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ORIGINAL PAPER

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Dehydrogenase activity in Mediterranean forest soils

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Abstract The dehydrogenase activity (DHA) in the upper 10 cm of forest soils was measured in three experimental plots (1 ha) in Los Alcornocales Natural Park (southern Spain). In each plot, a sylviculture treatment of thinning and shrub-clearing had been previously carried out in one half, while the other half was left as a forest control. Soil samples were taken during the dry season (July 2000) and after the first autumn rains (October 2000). The DHA of forest soil in autumn [527±165 nmol *p*-iodonitrotetrazolium formazan (INTF) g^{-1} h^{-1}] was almost double that in summer (289±95 nmol INTF g^{-1} h^{-1}), for one of the studied plots. During the dry season, DHA of forest control soils (324±85 nmol INTF g^{-1} h^{-1}) was higher than in the thinned and shrub-cleared forest (253±93 nmol INTF g^{-1} h^{-1}). During the autumn (wet season), however, the effects of the sylvicultural practices on the soil dehydrogenase were negligible. Significant differences in DHA were found between the three sites. Multiple regression analysis identified pH as the best predictor of DHA of these soils. Other soil properties (pH, K, Ca, Mg, and soil moisture) also showed significant correlations with DHA. In addition, clay content appeared to enhance the enzyme activity. Our results suggest that thinning and shrub-clearing in Mediterranean forests seem to affect negatively the soil DHA, and their impact is more marked during the dry season. However, season and site effects are better determinants of DHA than management practices.

Keywords Soil enzyme activity · Dehydrogenase activity · Shrub-clearing practices · Mediterranean forest · *Quercus suber*

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Introduction

Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances, and show a quick response to the induced changes (Dick 1997). Therefore, enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices. Enzyme activities have been found to be very responsive to different agricultural soil conservation practices such as non-tillage (Dick 1992; Bergstrom et al. 1998), organic amendments (Dick 1992; Perucci 1992; Miller and Dick 1995; Banerjee et al. 1997), crop rotation (Dick 1992; Miller and Dick 1995), and organic cultivation (Beyer et al. 1992). Likewise, Garcia et al. (1998) found that restoration practices of degraded arid soils in marginal areas strongly influenced soil enzyme activities. On the other hand, little information exists about enzyme activity in Mediterranean forest soils, with most studies being related to nutrient cycling and soil fertility (Schneider 1998).

To explore the potentiality of biochemical properties in the assessment of soil quality and sustainability, it is desirable to have a database of these properties in native soils with late-successional vegetation from different ecosystems around the world. Native soils supporting mature vegetation are used as the high-quality reference soils, since a balance exists between their physical, chemical, biological and biochemical properties that ensures the ecosystem sustainability (Dick 1997; Pankhurst et al. 1997). Trasar-Cepeda et al. (1999) and Leirós et al. (2000) thoroughly evaluated several biological and biochemical properties of an Atlantic forest ecosystem of *Quercus robur* in north-west Spain. However, basic information about biochemical properties of Mediterranean forest soils is still needed. Moreover, the effects of forest management on soil biology and biochemistry in Mediterranean ecosystems are not clearly understood.

The activity of dehydrogenase is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity (Skujins 1973), because, being exclusively intracellular, it is linked to viable cells. How-

Table 1 Mean values (±SD) of selected properties of soils sampled in autumn. *DHA* Dehydrogenase activity, *INTF p*-iodonitrotetrazolium formazan

	Panera	Buenas Noches	Tiradero
pH	6.22±0.2	5.15±0.2	5.59±0.33
Total soil C (%)	4.03±1.87	5.08±2.16	3.42±1.6
Total soil N (%)	0.36±0.13	0.36±0.13	0.37±0.09
Soil C:N ratio	11.32±3.83	14.06±3.4	9.22±3.38
Available P (mg kg^{-1})	4.33±3.22	6.00±3.64	4.52±1.32
Available K (mg kg^{-1})	178.4±65.9	140.1±55.9	131.6±39.1
Available Ca (mg kg^{-1})	2401.5±959.9	1787.5±593.0	1407.7±481.6
Available Mg (mg kg^{-1})	309.6±62.5	258.3±87.3	208.5±52.1
Clay content (%)[a]	39.1±12.3	12.2±5.6	23.1±12.5
DHA (nmol INTF g^{-1} h^{-1})	526.8±164.7	333.1±104.9	320.0±92.3

[a] n=24 except for clay (n=6)

ever, the relationship between an individual biochemical property and the total microbial activity is not always obvious, especially in the case of complex systems like soils, where the microorganisms and processes involved in the degradation of the organic compounds are highly diverse (Nannipieri et al. 1990). Nevertheless, dehydrogenase activity (DHA) has been used as an indicator of the microbiological activity in Mediterranean arid soils (Garcia et al. 1994), and in agricultural soils of more humid regions of north-west Germany (Beyer et al. 1992).

