Diversity and Distributions, (Diversity Distrib.) (2017) **23**, 209–220

BIODIVERSITY RESEARCH

Spatial congruence between taxonomic, phylogenetic and functional hotspots: true pattern or methodological artefact?

Iker Pardo[1]*, Cristina Roquet[2], Sébastien Lavergne[2], Jens M. Olesen[3], Daniel Gómez[1] and María B. García[1]

[1]*Instituto Pirenaico de Ecología (IPE-CSIC), Zaragoza-Jaca, PO Box 13034 Zaragoza, Spain* [2]*Laboratoire d'Écologie Alpine (LECA), CNRS - Université Grenoble Alpes (UMR 5553), F-38000 Grenoble, France* [3]*Department of Bioscience, Aarhus University, Ny Munkegade Building 1540, DK-8000 Aarhus C, Denmark*

*Correspondence: Iker Pardo, Instituto Pirenaico de Ecología (IPE-CSIC), Zaragoza-Jaca, PO Box 13034 Zaragoza, Spain.
E-mail: ik.pardo.g@gmail.com

ABSTRACT

Aim To assess the spatial congruence between hotspots based on taxonomic, phylogenetic and functional diversity, after accounting for the correlation between diversity metrics, and the spatial scale and sampling completeness of data.

Location The Ordesa and Monte Perdido National Park (Central Pyrenees, Spain), a species-rich area subjected to intensive botanical sampling.

Methods We selected hotspots using different diversity metrics and two different data sources (~49,000 occurrence records of 1379 vascular plants in 1 × 1 km grid cells and 1218 inventories of plant communities containing a total of 859 taxa) and compared their spatial congruence. The effect of sampling completeness of data was explicitly assessed. Phylogenetic diversity and functional diversity (measured with richness-dependent and richness-independent metrics) were based on a molecular phylogeny, and a functional dendrogram, respectively. The effectiveness of different types of hotspots in representing other diversity components was tested with permutation tests.

Results We found that spurious correlations between diversity metrics explained the congruence between taxonomic, phylogenetic and functional hotspots. When richness-independent metrics were used, diversity hotspots were no longer congruent regardless of the source of data. Hotspots were biased towards intensively sampled grid cells, and the amount of diversity they captured was exaggerated due to the coarse spatial scale of species-occurrence data. The efficiency of hotspots in terms of integrating different diversity components was lower at community scale and not significantly higher than expected at random, regardless of the sampling completeness.

Main conclusions Our results stress that the arbitrary use of diversity metrics and the scale of analyses along with the sampling bias in data can distort the true location of hotspots, and exaggerate their spatial congruence. After accounting for such methodological issues, we found a clear mismatch between diversity components that questions the utility of hotspots as a conservation tool of multiple diversity components.

Keywords
biodiversity database, functional traits, National Park, phylogeny, plant records, spatial bias.

INTRODUCTION

'Agony', 'crisis' and 'emergency' are terms repeatedly used in the scientific literature to depict the current status of biodiversity. In the face of this alarming scenario and limited conservation resources, priority is often given to hotspots, defined broadly as exceptionally rich areas containing a large number of species within a relatively small area (Myers,

DOI: 10.1111/ddi.12511
http://wileyonlinelibrary.com/journal/ddi

A Journal of Conservation Biogeography
Diversity and Distributions

1988). However, hotspots of species richness (SR) do not always capture other traditional conservation targets (e.g. threatened species or, endemisms) if the spatial distribution of target species and SR is not congruent (Prendergast *et al.*, 1993; Orme *et al.*, 2005; Ceballos & Ehrlich, 2006). Yet, it is unclear whether SR hotspots also present large gaps in the representation of other diversity components such as evolutionary or functional ones, whose relevance for biodiversity conservation is increasingly recognized (Winter *et al.*, 2013). Several authors have shown that maintaining high levels of phylogenetic diversity (i.e. the amount of evolutionary differences between species based on a phylogeny; PD; Faith, 1992) is not only crucial for preserving the evolutionary potential of diversity (Mace *et al.*, 2003; Forest *et al.*, 2007), but also for reducing the loss of evolutionary history, because extinction is phylogenetically non-random (Purvis *et al.*, 2000). Other contributions have emphasized instead the importance of functional diversity (FD), defined as trait complementarity between species (Tilman, 2001), in determining ecosystem functioning (Díaz & Cabido, 2001; Cadotte *et al.*, 2011). Although PD is a good surrogate of FD when target traits have evolved under the pattern of the common ancestor (i.e. when species retain their ancestral traits; e.g. Flynn *et al.*, 2011), this is not always so (e.g. Prinzing *et al.*, 2008; Pavoine *et al.*, 2013), and it is therefore advisable to measure FD directly from trait data (Cadotte *et al.*, 2013).

