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Relationship between vegetation diversity and soil functional diversity in native mixed-oak forests

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Abstract

Most studies on the interactions between aboveground vegetation and belowground soil diversity have been carried out in microcosms or manipulated field plots. In the current study, we investigated the relationship between forest vegetation diversity and soil functional diversity (calculated from the activity of soil enzymes) in naturally developed plant communities of native mixed-oak forests without imposing any disturbances to already existing plant-soil relationships. In order to do so, five different vegetation types, i.e., herbaceous plants, climbing plants, trees, shrubs, and ferns, were considered. Correlations between plant diversity, soil physicochemical properties, and soil enzyme activities were determined. Soil physicochemical parameters appeared strongly correlated with both enzyme activities (e.g., pH was positively correlated with amidase and arylsulphatase, and negatively with acid phosphatase; OM content was positively correlated with β-glucosidase, acid and alkaline phosphatase and urease, and negatively with amidase; total N was positively correlated with β-glucosidase, and acid and alkaline phosphatase, and negatively with amidase) and soil functional diversity. For ferns, strong correlations between enzyme activities and plant diversity indexes were found (i.e., dehydrogenase was positively correlated with species richness and Shannon's diversity; acid and alkaline phosphatase were negatively correlated with Shannon's diversity; acid phosphatase was also negatively correlated with species richness). Most interestingly, herbaceous plants and ferns showed a strong positive correlation between Shannon's plant diversity and soil functional diversity. Furthermore, herbaceous plants showed a strong positive correlation between species richness and soil functional diversity. Although these correlations between plant diversity and soil functional diversity might possibly be due to the fact that higher values of plant richness and diversity result in a greater habitat heterogeneity in the soil, current knowledge on the topic is

mixed and very incomplete and, then, one must be extremely cautious when interpreting such correlations.

Keywords: aboveground; belowground; functional diversity; mixed-oak forest; plant diversity; soil enzymes

1. Introduction

Until recently, those scientists involved in the study of the vast diversity of organisms that live on Earth have had an almost exclusively aboveground focus, with little effort being put into characterizing and understanding the significance of belowground biodiversity (Wardle, 2002; Bardgett, 2005). But aboveground and belowground components of terrestrial ecosystems are closely related, with soil organisms being intimately linked to plant communities (Bardgett, 2005). Indeed, plants provide a source of C and other nutrients for the soil decomposer community in the form of plant litter and root exudates and, in turn, the soil biota, particularly its microbiota, decomposes soil organic matter, stabilizes soil structure and, through its essential role in the cycling of elements, releases nutrients for plant growth (Porazinska et al., 2003). In addition, although abiotic factors have traditionally been interpreted as the drivers of the vegetation patterns observed in terrestrial ecosystems, more recently, biotic interactions in the soil have also been reported as major drivers of the composition of plant communities (Hooper et al., 2000; Wardle, 2005). Therefore, in order to understand the complex patterns of biodiversity in terrestrial ecosystems and, above all, their relationship to ecosystem function, a combined aboveground-belowground approach is required.

Biodiversity in soil is extremely high, particularly at the microbial scale (Torsvik et al., 1994), and, additionally, there are a large number of trophically equivalent organisms. In this respect, it has been suggested that most species in soil must be functionally redundant (Bardgett, 2005; O´Donnell et al., 2005). For that reason, measurements of functional diversity in soil communities are likely to provide information more relevant to the functioning of soil than species diversity (Zak et al., 1994). Besides, belowground, for microorganisms, a species approach is not always practical since the traditional species

concept is difficult to apply (Hooper et al., 2000). In this regard, soil enzyme measurements are very useful for the assessment of the status or the condition of the soil environment (Naseby and Lynch, 2002). Certainly, soil enzyme activities (i) control rates of nutrient cycling processes, (ii) are crucial to the availability of nutrients to both soil microbiota and plants, and (iii) can be valuable indicators of soil functional diversity (Bending et al., 2002; Naseby and Lynch, 2002). But soil functional diversity depends on numerous metabolic reactions and interactions of biota and hence it is certainly unrealistic to assume that a simple relationship might exist between a single enzyme activity and soil functional diversity (Nannipieri et al., 2002). Consequently, it is always crucial to measure simultaneously a range of enzyme activities.

The most important problem in interpreting data of soil enzyme activities is to discriminate among many components contributing to the overall activity. After all, the activity of any particular enzyme depends on enzymes that can have different locations such as: (i) in resting or dead cells, (ii) in cell debris, (iii) intracellularly in living cells, (iv) extracellularly in the soil solution, (v) absorbed by inorganic colloids and (vi) associated with humic molecules (Nannipieri et al., 2002). Although intracellular enzymes are present in plant, animal and microbial cells, enzyme activities are usually determined after removal of visible animals and plant debris and on sieved soil samples under laboratory conditions. Accordingly, and since those enzymes that have been released from lysed cells are rapidly degraded by microorganisms, the most important intracellular enzymes of soil are probably those in living microbial cells (Nannipieri et al., 2002).

Finally, it has been reported that both plant species identity and diversity are major factors affecting the abundance and diversity of soil organisms (Johnson et al., 2003; Wardle, 2005). But the mechanisms through which plant composition and diversity affect soil communities and trophic levels in soil food webs remain essentially unexplored (Wardle et

al., 2003). Furthermore, the few studies that have so far dealt with the interactions between vegetation structure and soil diversity have been restricted largely to a few ecosystem types and a few taxonomic groups of organisms within them (Bardgett, 2005). Most importantly, these studies on the interactions between aboveground and belowground communities have predominantly been carried out in microcosms or manipulated field plots (Porazinska et al., 2003; Van der Putten, 2005). To avoid the disturbances inherently associated with these two approaches, in the current study, the interactions between vegetation diversity and soil functional diversity have been investigated in naturally developed plant communities of native mixed-oak forests without imposing any disturbances to already existing plant-soil relationships.