Mediterranean forests, in particular *Quercus suber* L. (cork oak) forests, are usually subjected to shrub-clearing practices (Montoya 1988). This sylvicultural practice consists of the removal by slashing of the woody understorey vegetation with two main objectives: (1) to improve cork productivity by decreasing the competition by shrubs for soil nutrients, and (2) to reduce the risk of summer fires by avoiding the accumulation of fuel biomass. However, these sylvicultural practices may have detrimental side-effects on the forest ecology, by simplifying the ecosystem structure, decreasing the biodiversity, favouring opportunistic plant species, and limiting natural regeneration. Removal of the shrubs' aboveground biomass may also affect the forest soil properties, especially the biological and biochemical ones, since shrub-clearing modifies the microclimatic conditions at ground level, and the amount and quality of the potential organic inputs to soil.

The main objectives in this study were: (1) to measure DHA in Mediterranean forests soils, during conditions of two contrasting seasons (dry summer and wet autumn); (2) to study the relations between DHA and various soil properties; and (3) to evaluate the potential impact of the shrub-clearing management practice on DHA in forest soils.

Materials and methods

Study area

The study was carried out in Los Alcornocales Natural Park (provinces of Cádiz and Málaga, in southern Spain), where three experimental forest plots were established: Panera (36°31′54″N, 5°34′29″W), Buenas Noches (36°22′56″N, 5°34′58″W), and Tiradero (36°9′46″N, 5°35′39″W). The dominant tree species are *Q. suber* (cork oak) and *Quercus canariensis* Willd. (semi-deciduous oak). These forests have a dense and diverse woody understorey, e.g. 22 different shrub species were found in a 0.1-ha plot of cork oak forest (Ojeda et al. 2000). Dominant arborescent shrubs are *Arbutus unedo* and *Phillyrea latifolia*; heaths (*Erica* spp.) and leguminous shrubs (*Genista* spp.) are also abundant.

The soils are developed over Oligo-Miocene sandstone of the El Aljibe geological unit. Consequently, most of them show a sandy texture with good drainage, although soils with a considerable amount of clay can also be found scattered in the studied area. In general, soil samples had low pH values and high organic matter content (Table 1).

The climate is defined as mild Mediterranean type with warm, dry summers, and humid, cool winters. The influence of both the Atlantic Ocean and Mediterranean Sea acts as a temperature buffer, preserving the ecosystem from extreme temperature values and providing some humidity during the summer drought.

Soil sampling and soil analyses

Each forest plot (1 ha) was divided into two parts: one was subjected to the shrub-clearing treatment (in March 2000) whereas the other one was left untreated (control). Soil sampling was carried out in two different seasons: firstly during the dry season (end of July 2000; only in one plot), and secondly in autumn, just after the first rains (October 2000; in all three plots). Soil samples were taken from the upper 10-cm layer (once the litter had been removed), following a regular pattern. Each soil sample was composed by pooling three subsamples (3 cm in diameter×10 cm in depth) collected in a 1×1-m area. Soil samples were collected along each transect (established for vegetation studies, T. Marañón et al., unpublished study) at regular intervals of 6 m. A total of 24 samples (12 in treated and 12 in control zones) were collected from each forest plot. Samples were placed in plastic bags and kept in ice-boxes. Once in the laboratory, soil samples were sieved (<2 mm) and then stored at 4°C until the enzyme analysis was performed.

DHA was measured following the procedure described by Camiña et al. (1998) developed for acid forest soils rich in organic matter. Briefly, DHA was determined using 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) as the artificial electron acceptor (Benefield et al. 1977), which is reduced to the red-coloured *p*-iodonitrotetrazolium formazan (INTF). Then, the formazan was extracted with 1:1, ethanol:*N*,*N*-dimethylformamide and determined colorimetrically (at 490 nm). Each value of DHA activity is the mean of two replicates, and is expressed as nmol INTF g^{-1} dry equivalent soil h^{-1}. Data are expressed on an oven-dry soil basis (105°C).