While several studies have evidenced spatial mismatches between SR, PD and FD in fish, birds, mammals and plants (Forest *et al.*, 2007; Devictor *et al.*, 2010; Mouillot *et al.*, 2011), some others have not (Rodrigues & Gaston, 2002; Sechrest *et al.*, 2002; López-Osorio & Miranda-Esquivel, 2010). The spatial mismatch and congruence between diversity components are often attributed to ecological mechanisms and/or historical events (Orme *et al.*, 2005; Davies & Buckley, 2011; Fritz & Rahbek, 2012). However, the causes for divergent results may be multiple, including methodological ones. In fact, not all results from previous PD and FD studies are comparable, because they are based on different phylogenetic and functional metrics (Winter *et al.*, 2013). For instance, we may expect a spatial overlap between PD, FD and SR when phylogenetic and functional metrics are richness-dependent (Pavoine *et al.*, 2013). Another methodological issue affecting the degree of overlap between different diversity components is the spatial scale (i.e. the size of units used in analysis; Curnutt *et al.*, 1994; Reid, 1998), because richness patterns are often scale-dependent (e.g. Rahbek, 1995). Finally, the spatial congruence between diversity components may also be contingent upon the quality and quantity of distributional data (Rodrigues *et al.*, 2011). Species richest areas inferred from species-occurrence data tend to be biased towards well-sampled ones (Hortal *et al.*, 2007; Boakes *et al.*, 2010), but we do not know yet if other diversity components are also substantially biased. Therefore, it is unclear to what extent sampling biases can underlie the spatial congruence and mismatch between diversity congruence. Given the range of methodological issues that can potentially affect the outputs, it seems clear that we still need to improve and standardize methods before generating hypothesis about the spatial congruence and mismatch between diversity components.

In this study, we assess the spatial congruence between taxonomic, phylogenetic and functional diversity components in the Ordesa and Monte Perdido National Park (OMPNP; Central Pyrenees) and its implications for the utility of hotspots as a conservation tool of multiple diversity components. We examine the potential correlation between diversity metrics, and the effect of the spatial scale and sampling completeness on results using two data sources: species-occurrence data in grid cells of 1 × 1 km and a dataset based on local inventories of plant communities. We used the OMPNP as a case study, because aside from its extraordinary rich flora (nearly 20% of the Iberian Peninsula in only 0.07% of the territory), it has been subjected to intensive botanical sampling. In addition, the spatial resolution of data available is similar to that chosen for prioritization strategies, including hotspot-based ones, at small-scale elsewhere (Gjerde *et al.*, 2004; Laguna *et al.*, 2004).

METHODS

Study site

The OMPNP (42°N, 0°E) extends over a topographically complex area of 35,000 ha (including the buffer area) in the Central Pyrenees, with an elevational range between 700 and 3354 m. The main bedrock type is limestone, but flysch and sandstone outcrops are relatively abundant all across the National Park. Main habitats are, in order of decreasing abundance, grasslands, most of which have traditionally been used for summer pasturing; rocky habitat, including rocky grasslands, screes and cliffs; coniferous forests dominated by *Abies alba*, *Pinus sylvestris* or *P. uncinata;* deciduous forests, including those dominated by *Fagus sylvatica,* and mixed ones; Mediterranean forests, mainly dominated by *Quercus ilex*; and shrublands. Other habitats such as wetlands and anthropogenic habitat (vegetation occurring along pathways) cover < 1% of the OMPNP (see Appendix S1 in Supporting Information).

