COMPARISON OF ABSOLUTE BIOCHEMICAL PARAMETERS OF UNDISTURBED SOILS IN MEDITERRANEAN ENVIRONMENTS (NE SPAIN) WITH CORRESPONDING PARAMETERS RELATIVE TO SOIL ORGANIC CARBON

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Abstract

The study of soil quality requires the establishment of quality standards. To this end, several authors have highlighted the need to create databases of quality indicators, such as biochemical properties, for different types of undisturbed soils under various climates and to establish standardised methodologies for their development. In Spain, studies of the quality of native soils were initiated more than 15 years ago by several groups of authors from differing locations, but little is known regarding the biochemical characteristics of native soils in Catalonia (NE Spain). This study examines representative, minimally disturbed soils from Catalonia with a wide range of organic carbon contents. We examined the total and extractable organic carbon contents, total and extractable carbohydrates contents, enzyme activities (β-glucosidase, β-galactosidase, BAA-protease and urease), microbial biomass carbon and basal respiration of ten selected soils. Statistical analyses were applied to absolute values (i.e., per g of soil) and relative values (i.e., per g of soil organic carbon). The aim of this work was to determine the dependence of these properties on the organic matter content and the suitability of the relative values as soil quality indicators. The biochemical and microbiological parameter values of the native Catalan soils showed unusually wide ranges, although all of the values were similar to those already published for native soils in other Mediterranean climate areas. Overall, the sampled soils could be distinguished by their contents of organic carbon and total and extractable carbohydrates, rather than by their enzyme activities or microbiological variables; nevertheless, when the relative values were considered, the soils could be distinguished by their specific enzyme activities, particularly that of β-glucosidase, and by the labile proportion of organic matter. With the exception of the total carbohydrates/C ratio, the biochemical and microbiological parameters, expressed as functions of soil organic carbon content, were useful in distinguishing groups of native soils according to field observations and soil physicochemical properties.

Keywords

Soil quality

Mediterranean soils

Biochemical properties

Soil enzymes

1. Introduction

The lack of established quality standards is a critical issue for the study of soil quality. The choice of native (undisturbed) soils as references is based upon the association of maximum quality with a sustainable balance between soil components, under characteristic climate and vegetation conditions, and subject to little or no human disturbance (Doran and Parkin, 1994; Karlen et al., 1997). The biological and biochemical parameters of soils are particularly suitable as indicators of their quality because they respond to both natural and human-induced changes (Elliot et al., 1996; Gregorich et al., 1997; García et al., 2000; Filip, 2002; Gil-Sotres et al., 2005; Bastida et al., 2008b).

Early recommendations for basic indicators of soil quality already included biological characteristics. According to Melé and Crowley (2008), who examined 52 soil quality monitoring programmes developed worldwide through the end of 2003, 29% used biological indicators. Gil-Sotres et al. (2005) found that 40% of the publications on soil quality from 1990 to 2003 reported general biochemical parameters, while approximately 60% used specific ones (e.g., hydrolytic enzyme activities). More recently, 55-80% of studies (which considered only agricultural, forest and land use change) included biological indicators, according to a revision of the most common indicators used in soil quality assessment over the last 15 years (Zornoza et al., 2015).

The review by Bastida et al. (2008b) of the biological aspects of the quality of non-agricultural soils indicates that the most relevant works have been performed by Italian and Spanish authors. In Spain, studies of the quality of native soils were begun more than 15 years ago and have been undertaken by several groups of authors on soils from various geographic conditions (García et al., 2000, 2003). In Galicia (Spain), the study of native soils has focused on Umbrisols under Atlantic oak-woodland vegetation and a humid climate (Trasar-Cepeda et al., 1998, 2000, 2008a, 2008b; Leirós et al., 1999, 2000). In a Mediterranean climate, some authors studied soils in Murcia and Alicante (Spain), a rather heterogeneous territory, with high variability of climate and vegetation, including areas at risk of desertification (García et al., 1994; García and Hernández, 1997; Zornoza et al., 2007a, 2007b, 2008). All these studies of native soils from Spain have greatly contributed to the development and interpretation of soil quality data. However, no database exists that covers the whole Spanish territory and its lithological, climatic and vegetative diversity.

Scarce data are available concerning the biochemical characteristics of native soils in Catalonia (NE Spain) so we first performed a study of minimally disturbed soils of this territory in a previous work (Jiménez et al., 2012). Representative soils in our territory were studied including those covering a wide gradient of organic matter content. In this work, we provided preliminary information about biochemical properties; the results indicated that the studied biochemical parameters presented high and positive correlations between themselves, but analysis of organic carbon partial correlations indicated that these parameters were highly dependent on soil organic matter content. Consequently, we studied the same native soils, focusing on the behaviour of parameters expressed as a function of their soil organic carbon content (i.e., relative parameters), and present our conclusions herein.

The aim of this study was to elucidate, in non-modified soils developed under Mediterranean climate (NE Spain), the i) degree of influence of the soil organic carbon content on biochemical and microbiological parameters and ii) suitability of the relative parameters (i.e., per g of soil organic carbon) for distinguishing soils' characteristics. Thus, our hypothesis was that relative parameters would be able to group soils according to their general characteristics more accurately than absolute values.

2. Materials and Methods

2.1. Sites and soil sampling

Soil samples were collected from ten locations in Catalonia (NE Spain)**:** *Serres del Camp, Balaguer (BL), Serra del Corredor (CR), Conca d'Odena, Igualada (IG), Serra de la Picarda, La Granja d'Escarp (LG)*, *Serra Litoral (LT), Serra del Montnegre (MN), Serra de l'Ordal (OR), La Panadella* plateau *(PN), Segre* alluvial plain *(SG),* and *Plana de Vic (VC).* An overview of the sites and their soil characteristics is presented in Tables 1 and 2. We focused on native soils, under autochthonous vegetation (corresponding, as much as possible, to potential vegetation) which had not been disturbed by human action for decades. At all locations, forest and abandoned agricultural soils were distributed over wide zones in a landscape mosaic. To validate soil results, we selected four land uses: undisturbed (or subject to little disturbance) forest; abandoned agriculture field; dry grassland; and steppe.