Total C, total N, pH, available Ca^{2+}, Mg^{2+}, K^{+}, and P were determined in all (n=72) soil samples [see methods in Allen (1989)]. Soil water content was measured gravimetrically in summer samples, and by means of a Hydrosense sensor (Campbell) during autumn sampling. Soil water potential was determined (only in summer samples) as reported by Deka et al. (1995). Texture was measured (Gee and Bauder 1986) only in a limited number of samples (n=18).

Statistical analysis

Student's *t*-test was used to compare the treated half plot and the corresponding control plot to explore the possible impact of the shrub-clearing practice on soil DHA. However, in the Buenas Noches plot, where DHA values did not fit a normal distribution, a Mann-Whitney *U*-test was used instead. To compare soil DHA between the three sites, a two-way ANOVA was performed (DHA data was previously log-transformed). After a significant ANOVA, the post-hoc multiple comparisons were made with a Scheffé test. Relationships between soil variables and DHA were studied by means of correlation tests (Pearson's correlation coefficient). A multivariate linear regression model (forward stepwise) was fitted to test which soil properties could be used as predictors of DHA (data of some variables were log-transformed to meet the multiple linear regression assumptions). Statistical analyses were performed with Statistica 5.1 for Windows (Statsoft 1997).

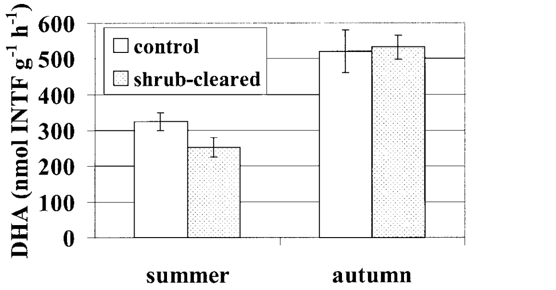

Fig. 1 Dehydrogenase activity (*DHA*) in forest soil (Panera site) measured in summer and autumn. Control and shrub-cleared zones are compared (n=12). *Vertical bars* represent ±SE. INTF *p*-Iodonitrotetrazolium formazan

Results and discussion

In general, the mean DHA measured (in autumn) in the forest soils of Los Alcornocales Natural Park was 393±155 nmol INTF g^{-1} h^{-1} in the upper 10-cm soil layer (range 192.6–976.4 nmol INTF g^{-1} h^{-1}). The literature contains only a few reports (Camiña et al. 1998; Leirós et al. 2000) of DHA in forest soils measured by using INT as electron acceptor. The values of DHA obtained are almost double those reported by Leirós et al. (2000) in an Atlantic forest of *Q. robur*, exposed to a temperate climate in north-west Spain, in soils containing a higher amount of soil organic matter than those of the Los Alcornocales forest. A reasonable hypothesis could be that the lower soil pH values, together with a higher C:N ratio, in northern forests leads to a slow decomposition rate and hence less DHA than in the southern soils studied.

Seasonal effects

DHA measured in autumn samples (527±165 nmol INTF g^{-1} h^{-1}) was almost double that measured in summer samples (289±95 nmol INTF g^{-1} h^{-1}) at the same location (Panera site; Fig. 1); accordingly, significant differences were detected between the DHA values in the two seasons (t=–6.52, P=0.000001, n=24).

The increase in soil water content in autumn would favour the increase in microbiological activity (and hence in DHA), especially considering the low water potential values measured in summer (–12.6 MPa and –10.1 MPa in the control and shrub-cleared zones, respectively). Garcia et al. (1994) also found that the rainy season enhanced the enzyme activity (DHA) of soils in the south-east arid region of Spain. Other authors also attributed the increase in microbial activity in forest (Görres et al. 1998) and in grassland soils (Banerjee et al. 2000) to higher soil moisture contents.

The higher inputs of organic residues during autumn (leaf-shedding season) in this forest ecosystem could also contribute to the observed seasonal increase in DHA. The litterfall inputs favour the overall soil oxidative activity; as the litterfall undergoes decomposition, smaller and simpler organic molecules are leached down from the litter layer to the surface soil horizon, as water-soluble organic matter, thus providing a labile organic substrate for soil microorganisms (McBrayer and Cromack 1980; Görres et al. 1998).

