**Effect of management and climate on biochemical properties of
grassland soils from Galicia (NW Spain)**

**Jorge Paz-Ferreiro¹, Carmen Trasar-Cepeda², M^a Carmen Leirós¹, Socorro
Seoane¹, Fernando Gil-Sotres¹**

¹*Departamento Edafología y Química Agrícola, Universidad de Santiago de Compostela. E-
15782. Santiago de Compostela, Spain.*

²*Departamento Bioquímica del Suelo, IIAG-CSIC, Apartado 122. E-15780. Santiago de
Compostela, Spain. E-mail: jpaz@udc.es*

Corresponding author: J. Paz-Ferreiro. Telephone number: +34 981563100; E-mail:
jpaz@udc.es Fax: +34 981494512.

Correspondence address: Departamento Edafología y Química Agrícola, Universidad de
Santiago de Compostela. E-15782. Santiago de Compostela, Spain.

Abstract

Biochemical properties are considered to be the best indicators available at present for
assessing soil quality. However, there are still many gaps in our knowledge about how
these properties are affected by abiotic factors and how these factors interact with soil
management. With the aim of understanding how climate and soil management affect
soil biochemical properties in grasslands soils from a temperate-humid area (Galicia,
NW Spain), a total of 60 soils were analyzed for several microbial and biochemical
properties. Grasslands were divided into groups according to the type of management
applied (native compared with intensive) and to the climate in the area where they were

sampled (Mediterranean subhumid with centroeuropean drift climate compared with Atlantic climate). We found that management had a greater influence on soil biochemical properties than climate. Altitude, which strongly influences climate in the region where the soils were analyzed, was found to be a significant factor that affected most soil biochemical properties. In conclusion, the results show that microbially-mediated processes are greatly affected by both, management and abiotic factors and that, for some properties (like net N mineralization and cellulase and casein-protease activities), abiotic factors can have an important influence on soil biochemical properties.

Keywords

Soil biochemical properties; soil enzymes; grassland soils; climate; management; altitude.

1. Introduction

Most soil scientists regard biochemical properties (principally oxidoreductases, different hydrolytic enzymatic activities related to the cycles of C, N, P and S, and other parameters, such as microbial biomass, soil N mineralization and respiration) as good environmental indicators of soil quality. This is because microbial activities and enzymatic activities are more sensitive to changes in soil quality than physical and chemical properties and can be easily and quickly quantified [35].

The use of soil biochemical properties as indicators of soil quality has been limited by the difficulty in interpreting the values obtained as both, soil management and abiotic factors influence these values. Moreover, the response of soil microorganisms to organic and inorganic amendments is often studied short term after addition of a single

dose to the soil [26], but less is known about their response in the field, after a significant number of years. Field studies have assessed soil biochemical properties in temperate areas by focusing on the effect of soil management in agricultural [2] and grassland soils [24]. These studies generally show that intensive managed systems suffer a decline in their biochemical activity and that this decline is related to a loss in organic matter.

Nevertheless, the contribution of specific enzymes to the overall enzyme activity depends on their arrangement with respect to various soil biotic and abiotic components [23]. Enzymatic activity depends in part on intracellular enzymes, which relies on soil microorganisms and thus, is affected by climate and management, and in part on the activity of extracellular enzymes immobilised by mineral colloids, which may not be as sensitive to environmental factors as microbial activity [23] and be dependent on soil management. Thus, it is difficult to evaluate the contribution of both activities, extracellular and intracellular, to overall soil enzymatic activity and how much of the biochemical activity responds to management or climatic factors. It is generally accepted that climate is one of the factors that has the greatest effect on soil biochemical properties [14, 29, 36]. Some authors even report that climatic factors and not management determine the size and activity of microbial biomass in grassland soils [39]. On the other hand, other authors report weak correlations between mean annual temperature or mean annual precipitation and soil biochemical properties [31]. As an example of this lack of consensus, in a review of worldwide papers about the effect of climate on soil C-biomass, Wardle [37] found that C-biomass was reported to increase, decrease or not be affected by temperature. It is noteworthy that, to date, most of the studies about biochemical properties have focussed in studying the effect of

management, while ignoring the effect of climate or the interaction between management and climate. To deepen more into knowing the dependence on climate and management of soil biochemical properties, we studied the effect of both, climate and management, on some biochemical properties of grassland soils from Galicia, NW Spain. Criteria for choosing enzyme assays were based on their importance in nutrient cycling and organic matter decomposition and the simplicity of the assay.