The climate in these areas is of the semiarid Mediterranean type. The average annual temperature ranges from 9 to 16 °C and rainfall varies from <400 to 825 mm/year (ICC & SMC, 2008). The common rocks in this area are carbonate rocks (limestone, marls, alluvial and colluvial deposits) together with silica rocks (shales and granodiorite). The dry climatic conditions promote erosion, physical degradation and salinisation of these soils. The vegetation developing on the sampled soils varies from site to site. The *BL* soil supports a xeric shrubland of *Rosmarinus officinalis* L. *IG* and *LG* soils were found in the lowland and midland dry grasslands, with rocky surfaces. The *LG* soil in particular corresponds to a steppe-like vegetation, well-adapted to low water availability, where the scarcity of rain prevents development of pastures and the vegetation is dominated by herbs and sparse shrubs. The *VC* soil is typical of *Aphyllanthes monspeliensis* L. grasslands, dominated by annual plants and gramineae. *CR* and *MN* soils support a Mediterranean woodland vegetation, dominated by holm-oak (*Quercus ilex* subsp. *rotundifolia* L.) and cork-oak (*Quercus suber* L.). The vegetation on *LT* and *PN* soils consists of holm-oaks (*Quercus ilex* L.). In contrast, the *OR* soil supports conifer-dominated woodlands, typically *Pinus pinea* L, *Pinus halepensis* Mill and *Pinus nigra* Arnold. The *SG* soil is associated with Mediterranean riparian woodlands where the most typical species are alder (*Almus glutinosa* (L.) Gaertn), ash (*Fraxinus excelsior* L.) and black poplar (*Populus nigra* L.).

A plot of approximately 100 m^2 was defined in each site, and a sample composed of 20-25 homogeneously mixed sub-samples was collected from the topsoil (0-10 cm) after litter removal. Samples were collected on two consecutive days in spring, then immediately sieved to obtain fine earth (<2 mm) and homogenised. One part was stored at 4 °C prior to biochemical and microbial analysis (within 15 days of sampling), while another was air-dried for a week and stored at room temperature before analysis of its chemical and physical properties.

2.2 Analytical methods

The main physical and chemical properties of the soil samples were characterised as follows. Texture was determined by the Bouyoucos method (Gee and Bauder, 1986). Electrical conductivity was measured in a 1/5 suspension, pH in a 1/2.5 (soil/water) suspension and total carbonates were measured using a Shimadzu TOC-V-Series analyser with a solid sample module SSM 5000A (Shimadzu Corporation, Kyoto, Japan) by adding diluted H_3PO_4 before heating at 200 °C. Total organic carbon was determined by potassium dichromate oxidation using the Walkley-Black procedure (Nelson and Sommers, 1982).

Carbohydrates were analysed in air-dried samples: total carbohydrates were determined by a double hydrolysis with H_2SO_4 (4 M and 0.5 M), as reported by Cheshire and Mundie (1966); and extractable carbohydrates (soluble in 0.5 M K_2SO_4) as described by Badalucco et al. (1992). Carbohydrate contents were measured by anthrone colourimetry (Brink et al., 1960).

Extractable organic carbon (extractable organic C) was obtained by extraction with 0.5 M K_2SO_4 (1:4 w/v dry soil: extractant ratio) and quantified using a Shimadzu TOC-V-Series analyser. Microbial biomass-C (MBC) was determined using the fumigation extraction procedure (Vance et al., 1987) in samples that had been pre-incubated for 7 days in the dark at 28 °C after being adjusted to 60% of their field capacity. Carbon dioxide emissions were determined in 100 g of soil previously adjusted to 60% field capacity and incubated for 7 days in the dark at 28 °C in sealed jars containing a vial with 10 mL of 0.5 M NaOH to absorb the gas; NaOH traps were removed daily during incubation. The quantity of CO_2 was determined by titration of NaOH with 0.5 M HCl (Hernández and García, 2003), and basal respiration (BR) values were obtained (after checking that daily CO_2 production was constant from the 5th day) by calculating the amount of CO_2 produced between the 6th and 7th days of incubation.

The method of Tabatabai and Bremner (1972) as modified by Nannipieri et al. (1978) was used to determine urease activity. BAA (N-benzoyl-L-argininamide) proteolytic activity was determined by the method of Ladd and Butler (1972) as modified by Bonmatí et al. (1998). β-glucosidase and β-galactosidase activities were determined as reported by Tabatabai (1982), with calibration plots of p-nitrophenol prepared by using individual soil samples, thus taking into account the relative adsorption of p-nitrophenol by each soil (Vuorinen, 1993).

Results were expressed on two bases: a) dry weight soil (absolute values); and b) total organic C measured in soil (relative values). We designated the relative values of enzyme activities as “specific activities”. For the analytical assays, mean values of three or four replicates per sample were used.

2.3 Statistical analyses

Total contents of the studied parameters in the sampled soils and their values relative to organic C content were statistically compared through (i) their coefficients of variation (CV), (ii) one-way analysis of variance (ANOVA), and (iii) factor analysis (FA). The Modified Bennett’s test was used to test the equality of pairs of CVs in order to compare their relative variability (Gupta and Ma, 1996). All properties were subjected to a one-way ANOVA to determine the differences between soils. Means were compared using the Student–Newman–Keuls (SNK) procedure (at a level of α=0.05). FA was performed to examine the structure of data by explaining the correlations among variables and to summarise data into a few dimensions by condensing the set of variables studied into a smaller set of latent variables (or factors). The Kaiser-Meyer-Olkin (KMO) test was used as a measure of data suitability for FA (Hair et al. 1998). To reach a KMO value of at least 0.6, three of the absolute variables (extractable organic C, microbial biomass-C and basal respiration) and three of the relative variables (total carbohydrates/C and specific β-glucosidase and specific urease activities) had to be removed before FA.

3. Results

3.1 Descriptive statistics of properties

Six of the ten measured variables varied approximately 10-fold (Table 3). Total carbohydrates, extractable carbohydrates contents and β-galactosidase activity, with CV over 90%, were the most dispersed parameters, whereas basal respiration and β-glucosidase activity, with CV< 65%, were the least. Nevertheless, in all cases, the modified Bennett’s test used to compare the different pairs of CV was not significant at the 5% level.

