



Effects of liming on soil properties and plant performance of temperate mountainous grasslands

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ABSTRACT

The application of lime or liming materials to acid-soil grasslands might help mitigate soil acidity, a major constraint to forage productivity in many temperate mountainous grasslands. Nowadays, in these mountainous grasslands, it is essential to promote agricultural practices to increase forage yield and nutritive value while preserving biodiversity and agroecosystem functioning. Two different field experiments were conducted in the Gorbeia Natural Park, northern Spain: (i) one in a calcareous mountainous grassland (Arraba) and (ii) the other in a siliceous mountainous grassland (Kurtzegan) to study the effects of a single application of two liming products, i.e. 2429 kg lime ($164.3\% \text{ CaCO}_3$) ha^{-1} and 4734 kg calcareous sand ($84.3\% \text{ CaCO}_3$) ha^{-1} , applied one month before the beginning of the sheep grazing season (May–October), on soil chemical (pH, organic C, total N, C/N ratio, %Al saturation, Olsen P, exchangeable K^+ and Ca^{2+}) and biological parameters (dehydrogenase, β -glucosidase, urease, acid phosphatase and arylsulphatase activity) as well as on botanical diversity (graminoids, forbs, shrubs) and forage yield and nutritive value (crude protein, modified acid detergent fibre, digestibility). Untreated control plots were also included in the experiment. Soil sampling was carried out at the end of the sheep grazing season (6 months after liming treatment), while botanical composition was determined one year after treatments application. Although no increase in soil pH was observed in Arraba, liming significantly increased dehydrogenase activity (an indicator of soil microbial activity) by 30.4 and 86.7% at Arraba and Kurtzegan site, respectively. Liming treatments significantly improved forage yield and nutritive value in Arraba but not in Kurtzegan. Furthermore, no differences in soil biological quality, evaluated using the “treated-soil quality index” as proposed in this work, were observed between treated and untreated soils, and between the two different lime treatments (lime, calcareous sand). It was concluded that, in acid-soil temperate mountainous grasslands, moderate liming treatments have no negative short-term effects either on soil quality or botanical composition, while resulting in improvements in forage yield and nutritive value under some conditions.

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1. Introduction

In the Basque Country (northern Spain), mountainous areas cover approximately 85% of the land surface, which provides an indication of the topographic and climatic constraints limiting the agricultural activity in this region. Likewise, mountainous grasslands, where grazing has been the most important economic activity since the Neolithic (Barandiaran and Manterola, 2000), cover approximately 20% of the Basque territory. In the last decades, the reduction in economic revenue is resulting in an abandonment of the agricultural activity in these Basque

mountainous grasslands. The progressive land abandonment, with concomitant livestock grazing reduction, in areas that have a long history of grazing is considered a disturbance, leading to loss of plant biodiversity and spread of shrubs (Montalvo et al., 1993). Grazing has been reported as a central, pivotal issue for mountainous grasslands, linking their conservation, productivity, economic use and management for biodiversity (Watkinson and Ormerod, 2001). With regard to botanical diversity, calcareous seminatural grasslands are among the most species-rich communities in temperate Europe (Kull and Zobel, 1991). In these mountainous grasslands, it is essential to promote agricultural practices that increase forage yield and nutritive value while preserving biodiversity and agroecosystem functioning and sustainability.

The high annual rainfall in these regions can increase the leaching of cations such as Ca^{2+} and Mg^{2+} , allowing the soil

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exchange complex to become dominated by H^+ and Al^{3+} , resulting in phytotoxicity. Numerous works can be found in the literature on the application of lime to acid-soil grasslands (McLean, 1971; Mombiola and Mateo, 1982). Calcium carbonate derived from different liming materials (lime, calcareous sand) provides OH^- ions to react with H^+ , thus reducing soil acidity problems (Ulrich, 1991). Besides, it favours the mineralization of accumulated organic matter and allows the release of sequestered P due to dissolution of aluminium and iron phosphates (Bardgett, 2005). Many agricultural practices aimed at increasing forage yield and nutritive value (tillage, intensive fertilization, introduction of more productive non-native forage species, etc.) are frequently restricted in protected areas, such as Natural Parks. Moreover, the complex orography of mountainous areas certainly complicates some intensive agricultural labours.

To our knowledge, there are not many reports on the effects of liming on soil biological parameters, such as enzyme activities, in temperate mountainous grasslands. As compared to soil physico-chemical properties, biological parameters are increasingly used as indicators of soil quality due to their sensitivity, rapid response to disturbances (which makes them tools of great potential to determine short-term effects) and capacity to provide information that integrates many environmental factors (Hernández-Allica et al., 2006; Mijangos et al., 2009).

The objectives of this work were (i) to study the short-term effects of the application of two different liming products (lime and calcareous sand) on soil chemical and biological parameters (enzyme activities) in a calcareous and a siliceous temperate mountainous grassland, (ii) to evaluate the sensitivity and response of soil biological indicators to soil disturbance and (iii) to determine the effects of liming on botanical composition and diversity (graminoids, forbs, shrubs) as well as on forage yield and nutritive value.

A new index of soil biological quality evaluation, called “treated-soil quality index” (T-SQI), is proposed for studies where the soil is intentionally treated to increase its biological activity, in an attempt to overcome the limitations of the “soil quality index” (SQI) (Bloem et al., 2006): the SQI needs an “ideal” soil for comparison purposes and, in our opinion, has limitations when applied to soils that have been intentionally subjected to treatment(s), for whatever reason, as it penalizes any type of variation (positive or negative) from the reference values shown by untreated soils in any of the studied parameters. In our study, control (no liming) soils were used as “reference” soils, based on the fact that in the mountainous grasslands studied here, grazing has been sustainably carried out since the Neolithic. Unfortunately, in the last decades, the reduction in economic revenue is threatening the maintenance of agricultural activity in these grasslands, leading to the current need to promote agricultural practices that increase forage yield and quality while preserving soil quality. In order to evaluate the effect of treatments, such as liming, on soil quality as reflected by the values of biological parameters, such as enzyme activities, a new index which does not penalize any type of variation from the reference values is needed. Here, we propose the T-SQI for those studies where the soil is intentionally treated to increase its biological activity (for example, to overcome a limiting factor such as soil acidity, nutrient deficiency, oxygen deficiency, water stress and so on; to increase the rate of degradation of an organic contaminant through bio-stimulation; etc.) while preserving the pattern shown by the “reference” soil among the values of those parameters used to determine biological activity (in the current work, for enzyme activities, the pattern of activity across the enzyme types characteristic of the “reference” soil, where grazing has been sustainably carried out since the Neolithic). For the T-SQI, the evenness of the “reference” soil is set to the maximum value, i.e. the mean value of

each biological parameter in the “reference” soil is considered as 100%. In our study, liming treatments were applied in an attempt to overcome the most limiting factor for forage production (soil acidity), but without having an effect on the original soil’s biological pattern, as reflected by the distribution of five soil enzyme activities involved in the major biogeochemical cycles (C, N, P, S), which were chosen as indicators of the soil biological activity/quality.

2. Materials and methods

2.1. Study area and experimental design

The study was conducted in the Gorbeia Natural Park (Basque Country, northern Spain), latitude $43^{\circ}07'N$ and longitude $2^{\circ}51'W$. The Gorbeia Natural Park is located in a mountain range where Gorbeia itself is the name given to the highest mountain (1482 m above sea level) found there. The climate is humid temperate, with an annual mean temperature of $10.1^{\circ}C$ and a mean precipitation of 2000 mm.