The major objective of the current work was to study the relationship between vegetation diversity (species richness, diversity, evenness) and soil functional diversity (calculated from the activity of soil enzymes which have a key function in the cycling of C, N, P and S) in native mixed-oak forests. Additionally, correlations between soil physicochemical properties and plant community diversity or soil enzyme activities were determined.

2. Materials and Methods

2.1 Study area

This study was carried out in the Urdaibai Biosphere Reserve (220 km^2) located in the north of the Iberian Peninsula (43º19´N, 02º40´W). Apart from the coastal landscape, within the Urdaibai Reserve, the potential vegetation in most of the territory consists of mixed-oak forests, dominated by Quercus robur L. with Fraxinus excelsior L. and Castanea sativa Miller (Onaindia et al., 2004). Throughout the 20th century, these native mixed-oak forests were

heavily fragmented and, as a result, nowadays they only cover around 6% of the total area of the Urdaibai Reserve (Rodríguez-Loinaz et al., 2006). In fact, most of the area initially covered by those mixed-oak forests is now occupied by forest plantations (Pinus radiata and Eucalyptus sp.) together with grasslands and crops.

2.2 Analysis of vegetation samples

For this study, 23 different stands (average size: 12 ha) of native mixed-oak forest located at an altitude of 50-300 m above sea level, and distributed throughout the Urdaibai Reserve, were selected. In order to avoid a possible edge effect, square sampling plots (25 x 25 m) were demarcated approximately in the centre of each stand (i.e., one sampling plot per stand). In each sampling plot, five different smaller (2 x 1 m) sub-plots, 12 m apart from each other, were delimited according to the method of the species/area curve (Kent and Coker, 1992). In each of the 115 sub-plots (23 stands x 5 sub-plots per stand), plant species identification, according to Flora del País Vasco (Aizpuru et al., 2000), and percentage cover for each plant species, calculated through visual estimation, were determined. Then, the following diversity indexes were calculated (Magurran, 2004): species richness (S), Shannon's diversity (H') and Shannon's evenness (J'). These three indexes were calculated for both (i) all the plant species present in the sampling plots considered as a whole and (ii) each of the five different growth forms here considered (i.e., herbaceous plants, climbing plants, trees, shrubs, and ferns).

2.3 Analysis of soil samples

After removing plant litter, soil samples were taken at the centre of each sub-plot (i.e., one core per sub-plot was taken from 0 to 15 cm depth using a 4.0 cm auger). The five soil

177 samples corresponding to each sampling plot were pooled to get a composite sample. Since
178 soil samples were taken 12 m apart from each other, the lack of spatial correlation between
179 them was warranted (Boerner et al., 2005).

180 For soil chemical analysis, soils were air-dried at 30ºC for 48 h, sieved to <2 mm, and
181 stored at room temperature. Soil pH was measured in the 1:2.5 (w/v) suspension of soil and
182 water. Soil organic matter content, total N, C/N ratio, particle size distribution, exchangeable
183 P (bicarbonate) and exchangeable K^+, Ca^{2+}, Mg^{2+} (ammonium nitrate) were determined
184 following standard methods (MAPA, 1994). Table 1 summarizes the data obtained during the
185 physicochemical characterization of the soils collected from the 23 studied stands.

186 For analysis of enzyme activities, soils were air-dried at 30ºC for 48 h, sieved to <2 mm
187 and stored at 4ºC. Dehydrogenase, β-glucosidase, acid and alkaline phosphatase, and
188 arylsulphatase activity were determined according to Dick et al. (1996) and Taylor et al.
189 (2002). Urease and amidase activity were determined according to Kandeler and Gerber
190 (1988) and Acosta-Martínez and Tabatabai (2000), respectively.

191 Dehydrogenase (EC 1.1) activity is an intracellular process that occurs in every viable
192 microbial cell and is measured to determine overall microbiological activity of soil
193 (Nannipieri et al., 2002). β-glucosidase (EC 3.2.1.21), a glycosidase important in the C cycle,
194 hydrolyzes carbohydrates with a β-D-glycoside bond by splitting off the terminal β-D-glucose
195 (Schinner et al., 1996) and then plays a central role in the hydrolysis of polymers of plant
196 residues, such as cellobiose, releasing glucose as an important energy source for soil
197 heterotrophic organisms. Phosphatases (alkaline: orthophosphoric monoester
198 phosphohydrolase, EC 3.1.3.1; acid: orthophosphoric monoester phosphohydrolase, EC
199 3.1.3.2) are important in the P cycle because they provide P for plant uptake by releasing PO_4
200 from organic P (Eivazi and Tabatabai, 1977). Arylsulphatase (arylsulphatase sulfohydrolase,
201 EC 3.1.6.1) is the enzyme that catalyzes the hydrolysis of organic sulphate ester ($R \cdot O \cdot SO_3 \cdot$

+ H_2O → R · OH + H^+ + SO_4^{2-}) releasing sulphate (SO_4^{2-}), the plant available form of S. Urease (urea amidohydrolase, EC 3.5.1.5) is an important enzyme in the N cycle that catalyzes the hydrolysis of urea to CO_2 and NH_3 (Tabatabai and Bremner, 1972). Amidase (acylamide amidohydrolase, EC 3.5.1.4) is another important enzyme in the N cycle that releases phytoavailable NH_4^+ from linear amides by acting on C-N bonds other than peptide bonds (Frankenberger and Tabatabai, 1981).

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 0.5 ml of iodonitrotetrazolium chloride-INT (0.5% w/v). The mixture was then incubated at 25ºC for 4 h and the reaction stopped with 8 ml of methanol. After centrifugation (1250 *x g*, 5 min), the absorbance value of the samples was read at 490 nm.