Six of the nine calculated relative parameters (expressed per unit of C) varied approximately 5-fold (Table 3). The metabolic activity of the organic matter (basal respiration/C) and the specific β-galactosidase activity, with CV over 50%, presented the highest dispersion, whereas the specific β-glucosidase activity and total carbohydrates content of organic matter, with CV< 25%, were the least dispersed. The pair constituted by the maximum (Basal respiration/C) and the minimum (Total carbohydrates/C) coefficients of variation was significant at the 5% level according the Modified Bennett's test.

By comparing the CV of the absolute parameters with those of the relative parameters, total carbohydrates and extractable carbohydrates were extremely variable, whereas total carbohydrates/C and extractable carbohydrates/C were those with the lowest variabilities, indicating that the variability of these parameters was mainly associated with the variation of total organic C content. The remaining assayed parameters (except basal respiration) seemed to be less dependent on the quantity of organic matter. In contrast, basal respiration was the only endpoint presenting a greater coefficient of variation, when considering the variability of values per C unit; this indicates that basal respiration was, as could be expected, highly associated with the composition of the organic matter.

3.2 Analysis of variance

The ANOVA revealed that all the parameters were significantly ($p<0.001$) influenced by soil location (Table 4 and Table 5). Comparison of the calculated F values indicated that the discriminant capabilities of the contents of total organic C, and total and extractable carbohydrates were higher than those of the other variables (extractable organic C, enzyme activities and microbial properties). The capability to discriminate soils based on the separation of means was very high in the case of total organic C, with complete differentiation of the ten samples; the lowest discriminant capability was related to microbial biomass and extractable organic C contents. BAA proteolytic activity was the least discriminatory enzyme activity.

The ANOVA showed, as in the case of the absolute endpoints, that all of the relative parameters were significantly ($p<0.001$) influenced by soil provenance (Table 6). Comparison of F values indicated that specific β-galactosidase activity had the highest, and microbial biomass-C/C the lowest, discriminant capabilities. Fewer significant differences between soils were observed in this case than in that of the absolute values.

Most of the absolute variables provided the same ranking of soils than that made by organic C content, except for *OR* and *PN* soils. All the parameters of *OR* soil showed lower values than expected, according to its organic C content. The same behaviour was observed in β-galactosidase and urease activities in *PN* soil. Inversely, in *LT* soil, three variables (extractable organic C, extractable carbohydrates and BAA proteolytic activity) had higher values than expected according to the soils organic C content; the same behaviour was observed in basal respiration and qCO_2 in *IG* and *SG* soils.

Of the relative endpoints, only total carbohydrates/C ranked soils in the same order as organic C, whereas extractable organic C/C, extractable carbohydrates/C, specific β-glucosidase activity and basal respiration/C displayed the opposite soils ranking to that of organic C.

3.3 Factor analysis

FA of absolute variables showed that the two first factors explained 96.7% of the variance (Table 7). Factor 1 contained the greatest degree of variability (52%), with total carbohydrates, extractable carbohydrates, total organic C and β-glucosidase having the most weight. Factor 2 explained 45% of the variability, with β-galactosidase, urease and BAA protease activities having most of the weight. FA placed *PN* and *OR* soils in the positive sector of Factor 1, separate from eight other soils, whereas *CR* and *MN* were isolated in the positive sector of Factor 2 (Figure 1).

In the case of relative variables, FA revealed that the two first factors explained 84% of the variance (Table 7). Factor 1 contained the greatest degree (54%) of variability; the four parameters with most weight being microbial biomass-C/C, extractable organic C/C, basal respiration/C and extractable carbohydrates/C. Factor 2 explained 30% of the variability, being associated with the specific activities of β-galactosidase and BAA-protease. In this case, FA placed *IG*, *BL* and *LG* soils on the positive axis of Factor 1, distinctly separated from the *OR* soil, whereas *LT*, *CR* and *MN* soils remained separated, on the positive axis of Factor 2, also distinctly separated from *OR* soil (Figure 2). *SG*, *PN* and *VC* soils occupied a central position, not clearly characterised by any factor.

4. Discussion

4.1 Ranges of properties

Total organic C content is a basic parameter for the characterisation of soils. In our case, C contents varied from 8 g kg^{-1} (*IG* soil) to 100 g kg^{-1} (*PN* soil), a wide range that was consistent with the variety of values previously reported for Catalan soils (Alcañiz et al., 2005) and, excluding the highest values, was normal in the framework of other native Mediterranean soils. Extractable organic C values indicated labile organic carbon pools in the studied soils, and varied from 189 mg kg^{-1} (*LG* soil) to 1423 mg kg^{-1} (*PN* soil), which could also be considered a wide range. Zornoza et al. (2007b) found 287 mg kg^{-1} and 455 mg kg^{-1} of extractable organic C in non-degraded soils from Mediterranean sites with organic C contents between 46 g kg^{-1} and 98 g kg^{-1}. The total carbohydrate contents were within the general range found in soils, from 1 g to 20 g glucose 100 g^{-1} (Folsom et al., 1974; Lowe, 1978; Cheshire, 1979; Gunina and Kuzyakov, 2015). Total carbohydrates content was the parameter most linked to soil organic C, and this dependence explained the similarity of total carbohydrates/C values across the different soils (Gunina and Kuzyakov, 2015). Extractable carbohydrate contents were higher than those reported from soils with a similar organic matter content, although most of those studies determined water-soluble carbohydrates (García et al., 2002; Saviozzi et al., 2001; Caravaca et al., 2002; Bastida et al., 2006). Extractable carbohydrate contents responded to differences in soil organic matter and also to soil microbial biomass contents; the response is consistent with the parameter’s being considered an indicator of carbon that is easily available for microorganisms and thus conditions microbial biomass and/or microbial activity (Badalucco et al., 1990, 1992; DeLuca and Keeney, 1993; Joergensen et al., 1996; García et al., 2000; Gunina and Kuzyakov, 2015).

The microbial biomass-C contents were similar to those reported by others from native soils under Mediterranean conditions in southern and SE Spain with similar organic matter levels (Miralles et al., 2007; Zornoza et al., 2007b). The values of MBC/C varied from 0.76 g 100 g^{-1} to 3.99 g 100 g^{-1} in the sampled soils and were generally similar to those obtained by others (Leirós et al., 2000; Trasar-Cepeda et al., 2000; Miralles et al., 2007); however, the highest values observed in the present study (in *LG*, *BL* and *IG* soils) were higher than those reported by those authors.

