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Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient

E. Gomez [a,*], J. Garland [b], M. Conti [c]

[a] *Cátedra de Microbiología Agricola, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Campo Experimental J. Villarino, 2123 Zavalla, Argentina*
[b] *Dynamac Corporation, Kennedy Space Center, FL 32899, USA*
[c] *Ca tedra do Edafologia, Facultad de Agronomia, Universidad de Buenos Aires, Av. San Martin 4453, 1417 Buenos Aires, Argentina*

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Abstract

This work assessed soil bacterial diversity through community-level physiological profiles (CLPP) over three consecutive years, at sites representing a gradient of land use intensification. The relationship between CLPP and soil physical and chemical properties, and the potential use of CLPP for soil quality monitoring was also evaluated. Samples were collected from sites under native vegetation (V0); naturalized prairie, cleared in 1982 (P16); conventional tillage, cleared in 1972 (T26); and direct drilling, cleared in 1958 (D40). Plate counts were performed to determine soil culturable bacterial density. Sample dilutions were inoculated on Biolog GN microplates, and optical density (OD) was recorded after 54 h of incubation. Richness (Ri), Shannon–Weaver index (*H*) and principal component analysis (PCA) on OD standardized data were performed. Soil aggregation evaluated through the variation between dry and wet average aggregate diameter (ΔAAD), soil organic carbon (SOC) and total nitrogen (TN) were determined. Richness and *H* differed significantly among the sites. Principal component analysis consistently differentiated the soils in all 3 years. The P16 site did not differ in ΔAAD from V0 and T26, while SOC and TN did not differentiate T26 from D40. Results showed a lower sensitivity of physical and chemical variables than CLPP to detect differences along the land use intensification gradient. Multiple correlations between Ri and *H* with ΔAAD, SOC and TN ($R > |0.70|$), suggested a high association between the soil aggregation condition and organic matter content with microbial diversity.

Keywords: Community-level physiological profiles; Microbial diversity; Land use intensification; Biolog assay

1. Introduction

Agricultural intensification has resulted in quality deterioration in several soils, affecting their productivity (Sanders, 1992). The evaluation of soil quality is complex, since it requires the consideration of physical, chemical and biological aspects (Wander and Bollero, 1999). Among biological properties,

* Corresponding author. Tel.: +54-341-49700801; fax: +54-341-49700805.
E-mail addresses: egomez@fcagr.unr.edu.ac, lenidelvallegomez@yahoo.com.ar (E. Gomez).

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microbiological ones have been considered as interesting attributes which are highly sensitive to changes (Elliott, 1997). Thus, microbiological properties have been pointed out as suitable indicators complementing physical and chemical properties in soil quality evaluation (Toresani et al., 1998; Stenberg, 1999).

Management practices have a significant impact on soil organism populations and their activity (Roper and Gupta, 1995); they should promote fertility and productivity in the long term to be considered sustainable (Beare, 1997). Preservation of soil microbial diversity appears to be essential in this context. Giller et al. (1997) suggested that the assessment of the effect of the anthropic disturbance on soil biodiversity could be performed through land use intensification gradients which allow exploring trends.

Garland and Mills (1991) developed a community-level redox technique based on sole C-source oxidation, originally designed for isolate identification, for estimating microbial community diversity. Since then, this approach has been increasingly used to differentiate microbial communities from diverse habitats (Garland, 1997; Gomez et al., 2000). While C-source patterns or community-level physiological profiles (CLPP) have been proposed as potential soil quality indicator (Stenberg, 1999), rigorous testing of this concept is lacking.

In Entre Ríos Province (Argentina), the area where this study was carried out, short-term agricultural profitability has promoted clearing of native vegetation, in many cases in an indiscriminate way, leading to soil structure deterioration and organic matter loss (Tasi, 2000). A previous research reported that different CLPP were obtained from sites with increasing time elapsed since clearing of native vegetation and different further managements, and from the undisturbed site. Bacterial community from the native condition showed the highest functional diversity, with respect to communities from the sites that were disturbed; the lowest functional diversity was found in the site with more time elapsed since clearing (Gomez et al., 2000).