Forestry management effects

In summer, the DHA activity was higher in the control zone (324±85.1 nmol INTF g^{-1} h^{-1}) than in the managed half-plot (253±93.2 nmol INTF g^{-1} h^{-1}), although this difference was only marginally significant (t=1.955, P=0.06). Spatial variability in soil properties is high in the studied experimental plots (T. Marañón et al., unpublished data) and this soil heterogeneity could mask the possible management effects on soil microbiological activity. On the other hand, when we analysed the effect of the shrub-clearing management practice on DHA, considering the sampling transect as a nested factor, we did find a significant management effect (F=9.2, P=0.008, df=1, 16). These results showed that shrub-clearing negatively affected the soil DHA during the dry season.

However, no significant effect of the management practice on soil dehydrogenase was found in autumn in any of the three sites studied. Only in one site (Tiradero) was a marginally significant trend (P=0.066) observed, with higher DHA values in the control zone than in the shrub-cleared zone (Fig. 2), as found in summer for dry soils. A possible interpretation of the different quantitative effects of the forest management practices on the DHA activity is that the negative effect of shrub-clearing on soil microbiological activity is produced under drought conditions, but is almost negligible when soils are moist. The microclimatic changes induced by the practice of shrub-clearing, such as higher radiation and a more dramatic oscillation in soil water content and soil temperature, would be more influential on soil processes during extreme conditions of drought. Besides, it may be also possible that in the shrub-cleared zones the practice of shrub-clearing provokes a shift in the composition of

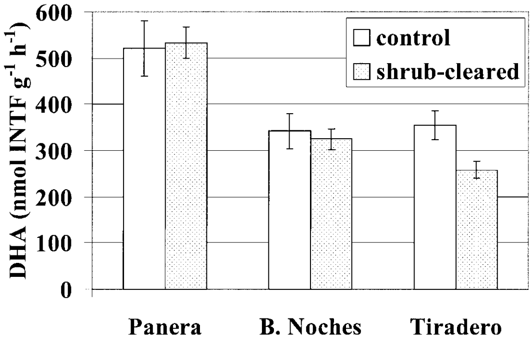

Fig. 2 DHA in forest soil measured in the three selected plots during autumn. Control and shrub-cleared zones are compared (n=12). *Vertical bars* represent ±SE. *B Noches* Buenas Noches; for other abbreviations, see Fig. 1

soil microorganisms with populations more sensitive to drought. The non-significant effects of shrub-clearing on soil DHA measured in autumn could also be due to the recovery of enzyme activity to background levels. The extent of soil recovery will depend on the degree of the disturbance caused by the management practice, the soil's resilience, and the time elapsed from the last time the practice was applied. Perucci (1992) found that the effect of compost addition on soil DHA was only shown 1 month after the compost was applied, and thereafter, no effect of this practice was observed. In this case, to assess better the impact of shrub-clearing on soil DHA, more frequent monitoring is needed.

Site effects

Significant differences were found between the soil DHA measured in the three forest plots in autumn, (Table 2). The Panera site showed the highest DHA values (Fig. 2).

The three experimental plots, located in the same forested area, were dominated by cork oak trees; but showed differences in plant species composition and soil properties (Table 1), and these differences could be responsible for the variations in DHA. Firstly, the species composition of the forest canopy plays an important role in determining the type of soil organic matter and its associated decomposition processes. Thus, the semi-deciduous oak *Q. canariensis* has a higher percentage cover than *Q. suber* in the Panera plot (the ratio of *Q. canariensis* to *Q. suber* is 1.29) whereas the opposite occurs in the Tiradero site (the ratio of *Q. canariensis* to *Q. suber* is 0.53), and in the Buenas Noches site *Q. canariensis* is totally absent (M. Diaz-Villa, personal communication). This varying proportion of semi-deciduous versus evergreen oaks in the forest canopy will be reflected in soil biological and biochemical properties. The leaf-shedding period of the semi-deciduous oak is mainly in autumn, while the evergreen cork oak sheds leaves throughout the year. Moreover, the decomposition rate of semi-deciduous oak litter is higher than that of

Table 2 Two-way ANOVA for DHA of forest soils, measured in autumn. F-values for main effects (management and site) and two-way interactions are presented

	df	*F*	*P*
Main effects			
Management	1	0.51	0.47 n.s.
Site	2	21.19	1.00×10^{-7}***
Two-way interactions			
Management×site	2	1.27	0.28 n.s.

***P<0.001, *n.s.* non-significant

cork oak, as shown by Gallardo and Merino (1993) in a litter bag experiment near the Panera site, because of the lower cutin content and the lower toughness of the semi-deciduous leaves. Indeed, in this particular Mediterranean ecosystem, the rate of litter decomposition is more affected by the leaf cutin content and leaf toughness than by other chemical constituents or physical features (Gallardo and Merino 1993).