Ranges of enzyme activity values were similar to those reported from undisturbed soils of SE Spain (Miralles et al., 2007; Zornoza et al., 2007b, 2008), but higher than those of denuded soils and arid zones (García et al., 1994, 2000, 2002; García and Hernández, 1997; Bastida et al., 2006, 2008a). When compared with Galician soils described as native, the soils we studied had much lower organic matter content, and the maximum values were particularly low in the cases of urease activity and BAA protease activity (Trasar-Cepeda et al., 2000, 2008b). The observed β-galactosidase activities were in agreement with those of Eivazi and Tabatabai (1988) and Bandick and Dick (1999), who found them to always be lower than β-glucosidase activities. In our study, the specific β-galactosidase activity was highest in acid forest soils (*MN*, *CR* and *LT*), thus explaining the high dispersion of this parameter.

4.2 Patterns of soil biochemical properties

The observed coefficients of variation were generally higher than those of other soils in similar studies. The high dispersion of values we observed was a consequence of the sampling regime, including soils from a variety of sources, from forest to grassland and drier areas with low plant cover (Miralles et al., 2007). It is worth noting that similar studies included only abandoned agricultural or forest lands, and soils presented narrower ranges of values for organic matter content (Saviozzi et al., 2001; Trasar-Cepeda et al., 2008b; Zornoza et al., 2008).

The C content did not present as wide a dispersion as other quality parameters, but showed a high discriminant capacity (as also found by Zornoza et al., 2007a, 2007b), with a high load on the first factor of the FA.

While the total carbohydrate contents indicated some similarity between the studied soils, the extractable carbohydrate contents seemed more useful for revealing differences between them. Total carbohydrates appeared linked to soil organic C and, as a consequence, total carbohydrates/C had a very weak discriminant capacity. In fact, the bibliography indicates that carbohydrate contents vary little among soils and that the profiles of monosaccharide composition are more variable (Lowe, 1978; Cheshire, 1979; Gunina and Kuzyakov, 2015). In contrast, extractable carbohydrates content displayed the highest discriminant capability, thus linked to Factor 1 in both FAs. This would be in agreement with the higher occurrence of determination of extractable carbohydrates (together with soluble C) in studies addressing soil quality (Ghani et al., 2003; Bongiovanni and Lobartini, 2006).

The absolute and specific β-glucosidase activities were the least dispersed parameters, but they enabled a remarkable distinction between soils, i.e., they were parameters of small dispersion and high discriminant capability. Others have also reported their small variation (Trasar-Cepeda et al., 2000, 2008b; Zornoza et al., 2007b). Moreover, the discriminant capacity of β-glucosidase activity was reinforced by its sensitivity to differences between treatments in different studies (Miller and Dick, 1995; Bandick and Dick, 1999; Monreal and Bergstrom, 2000; Badiane et al., 2001; Knight and Dick, 2004; Ceccanti et al., 2008). The observed activity of β-glucosidase was consistent with the link between it and the carbon cycle, and with its role in providing low molecular weight sugars as energy sources to microorganisms (Tabatabai, 1982; Eivazi and Tabatabai, 1988). β-glucosidase activity plays a role in defining soil quality indices where the predicting variable is soil organic C content (García et al., 1994; Zornoza et al., 2007a, 2007b).

We observed fewer differences in urease and BAA-protease activities between soils than in other parameters. We could conclude that there were more differences in the capacity of degradation of carbon compounds than in that of low molecular weight nitrogen-bound molecules, which coincides with the findings of Trasar-Cepeda et al. (2000) that urease and BAA-protease activities explain a very small proportion of the variability between native soils. In general, vegetation increases enzyme activities and these decrease as the plant cover diminishes (Bastida et al., 2006). However, according to García et al. (1997, 2002), urease and BAA-protease activities depend more on the type of vegetation than on plant cover. Those authors also found that BAA activity was less correlated with the other parameters, and the least affected by vegetation loss.

FA indicated that the most relevant absolute parameters for distinguishing the native soils studied were those associated with soil organic matter content (organic C, total and extractable carbohydrates content and β-glucosidase activity). However, in the case of relative parameters, the most relevant were those related with the fraction of labile organic matter of the soils. Therefore, characteristics related to microbial activity seem to provide more information about native soils than the absolute parameters. Specifically, the enzyme activities β–galactosidase and BAA-protease were useful to discern soils with low pH, suggesting that these activities would act synergistically in ecosystems characterised by a certain type of microbial biomass and/or organic matter.

4.3 Biochemical properties versus soil organic carbon content

Total carbohydrates content was the only parameter that increased with increasing organic matter content, indicating an important link between them, and consequently, the total carbohydrates/C content provided little additional information.

Extractable carbohydrates/C increased with decreasing organic matter content (with the exception of the *PN* soil) which could be related to the need for survival of the microbial biomass, considering that sugars maintain and stimulate microbial activities (Gunina and

Kuzyakov, 2015). Likewise, we found that soils with lower organic matter content exhibited higher proportions of extractable organic C. Nevertheless, the ratio of extractable carbohydrates/extractable organic C, which indicates the proportion of carbohydrates in the labile fraction, decreased with decreasing values of total organic matter content. The values of extractable carbohydrates/extractable organic C were in agreement with those reported in the bibliography, and the higher values from forest soils may be attributed to the accumulation of plant material (De Luca and Keeney, 1993; Joergensen et al., 1996).

Our results suggest that basal respiration is a biological characteristic that varies little and is relatively independent of the other absolute parameters, particularly organic matter content. Nevertheless, FA showed that the relative parameter BR/C is highly dependent on extractable organic C/C and MBC/C. Hence, the *PN* soil, with a C content 5-fold higher than the *IG* soil, exhibited a similar value of BR; since extractable organic C/C in the *PN* soil was significantly lower than in the *IG* soil, the BR value could be ascribed to the lower proportion of labile substrates, able to act as an energy source for the microorganisms.

An increment of organic matter roughly led to an increase in β-glucosidase activity but not in the corresponding specific activity. As an indicator of the organic matter decomposition capacity of the soil, β-glucosidase activity seemed proportionally higher in soils with less organic matter. This result was consistent with similar behaviour shown by extractable carbohydrates/C, which also presented a roughly inverse relationship with organic matter content.

