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Microbial Metabolic Potential Affected by Surplus Wastewater Irrigation in Tropical Soil Cultivated with Tifton 85 Bermuda Grass (*Cynodon dactylon* Pers. X C. *niemfuensis* Vanderyst)

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Abstract Agricultural reuse of treated sewage effluent (TSE) is an environmental and economic practice; however, little is known about its effects on the characteristics and microbial function in tropical soils. The effect of surplus irrigation of a pasture with TSE, in a period of 18 months, was investigated, considering the effect of 0% surplus irrigation with TSE as a control. In addition, the experiment consisted of three surplus treatments (25%, 50%, and 100% excess) and a nonirrigated pasture area (SE) to compare the soil microbial community level physiological profiles, using the Biolog method. The TSE application increased the average substrate consumption of the soil microbial community, based on the kinetic parameters of the average well color development curve fitting. There were no significant differences between the levels of surplus irrigation treatments. Surplus TSE pasture irrigation caused minor increases in the physiological status of the soil microbial community but no detectable damage to the pasture or soil.

Keywords Water reuse · Pasture · CLPPs · Bacterial communities · Effluent irrigation

Abbreviations

TSE	treated sewage effluent
CLPP	community level physiological profile
STP	sewage treatment plant
AWCD	average well color development
NMDS	non-metric multidimensional scaling
ANOSIM	analysis of similarity
AWC	available water capacity

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1 Introduction

Irrigation of crops with treated sewage effluent (TSE) is an usual and old practice in many countries (Bouwer and Chaney 1974; Ramirez-Fuentes et al. 2002); however, in Brazil, it is very recent, demanding more detailed studies (da Fonseca et al. 2007b). The TSE irrigation in agriculture poses an actual and relevant issue because it is a water reuse practice and a valuable source of plant nutrients and organic matter. In this context, Brazil presents a great potential for effluent reuse, based on its large extensions of agricultural land with low fertility soils and feasible weather conditions for irrigation systems (da Fonseca et al. 2007b).

TSE irrigation provides water for the soil-plant system and also involves inputs of salts and organic compounds, resulting in changes in environmental conditions (Bouwer and Chaney 1974; da Fonseca et al. 2007b). These modifications in the agrosystem affect the soil microbial communities, activities, functional group densities, and sanitary quality (Ibekwe et al. 2003; Gelsomino et al. 2006) and also chemical (Candela et al. 2007) and physical (Warrington et al. 2007) soil attributes.

Among the several techniques currently available to analyze soil microbial communities, the Biolog® (Biolog, Hayward, CA, USA) approach, based on utilization patterns of a wide range of single carbon sources (Garland and Mills 1991; Insam et al. 1996), provides the community level physiological profiles (CLPPs) by analysis of the number of substrates utilized (diversity of metabolic potential) or response levels to individual substrates or substrate guilds (Garland and Mills 1991 and Zak et al. 1994). This approach has been used to evaluate impacts of soil contamination (Ros et al. 2006), several crop management techniques (Govaerts et al. 2007) and wastewater irrigation (Zhang et al. 2008). However, until this moment, the application of Biolog systems to the study of microbial communities was not evaluated in tropical soils irrigated with TSE.

The Ecoplates were developed to analyze bacterial communities of environmental samples, containing 31 unique carbon sources with three replicates, and one control without substrate (Insam et al. 1996). Despite some criticisms (Preston-Mafham et al. 2002; Calbrix et al. 2005), this method has been indicated to evaluate microbial communities of different and contrasting environmental samples (Classen et al. 2003).

The most common approach to statistical analyses of the data comprehends multivariate techniques (Hitzl et al. 1997) like nonmetric multidimensional scaling (NMDS) (Classen et al. 2003). NMDS has been very useful in studies of microbial communities among the statistical methods currently in use (Nelson and Mele 2007; Classen et al. 2003). However, Biolog data can be further elaborated for the estimation of the kinetics of substrate consumption (Haack et al. 1995). The kinetic parameters are estimated by fitting the average well color development (AWCD) curve versus time to the density dependent logistic growth equation, proposed by Lindström et al. (1998). These parameters enable the comparison between samples without the interference of inoculum densities, resulting in a more accurate separation of the several substrate consumption patterns of environmental samples (Ros et al. 2006).

The aim of this study was to evaluate the effect of surplus irrigation of Tifton 85 Bermuda grass (*Cynodon dactylon* Pers. X C. *niemfuensis* Vanderyst) with secondary treated sewage effluent (TSE) for 18 months on the soil microbial community level physiological profiles, focusing on diversity and kinetics of substrate consumption and the correlation between these factors and the soil chemical attributes.