For β-glucosidase, acid and alkaline phosphatase, and arylsulphatase, 1 g of soil (dry weight) was mixed with 1.6 ml of buffer (i.e., 20 mM modified universal buffer-MUB, pH 6.0, for β-glucosidase; 20 mM MUB, pH 6.5, for acid phosphatase; 20 mM MUB, pH 11, for alkaline phosphatase; 500 mM acetate buffer, pH 5.8, for arylsulphatase) and 0.4 ml of substrate [i.e., 4-nitrophenyl-β-D-glucopyranoside (1.5% w/v) for β-glucosidase; 4-nitrophenyl phosphate disodium salt (1.85% w/v) for acid and alkaline 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 $CaCl_2$ and 1.6 ml of 500 mM NaOH. After centrifugation (1250 *x 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 10.0) and 0.25 ml of 820 mM urea. 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 *x g*, 5 min), 0.25 ml of the supernatant fraction was mixed with 3.75 ml of distilled water and 2 ml

of a reagent composed of a sodium salicylate/sodium nitroprusiate mixture (17% w/v and 0.12% w/v, respectively), 0.3 M NaOH and distilled water (1:1:1 v/v/v). Finally, 0.8 ml of sodium dichloroisocyanurate was added to the reaction mixture. After 30 min, the absorbance value of the samples was read at 670 nm.

For amidase activity, 1 g of soil (dry weight) was mixed with 1.5 ml of 100 mM THAM buffer, pH 8.0, and 0.5 ml of 8 mM L-leucine-β-naphthylamide. The mixture was then incubated at 37°C for 45 minutes and the reaction stopped with 3 ml of 95% ethanol. After centrifugation (1250 *x g*, 5 min), the absorbance of the samples was read at 540 nm.

From the values of these seven enzyme activities, soil functional diversity was determined using the Shannon's diversity index ($H'=-\sum p_i \log_2 p_i$), as indicated by Bending et al. (2004), where p_i = the ratio of the activity of a particular enzyme to the sum of activities of all enzymes. Since the seven enzymes here tested did show activity in all the analyzed samples, then, in this work, Shannon's diversity index reflects only the "evenness" or distribution of the enzyme activities (Bending et al., 2004). The order of magnitude of the values obtained for the different enzyme activities varied considerably depending on the specific activity being determined, thus leading to some enzyme activities having more weight than others during the calculation of the Shannon's diversity index. To resolve this problem, prior to the calculation of this index, a simple mathematical transformation was applied so that all enzyme activities had the same weight/relevance during such calculation, i.e. the value obtained for each enzyme activity was divided by the highest value found for that specific activity in the whole set of samples, and then multiplied by 100. In other words, for each enzyme activity, the percentage of the maximum value found for that specific activity in the whole set of samples was calculated.

2.4 Statistical analysis

Statistical analyses were performed using SPSS Programme (Inso Corporation). Pearson's correlations were calculated between soil physicochemical parameters, enzyme activities and vegetation data. When data did not adjust to a normal distribution, they were normalized using $\log_{10}$. When data could not be normalized, the Spearman's non-parametric correlation test was used.

3. Results

3.1 Vegetation structure

Table 2 shows the composition and percentage cover of those plant species found in the 23 stands of native mixed-oak forest here studied. Although altogether 110 different species of vascular plants were found in the studied stands, i.e., 53 herbaceous plants, 5 climbing plants, 18 trees, 23 shrubs, and 11 ferns, only those species appearing in at least more than one stand have been included in Table 2. At the time of sampling, gramineae were not flowered and then their identification was problematic. Consequently, all gramineae present in each sampling plot were identified only at the family level as Poaceae and quantified as 1 species. In any case, in our region, only two species of Poaceae (Brachypodium sylvaticum and Bromus ramosus) are usually found in this type of forests (Aseguinolaza et al., 1988). Hence, the fact that gramineae were not identified to species level in this study should have only a minor effect on diversity values.

In relation to herbaceous plants, gramineae showed a value of percentage cover of 42.36%. Hedera helix (52.67% cover) was the most frequent climbing plant found in our study area. As expected, the most common tree species found were Q. robur (98.11% cover), C. sativa (24.70% cover), Laurus nobilis (15.55% cover) and F. excelsior (10.49% cover). In

turn, the two most common shrubs were Rubus sp. (54.37% cover) and Corylus avellana (42.15% cover). Finally, the two dominant fern species were Pteridium aquilinum (22.24% cover) and Blechnum spicant (11.95% cover).

Table 3 shows the values of the different diversity indexes calculated for all the plant species considered as a whole and also according to growth forms (i.e., herbaceous plants, climbing plants, trees, shrubs, and ferns) in the 23 studied stands. The number of species per stand (S) ranged from 14 to 33. On average, there were 7.43, 3.17, 4.26, 5.52 and 3.43 species of herbaceous plants, climbing plants, trees, shrubs and ferns, respectively, per stand. The highest mean values of S and H' corresponded to herbaceous plants. Finally, according to Shannon's evenness index, ferns were the plants more uniformly distributed.