We hypothesized that soil under different management practices and climatic conditions would have effects on soil properties over a long period of time. Consequently, different enzyme activities were expected. It has been reported in many studies (for example Sariyildiz et al., 2005 [28]) that altitude can have strong influence on microclimate conditions (especially temperature and rainfall). Therefore, we also studied the effect of altitude on soil biochemical properties in the present study. This would allow scientist to select soil biochemical properties that do not depend on climatic parameters, instead responding to management, to measure soil quality.

2. Material and methods

2.1. Description of the study site

Galicia is a region located in the European temperate-humid zone (41°53′- 43°40′ N and 7°03′- 8°53′ W), where the mean annual precipitation ranges from 600 to 2600 mm, depending on the location, and the mean annual temperature varies from 6 to 15° C [7]. Grasslands cover approximately 12.5% of Galicia and traditionally managed native grasslands (with low grazing pressure, scarce or no use of mineral or organic amendments where the vegetation is adapted to the low fertility of Galician soils) coexist with intensively managed grasslands (high grazing pressure and intensive use of

amendments) areas. According to Carballeira et al. [7], Galicia can be divided into four phytoclimatic areas, although two of them account for the majority of the territory (see Figure 1) and thus, will be subject to study in this paper. Figure 2 shows a characteristic climatogram for each these two climatic areas that account for most of the territory. Firstly, Mediterranean subhumid with centroeuropean drift climate is characterized by an average temperature in the coldest month of the year lower than 6 °C and mean annual rainfall higher than 650 mm. This type of climate corresponds to most of the inner Galician region. Secondly, Atlantic climate has an average temperature in the coldest month higher than 6 °C and a mean annual rainfall higher than 650 mm. It covers most of the littoral of Galicia. The temperature changes are milder and the precipitation is higher in the Atlantic area than in the Mediterranean subhumid with centroeuropean drift (from now on Mediterranean subhumid) climatic area. In the Mediterranean subhumid climate area of Galicia altitude ranges from 300 to 2100 m.a.s.l., while altitude ranges from 0 to 600 m.a.s.l. in the Atlantic climatic area. Obviously, altitude and climatic parameters are correlated in Galicia. Mean annual temperature decreases 0.5 °C for each 100 m increase in altitude [8, 19]. On the other hand, along some transects there is a correlation between mean annual rainfall and altitude [8, 19], although the correlation does not go on for the whole area considered, as in the case of temperature. In the interior of Galicia (Mediterranean subhumid), annual rainfall usually increases with altitude, while, in the coastal areas (Atlantic area), rainfall increases with the proximity to the sea, as Galicia is swept by rainy fronts coming in from the Atlantic.

2.2. Soils

A total of 60 soils were collected during the spring of 2005 (between the months of March and May). Of these soils, 29 were sampled from native grasslands (16 in the Atlantic climate area and 13 in the Mediterranean subhumid climate area), and 31 were sampled from intensively managed grasslands (20 in the Atlantic climatic area and 11 in the Mediterranean subhumid area). The native grasslands are subject to an extensive or low intensity management regime, which means that they are rotationally grazed with a stocking rate between 0.6 and 1.1 cows ha⁻¹. The sward is cut once or twice a year. The grasslands generally remain unfertilised, with the only input being the dung excreted by grazing cattle, although in some isolated cases, they receive small quantities of organic fertilisers (<20 kg N ha⁻¹ of cattle slurry). The botanical community of native grasslands is dominated by low productivity plant species, in particular, few grass species, such as *Agrostis stolonifera* L., *Holcus lanatus* L., *Lolium multiflorum* Lam., *Agrostis capillaris* L., *Lolium perenne* L. and *Poa annua* L., with a presence of a small number of legumes, mainly *Trifolium repens* L. [27]. On the contrary, the management practices used in the intensively managed grasslands include liming to correct their pH, periodic re-seeding (usually annual or biannual) with removal of the existing grassland, surface ploughing and seeding, as well as periodic fertilization with cattle slurry and inorganic fertilizers. Intensively managed grasslands receive over 200 kg N ha⁻¹ and 50 kg P ha⁻¹ annually. Vegetation in the intensive managed grasslands is dominated by *Lolium multiflorum* Lam. and *Trifolium repens* L. [12]. Grassland management and grassland botanic are described in more detail for native grasslands [24] and for intensively managed grasslands [25].