2 Materials and Methods

The experiment was carried out at the municipal district of Lins, State of São Paulo, Brazil (49°50′ W; 22°21′ S; 440 m; average slope 10%), close to the sewage treatment plant (STP), which is operated by Sabesp (Company for basic sanitation of the State of São Paulo). The regional climate is characterized as mesothermic with a dry winter, with mean temperatures between 18°C and 26°C and an annual rainfall level from 1,100 to 1,300 mm (Ciiagro 2008).

The soil of the experimental area is a Typic Haplustult, sandy clay loam, cropped with Tifton 85 Bermuda grass, with a 12 m depth of groundwater table on the experimental area level. The grass cultivar was sown in January 2002 and the experiment started in December 2005. The soil at the experimental area was characterized by its acidity [pH ($CaCl_2$ 0.01 M) 4.5], low cation exchange capacity (CEC 32.49 $mmol_c\ kg^{-1}$), total carbon (TC 5.97 $mg\ kg^{-1}$), and total nitrogen (TN 0.46 $mg\ kg^{-1}$), phosphorus (P 6.23 $mg\ kg^{-1}$), and no detectable exchangeable sodium (Na^+). Greater details on experimental area and soil fertility can be found in da Fonseca et al. (2007c).

The experiment was arranged in a strip plot design with four treatments and four replications. Each strip was located at the same level. The treatments consisted of irrigation with TSE on the control treatment (named 0% of excess) and three other surplus treatments (25%, 50%, and 100% of excess) and a nonirrigated Tifton 85 Bermuda grass area (SE). Plot size was 82 m^2 (7.0×12.0 m), and the border was 1.0 m at each side. This was necessary because the conventional sprinklers were located at the border of each plot at a height of 90 cm, and the irrigation was unequally distributed over the whole area.

The irrigation management was based on the critical volumetric water content in the 0–60-cm layer. Tensiometers were installed at 0–20, 20–40, and 40–60 cm soil layers in all plots in the harvested area of the control treatment; moreover, the matrix potential (ψ_m) was monitored every day, in the morning. The ψ_m values obtained and the water retention curve data (data not shown) were fitted to the van Genuchten equation (Van Genuchten 1980) to calculate the volumetric water content, using a computer program—Soil Water Retention Curve (Dourado-Neto et al. 2000).The available water capacity (AWC) for the Tifton 85 Bermuda grass in the control treatment (0% of excess), at the 0–60-cm layer, was estimated by the difference between the volumetric water content at ψ_m=−19.6 and −1,470 kPa. Irrigation started when soil volumetric water content was 50% of AWC. Corresponding amount of effluent necessary to reach the AWC was applied to the soil-plant system in control treatment, and 25%, 50%, and 100% in excess of this amount were applied to the other treatments.

The effluent used in irrigation was derived from STP. This plant consists of three anaerobic and three facultative stabilization ponds. This system presents high efficiency in pathogen and parasite remotion and is considered as the most capable to produce effluents with compatible characteristics to agriculture reuse, as recommended by the World Health Organization (WHO 1989). The effluent stays for 5 days in the anaerobic ponds and 15 days in the facultative ponds. The effluent was sampled each two months and its composition is presented at Table 1. According to da Fonseca et al. (2007c), the effluent that we used in the experiment can be considered an adequate source of nutrients (mainly N and K), poor in heavy metals but rich in sodium (Na). The accumulated rainfall during the experimental period (January 2006 to July 2007) was 2,422 mm, and the accumulated TSE irrigation ranged from 1,254 to 2,297 mm for the lowest (control) and the highest (100% excess) irrigation level.

In the course of the experiment, according to TSE constitution, the irrigation resulted in the addition of organic matter, whose contents of total particulate carbon (TPC) and dissolved organic carbon ranged from 784 kg ha^{-1} for the control treatment to 1441 kg ha^{-1} for the highest amount of TSE irrigation (100% excess). Of these organic materials, 350 and 66 g kg^{-1} corresponded respectively to carbon and nitrogen. The quantity of mineral nitrogen was 443 kg ha^{-1} for the control treatment and 813 kg ha^{-1} for the highest amount of TSE irrigation (100% excess).