The aims of the study reported here, conducted over three consecutive years, were: to determine whether physiological profiles based on the use of carbon sources by bacterial community produced a consistent pattern over the time associated to land use intensification; and to relate the profiles obtained to other soil properties used to evaluate soil quality. The suitability of the carbon-source utilization profiles to be used for monitoring soil quality was also analyzed.

2. Materials and methods

2.1. Site description and sampling

This work was carried out in a Vertic Argiudoll from Entre Ríos, Argentina (31°30′S latitude; 59°45′W longitude). In October 1998, 1999 and 2000, three samples composed by 20 sub-samples were taken in the 0–7.5 cm layer from each one of the plots, sieved (<2 mm), stored at 4 °C and analyzed within a week to evaluate microbiological properties. In October 1998 and 2000, undisturbed and composite samples were taken to evaluate physical and chemical properties, respectively. Due to the mensurative nature of the experiment, randomized assignment of treatments was not possible. Several researches faced this frequent difficulty in field studies (Kennedy and Smith, 1995; Staddon et al., 1998). Like in other works of this kind (Bardgett et al., 2001), the sources of error associated with this problem were minimized by sampling randomly located sectors from each site (Gomez et al., 2000).

Samples were taken from sites that were named with a letter and a number which refer to the condition or management, and to the years elapsed since clearing of the native vegetation at the beginning of the study (1998), respectively. They were: (1) a site under xerophytic and herbaceous native vegetation, with Prosopis and Celtis as dominating bush genera, and Stipa, Setaria, Bothriochloa, Paspalum, Stenandrium, Scoparia and Trifolium as main herbaceous genera (V0); (2) a site that had been cleared in 1982, conventionally cropped (moldboard plowing as the main labor) for 8 years and then under naturalized prairie (P16); (3) a site cleared in 1972, conventionally cropped with corn and soybean (T26); and (4) a site cleared in 1958, conventionally cropped till 1994, and then under direct drilling (D40). More detailed information about sites and soil type is described in Gomez et al. (2000). Monthly mean temperature and rainfall data in October 1998, 1999 and 2000 were: 22, 18, 20 °C, and 50, 25, 140 mm, respectively.

2.2. Laboratory analysis

2.2.1. Heterotrophic bacterial counts

Since the CLPP is a method based on culturability, plate counts seem to be the most suitable enumeration technique to relate with CLPP (Garland and Lehman, 1999). Tenfold dilution series from soil suspensions (soil 10 g; sterile deionized water 100 ml; shaken for 1 h) were performed and aliquots of 1 ml were plated on tryptic soy agar (Difco Lab, Detroit, MI). Counts were done after 5 days of incubation at 25 °C and results expressed as colony forming units (CFU) g^{-1} soil (on a dry-weight basis).

2.2.2. Community-level physiological profiles

Biolog GN microplates were used (Biolog Inc., 1993 Hayward CA). Soil suspensions (soil on a dry-weight basis 10 g; sterile saline 0.85% NaCl 100 ml) were prepared according to Biolog GN supplier instructions. Some researches reported, after the present work, that deionized water could be used to make the suspensions for inoculating Biolog GN microplates (Garland and Lehman, 1999; Frankilin et al., 2001). Soil suspensions were then shaken for 1 h prior to pre-incubation for 18 h to allow microbial utilization of any soluble organic carbon derived from the soil that could interfere in the sole-C-source-use response (Dick et al., 1996). Biolog GN microplates were inoculated with aliquots of 100 μl from 10^{-4} dilution (10^4–10^5 culturable cells ml^{-1}) and incubated at 25 °C. Color development in the wells, which is indicative of carbon-source utilization, was recorded as OD with a plate reader at regular time intervals of incubation.

2.2.3. Physical and chemical properties

Intensification in the area under study was reported to promote soil aggregation and organic matter losses, as it was mentioned above. Soil structure and organic matter evaluated through organic C and total N were reported as useful variables to assess soil quality (Gregorich et al., 1994; Doran and Parkin, 1994). Soil aggregation was determined according to the method of De Leenheer and De Boodt (1958). Soil samples were dry and wet sieved, and the variation between dry and wet average aggregate diameter (ΔAAD) after sieving, which is indicative of structural stability, was used for analysis. Organic carbon and total nitrogen were determined by Walkley-Black (Nelson and Sommers, 1982) and Kjeldahl (Bremner and Mulvaney, 1982) methods, respectively.