Two different field experiments were conducted: (i) one in a calcareous grassland (soil: pH = 4.6, clay loam, Typic Dystrudept; SSS, 2006) belonging to an area called “Arraba”, located at 1050 m above sea level, and (ii) the other in a siliceous grassland (soil: pH = 4.2, sandy loam, Aeris Humaquept; SSS, 2006) in an area called “Kurtzegan”, located at 950 m above sea level (note: here the terms “calcareous” and “siliceous” refer to the soil parent material). The most common plant species present in the seminatural grasslands of “Arraba” is *Festuca gr. rubra* L., in association with *Agrostis capillaris* L., *Trifolium repens* L., *Hieracium pilosella* L., and shrubs such as *Ulex gallii* Planchon and *Erica vagans* L. The most common plant species present in the seminatural grasslands of “Kurtzegan” is *Agrostis curtisii* L., in association with *A. capillaris* L., *F. gr. rubra* L., *Galium saxatile* L., and shrubs such as *E. vagans* L. and *Erica tetralix* L.

In both areas (Arraba and Kurtzegan), a randomized complete block design with three replicates was established, with each experimental plot measuring 10×10 m: a total of 9 experimental plots (3 treatments: lime, calcareous sand, control-no liming; 3 replicates per treatment). Both areas were exposed to natural grazing throughout the experiment.

Before treatment application, soil was sampled and its chemical (pH, extractable Ca^{2+} and K^+ , aluminium saturation, organic C, C/N, Olsen P) and biological (dehydrogenase, acid phosphatase, aryl-sulphatase, β -glucosidase and urease activities) properties determined to verify homogeneity among experimental plots (see “Initial soil” column in Tables 1 and 2). No significant differences were found between control plots and those to be treated.

One month before the beginning of the sheep grazing season (in this area, sheep grazing season extends from May through October), the following liming treatments were applied manually (broadcast spreading by hand on soil surface as it is usually done by shepherds and farmers in these mountainous areas): (i) 2429 kg lime ha^{-1} (92% CaO ; 164.3% $CaCO_3$; as 1.5–4 mm granules) and (ii) 4734 kg calcareous sand ha^{-1} [47.2% CaO ; 84.3% $CaCO_3$; as 58.96% coarse sand (0.5–2 mm), 25.68% fine sand (0.05–0.5 mm), 8.21% silt (0.002–0.005 mm) and 7.15% clay (<0.002 mm)]. Thus, the same amount of Ca^{2+} was applied under both treatments. Lime was chosen for its being the liming product most commonly used in northern Spain. Calcareous sand was studied as an alternative liming product cheaply obtainable in a nearby quarry (Nafarrondo S.A., Orozko, Biscay; this calcareous sand is derived from Early Cretaceous calcareous algae).

Soil sampling was carried out at the end of the sheep grazing season (10th October 2003). Fifteen soil sub-samples (10 cm depth, 3 cm diameter) were randomly collected from each plot and then

Table 1

Effect of liming treatments on soil chemical parameters. Values followed by different letters are significantly different among treatments within each grassland ($P < 0.05$) according to one-way ANOVA and Fisher's PLSD-test. Values followed by * indicate significant differences between grasslands within each treatment according to Student's t-test. Mean values ($n = 3$).

		Initial soil	Control	Lime	Sand
pH	Arraba	4.7	4.6*	4.7	4.6
	Kurtzegan	4.2	4.2a*	4.5b	4.5b
Ca ²⁺	Arraba	961	776*	905	952
	Kurtzegan	380	418a*	1067b	865b
Al saturation	Arraba	44	53*	47	48
	Kurtzegan	66	70a*	40b	49b
OC	Arraba	6.4	5.2*	5.8*	6.4*
	Kurtzegan	17.4	18.6*	16.9*	18.0*
C/N	Arraba	12	12*	12*	12*
	Kurtzegan	20	20*	18*	18*
Total N	Arraba	0.50	0.46*	0.49*	0.50*
	Kurtzegan	0.89	0.94*	0.93*	0.97*
Olsen P	Arraba	9.7	7.0	10.0	11.7
	Kurtzegan	8.4	8.7	6.7	6.7
K ⁺	Arraba	115	97	107	118
	Kurtzegan	101	108	90	95

Units: Ca²⁺, Olsen P and K⁺ in mg kg⁻¹; Al saturation, OC and total N in %.

thoroughly mixed to give a composite sample. All laboratory analyses (chemical and biological) were carried out in duplicate.

2.2. Analysis of soil samples

For soil chemical analysis, soils were air-dried at 30 °C for 48 h, sieved to <2 mm and stored at room temperature. Soil pH was measured in H₂O using a 1:2.5 soil:solution ratio and extractable acidity was determined by displacing exchangeable cations with a 0.6 N BaCl₂ extract and titrating the displaced solution with 0.01 N NaOH until pH 4.0. Soil organic C (OC) was determined following the Walkley–Black method (Hesse, 1971) and total N was determined following the Kjeldahl method (AOAC, 1980). Olsen P was determined according to Olsen et al. (1954). Exchangeable cations (Ca²⁺, K⁺) were determined after displacement with 1 N NH₄OAc at pH 7. Calcium was determined by Varian SpectraAA-220 FS atomic absorption (Varian Ibérica S.L., Barcelona, Spain) and K⁺ by flame emission using the same equipment.

Table 2

Effect of liming treatments on soil enzyme activities. Values followed by different letters are significantly different among treatments within each grassland ($P < 0.05$) according to one-way ANOVA and Fisher's PLSD-test. Values followed by * indicate significant differences between grasslands within each treatment according to Student's t-test. Mean values ($n = 3$).

		Initial soil	Control	Lime	Sand
Dehydrogenase	Arraba	5.9	5.6a*	7.1ab*	7.3b*
	Kurtzegan	1.6	1.5a*	2.8b*	2.2ab*
Acid phosphatase	Arraba	1501	1798	1954	1634*
	Kurtzegan	2093	1504	2333	2224*
Arylsulphatase	Arraba	919	765	875*	922*
	Kurtzegan	522	528	659*	681*
β-Glucosidase	Arraba	217	239*	269*	250*
	Kurtzegan	158	150*	165*	162*
Urease	Arraba	106	89	98*	103*
	Kurtzegan	89	118	176*	188*

Dehydrogenase: $\mu\text{g INTF g}^{-1}$ dry soil h⁻¹; Acid phosphatase: $\mu\text{g 4-NP g}^{-1}$ dry soil h⁻¹; Arylsulphatase: $\mu\text{g 4-NP g}^{-1}$ dry soil h⁻¹; β-Glucosidase: $\mu\text{g 4-NP g}^{-1}$ dry soil h⁻¹; Urease: $\mu\text{g N-NH}_4 \text{ g}^{-1}$ dry soil h⁻¹.

For the analysis of β-glucosidase, acid phosphatase, arylsulphatase and urease activity, soils were air-dried at 30 °C for 48 h, sieved to <2 mm and stored for <2 months at 4 °C. For dehydrogenase activity, soils were sieved to <2 mm and stored fresh for <2 months at 4 °C. β-glucosidase, acid phosphatase and arylsulphatase activity were determined according to a modification of Tabatabai (1994), as previously described (Rodríguez-Loinaz et al., 2008). Urease and dehydrogenase activity were determined following a modification of Kandeler and Gerber (1988) and Von Mersi and Schiner (1991), respectively, as previously described (Rodríguez-Loinaz et al., 2008).