Plant distribution data

All analyses were separately conducted on the basis of two information sources: species-occurrence data in grid cells and local inventories of plant communities. The former consists of ~49,000 records of species and subspecies of vascular plants (ferns, gymnosperms and angiosperms) obtained from herbarium collections and inventories and aggregated in sampling units of 1 × 1 km (http://proyectos.ipe.csic.es/floragon/index.php). More than 95% of these records were gathered in the last two decades. Although the OMPNP stands out in terms of density of plant records in the Iberian

Peninsula (Font *et al.*, 2010), the knowledge about the spatial distribution of taxa (including species and subspecies) is still incomplete and spatially biased due to uneven sampling effort (i.e. some grid cells have been subjected to more intense sampling than others; Pardo *et al.*, 2013).

The second set of data was based on local inventories of plant communities collected following the phytosociological method, which were compiled from the SIVIM website (http://www.sivim.info/sivi/). We initially retrieved 1962 inventories, from which only 1218 were selected for analysis after filtering for taxonomic accuracy and source reliability. Most of these inventories (80%) were relatively recent (collected between 1990 and 2010), and their size ranged between 0.001 and 0.03 ha (median size was 0.004 ha). In the phytosociological sampling, the size of the inventory is associated with the density of species, for example it is, on average, larger in forests than in grasslands. For subsequent analyses, plant inventories were grouped into main habitat classes according to their syntaxonomy. Although inventories were performed in scattered localities (Fig. 1), main habitats were proportionally represented regarding their area (see Appendix S2).

Quantifying diversity components

Species richness was measured as the number of species and subspecies at each sampling unit (i.e. grid cells and inventories) and endemism richness (ER) as the number of taxa whose distribution is restricted to the Pyrenees. For PD estimation, we first generated a molecular phylogeny of the flora of the OMPNP resolved to the genus level, following Roquet *et al.* (2013). DNA sequences for 10 regions were downloaded from GenBank: three conserved regions (*matK*, *ndhF* and *rbcL*), plus seven regions less conserved that were clustered to the family or order level for the alignment (*atpB*, *ITS*, *psbA-trnH*, *rpl16*, *rps16*, *rps4-trnS* intergenic spacer, *trnL-F*). Alignment for each region was performed with three methods: KALING (Lassmann & Sonnhammer, 2005), MAFFT (Katoh *et al.*, 2005) and MUSCLE (Edgar, 2004). The best alignment was determined with MUMSA (Lassmann & Sonnhammer, 2006), checked visually with SEAVIEW (Gouy *et al.*, 2010) and depurated later on with TRIMAL software (Capella-Gutiérrez *et al.*, 2009). All regions were concatenated with FASconCAT (Kück & Meusemann, 2010). For phylogenetic inference, we conducted a maximum likelihood (ML) using RAXML (Stamatakis *et al.*, 2008) with the model GTR+Gamma, applying a supertree constraint at the family level on the basis of Davies *et al.* (2004) and Moore *et al.* (2010), and setting one partition for each DNA region. Node support was estimated using bootstrap values. Once the topology of the best ML tree was obtained, we dated the tree with penalized-likelihood as implemented in r8s (Sanderson, 2003) and used a wide range of fossil data to calibrate the tree (25 fossils extracted from Bell *et al.*, 2010; Smith *et al.*, 2010). Finally, we transformed polytomies at the genus level into dichotomies of branches of length zero at random with the *MULTI2DI* function in PICANTE (Kembel *et al.*, 2010).