3.2 Vegetation and soil physicochemical parameters

Table 4 shows the Pearson's correlation values obtained between some soil physicochemical parameters (pH, OM, total N, K^+, Ca^{2+}) and diversity indexes for all the plant species considered as a whole and also according to growth forms. Since no correlations were found between the other physicochemical properties here determined (i.e., C/N ratio, particle size distribution, and P and Mg^{2+} contents) and plant diversity indexes, their data have not been included in Table 4. Similarly, no significant correlations ($P<0.05$* or $P<0.01$**) were found between soil physicochemical parameters and diversity indexes for climbing plants, trees or shrubs, and then their data have not been included either in Table 4. Although some significant correlations ($P<0.05$) were indeed found between physicochemical parameters and diversity indexes for all three categories included in Table 4 (all the plant species considered as a whole, ferns and herbaceous plants), strong significant correlations ($P<0.01$) were only detected for herbaceous plants (a strong positive correlation between soil

303 pH and both species richness and Shannon's diversity) and when all the plant species were
304 considered as a whole (a strong positive correlation between soil pH and species richness).
305 Ferns also showed a significant, though not strong, positive correlation between soil pH and
306 species richness and Shannon's diversity. In addition, for ferns, a significant, though not
307 strong, negative correlation was found between species richness and OM, total N and K^+
308 content (in turn, ferns also showed a significant, though not strong, negative correlation
309 between Shannon´s diversity and OM and total N content). There was a strong positive
310 correlation between Ca^{2+} content and richness of herbaceous plants. Calcium content was also
311 positively (not strongly) correlated with Shannon´s diversity for herbaceous plants and with
312 species richness when all the plant species were considered as a whole. Finally, a significant,
313 not strong, correlation was found between soil pH and Shannon´s diversity (positive) and
314 between OM content and species richness (negative) when all the plant species were
315 considered as a whole (Table 4).
316
317 3.3 Soil enzyme activities and physicochemical parameters
318
319 Table 5 shows the Pearson's correlation values obtained between some soil
320 physicochemical parameters (pH, OM, total N, C/N ratio, and P, Ca^{2+} and Mg^{2+} contents) and
321 soil enzyme activities and functional diversity (calculated through the application of
322 Shannon's diversity index to the seven enzyme activities here determined). Since no
323 correlations were found between the other soil physicochemical properties here determined
324 (i.e., particle size distribution and K^+ content) and soil enzyme activities or functional
325 diversity, their data have not been included in Table 5. Soil pH appeared strongly correlated
326 with three enzyme activities (i.e., amidase and arylsulphatase were positively correlated with
327 soil pH; acid phosphatase was negatively correlated with soil pH) and soil functional

328 diversity. Similarly, soil OM content was strongly and positively correlated with β-
329 glucosidase, acid and alkaline phosphatase, and urease, and negatively with amidase and
330 functional diversity. Total N was also strongly and positively correlated with β-glucosidase,
331 and acid and alkaline phosphatase, and negatively with amidase. C/N ratio was strongly and
332 positively correlated with acid phosphatase (and also correlated, though not strongly, with β-
333 glucosidase and alkaline phosphatase). A strong positive correlation was found between P
334 content and β-glucosidase and alkaline phosphatase. Finally, Ca^{2+} and Mg^{2+} contents were
335 strongly and positively correlated with amidase and arylsulphatase, respectively.
336
337 3.4 Soil enzyme activities and vegetation
338
339 Table 6 shows the Pearson's correlation values obtained between soil enzyme activities
340 and diversity indexes for all the plant species considered as a whole and also according to
341 growth forms (i.e., herbaceous plants and ferns). Again, no significant correlations ($P<0.05$*
342 or $P<0.01$**) were found between soil enzyme activities and diversity indexes for climbing
343 plants, trees or shrubs, and then their data have not been included in Table 6. In the case of
344 herbaceous plants, no strong significant correlations were found. By contrast, some strong
345 significant correlations ($P<0.01$) were found between enzyme activities and diversity indexes
346 for ferns (acid and alkaline phosphatase were negatively correlated with Shannon´s diversity;
347 dehydrogenase was positively correlated with species richness and Shannon´s diversity; acid
348 phosphatase was negatively correlated with species richness) and when all the plant species
349 were considered as a whole (dehydrogenase activity was positively correlated with species
350 richness; amidase was positively correlated with Shannon's diversity).
351
352 3.5 Soil functional diversity and vegetation

353

354 Table 7 shows the Pearson's correlation values obtained between soil functional diversity
355 (calculated through the application of Shannon's diversity index to the seven enzyme
356 activities here determined) and diversity indexes for all the plant species considered as a
357 whole and also according to growth forms (i.e., herbaceous plants and ferns). As above, no
358 significant correlations ($P<0.05$* or $P<0.01$**) were found between soil functional diversity
359 and diversity indexes for climbing plants, trees or shrubs, and therefore their data have not
360 been included in Table 7. Most interestingly, strong correlations were found between plant
361 Shannon´s diversity and soil functional diversity for all three categories (all the plant species
362 considered as a whole, herbaceous plants, ferns). There was also a strong correlation between
363 plant species richness and soil functional diversity for herbaceous plants and when all the
364 plant species were considered as a whole. Finally, soil functional diversity was positively
365 correlated, though not strongly, with ferns species richness.

366

367 **4. Discussion**

368

369 4.1 Vegetation structure

370

371 The specific plant species found in a forest have been reported to be good indicators of its
372 conservation condition as well as of the effect of disturbances and management practices on
373 such conservation (Ferris-Kaan et al., 1998; Kneeshaw et al., 2000). In the current study, most
374 of the plant species found in the studied stands are characteristic of Atlantic mixed-oak forests
375 (Aseginolaza et al., 1988; Aizpuru et al., 2000). Moreover, some of the species observed in
376 the undergrowth, e.g. Lamiastrum galeobdolon and Dryopteris affinis, due to their having a
377 small seed bank and being then difficult to maintain after certain management practices such
378 as clear-cutting of undergrowth (Amezaga and Onaindia, 1997), can be considered good