For the determination of dehydrogenase activity, 1 g of soil (wet weight) was mixed with 0.4 ml of 100 mM Tris (hydroxymethyl) aminomethane buffer – THAM, pH 7.0, and 0.4 ml of iodonitrotriazolium chloride – INT (0.5% w/v). The mixture was then incubated at 25 °C for 4 h and the reaction stopped with 8 ml of methanol. After centrifugation (1250 × g, 5 min), the absorbance value of the samples was read at 490 nm. Regarding dehydrogenase activity, a laboratory experiment was performed to evaluate the effect of Ca²⁺ on the determination of dehydrogenase activity, finding out that this activity significantly increased as a result of the addition of both 1 and 0.5% CaCO₃. However, the addition of 0.2% CaCO₃ (=0.08% Ca²⁺) or lower (in our experimental plots, the addition of lime and calcareous sand led to increases in soil Ca²⁺ well below 0.08%) did not result in higher values of dehydrogenase activity.

For β-glucosidase, acid phosphatase and arylsulphatase activity, 1 g of soil (dry weight) was mixed with 1.6 ml of buffer (20 mM modified universal buffer-MUB, pH 6.0, for β-glucosidase; 20 mM MUB, pH 6.5, for acid phosphatase; 500 mM acetate buffer, pH 5.8, for arylsulphatase) and 0.4 ml of substrate [4-nitrophenyl-β-D-glucopyranoside (1.5% w/v) for β-glucosidase; 4-nitrophenyl phosphate disodium salt (1.85% w/v) for acid phosphatase; potassium 4-nitrophenyl sulphate (1.3% w/v) for arylsulphatase]. The mixture was incubated at 37 °C for 45 min and the reaction stopped with 0.4 ml of 500 mM CaCl₂ and 1.6 ml of 500 mM NaOH for arylsulphatase and acid phosphatase, and with 0.4 ml of 500 mM CaCl₂ and 1.6 ml of 100 mM THAM, pH 12, for β-glucosidase. After centrifugation (1250 × g, 5 min), the absorbance value of the samples was read at 410 nm.

For urease activity, 1 g of soil (dry weight) was mixed with 1.75 ml of 100 mM borate buffer (pH 10) and 0.25 ml of 820 mM urea (substrate). The mixture was then incubated at 37 °C for 1 h and the reaction stopped with 6 ml of acidified 2 M KCl. After centrifugation (1250 × g, 5 min), 0.25 ml of the supernatant fraction was mixed with 3.75 ml of distilled water and 2 ml of a solution consisting of a sodium salicylate–sodium nitroprussiate mixture (17% and 0.12% w/v, respectively), 0.3 M NaOH and water (1:1:1). Finally 0.8 ml of sodium dichloroisocyanurate (0.1% w/v) was added. After 30 min, the absorbance value of the samples was read at 670 nm.

2.3. Vegetation analysis

Regarding botanical composition, 10 randomly placed quadrats (0.5 m × 0.5 m) were sampled within each plot (Kent and Coker, 1992) in spring of the next sheep grazing season (10th May 2004) (in many cases, for accurate identification, plants must be sampled during the flowering period; in any event, in our experimental area, plant development comes to a halt during the winter season) and identified according to Flora del País Vasco (Aizpuru et al., 2000). The abundance of each species was assessed by visual estimate of foliar cover in terms of occupied surface. Species cover was expressed as percentage of total cover.

Forage yield throughout the sheep grazing season (six months; from May to October 2003) was determined from the sum of the observed monthly yields using grazing-exclusion cages (0.5×1 m) which were placed in each of the experimental plots (2 grazing-exclusion cages per plot). Each month, forage was harvested manually from inside and outside (same surface; 0.5×1 m) grazing-exclusion cages, oven-dried at 70°C for 48 h and its dry matter (DM) recorded. Monthly forage yield was calculated by subtracting the amount of forage DM obtained outside grazing-exclusion cages from that obtained inside the cages. With regard to forage nutritive value, dried forage samples were grounded and homogenized by sieving (1 mm), and then crude protein, modified acid detergent fibre (MADF) and digestibility were determined according to Zasoski and Burau (1977).

2.4. Data analysis

Five indices were used to determine plant diversity (Magurran, 2004): species richness (S), Shannon's diversity index ($H' = -\sum p_i \ln p_i$), Pielou's evenness index ($J = H' / \ln S$), Simpson's diversity index ($1 - D$; $D = \sum p_i^2$), where p_i = the proportion of individuals in the i th species and, finally, Berger–Parker's diversity index ($1 - d$), where d describes the relative importance (dominance) of the most abundant species.

Differences among treatments within each site were performed as one-way analysis of variance (ANOVA) and Fisher's PLSD-test was used to establish the significance of the differences among means (except for acid phosphatase in Arraba). For soil enzyme data, a two-way ANOVA–MANOVA for the whole set of soil enzyme activities ($P < 0.05$) was also undertaken. Differences between sites within each treatment were determined according to Student's t -test (except for acid phosphatase in Arraba). Pearson's correlations were calculated between values of soil chemical parameters and enzyme activities (except for acid phosphatase in Arraba). When data did not adjust to a normal distribution (urease in Arraba), they were normalized using \log_{10} . When data could not be normalized (acid phosphatase in Arraba), non-parametric tests were used to detect correlations (Spearman) and differences among treatments (Kruskal–Wallis) and between sites (U–Mann Whitney).

Sun-ray plots were used to provide a visual illustration of the overall effect of liming treatments on soil parameters. The order of magnitude of the values obtained for the different enzyme activities varied considerably depending on the specific activity being determined. Therefore, for sun-ray plots, enzyme activity data were normalized by dividing the value obtained for each enzyme activity by the mean value obtained for that specific activity in control (no liming) plots and then multiplied by 100. From these sun-ray plots, the soil quality index (SQI) described by Bloem et al. (2006) was calculated using the average factorial deviation from the reference value (Ten Brink et al., 1991):

$$\text{SQI} = 10^{\log m - \frac{\sum_{i=1}^n |\log m - \log n_i|}{n}}$$

where m is the reference (mean value of control “no-liming” plots, set to 100%) and n are the measured values as percentages of the reference. Here, we propose the T-SQI which takes into account both (i) the magnitude of the increment of each enzyme activity, as compared to the value for that specific enzyme activity shown by the “reference” soil (100%; first Σ of the numerator), and (ii) the maintenance of the evenness among the studied enzyme activities shown by the “reference soil” (second Σ of the numerator):

$$\text{T-SQI} = 10^{\log m + \frac{\sum_{i=1}^n (\log n_i - \log m) - \sum_{i=1}^n |\log n_i - \log \bar{n}|}{n}}$$

where m is the reference (mean value of enzyme activity in the control, untreated, “reference” soil, set to 100%) and n are the measured values for each enzyme activity as percentages of the reference.

3. Results

3.1. Soil chemical parameters

Table 1 shows the short-term effects of liming treatments on soil chemical parameters in both grasslands. In Arraba, no significant differences were observed between control plots and those subjected to liming treatment (lime or calcareous sand) regarding soil chemical parameters. On the other hand, in Kurtzegan, liming treatment resulted in significantly higher values of soil pH and exchangeable Ca^{2+} , and significantly lower values of %Al saturation.

Pertaining to differences between Arraba and Kurtzegan, control plots showed significantly lower values of soil pH and exchangeable Ca^{2+} , and significantly higher values of %Al saturation, OC content, C/N ratio and total N in Kurtzegan than in Arraba.