These results could be related with the fact that soils with low organic matter were able to maintain their mineralization capacity. Ceccanti and Pezzarossa (1994) and Masciandaro and Ceccanti (1999) found a similar pattern in the soils they studied, explaining that soils with lower organic matter content were better able to preserve the activity of the humus-enzyme complexes which underlie soil resilience. Trasar-Cepeda et al. (2008b) reflected deeply on the relation between specific β-glucosidase and the organic C content for six groups of soils from Galicia under different types of use. With the exception of a group of typical native oak soils, they found an inverse relationship between these variables in each group. Their hypothesis focused on the existence of an ecological mechanism to maintain soil metabolic activity, such as the stabilisation of enzymes.

The specific β-galactosidase activity was highest in our acid forest soils (*LT*, *CR* and *MN*). This was in agreement with the findings of Jolivet et al. (2006), who reported a higher proportion of galactose in acid forest soils than in grassland-type soils. Moreover, the ratios β-galactosidase/BMC, Urease/BMC and BAA-protease/BMC were also significantly higher in these three soils (data not shown). Using FA, we discerned a link between β-galactosidase activity and BAA-protease activities. All these results seem to indicate that in this study's acid forest soils, the proportion of a particular microbial community might be important. Joergensen et al. (1996) argued that the organic matter of acid soils corresponds to plant material that is resistant to decomposition, so the excretion of extracellular polysaccharides could be important. Therefore, we hypothesise that this soil's microbial community would be characterised by the presence of galactose in mixed polysaccharide-peptide or polysaccharide-protein components. Two aspects would strengthen our hypothesis: i) glomalins (glycoproteins with galactose as a sugar component) from micorrhyzal fungi are found in high concentrations, especially in acidic and undisturbed soils (Nichols and Wright, 2004); and ii) based on their sugar composition, actinomyces cells have high galactose contents (Gunina and Kuzyakov, 2015).

4.4 Relationships among relative biochemical properties and soil properties

The groups of relative parameters obtained by FA were more distinct than those of absolute parameters, and those resulting from the cluster analysis presented by Jiménez et al. (2012). The groupings were consistent, as a whole, with the field observations and the soil physicochemical properties. In fact, we discerned a group consistent with that obtained from the absolute variables, also indicating that soils developed on calcareous rock varied more among themselves and showed more distinct from acid soils.

The group of soils with low organic matter content, *IG*, *BL* and *LG*, corresponded to calcareous soils from the Central Depression in Catalonia and also to the most arid part of the area; *BL* and *LG* were gypsum or saline soils. Soils with the lowest organic matter content exhibited high proportions of labile organic matter and microbial activity, and also higher β-glucosidase and urease specific activities. The group formed by the *MN*, *CR* and *LT* soils, being forest soils developed on non-calcareous materials, displayed a biochemical specificity characterised by high β-galactosidase and BAA-protease specific activities, which could be involved in the degradation of complex carbohydrate-protein substrates. The third group includes only *OR*, a forest soil developed on calcareous rock but also a typical Mediterranean red soil with a decarbonated A horizon over a carbonated B. This soil presented unique biochemical characteristics. We believe that the vegetation type (pine woodland), being rich in lignified material and lacking degradable substrate, explains the low values of the extractable organic C/C ratio, its low relative microbial properties (MBC/C and BR/C), and all specific enzyme activities (especially β-glucosidase). These results are in agreement with the findings of several authors on the effect of vegetation type on labile organic matter content, soil enzymatic activity, and on the content and degradation speed of carbohydrates (Folsom et al., 1974; García et al. 1994, Martens and Loeffelmann, 2002; Miralles et al., 2007; Bastida et al., 2008a).

The three remaining soils (*PN*, *SG* and *VC*), developed from calcareous rock, presented intermediate characteristics between the aforementioned groups and were therefore difficult to define. The *PN* soil had distinctive characteristics because it was decarbonated and located in a dry area, although the wet microclimate conditions allowed the development of a deep organic horizon. This soil presented significantly higher values of total carbohydrates/C, which might indicate important inputs of plant material, or environmental conditions unfavourable to decomposition due to their location, or an intimate association with a particle size that is not suitable for microbial decomposition (Sanger et al., 1997; Marando et al., 2012). The *SG* soil was also unique because it was a fluvisol exposed to yearly flooding and had the highest values of qCO_2 and specific β-glucosidase activity, which would indicate high microbial and organic matter activities. In flooded soils, chemical changes may alter soil properties over time, including soil nutrient availability, enzyme activities, organic matter dynamics and structure and microbial function (Unger et al., 2009; Wilson et al., 2010). The characteristics of the *VC* soil, a priori similar to the *IG* soil, could be attributed to its development under a wetter and colder climate, resulting in a higher and more stable content of organic matter.

Overall, the results indicated that the sampled soils could be more readily distinguished by their total organic and carbohydrate contents (total and extractable) than by their extractable organic C content, enzyme activities or microbiological variables (MBC and BR). However, if the influence of C content was excluded, the most relevant relative parameters were those related to the fraction of labile organic matter. Carbonated soils could be distinguished from each other through relative parameters; and acid soils seemed to contain a type of organic matter that differentiated them from each other.

We conclude that i) in soils with higher contents of organic C, factors such as pH or vegetation type influenced the magnitudes of enzyme activity; ii) soils with low organic matter content had higher relative capacities to maintain microbial activity, thus ensuring the survival of the microbial biomass; and iii) the studied relative biochemical and microbiological parameters, except total carbohydrates/C, are useful in detecting the main differences between the native soils studied. Therefore, the relative parameters studied might contribute to the study of soil quality.

Finally, we suggest that further studies of reference values for native soils in Catalonia consider, using our described soil grouping as a starting point, separating non-calcareous and calcareous soils (with a subgroup of carbonated soils with low organic matter content). Within each of these soil groups, the organic matter content and the dispersion values of the biochemical and microbiological parameters would be lower; therefore, establishing quality standards would be easier.

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6. References

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Table 1. Characteristics of the soil sampling sites.