Soils were collected, with a shovel, before any fertilizer was applied to the land. At each of the 60 plots, a representative sample was obtained from the top 10 cm of the soil, at

10-15 points distributed uniformly over an area of approximately 1 ha. Samples were pooled in the field to obtain composite samples representative of each site. Approximately five kilograms of soil were sampled in each plot and transported to the laboratory in isothermal bags, where they were sieved (< 4 mm). Moisture content was determined gravimetrically. A sub-sample of the soil was air-dried to ascertain general soil properties, and the remaining soil was stored at 4 °C pending biochemical analyses. All biochemical analyses were carried out within two weeks of sampling.

2.3. Soil physical and chemical properties

Total organic C (wet oxidation) and N (Kjeldahl digestion) contents and pH in water and in 1 M KCl (in both cases using a 1:2.5 soil: solution ratio) were determined following the methods described by Guitián and Carballas [13]. Available P was extracted with 0.5 M sodium bicarbonate (pH 8.2), according to Bowman and Cole [5] and inorganic P in the extracts was determined according to Murphy and Riley [21], after flocculation to pH 1.5 with sulphuric acid, centrifugation and filtration of supernatants. Amorphous Al and Fe were extracted with 0.2 M ammonium oxalate/oxalic acid solution of pH 3.0 [13] and were determined by atomic absorption spectrometry (Varian SpectrAA 220 FS spectrometer, UK). Particle size distribution was determined with a Robinson pipette using Calgon as dispersant [13].

2.4. Microbial biomass carbon, basal respiration and net nitrogen mineralization

Microbial biomass C (C biomass) was determined by the chloroform fumigation-extraction method [34]. The difference in C content of the fumigated and unfumigated extracts was converted to microbial biomass C (expressed in mg kg⁻¹ of dry soil)

applying a factor (K_c) of 0.45. The C extracted with K_2SO_4 from the unfumigated samples was used as a measure of the labile pool of C.

Soil basal respiration was determined by static incubation [13]. The CO_2 produced during a 10-day period by 25 g soil samples incubated at field moisture content at 25 °C was collected in 10 ml of a 1 M NaOH solution, which was then titrated against HCl with an automatic titrator. qCO_2 (μg CO_2 -C released mg^{-1} biomass carbon h^{-1}) was calculated as the ratio between basal respiration and microbial biomass C.

To determine net nitrogen mineralization, 10 g soil samples were extracted for 30 min with 50 ml of 2 M KCl before and after incubation for 10 days at 25 °C at field moisture content. Total inorganic nitrogen was determined in the extracts by Kjeldahl distillation [3]. Net nitrogen mineralization (mg kg^{-1} 10 d^{-1}) was calculated as the difference between the values obtained before and after incubation.

2.5. Enzymatic activities

Dehydrogenase activity was determined by a modification of the method of von Mersi and Schinner by Camiña et al. [6], with iodonitrotetrazolium violet (INT) as substrate, and the results are expressed as μmol iodonitrotetrazolium formazan (INTF) g^{-1} h^{-1} ; catalase activity was determined according to the method of Trasar-Cepeda et al. (1999) [33] and results are expressed as $mmol$ H_2O_2 consumed g^{-1} h^{-1} .

Acid phosphomonoesterase, β -glucosidase, phosphodiesterase and arylsulphatase activities were determined after first incubating the soils with a substrate containing a *p*-nitrophenyl moiety and then measuring the amount of *p*-nitrophenol released during enzymatic hydrolysis, by spectrophotometry. Phosphomonoesterase was determined following the method of Tabatabai and Bremner [22], with 16mM *p*-nitrophenyl

phosphate as substrate. β -Glucosidase activity was determined similarly as described for phosphomonoesterase activity using 25mM *p*-nitrophenyl- β -D-glucopyranoside as substrate [11]. Phosphodiesterase activity was measured by incubating soil samples with 10mM bis-*p*-nitrophenyl phosphate as substrate [4]. Arylsulphatase activity was determined by incubating the samples with 5mM *p*-nitrophenyl sulphate as substrate [32]. The activity of each of the above four enzymes is expressed as $\mu\text{mol } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$.

The activities of urease and protease hydrolysing benzoylargininamide (BAA-protease) were determined as described by Nannipieri [22]. Briefly, urease activity was determined by incubating the samples with 1065.6 mM urea as substrate and measuring the NH_4^+ released with an ammonia electrode. The BAA-protease activity was determined under the same incubation conditions and by the same method used to determine NH_4^+ , but with 30 mM α -N-benzoyl-L-argininamide (BAA) as substrate. In both cases, enzyme activity is expressed as $\mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$.