379 indicators of the degree of organization and naturalness of this kind of forests. In
380 consequence, the stands here studied appear to be in a good condition of conservation and are
381 thus suitable representatives of a mixed-oak forest ecosystem.
382
383 4.2 Vegetation and soil physicochemical parameters
384
385 In this study, for herbaceous plants, strong correlations ($P<0.01$) were found between soil
386 physicochemical parameters and plant diversity (e.g., soil pH and Shannon´s diversity). Ferns
387 showed several positive and negative, not strong ($P<0.05$), correlations between soil
388 physicochemical parameters and plant diversity. For the other three growth forms (climbing
389 plants, trees and shrubs), no similar correlations were found. This is most likely due to ferns
390 and herbaceous plants having their root systems within the area of the soil sampled in this
391 study (upper 0-15 cm). After all, soil physicochemical and biological properties are known to
392 change with soil depth (Agnelli et al., 2004). Trees, shrubs and climbing plants have usually
393 deeper root systems than ferns and herbaceous plants and, since plants are affected by the
394 characteristics of the soil around their roots, then the lack of correlations between soil
395 physicochemical parameters and plant diversity indexes for these three growth forms is not
396 surprising.
397 Regarding pH, many authors have also reported higher values of plant diversity at higher
398 soil pH values (Pärtel et al., 2004; Lenière and Houle, 2006). In addition, soil pH is known to
399 affect many enzyme activities involved in the mineralization of essential nutrients such as N,
400 S and P (Tabatabai and Bremner, 1970, 1972; Acosta-Martínez and Tabatabai, 2004). Finally,
401 the fact that, in herbaceous plants, similar correlations were found between plant diversity
402 indexes and both soil pH and Ca^{2+} content, is most likely due to the positive correlation
403 between pH and Ca^{2+} usually found in soil.

404

405 4.3 Soil enzyme activities and physicochemical parameters

406

407 Soil physicochemical properties, such as pH, OM content, total N, texture, etc. have
408 been reported to affect soil enzyme activities (Acosta-Martínez and Tabatabai, 2000). In our
409 study, soil pH, OM content, total N, C/N ratio, and P, Ca^{2+} and Mg^{2+} content have shown
410 strong significant correlations with some enzyme activities and, most importantly, with the
411 soil functional diversity calculated from those enzyme activities.

412 pH is indeed a very important soil property that affects, among other parameters, the
413 diversity and composition of the soil microbial community (Bardgett, 2005). In turn, soil pH
414 has been reported to affect the activity of soil enzymes through different mechanisms.
415 Ionization or protonation of the acidic or basic groups in the enzyme active center is thought
416 to account for most of the decrease in enzyme activity observed when pH deviates from
417 optimum (Wang et al., 2006). Similarly, soil pH can alter the concentration of inhibitors,
418 activators, and substrates (Wang et al., 2006). In agricultural soils, Acosta-Martínez and
419 Tabatabai (2000) found the same correlations between soil pH and acid phosphatase and
420 arylsulphatase here observed. Nonetheless, these authors also found a positive correlation
421 between soil pH and β-glucosidase and alkaline phosphatase. Dick et al. (2000) and Wang et
422 al. (2006) also found a negative correlation between pH and acid phosphatase. On the other
423 hand, others authors reported a positive correlation between acid phosphatase and soil pH
424 (Hinojosa et al., 2004; Klose et al., 2004). Many authors have also described a positive
425 correlation between arylsulphatase and soil pH (Hinojosa et al., 2004; Klose et al., 2004;
426 Wang et al., 2006).

427 In addition, we found positive correlations between OM content and β-glucosidase, acid
428 and alkaline phosphatase, and urease. Many studies have formerly described positive

429 correlations between β-glucosidase, arylsulphatase, phosphatase, amidase, urease, etc. with
430 organic C (Frankenberger and Tabatabai, 1981; Dick et al., 1988; Eivazi and Tabatabai, 1990;
431 Deng and Tabatabai, 1997; Šantrůčková et al., 2004). Turner et al. (2002) determined β-
432 glucosidase activity over a range of soils with different C contents and found a positive
433 correlation between this activity and total C content. OM content and C content are correlated
434 and then the results of Turner et al. (2002) also agree with our data. In agreement with our
435 results, Dick et al. (1988) also found a positive correlation between β-glucosidase and total N
436 content. The strong positive correlation found in our study between β-glucosidase and both
437 OM content and total N is not surprising, as soil organic C and N content in soils have been
438 reported to be correlated themselves (Acosta-Martínez and Tabatabai, 2001).

439 On the contrary, the negative strong correlations here reported between amidase and both
440 OM content and total N are somewhat unusual. Sowerby et al. (2005) found a remarkable
441 inverse exponential relationship between soil OM and β-glucosidase, sulphatase, phosphatase,
442 and leucine amino peptidase. These authors suggested that this inverse relationship could be
443 due to the inhibition of phenol oxidase in anoxic conditions (part of their study was carried
444 out in Wales where soils receive heavy rainfall and commonly experience water-logged
445 anoxic conditions). Indeed, phenolic compounds are thought to inhibit soil enzyme activities
446 and, in general terms, are found in greater quantities in soils with higher levels of OM
447 (Sowerby et al., 2005). But, in our case, only one enzyme (amidase) showed a negative
448 correlation with soil OM (besides, four different enzymes showed a strong positive
449 correlation with OM content). Therefore, further work is needed to explain the nature of this
450 negative relationships between amidase and OM content and total N.

451 The positive correlations here found between phosphatases and P content are unexpected
452 since increased available (soluble) inorganic phosphate is known to decrease soil phosphatase
453 activity (Tabatabai, 1982). In this respect, Naseby et al. (1998) found the activities of

454 enzymes from the P cycle (acid phosphatase, alkaline phosphatase, and phosphodiesterase) to
455 be negatively correlated with the amount of readily available P.

