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A comparison of indexing methods to evaluate quality of soils: the role of soil microbiological properties

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Abstract. The study evaluates and compares two procedures for selecting soil quality indicators (used for the construction of soil quality indices, SQI) by using diverse chemical, physical, and biological properties, and evaluates the role of soil microbiological properties in the construction of SQI. Different soil environments were selected from an extensive agricultural production site in the rolling pampa, Buenos Aires, Argentina, The plots included an undisturbed soil, a grassland soil, and continuous tilled soils with four different surface horizon depths (25, 23, 19, and 14 cm). Various properties were measured, and a minimum dataset was chosen by principal component analysis (PCA) considering all measured soil properties together (procedure A), or the PCA was performed separately according to classification as physical, chemical, or biological soil properties (procedure B). The measured soil properties involved physical, chemical, and biochemical properties determined by standard protocols used in routine laboratory analysis (simple SQI, SSQI) or more laborious protocols to determine microbial community structure and function by phospholipid fatty acid (PLFA) and catabolic response profile (CRP), respectively (complex SQI, CSQI). The selected properties were linearly normalised and integrated by the weight additive method to calculate SSQI A, SSQI B, CSQI A, and CSQI B indices. Two microbiological SOI (MSOI) were also calculated; MSOI 1 considered only biological properties according to the procedure used for calculating SQI; MSQI 2 was calculated by considering three selected microbiological parameters representing the size (microbial biomass carbon), activity (soil basal respiration), and functional diversity (evenness, determined by CRP) of the microbial communities.

All of the constructed indices show the same differences among the study sites. The inclusion of CRP and PLFA data in the indices slightly increased, or did not increase, the index sensitivity. Microbiological indices had the same sensitivity as the indices integrated by physical, chemical, and biological properties. An evaluation of the SQI constructed by both procedures found no difference in sensitivity. However, SQI constructed by procedure B allowed evaluation of the effects of management practices on physical, chemical, and biological soil properties.

Additional keywords: biological indicators, chemical indicators, physical indicators, soil quality, soil quality indices.

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Introduction

Soil quality assessment is needed to evaluate soil conditions and the sustainability of soil and crop management practices. Evaluation of soil quality requires identification and measurement of specific parameters or 'indicators' that are sensitive to changes in soil functions (Doran and Parkin 1996; Karlen *et al.* 2003). These indicators should be readily determined by routine protocols in soil analysis laboratories. For a quantitative and integrated assessment of soil functioning, soil quality indexing is a method that can be easily modified for different soils and used to assess dynamic soil quality ratings and determine trends in those ratings, and thus be used to quantify long-term effects of various land uses or soil management decisions (Xu *et al.* 2006). According to Bastida *et al.* (2008), among the 14 000 contributions published after 1940 with reference to the term 'soil quality', only 934 are related to soil quality indices, and few of those provide a quantitative index of soil quality. A soil quality index (SQI) should include a minimum dataset, and the chosen indicators should be limited to the interrelated parameters, providing numerical data of the main soil functions (Acton and Padbury 1993). Several indexing methods have been used to calculate an integrated index of soil quality. The approach proposed by Andrews *et al.* (2002*b*) is the most used and it is based on the selection of a minimum dataset

of indicators (MDS) by principal component analysis (PCA) and successive multiple regression with indicators representing objective functions. Then, the selected indicators are normalised and integrated by a weighted additive index (WAI). By this procedure, the indicators are weighted using the PCA results and then summed to obtain a final score for each observation.

When PCA is performed, the indicators are selected from a diverse group of soil properties, which usually include chemical, physical, and biological properties. However, the indicators finally included in the SQI cannot represent all of these aspects. A modification of the indicator selection step could be made to avoid this problem if the PCA were performed separately for the physical, chemical, and biological properties, including in the final SQI the more-sensitive parameters of these groups of soil properties. Qi *et al.* (2009) compared indices calculated with different selection criteria and integration methods, concluding that WAI and MDS were the best approaches in defining soil quality. It was suggested that future research should address the improvement of selection of MDS and calculation of WAI rather than studying other methods of soil quality indexing.