The grass was harvested bimonthly, cut 5 cm above ground (nine cuts between December 2005 and June 2007) using a portable motorized grass trimmer. After cutting, the grass was manually collected from the plot. The fertilization of the experiment plots was realized for a forage/hay production system (Werner et al. 1996). All plots received the same rates of nitrogen (50 kg N ha^{-1} as ammonium nitrate), potassium (66 kg K ha^{-1} as potassium chloride), applied just after every grass cut and phosphorus (44 kg P ha^{-1} as simple superphosphate), applied every 6 months, starting after the first phosphorus fertilization in the beginning of the experiment. All fertilizers were manually applied, over the cut grass. More details about fertilization recommendations for Tifton 85 Bermuda grass irrigated with this effluent are available in da Fonseca et al. (2007c).

To perform the analysis, soil samples were randomly taken at 0 to 10 cm depth using a stainless steel sampler with six simple samples combined into one composite sample 18 months after the setting up of the experiment. Soil samples from two Tifton 85 Bermuda grass plots without fertilization and irrigation, defined as nonirrigated area (SE), were also obtained. All composite samples were stored at 4°C until the microbial analyses were performed (maximum 5 days). For the chemical analyses, subsamples were air-dried and sieved (<2.0 mm).

The soil chemical properties were as follows: (1) total carbon (TC) and total nitrogen (TN); (2) active acidity (pH; in 0.01 calcium chloride mol L^{-1} solution); (3) exchangeable aluminum (Al), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), and cation exchange capacity (CEC), calculated as the sum of Ca, Mg, K, Na and total acidity (H + Al). These determinations were performed according to regional methods suggested by van Raij et al. (2001).

The community level physiological profile assay was performed by using Biolog Ecoplates® (Biolog, Hayward, CA, USA). The concentration of the inocula was previously estimated, by total bacterial count using a dilution plate method, on nutrient agar

Table 1 Treated sewage effluent characteristics and WHO (2006) orientation values for wastewater agricultural irrigation

Characteristics	mg L^{-1}	EET	Restriction use level[a]		
			low	Low to moderate	Severe
ST[b]	mg L^{-1}	127.8±20.4	<50	50-100	>100
Salinity					
EC[c]	dS m^{-1}	0.85±0.1	<0.7	0.7–3.0	>3.0
TDS[d]	mg L^{-1}	691±70	<450	450–2000	>2000
Infiltration				CE	
SAR[e, f] de 6–12	$(\text{mmol L}^{-1})^{0.5}$	11.9±2.9	>1.9	1.9–0.5	<0.5
Toxicity					
Na^+	mg L^{-1}	131.7±6.6	<69	>69	
Cl^-	mg L^{-1}	63.4±7.9	<106	>106	
B[f]	mg L^{-1}	0.17±0.08	<0.7	0.7–3.0	>3.0
$N–NO_3^-$	mg L^{-1}	0.30±0.43	<5	5–30	>30
HCO_3^-	mg L^{-1}	449.0±79.1	<92	92–519	>519
pH		7.5	Normal interval 6.5–8.0		
Other elements			Limit values		
$P–H_2PO_4^-$	mg L^{-1}	4.3±1.1			
$N–NH_4^+$	mg L^{-1}	23±3.5			
Ca	mg L^{-1}	8.1±1.1			
Mg	mg L^{-1}	1.9±0.5			
K	mg L^{-1}	17±1.8			
Al	mg L^{-1}	0.03±0.02	5.0		
Cd[g]	mg L^{-1}	Nd	0.01		
Cr[g]	mg L^{-1}	Nd	0.1		
Cu	mg L^{-1}	0.002±0.001	0.2		
F	mg L^{-1}	0.48±0.32	1.0		
Fe	mg L^{-1}	0.08±0.06	5.0		
Mn	mg L^{-1}	0.015±0.006	0.2		
Ni[g]	mg L^{-1}	Nd	0.2		
Zn	mg L^{-1}	0.02	2.0		

[a] Ayers and Westcot (1985)

[b] Total solids

[c] Electrical conductivity

[d] Total dissolved solids (Gloaguen et al. 2007)

[e] Sodium adsorption ratio

[f] da Fonseca et al. (2007c)

[g] Concetration under the dectection limit

medium, at 25°C for 48 h, to standardize the inoculum density. Microplates were inoculated with 120-μL soil suspensions prepared with 10 g of soil diluted in sterile sodium chloride (NaCl; 0.85% *w*/*v*), to contain approximately 100 cells mL^{-1}. Then, the microplates were incubated at 28°C and analyzed after 12 h, and then every 24 h, for 7 days, using an automated plate reader (Model 550, Biorad Laboratories, Hercules, CA, USA), set to 590ηm. Optical density (OD_{590nm}) data were corrected by nullifying each response well against its own first reading and also against the control well of each microplate.