3.2. Soil enzyme activities

A two-way ANOVA–MANOVA showed significant differences between sites for the whole set of soil enzyme activities ($P < 0.05$), but not between treatments. No “site-treatment” interaction was observed. Table 2 shows the short-term effects of liming treatments on individual enzyme activities in both grasslands, according to one-way ANOVA. In Arraba, the addition of calcareous sand led to significantly higher values of dehydrogenase activity, as compared to controls (30.4% higher). Similarly, in Kurtzegan, the addition of lime resulted in significantly higher values of dehydrogenase activity, as compared to controls (86.7% higher). No significant differences were observed between control plots and those subjected to liming treatment for the other soil enzyme activities here tested. No significant differences were observed between lime-treated and calcareous sand-treated plots.

Control plots showed significantly lower values of dehydrogenase and β -glucosidase activity in Kurtzegan than in Arraba (Table 2). In lime-treated soils, significantly lower values of dehydrogenase, arylsulphatase and β -glucosidase activity, and significantly higher values of urease activity were found in Kurtzegan than in Arraba. In soils treated with calcareous sand, significantly lower values of dehydrogenase, arylsulphatase and β -glucosidase activity, and significantly higher values of acid phosphatase and urease activity were found in Kurtzegan than in Arraba.

In Kurtzegan, significant positive correlations were found between soil pH and dehydrogenase ($r = 0.659$; $P = 0.0001$), urease ($r = 0.641$; $P = 0.0002$) and acid phosphatase ($r = 0.493$; $P = 0.0081$) activity. Likewise, in Arraba, a significant positive correlation between soil pH and acid phosphatase ($r = 0.392$; $P = 0.0425$) was found.

In Arraba, urease, an enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_3 , was positively correlated with total N ($r = 0.890$, $P < 0.0001$) and OC content ($r = 0.625$, $P < 0.0001$). Finally, in Kurtzegan we found a significant negative correlation ($r = -0.378$; $P = 0.050$) between acid phosphatase, an enzyme that releases PO_4 from organic P, and Olsen P.

In Fig. 1, sun-ray plots are presented to provide a visual illustration of the overall effect of liming treatments on soil enzyme activities in Arraba (Fig. 1A) and Kurtzegan (Fig. 1B). In these sun-ray plots, 100% corresponds to mean values of control (no liming) soils. In Arraba, values of the SQI calculated from sun-ray plots were 86.8 and 85.6% for soils treated with lime and calcareous sand, respectively. In Kurtzegan, values of the SQI were 71.1 and 73.4% for

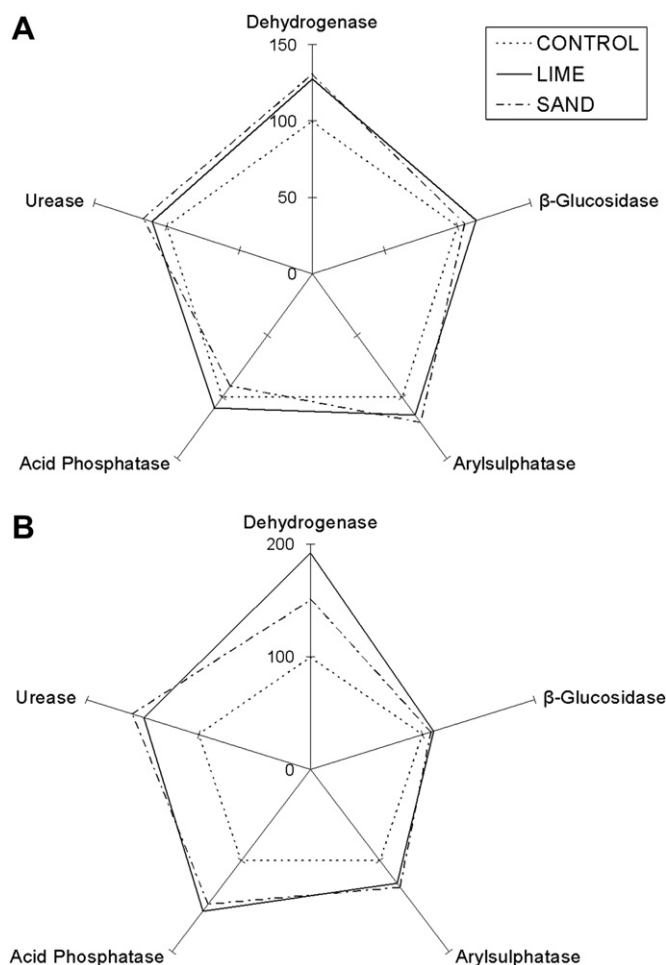


Fig. 1. Effect of liming treatments on soil enzyme activities in Arraba (A) and Kurtzegan (B). 100% corresponds to mean values of control (no liming) soils.

soils treated with lime and calcareous sand, respectively. Correspondingly, values of the here proposed T-SQI were: in Arraba, 104.3 and 99.7% for soils treated with lime and calcareous sand, respectively; in Kurtzegan, 118.0 and 117.6% for soils treated with lime and calcareous sand, respectively. Regarding SQI and T-SQI, no significant differences were observed between control and treated soils within each site (Arraba, Kurtzegan).

3.3. Vegetation

Table 3 shows the short-term effects of liming treatments on botanical composition and percentage cover of plant species, considering three categories of growth forms (graminoids, forbs and shrubs). More species of forbs were found in Arraba (18 species) than in Kurtzegan (7 species). In Kurtzegan, 7 species of shrubs were identified: *Calluna vulgaris* L. Hull., *Daboecia cantabrica* (Huds) K. Koch, *Erica ciliaris* L., *Erica cinerea* L., *E. tetralix* L., *E. vagans* L., and *U. gallii* Planchon (by contrast, no shrubs were found in Arraba).

In Arraba, *F. rubra* L. was the dominant species (29.4–32.9%), together with *A. capillaris* L. (17.0–19.6%) and *Anthemis nobilis* L. (4.1–5.0%). In Kurtzegan, *A. curtisii* Kerguelen was the dominant species (36.3–39.2%), together with *E. vagans* L. (9.8–19.9%) and *Danthonia decumbens* (L.) D.C. (10.7–12.2%). With regard to forbs, in Arraba, highest values of percentage cover were observed for *T. repens* L. (9.0–11.9%), *Taraxacum officinale* Weber (3.8–4.5%) and *Leontodon hispidus* L. (2.9–3.9%).

Regarding the short-term effects of liming treatments on botanical composition and percentage cover, in general, no significant differences were found between control plots and those subjected to liming treatment, neither in Arraba nor in Kurtzegan (Table 3). In any case, in Arraba, significantly lower values of percentage cover were found in lime-treated versus control plots for *Bellis perennis* L. and *Plantago minor* L. In Kurtzegan, liming treatment (lime and calcareous sand) prevented the growth of *A. capillaris* L. In Kurtzegan, values of percentage cover for *E. vagans* L. and *U. gallii* Planchon were significantly lower in plots subjected to liming treatment than in controls. By contrast, in Kurtzegan, the addition of lime led to significantly higher values of percentage cover of *Nardus stricta* L.

Table 4 shows the short-term effects of liming treatments on the values of diversity indices calculated from botanical data in both grasslands for the different growth forms (graminoids, forbs, shrubs) and also for the total number of plant species considered as a whole. In general, liming treatments had no significant effect on plant diversity. In any case, in Arraba, the addition of lime led to significantly lower values of Shannon's and Simpson's diversity for forbs. In Kurtzegan, significantly higher values of Berger–Parker's evenness for shrubs were observed in lime-treated plots, as compared to controls. As far as graminoids and forbs are concerned, in general, apart from Pielou's, significantly higher values of plant diversity were found in Arraba than in Kurtzegan. Likewise, apart from Pielou's, significantly higher values of total plant diversity were found in Arraba than in Kurtzegan.