Secondly, differences in soil DHA between sites could also be due to the different soil texture (Table 1). The Panera site showed both the highest clay content and highest DHA. Soil texture has been reported as a key determinant of microbial ecology (Stotzky 1986). Ladd et al. (1996) found a positive relationship between clay content and the soil microbial biomass content, and Beyer et al. (1992) found a positive correlation between clay content and enzyme activity (DHA). Soil texture affects other soil properties, such as water availability, nutrient supply (especially cations) and, to some extent, pH values, which in turn determine microbial growth and activity (Stotzky 1986; Ladd et al. 1996; Görres et al. 1998; Leirós et al. 2000; Zeller et al. 2001). In general, fine-textured soils have more micropores than sandy soils. Soil micropores protect mineralising microorganisms against grazers (Killham 1994) and this can be one of the reasons for the higher microbial biomass and enzyme activity of fine-textured soils.

Relationships with soil variables

Soil DHA was positively and significantly correlated with soil pH, Ca, Mg, K and water content (Table 3). On the other hand, no correlation was found between dehydrogenase enzyme activity and total soil C and N. These results agree with those reported by Beyer et al. (1992). However, Leirós et al. (2000) reported a clear positive relationship between soil DHA and soil C. Probably in these forest ecosystems soil microorganisms are nutrient rather than C limited, since DHA did not respond to the variation of C contents or to the C:N ratio. The observed increase in DHA from dry (summer) to wet (autumn) season, shows a positive relationship between DHA and water content. The positive effect of increasing water content and nutrient addition on soil DHA has been already reported (Nannipieri et al. 1990).

Table 3 Pearson correlation coefficients between some selected soil properties and DHA

	DHA	pH	K	N
pH	0.55**	1.00	0.35**	0.04
Water content	0.35**	0.58**	0.14	–0.18
Total C	–0.12	–0.26*	0.33**	0.68**
Total N	–0.04	0.04	0.53**	1.00
C/N ratio	–0.08	–0.36**	–0.03	0.02
Available Ca	0.32**	0.41**	0.58**	0.46**
Available Mg	0.32**	0.40**	0.65**	0.55**
Available K	0.32**	0.35**	1.00	0.53**
Available P	–0.06	–0.17	0.05	0.39**

*P<0.05, **P<0.01, n=72

Table 4 Predictors of DHA in autumn based on multiple regression analysis

Multiple R	R^2 Adjusted	F	P (F)
Goodness-of-fit parameters			
0.587	0.316	11.957	2.00×10^{-6}***

Selected variables	t	P (t)	β Coefficient
Significance of regression components			
pH	4.31	0.00005***	0.461
Available K	2.09	0.04*	0.264
Total N	–1.73	0.08 n.s.	–0.204

*P<0.05, ***P<0.001

Multiple linear regression parameters for dehydrogenase are shown in Table 4. Several soil properties (pH, water content, C, N, C/N, Ca, Mg, K and P) measured in autumn samples (n=72) were considered to fit the multivariate linear regression model. The forward stepwise regression analysis included in the model the following variables: pH, K, and N. However, only pH and K variables were significant regression components, while N (although included in the regression model) made no significant contribution to R^2 (Table 4). According to these results, soil pH is the best predictor of DHA, and accounts for the highest proportion of the overall variance explained.

In conclusion, the DHA in forest soils of Los Alcornocales Natural Park (S. Spain) is high, compared to the few reported data. In general, DHA was higher in autumn (with a higher soil moisture content) than during the dry summer. The shrub-clearing management practice induced a decrease in the soil DHA during summer (dry season), but this effect was not apparent during the autumn (wet season). However, site factors (forest canopy species composition, soil texture, pH, and available nutrients) and seasonal sampling date were greater determinants of the variation in DHA than management factors. Nutrient supply and soil pH were better predictors of DHA than the amount and quality (based on its C:N ratio) of the soil organic matter.