The corrected data were transformed by dividing each value by the average well color development (Garland and Mills 1991). The corrected values for the whole plate and for the six groups of substrates according to their chemical nature (carboxylic acids, polymers, carbohydrates, phenols, amino acids, and amines) were used to evaluate the average heterotrophic metabolism and for estimation of kinetic parameters, by fitting the OD_{590nm} curve against time to the density dependent logistic growth equation proposed by Lindström et al. (1998):

$$OD_{590nm} = K \div \left[1 + e^{-R(T-S)}\right] \quad (1)$$

Where K (asymptote) is the maximum degree of color development (OD_{590nm}), R (degradation rate) the exponential rate of OD change (h^{-1}), T the time following inoculation of the plates (h), and S the time when the mid-point of the exponential portion of the curve (i.e., when $Y=K/2$) has been reached (h).

Shannon's diversity index (H') and substrate consumption richness (Ss) were calculated according to Zak et al. (1994), considering the AWCD for each reading time, during the incubation period.

The kinetic parameters estimated for the average heterotrophic metabolism and for the groups of substrates, Shannon's diversity index (H'), substrate consumption richness (Ss), and chemical parameters were submitted to analysis of variance (ANOVA), and the means were compared using the LSD test ($P<0.05$). These parameters were also correlated with soil chemical attributes, with the particulate contents of C and N and the respectively dissolved fractions, which were added to the soil via TSE irrigation, by means of Pearson's correlation. The statistical analyses were carried out using the SAS program, version 8.02 (SAS System 1999).

Nonmetric multidimensional scaling (NMDS) ordination was carried out to test differences in CLPPs based on sample similarities. The estimated kinetic parameters were transformed by log (x+1) and used to elaborate the dissimilarity matrix, using the Bray–Curtis model. Additionally, differences among the treatments were evaluated using an analysis of similarity (ANOSIM), according to the Primer 5 program (Primer-E Ltda 2001).

3 Results

3.1 Chemical Parameters

TSE irrigation of Tifton 85 pasture, after 18 months, increased soil pH, exchangeable Ca and Na, CEC and decreased exchangeable Mg, Al and the carbon/nitrogen ratio (C/N; Table 2). However, the nonirrigated pasture showed similar results to those of TSE-irrigated areas, for TC (8.16 g kg^{-1}), TN (0.55 g kg^{-1}), and K (1.68 $mmol_c$ kg^{-1}).

Exchangeable Na and Al were the chemical parameters most affected by TSE irrigation. Na could be considered irrelevant in the Tifton 85 experimental area, since the value observed in the non-irrigated pasture (0.90 $mmol_c$ kg^{-1}) is extremely low. However, this value increased to 6.03 $mmol_c$ kg^{-1} at the 25% surplus TSE irrigation treatment. The decrease of exchangeable Al is coherent with the pH increase

Table 2 Chemical soil parameters from a Tifton 85 Bermuda grass area irrigated with TSE, for 18 months (0 (control), 25%, 50%, and 100% of excess) and from a nonirrigated Tifton 85 Bermuda grass area (SE), at Lins, SP, Brazil

	pH	C/N	Na	Ca	Mg	Al	CEC
0	5.21 a	14.38 c	4.30 b	13.77 a	5.72 b	0.95 b	25.57 ab
25	5.06 b	14.70 bc	6.03 a	11.12 b	5.43 b	0.87 b	24.00 b
50	5.30 a	15.08 ab	6.02 a	13.17 a	5.77 b	0.85 b	26.80 a
100	5.26 a	14.78 bc	5.16 ab	13.84 a	6.03 b	0.80 b	26.80 a
SE	4.90 c	15.33 a	0.90 c	8.34 c	7.31 a	6.03 a	18.12 c

Average values (n=4). Means followed by same letters, for each column, do not differ using the LSD test ($P<0.05$)

pH active acidity, *C/N* carbon/nitrogen ratio, *Na* exchangeable sodium, *Ca* calcium, *Mg* magnesium, *Al* aluminum, *CEC* cation exchange capacity

(Table 2), reducing from 6.03 $mmol_c\ kg^{-1}$ in the nonirrigated pasture to 0.8 $mmol_c\ kg^{-1}$ in the 100% surplus TSE irrigation treatment. The exchangeable Ca increased with the TSE irrigation and for exchangeable Mg, the treatments irrigated with STE presented lower values than the nonirrigated pasture.

Despite of the absence of TSE irrigation effects on TC and TN, C/N ratio in TSE-irrigated areas was lower than in the nonirrigated area. This effect was probably due to the nitrogen added via fertilization and TSE irrigation.