Table 5 shows the short-term effects of liming treatments on forage yield (tonnes of dry matter per hectare) and nutritive value (crude protein, modified acid detergent fibre, digestibility) in both grasslands. In Arraba, the addition of lime led to significantly higher values of forage yield and digestibility, and significantly lower values of modified acid detergent fibre, as compared to controls. The addition of calcareous sand in Arraba led to significantly higher values of digestibility, and significantly lower values of modified acid detergent fibre, as compared to controls. No significant differences were found in Kurtzegan as a result of liming treatments. In general, significantly higher values of forage yield, crude protein and digestibility were found in Arraba than in Kurtzegan. By contrast, significantly higher values of modified acid detergent fibre were found in Kurtzegan than in Arraba.

4. Discussion

4.1. Soil chemical parameters

The two grasslands here studied are characterized by a very low value of soil pH, most likely due to the high annual rainfall typical of these mountainous areas, which results in the leaching of mono-valent and divalent cations and, concomitantly, elevated, phytotoxic values of %Al saturation that negatively affect forage productivity.

The calcareous origin of Arraba's soil results in a natural CaCO_3 supply from chemical weathering, contributing to the significantly higher values of soil pH and exchangeable Ca^{2+} and significantly lower values of %Al saturation observed in Arraba as compared to Kurtzegan (Table 1). This phenomenon can also explain the smaller increase in soil pH obtained in Arraba as a result of liming, possibly due to the higher soil pH buffering capacity of the Arraba's soil.

A lower soil pH might cause an inhibition of soil microbial activity which could contribute to the accumulation of organic C in Kurtzegan. On the other hand, one would expect that significantly higher values of soil pH, as a result of liming treatments, would contribute, at least to some extent, to faster rates of organic matter decomposition and higher values of Olsen P due to dissolution of

Table 3

Effects of liming treatments on botanical composition and percentage cover of plant species, considering three categories of growth forms (graminoids, forbs and shrubs). Values followed by different letters are significantly different among treatments within each grassland ($P < 0.05$) according to one-way ANOVA and Fisher's PLSD-test. Mean values ($n = 3$) \pm standard errors.

Plant species	Arraba			Kurtzegan		
	Control	Lime	Sand	Control	Lime	Sand
<i>Graminoids</i>						
<i>Agrostis capillaris</i> L.	17.0 \pm 1.1	19.6 \pm 1.1	19.0 \pm 1.8	2.8 \pm 1.1	0.0 \pm 0.0	0.0 \pm 0.0
<i>Agrostis curtisii</i> Kerguelen	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	36.3 \pm 3.3	36.5 \pm 2.1	39.2 \pm 3.3
<i>Aira praecox</i> L.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0
<i>Anthemis nobilis</i> L.	5.0 \pm 1.1	4.1 \pm 1.2	4.2 \pm 1.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Carex caryophyllaea</i> Latourr.	3.4 \pm 0.4	3.0 \pm 0.6	1.8 \pm 0.5	1.8 \pm 0.9	2.7 \pm 1.4	4.8 \pm 1.2
<i>Carex</i> sp.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	3.8 \pm 1.8	5.5 \pm 1.5	6.3 \pm 1.5
<i>Dactylis glomerata</i> L.	3.0 \pm 0.8	1.7 \pm 0.5	1.8 \pm 0.5	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Danthonia decumbens</i> (L.) D.C.	2.5 \pm 0.2	1.7 \pm 0.6	1.4 \pm 0.6	10.7 \pm 2.0	12.2 \pm 2.0	11.5 \pm 2.6
<i>Festuca rubra</i> L.	29.4 \pm 2.6	30.3 \pm 2.7	32.9 \pm 0.7	2.2 \pm 1.2	0.6 \pm 0.4	0.0 \pm 0.0
<i>Luzula campestris</i> (L.) D.C.	3.1 \pm 0.5	3.0 \pm 0.5	3.5 \pm 0.6	0.1 \pm 0.1	0.0 \pm 0.0	0.7 \pm 0.7
<i>Nardus stricta</i> L.	0.2 \pm 0.2	0.0 \pm 0.0	0.1 \pm 0.1	0.2 \pm 0.2a	5.8 \pm 2.2b	1.3 \pm 0.9a
<i>Poa annua</i> L.	3.0 \pm 1.1	3.8 \pm 1.7	2.2 \pm 0.7	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Pseudoarrenatherum longifolium</i> (Thore) Rouy	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.2 \pm 0.2
<i>Scilla verna</i> Hudson	0.2 \pm 0.2	0.1 \pm 0.1	0.9 \pm 0.8	2.3 \pm 0.5	2.3 \pm 0.6	2.5 \pm 0.6
Total graminoids	66.7 \pm 2.3	67.3 \pm 1.6	67.9 \pm 0.7	60.4 \pm 5.1	65.5 \pm 2.6	66.5 \pm 5.7
<i>Forbs</i>						
<i>Bellis perennis</i> L.	1.6 \pm 0.4a	0.6 \pm 0.2b	0.7 \pm 0.3ab	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Cerastium fontanum</i> Baumg.	0.9 \pm 0.3	2.4 \pm 1.3	2.5 \pm 0.9	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Cirsium eriophorum</i> (L.) Scop	0.0 \pm 0.0	0.1 \pm 0.1	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Eryngium bourgatii</i> Gouan	0.4 \pm 0.3	0.4 \pm 0.3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Galium saxatile</i> L.	1.4 \pm 0.3	1.1 \pm 0.6	1.3 \pm 0.3	0.4 \pm 0.4	0.0 \pm 0.0	0.1 \pm 0.1
<i>Hieracium pilosella</i> L.	3.1 \pm 1.1	4.5 \pm 1.8	3.3 \pm 0.6	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Hypochoeris radicata</i> L.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.1 \pm 0.1	0.0 \pm 0.0
<i>Jasione laevis</i> Lam.	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1
<i>Leontodon hispidus</i> L.	3.5 \pm 2.1	2.9 \pm 1.0	3.9 \pm 1.0	0.1 \pm 0.1	0.2 \pm 0.1	0.4 \pm 0.2
<i>Lotus corniculatus</i> L.	1.9 \pm 0.8	2.2 \pm 0.5	3.4 \pm 1.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Plantago lanceolata</i> L.	0.0 \pm 0.0	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Plantago minor</i> L.	2.3 \pm 1.0a	0.1 \pm 0.1b	0.5 \pm 0.2ab	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Potentilla erecta</i> (L.) Rauschel	0.8 \pm 0.5	0.1 \pm 0.0	0.1 \pm 0.1	1.3 \pm 0.7	3.0 \pm 0.7	2.8 \pm 0.8
<i>Potentilla montana</i> Brot.	0.9 \pm 0.4	0.6 \pm 0.3	1.4 \pm 0.9	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Prunella vulgaris</i> L.	0.2 \pm 0.1	0.0 \pm 0.0	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Ranunculus repens</i> L.	0.1 \pm 0.1	0.0 \pm 0.0	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Rumex acetosella</i> L.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.3	0.0 \pm 0.0	1.9 \pm 1.6
<i>Sedum</i> sp.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.2 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0
<i>Serratula seaneii</i> (Willk.)	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Taraxacum officinale</i> Weber	4.5 \pm 0.6	3.8 \pm 0.7	3.9 \pm 1.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Trifolium repens</i> L.	9.0 \pm 0.8	11.9 \pm 1.2	9.1 \pm 3.2	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
<i>Veronica arvensis</i> L.	0.8 \pm 0.3	0.3 \pm 0.2	0.8 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
Total forbs	31.3 \pm 2.4	31.2 \pm 1.4	31.3 \pm 1.1	2.3 \pm 0.5	3.4 \pm 0.6	5.3 \pm 2.1
<i>Shrubs</i>						
<i>Calluna vulgaris</i> (L.) Hull.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	4.2 \pm 1.9	6.2 \pm 1.7	4.3 \pm 1.0
<i>Daboecia cantabrica</i> (Huds) K. Koch.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.6 \pm 0.5	0.9 \pm 0.5	0.5 \pm 0.5
<i>Erica ciliaris</i> L.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	1.2 \pm 0.7	1.7 \pm 1.1	1.8 \pm 0.7
<i>Erica cinerea</i> L.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	1.8 \pm 1.0	2.6 \pm 1.4	2.2 \pm 0.3
<i>Erica tetralix</i> L.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	1.3 \pm 1.2	0.7 \pm 0.4	0.1 \pm 0.1
<i>Erica vagans</i> L.	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	19.9 \pm 1.5a	9.8 \pm 1.2b	13.0 \pm 2.5b
<i>Ulex gallii</i> Planchon	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	2.9 \pm 0.5a	1.1 \pm 0.7b	0.7 \pm 0.5b
Total shrubs	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	32.0 \pm 5.1	22.8 \pm 3.1	22.7 \pm 4.4