The activity and composition of soil microbial communities is important in determining soil quality (Saggar et al. 2001; Beck et al. 2005) because changes in microbial properties are more sensitive to variations in soil quality than changes in chemical and physical properties (Nannipieri et al. 2003). Indeed, the measurements of some microbial properties can provide an integrated and relevant view of soil health (Grayston et al. 2004; Breure et al. 2005). Harris (2003) proposed measurement of size, activity, and diversity of microbial communities to characterise soil quality. Winding et al. (2005) recommended the determination of respiration, microbial biomass, and microbial diversity by using the catabolic response profile (CRP) and phospholipid fatty acid (PLFA) techniques. As has been presented, most of the literature on soil quality in recent years recommends biological indicators to assess soil quality in place of chemical or physical indicators, based on the assumption that biological indicators respond to physical and chemical changes in soil. Others researchers (Wander et al. 2002) consider that an overall assessment of soil quality must include all soil aspects, and not only the biological component. However, at present, there is no research that compares these two methods of assessing soil quality.

The aims of this work were (i) to evaluate and compare two procedures to select indicators of soil quality for the construction of SQI considering diverse chemical, physical, and biological properties; and (ii) to evaluate the role of soil microbiological properties in the construction of SQI.

Materials and methods

Field site, treatments, and soil sampling

The site is an agricultural cattle field $(34^{\circ}01 \text{ S}, 60^{\circ}20 \text{ W})$ in Arrecifes, Buenos Aires province, Argentina. The soil is classified as series Arroyo Dulce (Typic Argiudoll), is a silt loam dark, deep, and well-drained hill soil, with good fertility conditions. Three different environments were studied: (1) pristine undisturbed soil (UN); (2) a pasture grassland soil

(GL); and (3) soil under continuous cultivation (NT) with four different surface horizons (INTA 1974): plot 1 with an A horizon of 25 cm (NT 25), plot 2 with an A horizon of 23 cm (NT 23), plots 3 and 4 with A horizons of 19 and 14 cm, respectively (NT 19 and NT 14). These NT plots were previously managed by reduced tillage (subsoiling and chiselling), but since 1990 have been cultivated by the no tillage system. Crop rotation was the same over the study area, consisting of wheat, corn, wheat/soybean, and soybean.

The UN environment had not been cultivated for least a 100 years. However, the original vegetation has undergone a profound transformation as a result of intensive farming. The vegetation completely covers the soil and the predominant species are *Ranunculus platenses*, *Plantago myosorus*, *Dichondra microcalyx Oxalis mallobolb*, *Spergularis platense*, *Oxalis articulata*, *Geranium albicans*, *Panicum milioides*, *Piptochaetium montevidense*, *Stipa neesiana*, *Stipa papposa*, *Paspalum dilatatum*, *Paspalum distichum*, *Melica brasiliana*, *Bromas unioloides*, *Lolium multiflorum*, and *Trifolium repens*.

Grassland (the GL environment) was introduced in 1998, with *Festuca arundinacea* Schreb. and *Paspalum dilatatum* Poir. the dominant species. Previously, the area was under cereal and oilseed agricultural production with a rotation system similar to that detailed for the NT system.

Soil sampling was performed in July 2005 from an area of 0.5 ha. Each area was divided into three smaller areas (20 by 80 m), along which composite samples were taken (Erkossa *et al.* 2007; Masto *et al.* 2007) at two depths: 0-10 and 10-20 cm. Soil was air-dried, sieved (<2 mm), and stored at room temperature prior to chemical and physical analysis, or stored at 4°C prior to being analysed for microbiological properties.

Soil physical analyses

Bulk density was determined by the core method (Blake 1965) and particle size analysis by the sedimentation procedure (Bouyoucos 1927); the later property was expressed in percentage of clay, silt, and sand. Structural stability was determined as reported by De Leenheer and De Boodt (1958), and results were expressed as mean weight diameter (MWD) (Kemper and Rosenau 1986), which is inversely related to soil aggregate stability. Saturated hydraulic conductivity (K) was determined only for the 0–0 cm soil sample by using the constant head method (Klute 1965).