3.2 Carbon Substrate Utilization Patterns

The carbon substrate consumption, evaluated by the average heterotrophic metabolism, showed no differences between the control TSE irrigated treatment (0% excess) and the surplus TSE irrigation (25%, 50%, and 100% excess; Fig. 1). However, the irrigated areas presented a higher average heterotrophic metabolism than nonirrigated pasture. The lag phase took the first 20 h for all treatments, followed by the exponential growth phase. The average heterotrophic metabolism of the nonirrigated area presented a longer exponential growth phase than TSE-irrigated areas. Only at the stationary phase, the results for the control TSE irrigation (0% excess) differed from surplus TSE-irrigated areas.

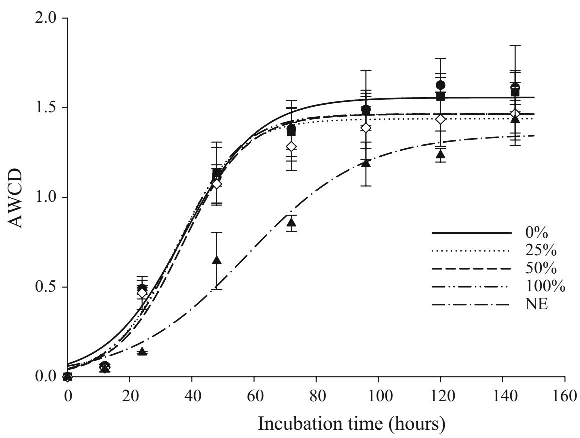

Fig. 1 Kinetics of AWCD curve fitting of soil suspensions from Tifton 85 Bermuda grass irrigated with TSE, for 18 months (*filled circles* 0% (control), *filled stars* 25%, *filled squares* 50%, and *empty diamonds* 100% of excess) and for a nonirrigated Tifton 85 Bermuda grass area (*filled triangles* SE), at Lins, SP, Brazil. The *lines* represent the fitted equations and the *dots* represent the means of each treatment (n=4)

The maximum degree of substrate consumption, represented by the K parameter, was significantly higher for TSE irrigation areas (Table 3). The lowest K was observed for nonirrigated areas. Likewise, the maximum exponential rate, r, showed different effects for TSE irrigation, compared to nonirrigated areas, which also took more time to reach the midpoint of exponential growth, represented by the s parameter.

For nonmetric multidimensional scaling ordination followed by ANOSIM analyses of the kinetic parameters (Fig. 2), the stress value (0.01) shows that the graphic representation has a greater probability to correspond to the correct interpretation of the data. The ANOSIM analyses showed significant differences (global r=0.468, P<0.001) for average heterotrophic metabolism and the *Pairwise* test results showed that the TSE-irrigated areas were significantly different from the nonirrigated areas (Table 4), but there was no difference to the surplus TSE irrigation.

Kinetic parameters and soil chemical parameters showed some significant relationships (Table 5). The K value was positively correlated with TPC and total particulate nitrogen (TPN) added by TSE irrigation. The low carbon/nitrogen ratio of TSE was probably the major factor that stimulated the increase of the K value.

The maximum exponential rate (r value) was positively correlated with all attributes, except exchangeable Al, which presented a negative correlation. TSE irrigation increased soil pH (5.5) compared with nonirrigated areas (4.8). These results, in addition to higher cation exchange capacity, can be associated with an increase in microbial growth. A positive correlation with the r value was observed for TPC, TPN, TC, TN, and exchangeable Na. Furthermore, the reduction of exchangeable Al was positively correlated with the r value. In contrast to the r value, negative correlations were observed for the s value in relation to the same attributes.

Shannon's diversity index (H') and substrate consumption richness (Ss) were altered by TSE irrigation. Ss was higher for TSE-irrigated areas than for nonirrigated areas after 48 h of incubation (Fig. 3a). Shannon's diversity index showed a significant effect of TSE irrigation from 12 h incubation on, and up to 96 h (Fig. 3b), with higher values for TSE-irrigated areas (3.22) compared with nonirrigated areas (2.43). However, the nonirrigated areas showed H' values increase during the incubation

Table 3 Kinetic parameters from AWCD curve fitting of soil suspensions from Tifton 85 Bermuda grass irrigated with TSE, for 18 months (0% (control), 25%, 50%, and 100% of excess) and for a nonirrigated Tifton 85 Bermuda grass area (SE), at Lins, SP, Brazil

Treatment	*K*	*r*	*s*
0	1.557±0.06 a	0.082±0.014 b	37.079±3.01 b
25	1.438±0.04 ab	0.098±0.015 a	34.910+2.27 b
50	1.464±0.05 ab	0.096±0.027 a	36.856±2.77 b
100	1.465±0.05 ab	0.097±0.016 a	35.579±2.55 b
SE	1.353±0.08 b	0.053±0.099 c	57.679±5.01 a

Average values and standard deviation ($n=4$). Means followed by same letters, for each column, do not differ using the LSD test ($P<0.05$)

K maximum degree of substrate consumption, *r* rate of substrate consumption and *s* the time to reach the midpoint of the rate of substrate consumption

period, reaching the highest H' value after 144 h of incubation, while the TSE-irrigated areas presented a lower variation.