2.3. Data analysis

Richness (Ri), as the number of oxidized C substrates, and the Shannon–Weaver index (*H*) (i.e., the richness and evenness of response) were calculated from the 54 h plate readings. An OD of 0.25 was used as threshold for positive response (Garland, 1997). Shannon–Weaver index was calculated as follows (Gomez et al., 2000).

$$H = -\Sigma p_i (\ln p_i)$$

where p_i is the ratio of the activity on each substrate to the sum of activities on all substrates.Richness, *H*, CFU g^{-1} soil ($\log_{10}$ transformed), ΔAAD, SOC and TN were analyzed by ANOVA and Duncan test for mean comparisons. Principal component analysis was performed on OD data from the 95 carbon sources at 54 h of incubation, that were standardized by the average well color development in each microplate to remove inoculum density effect (Garland, 1997). Correlation analysis (Pearson coefficient) was used to test the relationships between Ri and *H*, and ΔAAD, SOC and TN. All statistical analysis was performed with SAS (1990), version 6.12.

3. Results

3.1. Heterotrophic bacterial counts

Bacterial counts varied from year to year. In 1998, they were significantly higher ($P < 0.05$) in D40 than in V0, P16 and T26; while T26 showed lower counts in 1999 in relation to the other sites. Counts in all the sites differed in 2000 (Fig. 1).

3.2. Community-level physiological profiles

The 54 h incubation time, which data were used to perform the statistical analysis was the shortest incubation time that allowed the best resolution among sites (i.e. the largest differentiation with the highest amount of variance explained by the first two axes), over the 3 years.

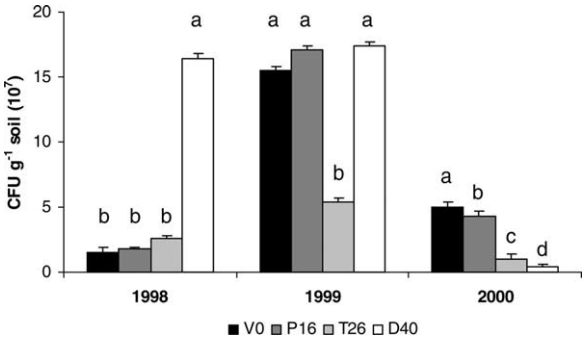

Fig. 1. Bacterial counts on tryptic soy agar in soils from V0 (native condition); P16 (naturalized prairie, cleared in 1982); T26 (conventionally tilled, cleared in 1972), and D40 (no tilled, cleared in 1958). Means followed by the same letter are not significantly different (Duncan; $P < 0.05$); $n = 3$.

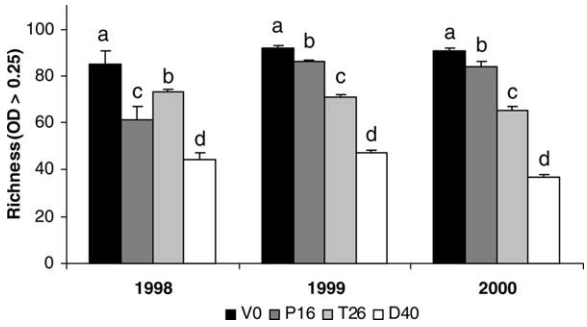

Fig. 2. Richness of carbon substrates metabolized in Biolog GN by microbial communities from V0 (native condition), P16 (naturalized prairie; cleared in 1982), T26 (conventionally tilled; cleared in 1972) and D40 (no tilled; cleared in 1958). Means followed by the same letter are not significantly different (Duncan; $P < 0.01$); $n = 3$.

Richness of metabolized substrates was significantly different ($P < 0.01$) among V0, P16, T26 and D40 in all the three sampling years. Richness was always significantly higher in V0 with respect to the other sites, while the lowest values of Ri were found in D40 (Fig. 2).The Shannon–Weaver index (H) was significantly higher in V0 ($P < 0.01$) and significantly lower in D40 with respect to the other sites in all 3 years. In 1998, P16 and T26 did not differ in H. In 1999 and 2000, H was significantly greater in P16 compared to T26 (Fig. 3).