The activity of protease hydrolysing casein (casein-protease) was determined by incubating the samples with 1% casein as substrate, in 0.05 M Tris(hydroxymethyl)aminomethane-HCl (TRIS-HCl) buffer (pH 9.0) and measuring the released amino acids by the Folin-Ciocalteu colorimetric method described by Ladd and Butler [15]. Enzyme activity is expressed as $\mu\text{mol tyrosine g}^{-1} \text{ h}^{-1}$.

Invertase activity was determined by incubating the samples with 35.06 mM saccharose in 2 M acetate buffer (pH 5.5) and measuring the released reducing sugars following the method of Schinner and von Mersi [30]. Carboxymethylcellulase (CM-cellulase) activity was determined in a similar way using 0.7% carboxymethyl-cellulose

as substrate [30]. In both cases, the enzyme activities are expressed as $\mu\text{mol glucose g}^{-1}$ h^{-1} . All enzyme activities were determined in triplicate.

2.6. Statistical analysis

All statistical analyses were performed with SPSS, version 15.0. An ANCOVA (analysis of covariance) was performed using management and climate as factors and altitude as a covariable. Variables were log-transformed when it was required to meet requirements of ANOVA (normality and heteroscedasticity of errors).

3. Results

The mean values and standard deviations of physical and chemical soil properties in the different samples considered in the present study are shown in Table 1. The levels of organic matter were significantly lower ($P < 0.05$ for total carbon and $P < 0.01$ for total nitrogen) in the intensively managed soils from the Mediterranean subhumid area than in the native grassland soils located in the same area (see Table 1). In the case of the Atlantic area, differences in organic matter content for intensive and native grasslands were not statistically significant (see Table 1). In both climatic areas, the concentrations of available P were higher in the intensively managed grasslands than in the native grasslands. For both, organic matter and available P, the mean values showed no statistically significant difference when areas under the same management but different climate were considered.

Other properties (clay content, pH in water and in KCl, iron and aluminium content) showed similar average values (no statistical difference of mean values) for soils located in a different climatic area or under different management systems.

The mean values (and standard deviations) of the biochemical properties analyzed for each of the four groups of soils studied are shown in Tables 2 and 3, respectively. The values of the biochemical properties varied widely, even under the same climatic conditions or in grasslands under the same management. As an example, urease values, in soils under the same management and climate, always had a coefficient of variation higher than 50 %. In general, the values of soil biochemical properties were similar regardless of the climatic area considered. As an exception, when native grassland soils were considered, invertase and BAA-protease showed significant higher values in the Mediterranean climatic area than in the Atlantic area. More differences were verified for different management systems. Biomass-C, catalase, dehydrogenase, β -glucosidase, invertase, BAA-protease, phosphodiesterase, phosphomonoesterase and arylsulphatase activities showed mean values statistically lower in intensive grasslands compared with native grasslands, but this fact was only verified for the Mediterranean region. While for qCO_2 the situation was the reverse (higher values in intensive grasslands than in native grasslands in the Mediterranean area).

Table 4 shows the correlations among annual precipitation, annual mean temperature, altitude, and soil biochemical properties. No biochemical property showed statistically significant correlations with mean annual rainfall, while only cellulase ($r=0.34$, $P<0.01$) and BAA-protease ($r=0.32$, $P<0.05$) did with mean annual temperature. Nevertheless, altitude showed statistically significant correlations with C-biomass, catalase, dehydrogenase, invertase, cellulase, β -glucosidase, BAA-protease, urease and phosphomonoesterase. Correlations among altitude and catalase, invertase and BAA-protease were particularly high ($P<0.001$). Thus, altitude was used in the

ANCOVA analysis as a covariable, as it has showed more influence on soil biochemical properties than mean annual rainfall or mean annual temperature (see Table 4).

The results of the ANCOVA showed that biochemical properties were affected by management (Table 5, Table 6). As an exception, we have found that soil management did not affect labile carbon or N mineralization (Table 5). On the other hand, most of the hydrolytic enzymes studied were also affected by management (Table 6). Specifically, all of the enzymatic activities studied related to the P (phosphodiesterase and phosphomonoesterase activities) and S cycles (arylsulphatase activities) were highly affected by management ($P \leq 0.01$), with lower values in managed compared to native grasslands. Some enzymes involved in the C cycle (invertase, β -glucosidase) and N cycle (BAA-protease, urease) were also affected by management ($P \leq 0.05$). Net N mineralization, and CM-cellulase, casein-protease, phosphomonoesterase and arylsulphatase activities were significantly affected by climate (see Tables 5 and 6), being higher in the Atlantic than in the Mediterranean subhumid area. Altitude affected all biochemical properties but labile C. On the other hand, no interaction was found between management and climate for any of the biochemical properties studied.

