View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Digital.CSIC

Biochemical properties in managed grassland soils in a temperate humid zone: modifications of soil quality as a consequence of intensive grassland use

J. Paz-Ferreiro[a*], C. Trasar-Cepeda[b], M.C. Leirós[a], S. Seoane[a], F. Gil-Sotres[a]

[a]Departamento de Edafología y Química Agrícola, Universidad de Santiago de Compostela, E-15782, Santiago de Compostela, Spain

[b]Departamento de Bioquímica del Suelo, IIAG-CSIC, Apartado 122, E-15780, Santiago de Compostela, Spain

*Corresponding author

E-mail: jpaz@udc.es

Phone number: 0034981 563100 Ext. 14984

Fax number: 0034981594912

Abstract

The maintenance of soil quality is critical for ensuring the sustainability of the environment and the biosphere. Although soil biochemical properties are considered good indicators of changes in soil quality, few studies have been made of the changes in biochemical properties brought about by anthropogenic disturbance of grassland ecosystems. In the present study, several biochemical properties were analysed in 31 grassland soils subjected to a high level of management, and the values obtained were compared with known values corresponding to native grasslands from the same region (Galicia, NW Spain). The 31 managed grasslands were divided in two groups (re-sown grasslands and improved grasslands) according to their management and past land use. The biochemical properties studied were: labile carbon, microbial biomass carbon, microbial respiration, metabolic quotient, net nitrogen mineralization, and the activities of the following enzymes: dehydrogenase, catalase, phosphodiesterase, phosphomonoesterase, casein-protease, BAA-protease, urease, cellulase, ß-glucosidase, invertase and arylsulphatase. Managed grasslands exhibited lower values of soil biochemical properties than native grasslands. Three biochemical equilibrium equations were used to compare soil quality in managed and native grasslands. One of the equations did not show any significant difference between the groups of grassland soils considered. On the contrary, two of the equations showed similar soil quality for improved and native grasslands, while re-sown grasslands exhibited a loss of soil quality when compared to native grassland soils.

Keywords: soil biochemical properties; soil quality; grassland management; soil enzymes; soil organic matter

Introduction

Anthropogenic land management is one of the main causes of soil degradation, and there is, therefore, worldwide interest in quantifying the loss of soil quality generated by agricultural operations (Dick, 1992). As indicated recently (Gil-Sotres et al., 2005), there are two basic requirements for the correct evaluation of soil quality. First of all, properties used to evaluate soil status must be established. Although some authors consider that soil physical and chemical properties may be used, biochemical properties are generally preferred (Dick, 1992) because they are more sensitive and respond more quickly than physical or chemical properties to the effects of management practices (Nannipieri et al., 2002). Furthermore, biochemical properties can be determined rapidly and easily in the laboratory (Nannipieri et al., 2002). However, individual biochemical properties are of limited use as soil quality indicators (Nannipieri et al., 1990; Gil-Sotres et al., 2005) because soil processes cannot be reflected adequately by the measurement of individual properties. At present, it appears that using equations that include several biochemical properties reflects better the complexity of the soil system and it is the most suitable and accurate method of evaluating soil quality (Nannipieri et al., 2002; Gil-Sotres et al., 2005). Secondly, the soil that represents the maximum quality must be established so that relevant comparisons can be made. For forest soils, soils under *climax* ecosystems are usually used for comparative purposes (Trasar-Cepeda et al., 1998). But, in the case of grassland soils, native grassland soils (with no or minimal human intervention) should be used as reference soils, as grassland ecosystems have very different soil microclimates and organic matter dynamics from forest soils, which results in different values in soil biochemical properties (Saviozzi et al 2001).

Herbaceous vegetation constitutes a quarter of Earth´s land surface (Snaydon, 1981). Only some of these areas correspond to natural formations, whereas most of it has been converted from forest or from shrubland to managed grasslands. In this latter case, large amounts of fertilizers have been added to the land. Until recently, these types of management practices were considered essential and were justified by the highly productive soils created. However, it is now considered that management practices must favour recycling of nutrients to minimise the use of external inputs and must also promote conservation of biodiversity (Yeates et al., 1997). Management practices involving the use of large amount of fertilizers, ploughing and periodic re-seeding can cause soil degradation (Dick, 1992). Thus, it has been proved that grassland management results in decreased soil organic matter contents (Sparling and Schipper, 2004) and specific studies carried out in the United Kingdom (Lovell et al., 1995) and in the Alps (Zeller et al., 2001) have showed that managed grasslands support smaller populations of microbes than native grasslands soils, alterations that should reflect a diminishing in soil quality. Sensitivity of soil biochemical properties to management suggests the possibility of using such properties as indicators of soil quality in grassland ecosystems, similarly as used in other ecosystems [see, for example, Masto et al. (2007) for crops or Trasar-Cepeda et al. (1998) for forests]. It is noteworthy, that, although soil biochemical properties are considered to be sensitive indicator to assess soil quality, only a few equations that estimate soil quality from soil biochemical properties are available, as it can be seen in a recent review (Bastida et al., 2008).

The aim of the present study was to characterize the biochemical properties of managed grassland soils in Galicia and to compare their values with those of native grasslands located in the same region (Paz-Ferreiro et al., 2007). This comparison will allow us to determine the degree to which grassland management causes changes in the biochemical status of the soils, and, where changes have occurred, to evaluate their importance.

Material and Methods

Soils

Galicia (NW Spain) has a temperate-humid climate with an average temperature of 14.4 °C and an annual average rainfall of 1405 mm. Herbaceous vegetation covers approximately 12.5% of Galicia. It is estimated that 65% of Galician grasslands are not native. The botanical community in these grasslands is dominated by *Lolium multiflorum* Lam. and *Trifolium repens* L. (Fraga et al., 2002). Two types of managed grassland soils that differ in terms of past land use were considered in our study: improved grasslands and re-sown grasslands. The improved grasslands have been under grassland use for, at least, the last 100 years, whereas the re-sown grasslands were originally forests which have been cleared 30-40 years ago. The management practices used in both types of grasslands include liming to correct their pH, periodic re-seeding (usually annual or biannual) with removal of the existing grassland, surface ploughing and seeding, as well as periodic fertilization, mainly with cattle slurry (for the improved grasslands) and inorganic fertilizers (for the re-sown grasslands). In the present study, thirty-one managed grassland soils throughout Galicia were analysed. Sixteen of these correspond to re-sown grasslands and 15 to improved grasslands. Table 1 and Table 2 indicate the location and parent material of the soils sampled.

Samples were collected during the spring of 2005 (before soils were managed or fertilized). The utilization of the same sampling procedure and the same sampling period as in a previous work (Paz-Ferreiro et al., 2007) allowed us to do valid comparisons between the soils described here and those of native grasslands.