Location	Soil sample	Parent material	Soil type†	Soil use	Rainfall mm/year	UTM Coordinates‡		
						X (m)	Y (m)	Z (m)
Balaguer	BL	Gypseous marls	Regosol	Dry grassland	400	320964	4629954	247
Corredor	CR	Shale	Umbrisol	Forest	650	468616	4613264	520
Igualada	IG	Marls	Cambisol	Abandoned fields	600	389094	4603626	321
La Granja	LG	Marls	Cambisol	Steppe	< 400	279933	4588381	177
Litoral	LT	Granodiorite	Cambisol	Forest	575	438215	4596142	200
Montnegre	MN	Granodiorite	Umbrisol	Forest	825	469095	4614800	440
Ordal	OR	Limestone	Luvisol	Forest	675	402104	4582750	382
Panadella	PN	Limestone	Leptosol	Forest	625	367214	4606841	784
Segre	SG	Alluvial deposits	Fluvisol	Forest	< 400	281929	4590996	090
Vic	VC	Marls	Cambisol	Dry grassland	800	442007	4647012	468

†IUSS, 2015

‡31N (ETRS89geodesic datum)

Table 2. Sampled soil characteristics.

Soil sample	Textural class (USDA)	Sand %	Silt %	Clay %	pH	EC (25 ºC) dS·m^{-1}	$CaCO_3$ %
BL	Sandy Loam	60.1	33.2	6.6	8.15	2.000	12
CR	Loam	37.7	38.9	23.3	6.45	0.129	<0.3
IG	Clay Loam	28.6	33.6	37.8	8.50	0.159	64
LG	Clay Loam	36.4	29.9	33.7	8.40	1.377	35
LT	Loamy Sand	85.8	5.7	8.5	6.95	0.064	<0.3
MN	Sandy Loam	63.9	21.7	14.4	6.45	0.092	<0.3
OR	Clay	3.7	34.0	62.3	8.00	0.191	<0.3
PN	Sandy Clay Loam	45.2	19.9	34.9	7.80	0.243	<0.3
SG	Sandy Loam	72.2	15.5	12.4	8.65	0.121	33
VC	Loam	48.5	28.1	23.4	8.50	0.163	37

Table 3. Mean, minimum, maximum and coefficient of variation (CV) of the studied parameters in the ten sampled soils, ranked in order of descending CV.

Absolute parameters	Units†	Mean±SD	Min	Max	CV %	Relative parameters	Units†	Mean±SD	Min	Max	CV %
β-Galactosidase	μmol pNP g^{-1} h^{-1}	0.35±0.34	0.03	0.98	97	Basal respiration/C	mg C-CO_2 100 g^{-1} C h^{-1}	1.33±0.81	0.39	2.55	61
Extractable carbohydrates	g glucose kg^{-1}	0.44±0.42	0.11	1.50	95	Specific β-Galactosidase	μmol pNP g^{-1} C h^{-1}	7.34±4.20	2.11	14.87	57
Total carbohydrates	g glucose kg^{-1}	7.31±6.76	0.99	22.99	93	Microbial biomass C/C	mg C 100 mg^{-1} C	2.20±1.01	0.76	3.99	46
BAA-Protease	μmol NH_3 g^{-1} h^{-1}	2.71±2.15	0.45	5.65	80	Specific BAA-Protease	μmol NH_3 g^{-1} C h^{-1}	63.91±27.44	10.44	114.58	43
Urease	μmol NH_3 g^{-1} h^{-1}	2.56±1.88	0.71	6.21	73	Extractable organic C/C	g C 100 g^{-1} C	1.42±0.58	0.54	2.22	41
Total organic C	g C kg^{-1}	45.3±33.1	8.5	107.4	73	Specific Urease	μmol NH_3 g^{-1} C h^{-1}	65.44±26.14	20.91	95.96	40
Microbial biomass C	mg C kg^{-1}	813±571	338	2170	70	Extractable carbohydrates-C/C	g C-glucose 100 g^{-1} C	0.39±0.12	0.24	0.56	30
Extractable organic C	mg C kg^{-1}	527±368	189	1423	70	Specific β-Glucosidase	μmol pNP g^{-1} C h^{-1}	42.31±9.57	22.15	58.46	23
β-Glucosidase	μmol pNP g^{-1} h^{-1}	1.71±1.07	0.39	3.83	62	Total carbohydrates-C/C	g C-glucose 100 g^{-1} C	5.90±1.08	4.68	8.57	18
Basal respiration	mg C-CO_2 kg^{-1}	0.41±0.18	0.15	0.67	43						

†pNP: p-nitrophenol

Table 4. Results of one-factor ANOVA for organic carbon and carbohydrate parameters in the ten sampled soils (identified as in Table 1), ranked in order of descending total organic C content

	Organic C		Carbohydrates		Ratio
	Total	Extractable	Total	Extractable	Extractable Carbohydrates/ /Extractable Organic C
units	g C kg^{-1}	mg C kg^{-1}	g glucose kg^{-1}	g glucose kg^{-1}	g glucose-C 100 g^{-1} C
Soil	Mean values				
PN	107.3a	1423a	23.00a	1.50a	42.2b
OR	78.1b	424cd	11.64b	0.48d	45.8a
MN	72.3c	871b	11.87b	0.69b	31.9c
CR	62.0d	518c	8.60c	0.57c	44.0ab
VC	41.5e	520c	5.19d	0.25e	19.3f
LT	30.7f	283e	4.13e	0.22f	30.8c
IG	23.1g	433cd	3.33f	0.27e	25.0d
SG	18.2h	361d	2.51g	0.16g	17.3g
BL	12.1i	250ef	1.81h	0.17g	26.5d
LG	8.5j	189f	0.99i	0.11h	22.6e
F value	3835***	212***	1688***	2685***	247***

***Significant at P <0.001. Means within a column followed by the same letter are not significantly different at P=0.05 SNK.

Table 5. Results of one-factor ANOVA for enzyme activities and microbial properties in the ten sampled soils (identified as in Table 1), ranked in order of descending total organic C content.