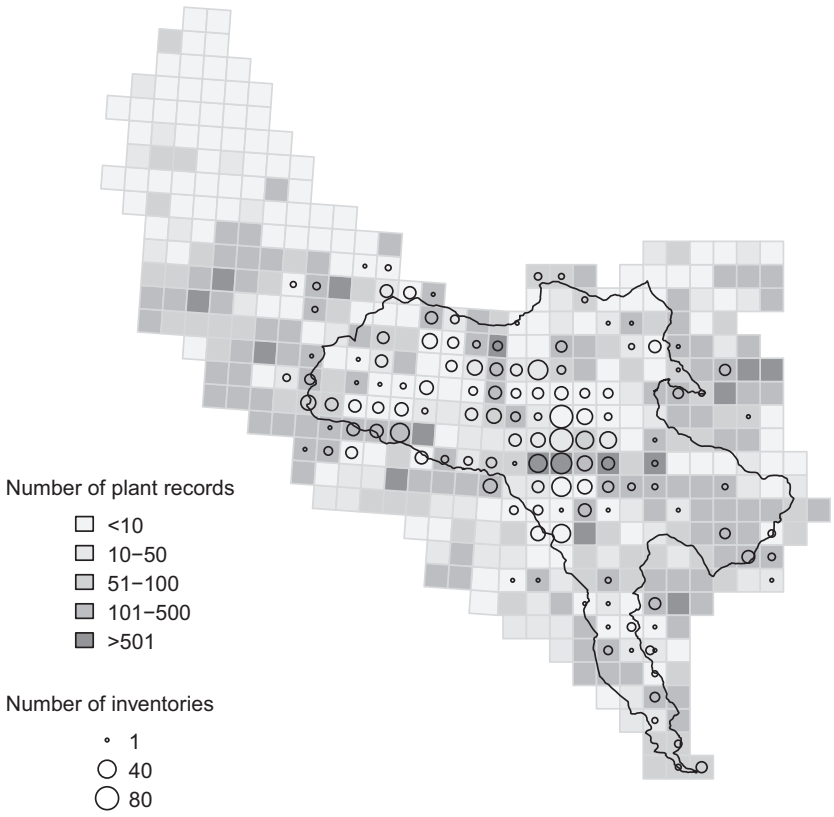

Figure 1 Distribution of plant records and inventories of plant communities across 1 × 1 km grid cells in the Ordesa and Monte Perdido National Park (core and buffer areas are separated by a black line).

On the basis of this phylogeny, we calculated PD as the sum of the branch lengths of the co-occurring taxa for each sampling unit (grid cells and inventories). Among existing metrics of PD, we selected the one by Faith (1992) because it is widely used in similar studies (Sechrest *et al.*, 2002; Forest *et al.*, 2007; Fritz & Rahbek, 2012), it provides a more robust basis for conservation than other metrics (Pio *et al.*, 2011), and it is probably the most intuitive one for interpretation. All phylogenetic analyses were carried out in R 3.3.0 (R Development Core Team, 2016) using PICANTE (Kembel *et al.*, 2010), APE (Paradis *et al.*, 2004) and GEIGER (Harmon *et al.*, 2008) R-packages.

Functional diversity was estimated on the basis of eight traits related to life history (Raunkiaer's life form, life span), plant propagation, dispersal syndrome, pollination system, sexual expression, inflorescence architecture and floral colour (Table 1), plus regional mean population size of adults (a few individuals; < 25 individuals; < 100 individuals; < 1000 individuals; and > 1000 individuals). Trait information was compiled from the literature and online databases (Table 1). Taxa with no trait information (< 10%) were excluded from calculations of FD. Correlation of traits along the phylogeny (i.e. phylogenetic signal) was tested with '*phylo.signal.disc*' function, a phylogenetic permutation test written in R by E. Rezende, which indicated that all traits were significantly correlated ($P < 0.05$). Following Petchey & Gaston (2002), we calculated functional distance based on Gower's metric (Gower, 1971) and performed a hierarchical clustering analysis to produce a functional dendrogram using *daisy* (Maechler *et al.*, 2013) and HCLUST R-functions, respectively. Next, we used *treedive* function in the VEGAN R-package (Oksanen *et al.*, 2016) to calculate FD of sampling units as the sum of the total branch lengths connecting recorded along the dendrogram (Petchey & Gaston, 2002).

These phylogenetic and functional metrics are not independent from SR (Pavoine *et al.*, 2013). To measure richness-independent phylogenetic and functional diversity, we performed quadratic models between SR and PD, and FD, respectively, and used residuals of these models (PD_R and FD_R; Davies *et al.*, 2008; Devictor *et al.*, 2010; Fritz & Rahbek, 2012; see Appendix S3). As model residuals were not spatially correlated, we did not consider models with autocorrelation structures.

Table 1 Description of biological and ecological traits used for the calculation of functional diversity.