4. Discussion

Some biochemical properties were affected by climate, although, overall, climate had less influence on soil biochemical properties than management. Differences due to management in grassland soils have been discussed at length [9,10,25] and attributed them to a range of factors such as changes in substrate availability and root growth, microbial competition and community structure and the build up of recalcitrant compounds in fertilized soils. Overall these differences were probably driven by

changes in organic matter content among soils under different management systems [25].

Nitrogen mineralization, and casein-protease, phosphomonoesterase and arylsulphatase activities were lower in soils from the Mediterranean subhumid area than in those from the Atlantic area ($P \leq 0.05$) reflecting that these enzymes could be particularly sensitive to different soil climate. As one of the main differences among both climatic areas is water availability (being the other one temperature) it is reasonable to suppose that the differences in N mineralization, and casein-protease, phosphomonoesterase and arylsulphatase activities between both types of soils could be due to soil water content, as differences in temperature would not explain a lower microbial activity in the warmer area (Mediterranean subhumid area). In fact, soil water content is known to limit the biological processes in soils [14, 16]. In fact, other studies [1] have shown that drying reduces the total microbial abundance and also suppress the relative activity of certain groups of fungi, leading to a decrease in enzymatic activities and soil respiration. This decrease could account for the lower soil biochemical values measured in soils under drier conditions.

Although the relation among climatic parameters (precipitation and temperature) and soil biochemical properties was only significant in a few occasions, it is noteworthy that altitude was more closely related to soil biochemical properties than climatic parameters. It is accepted that topography, and in particular altitude, influences local microclimates in the ecosystem by changing the pattern of precipitation, temperature and relative humidity [38]. About the effect of altitude on soil biochemical properties, we should stress that in the present study we considered areas located within a wide range of altitude (from 10 to 950 m.a.s.l.), but the range of altitude differed for each

climatic region. Samples in the Mediterranean subhumid area were taken at a mean altitude of 559 ± 172 m.a.s.l. while samples in the Atlantic region were taken at a mean altitude of 281 ± 170 m.a.s.l. which was significantly lower ($P < 0.001$). Most samples in the Mediterranean subhumid area (22 out of 24) were taken at an altitude above 400 m.a.s.l. and the most frequent group were soils taken at an altitude between 400 and 500 m.a.s.l. (10 soils). On the other hand, soils in the Atlantic area were sampled under 400 m.a.s.l (28 out of 36 soils), with most of the soils sampled at an altitude between 300 and 400 m.a.s.l. (12 soils). In the present study we found that altitude clearly affected all biochemical properties but labile C. The values of biochemical properties tended to increase with altitude, regardless of the climatic area considered. The fact that altitude can take account for some part of the variability on soil biochemical properties, due to abiotic factors, is, in our opinion, due to four reasons. Firstly, as stated before, the altitude in the study area is a good indication of the climatic condition, as, in Galicia, is related to both, temperature and precipitation. Secondly, we know exactly the altitude on the study sites, while it was sometimes difficult to find a meteorological station in the proximity of the studied soils. Thirdly, altitude is invariable, while precipitation and temperature are modified both, through the year and across different years, making difficult to decide how variability in soil microbial properties should be attributed to short or long term changes in climatic parameters. Finally, altitude was significantly different in both climatic areas ($P < 0.001$).

In spite of its obvious influence on climatic parameters, altitude appears to have been overlooked in most of these types of studies, and we only know of few papers [17, 18, 20] in which the effect of altitude on soil biochemical properties was investigated. Löffler et al. [17] studied chitinase and phosphatase activity in a high altitudinal