		Enzyme activities				Microbial properties		
		β-glucosidase	β-galactosidase	Urease	BAA-protease	Basal respiration	Microbial biomass C	qCO_2
	Units*	µmol pNP† g^{-1} h^{-1}	µmol pNP g^{-1} h^{-1}	µmol NH_3 g^{-1} h^{-1}	µmol NH_3 g^{-1} h^{-1}	mg C-CO_2 kg^{-1} h^{-1}	mg C kg^{-1}	µg C-CO_2 mg^{-1} C-biomass h^{-1}
Mean values	Soil							
	PN	3.83a	0.56c	3.72c	5.64a	0.61b	2170a	0.28d
	OR	1.73e	0.16f	1.63f	0.82e	0.31d	592de	0.52cbd
	MN	2.84b	0.98a	6.21a	5.52a	0.56b	1344b	0.42cd
	CR	2.37c	0.89b	5.20b	5.46a	0.67a	946c	0.71bc
	VC	1.89d	0.24e	2.18d	2.25c	0.33d	802cd	0.41cd
	LT	1.34f	0.33d	1.96e	3.52b	0.25e	439e	0.56cbd
	IG	1.15g	0.16f	2.07de	1.64d	0.59b	741cd	0.81b
	SG	1.06g	0.12f	0.80h	0.90e	0.40c	343e	1.20a
	BL	0.53h	0.05g	1.16g	0.83e	0.28de	415e	0.70bc
	LG	0.39i	0.03g	0.71h	0.45f	0.15f	338e	0.50cd
F value		717***	473***	888***	601***	136***	78***	14***

†pNP: p-nitrophenol ***Significant at P <0.001. Means within a column followed by the same letter are not significantly different at P=0.05 SNK.

Table 6- Results of one-factor ANOVA of extractable organic C, carbohydrates, enzyme activities and microbial properties expressed per unit of organic C in the ten sampled soils (identified as in Table 1), ranked in descending total organic C content.

			Carbohydrates		Specific enzyme activities				Microbial properties	
		Extractable organic C/C	Extractable carbohydrates/C	Total carbohydrates/C	β-glucosidase	β-galactosidase	Urease	BAA-protease	BR/C	MBC/C
Units		g C 100 g^{-1} C	g C-glucose 100 g^{-1} C	g C-glucose 100 g^{-1} C	µmol pNP† g^{-1} C h^{-1}	µmol pNP† g^{-1} C h^{-1}	µmol NH_3 g^{-1} C h^{-1}	µmol NH_3 g^{-1} C h^{-1}	mg C 100 mg^{-1} C	mg C-CO_2 100 g^{-1}C h^{-1}
Mean values	Soil									
	PN	1.33c	0.56a	8.57a	35.64e	5.20e	34.64d	52.56d	0.57ef	2.02bc
	OR	0.54e	0.25g	5.96c	22.15f	2.11g	20.91e	10.44e	0.39f	0.76c
	MN	1.21c	0.38d	6.57b	39.30d	13.58a	85.84a	76.37c	0.77de	1.86bc
	CR	0.84d	0.37de	5.54cd	38.31d	14.39a	83.91a	88.16b	1.08d	1.53c
	VC	1.25c	0.24g	5.00ef	45.43c	5.83de	52.51c	54.20d	0.79de	1.93bc
	LT	0.92d	0.28f	5.37de	43.55c	10.85b	63.92b	114.58a	0.81de	1.43c
	IG	1.87b	0.47c	5.76cd	49.94b	6.99c	89.36a	70.92c	2.55a	3.21ab
	SG	1.98ab	0.34e	5.52cd	58.46a	6.43cd	43.92c	49.75d	2.20b	1.89bc
	BL	2.07ab	0.55a	5.99c	43.98c	4.28f	95.97a	68.65c	2.32ab	3.42ab
	LG	2.22a	0.50b	4.67f	46.41c	3.71f	83.40a	53.47d	1.82c	3.99a
F values		71***	131***	63***	144***	221***	74***	62***	103***	8***

†pNP: p-nitrophenol ***Significant at P <0.001. Means within a column followed by the same letter are not significantly different at P=0.05 SNK

Table 7. Factor loadings matrix after varimax rotation.

Absolute parameters			Relative parameters		
Variable	Factor 1	Factor 2	Variable	Factor 1	Factor 2
Total carbohydrates	0.96	0.29	Microbial biomass C/C	0.95	-0.06
Extractable carbohydrates	0.92	0.33	Extractable organic C/ C	0.95	-0.15
Organic C	0.90	0.38	Basal respiration/C	0.87	0.01
β-Glucosidase	0.81	0.56	Extractable carbohydrates-C/C	0.79	-0.01
BAA-Protease	0.47	0.85	Specific β-Galactosidase	-0.25	0.92
Urease	0.33	.093	Specific BAA-Protease	0.13	0.95
β-Galactosidase	0.30	0.95			
Explained variance	52%	45%	Explained variance	54%	30%

