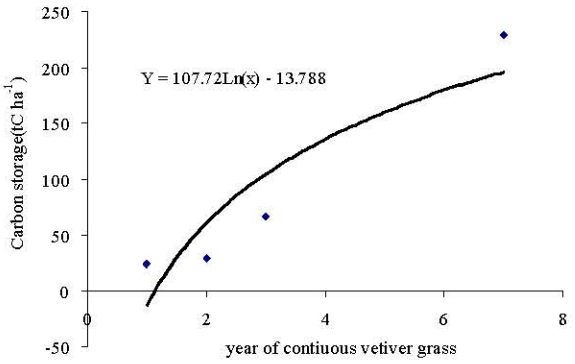

Fig. 2: Changes in the mean C storage in soil at 120 cm depth under continuous vetiver grass (the equation excluded the 5 yr site because of more variation).

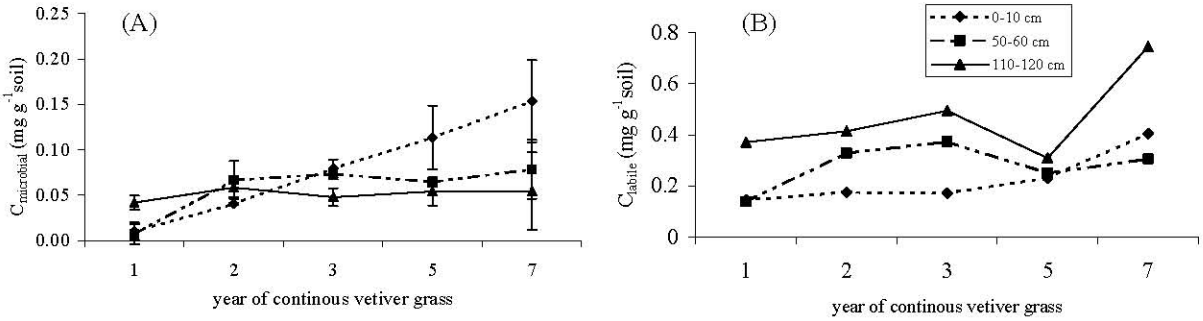

Fig. 3: Effect of continuous vetiver grass on soil microbial biomass C (A) and soil labile organic C (B). Error bars indicate standard deviation (n = 3).

$$Y = 107.72\,Ln(x) - 13.788 \quad (7)$$

Where Y is C storage at 120 cm depth (ton C ha^{-1}) and x is the year of vetiver grown.

Some environmental factors such as soil characteristics, soil profile, vegetation cover and geography have been attributed to SOC and organic N accumulation. Solomon *et al.* [13] showed that most of SOM (stable soil C) was bound to the clay-size separates (40 to 63% of SOC and 56 to 71% of organic N), while only 4 to 13% of SOC and 2 to 9% of organic N were found in the sand-size separates and 29 to 52% of SOC and 25 to 40% of organic N was contained in the silt. As in this study, the 7 yr site enriched with SOC and organic N contained more clay particles than other sites. Plant decomposition leads to the selective preservation of some resistant plant constituents, such as lignin. In addition, the turnover of microorganisms produces compounds, which are precursors of SOM [14]. High lignin, tannin and polyphenol contents of leaves and roots may have inhibited mineralization through phytotoxic interactions and chemical interactions with both organic and inorganic N sources, [15] which means more C and N is retained in subsoils. However, the labile cytoplasm components are readily leached from plant residues and provide the initial energy and nutrients to start the decomposition process [14]. Sediment deposition from soil erosion may be another possible reason for the SOC and total N measurements at the 7 yr site.

Soil Microbial Biomass C and Soil Labile Organic C: The levels of $C_{microbial}$ and C_{labile} are given in Fig. 3 and Table 2. The $C_{microbial}$ and C_{labile} contents were greatest at the 7 yr site in 0-10 and 60-120 cm layer, respectively. Averaged across the sites, the $C_{microbial}$ content in 0-10 cm layer ranged between 0.01 and 0.15 mg g^{-1} soil, in 10-60 cm layer was between 0.01 and 0.08 mg g^{-1} soil and in 60-120 cm layer was between 0.04 and 0.06 mg g^{-1} soil. The C_{labile} content in the 0-10 cm layer varied between 0.144 and 0.404 mg g^{-1} soil, the10-60 cm layer ranged between 0.137 and 0.372 mg g^{-1} soil and the C_{labile} content in the 60-120 cm layer was between 0.308 and 0.744 mg g^{-1} soil. The effect of continuous vetiver grass on $C_{microbial}$ was significant, reflecting the steep increment in the surface layers (0.15 mg g^{-1} soil). Also, the growth of vetiver grass affected the deeper soils, with a C_{labile} of 0.744 mg g^{-1} soil.