456 Regarding the correlations found between physicochemical parameters and soil
457 functional diversity (i.e., strong positive for pH; strong negative for OM content; negative for
458 total N; positive for Ca^{2+}), firstly, it is important to emphasize that, as abovementioned,
459 although Shannon´s diversity values (H') usually account for both species richness and
460 evenness, in this work, our H' values for soil functional diversity reflect only "evenness". Soil
461 is a very biodiverse, complex environment that (i) shows an inherent degree of fluctuation, (ii)
462 has several trophic levels, (iii) an overwhelming number of different species, and (iv) also
463 exhibits a high level of functional redundancy. As a consequence, it is always difficult to
464 interpret correlations such as those here described.

465

466 4.4 Interactions between aboveground vegetation and belowground enzymes and functional
467 diversity

468

469 Aboveground and belowground components of terrestrial ecosystems are implicitly
470 dependent on each other (Porazinska et al., 2003). For instance, the loss of plant species in a
471 certain ecosystem can lead to changes in the community of soil decomposers which, in turn,
472 affects the mineralization of OM with consequences for other ecosystem processes (Spehn et
473 al., 2000). Lately, an increasing number of reports are appearing in the literature on the
474 relationship between vegetation structure and the activity and diversity of soil biota (Johnson
475 et al., 2003; Porazinska et al., 2003; De Deyn et al., 2004; Salamon et al., 2004). Although,
476 occasionally, positive and negative correlations have been found between aboveground and
477 belowground components of terrestrial ecosystems, these correlations do not neccesarily

imply mechanistic linkages, but they are a first, fundamental step in assessing whether such linkages exist (Hooper et al., 2000).

The positive correlation here found between dehydrogenase and diversity indexes for ferns (strong) and herbaceous plants (not strong) could be due to the possibility that a higher plant diversity might have led to higher values of plant productivity, resulting in higher amounts of organic C entering the soil system. Although the relationship between plant species diversity and plant productivity is still controversial, there are many works in the literature reporting a higher productivity in species-rich than in species-poor plant communities (Tilman and Downing, 1994; Naeem et al., 1996; Tilman et al., 1996, 1997).

Ferns showed a strong negative correlation between acid phosphatase and S and H'. This negative correlation could be explained by the fact that, under favourable conditions (such as P sufficiency), plant communities regulated by competition are thought to have a lower biodiversity owing to competitive exclusion.

Most importantly, we have found a strong positive relationship between soil functional diversity and the diversity of ferns and herbaceous plants. The lack of correlations for the other three growth forms (climbing plants, trees, and shrubs) is again most likely due to our sampling procedure (see above). Although, in our study, the H' values for soil functional diversity reflect only evenness, interestingly enough, we have not found any correlations between plant evenness and soil functional diversity.

Stephan et al. (2000) found a positive correlation between plant diversity and soil microbial functional diversity in grasslands. These authors concluded that the increase in the amount and diversity of resources that entered the soil was responsible for the higher values of soil microbial functional diversity. Similarly, Benizri and Amiaud (2005) found a positive correlation between plant diversity and soil microbial functional diversity, inferring that it

was probably due to differences in the composition of the rhizosphere between plant species and also between different phenological stages within the same species.

Much further research is still needed to unravel the linkages that might exist between aboveground vegetation structure and belowground soil functional diversity. The understanding of the linkages between biodiversity aboveground and belowground may provide crucial information for the conservation of the soil ecosystem and the functions and services it freely bestows to humanity. After all, those linkages might be essential for the sustainable functioning of terrestrial ecosystem processes.

We conclude that there are strong positive and negative correlations between (i) soil physicochemical parameters and vegetation diversity, (ii) soil physicochemical properties and soil enzyme activities, (iii) soil physicochemical properties and soil functional diversity, (iv) vegetation diversity and soil enzyme activities, and, most importantly, (v) vegetation diversity and soil functional diversity. Although these correlations between vegetation diversity and soil functional diversity might possibly be due to the fact that higher values of plant richness and diversity result in a greater habitat heterogeneity in the soil, current knowledge on the topic is mixed and very incomplete and, then, one must be extremely cautious when interpreting such correlations. Certainly, much further research is still needed to understand the linkages that exist between aboveground vegetation and belowground soil communities.

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	Mean ± SEM	Interval
pH	4.9±0.1	4.1-5.6
Organic matter (%)	5.87±0.35	3.25-10.14
Total N (%)	0.23±0.01	0.15-0.37
C/N ratio	14.8±0.3	11.8-17.5
Coarse sand (%)	9.2±1.7	0.7-34.7
Fine sand (%)	34.2±2.1	16.7-52.1
Silt (%)	27.2±1.4	15.6-41.0
Clay (%)	29.4±1.7	17.6-49.1
P (mg kg^{-1})	3.6±0.3	2.0-6.0
K^+ (mg kg^{-1})	95±5	54-166
Ca^{2+} (mg kg^{-1})	707±124	160-1,940
Mg^{2+} (mg kg^{-1})	231±73	44-1,241

Table 1

Physicochemical properties of the 23 studied soils. Mean ± SEM (n=23). Interval: maximum and minimum values.