TSE irrigation altered the kinetic parameters for each group of substrates. The greater differences in *K* and *r* values were observed for amines and phenols. For the *s* value, the greater differences were observed for carbohydrates. The *K* value increased for amines, with the 25% excess TSE irrigation significantly higher than the others. Lower *K* values were observed for phenols, with the control (0% excess) TSE irrigation significantly higher than the others. For the *r* value, all substrate groups were affected by TSE irrigation, except for amino acids. The highest variation was observed for the phenol group, which also presented the lowest *K* values. For the *s* value, carbohydrates, amines, and phenols presented the higher variation, with lower values for phenols and higher values for carbohydrates (not shown).

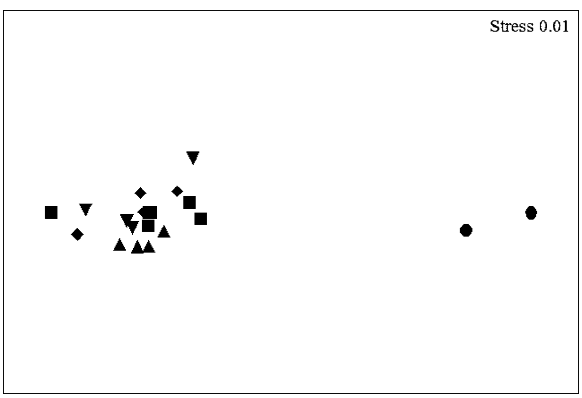

Fig. 2 Nonmetric multidimensional scaling of AWCD kinetic parameters of soil suspensions from Tifton 85 Bermuda grass irrigated with TSE, for 18 months (*filled triangles* 0%, *filled inverted triangles* 25%, *filled squares* 50%, and *filled diamonds* 100% excess) and for a nonirrigated Tifton 85 Bermuda grass area (*filled circles* SE), in Lins, SP, Brazil

4 Discussion

The particulate phase of effluents from stabilization ponds is composed mostly by algae (von Sperling 2002), which have a low C/N ratio. TSE irrigation, along with the available nutrients and moisture, as well as the organic matter of easy degradation provided by the algae, can improve soil microbial activity (Robertson and Groffman 2007). This higher soil microbial activity at the irrigated areas could be related with the increase of pasture yield observed in the surplus irrigated treatments (data not shown). The pasture yield increase with effluent irrigation was also observed by da Fonseca et al. (2007c). The authors verified that effluent irrigation increased the dry matter yield (38.5 Mg ha^{-1} $year^{-1}$) compared with water irrigation (32.7 Mg ha^{-1} $year^{-1}$), with the same fertilization and management practices.

The evaluation of the effects of effluent irrigation on microbial communities using Ecoplates® is still restricted to a few studies (Zhang et al. 2008; Gelsomino et al. 2006). The microbial community physiological potential, estimated by using Ecoplates®, has been considered a useful and quick method to get information about the physiological

Table 4 *Pairwise* test (R Statistics) of kinetics from AWCD curve fitting of soil suspensions from Tifton 85 Bermuda grass irrigated with TSE, for 18 months (0% (control), 25%, 50%, and 100% of excess) and for a nonirrigated Tifton 85 Bermuda grass area (SE), at Lins, SP, Brazil

Treatment	*R* statistical
0, 25	0.177
0, 50	0.292
0, 100	0.406
0, SE	1.000**
25, 50	−0.031
25, 100	−0.167
25, SE	1.000**
50, 100	−0.146
50, SE	1.000**
100, SE	1.000**

** $p<0.001$

Table 5 Pearson's correlation between metabolic kinetic parameters and chemical soil properties, total particulate carbon (TPC), and total particulate nitrogen (TPN) added by TSE

	pH	Al	CEC	Na	TC	TN	TPC	TPN
K	0.290	−0.377	−0.010	0.226	0.103	0.127	0.480*	0.480*
r	0.840**	−0.787**	0.499*	0.803**	0.546*	0.587**	0.643**	0.643**
s	−0.841**	0.800**	−0.339	−0.940**	−0.608**	−0.661**	−0.818**	−0.818**

K maximum degree of substrate consumption, *r* rate of substrate consumption, *s* time to reach the midpoint of the rate of substrate consumption, *pH* active acidity, *Al* exchangeable aluminum, *CEC* cation exchange capacity, *Na* exchangeable sodium, *TC* total carbon, *TN* total nitrogen

dynamics of microbial communities for different kinds of soils (Goberna et al. 2005; Classen et al. 2003), land management practices (Govaerts et al. 2007), or soil contamination with pesticides (Ros et al. 2006).