335 gradient in Norway and did not find any consistent trend for the variation of enzymatic
336 activities with altitude in a range from 1000 to 1605 m.a.s.l. Margesin et al. [18] studied
337 a range of altitude between 1500 to 2500 m.a.s.l. and found that microbial activity
338 decreased with altitude. In a similar study, Miralles et al. [20] covered a larger range of
339 altitudes (from 1000 to 3000 m.a.s.l.) than Margesin et al. [18]. At low altitudes,
340 biological processes appear to be determined by summer drought [20], whereas at high
341 altitudes, they are limited during most of the year by low temperature. This combination
342 of factors increases soil biochemical properties in areas situated at a moderate altitude.
343 In spite of being in a different climatic region our results could be explained in the same
344 way as [20], as in our study we have a range of altitude from 10 to 950 m.a.s.l. The
345 mean annual soil temperature is higher at low altitudes, while precipitation is lower at
346 low altitudes which may lead to a decrease of the biochemical properties of soils located
347 in such areas, because of drought. In contrast, soils located at higher altitudes although
348 subjected to lower temperatures would receive higher levels of precipitation, which
349 would favour soil biochemical properties. However, we did not find any effect of
350 rainfall on soil biochemical activity and only few of the biochemical properties were
351 affected by the temperature of the site. This suggests that the effect of altitude on soil
352 biochemical properties could be caused by an interaction between precipitation and
353 temperature or by other factors, such as changes in substrate composition or availability.
354 In our study and for the range of altitudes considered, we observed that the level of
355 biochemical activity increased with altitude. Nevertheless, we hypothesize that if a
356 range including higher altitudes were considered (taking samples above 1000 m.a.s.l.)
357 we would observe that, from a certain altitude, a decrease in the level of soil

biochemical properties would be produced. This decrease would be caused by temperature limitation.

On the whole, it is important to notice that some biochemical properties were not affected by management but were affected by other factors such as climate, altitude and soil moisture. This was true for respiration and for net N mineralization, cellulase and casein-protease activities. Thus, it is important to have our findings into account if biochemical properties want to be used in soil quality used, as their dependence with climatic factors would interfere with their role as soil quality indicators. It is important to notice that there might be some limitations in our study, as our samplings from each site were taken only once (in one year and one season). Therefore, future investigation should also consider the temporal dynamics of soil biochemical properties.

5. Conclusions

Our results emphasize the importance of climate when evaluating the modifications in soil microbiological and biochemical status caused by grassland management. Although grassland management was found to play an important role in determining the size and activity of the microbial communities, some biochemical properties (net N mineralization and cellulase and casein-protease activities) did not reflect these changes, and were more sensitive to abiotic factors, such as climate and altitude.

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485 Table 1 Mean values (and standard deviations) of the general characteristic of the soils from the different climatic areas and subjected to
486 the different management systems studied. Different capital letters indicate statistically significant differences for management ($P \leq 0.05$),
487 while different lowercase letters indicate statistically significant differences for climate ($P \leq 0.05$).

Parameter	Native grasslands		Intensively managed grasslands	
	Atlantic	Mediterranean subhumid	Atlantic	Mediterranean subhumid
Total carbon (C) (%)	5.5±2.7Aa	5.9±3.1Aa	4.9±2.3Aa	3.6±1.4Ba
Total nitrogen (N) (%)	0.43±0.25Aa	0.47±0.20Aa	0.38±0.16Aa	0.27±0.10Ba
C/N	13±2Aa	12±1Aa	13±1Aa	13±2Aa
pH (H ₂ O)	5.3±0.5Aa	5.3±0.5Aa	5.3±0.4Aa	5.4±0.5Aa
pH (KCl)	4.1±0.6Aa	4.2±0.5Aa	4.1±0.4Aa	4.2±0.4Aa
Clay (%)	20±7Aa	21±8Aa	19±6Aa	18±5Aa
Sand (%)	52±17Aa	45±18Aa	52±13Aa	57±15Aa
Available inorganic P (mg kg ⁻¹)	18±7Aa	20±8Aa	59±8Ba	56±14Ba
Al ₂ O ₃ (%)	0.70±0.48Aa	0.62±0.58Aa	0.73±0.51Aa	0.38±0.30Aa
Fe ₂ O ₃ (%)	0.76±0.59Aa	0.66±0.41Aa	0.61±0.47Aa	0.38±0.17Aa

488 Table 2 Mean values (and standard deviations) of some biochemical properties for the different climatic areas and different management
489 systems studied. Different capital letters indicate statistically significant differences for management ($P \leq 0.05$), while different lowercase
490 letters indicate statistically significant differences for climate ($P \leq 0.05$).