Soil physical and chemical properties

Total organic C (wet oxidation) and N (Kjeldahl digestion) contents and pH in 1 M KCl (1:2.5 soil: solution ratio) were determined following the methods described by Guitián and Carballas (1976). Available P was extracted with 0.5 M sodium bicarbonate (pH 8.2), according to Bowman and Cole (1978), and inorganic P in the extracts was determined according to Murphy and Riley (1962). Amorphous Al and Fe were extracted with 0.2 M ammonium oxalate/oxalic acid buffer of pH 3.0 (Guitián and Carballas, 1976) and were determined by atomic absorption spectrometry. Particle size distribution was determined with a Robinson pipette using Calgon as dispersant (Guitián and Carballas, 1976).

Microbial biomass carbon, basal respiration and nitrogen mineralization

Microbial biomass C was determined by the chloroform fumigation-extraction method (Vance et al., 1987). The difference in C content of the fumigated and unfumigated extracts was converted to microbial biomass C (expressed in mg kg^{-1} of dry soil) applying a factor (K_c) of 0.45. The C extracted with K_2SO_4 from the unfumigated samples was used as a measure of the labile pool of C (Haynes, 2005).

Soil basal respiration was determined by static incubation (Guitián and Carballas, 1976). The CO_2 produced during a 10-day period by 25 g soil samples incubated at field moisture content at 25 ºC was collected in 10 ml of a 1 M NaOH solution, which was then titrated against HCl with an automatic titrator. $q\text{CO}_2$ (µg CO_2-C released mg^{-1} biomass carbon h^{-1}) was calculated as the ratio between basal respiration and microbial biomass C.

To determine net N mineralization, 10 g soil samples were extracted for 30 min with 50 ml of 2 M KCl before and after incubation for 10 days at 25 ºC at field moisture content. Total inorganic N was determined in the extracts by Kjeldahl distillation (Bremner, 1965). Net nitrogen mineralization (mg kg^{-1} 10 d^{-1}) was calculated as the difference between the values obtained before and after incubation.

Enzymatic activities

The activities of oxidorreductases (dehydrogenase and catalase) and hydrolytic enzymes of the C (cellulase, β-glucosidase and invertase), N (casein-protease, BAA-protease and urease), P (phosphodiesterase and phosphomonoesterase) and S (arylsulphatase) cycles were determined. The methods used to determine specific biochemical parameters are shown in Table 3. All enzyme activities were determined in triplicate.

Soil quality indexes

To assess soil quality several equations that theoretically give indication of biochemical equilibrium in unaltered soils were used:

a) Trasar-Cepeda et al (1998) index: These authors chose a series of soils covered by climax vegetation in Galicia (the same region as in our study) and established an equation to define total N from several microbial parameters (microbial biomass C, mineralized N, phosphatase, β-glucosidase and urease) (Eq. 1). With the resulting equation, they found that there was a balance between total N and several biochemical parameters.

$$\text{Total N} = (0.38\cdot 10^{-3}\cdot \text{ microbial biomass C}) + (1.410^{-3}\cdot \text{ mineralized N}) + (13.6\cdot 10^{-3}\cdot \text{ phosphomonoesterase}) + (8.9\cdot 10^{-3}\cdot \beta\text{-glucosidase}) + (1.6\cdot 10^{-3}\cdot \text{ urease}) \quad (1)$$

where: total N is expressed as percentage, microbial biomass C and mineralized N (mg kg^{-1}), and the enzymatic activities in μmol of liberated product g^{-1} h^{-1}. This model points to the closeness or nearness to an ideal quality state in the chosen soils, for which they used the quotient between the N estimated by the model and the total N calculated by the Kjedahl method. This equation was tested in soils contaminated by heavy metals, mine soils and arable soils (Leirós et al., 1999). The ratio 100 Nt/Nr enables quantification of the degree to which soil biochemical status differs from biochemical equilibrium. Soils with biochemical equilibrium should have a Nt/Nr ratio equal to 100 and values far from 100 represent degraded soils.

b) Puglisi et al. (2006) index: These authors developed an index (AI 3) of soil quality which was validated using 17 previous data sets. AI 3 was able to discriminate between altered and control soils under heavy metal contamination, intensive agricultural exploitation, contamination from tannery and landfill effluents, mining activities and erosion. Puglisi et al. (2006) found that the lower AI 3 was the more quality the soil had.

$$\text{AI 3} = (7.87 \cdot \beta\text{-glucosidase}) - (8.22 \cdot \text{phosphomonoesterase}) - (0.49 \cdot \text{urease}) \quad (2)$$

where the enzymatic activity are expressed in μmol of liberated product g^{-1} h^{-1}.

c) Paz-Ferreiro et al. (2007) equation: These authors studied a series of native grassland soils in Galicia and found out an equation to define total C from several microbial parameters (microbial biomass C, catalase, phosphomonoesterase and urease) (Eq. (3)). With the resulting equation, they found that there was a balance between total C and several biochemical parameters. The following equation explained 96% of the total variance in the estimation of total C:

$$\text{Total carbon} = 0.764 + 2.304 \cdot 10^{-3} \cdot \text{biomass-C} + 0.936 \cdot \text{catalase} + 0.017 \cdot \text{urease} + 0.206 \cdot \text{phosphomonoesterase} \quad (3)$$

where: total C is expressed as percentage, microbial biomass C as mg kg^{-1}, and the enzymatic activities in μmol of liberated product g^{-1} h^{-1} for urease and phosphomonoesterase and as mmol H_2O_2 evolved g^{-1} h^{-1} for catalase. For this index, soil quality was estimated comparing the total carbon content measured by dichromate oxidation (Cr), with the total carbon content estimated from equation 3. Soils with biochemical equilibrium should have a Ct/Cr ratio equal to 100. This expression has never been validated and has, so far, only been used for native grasslands, but, the wide range of organic matter and the large number of soils used for calibration (total carbon in the 29 native grassland soils ranged from 2.2 to 15.1%) suggest that the model is also applicable to managed soils, as they present a similar range of total carbon contents.

Statistical analysis

All statistical analyses were performed with SPSS version 15.0. Differences between means were tested by analysis of variance (ANOVA).

Because of the vast amount of soils sampled in the study, only mean values for biochemical properties are provided. Data for individual soils can be provided to readers on request.

Results

Table 4 includes the mean values for general properties in Galician managed grasslands; data for native grassland soils (obtained from Paz-Ferreiro et al., 2007) are also shown. The most significant finding as regards the differences between managed and native grasslands was the lower organic matter content in the former, especially in the case of re-sown grasslands. Native grasslands had an average C content of 5.70±2.90 % while it was 5.26±2.00 % for improved grasslands and 3.32±1.20 % for re-sown grasslands. The difference in C content was not statistically significant for improved grasslands, but was significant for re-sown grasslands. Extractable P was higher in the managed grasslands

(60±9 mg kg^{-1} for improved grasslands and 54±10 mg kg^{-1} for re-sown grassland) than in the native grasslands (19±7 mg kg^{-1}), reflecting the effects of fertilization. The managed grasslands have similar clay and sand content, as well as pH than the native grasslands.