Table 2: Soil microbial biomass C and pool sizes of soil labile organic C.

Site	Soil depth (cm)	$C_{microbial}$ (mg g^{-1})	C_{labile} (mg g^{-1})	k ($cycle^{-1}$)	10/k (day)	$C_{microbial}/C_{labile}$ (%)	$C_{microbial}$/SOC (%)
1 yr	0-10	0.01^{Cb}	0.144	0.543	18.4	6.94	0.73
	10-60	0.01^{Bb}	0.137	0.348	28.7	7.28	0.51
	60-120	0.04^{a}	0.370	0.424	23.6	10.82	2.71
2 yr	0-10	0.04^{Cc}	0.174	0.196	51.1	23.22	2.05
	10-60	0.07^{Aa}	0.327	0.146	68.4	20.41	3.60
	60-120	0.06^{b}	0.413	0.156	64.1	14.13	3.39
3 yr	0-10	0.08^{Ba}	0.171	0.564	17.7	46.70	0.96
	10-60	0.07^{Aa}	0.372	0.228	43.8	18.82	1.52
	60-120	0.05^{b}	0.494	0.481	20.8	10.11	0.95
5 yr	0-10	0.11^{Aa}	0.230	0.240	41.7	47.90	1.26
	10-60	0.06^{Ab}	0.249	0.359	27.8	24.07	1.61
	60-120	0.05^{b}	0.308	0.332	30.2	16.24	1.37
7 yr	0-10	0.15^{A}	0.404	0.214	46.7	37.17	1.40
	10-60	0.08^{A}	0.304	0.285	35.1	26.31	1.02
	60-120	0.05	0.744	0.093	107.5	6.72	0.09

Different higher and lower case letters represent significant differences ($p < 0.05$) of the treatment among sites and among treatment within the same site, respectively.

The potential C turnover rates for each site and soil layer are shown in Table 2. The fastest C turnover was found at the 0-10 cm layer of the 3 yr site (18 days) and the slowest was at 60-120 cm layer of the 7 yr site (108 days). The proportion of $C_{microbial}$ to C_{labile} was found to decrease from soil surface to deep layers in all sites, except the 1 yr site, by the highly found in 0-10 cm layer of the 5 yr site (47.90%). The $C_{microbial}$ to SOC ranged between 0.09% and 3.60%, with the maximum at the 10-60 cm level of the 2 yr site.

$C_{microbial}$ and other active fractions suggest regulated by soil textures. This study found that the C_{labile} content enriched in the deep soil of the 7 yr site (0.744 mg g^{-1} soil), comprising high clay particles (Table 2). O-alkyl-C structures (mainly carbohydrate-derived), is known to be the moiety preferentially used by soil microorganisms, found enriched in clay-size separates rather than silt-size [13]. Moreover, SOM from Scanning Electron Microscope study associated with the clay-size separates was composed of completely decomposed organic structures, while SOM associated with the sand-size separates was composed of undecomposed and macromorphologically identifiable particulate plant residues [13].

A particular proportion of the SOM input is readily utilized by the organisms, such that the biomass C generally comprises only 1-5% of SOC in 0-10 cm layer [16, 17]. In this study, the proportions of $C_{microbial}$ to SOC in the topsoil were in the range (between 0.73 and 2.05%) and similar to that of *Acacia nilotica* (1.84%), *Eucalyptus tereticornis* (1.81%) and *Populus deltoids* (1.88%) [18]. The SOC values in this study of years 3, 5 and 7 were 8.37, 8.7 and 10.74 mg g^{-1} soil, respectively, which all are greater than that of 6 yrs of *A. nilotica*, *E. tereticornis* and *P. deltoids* (6.8, 4.8 and 5 mg g^{-1} soil, respectively). Anderson [19] considered the $C_{microbial}$: C_{org} ratio <2.0 for neutral arable soils. The $C_{microbial}$:C_{org} ratio could be used as an indicator of stability to recognize environmental changes, givens an "eco-physiological profile" of a site [19]. Ross *et al.* [20] stated that higher values of $C_{microbial}$ could be found in soil samples with a pH above 6.0. The level of $C_{microbial}$ was significant larger in soil sampled under the canopy of shrubs, such as "huisache" (*Acacia tortuosa*; 0.32 mg g^{-1} soil) and "mesquite" (*Prosopis laevigata*; 0.373 mg g^{-1} soil), compared to the soil cultivated with "maize" (*Zea mays*) for >19 years (0.122 mg g^{-1} soil).

CONCLUSION

Continuous vetiver grass in clay particles could increase large amounts of soil organic C, soil microbial biomass C and labile organic C. Vetiver grass can also enhance general soil quality, as well as sequestration of C.

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