Soil chemical analyses

Soil pH was measured in a 1:2 soil/distilled water suspension using a pre-calibrated glass electrode (McLean 1982). Electrical conductivity of saturated soil paste was determined as reported by Rhoades (1996). Extractable phosphorus (P) was determined as reported by Bray and Kurtz (1945). Total N (TN) was determined using the Kjeldhal method proposed by Bremner and Mulvaney (1982). The total organic carbon (TOC) content of soil was evaluated using the wet oxidation method of Walkley and Black (Nelson and Sommers 1996). Stock C (SC) was calculated from the thicknesses and bulk densities of each soil depth as described by Ellert and Bettany (1995) and the results were expressed in Mg/ha.

Soil biological analyses

Biochemical properties

These methods can be easily used in routine laboratory analysis. The determination of particulate and soluble C forms was included among these properties and not in the chemical properties, since they represent organic C sources for soil microbial communities, which are generally carbonlimited (Soon et al. 2007). Particulate organic C (POC) was measured as described by Cambardella and Elliott (1992), and the C content was determined by dichromate oxidation as previously reported. Soil basal respiration (Resp) was measured according to Jenkinson and Powlson (1976). Soil microbial biomass C (MBC) was measured by the chloroform fumigation-extraction method (Vance et al. 1987). The difference between the C contents of the fumigated and unfumigated extracts was converted MBC by a conversion factor of 0.33 (Sparling and West 1988). The C extracted with K_2SO_4 from the unfumigated soil samples was used as a measure of the labile C pool (SOC) (Haynes 2005). Both the respiration and microbial biomass were used to calculate the metabolic quotient (qCO_2), which expresses the quantity of CO_2 emitted per microbial biomass unit and time. We also calculated the microbial coefficient MBC/TOC where TOC is the total organic carbon (Anderson and Domsh 1990).

Microbiological properties

These analyses include the determination of PLFA and CRP. The PLFA is used to evaluate microbial community structure, and the CRP the microbial functional diversity. These microbiological soil properties were measured only for the 0-10 cm depth.

Catabolic responses profiles were measured by short-term respiration responses of soil to the addition of a range of simple organic compounds (Degens and Harris 1997). The sieved soil was conditioned for 7 days (20°C) at field moisture content (18% gravimetric water content) prior to analysis. The substrates used were two amines (D-glucosamine, L-glutamine), five amino acids (L-arginine, L-glutamic acid, L-histidine, L-lysine, L-serine), two carbohydrates (D-glucose, D-mannose), and 11 carboxylic acids (L-ascorbic acid, citric acid, tartaric acid, gluconic acid, α ketobutaric acid, α -ketoglutaric acid, DL-malic acid, malonic acid, panthotenic acid, quinic acid, uric acid). Functional evenness (*E*) was calculated from the responses profile as:

$$E = \frac{1}{\sum p_i^2}$$

where p_i is the percentage of total respiration obtained by the sum of all respiration rates expressed as μ g CO₂-C g/soil, due to the respiration rate of each substrate (Magurran 1988).

Methyl ester fatty acid phospholipid profiles (FAMEs) were determined as reported by Schutter and Dick (2000). FAMEs were determined by a gas chromatograph (Hewlett-Packard 5890 Series II, Agilent, Palo Alto, CA) equipped with capillary column HP Ultra 2 (5% difenil–95% dimetilpolisiloxane, 25 m by 0.2 m) and compounds were revealed by using a flame ionisation detector. The oven temperature was initially set at 150°C for 1 min, then raised to 210°C at a rate of 5°C/min and held for 20 min. Identification