Table 5 shows the values for biochemical properties in managed grasslands; data for native grasslands (obtained from Paz-Ferreiro et al., 2007) are also shown. It is important to note that, generally, soil biochemical properties lowered their values in managed grasslands in relation to native grasslands. On a whole, re-sown grasslands exhibited even lower values of biochemical properties than both improved and native grasslands. However, when the values for the biochemical properties were expressed in terms of organic C content, the mean values for native and anthropogenic grasslands were generally very similar (Table 6) and for most of the properties there were no significant differences for the three groups of grassland soils.

Table 7 and Table 8 show the correlation coefficient for the different properties analyzed in improved grasslands and in re-sown grasslands, respectively. Correlations between biochemical properties were similar in both types of managed grassland soils and will be discussed together. Total C and N were both closely correlated with many biochemical properties. The properties that were least correlated with others were BAA-protease, urease and cellulase activities. It is noteworthy that there were no correlations between the activities of β-glucosidase and cellulase, as both enzymes are involved in the break up of cellulose, and are generally strongly correlated. The lack of correlations suggests a decoupling of enzyme activities involved in the carbon cycle, which may indicate that, in managed grasslands, the processes of degradation of different sized carbon compounds are regulated by different mechanisms, probably because organic amendments increase the input of labile organic matter into the soil.

Figure 1 shows the values of 100 (Nt/Nr), AI 3 and 100 (Ct/Cr), according to equations 1, 2 and 3, respectively, by comparing both types of managed grasslands with the native grasslands. Using equation 1, we obtained a 100 (Nt/Nr) of 93±27 for native grasslands, 81±22 for improved grasslands and 88±23 for re-sown grasslands. According to equation 2, native grassland soils exhibited an AI 3 value of -50±35. This value was -37±6 for improved grasslands and -29±12 for re-sown grasslands. According to equation 3, the mean values of 100(Ct/Cr) were 100±10 for native grasslands, 87±22 for improved grasslands, and 126±21 for re-sown grasslands.

Discussion

General and biochemical properties

Similar pH values in the managed and native grasslands suggest that the action of liming material applied to managed grasslands disappears over time. This effect is possibly due to the climate in Galicia, which is generally characterized by annual rates of precipitation of more than 1000 mm per year. Such high precipitations promote loss of liming material and originate gradual acidification of the soil. The fact that the soils analyzed have a similar pH allows us a better comparison of their biochemical properties, as it is known that pH can influence soil enzymatic activities (Tabatabai, 1994).

The lower organic matter content in managed grasslands relative to native grasslands is consistent with other studies (see, for example, Nsabinama et al., 2004) and could be due to the action of two basic factors. Firstly, grassland management is associated with a decrease in root exudates (Mawdsley and Bardgett, 1997) due to decreases in the amount of root mass brought about by the use of inorganic fertilizers (Stewart and Metherell, 1999). Secondly, the effect of ploughing in managed grasslands creates a lower degree of soil aggregation (Gupta and Germida, 1988), which causes greater physical exposure of organic matter to the atmosphere, thereby favouring oxidation of organic matter (van Veen and Paul, 1981). It is important to stress that the re-sown grassland soils contained lower amounts of organic matter than both improved grassland soils and native grasslands. This can be attributed to the clearing of pre-existing forest vegetation cover in re-sown grasslands. Clearing has been associated with rapid mineralization of the organic matter pool (Okore et al., 2007), although cultivation also dilutes soil C because it leads to mixing of subsoil with a relatively low organic matter content and surface soil with a higher organic matter content (Gregorich et al., 1998). However, the C/N ratios were similar in all three types of grassland studied, which suggests that the loss of carbon and of nitrogen occur at the same rate in managed grassland soils, regardless of soil management.

A decrease in the size of the microbial populations in managed grassland compared to native grasslands was found, as can be seen from the microbial biomass values (Table 5). This decline could be attributed to a reduction in microhabitats generated by the loss of organic matter. Enzymatic activity values were lower in managed grasslands than in native grasslands. This decrease of enzymatic activities is more manifest in re-sown grassland soils, where it can be attributed to reduced litter input and changes in soil climate after clear-cutting of the pre-existing forest soil, as suggested in other studies (Tan et al., 2008).

Phosphomonoesterase and casein-protease activities were lower in the re-sown grasslands than in the native grasslands. It is known that the application of large amounts of inorganic phosphorus to grasslands may restrict synthesis of phosphomonoesterases (Uhlirova et al., 2005) and that N fertilizer may have a similar effect on casein-protease (Dick, 1992). However, as the decrease in both activities was similar to that found for organic matter content, it is possible that the reduction in enzymatic activity may be largely due to the loss of organic matter that occurs in these grassland soils.

When the values for the biochemical properties were expressed in terms of organic C content, the mean values for native and anthropogenic grasslands were very similar and, generally, their average values were not significantly different (Table 6). This shows that the same factors that cause the reduction in the organic matter content in managed grasslands also generate lower biochemical activity.

Interestingly, managed and native grasslands did not exhibit significantly different values of $q\mathrm{CO}_2$, although higher values of this property have been reported for managed ecosystems (Insam and Domsch, 1988). It seems that, in contrast to other reports (Insam and Domsch, 1988), the managed grassland soils studied in our work are not in a situation of metabolic stress.

In summary, our results indicate that grassland management generates a quantitative change in soil biochemical properties, when compared to native grasslands. These changes were more apparent in re-sown than in improved grasslands. This implies that forest clearing for grassland establishment has had a stronger impact on soil biochemical properties than intensive grassland management.

Correlations between properties: Principal Component Analysis

Correlations between total N content and each of the different biochemical properties were generally higher than those between total C content and soil biochemical properties, which is in contrast to recent findings for native Galician grasslands (Paz-Ferreiro et al., 2007). Furthermore, for improved grassland soils, biomass-C was related to neither total C nor total N, which suggests that the organic matter in these soils no longer played a predominant role in determining the size of the microbial populations (Carter et al., 1999). Moreover, many of the biochemical properties measured in the present study were significantly related to each other, as also reported by Ross et al. (1995) and Banerjee et al. (2000) for other grassland soils.

In order to clarify the structure of the interdependence between properties in the managed grasslands, principal component analysis (PCA) was applied to the data corresponding to all the soil properties studied. The structure of PCA for re-sown and for improved grasslands differed from that corresponding to native grassland soils (Paz-Ferreiro et al., 2007), which suggests that managed grasslands have suffered qualitative changes in their biochemical properties. The two first principal components in the three groups of soils considered are shown in Figure 2. Native grassland soils (Paz-Ferreiro et al., 2007) and forest climax soils (Trasar-Cepeda et al., 2000) have a high interdependence between organic matter and biochemical properties and among biochemical properties, but, in contrast, managed grassland exhibit less interdependence among these properties (Figure 2). PCA analysis shows that the more intensive the management is the more dispersed the cluster of properties is.