PLANT SPECIES	% COVER (Mean ± SEM)	PLANT SPECIES	% COVER (Mean ± SEM)
Herbaceous plants		**Trees**	
Ajuga reptans L.	1.45 ± 0.44	Acer campestre L.	2.78 ± 1.61
Anemone nemorosa L.	0.45 ± 0.39	Alnus glutinosa (L.) Gaertner	1.94 ± 1.52
Angelica sylvestris L.	1.18 ± 0.89	Arbutus unedo L.	1.39 ± 0.79
Arum italicum Miller	0.24 ± 0.14	Betula alba L.	3.42 ± 1.98
Cardamine pratensis L.	0.42 ± 0.34	Castanea sativa Miller	24.70 ± 5.77
Cardamine raphanifolia Pourret	0.25 ± 0.19	Fagus sylvatica L.	0.52 ± 0.43
Carex pendula Hudson	0.82 ± 0.52	Fraxinus excelsior L.	10.49 ± 3.07
Cirsium sp.	0.09 ± 0.06	Laurus nobilis L.	15.55 ± 7.39
Eupatorium cannabinum L.	0.07 ± 0.06	Prunus avium L.	1.79 ± 0.91
Euphorbia amygdaloides L.	1.82 ± 0.84	Quercus ilex L.	2.18 ± 1.46
Euphorbia dulcis L.	0.61 ± 0.44	Quercus robur L.	98.11 ± 5.12
Geranium robertianum L.	0.56 ± 0.24	Salix atrocinerea Brot.	8.64 ± 3.65
Glechoma hederacea L.	0.33 ± 0.19	**Shrubs**	
Helleborus viridis L.	0.12 ± 0.09	Calluna vulgaris (L.) Hull	0.79 ± 0.59
Hypericum pulchrum L.	0.34 ± 0.13	Cornus sanguinea L.	9.22 ± 3.40
Lamiastrum galeobdolon (L.) Ehrend. & Polatschek	4.13 ± 1.79	Corylus avellana L.	42.15 ± 8.73
Lathyrus linifolius (Reichard) Bässler	0.49 ± 0.16	Crataegus monogyna Jacq.	3.14 ± 1.74
Mercurialis perennis L.	0.07 ± 0.06	Daboecia cantabrica (Hudson) C. Koch	1.13 ± 0.51
Oxalis acetosella L.	0.73 ± 0.45	Erica vagans L.	0.25 ± 0.14
Picris sp.	0.04 ± 0.03	Euonymus europaeus L.	1.06 ± 0.66
Poaceae Barnhart (Gramineae Juss.)	42.36 ± 6.09	Frangula alnus Miller	2.65 ± 0.97
Potentilla erecta (L.) Raeuschel	1.01 ± 0.60	Hypericum androsaemum L.	1.95 ± 0.61
Potentilla sterilis (L.) Garcke	0.68 ± 0.33	Ilex aquifolium L.	2.06 ± 1.46
Pulmonaria longifolia (Bast,) Boreau	0.55 ± 0.38	Ligustrum vulgare L.	1.97 ± 1.68
Ranunculus tuberosus Lapeyr.	1.19 ± 0.50	Prunus spinosa L.	0.97 ± 0.94
Rubia peregrina L.	3.04 ± 0.99	Rosa sp.	8.41 ± 2.07
Rumex sp.	0.19 ± 0.13	Rubus sp.	54.37 ± 5.88
Saxifraga hirsuta L.	0.58 ± 0.41	Ruscus aculeatus L.	3.70 ± 1.48
Solidago virgaurea L.	0.82 ± 0.37	Ulex sp.	1.39 ± 0.65
Stachys officinalis (L.) Trevisan	4.86 ± 1.44	**Ferns**	
Stellaria holostea L.	0.67 ± 0.51	Asplenium adiantum-nigrum L.	0.67 ± 0.30
Symphytum tuberosum L.	1.36 ± 0.63	Asplenium scolopendrium L.	0.27 ± 0.15
Teucrium scorodonia L.	6.77 ± 1.84	Athyrium filix-femina (L.) Roth	8.49 ± 3.06
Vicia sepium L.	0.97 ± 0.45	Blechnum spicant (L.) Roth	11.95 ± 3.04
Viola riviniana Reichenb.	3.20 ± 0.99	Dryopteris affinis (Lowe) Fraser- Jenkins	9.54 ± 2.81
Climbing plants		Dryopteris carthusiana (Vill.) H. P. Fuchs	0.15 ± 0.12
Clematis vitalba L.	3.24 ± 3.09	Polystichum setiferum (Forsskål) Woynar	7.48 ± 2.46
Hedera helix L.	52.67 ± 5.42	Pteridium aquilinum (L.) Kuhn	22.24 ± 4.89
Lonicera periclymenum L.	19.30 ± 3.04		
Smilax aspera *L.*	20.62 ± 4.88		
Tamus communis *L.*	4.84 ± 2.90		

Table 2

Plant species composition and percentage cover (mean ± SEM; n=23) of the plant species found in the 23 studied stands of native mixed-oak forest. Only those species found in at least more than one stand have been included in this Table.

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Growth form	S Mean ± SEM	H' Mean ± SEM	J' Mean ± SEM
Herbaceous plants	7.43±0.82	1.76±0.17	0.64±0.03
Climbing plants	3.17±0.16	1.16±0.11	0.69±0.05
Trees	4.26±0.28	1.29±0.11	0.64±0.03
Shrubs	5.52±0.28	1.66±0.08	0.69±0.03
Ferns	3.43±0.39	1.28±0.15	0.80±0.03
Total	**23.83±1.16**	**3.59±0.06**	**0.79±0.01**

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738 Table 3

739 Diversity indexes calculated for all the plant species considered as a whole (total) and also according to growth forms (i.e., herbaceous plants,
740 climbing plants, trees, shrubs, and ferns) in the 23 studied stands of native mixed-oak forest. S: species richness; H': Shannon's diversity; J':
741 Shannon's evenness.