The PCA based on intensity in the utilization of the 95 carbon substrates present in Biolog GN plates, showed a consistent separation of samples coming from the sites with increasing time since clearing and the native condition. The first and second principal components explained 62.8, 73.1 and 68.3%, in 1998, 1999 and 2000, respectively (Fig. 4A–C).

Although PCA showed a clear differentiation of samples from the four sites, the carbon sources that significantly ($R > |0.69|$) contributed to the discrimination in the two-dimensional space varied among the 3 years. However, 23 from the 95 substrates present in Biolog GN microplates were consistently correlated to PC1 in the 3 years, as denoted in Table 1. Substrates like Tween 40, Tween 80, *cis*-aconitic acid, citric acid, β-hydroxybutyiric acid, α-ketoglutaric acid, D,L-lactic acid, D-saccharic acid, L-aspartic acid, L-glutamic acid, D,L-carnitine and γ-aminobutyric acid had a

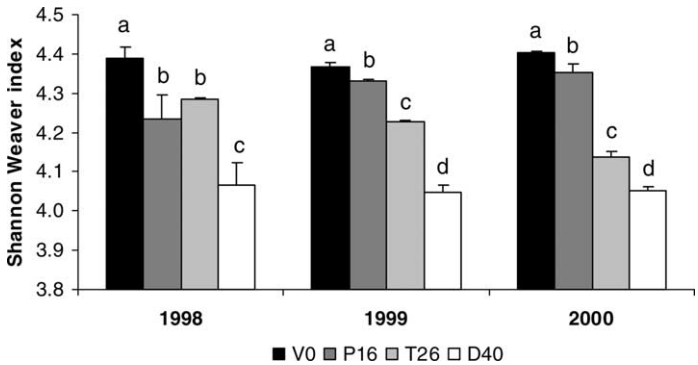

Fig. 3. Shannon–Weaver diversity index (H) calculated from carbon substrates in Biolog GN microplates metabolized by microbial communities from V0 (native condition), P16 (naturalized prairie; cleared in 1982), T26 (conventionally tilled; cleared in 1972) and D40 (no tilled; cleared in 1958). Means followed by the same letter are not significantly different (Duncan; $P < 0.01$); $n = 3$.

strong response in D40 samples and showed high negative correlations with PC1. In turn, substrates with high correlation with PC1, like α-cyclodextrin, gentiobiose, D-melibiose, β-methyl-glucoside, succinamic acid, glycil-L-aspartic acid, L-serine and thymidine, were highly oxidized by microbial communities from V0 (Table 1).

3.3. Physical and chemical properties

The lowest variation in ΔAAD was observed in V0 in 1998 and 2000, indicating the undisturbed site as showing the highest structural stability, though it was not significantly different ($P < 0.05$) from P16, but differed from T26 and D40; P16 did not differ