Trait	Description	Categories	Source
Raunkiaer's life form	Position of renewal buds during unfavourable seasons for growing	Terophythe; Geophyte; Hemicryptophyte; Chamaephyte; Phanerophyte	1
Life span		Annual; short-lived (< 5 year); long-lived (≥ 5 year)	1
Plant propagation	Main system of recruiting new individuals	Sexual; Vegetative; Mixed	1, 2
Dispersal syndrome	Seed dispersal agent according to morphological features	Autochory; Endochory; Exochory; Anemochory assumed due to small seed size (<1 mm and without special morphological characters); None	1, 3, 4
Pollination system	Flower shape was used as a proxy of insect accessibility	Insect and wind pollination; insect pollination (flowers can only be pollinated by specialized insects); no insect pollination	1, 5
Sexual expression	Spatial pattern of male and female organs	Complex; Dioecious; Hermaphroditic; Monoecious	1, 5
Inflorescence architecture	Abundance and arrangement of flowers in the inflorescence	Dense; Specialized; Inconspicuous; Lax; Solitary	1, 5
Floral colour		Colourless; White; Yellow; Blue; Pink; Red; Multiple colours	1, 5

Source: (1) Knowledge of authors and online databases: http://atlasflorapyrenaea.org/florapyrenaea/index.jsp, and http://proyectos.ipe.csic.es/floragon/index.php; (2) Klimeš *et al.* (1997); (3) Poschlod *et al.* (2003); (4) Kleyer *et al.* (2008); (5) Kuhn *et al.* (2004).

Spatial congruence between hotspots and their utility for conservation

Hotspots were initially defined as the top 5% sampling units ($n = 16$ grid cells, and $n = 64$ inventories) of each diversity component (SR, ER, PD, FD, PD_R and FD_R). The spatial congruence between different types of hotspots was measured as

$$\frac{A \cap B \cap C \cap D}{A + B + C + D} \quad (1)$$

where A, B, C and D are the set of hotspots of each diversity component. Dividend was substituted by (A∩B∩C) and (A∩B) to calculate the overlap between all possible combinations of three and two types of hotspots, respectively.

We evaluated the utility of hotspots as a conservation tools in terms of their representation of multiple diversity components, by comparing the percentage of each diversity component captured at each type of hotspots with that found in the same number of sampling units selected at random. Differences between observed diversity values in hotspots and those expected at random were contrasted at the

0.05 significance level with a permutation test (1000 iterations). To assess the consistency of results regarding the percentage of sampling units selected as hotspots (hereafter hotspot definition criterion), all analyses were repeated by gradually relaxing the criterion from 5% to 30% top sampling units.

The effect of sampling completeness

We estimated the sampling completeness in grid cells following Pardo *et al.* (2013), as the first derivative of a generalized additive model fitted to randomized species accumulation curves at the end of the curve. For the sake of interpretation, values obtained with this procedure were rescaled by subtracting initial values from one so that values close to one indicate almost complete sampling. The relationship between estimates of sampling completeness and diversity was then tested by means of quantile regression (Koenker & Bassett, 1978). This method was applied to parse out the strength of spatial biases in data across quantiles of interest (Cade & Noon, 2003), which in the case of this study are the highest ones (0.8, 0.9, 0.95). Quantile regressions with bootstrapped standard errors were performed with *qr* function from QUANTREG R-package (Koenker, 2016),

Inventories are virtually complete samples of plant communities; however, this source of information was incomplete in the sense that not all communities and taxa of the OMPNP were included. To assess whether our incomplete knowledge about plant diversity affected the consistence of our results, we repeated analyses with three subdatasets created by selecting 75%, 50% and 25% of total inventories at random (see Appendix S4 for further details).