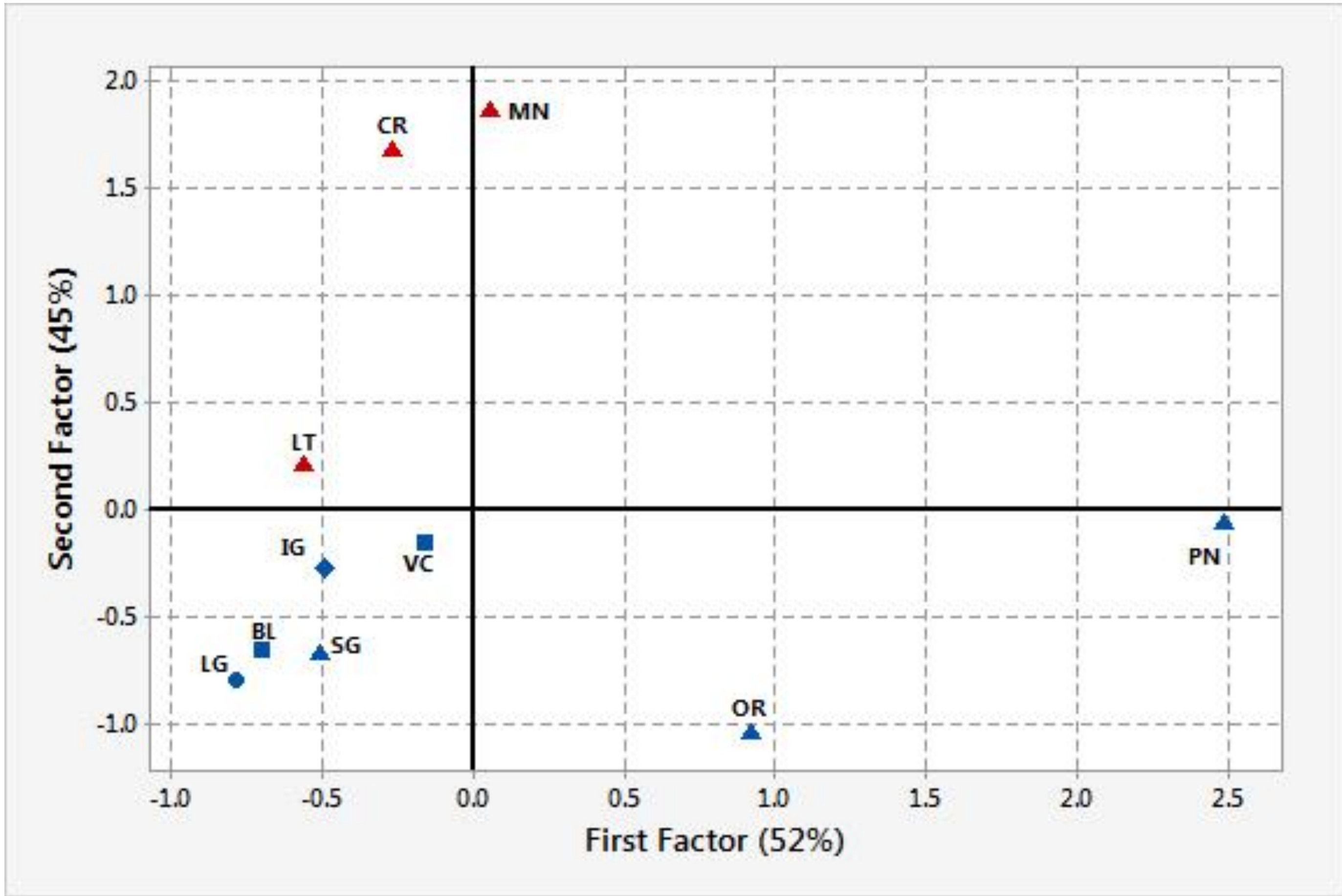

Figure 1- Score plot for the first two factors (from absolute parameters) for the ten sampled soils (identified as in Table 1).
Legend: blue symbol (calcareous soil), red symbol (non-calcareous soil), triangle (forest), square (dry grassland), rhombus (abandoned field) and circle (steppe).

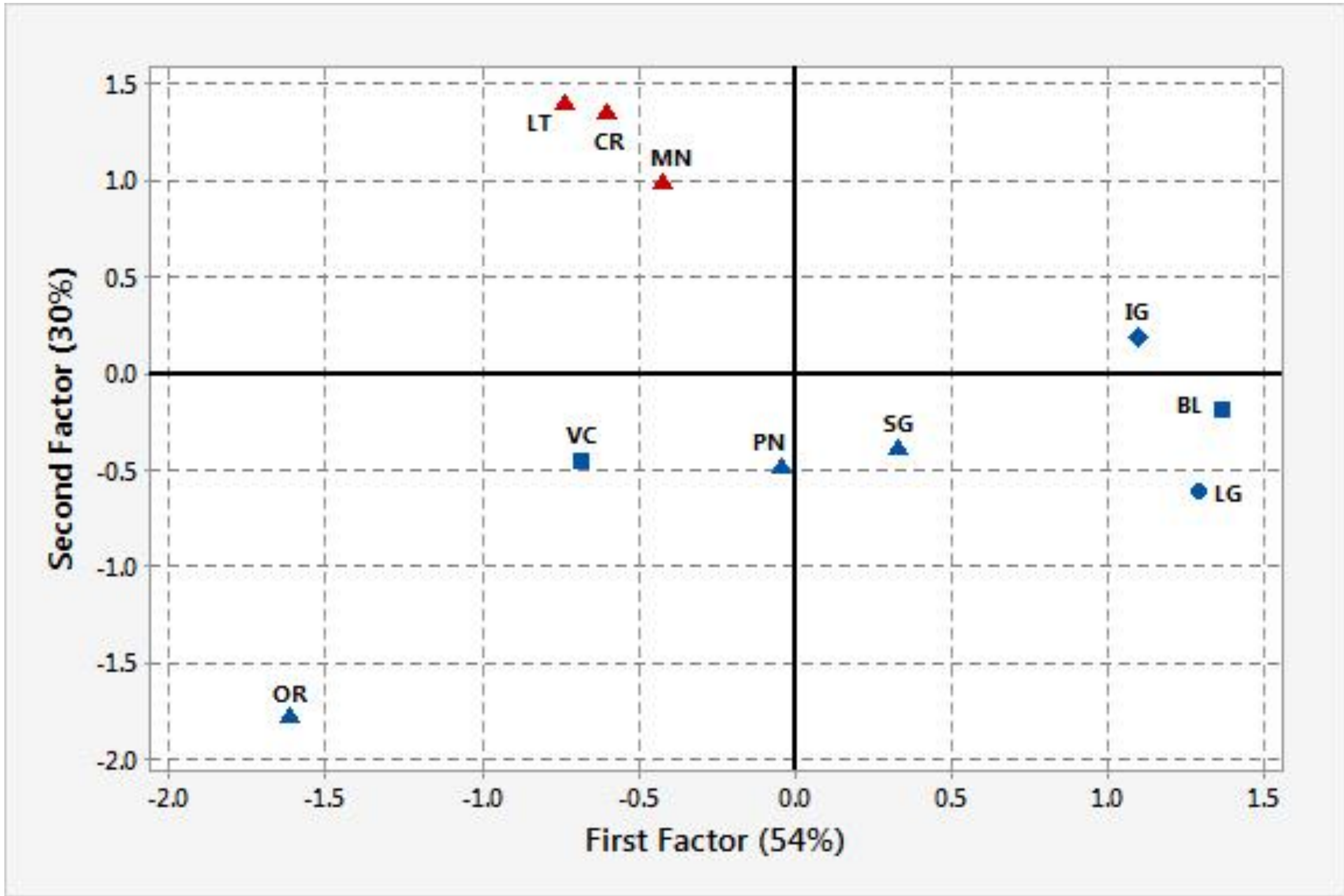

Figure 2. Score plot for the first two factors (from relative parameters) for the ten sampled soils (identified as in Table 1).

Legend: blue symbol (calcareous soil), red symbol (non-calcareous soil), triangle (forest), square (dry grassland), rhombus (abandoned field) and circle (steppe).