aluminium and iron phosphates (Bardgett, 2005). However, liming treatments did not result in any significant changes in OC content, total N, C/N ratio, Olsen P or exchangeable K^+ , either in Kurtzegan or in Arraba, probably due to the relatively small increase in soil pH caused by liming in our experiment. In any case, OC content in soils is known to change very slowly over time and, therefore, many years may be required to detect significant changes in such a parameter (Powelson et al., 1987). In agreement with our data, Matsi and Keramidas (1999) did not find any significant changes in Olsen P as a result of fly ash application in a 300-day experiment with soils characterized by high OC contents and low pHs such as ours. Finally, it must be taken into account that, in our study, liming treatments were not severe, due to fact that both grasslands are

included in a Natural Park where agricultural practices must be carefully controlled to minimize risks of negative environmental impact.

4.2. Soil enzyme activities

The significantly higher values of dehydrogenase observed in lime-treated plots in Kurtzegan, as compared to controls, might be explained by the fact that this activity is a measure of the activity of physiologically active microorganisms (Nannipieri et al., 2002) and, consequently, more sensitive to short-term changes in soil conditions than the other four enzyme activities here tested (Mijangos et al., 2006) which can, by contrast, be accumulated in soil by

Table 4

Effect of liming treatments on the values of plant diversity indices, for the different growth forms (graminoids, forbs, shrubs) and also for the total number of plant species considered as a whole. Values followed by different letters are significantly different among treatments within each grassland ($P < 0.05$) according to one-way ANOVA and Fisher's PLSD-test. Values followed by * indicate significant differences between grasslands within each treatment according to Student's t-test. Mean values ($n = 3$).

		Control	Lime	Sand
<i>All species</i>				
Species richness (<i>S</i>)	Arraba	21.3*	19.5*	20.5*
	Kurtzegan	14.0*	14.3*	13.8*
Shannon's (<i>H'</i>)	Arraba	2.37*	2.19	2.22*
	Kurtzegan	1.90*	1.97	1.92*
Pielou's (<i>J</i>)	Arraba	0.11*	0.11*	0.11*
	Kurtzegan	0.14*	0.14*	0.14*
Berger–Parker's ($1 - d$)	Arraba	0.70	0.69	0.67*
	Kurtzegan	0.63	0.62	0.59*
Simpson's ($1 - D$)	Arraba	0.85*	0.83	0.83*
	Kurtzegan	0.77*	0.78	0.77*
<i>Graminoids</i>				
Species richness (<i>S</i>)	Arraba	8.50	8.25*	8.75*
	Kurtzegan	7.00	6.50*	6.00*
Shannon's (<i>H'</i>)	Arraba	1.60*	1.47	1.43*
	Kurtzegan	1.22*	1.30	1.23*
Pielou's (<i>J</i>)	Arraba	0.19	0.18*	0.17*
	Kurtzegan	0.18	0.20*	0.21*
Berger–Parker's ($1 - d$)	Arraba	0.56*	0.55*	0.51*
	Kurtzegan	0.40*	0.44*	0.41*
Simpson's ($1 - D$)	Arraba	0.72*	0.70	0.67*
	Kurtzegan	0.58*	0.63	0.59*
<i>Forbs</i>				
Species richness (<i>S</i>)	Arraba	12.75*	11.25*	11.75*
	Kurtzegan	2.00*	2.00*	3.00*
Shannon's (<i>H'</i>)	Arraba	2.06a*	1.80b*	1.96ab*
	Kurtzegan	0.52*	0.37*	0.70*
Pielou's (<i>J</i>)	Arraba	0.17*	0.16*	0.17*
	Kurtzegan	0.04*	0.03*	0.05*
Berger–Parker's ($1 - d$)	Arraba	0.71	0.62*	0.72
	Kurtzegan	0.48	0.12*	0.34
Simpson's ($1 - D$)	Arraba	0.83a*	0.77b*	0.81ab*
	Kurtzegan	0.32*	0.21*	0.40*
<i>Shrubs</i>				
Species richness (<i>S</i>)	Arraba	—	—	—
	Kurtzegan	5.00*	5.75*	4.75*
Shannon's (<i>H'</i>)	Arraba	—	—	—
	Kurtzegan	1.03*	1.32*	1.15*
Pielou's (<i>J</i>)	Arraba	—	—	—
	Kurtzegan	0.21*	0.23*	0.25*
Berger–Parker's ($1 - d$)	Arraba	—	—	—
	Kurtzegan	0.35a*	0.56b*	0.42ab*
Simpson's ($1 - D$)	Arraba	—	—	—
	Kurtzegan	0.52*	0.67*	0.59*

complexing humic colloids and clay (Dick et al., 1996). Indeed, although most soil enzymes have a significant portion of the enzymatic activity associated with abiotic enzymes, dehydrogenase requires the organization of the living intracellular environment to express its activity and then exists only in viable cells (Dick, 1997; Nannipieri et al., 2002). No significant differences were observed between control and treated plots for these four enzymes although non-significantly higher values were always observed in treated

Table 5

Effect of liming treatments on forage yield and nutritive value. Values followed by different letters are significantly different among treatments within each grassland ($P < 0.05$) according to one-way ANOVA and Fisher's PLSD-test. Values followed by * indicate significant differences between grasslands within each treatment according to Student's t-test. Mean values ($n = 3$).

		Control	Lime	Sand
Yield ($t\ DM\ ha^{-1}$)	Arraba	3.84a	4.91b*	4.26ab*
	Kurtzegan	2.65	2.22*	2.30*
Crude protein (% DM)	Arraba	17.42*	16.79*	17.78*
	Kurtzegan	11.19*	10.94*	10.42*
MADF (% DM)	Arraba	30.57a*	26.91b*	26.86b*
	Kurtzegan	38.65*	39.22*	39.97*
Digestibility (% DM)	Arraba	51.22a*	54.22b*	54.46b*
	Kurtzegan	34.34*	35.60*	34.92*

MADF: modified acid detergent fibre.

versus control plots (apart from acid phosphatase activity in calcareous sand-treated Arraba plots).