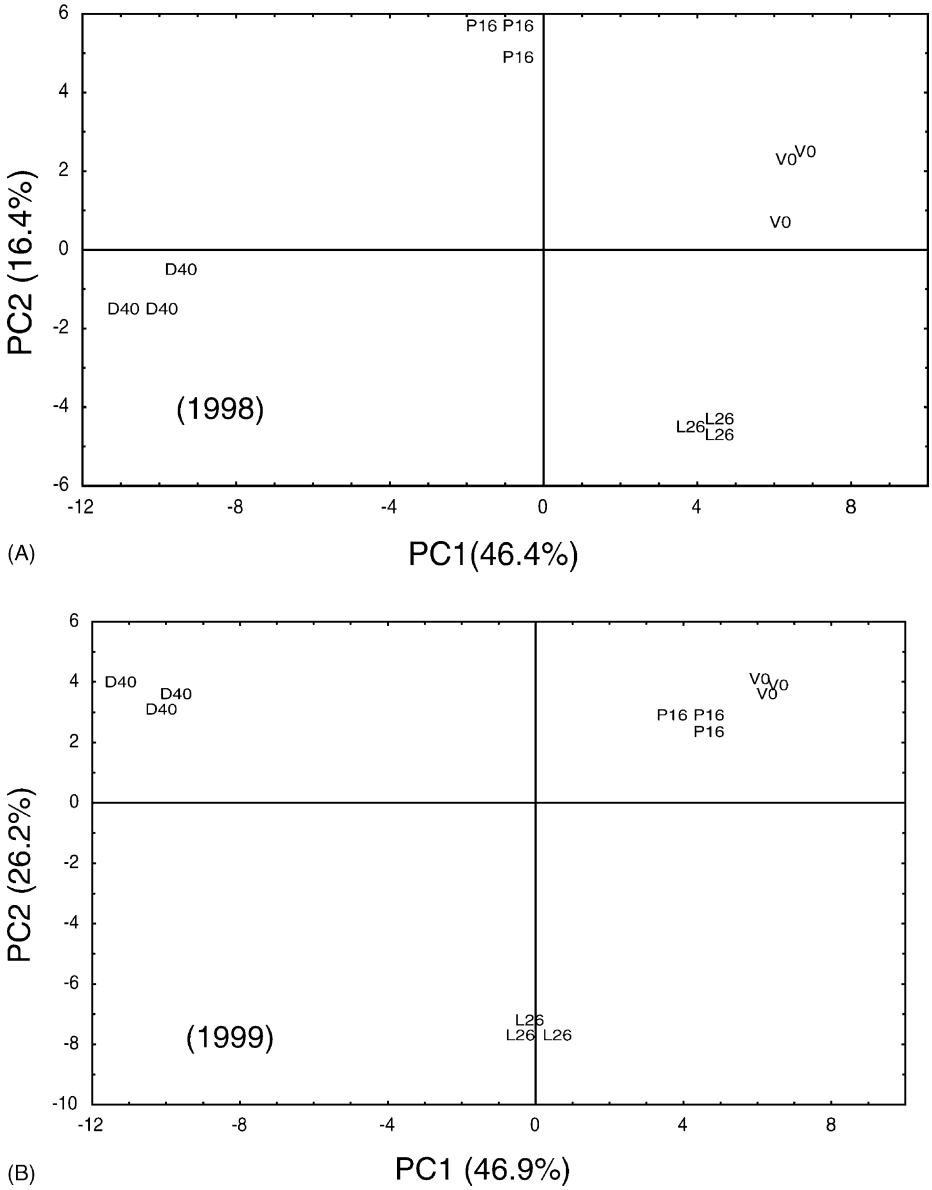

Fig. 4. Principal components on standardized data of activity on carbon sources in Biolog GN, of samples coming from V0 (native condition), P16 (naturalized prairie; cleared in 1982), T26 (conventionally tilled; cleared in 1972) and D40 (no tilled; cleared in 1958) in 1998 (A), 1999 (B) and 2000 (C).

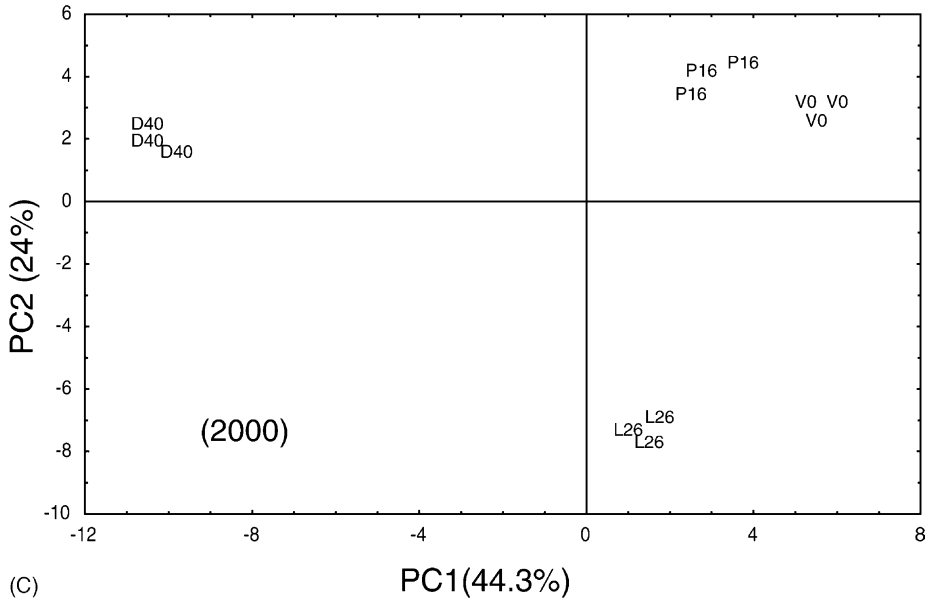

Fig. 4. (*Continued*).

from T26, while the highest value was found in D40 (Fig. 5). The organic carbon and TN was significantly higher ($P < 0.05$) in soils from the V0 sites relative to all other sites, and in P16 versus T26 and D40, in both 1998 and 2000 (Table 2). When Ri and H were correlated with physical and chemical properties, a significant negative correlation with ΔAAD was found (−0.83 and −0.84, respectively), while those variables were positively correlated with SOC (0.73 and 0.71, respectively) and TN (0.74 and 0.74, respectively).