of peaks was done by comparing retention times of samples to those of known standards (bacterial acid methyl ester standard in methyl caproate, Sigma-Aldrich Cat. No. 47080-U, Sigma-Aldrich Co, St. Louis, MO). Fatty acids were categorised according to the convention: A, B, and w C, where 'A' is the number of carbon atoms in the chain. 'B' is the number of unsaturations, and 'w' preceding 'C' is the number of carbon atoms between the methyl end of the molecule and the first unsaturation. Prefixes used are: 'i' for iso-branched; 'a' for anteiso-branched; 'cy' for cyclopropil. Individual FAMEs were reported as ratios of peak area to methyl hexadecanoate (C16:0) (Drijber et al. 2000; Spedding et al. 2004), which is often the most abundant FAME in samples and it significantly correlates with microbial biomass (Zelles et al. 1992). For the calculation of the fungal/bacterial PLFA (F/B) ratio, the saturated FAMEs (S) i15:0, a15:0, i16:0, 16:0, 17:0, 20:0, 22:0 and the monounsaturated (M) 16:1w9, cy17:0, cy19:0, 18:1w9 were chosen to represent bacterial biomass, and 18:2w6 was taken as indicator of fungal biomass. FAMEs 11:0, 12:0, 13:0, 14:0 were also considered for the total PLFA. The cy17:0 and 16:1w9 FAMEs were considered for calculating the cyclopropil/precursors (cy/pre) ratio, and i15:0 and a15:0 FAMEs for the iso/anteiso (i/a) ratio. In bacteria, the cy/pre and iso/ anteiso PLFA ratios have been proposed as indicators of stress conditions, as both ratios has been shown to increase under situations such as acidic conditions, low oxygen, high temperature, and low nutrient availability (Guckert et al. 1986; Kieft et al. 1994), and has been associated with nutrient stress or physical or chemical disturbance (Pinkart et al. 2002).

Soil quality index

Data were processed using the InfoStat statistics program (InfoStat 2007). Twenty-four soil properties were measured for each soil layer; the data were first checked for normality and then subjected to analysis of variance (ANOVA). Variables with statistically significant F-values (P < 0.05), and with CV <40% (Wander and Bollero 1999), were further analysed by PCA. The separation of treatment means was carried out by the Rienzo, Guzmán, and Casanoves (DGC) test (Di Rienzo et al. 2002). The PCA is a mathematical procedure giving a small number of uncorrelated variables (PC) from several correlated, and thus it can reduce the size of the parameter dataset. The first PCs account for most of the remaining variability. We have assumed that PC 1, receiving high eigenvalues, best represented variation of the system. Therefore, only PCs with eigenvalues >1 (Brejda *et al.* 2000), and those that explained at least 10%of the variation in the data (Wander and Bollero 1999), were included. Under a particular PC, each soil property was given a weight or factor loading that represents the contribution of the variable to the composition of the PC. Factors loadings close to +1 or -1 are the most important in explaining the variability of the results. Within each PC, only highly weighted factors were retained for MDS. We have defined highly weighted factor loadings as those having absolute values within 10% of the highest factor loading. Multivariate correlation coefficients were carried out when more than one factor was retained under a single PC (Andrews et al. 2002a). The variable with the highest correlation sum was considered for the MDS. When highly

weighted variables were not correlated (correlation coefficient <0.7), each of them was retained in the MDS.

The inclusion of variables in the PCA was performed by two different procedures (Table 1). The traditional one (procedure A) considers variables together (Andrews *et al.* 2002*a*), whereas in the other (procedure B), the PCA is performed separately for the physical, chemical, and biological properties (Wander and Bollero 1999). The latter procedure ensures the inclusion of at least one physical, chemical, and biological indicator in the MDS. Both procedures consider chemical, physical, and biochemical variables (simple SQI, SSQI) or microbiological variables (complex SQI, CSQI).

In order to study the sensitivity of SQIs composed of different properties, two types of microbiological index were constructed. The first microbiological SQI (MSQI 1) was based on the measurement of biological variables according to the procedure used for SQI; the second (MSQI 2) was based on the determination of three selected microbiological parameters representing the size (MBC), activity (soil basal respiration), and functional diversity (eveness, determined by CRP) of the microbial communities.