The physiological profile, obtained using Ecoplates®, detected differences between TSE irrigated pasture and nonirrigated pasture. Similar findings were reported by Gelsomino et al. (2006), who found that CLPPs, among other molecular techniques used to evaluate microbial communities, were useful to discriminate between areas repeatedly subjected to flooding with wastewaters from those who were not affected by flooding.

In this study, TSE irrigation modified the soil microbial community physiological response, in comparison to nonirrigated pasture, affected by inputs of

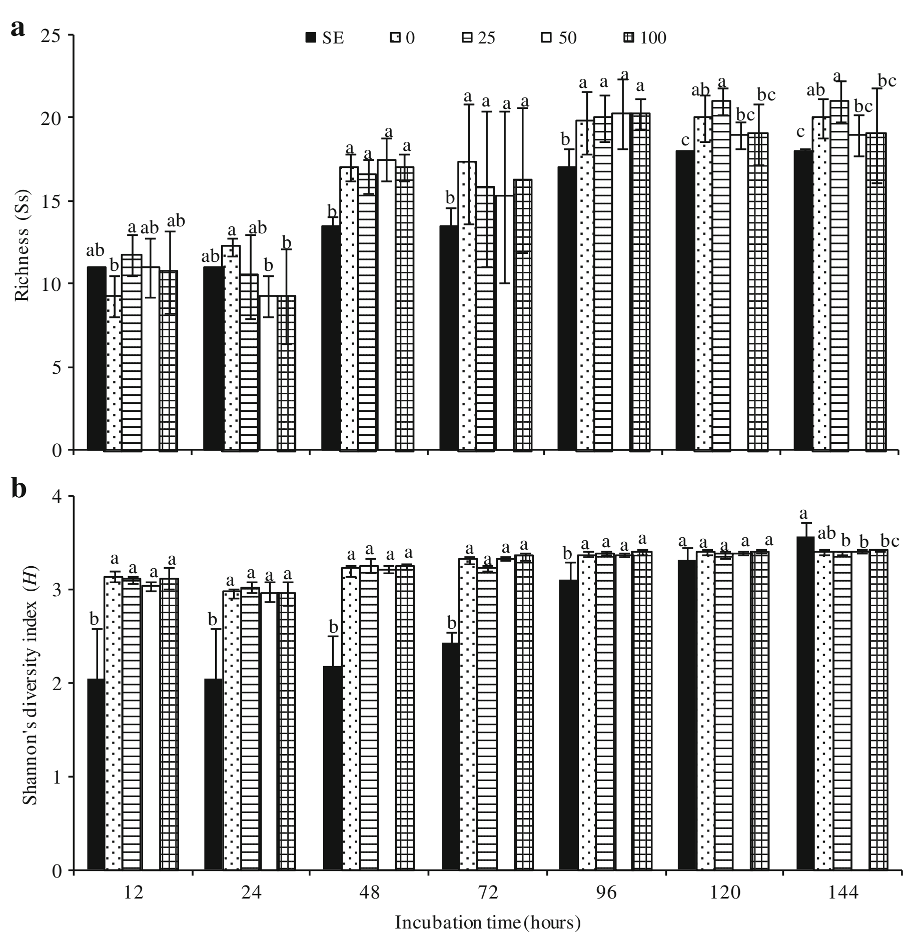

Fig. 3 **a** Richness (Ss) and **b** Shannon's diversity index (*H*′) of carbon substrate diversity, derived from CLPPs analysis of Tifton 85 Bermuda grass irrigated with TSE, for 18 months (0% (control), 25%, 50%, and 100% of excess) and for a nonirrigated Tifton 85 Bermuda grass area (SE), in Lins, SP, Brazil. *Means followed by same letters*, for each incubation time, do not differ when using the LSD test ($P<0.05$)

carbon and nitrogen from TSE that possibly stimulated the microbial activity, increasing the potential heterotrophic metabolism of the soil microbial community. This is in agreement with Zhang et al. (2008). These authors found that effluent irrigation increased significantly the carbon substrate utilization, evaluated using Ecoplates, in comparison with water irrigated areas. However, the authors found no differences for Shannon's diversity index, although effluent irrigated areas presented higher values (3.15) than water irrigated areas (2.85).