Our results suggest that anthropogenic management not only causes a decrease in soil organic matter and in soil biochemical properties but also changes in the relationships among biochemical properties. In other words, grassland management generates both qualitative and quantitative changes in the biochemical status of the soil.

Changes in the biochemical equilibrium of the soil caused by intensive grassland management: evaluation of soil quality

The three equations utilized resulted in different information about soil quality. Thus, using equation 1, we obtained a 100 (Nt/Nr) of 93±27 for native grasslands, 81±22 for improved grasslands and 88±23 for re-sown grasslands (Figure 1). Hence, equation 1 was unable to distinguish the effect of management on soil biochemical quality among the three types of soils ($P<0.05$), which would appear to contradict our finding that managed grassland soils showed quantitative and qualitative differences in their biochemical properties (see above discussion). These divergences can be accounted for by the fact that equation 1 was developed from forest climax soils and, as other authors have found, grassland and forest soils exhibit different behaviour for different biochemical properties (Saviozzi et al., 2001), especially for enzymes involved in the N cycle (Paz-Ferreiro et al., 2007).

According to equation 2, native grassland soils exhibited an AI 3 value of -50±35 (Figure 1). This value was -37±6 for improved grasslands and -29±12 for re-sown grasslands. A problem in using this equation to evaluate soil quality is that Puglisi et al. (2006) did not define any threshold value and, indeed, they found that reference soils exhibited AI3 values from -350 to -47, while AI3 could range from -180 to -94 for eroded soils and from -166 to 12 in hydrocarbon polluted soils. In our work we also found high data variability, with values that ranged from -169 to -1 in the case of native grasslands, from -53 to -17 in the case of improved grasslands and from -50 to -14 in the case of re-sown grasslands. Although AI 3 values for native grasslands are lower than for intensive grasslands (which implies more soil quality for the former than for the later), the values obtained are not significantly different ($P<0.05$). Most importantly, our results also showed that native grasslands and re-sown grassland soils exhibit statistically significant different values of AI 3. Equation 2 seems to be useful for assessing soil quality in temperate grassland ecosystems and suggests that re-sown grassland soils are degraded in comparison with native grassland soils, while improved grasslands have a similar quality to that of native grasslands. This result seems to be coherent with two findings discussed above: (1) in comparison to native grasslands, re-sown grassland soils appeared to have suffered a loss of biochemical properties; and (2) managed grasslands (especially re-sown grassland soils) appear to exhibit a qualitative change in their biochemical properties. Unfortunately, the absence of threshold values for equation 2 does not allow us to quantify how many of the soils studied here have suffered an important alteration in soil quality.

According to equation 3, the mean values of 100(Ct/Cr) were 87±22 for improved grasslands, 100±10 for native grasslands and 126±21 for re-sown grasslands (Figure 1). The mean values of 100(Ct/Cr) ratio differed significantly ($P<0.05$) between re-sown grassland soils, on one hand, and both, native grasslands and improved grasslands, on the other. It is noteworthy, that, as found for equation 2, the values for re-sown grassland differed from those of native grasslands.

The results obtained with equation 3 indicate that many managed grasslands in Galicia have adequate levels of biochemical activity, as they have 100(Ct/Cr) values of between 85 and 115%. This represents a variation in the real value compared with the calculated value of ±15%, a degree of dispersion that may be considered acceptable for studies based on soil biochemical properties. This variation is in agreement with studies of soils subjected to minimal anthropogenic disturbance (native grassland soils).

Our data showed that 40% of the improved grasslands exhibited 100(Ct/Cr) values lower than 85%, and that 44% of re-sown grassland soils exhibit 100(Ct/Cr) values higher than 115%, which suggests that the biochemical equilibrium characteristic of native grassland ecosystems has been altered in managed grasslands. These results are in accordance with the quantitative and qualitative changes found in soil biochemical properties, as discussed above. That is, it was shown that re-sown and native grasslands exhibit substantially greater divergence in biochemical properties than the improved and native grasslands.

Our results suggest that some of the improved grasslands are suffering a loss of soil quality, probably due to a slowing of the biogeochemical cycling of elements. The results suggest that in this type of soil the microbial pool is more sensitive to intense management than the organic matter pool as the former diminishes faster than the latter. In the case of the re-sown grasslands, our results suggest that some of them are in a state of high microbiological and biochemical activity (Leirós et al., 1999). This transitory state may be explained by the effect of fertilization on soil biochemical properties, which may lead to higher values of enzymatic activities with respect to organic matter content (Simek et al., 1999).

In summary, the results suggest that the biochemical equilibrium characteristic of native (mature) grassland ecosystems has been altered in managed grasslands, especially in re-sown grassland soils, and suggests that managed grasslands are suffering a loss of soil quality. Two biochemical equilibrium equations have been proved to be useful for measuring the loss of soil quality in temperate grassland ecosystems. Equations 2 and 3 showed lower soil quality in re-sown than in native grasslands. Moreover, equation 3 was found to discriminate between the 100(Ct/Cr) values of improved and re-sown grassland soils, evidencing a different range of value for both types of soil.

Conclusions

The data revealed a lower biochemical activity associated with the low total C content in managed grasslands. Biochemical equilibrium indexes proved to be useful in evaluating soil quality in temperate grassland soils. AI3 proved to be useful in temperate grassland soils to measure soil quality, although its use was limited by the lack of threshold values. The use of Ct/Cr as an index of deviation of a soil from biochemical equilibrium (and hence a loss of soil health or quality) was validated. Re-sown grasslands have been proved to have lower soil quality than other types of grassland soils. Thus, from the point of view of soil quality, managed grasslands, where possible, should be established on previous grasslands, instead of being established through forest clear cutting.

Acknowledgements

The present study was financed by the Spanish Ministry of Science and Technology, under Project No. BTE2001-0987. J. Paz-Ferreiro thanks the Spanish Ministry of Education for financial support (FPU programme).

References

Banerjee MR, Burton DL, McCaughey WP, Grant CA (2000) Influence of pasture management on soil biological quality. Journal of Range Management 53:127–133.

Bastida F, Zsolnay A, Hernández T, García C (2008) Past, present and future of soil quality indices: A biological perspective. Geoderma 147:159–171

Bremner JM (1965) Inorganic forms of nitrogen. In: *Methods of Soil Analysis, Part 2, Microbiological and biochemical properties.* (eds CA Black, DD Evans, JL White, LE Ensminger, FE Clark. Madison, ASA-SSSA. pp. 1179-1237.

Bowman MG, Tabatabai MA (1978) Phosphodiesterase activity of soils. Soil Sci Soc Am J 42:284–290.

Bowman RA, Cole CV (1978) An exploratory method for fractionation of organic phosphorus from grassland soils. Soil Sci 125:49–54.