After selection of the MDS indicators, each indicator was transformed by the linear scoring method (Andrews *et al.* 2002*b*; Sharma *et al.* 2005, 2008). Indicators were arranged depending on whether a higher value was considered 'good' or 'bad' in terms of soil functions. For 'more is better' indicators, each observation was divided by the highest observed value such that the highest observed value received a score of 1. For 'less is better' indicators, the lowest observed value was divided by each observation such that the lowest observed value received a score of 1. Once transformed, the indicators were weighted by the PCA. Each PC gave the percentage of the variation with respect to the total dataset. This percentage, divided by the total percentage of variation of all PCs with eigenvectors >1,

Table 1. Soil properties and indexing procedures

SQI, Soil quality index; A, All properties are considered together in the principal components analysis (PCA); B, groups of properties considered separately in the PCA; Biological properties: biochemical soil properties and microbiological soil properties; Resp, Basal soil respiration; MBC, microbial biomass carbon; E, functional evenness

| Quality index | Properties | Procedure | | |
|-----------------------------------|-------------|------------------|--|--|
| Simple SQI A | Physical | А | | |
| (SSQI A) | Chemical | | | |
| | Biochemical | | | |
| Simple SQI B | Physical | В | | |
| (SSQI B) | Chemical | | | |
| | Biochemical | | | |
| Complex SQI A | Physical | А | | |
| (CSQI A) | Chemical | | | |
| | Biological | | | |
| Complex SQI B | Physical | В | | |
| (CSQI B) | Chemical | | | |
| | Biological | | | |
| Microbiological SQI 1 (MSQI 1) | Biological | В | | |
| Microbiological SQI 2 | MBC | Expert variables | | |
| (MSQI 2) | Resp | selection | | |
| | E | | | |

provided the weighted factor for the chosen indicator. Then the scored indicators for each observation were summed by the following equation:

$$SQI = \sum_{i=1}^{n} W_i S_i$$

where *S* is the score of the indicator, and *W* the weighted factor derived from the PCA. For the SQI constructed by procedure B, the physical, chemical, and biological indicators were given the same weights in the index. Higher index scores were assumed to give the best soil quality. It is known that soil properties are linked to soil functions (Andrews *et al.* 2004), and then a higher value of the indicators integrating the SQI could be interpreted as a better functioning of soil.

The calculated SQI values were tested for their significance at P=0.05 by ANOVA, and the means were compared by the DGC procedure. For a validation approach, values of indices were finally correlated with the varimax-rotated scores of PC 1 obtained by considering all significant data (Wander and Bollero 1999) to compare the sensitivity among systems that use all significant indicators as opposed to using an MDS. The SQI that best differentiate among management system (UN, GR, NT), depth level of the A horizon (for the NT plots), and with high correlation coefficients with PC 1 scores, were considered accurate to represent the soil functioning.

Results

Selection of indicators

Three physical parameters, % silt, % sand, and bulk density of the soil depth 1 (0–10 cm), were excluded according to the screening criteria (CV <40%,; P < 0.05). In the10–20 cm soil layer (soil depth 2), MWD was the only considered indicator among the measured physical properties. Only TOC, TN, and SC of the 0–10 cm soil layer (soil depth 1) were selected among the analysed chemical properties. All of the biological properties were selected for the surface soil layer, whereas no biochemical property was selected for the deepest soil layer.

Table 2 presents the results of PCA analysis. The PC1 was the only PC selected for physical properties, with the final selection of hydraulic conductivity (K 1) among the measured physical properties. Also in the case of chemical parameters, PC 1 was the only PC selected, with TOC 1 representing the chemical properties. Among the biochemical properties, MBC 1 was selected from PC 1 after correlation analysis.

When both biochemical and microbiological parameters were considered, PC 1 and PC 2 were selected. According to PC 1, MBC 1, POC 1, and i/a PLFA ratio for the first soil layer were selected after correlation analysis. For PC 2, the final indicators selected were the functional diversity index (E) and the cy/pre PLFA ratio.