RESULTS

Species-occurrence data

Seventy percentage of the 321 grid cells included in the complex topography of the OMPNP contained plant records. After filtering for synonyms, we listed 1379 taxa (3% ferns, 1% gymnosperms, 96% angiosperms), of which 73 (5%) were endemic to the Pyrenees. Phylogenetic and functional trees were based on 98% of these taxa (see Appendix S5). Values of SR, ER, PD and FD were highly correlated to each other (Spearman coefficient > 0.77; Table 2), and their spatial distribution was similar (Appendix S1). As SR significantly explained the variation in PD and FD across grid cells ($r^2 = 0.95$, P-value < 0.001; $r^2 = 0.98$, P-value < 0.001, respectively; see Appendix S3), these metrics provided almost the same values of diversity and identical selection of hotspots. We therefore choose to present results based on PD and FD in Appendix S6. Measures of PD_R and FD_R were instead uncorrelated with SR and ER (Table 2), and accordingly, their corresponding hotspots were no longer spatially congruent with each other (Fig. 2). This general mismatch between diversity hotspots was relatively consistent even if the percentage of grid cells considered as hotspots increased from 5% till 22% (i.e. 71 grid cells; Fig. 2).

The effect of sampling completeness on SR, ER and PD_R was significant (Fig. 3) and increased towards highest conditional quantiles, as indicated by increasing slopes of regression lines (Fig. 3; see coefficient and statistics of regressions in Appendix S7). Accordingly, the set of hotspots of these metrics were located in intensively sampled grid cells (sampling completeness above 0.95). In contrast, values of FD_R were not statistically related to sampling completeness (Fig. 3), and hotspots of FD_R were found in both poorly and excellently surveyed grid cells (sampling completeness values ranging from 0.75 to 1).

In spite of the spatial mismatch between hotspots, the amount of each diversity component captured in SR hotspots was high (> 74%) and, on average, 15% significantly higher than expected in hotspots selected at random (Fig. 4). Other types of hotspots included diversity components in a lower proportion and not always significantly higher than expected at random (Fig. 4). For instance, hotspots of PD_R did not efficiently capture any other diversity component, and FD_R and ER hotspots also failed in integrating endemism and phylogenetic diversity, respectively (Fig. 4). Importantly, the efficiency of hotspots for diversity representation was similar when the definition of hotspots was relaxed (Fig. 4).

Plant inventories

Inventories selected for analyses included 859 plant species (62% of total pool), 40 (55%) endemisms and 79% of the PD and FD known in the OMPNP. Most missing taxa were locally rare (occurring in < 1% of the territory). As observed

Table 2 Coefficients of Spearman correlation between species richness (SR), endemism richness (ER), phylogenetic diversity (PD), functional diversity (FD), richness-independent PD (PD_R) and richness-independent FD (FD_R) from two sources of data.

Species-occurrence data	SR	ER	PD	PD_R	FD	FD_R	Plant inventories
SR	–	0.15	0.87	−0.12	0.96	−0.05	SR
ER	0.81	–	0.07	−0.11	0.07	−0.29	ER
PD	0.98	0.77	–	0.34	0.91	0.19	PD
PD_R	0.13	−0.05	0.30	–	0.18	0.48	PD_R
FD	0.99	0.78	0.99	0.13	–	0.22	FD
FD_R	0.13	−0.11	0.25	0.77	0.23	–	FD_R