Camiña F, Trasar-Cepeda C, Gil-Sotres F, Leirós MC (1998) Measurement of dehydrogenase activity in acid soils rich in organic matter. Soil Biol Biochem 30: 1005–1011.

Carter MR, Gregorich EG, Angers DA, Beare MH, Sparling GP, Wardle DA, Voroney RP (1999). Interpretation of microbial biomass measurements for soil quality assessment in humid temperate regions. Can J Soil Sci 79:507–520.

Dick RP (1992) A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agric Ecosyst Environ 40:25–36.

Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. Soil Biol Biochem 20:601-606.

Fraga MI, Calvo LR, Baleato JC (2002) Comparative study of floristic diversity in sown grasslands and permanent pastures from Galicia (Northwest Spain). In: Lowland Grasslands of Europe: Utilization and Development (eds G Fisher, B Frankow-Lindberg), pp 281–283. FAO, Roma.

Gil-Sotres F, Trasar-Cepeda C, Leirós MC, Seoane S (2005) Different approaches to evaluating soil quality using biochemical properties. Soil Biol Biochem 37:877–887.

Gregorich EG, Greer KJ, Anderson DW, Liang BC (1998) Carbon distribution and losses: Erosion and deposition effects. Soil Till Res 47:291–302.

Guitián F, Carballas T (1976) Técnicas de Análisis de Suelos. Santiago de Compostela, Editorial Pico Sacro.

Gupta VVSR, Germida JJ (1988) Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol Biochem 20:777–786.

Insam H, Domsch KH (1988) Relationship between organic soil carbon and microbial biomass on chronosequences of reclamation sites. Microbial Ecology 15:175–188.

Ladd JN, Butler JHA (1972). Short-term assays of soil proteolytic enzyme activities using protein and dipeptide derivatives as substrates. Soil Biol Biochem ***4***:19-30.

Leirós MC, Trasar-Cepeda C, García-Fernández F, Gil-Sotres F (1999) Defining the validity of a biochemical index of soil quality. Biol Fertil Soils 30:140–146.

Lovell RD, Jarvis SC, Bardgett RD (1995) Microbial biomass and activity in long term grassland: effects of management changes. Soil Biol Biochem 27:969–975.

Masto RE, Chhonkar PK, Singh D, Patra AK (2007) Soil quality response to long-term nutrient and crop management on a semi-arid Inceptisol. Agric Ecosyst Environ 18, 130–142.

Mawdsley JL, Bardgett RD (1997) Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the microbial population of an upland grassland soil. Biol Fertil Soils 27:969–975.

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Analytical Chimica Acta 27:31-36.

Nannipieri P, Ceccanti B, Cervelli S, Materese E (1980) Extraction of phosphatase, urease, proteases and organic carbon from soil. Soil Sci Soc Am J 44:1011-1016.

Nannipieri P, Ceccanti B, Grego S (1990) Ecological significance of biological activity in soil. In: Soil Biochemistry, vol. 6. (eds JM Bollag, G Stotzky), pp. 293-355. Marcel Dekker Inc., New York.

Nannipieri P, Kandeler E, Ruggiero P (2002) Enzyme activities and microbiological and biochemical processes in soils. In: Enzymes in the Environment: Activity, Ecology and Applications. (eds RG Burns, RP Dick), pp 1-34. Marcel Dekker Inc, New York.

Nsabimana D, Haynes RJ, Wallis FM (2004) Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Appl Soil Ecol 26:81–92.

Okore IK, Tijani-Eniola H, Agboola AA, Aiyelari EA (2007) Impact of land clearing methods and cropping systems on labile soil C and N pools in the humid zone Forest of Nigeria. Agric Ecosyst Environ 120:250–258

Paz-Ferreiro J, Trasar-Cepeda C, Leirós MC, Seoane S, Gil-Sotres F (2007) Biochemical properties of acid soils under native grassland in a temperate humid zone. New Zeal J Agr Res 50:537–548.

Puglisi E, Del Rea AAM, Rao MA, Gianfreda L (2006) Development and validation of numerical indexes integrating enzyme activities of soils. Soil Biol Biochem 38:1673–1681.

Ross DJ, Speir TW, Kettles HA, Mackay AD (1995) Soil microbial biomass C and N mineralization and enzyme activities in a hill pasture: influence of season and slow-release P and S fertilizer. Soil Biol Biochem 27:1431–1443.

Saá A, Trasar-Cepeda MC, Gil-Sotres F, Carballas T (1993) Changes in soil phosphorus and acid phosphatase activity immediately following forest fires. Soil Biol Biochem 25:1223-1230.

Saviozzi A, Levi-Minzi R, Cardelli R, Rifaldi R (2001) A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant Soil 233:251–259.

Schinner F, von Mersi W (1990) Xylanase-, CM-cellulase- and invertase activity in soil: an improved method. Soil Biol Biochem 22:511-515.

Simek M, Hopkins DW, Kalcik J, Picek T, Santruckova H, Stana J, Trávník K (1999) Biological and chemical properties of arable soils affected by long-term organic and inorganic fertilizer applications. Biol Fertil Soils 29:300–308.

Snaydon RW (1981) The ecology of grazed pastures. In: *Grazing Animals*. (ed. FHW Morley), pp 13-31Amsterdam, Elsevier.

Sparling GP, Schipper L (2004) Soil quality monitoring in New Zealand: trends and issues arising from a broad-scale survey. Agric Ecosyst Environ 104:545–552.

Stewart DPC, Metherell AK (1999) Carbon (^{13}C) uptake and allocation in pasture plants following field pulse labelling. Plant Soil 210:61–73.

Tabatabai MA (1994) Soil Enzymes. In: Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. (eds. RW Weaver, JS, Angle, PS Bottomley), pp 775-833. ASA-SSSA. Madison.

Tabatabai MA, Bremner JM (1970) Arylsulfatase activity of soils. Soil Sci Soc Am Proceedings 34:225-229.

Tan X, Chang SX, Kabzems R (2008) Soil compaction and forest floor removal reduced microbial biomass and enzyme activities in a boreal aspen forest soil. Biol Fertil Soils 44:471–479.

Trasar-Cepeda C, Leirós MC, Gil-Sotres F, Seoane S (1998) Towards a biochemical index for soils: an expression relating several biological and biochemical properties. Biol Fertil Soils 26:100–106.

Trasar-Cepeda C, Camiña F, Leirós MC, Gil-Sotres F (1999) An improved method to measure catalase activity in soils. Soil Biol Biochem 31:483-485.

Trasar-Cepeda C, Leirós MC, Gil-Sotres F (2000) Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperate-humid zone (Galicia, N.W. Spain): specific parameters. Soil Biol Biochem 32:747–755.

Uhlirova E, Simek M, Santruckova H (2005) Microbial transformation of organic matter in soils of montane grasslands under different management. Appl Soil Ecol 28:225–235.

Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass carbon. Soil Biol Biochem 19:703-707.

Van Veen JA, Paul EA (1981) Organic carbon dynamics in grassland soils. I. Background information and computer simulation. Can J Soil Sci 61:181–186.

Yeates GW, Bardgett RD, Cook R, Hobbs PJ, Bowling PJ, Potter JF (1997) Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. J Appl Ecol 34:453–471.

Zeller V, Bardgett RD, Tappeiner U (2001) Site and management effects on soil microbial properties of subalpine meadows: a study of land abandonment along a north-south gradient in the European Alps. Soil Biol Biochem 33:639–649.

Table 1 Geographical location and parent material of the 15 improved grasslands under study.

Sample	Latitude (N)	Longitude (W)	Altitude asl (m).	Parent material
IG1	43º09´50´´	8º49´20´´	330	Schists and gneisses
IG2	42º57´52´´	8º45´30´´	380	Granite
IG3	43º10´48´´	8º20´31´´	450	Schists
IG4	43º03´35´´	8º00´35´´	510	Gabbres
IG5	43º04´45´´	8º11´40´´	398	Schists
IG6	43º34´41´´	8º59´40´´	215	Paragneisses
IG7	43º37´29´´	7º56´55´´	330	Basic rocks
IG8	43º23´08´´	7º48´12´´	520	Schists
IG9	43º53´00´´	7º55´20´´	430	Granodiorite
IG10	43º02´20´´	8º25´30´´	306	Schists
IG11	43º32´24´´	7º26´40´´	80	Granite
IG12	43º45´43´´	7º59´20´´	580	Schists
IG13	42º48´01´´	8º51´12´´	70	Schists
IG14	42º32´33´´	8º18´20´´	615	Schists
IG15	42º49´06´´	8º24´54´´	280	Schists and paragneisses

Table 2 Geographical location and parent material of the 16 re-sown grasslands studied.

Sample	Latitude (N)	Longitude (W)	Altitude asl (m)	Parent material
RG1	43º10´50´´	8º38´31´´	180	Schists and gneisses
RG2	43º14´08´´	8º16´57´´	160	Schists
RG3	43º21´55´´	8º10´00´´	100	Schists
RG4	43º15´21´´	7º42´45´´	425	Schists
RG5	43º04´42´´	7º10´15´´	780	Slates
RG6	43º03´09´´	7º15´45´´	620	Slates
RG7	42º34´38´´	7º29´26´´	348	Granite
RG8	42º26´36´´	7º54´34´´	440	Granite
RG9	43º31´02´´	8º00´31´´	549	Granite
RG10	42º41´54´´	8º16´04´´	355	Granite
RG11	42º11´25´´	7º45´40´´	490	Granite
RG12	42º04´45´´	7º41´40´´	622	Granite
RG13	41º53´10´´	7º28´20´´	385	Granite
RG14	42º05´50´´	8º20´20´´	110	Granite
RG15	42º07´10´´	8º35´40´´	100	Granite
RG16	42º13´50´´	8º35´40´´	105	Paragneisses

Table 3 Enzymatic assays performed on the 31 soil samples.

Enzymatic activity	Substrate (concentration)	pH	Units	Reference
Dehydrogenase	INT (0.5 %)	7.5	μmol INTF g^{-1} h^{-1}	Camiña et al. (1998)
Catalase	H_2O_2 (1 %)	7.4	mmol consumed g^{-1} h^{-1}	Trasar-Cepeda et al. (1999)
Urease	Urea (1065.6 mᴍ)	8.0	μmol NH_3 g^{-1} h^{-1}	Nannipieri et al. (1980)
BAA-protease	BAA (0.03 ᴍ)	8.0	μmol NH_3 g^{-1} h^{-1}	Nannipieri et al. (1980)
Casein-protease	Casein (1 %)	9.0	μmol tyrosine g^{-1} h^{-1}	Ladd and Butler (1972)
β-glucosidase	*p*-nitrophenyl-β-D-glucopyranoside (25 mᴍ)	5.0	μmol *p*-nitrophenol g^{-1} h^{-1}	Eivazi and Tabatabai (1988)
CM-cellulase	carboxymethyl-cellulose (0.7 %)	5.5	μmol glucose g^{-1} h^{-1}	Schinner and von Mersi (1990)
Invertase	Sucrose (35.06 mᴍ)	5.5	μmol glucose g^{-1} h^{-1}	Schinner and von Mersi (1990)
Phosphodiesterase	bis-*p*-nitrophenyl phosphate (10 mᴍ)	5.0	μmol *p*-nitrophenol g^{-1} h^{-1}	Bowman and Tabatabai (1978)
Phosphomonoesterase	*p*-nitrophenyl phosphate (16 mᴍ)	5.0	μmol *p*-nitrophenol g^{-1} h^{-1}	Saá et al. (1993)
Arylsulphatase	*p*-nitrophenyl sulphate (5 mᴍ)	5.0	μmol *p*-nitrophenol g^{-1} h^{-1}	Tabatabai and Bremner (1970)

Table 4 Values of the chemical and physical properties of the improved grasslands, the re-sown grasslands and the native grassland soils (mean ± standard deviation and range of variation) under study.

Property	**Improved grasslands**		**Re-sown grasslands**		**Native grasslands**	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
pH H_2O	5.43±0.47a	4.90-6.52	5.21±0.27a	4.74-5.58	5.29±0.50a	4.50-6.40
pH KCl	4.26±0.48a	3.72-5.23	3.97±0.26a	3.59-4.52	4.16±0.52a	3.35-5.50
Clay	21±7a	12-39	17±4a	11-23	21±7a	9-42
Silt	27±8a	17-46	26±12a	10-55	30±13a	11-60
Sand	52±13a	21-71	57±14a	33-79	49±17a	18-80
Total C (%)	5.26±2.02a	2.5-9.5	3.32±1.20b	1.8-5.4	5.7±2.9a	2.2-15.1
Total N (%)	0.398±0.135ab	0.22-0.66	0.260±0.100b	0.15-0.45	0.45±0.23a	0.15-1.05
C/N	13±1a	11-15	13±2a	10-16	13±2a	10-18
Available P (mg kg^{-1})	60±9b	50-77	54±10b	39-78	19±7a	6-31
Al_2O_3 (%)	0.80±0.48a	0.31-2.04	0.31±0.16b	0.09-0.67	0.67±0.52a	0.07-2.25
Fe_2O_3 (%)	0.68±0.22a	0.36-1.11	0.35±0.17b	0.11-0.73	0.72±0.52a	0.11-2.22

Table 5 Values of the biochemical properties of the improved grasslands, the re-sown grasslands and the native grassland soils (mean ± standard deviation and range of variation) under study. For the same property different letters indicate significant differences ($P<0.05$).