The PCA analysis of physical, chemical, and biochemical properties was represented by PC 1. Among these properties, MBC 1 was further considered with POC 1 and MWD. When all soil properties were considered together, only the PC 1 was selected, and after correlation analysis, MBC 1, POC 1, and MWD 1 were included in the MDS.

| Soil properties | Physical chemical | Physical chemical biochemical | Physical | Chemical | Biological | | |
|-------------------------------|-------------------|-------------------------------|---------------|----------|-------------|--------------|--------------|
| considered | biological | | | | Biochemical | Micro | biological |
| PC | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| Eigenvalues | 15.75 | 11.4 | 3.5 | 2.78 | 5.16 | 9.90 | 1.18 |
| Proportion Weighted factor | 0.83 | 0.86 | 0.88 | 0.93 | 0.86 | 0.82 0.89 | 0.10 0.11 |
| | | Fa | ctor loadings | | | | |
| %Clay 1 | -0.23 | -0.28 | -0.51 | | | | |
| MWD 1 | -0.23 | -0.27 | -0.46 | | | | |
| MWD 2 | -0.22 | -0.27 | -0.50 | | | | |
| K | 0.24 | 0.29 | 0.53 | | | | |
| SC 1 | 0.24 | 0.29 | | 0.58 | | | |
| TOC 1 | 0.24 | 0.29 | | 0.59 | | | |
| TN 1 | 0.23 | 0.27 | | 0.56 | | | |
| SOC 1 | 0.22 | 0.26 | | | 0.40 | 0.29 | -0.07 |
| POC 1 | 0.23 | 0.27 | | | 0.38 | 0.28 | 0.44 |
| MBC 1 | 0.25 | 0.30 | | | 0.43 | 0.31 | -0.0047 |
| Resp 1 | 0.24 | 0.29 | | | 0.42 | 0.3 | 0.19 |
| qCO ₂ 1 | -0.21 | -0.24 | | | -0.37 | -0.27 | 0.39 |
| MBC/TOC 1 | 0.24 | 0.29 | | | 0.43 | 0.31 | -0.08 |
| Е | 0.21 | | | | | 0.26 | 0.43 |
| TPLFA | 0.24 | | | | | 0.30 | 0.09 |
| S/M | -0.23 | | | | | -0.28 | -0.34 |
| F/B | -0.23 | | | | | -0.29 | 0.23 |
| i/a 1 | -0.22 | | | | | -0.30 | 0.25 |
| cy/pre | -0.19 | | | | | -0.26 | 0.43 |

Table 2. Results of principal components analysis

MWD, Mean weight diameter; *K*, saturated hydraulic conductivity; TN, total N; TOC, total organic carbon; SC, stock C; POC, particulate organic C; Resp, basal soil respiration; MBC, microbial biomass C; SOC, soluble organic carbon; qCO₂, metabolic quotient; MBC/TOC, microbial coefficient; E, functional evenness; TPLFA, total methyl ester fatty acids phospholipids; F/B, fungal/bacterial PLFA; S/M, saturated/ monounsaturated PLFA ratio: cv/pre. cvclopropil/precursors PLFA ratio: i/a. iso/anteiso PLFA ratio

Transformation and integration of properties

To carry out linear scores of selected properties, values of each observation of K, TOC, POC, MBC, Resp, and E were divided by the highest observed value; and values of MWD, cy/pre, and i/a were divided by the lowest observed value.

Selected properties for a given PC have the same weight on the index, and for this reason, the properties were not weighted when only one PC was considered. In the CSQI B index, two PCs were retained for the biological properties, and thus biological properties were weighted according to the weighted factor (Table 2). Therefore, they were divided by the number of properties selected for each PC.

Soil quality indices were: SSQI A (MBC 1 + POC 1 + MWD 1); SSQI B (K 1 + TOC 1 + MBC 1); CSQI A (MBC 1 + POC 1 + MWD 1); CSQI B [K 1 + TOC 1 + 0.3 * (MBC 1 + i/a + POC 1) + 0.05 * (E + cy/pre)]; MSQI 1 [0.3 * (MBC 1 + i/a + POC 1) + 0.05 * (E + cy/pre)]; and MSQI 2 (MBC 1 + Resp 1 + E).

Comparison of the soil quality indices

Figure 1 shows the values of SQIs. The SSQI A index differentiated the UN and GL soils from those under continuous cultivation (NT) (Fig. 1*a*). Values were similar in

NT plots with surface horizon depths of 25 and 23 cm (NT 23 and NT 25) and higher than values of plots with surface horizon depths of 19 and 14 cm (NT 19 and NT 14). The index values were highly influenced by MBC and MWD from the first soil layer (0-10 cm depth).