As abovementioned, we found some positive correlations between soil pH and enzyme activities. Previous reports have also described positive correlations between soil pH and urease (Ekenler and Tabatabai, 2004), acid phosphatase (Klose et al., 2004), arylsulphatase (Wang et al., 2006), dehydrogenase (Rodríguez-Loinaz et al., 2008) and β -glucosidase (Acosta-Martínez and Tabatabai, 2000; Wang et al., 2006). In contrast, many authors (Acosta-Martínez and Tabatabai, 2000; Dick et al., 2000; Wang et al., 2006; Rodríguez-Loinaz et al., 2008) have reported a negative correlation between soil pH and acid phosphatase. The absence of correlation between soil pH and β -glucosidase activity in our study may be explained by the fact that this enzyme hydrolyzes organic compounds with a β -D-glycoside bond by splitting off the terminal β -D-glucose, and thus its activity may have been stimulated by organic substrates accumulated at low pH. In this respect, Martens et al. (1992) reported that the release, by decomposing organic compounds, of trigger molecules or promoters stimulates the production of hydrolytic enzymes.

In agreement with Kurtzegan results, Wright and Reddy (2001) reported a negative correlation between acid phosphatase and Olsen P. Tabatabai (1982) reported that increased levels of available (soluble) inorganic phosphate inhibit the activity of soil phosphatase through a feed-back mechanism. Phosphatase production and activity are linked to biotic demand for P (Olander and Vitousek, 2000) which explains the high values of acid phosphatase found in both grassland soils, as levels of available soil P in Arraba and Kurtzegan are certainly low.

Finally, many studies have reported positive correlations between urease and organic C (Dick et al., 1988; Deng and Tabatabai, 1997; Turner et al., 2002) and N content (Li et al., 2008; Rodríguez-Loinaz et al., 2008), similar to those described above for Arraba.

In both grassland soils, values of arylsulphatase activity were certainly high. Arylsulphatase, the enzyme that catalyses the hydrolysis of organic sulphate ester releasing sulphate, is considered an indirect indicator of the presence of fungi (ester sulphates are present only in fungal cells) (Dick et al., 1996). Fungi are more tolerant to acid-soil pH than bacteria, suggesting that the high values of arylsulphatase found in Arraba and Kurtzegan might be due to the presence of acid-tolerant soil fungi.

Values of T-SQI were higher in Kurtzegan than in Arraba, owing to the higher increments of soil enzyme activity found in the siliceous grassland versus controls. On the other hand, within each site (Arraba or Kurtzegan), values of SQI for lime-treated and calcareous sand-treated soils were non-significantly lower than those of

control soils (100%), because SQI always penalizes any type of variation from the reference values shown by untreated soils in any of the studied parameters. In any case, although sun-ray plots and derived indices can be used as tools for comparison purposes and for relatively simple presentation of complicated results, it must be remembered that they are only simplified reflections of complex ecosystems and should not be taken as absolute values (Bloem et al., 2006).

4.3. Vegetation

The lower values of soil pH in Kurtzegan might have possibly contributed, among other factors (for instance, parent material, history of grazing, landscape features, slope, etc.) to the absence of some forb species, for example, *T. repens* L., *T. officinale* Weber, *H. pilosella* L., and *Lotus corniculatus* L., which were, by contrast, present in Arraba. The lack of shrubs in Arraba is mainly caused by the regular presence of livestock in that area, unlike in Kurtzegan. Indeed, as reflected by the dominance of forage plant species of high nutritive value such as *F. gr. rubra* L., *A. capillaris* L. and *T. repens* L., Arraba is a mountainous grassland of much interest for livestock grazing. On the contrary, the dominant plant species in Kurtzegan, *A. curtisii* Kerguelen, *E. vagans* L. and *D. decumbens*, are characteristic of acid grasslands and have low nutritive value for grazing animals.

Liming treatments did not have much effect on botanical composition, possibly due to the low amount of lime/calcareous sand applied to the soil in our study and also to the somewhat short period of time passed between liming application and plant identification. In any event, in Kurtzegan, the percentage cover of two plant species which are commonly found under acid-soil pHs, *E. vagans* L. and *U. gallii* Planchon, significantly decreased with liming.

Regarding plant diversity, apart from Pielou's, higher values of plant diversity for forbs, graminoids and total plant species were found in Arraba than in Kurtzegan. Again, this is probably partly due, among other factors (for instance, parent material, history of grazing, landscape features, slope, etc.) to the higher values of soil pH found in Arraba and also to the positive effect of grazing on plant diversity. It is important to emphasize that many of these parameters are interrelated. Thus, the kind of parent material influences soil pH which, in turn, might negatively affect forage quality (species of lower palatability) and, in this way, decrease grazing pressure and then increase plant diversity. On the other hand, in lime-treated Kurtzegan plots, the significant increase in Berger–Parker's evenness found for shrubs was due to the observed reduction in %cover of the most dominant shrub species (*E. vagans*).

Calcareous seminatural grasslands, such as that of Arraba, are among the most species-rich communities in temperate Europe (Kull and Zobel, 1991). The different behaviour of the Pielou's index is not surprising since this index only considers evenness of species abundances, not species richness.

Forage production was higher in Arraba, most likely due to the lower soil pH found in Kurtzegan leading to somewhat phytotoxic levels of aluminium. In Arraba, lime-treated plots showed significantly higher values of forage yield than controls. This lime-induced increase in forage yield did not occur in Kurtzegan where liming treatment was more effective, probably due to the significant decrease of *E. vagans* and *U. gallii* observed in Kurtzegan in treated plots. On the other hand, forage nutritive value was better in Arraba (higher values of crude protein and digestibility, and lower values of modified acid detergent fibre) as reflected by the differences in botanical composition between both grasslands. Many forb species are usually associated with good values of crude protein and digestibility (García-González et al., 2005).

5. Conclusions

Soil acidity is the major limiting factor negatively affecting forage yield and nutritive value in many temperate mountainous grasslands of the Basque Country. When liming treatments are considered to correct soil acidity problems of temperate mountainous grasslands located within Natural Parks, only moderate amounts of liming products are allowed, to minimize risks of negative environmental impact. Regarding the short-term effects of liming on soil parameters, the application of a moderate amount of Ca^{2+} , as lime or calcareous sand, to a calcareous and siliceous temperate mountainous grassland, although not very effective at increasing soil pH, had a stimulatory effect on dehydrogenase activity (an indicator of microbial activity). This enzyme activity, which exists only in viable cells, was more sensitive than the other four enzyme activities studied here (β -glucosidase, urease, acid phosphatase and arylsulphatase activity). In relation to the effects of liming on vegetation (graminoids, forbs, shrubs), the application of a moderate amount of Ca^{2+} did improve forage yield and nutritive value in the calcareous grassland, without affecting botanical composition and diversity (neither in Arraba nor in Kurtzegan). Finally, no differences in soil biological quality, estimated with the here proposed “treated-soil quality index” (T-SQI) were observed between treated and untreated soils, nor between lime-treated and calcareous sand-treated soils, within each site. The T-SQI is a most suitable tool for the assessment of soil quality in those studies where the soil has been intentionally treated to increase its biological activity. These findings are most useful to land managers and decision-makers, especially those involved in the conservation of protected areas where sustainable agricultural practices must be implemented in order to preserve ecosystem functioning and biodiversity. Further research is needed to evaluate the long-term effects of liming on soil quality and forage productivity in acid-soil temperate mountainous grasslands as well as to verify the suitability of the T-SQI in different soils.