Property	Improved grasslands		Re-sown grasslands		Native grasslands	
	Mean	Range	Mean	Range	Mean	Range
Labile C[1]	268±69a	142-383	245±143a	105-728	279±112a	121-665
Microbial biomass C[1]	429±123ab	266-628	310±135b	132-559	688±462a	114-2003
Basal respiration[2]	385±131ab	211-604	250±130b	43-467	432±227a	125-1190
$q\mathrm{CO}_2$[3]	3.85±1.33a	2.55-7.01	3.53±1.67a	1.12-7.41	3.1±1.3a	1.0-6.8
Catalase[4]	1.56±0.40a	0.96-2.25	1.76±0.44a	0.94-2.35	1.72±0.84a	0.44-4.16
Dehydrogenase[5]	0.60±0.21ab	0.28-1.16	0.49±0.22b	0.19-1.14	0.80±0.43a	0.24-2.32
Net N mineralization[1]	23±12b	7.26-54	12±13a	-3.5-41.5	12.9±12.1a	-1.1-50.9
CM-Cellulase[6]	0.214±0.081a	0.036-0.412	0.187±0.920a	0.064-0.377	0.221±0.106a	0.099-0.570
β-glucosidase[7]	1.48±0.56a	0.67-2.60	1.25±0.52a	0.45-2.20	1.74±0.81a	0.50-3.96
Invertase[6]	6.00±2.14ab	3.52-10.23	4.41±2.13b	1.68-10.80	7.43±4.43a	1.39-19.50
Casein-protease[8]	1.22±0.38ab	0.62-1.88	1.01±0.44b	0.43-1.87	1.43±0.59a	0.45-2.83
BAA-protease[9]	18±8a	6-38	17±8a	7-33	23±13a	5-53
Urease[9]	19±9a	3-33	14±9a	4-29	35±46a	3-193
Phosphodiesterase[7]	0.68±0.30ab	0.29-1.42	0.45±0.18b	0.22-0.77	0.82±0.36a	0.16-1.65
Phosphomonoesterase[7]	4.84±1.30ab	2.06-7.07	3.93±1.25b	2.60-6.45	5.67±2.56a	1.45-12.84
Arylsulphatase[7]	0.42±0.16ab	0.12-0.66	0.29±0.24b	0.09-1.09	0.59±0.42a	0.07-1.98

[1]mg kg^{-1}, [2]mg CO$_2$-C evolved 10 days kg^{-1} soil, [3]µg CO$_2$-C mg biomass carbon^{-1} h^{-1}, [4]mmol H$_2$O$_2$ consumed g^{-1} h^{-1}, [5]µmol INTF g^{-1} h^{-1}, [6]µmol glucose g^{-1} h^{-1}, [7]µmol *p*-nitrophenol g^{-1} h^{-1}, [8]µmol tyrosine g^{-1} h^{-1}, [9] µmol NH$_3$ g^{-1} h^{-1}.

Table 6 Values of the biochemical properties, expressed as unit of total carbon content (TC), of the native grassland soils, the improved grassland soils and the re-sown grassland soils (mean ± standard deviation and range of variation) under study. For the same property different letters indicate significant differences (P<0.05).

	Native grasslands		Improved grasslands		Re-sown grasslands soils	
	Mean	Range		Range	Mean	Range
Labile C/TC[1]	0.53±0.14a	0.28-0.87	0.55±0.14a	0.25-0.78	0.76±0.37a	0-46-1.95
Microbial biomass/TC[1]	1.16±0.43a	0.53-2.55	0.90±0.33a	0.30-1.57	0.99±0.45a	0.36-2.26
Microbial respiration/TC[1]	0.79±0.29a	0.38-1.55	0.77±0.23a	0.36-1.42	0.77±0.40a	0.21-1.89
Catalase/TC[2]	31±9a	17-48	33±11a	13-56	49±17b	20-88
Dehydrogenase/TC[3]	14±5a	6-26	12±4a	5-19	15±5a	9-26
Net N mineralization/TC[4]	0.25±0.21a	-0.23-0.73	0.45±0.19a	0.19-0.92	0.36±0.35a	-0.14-0.90
CM-cellulase/TC[5]	4.1±1.4a	1.7-7.5	4.4±2.0ab	1.4-8.9	5.7±2.1b	2.8-9.6
β-glucosidase/TC[6]	32±11a	16-62	30±12a	11-60	41±18a	8-89
Invertase/TC[5]	131±51a	45-263	123±52a	63-275	134±37a	70-205
Casein-protease/TC[7]	27±7a	15-61	25±6a	12-36	31±10a	18-54
BAA-protease/TC[8]	428±199a	178-805	367±185a	98-722	515±155b	163-726
Urease/TC[8]	519±414a	64-1893	379±206a	102-852	404±145a	218-902
Phosphodiesterase/TC[6]	15±6a	8-28	14±5a	7-22	14±5a	9-27
Phosphomonoesterase/TC[6]	103±30a	42-176	99±30a	49-148	125±36b	86-218
Arylsulphatase/TC[6]	10±5a	3-24	8±3a	4-15	8±5a	2-20

[1] g 100 g^{-1}, [2]mmol H_2O_2 consumed g^{-1} C h^{-1}, [3]µmol INTF g^{-1} C h^{-1}, [4]mg N g^{-1} C 10 d^{-1}, [5]µmol glucose g^{-1} C h^{-1}, [6]µmol *p*-nitrophenol g^{-1} C h^{-1}, [7]µmol tyrosine g^{-1} C h^{-1}, [8]µmol NH_3 g^{-1} C h^{-1}.

Table 7 Correlations among all the biochemical properties and total C (TC) and N (TN) contents for improved grassland soils (***P<0.001; ** P<0.01; * P< 0.05)