Parameter	Native grasslands		Intensively managed grasslands	
	Atlantic	Mediterranean subhumid	Atlantic	Mediterranean subhumid
Biomass-C*	596±410Aa	801±514Aa	398±129Aa	315±136Ba
Labile C*	275±98Aa	284±131Aa	286±126Aa	222±71Aa
Basal respiration [#]	404±172Aa	465±285Aa	365±135Aa	287±152Aa
$q\text{CO}_2^{\$}$	3.55±1.54Aa	2.52±0.77Aa	4.00±1.69Aa	4.02±2.19Ba
Mineralized N*	14.14±12.30Aa	11.47±12.23Aa	21.31±13.36Aa	13.03±10.41Aa
Catalase ^{&}	1.46±0.71Aa	2.05±0.91Aa	1.55±0.48Aa	1.34±0.30Ba
Dehydrogenase ⁺	0.70±0.30Aa	0.91±0.53Aa	0.61±0.23Aa	0.44±0.16Ba

491 * mg kg⁻¹, # mg CO₂-C evolved kg⁻¹ soil 10 d⁻¹, \$ μg de CO₂-C evolved h⁻¹ mg biomass-C⁻¹, & mmol of H₂O₂ consumed g⁻¹ h⁻¹, + μmol of
492 INTF g⁻¹ h⁻¹.

493

494 Table 3 Mean values (and standard deviations) of the hydrolytic enzymatic activities of the C, N, P and S cycles for the different climatic
495 areas and different management systems studied. Different capital letters indicate statistically significant differences for management
496 ($P \leq 0.05$), while different lowercase letters indicate statistically significant differences for climate ($P \leq 0.05$).

Parameter	Native grasslands		Intensive managed grasslands	
	Atlantic	Mediterranean subhumid	Atlantic	Mediterranean subhumid
Cellulase*	0.215±0.099 Aa	0.227±0.118 Aa	0.205±0.089 Aa	0.212±0.091 Aa
β-glucosidase [#]	1.53±0.71 Aa	2.01±0.88 Aa	1.47±0.59 Aa	1.35±0.54 Ba
Invertase*	5.94±3.41 Aa	9.27±4.96 Ab	5.88±2.74 Aa	4.79±2.36 Ba
Casein-protease ^{\$}	1.52±0.65 Aa	1.32±0.51 Aa	1.22±0.48 Aa	1.14±0.40 Aa
BAA-protease ^{&}	18.02±9.25 Aa	29.65±13.84 Ab	16.05±8.08 Aa	17.64±8.82 Ba
Urease ^{&}	27.76±41.27 Aa	43.82±50.52 Aa	15.71±9.53 Aa	15.66±8.40 Aa
Phosphodiesterase [#]	0.77±0.35 Aa	0.88±0.37 Aa	0.64±0.29 Aa	0.48±0.18 Ba
Phosphomonoesterase [#]	5.51±2.32 Aa	5.86±2.91 Aa	4.73±1.33 Aa	3.76±1.35 Ba
Arylsulphatase [#]	0.54±0.32 Aa	0.65±0.52Aa	0.42±0.22 Aa	0.26±0.14 Ba

497 * μmol glucose g⁻¹ h⁻¹, [#] μmol *p*-nitrophenol g⁻¹ h⁻¹, ^{\$} μmol tyrosine g⁻¹ h⁻¹, & μmol NH₃ g⁻¹ h⁻¹

498

499 Table 4 Correlations among all the biochemical properties and climatic parameters for Galician native grasslands (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).