The SSQI B shows the same differences among plots as showed in SSQI A (Fig. 1*b*), and differences were mainly attributed to MBC and hydraulic conductivity (*K*) of the first 10 cm soil depth.

Complex soil quality indices (Fig. 1*c* and *d*) showed differences among plots similar to those observed by considering SSQIs. The CSQI A was integrated by the same indicators as SSQI A. The contribution of the measured properties to CSQI B was mainly due to K and the biological properties. The contribution of both E and the ratio of cy/pre FAMEs was not relevant due to their low weight in to the index.

Both microbiological indices (Fig. 1e and f) showed the same differences among plots, similar to that of the integrative indices (simple and complex soil quality integrated by chemical, physical, and biological indicators).

The ANOVA of PC 1 scores and the constructed soil quality indices showed the same differences among plots (P < 0.005). Correlation coefficients of soil quality indices with varimax-

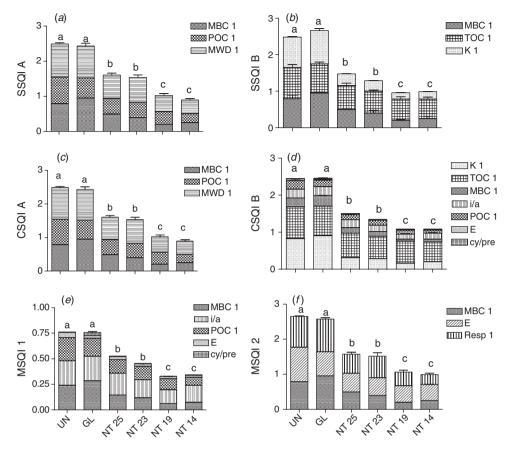


Fig. 1. Values of soil quality indices. Different letters denote significant differences between situations at $\alpha = 0.05$. UN, Undisturbed plot; GL, grassland plot; NT, no-tillage plots (25, 23, 19, and 14 are the A horizon depths of NT plots). MWD, Mean weight diameter; *K*, saturated hydraulic conductivity; TOC, total organic carbon; POC, particulate organic C; Resp, basal soil respiration; MBC, microbial biomass C; E, functional evenness; cy/pre, cyclopropil/precursors PLFA ratio; i/a, iso/anteiso PLFA ratio.

rotated scores of PC 1 obtained from all significant data were 0.97 (for SSQI A and CSQI A), 0.98 (for SSQI B, CSQI B, and MSQI 1), and 0.99 (for MSQI 2).

Discussion

Several authors (Cambardella et al. 2004; Sharma et al. 2005; Bastida et al. 2006; Dawson et al. 2007; Sharma et al. 2008; Karlen et al. 2008; Qi et al. 2009) have implemented the approach proposed by Andrews et al. (2002a) in the construction of SQI. Although this approach has been able to evaluate different soil systems and it has been more successful than other SQI construction techniques (Qi et al. 2009), it presents some problems. First, the lack of indicators representing all soil properties (physical, chemical, and biological) could decrease the SQI sensitivity with changes in management, and the same may happen when physical, chemical, and biological properties present different weights in the calculated SQI. In addition, the selection of the properties included in the MDS is carried out after regression with crop yield, which is often closely related with the levels of available nutrients in soil, and this may give erroneous conclusions in assessing the overall soil quality, which may depend on other

factors. Sharma *et al.* (2005, 2008) used regression with crop yields to select soil properties and construct an index differentiating the effects of tillage and fertilisation rates on soil quality. The index was capable of differentiating the effects of fertilisation rates, but not those of different tillage, probably because physical properties, which were probably affected by tillage, had low weights in the constructed index. Our approach, based on the separate selection of soil physical, chemical, and biological properties, which are equally weighted in the SQI, can avoid the problems presented by Andrews *et al.* (2002*a*, 2002*b*) in constructing SQIs.