4. Discussion

4.1. CLPP methodological considerations

Previous researches reported that C-substrate oxidation depends on both community composition and inoculum density (Garland and Mills, 1991; Smalla et al., 1998). Thus, samples with different cell density could appear to be distinct in the analysis due to variations in the intensity of substrate oxidation produced by a density effect rather than by real differences in metabolic patterns. Also, plate counting had been suggested as the most suitable enumeration technique for measuring microbial density inoculated in Biolog GN microplates (Garland and Lehman, 1999). In the present work, contrary to findings from other researches, C-source use profiles in D40 were the lowest, despite this site showed the highest heterotrophic bacterial populations developed on tryptic soil agar in 1998 and 1999 sampling. Nevertheless, in order to remove the effect of inoculum density, data from OD corresponding to 54 h incubation time were standardized for the multivariate analysis. This approach, besides showing the relationship among samples coming from the different sites independently of microbial density, allowed a better visualization of those C-sources being used with higher intensity by communities from D40, in which most of the substrates were poorly oxidized.

Several substrates that significantly contributed to the PCA separation varied year by year, although 24 and 50% of the C-sources present in Biolog GN were the same contributing to the separation in the PC1 in the 3 years and in at least 2 years, respectively. Several studies found different substrates as responsible for the separation of samples (Bossio and Scow, 1995; Haack et al., 1995). Also, selective growth was reported to occur in microplate wells (Haack et al., 1995; Smalla et al., 1998), so, it should not be emphasized on particular substrates, since they can not be related to “in

Table 1
Correlations with PC1 (A) and PC2 (B) of carbon substrates in Biolog GN which significantly contributed ($R > |0.69|$) to the separation in the two-dimensional space in three (∗) or at least two sampling dates

C-source	1998	1999	2000	C-source	1998	1999	2000
(A) PC1							
α-Cyclodextrin*	0.77	0.81	0.74	γ-Hydroxy-butyric acid	0.79	0.79	
Tween 40*	−0.76	−0.96	−0.95	*p*-Hydroxy-phenylacetic acid	−0.77	−0.73	
Tween 80*	−0.91	−0.98	−0.96	α-Ketoglutaric acid*	−0.95	−0.97	−0.95
N-Acetyl-D-glucosamine		0.73	0.88	D,L-Lactic acid*	−0.90	−0.94	−0.94
L-Arabinose		−0.97	0.76	Quinic acid	−0.87		−0.78
Cellobiose	0.95		0.87	D-Saccharic acid*	−0.99	−0.97	−0.96
Gentiobiose*	0.72	0.97	0.83	Succinic acid*	−0.98	−0.94	−0.85
α-D-Glucose	−0.78		−0.73	Bromosuccinic acid	−0.88		−0.84
Maltose	0.70	0.72		Succinamic acid*	0.89	0.71	0.73
D-Mannitol	−0.71	0.79		D-Alanine	0.72		−0.74
D-Mannose	−0.74		0.76	L-Asparagine	−0.74	−0.84	
D-Melibiose*	0.89	0.71	0.76	L-Aspartic acid*	−0.88	−0.71	−0.98
β-Methyl-glucoside*	0.85	0.90	0.93	L-Glutamic acid*	−0.85	−0.86	−0.83
D-Raffinose	0.93	0.84		Glycil-L-Aspartic acid*	0.78	0.75	0.71
L-Rhamnose		0.77	0.80	L-Histidine*	0.81	−0.86	−0.82
D-Sorbitol		0.77	0.74	L-Serine*	0.82	0.89	0.93
D-Trehalose*	0.84	0.79	0.85	D,L-Carnitine*	−0.78	−0.75	−0.82
Turanose	0.83	0.74		γ-Aminobutyric acid*	−0.80	−0.82	−0.74
Mono-methyl-succinate	−0.89	−0.77		Urocanic acid		−0.90	−0.97
cis-Aconitic acid*	−0.99	−0.96	−0.90	Uridine	0.94	−0.92	
Citric acid*	−0.94	−0.95	−0.98	Thymidine*	0.83	0.87	0.95
D-Galacturonic acid	0.85	−0.99		Phenyl-ethylamine	0.80	0.70	
D-Gluconic acid	−0.72		−0.70	D,L-α-glycerolphosphate		0.75	−0.81
β-Hydroxybutyric acid*	−0.93	−0.83	−0.90				
(B) PC2							
N-Acetyl-D-glucosamine		−0.73	−0.83	L-Alanyl-glycine	0.77	−0.70	
D-Fructose		−0.92	−0.90	L-Pyroclutamic acid		0.73	0.77
D-Galactose		0.78	0.70	Glucose-6-phosphate	0.77	−0.75	