The NMDS ordination followed by ANOSIM pointed out significant differences between TSE-irrigated areas and nonirrigated areas. NMDS attempts to place the fitting similarities calculated in a triangular matrix of similarity coefficients computed between every pair of samples on a plot of two dimensions (Manly 2005). This statistical analysis has also the advantage of using the *Pairwise* statistical test, that gives the *R* statistics to compare pairs of variables and shows the level of significance between them.

Soil pH increase observed after 18 months of TSE irrigation is in agreement with Fonseca et al. (2007a). This result may be a consequence of the high pH value of TSE [pH (H_2O) 7.5], stimulation effect of TSE irrigation on microbial activity, suitable moisture conditions, substrate addition, as well as organic matter mineralization. However, no soil pH variation after effluent irrigation was detected by Rusan et al. (2007), which is probably related with the soil buffering capacity. The practice of TSE irrigation enhanced soil cation exchange capacity and decreased exchangeable Al, when compared with the nonirrigated area, in accordance with the results described by other authors (da Fonseca et al. 2007a, b). These results were related with the effect of the STE constituents, which acted on the replacement of the exchangeable Al, and part of the Mg^{2+}, by Ca^{2+} and in minor quantity by Na^{+} in the cation exchange complex, and the alkalinity (HCO_3^-) that neutralized part of the potential acidity (H^+) of the cation exchange complex.

The positive correlation between Na and the *r* value is in contrast with the other findings. Rietz and Haynes (2003) showed a negative effect of increasing exchangeable Na concentrations on microbial activity, evaluated by ammonification rate and fluorescein diacetate hydrolysis rates. A possible explanation for our results is that TSE irrigation increased both exchangeable Na and TN, and this fact may have caused a "confounding" effect on this correlation value. TN and TPN presented significant positive correlations (0.768 and 0.870, respectively) with exchangeable Na. Therefore, we hypothesize that the positive effect observed between exchangeable Na and the *r* value is a consequence of a concomitant high addition of TPN and should not be considered as a cause and effect relation.

TSE irrigation research has been stimulated by raising concerns about water supplies for agriculture (Marris 2008). Furthermore, questions related to soil chemical (Rusan et al. 2007) and physical (Warrington et al. 2007) attributes, impact studies of this activity on microbial communities, and their physiological potentials can add useful information for future agriculture effluent reuse guidelines.

Economic gains with nutrient supplies by TSE irrigation is not only the major reason for farmers to choose this practice (da Fonseca et al. 2007b) but it also represents an environmental issue. Water reuse in agriculture can reduce demands on water resources for irrigation purposes; moreover, wastewaters are a constant water reuse resource and could also contribute to the market value of crops (Toze 2006).

The considerations about the impacts related with the wastewater irrigation can only be pointed out for a short term since they are based on possible modifications of the soil microbial community evaluated by the community level physiological profiles. However, except for the concern about a possible salts accumulation, especially Na^+, after long-term irrigation, all the other results suggest mostly positive modifications of the soil for Tifton 85 Bermuda grass cropping.

Monitoring soil attribute shifts in short-term experiments under a specific management practice, like wastewater irrigation, is crucial to evaluate the viability of this reuse. Choosing variables for evaluation of the sustainability of the effluent irrigation should consider not only chemical and physical attributes but also variables that respond quickly to changes in soil management, like microbiological attributes (Bending et al. 2004). Also, continuous evaluation of long-term TSE irrigation trials should be taken into account, as a way of learning the supporting capacity of the plant–soil–environment system. The viability of wastewater reuse in agriculture has been shown by well managed and monitored areas (Rusan et al. 2007), stimulating the development of models and tools capable to help in decision making (Hidalgo et al. 2007), without forgetting to take into account the risks related to public health (Salgot et al. 2006).

5 Conclusions

The surplus TSE pasture irrigation for 18 months, evaluated by CLPPs, with Ecoplates, caused no significant effects on soil microbial physiological capacity compared with control TSE irrigation, evaluated by diversity and kinetics of substrate consumption, and so possibly it would not cause any damage to pasture or to the soil.

Pasture TSE irrigation stimulated the soil microbial community metabolic capacity in comparison to nonirrigated pasture, which also had an effect on soil fertility improvement. Changes in soil microbial physiological status as a consequence of short term managements, such as TSE irrigation, can be well suited to evaluate and monitor these areas.

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