500

	Bio.	C lab.	Resp.	N min.	Cat.	DH	Cel.	β-gluc.	Inv.	Cas.	BAA	Ure.	Dies.	Mono.	Aryl.	T	P
C-biomass	1																
Labile C	0.49***	1															
Respiration	0.69***	0.64***	1														
N mineralized	0.15	0.38**	0.33**	1													
Catalase	0.78***	0.62***	0.76***	0.25*	1												
Dehydrogenase	0.79***	0.62***	0.76***	0.33**	0.80***	1											
Cellulase	0.49***	0.53***	0.53***	0.21	0.58***	0.57***	1										
β-glucosidase	0.74***	0.49***	0.71***	0.29*	0.78***	0.79***	0.51***	1									
Invertase	0.78***	0.56***	0.69***	0.18	0.85***	0.78***	0.64***	0.82***	1								
Casein-protease	0.57***	0.55***	0.71***	0.31*	0.67***	0.64***	0.59***	0.66***	0.58***	1							
BAA-protease	0.70***	0.46***	0.60***	0.28*	0.63***	0.68***	0.50***	0.56***	0.62***	0.45***	1						
Urease	0.74***	0.57***	0.60***	0.36**	0.58***	0.76***	0.56***	0.57***	0.66***	0.46***	0.67***	1					
Phosphodiesterase	0.74***	0.55***	0.82***	0.35**	0.73***	0.77***	0.41***	0.80***	0.71***	0.71***	0.57***	0.52***	1				
Phosphomonoesterase	0.78***	0.59***	0.76***	0.36**	0.75***	0.87***	0.63***	0.74***	0.73***	0.78***	0.70***	0.68***	0.78***	1			
Arylsulphatase	0.77***	0.60***	0.74***	0.28*	0.75***	0.90***	0.49***	0.68***	0.70***	0.66***	0.67***	0.73***	0.76***	0.87***	1		
Mean annual T	-0.16	-0.09	-0.11	-0.03	-0.24	-0.15	-0.34**	-0.14	-0.24	-0.04	-0.32*	-0.22	-0.04	-0.13	-0.12	1	
Mean annual rainfall	-0.03	0.07	0.10	0.22	-0.12	0.02	0.06	-0.03	0.06	0.12	-0.07	-0.04	0.11	0.16	0.01	0.09	1
Altitude	0.31*	0.12	0.23	0.09	0.41***	0.31*	0.37**	0.34**	0.46***	0.11	0.50***	0.35**	0.22	0.26*	0.25	-0.71***	-0.12

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Table 5 Effects of management, climate, the interaction between management and climate, and altitude on some soil biochemical parameters.

Source of variation	Df	F	P
Biomass			
Altitude	1	7.137	0.010
Management	1	17.538	0.000
Climate	1	1.347	0.251
Management*Climate	1	1.140	0.290
Labile C			
Altitude	1	3.056	0.086
Management	1	0.872	0.354
Climate	1	3.369	0.072
Management*Climate	1	0.676	0.415
Respiration			
Altitude	1	5.394	0.024
Management	1	5.481	0.023
Climate	1	2.679	0.107
Management*Climate	1	0.776	0.282
N mineralization			
Altitude	1	4.940	0.030
Management	1	1.492	0.199
Climate	1	7.602	0.008
Management*climate	1	0.125	0.735
Catalase			
Altitude	1	9.151	0.004
Management	1	4.282	0.043
Climate	1	1.012	0.319
Management*climate	1	3.058	0.086
Dehydrogenase			
Altitude	1	8.341	0.006
Management	1	12.565	0.001
Climate	1	2.672	0.107
Management*climate	1	2.479	0.121

514 Table 6 Effects on some enzymatic activities, of management, climate, the interaction
515 between management and climate, and altitude

Source of variation	Df	F	P
Cellulase			
Altitude	1	14.270	0.000
Management	1	0.444	0.508
Climate	1	4.512	0.038
Management*climate	1	0.559	0.458
β -glucosidase			
Altitude	1	6.108	0.017
Management	1	4.579	0.037
Climate	1	0.631	0.431
Management*climate	1	1.177	0.283
Invertase			
Altitude	1	14.417	0.000
Management	1	8.387	0.005
Climate	1	1.944	0.169
Management*climate	1	3.060	0.086
Casein-protease			
Altitude	1	4.791	0.033
Management	1	3.523	0.066
Climate	1	4.902	0.031
Management*climate	1	0.874	0.354
BAA-protease			
Altitude	1	10.966	0.002
Management	1	8.896	0.004
Climate	1	0.003	0.957
Management*climate	1	1.553	0.218
Urease			
Altitude	1	7.960	0.007
Management	1	6.703	0.012
Climate	1	1.115	0.296
Management*climate	1	0.105	0.747
Phosphodiesterase			
Altitude	1	6.004	0.017
Management	1	11.954	0.001
Climate	1	3.389	0.071
Management*climate	1	1.176	0.283
Phosphomonoesterase			
Altitude	1	10.317	0.002
Management	1	8.904	0.004
Climate	1	6.489	0.014
Management*climate	1	0.299	0.587
Arylsulphatase			
Altitude	1	7.602	0.008
Management	1	10.550	0.002
Climate	1	4.112	0.047
Management*climate	1	0.855	0.359

Figure captions

Figure 1. Location of the two climatic areas (out of a total of four present in Galicia) considered in this study. Darker areas represents Mediterranean subhumid with centroeuropean drift, while lighter ones represent Atlantic climate.

Figure 2. Characteristic climatogram, showing temperature (T) and precipitation (P) for the two climatic regions of Galicia considered in this study.

Figure 1.

556 Figure 2.

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