Table 2
Soil organic carbon (SOC) and total nitrogen (TN) in V0 (native condition), P16 (naturalized prairie; cleared in 1982), T26 (conventionally tilled; cleared in 1972) and D40 (no tilled; cleared in 1958)

Sampling sites	1998		2000	
	SOC ($g\,kg^{-1}$)	TN ($g\,kg^{-1}$)	SOC ($g\,kg^{-1}$)	TN ($g\,kg^{-1}$)
V0	55.3 ±4.2 a	4.3 ± 0.3 a	64.7 ± 4.9 a	5.9 ± 0.4 a
P16	31.1 ± 0.4 b	3.9 ± 0.1 b	36.6 ± 1.5 b	4.0 ± 0.1 b
T26	26.9 ±3.5 c	2.1 ± 0.2 c	20.2 ± 2.0 c	2.1 ± 0.1 c
D40	24.0 ± 3.4 c	2.0 ± 0.1 c	19.4 ± 2.0 c	2.0 ± 0.1 c

Values are mean ± standard deviation. Different letters within a column indicate significant difference (Duncan; $P < 0.05$); $n = 3$.

situ" utilization. The 95 substrates in Biolog GN microplates provide a wide set of compounds that enable to estimate relative potential metabolic versatility.

Color development in microplates followed a sigmoid pattern (data not shown) as observed by other researchers. Kinetic analysis of data from multiple plate readings has been recommended as a suitable approach to the observed non-linear responses (Lindstrom et al., 1998; O'Connell and Garland, 2002), but single-point plate readings offer a simpler approach. Data from the 54 h incubation time differentiated samples better in terms of richness, diversity and pattern compared to

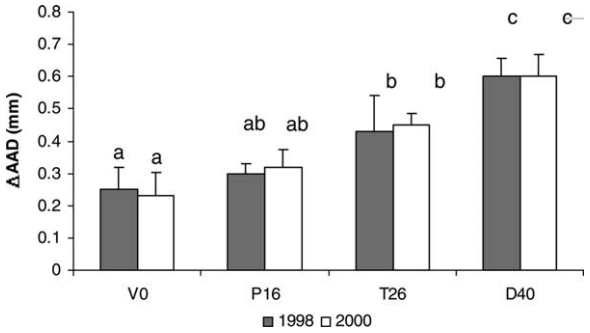

Fig. 5. Variation in aggregate weighed average diameter measured in samples from V0 (native condition), P16 (naturalized prairie; cleared in 1982), T26 (conventionally tilled; cleared in 1972) and D40 (no tilled; cleared in 1958) in 1998 and 2000. Means followed by the same letter are not significantly different (Duncan; $P < 0.05$); $n = 3$.

36 and 48 h of incubation (data not shown). Similar results were reported by Bossio and Scow (1995), who studied microbial communities from a vertic soil under similar incubation conditions and found the 50 h OD measures as the best resolution reading point.

4.2. CLPP related to land use intensification gradient

Various studies have differentiated soil samples based on CLPP, including soil types (Winding, 1994), alternative cropping systems with rice (Bossio and Scow, 1995), and previous management history (Grayston and Campbell, 1996). The present work found consistently distinctive CLPP in samples from sites with increasing time elapsed since clearing of the native vegetation and different further managements over three consecutive years. A high proportion of the information contained in the original 95-dimensional data was displayed in a two-dimensional space, indicating that there was a consistent pattern in the data structure (Pielou, 1984).