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References

Allen SE (1989) Chemical analysis of ecological materials. Blackwell, Oxford

Banerjee MR, Burton DL, Depoe S (1997) Impact of sewage sludge application on soil biological characteristics. Agric Ecosyst Environ 66:241–249

Banerjee MR, Burton DL, McCaughery WPP, Grant CA (2000) Influence of pasture management on soil biological quality. J Range Manage 53:127–133

Benefield CB, Howard PJA, Howard DM (1977) The estimation of dehydrogenase activity in soil. Soil Biol Biochem 9:67–70

Bergstrom DW, Monreal CM, King DJ (1998) Sensitivity of soil enzyme activity to conservation practices. Soil Sci Soc Am J 62:1286–1295

Beyer L, Wachendorf C, Balzer FM, Balzer-Graf UR (1992) The effect of soil texture and soil management on microbial biomass and soil enzyme activities in arable soils of Northwest Germany. Agrobiol Res 45:276–283

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

Deka RN, Wairiu M, Mtakwa PW, Mullins CE, Veenendaal EM, Townsend J (1995) Use and accuracy of the filter-paper technique for measurement of soil matric potential. Eur J Soil Sci 46:233–238

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

Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Biological indicators of soil health. CAB International, New York, pp 121–156

Gallardo A, Merino J (1993) Leaf decomposition in two Mediterranean ecosystems of southwest Spain: influence of substrate quality. Ecology 74:152–161

Garcia C, Hernandez T, Costa F (1994) Microbial activity in soils under Mediterranean environmental conditions. Soil Biol Biochem 26:1185–1191

Garcia C, Hernandez T, Albaladejo J, Castillo V, Roldán A (1998) Revegetation in semiarid zones: influence of terracing and organic refuse on microbial activity. Soil Sci Soc Am J 62:670–676

Gee GW, Bauder JW (1986) Particle-size analysis. In: Klute A (ed) Methods of soil analysis. Part 1. Physical and mineralogical methods. American Society of Agronomy, Madison, Wis. pp 383–411

Görres JH, Dichiaro MJ, Lyons JB, Amador JA (1998) Spatial and temporal patterns of soil biological activity in a forest and an old field. Soil Biol Biochem 30:219–230

Killham K (1994) Soil ecology. Cambridge University Press, Cambridge

Ladd JN, Foster RC, Nannipieri P, Oades MJ (1996) Soil structure and biological activity. In: Bollag JM, Stotzky G (eds) Soil biochemistry, vol 9. Dekker, New York, pp 23–77

Leirós MC, Trasar-Cepeda C, Seoane S, Gil-Sotres F (2000) Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperate-humid zone (Galicia, NW Spain): general parameters. Soil Biol Biochem 32:733–745

McBrayer JF, Cromack K Jr (1980) Effect of snow-pack on oak-litter breakdown and nutrient release in a Minnesota forest. Pedobiologia 20:47–54
Miller M, Dick RP (1995) Thermal stability and activities of soil enzymes as influenced by crop rotations. Soil Biol Biochem 27:1161–1166
Montoya JM (1988) Los alcornocales. Ministry of Agriculture, Fisheries and Food, Madrid
Nannipieri P, Grego S, Ceccanti B (1990) Ecological significance of biological activity. In: Bollag J-M, Stotzky G (eds) Soil biochemistry, vol 6. Dekker, New York, pp 293–355
Ojeda F, Marañón T, Arroyo J (2000) Plant diversity patterns in the Aljibe Mountains (S. Spain): a comprehensive account. Biodiv Conserv 9:1323–1343
Pankhurst CE, Doube BM, Gupta VVSR (1997) Biological indicators of soil health: synthesis. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Biological indicators of soil health. CAB International, New York, pp 419–435
Perucci P (1992) Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biol Fertil Soils 14:54–60
Schneider K (1998) P availability in forest soils and its role for the nutrition of *Quercus pyrenaica* Willd. in the Sierra de Gata, western Spain. Thesis. Universitaet Hohenheim of Stuttgart, Stuttgart
Skujins J (1973) Dehydrogenase: an indicator of biological activities in arid soils. Bull Ecol Res Commun [Stockh] 17:235–241
StatSoft (1997) Statistica for Windows, version 5.1. Statsoft, Tulsa, Okla.
Stotzky G (1986) Influence of soil mineral colloids on metabolic processes, growth, adhesion and ecology of microbes and viruses. In: Huang PA, Schnitzer M (eds) Interactions of soil minerals with natural organics and microbes. Soil Science Society of America, Madison, Wis. pp 305–428
Trasar-Cepeda C, Leirós C, Gil-Sotres F (1999) Biochemical properties of acid soils under climax vegetation (Atlantic oak-wood) in an area of the European temperate-humid zone (Galicia, NW Spain): specific parameters. Soil Biol Biochem 32:747–755
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