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		TOTAL			HERBACEOUS PLANTS			FERNS		
		S	**H'**	**J'**	**S**	**H'**	**J'**	**S**	**H'**	**J'**
pH	R	0.732**	0.519*	-0.311	0.772**	0.672**	0.201	0.446*	0.419*	0.047
	P	<0.001	0.011	0.148	0.000	0.000	0.370	0.033	0.046	0.839
OM (%)	R	-0.451*	-0.469	-0.135	-0.256	-0.095	0.100	-0.479*	-0.522*	-0.321
	P	0.031	0.024	0.538	0.238	0.667	0.659	0.021	0.011	0.157
Total N (%)	R	-0.394	-0.409	-0.167	-0.217	-0.038	0.130	-0.427*	-0.466*	-0.235
	P	0.063	0.053	0.446	0.321	0.863	0.564	0.042	0.025	0.305
K^+ (mg kg^{-1})	R	-0.191	-0.203	-0.162	0.052	0.094	0.097	-0.415*	-0.300	-0.065
	P	0.393	0.352	0.461	0.815	0.669	0.667	0.049	0.164	0.780
Ca^{2+} (mg kg^{-1})	R	0.434*	0.336	-0.139	0.574**	0.419*	0.073	0.076	-0.004	-0.261
	P	0.039	0.117	0.528	0.004	0.047	0.748	0.729	0.985	0.253

Table 4

Pearson's correlations between soil physicochemical parameters and diversity indexes for all the plant species considered as a whole (total) and also according to growth forms (i.e., herbaceous plants and ferns). S: species richness; H': Shannon's diversity; J': Shannon's evenness. R: Pearson's coefficient of correlation; P: significance; *P<0.05; **P<0.01.

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		β-glucosidase	Acid phosphatase	Alkaline phosphatase	Urease	Amidase	Dehydrogenase	Arylsulphatase	Soil H'
pH	R	-0.030	-0.630**	-0.315	-0.075	0.577**	0.479*	0.553**	0.706**
	P	0.890	0.001	0.143	0.735	0.004	0.021	0.006	0.000
OM (%)	R	0.723**	0.762**	0.944**	0.532**	-0.670**	-0.300	-0.022	-0.570**
	P	0.000	0.000	0.000	0.009	0.000	0.165	0.919	0.005
Total N (%)	R	0.658**	0.647**	0.861**	0.464*	-0.584**	-0.308	0.063	-0.442*
	P	0.001	0.001	0.000	0.026	0.003	0.153	0.776	0.035
C/N	R	0.439*	0.530**	0.474*	0.334	-0.346	-0.105	-0.087	-0.386
	P	0.036	0.009	0.022	0.120	0.106	0.634	0.693	0.069
P (mg kg^{-1})	R	0.620**	0.487*	0.705**	0.449*	-0.526*	-0.285	0.141	-0.323
	P	0.002	0.018	0.000	0.032	0.010	0.188	0.521	0.133
Ca^{2+} (mg kg^{-1})	R	0.167	-0.417*	-0.126	-0.067	0.565**	0.098	0.504*	0.479*
	P	0.446	0.048	0.565	0.760	0.005	0.658	0.014	0.021
Mg^{2+} (mg kg^{-1})	R	0.433*	-0.160	0.242	0.122	0.272	0.066	0.648**	0.396
	P	0.039	0.465	0.265	0.581	0.209	0.767	0.001	0.062

752 Table 5

753 Pearson's correlations between soil physicochemical parameters and soil enzyme activities and soil functional diversity (soil H'). R: Pearson's

754 coefficient of correlation; P: significance; *P<0.05; **P<0.01.

		TOTAL			HERBACEOUS PLANTS			FERNS		
		S	**H'**	**J'**	**S**	**H'**	**J'**	**S**	**H'**	**J'**
β-glucosidase	R	-0.047	0.068	0.117	0.140	0.143	0.138	-0.406	-0.463*	-0.363
	P	0.831	0.758	0.594	0.523	0.515	0.539	0.054	0.026	0.106
Acid phosphatase	R	-0.487*	-0.377	0.058	-0.453*	-0.392	-0.266	-0.594**	-0.587**	-0.113
	P	0.019	0.076	0.793	0.030	0.064	0.232	0.003	0.003	0.627
Alkaline phosphatase	R	-0.324	-0.347	-0.141	-0.125	0.049	0.165	-0.492*	-0.534**	-0.374
	P	0.131	0.105	0.522	0.570	0.826	0.464	0.017	0.009	0.095
Urease	R	-0.012	-0.025	-0.113	0.006	-0.008	-0.115	-0.215	-0.239	-0.230
	P	0.956	0.911	0.607	0.979	0.971	0.611	0.325	0.272	0.316
Amidase	R	0.471*	0.606**	0.353	0.388	0.282	0.249	0.400	0.392	0.160
	P	0.023	0.002	0.098	0.068	0.192	0.264	0.059	0.064	0.490
Dehydrogenase	R	0.592**	0.325	-0.295	0.500*	0.431*	0.183	0.635**	0.628**	0.169
	P	0.003	0.130	0.172	0.015	0.040	0.414	0.001	0.001	0.463
Arylsulphatase	R	0.387	0.435*	0.100	0.441*	0.493*	0.449*	0.237	0.282	-0.061
	P	0.068	0.038	0.649	0.035	0.017	0.036	0.276	0.193	0.794

Table 6

Pearson's correlations between soil enzyme activities and diversity indexes for all the plant species considered as a whole (total) and also according to growth forms (i.e., herbaceous plants and ferns). S: species richness; H': Shannon's diversity; J': Shannon's evenness. R: Pearson's coefficient of correlation; P: significance; *P<0.05; **P<0.01.

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763

		TOTAL			HERBACEOUS PLANTS			FERNS		
		S	**H'**	**J'**	**S**	**H'**	**J'**	**S**	**H'**	**J'**
Soil H'	R	0.589**	0.630**	0.116	0.560**	0.545**	0.373	0.470*	0.554**	0.113
	P	0.003	0.001	0.597	0.005	0.007	0.087	0.023	0.006	0.625

764

765 Table 7

766 Pearson's correlations between soil functional diversity (soil H') and diversity indexes for all the plant species considered as a whole (total) and

767 also according to growth forms (i.e., herbaceous plants and ferns). S: species richness; H': Shannon's diversity; J': Shannon's evenness. R:

768 Pearson's coefficient of correlation; P: significance; *P<0.05; **P<0.01.