Samples from V0 had consistently higher values of richness and diversity of metabolized substrates with respect to the cleared sites, a result coincident with research from other habitats that found higher potential in the use of C-sources in undisturbed versus disturbed sites (Staddon et al., 1998; Goodfriend, 1998).

The P16 site had lower Ri and *H* values in 1998 as related to 1999 and 2000. The lower grass cover due to intensive grazing observed (not measured) in the first year could be a factor explaining that finding. As regards intensive grazing, some authors have reported it might reduce underground plant biomass (Holland and Detling, 1990; Pandey and Singh, 1992), and the functional abilities of bacterial communities could be affected by changes in plant biomass as a consequence of qualitative and quantitative modifications in organic matter (Insam et al., 1996). The increase in Ri and *H* of the P16 soils in 1999 and 2000 also could be viewed as the beginning of a recovering effect of the soil under naturalized prairie, since even a few years of disturbance were enough to adversely alter the organic matter content, and the functional diversity as measured in 1998 in relation to the undisturbed site. The greater similarity in the CLPP between P16 and V0 during the last 2 years may be indicative of a recovery effect, as seen by Lupwayi et al. (1998) when comparing conservationist and conventional soil management practices.

Results from the two conventionally cropped systems need to be viewed as a function of both time from vegetation clearing and recent management practices. Even though the D40 site had been cleared much longer ago than the T26 site, organic carbon and total nitrogen were similar between the sites, potentially as a result of the compensatory effect of no till practices at the D40 site since 1994. However, functional richness and diversity were lower at the D40 site, which is in contrast to previously observed positive (Lupwayi et al., 1998) or neutral (Hassink et al., 1991) effect of no till on microbial diversity. The high silt and montmorillonitic clay content, together with the long history of continual agriculture under a conventional management, generated compaction and aggregation loss at the D40 site, as reflected in the ΔAAD measures, and this could be influencing composition and activity of the microbial communities. Previous crop history and soil textural characteristics have been reported as countering potential beneficial effects of conservation practices in Vertic Argiudolls (Gomez et al., 2001).

Comparison of the CLPP results with other physical and chemical properties showed that the CLPP were more sensitive to distinguish the sites under study. The CLPP differentiated among all the sites within the land use intensification gradient, while ΔDDA did not differentiate between P16 from V0 and T26 from P16, and SOC and TN could not distinguish between the two agricultural sites. However, crop production data

as regards these last two sites such as average soybean production in T26 and D40, that was 2100 and 950 kg ha^{-1}, respectively, indicates that there are substantial differences in the productivity between both sites.

The high correlation between physical (ΔDDA) and chemical (SOC and TN) measurements with CLPP reflects a strong association of soil aggregation condition and organic matter content with microbial diversity. As regards soil physical condition, Young and Ritz (1998) pointed out the close relationship between the physical environment and biodiversity, especially with respect to soil structure, since it constitutes the three-dimensional space where soil biota develops, conditioning other soil properties such as porosity, gas fluxes and substrate protection.

A proper soil quality indicator must be sensitive to disturbance produced as a consequence of soil management (Edwards, 1996), but it must be stable enough as not to be influenced by short-term weather fluctuations or crop development (Doran et al., 1996). The consistency and reproducibility in the differentiation of sites from community-level physiological profiles obtained in the present study, yet under fluctuating conditions of temperature and rainfall, suggest that CLPP could be a useful tool for soil quality monitoring, particularly given its relative simplicity. The CLPP approach has bias and limitations as well as other methods for studying microbial communities (Garland, 1997). It provides information about a portion of the community, that which is able to grow under the assay conditions. Nevertheless, this technique has been recognized as a useful tool for comparison of microbial communities (Haack et al., 1995; Smalla et al., 1998). Since it was reported that CLPP detected functional changes in microbial communities as a result of differing carbon availability in bioreactors and in soil (Garland et al., 1997; Grayston et al., 1998), its physiological basis would provide an ecologically relevant overview as long as the results are interpreted as a profile of phenotypic potential and not in situ activity.

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