	Labile C	Bio.	Resp.	$q\mathrm{CO_2}$	Cat.	Deh.	N min.	Cel.	Glu.	Inv.	Cas.	BAA.	Ure.	Dies.	Mono.	Aryl.	TC
Biomass C	NS	1															
Respiration	NS	0.73^{***}	1														
$q\mathrm{CO_2}$	NS	NS	NS	1													
Catalase	NS	0.64	0.79^{***}	NS	1												
Dehydrogenase	NS	0.67^{**}	0.66^{**}	NS	0.64^{**}	1											
Net N mineralization	0.54^{*}	NS	0.67^{**}	NS	0.71^{**}	0.74^{**}	1										
CM-cellulase	0.59^{*}	NS	$^{-}0.17$	NS	NS	NS	NS	1									
β-glucosidase	NS	NS	0.79^{***}	NS	0.66^{**}	0.86^{***}	0.65^{**}	NS	1								
Invertase	NS	NS	0.64^{**}	NS	0.65^{**}	0.66^{**}	NS	NS	0.75^{**}	1							
Casein-protease	NS	NS	0.68^{**}	0.76^{**}	NS	NS	NS	NS	0.62^{**}	NS	1						
BAA-protease	0.57^{*}	NS	0.15	NS	NS	NS	NS	NS	NS	NS	NS	1					
Urease	NS	0.52^{*}	0.26	NS	NS	NS	NS	NS	NS	NS	NS	0.81^{***}	1				
Phosphodiesterase	NS	NS	0.75^{**}	NS	0.62^{**}	0.79^{***}	0.80^{***}	NS	0.86^{***}	0.61^{*}	0.67^{**}	NS	NS	1			
Phosphomonoesterase	NS	0.62^{*}	0.68^{**}	NS	NS	0.83^{***}	0.57^{*}	NS	0.78^{***}	NS	0.64^{**}	NS	0.58^{*}	0.74^{**}	1		
Arylsulphatase	NS	0.62^{*}	0.60^{*}	NS	NS	0.80^{***}	NS	NS	0.74^{**}	0.52^{*}	0.59^{*}	0.62^{*}	0.78^{***}	0.77^{***}	0.87^{***}	1	
TC	NS	NS	0.66^{**}	NS	NS	NS	NS	NS	NS	NS	0.70^{**}	NS	NS	NS	NS	NS	1
TN	0.57^{*}	NS	0.71^{**}	-0.38^{*}	NS	NS	0.55^{*}	NS	NS	NS	0.68^{**}	NS	NS	0.59^{*}	0.52^{*}	NS	0.98^{***}

Table 8 Correlations among all the biochemical properties and total C (TC) and N (TN) contents for re-sown grassland soils (***P<0.001; ** P<0.01; * P< 0.05)

	Labile C	Bio.	Resp.	$q\mathrm{CO}_2$	Cat.	Deh.	N min.	Cel.	Glu.	Inv.	Cas.	BAA.	Ure.	Dies.	Mono.	Aryl.	TC
Biomass C	NS	1															
Respiration	0.62^{**}	0.75^{***}	1														
$q\mathrm{CO}_2$	NS	NS	NS	1													
Catalase	0.54^{*}	0.56^{*}	0.68^{**}	NS	1												
Dehydrogenase	0.61*	0.61^{**}	0.55^{*}	NS	0.61^{*}	1											
Net N mineralization	NS	0.52^{*}	0.65^{**}	NS	0.56^{*}	0.71^{**}	1										
CM-cellulase	NS	NS	NS	NS	NS	0.50^{*}	NS	1									
β-glucosidase	NS	0.57^{*}	NS	NS	NS	NS	NS	NS	1								
Invertase	NS	0.65^{**}	0.62^{*}	NS	NS	NS	NS	0.84^{***}	0.55^{*}	1							
Casein-protease	0.50^{*}	0.72^{**}	0.73^{**}	NS	0.82^{***}	0.82^{***}	0.71^{**}	0.73^{**}	0.66^{**}	0.77^{***}	1						
BAA-protease	NS	0.63^{**}	0.58^{*}	NS	NS	NS	NS	0.55^{*}	NS	0.67^{**}	0.51^{*}	1					
Urease	NS	NS	NS	NS	NS	0.59^{*}	NS	0.75^{**}	NS	0.61^{*}	0.62^{*}	0.73^{**}	1				
Phosphodiesterase	NS	0.82^{***}	0.83^{***}	NS	0.58^{*}	0.76^{***}	0.79^{***}	NS	0.52^{*}	0.54^{*}	0.76^{***}	0.61^{*}	NS	1			
Phosphomonoesterase	NS	0.85^{***}	0.82^{***}	NS	0.50^{*}	0.73^{**}	0.74^{***}	0.60^{*}	0.53^{*}	0.73^{*}	0.80^{***}	0.78^{***}	0.69^{**}	0.90^{***}	1		
Arylsulphatase	NS	0.72^{**}	0.58^{*}	NS	0.53^{*}	0.90^{***}	0.83^{***}	NS	NS	NS	0.73^{**}	NS	NS	0.80^{***}	0.74^{***}	1	
TC	NS	0.54^{*}	0.67^{**}	NS	NS	0.69^{**}	NS	0.71^{**}	NS	0.76^{***}	0.78^{***}	0.69^{**}	0.84^{***}	0.64^{**}	0.75^{***}	0.59^{*}	1
TN	NS	0.59^{*}	0.71^{**}	NS	0.55^{*}	0.78^{***}	0.61^{*}	0.72^{**}	NS	0.74^{***}	0.82^{***}	0.75^{***}	0.87^{***}	0.72^{**}	0.84^{***}	0.68^{**}	0.98^{***}

Figure captions

Fig. 1 Soil quality in Galician grassland soils according to three different soil quality equations

Fig. 2 PCA analysis for several types of grasslands in Galicia.

Fig. 1 Soil quality in Galician grassland soils according to three different soil quality equations.

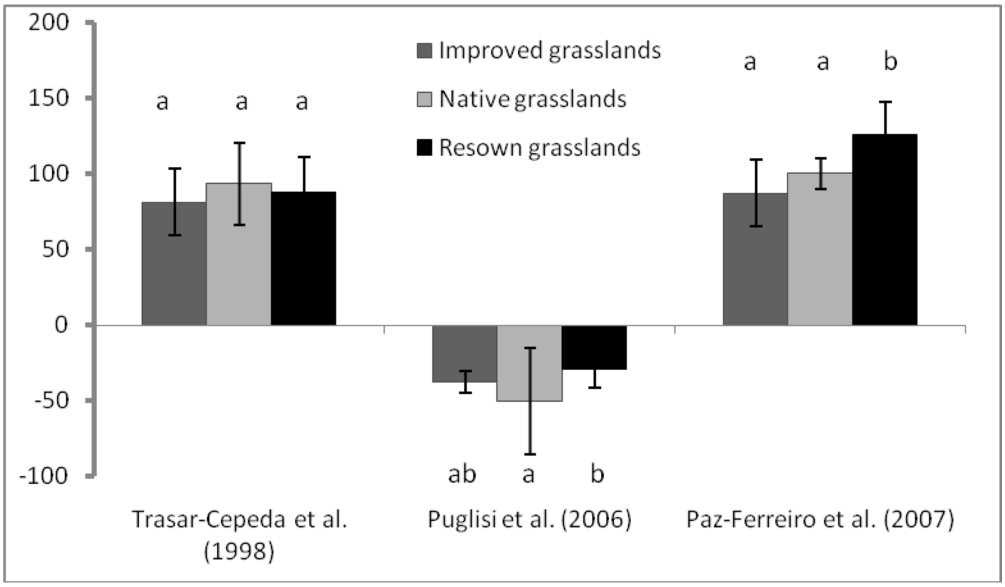

Fig. 2 PCA analysis for several types of grasslands in Galicia. Resp, N min, DH, Gluco, BAA, Pmono, Pdies and Aryl stands for respiration, N net mineralization, dehydrogenase, β-glucosidase, phodphomonoesterase, phosphodiesterase and arylsulphatase respectively.