Analysis of the measured properties incorporated in the PCA by groups (procedure B) did not affect the sensitivity of the index to differentiate among the tested situations, in comparison with the indices constructed by considering all soil properties together in the PCA (procedure A). The incorporation of TOC 1 (Fig. 1b and d) differentiated the effects of management practices and erosion on the soil organic matter content, which can affect various soil properties. However, MBC 1 was more sensitive than TOC 1 to differences among NT plots; probably labile organic C pools, which can affect MBC, may be more sensitive than TOC as an indicator of soil quality (Gregorich *et al.* 1994; Ghani *et al.* 2003; Soon *et al.* 2007). The low contribution of both E and cy/pre to the final value of the CSQI B index was due to their low weighted factor (Table 2). We suggest considering only PCs which explain \geq 20% of variability to avoid this problem when PCA of soil chemical, physical, and biological soil properties is performed separately.

The inclusion of the microbiological properties in the SQI, which already included chemical, physical, and simple biological indicators, did not increase the sensitivity of the index to differentiate the studied plots, independently of the applied procedure, despite the high sensitivity of the complex microbiological properties, especially E, to changes in soil quality (data not shown) (Degens *et al.* 2000; Sparling *et al.* 2000). Probably, the information covered by the 'complex microbiological properties' was already covered by physical, chemical, or simple microbiological properties. To avoid this problem, a previous step of correlation analysis between the selected indicators would summarise the number of correlated variables entering the PCA.

The MSQIs presented the same sensitivity as the integrated indices to differentiate the studied situations, and this confirms the sensitivity of microbial properties to changes in soil quality and the close link between microbial properties and soil functionality (Nannipieri *et al.* 2003; Winding *et al.* 2005). Bastida *et al.* (2006) calculated an index of soil microbiological degradation by applying the same approach that we have used for constructing the MSQI 1, but weighting the indicators of the same PC by considering the eigenvalues. Their procedure is probably more suitable than ours to maximise treatment differences and to express the sensitivity of the measured properties.

Influence of soil management on size, activity, and functional diversity of microbial communities can be analysed by the MSQI 2. Functional diversity (E) of GL was lower than in UN, probably due to the higher vegetal diversity of UN; for this reason, microbiological communities of UN soil were probably more adapted than those of GL soil to use different organic C sources (Bardgett and Shine 1999). The NT plots had the lowest values of E, probably because rates of organic C inputs by crop plants (wheat, bean, and corn) were lower than those from grassland plants; this may have affected not only the functional diversity (E) of soil microbial communities, but also size and activity, since MBC and Resp were also the lowest in NT plots. The NT plots were under continuous cropping systems, but most of the plant biomass was removed by the harvest. Therefore, higher vegetal cover and root density in reference situations (UN and GL) than in NT plots (data not shown) mean quantitative and qualitative differences in the organic C inputs, which can affect the size and activity of microbial communities. The MSQI 2 could differentiate plots as well as the other indices, despite it only integrating three microbiological properties. Influence of seasonal variations in temperature and moisture on soil properties should be considered when applies SQIs, especially for biological indicators (Bell et al. 2008).

All index values showed high correlation coefficients with varimax-rotated scores of PC 1, confirming that a minimum group of carefully selected indicators can be used to evaluate soil quality changes. However, by considering physical, chemical,

and biological properties, we have more information about soil functioning, because it is not only the value of the index which informs about the soil quality, it is knowing what part of soil quality is being affected. The use of microbial indices was as sensitive as of integrative ones, but they did not provide information about which part of the soil function is most damaged. For example, a low value of a microbial indicator can be a signal of a decrease in the soil quality, but why? Is it because of lack of nutrients, because of damage to the soil structure and the pore system, or because of decreasing soil organic matter pool? Although soil biological properties, especially measurements of microbiological diversity, have shown their potential as indicators of soil quality in numerous soil studies, and thus are in concordance with our finding, many questions cannot be answered if the soil quality evaluation considers only the biological indicators.

Conclusions

The soil quality indices constructed by the different methodological approaches showed the same differences among the study situations. Microbiological indices presented the same sensitivity as the integrated indices to assess the soil quality. However, a construction technique that includes physical, chemical, and biological indicators in the final index of soil quality gave a more complete picture of the impact of soil management practices and erosion on soil properties.

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