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References

- Acosta-Martínez, V., Tabatabai, M.A., 2000. Enzyme activities in a limed agricultural soil. *Biol. Fert. Soils* 31, 85–91.
- Aizpuru, I., Aseginolaza, C., Uribe-Echebarria, P.M., Urrutia, P., Zorrakin, I., 2000. Claves Ilustradas de la Flora del País Vasco y Territorios Limitrofes. Servicio de Publicaciones del Gobierno Vasco, Vitoria-Gasteiz, 831 pp.
- AOAC, 1980. In: Harwitte, W. (Ed.), *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed. AOAC, Washington DC, pp. 127–129.
- Barandiaran, J.M., Manterola, A., 2000. *Ganadería y Pastoreo en Vasconia*. Etniker Euskalerria-Eusko Jaurlaritza, Bilbao, 1020 pp.
- Bardgett, R.D., 2005. *The Biology of Soil*. Oxford University Press, Oxford, 242 pp.
- Bloem, J., Schouten, A.J., Sørensen, S.J., Rutgers, M., van der Werf, A., Breure, A.M., 2006. Monitoring and evaluating soil quality. In: Bloem, J., Hopkins, D.W., Benedetti, A. (Eds.), *Microbiological Methods for Assessing Soil Quality*. CAB International, Wallingford, pp. 23–49.
- Deng, S.P., Tabatabai, M.A., 1997. Effects of tillage and residue management on enzyme activities in soils: III. Phosphatases and arylsulphatase. *Biol. Fert. Soils* 24, 141–146.
- Dick, R.P., 1997. Soil enzyme activities as integrative indicators of soil health. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (Eds.), *Biological Indicators of Soil Health*. CAB International, Wallingford, pp. 121–156.
- Dick, R.P., Rasmussen, P.E., Kerle, E.A., 1988. Influence of long-term residue management on soil enzyme activity in relation to soil chemical properties of a wheat-fallow system. *Biol. Fert. Soils* 6, 159–164.

- Dick, R.P., Breakwell, D.P., Turco, R.F., 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran, J.W., Jones, A.J. (Eds.), *Methods for Assessing Soil Quality*. SSSA, Madison, WI, pp. 247–271.
- Dick, W.A., Cheng, L., Wang, P., 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biol. Biochem.* 32, 1915–1919.
- Ekenler, M., Tabatabai, M.A., 2004. Arylamidase and amidohydrolases in soils as affected by liming and tillage systems. *Soil Till. Res.* 77, 157–168.
- García-González, R., Aldezabal, A., Garin, I., Marinas, A., 2005. Valor nutritivo de las principales comunidades de pastos de los puertos de Góriz (Pirineo Central). *Pastos* 35, 77–103.
- Hernández-Allica, J., Becerril, J.M., Zárate, O., Garbisu, C., 2006. Assessment of the efficiency of a metal phytoextraction process with biological indicators of soil health. *Plant Soil* 281, 147–158.
- Hesse, P.R., 1971. *A Test Book of Soil Chemical Analysis*. John Murray, London.
- Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol. Fert. Soils* 6, 68–72.
- Kent, M., Coker, P., 1992. *Vegetation Description and Analysis. A Practical Approach*. Belhaven Press, London, 363 pp.
- Klose, S., Wernecke, K.D., Makeschin, F., 2004. Microbial activities in forest soils exposed to chronic depositions from a lignite power plant. *Soil Biol. Biochem.* 36, 1913–1923.
- Kull, K., Zobel, M., 1991. High species richness in an Estonian wooded meadow. *J. Veg. Sci.* 2, 711–714.
- Li, J., Zhao, B.-Q., Li, X.-Y., Jiang, R.-B., So, H.B., 2008. Effects of long-term combined application of organic and mineral fertilizers on microbial biomass, soil enzyme activities and soil fertility. *Agric. Sci. China* 7, 336–343.
- Magurran, A.E., 2004. *Measuring Biological Diversity*. Blackwell Publishing, Oxford, 256 pp.
- Martens, D.A., Johanson, J.B., Frankenberger, W.T., 1992. Production and persistence of soil enzymes with repeated additions of organic residues. *Soil Sci.* 153, 53–61.
- Matsi, T., Keramidas, V.Z., 1999. Fly ash application on two acid soils and its effect on soil salinity, pH, B, P and on ryegrass growth and composition. *Environ. Pollut.* 104, 107–112.
- McLean, E.O., 1971. Potentially beneficial effects from liming: chemical and physical. *Soil Crop Sci. Soc. Fla. Proc.* 31, 189–196.
- Mijangos, I., Pérez, R., Albizu, I., Garbisu, C., 2006. Effects of fertilization and tillage on soil biological parameters. *Enz. Microb. Technol.* 40, 100–106.
- Mijangos, I., Becerril, J.M., Albizu, I., Epelde, L., Garbisu, C., 2009. Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies. *Soil Biol. Biochem.* 41, 505–513.
- Mombiola, F.A., Mateo, M.E., 1982. Respuesta a seis dosis de P y cal en el establecimiento de praderas permanentes en dos tipos de suelos gallegos a monte. *Pastos* 12, 187–201.
- Montalvo, J., Casado, M.A., Levassor, C., Pineda, F.D., 1993. Species diversity patterns in Mediterranean grasslands. *J. Veg. Sci.* 4, 213–222.
- Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns, R.G., Dick, R.P. (Eds.), *Enzymes in the Environment*. Marcel Dekker, New York, pp. 1–33.
- Olander, L.P., Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49, 175–190.
- Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circ. 939. USDA, Washington, DC.
- Powlson, D.S., Brookes, P., Christensen, B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biol. Biochem.* 19, 59–164.
- Rodríguez-Loinaz, G., Onaindia, M., Amezcaga, I., Mijangos, I., Garbisu, C., 2008. Relationship between vegetation diversity and soil functional diversity in native mixed-oak forests. *Soil Biol. Biochem.* 40, 49–60.
- SSS (Soil Survey Staff), 2006. *Keys to Soil Taxonomy*, 10th ed. USDA Natural Resources Conservation Service, Washington, DC.
- Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. SSSA and ASA, Madison, WI, pp. 903–947.
- Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), *Methods of Soil Analysis. Part 2. Microbiological and Biological Properties*. SSSA and ASA, Madison, WI, pp. 775–833.
- Ten Brink, B.J.E., Hosper, S.H., Colijn, F., 1991. A quantitative method for description and assessment of ecosystems: the AMOEBA-approach. *Mar. Pollut. Bull.* 23, 265–270.
- Turner, B.L., Hopkins, D.W., Haygarth, P.M., Ostle, N., 2002. β -Glucosidase activity in pasture soils. *Appl. Soil Ecol.* 20, 157–162.
- Ulrich, B., 1991. An ecosystem approach to soil acidification. In: Ulrich, B., Sumner, M.E. (Eds.), *Soil Acidity*. Springer-Verlag, Berlin, pp. 28–79.
- Von Mersi, W., Schiner, F., 1991. An improved and accurate method for determining the dehydrogenase activity of soils with iodinitrotetrazolium chloride. *Biol. Fert. Soils* 11, 216–220.
- Wang, A.S., Angle, J.S., Chaney, R.L., Delorme, T.A., McIntosh, M., 2006. Changes in soil biological activities under reduced soil pH during *Thlaspi caerulescens* phytoextraction. *Soil Biol. Biochem.* 38, 1451–1461.
- Watkinson, A.R., Ormerod, S.J., 2001. Grassland, grazing and biodiversity: editors' introduction. *J. Appl. Ecol.* 38, 233–237.
- Wright, A.L., Reddy, K.R., 2001. Phosphorus loading effects on extracellular enzyme activity in Everglades wetland soils. *SSSAJ* 65, 588–595.
- Zasoski, R.J., Burau, R.G., 1977. A rapid nitric-perchloric acid digestion method for multielement tissue analysis. *Commun. Soil Sci. Plant Anal.* 8, 425–436.