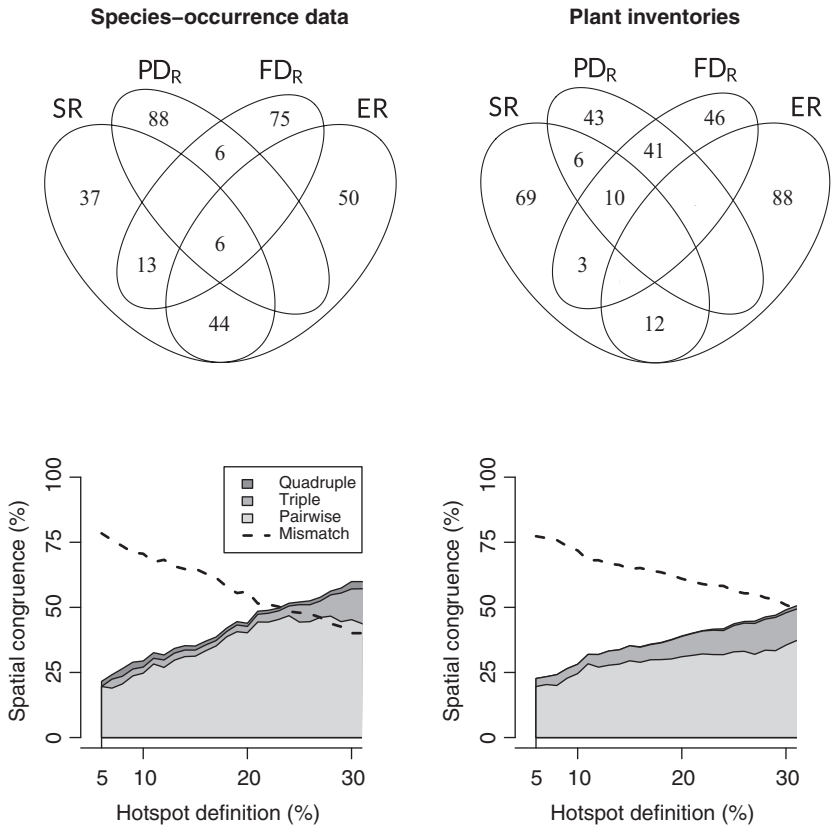

Figure 2 Spatial congruence between hotspots based on species richness (SR), endemism richness (ER) and richness-independent measures of phylogenetic and functional diversity (PD_R and FD_R, respectively) in the Ordesa and Monte Perdido National Park, according to two sources of information. Upper panel shows the spatial congruence between all possible combinations of hotspots defined as 5% top sampling units. Lower panel shows the percentage of overlap and mismatch between two, three and four types of hotspots by relaxing the definition criterion.

in grid cells, SR and PD and FD were correlated (Table 1), although in this case, the variance of PD explained by SR was lower ($r^2 = 0.71$, 1215 d.f., $P < 0.001$) than that of FD ($r^2 = 0.93$, 1215 d.f., P-value < 0.001). Consequently, PD complemented SR for hotspots identification when using this data source, whereas FD did not (for the sake of coherence with results based on species-occurrence data, results based on both PD and FD are shown in the Appendix S6).

Different types of hotspots were spatially non-congruent, except those based on PD_R and FD_R that partially overlapped (Fig. 2). Although the overlap between hotspots increased with the number of grid cells used for hotspot definition, by no means it was higher than the mismatch (Fig. 2). The spatial congruence was particularly low between hotspots based on SR and ER, even if more than 80% of hotspots were located in the same habitat, that is grasslands**.** Hotspots based on PD_R and FD_R were frequent in forests (87% and 67% of hotspots, respectively), especially in deciduous ones, where their spatial overlap was moderate (Fig. 2).

When using inventories, the representation of multiple diversity components in hotspots was between 25% and 42% lower than observed in hotspots in grid cells, and none of the different types of hotspots performed statistically better than expected at random (Fig. 4). Although the percentage of diversity incidentally captured in hotspots increased with the number of inventories considered as hotspots, it was not significantly different from that expected at random (Fig. 4). Very similar results were found regarding the spatial congruence and diversity representation of hotspots when the spatial overlap was inferred from subdatasets, and hence, results are not shown here (see Appendix S4).

DISCUSSION

Our study demonstrated that methodological aspects such as the choice of diversity metrics and spatial bias in species-occurrence data can determine the spatial congruence between taxonomic, phylogenetic and functional diversity hotspots. Analysing two sources of data of plant diversity in the OMPNP, we found a spurious spatial overlap between taxonomic, phylogenetic and functional hotspots, because the influence of SR overrode almost completely the contribution of the phylogeny and functional variability to PD and FD (Pavoine *et al.*, 2013). In contrast, the congruence between different types of hotspots in the OMPNP dissipated when richness-independent phylogenetic and functional metrics were used, regardless of the data source used and the number of sampling units considered for hotspot selection. Rodrigues & Gaston (2002) anticipated such redundancy between SR and PD metrics when phylogenies are balanced (i.e. similar ramification across branches), and this may apply to FD too. However, mathematical correlation should not be systematically discarded, unless it is explicitly tested. (Pavoine *et al.*, 2013). Indeed, such spurious correlation may be scale-dependent as in this study, which makes even harder to anticipate when richness-dependent PD and FD are certainly more informative than SR for conservation.

Geographical differences in sampling completeness clearly affected and confounded the identification of hotspots

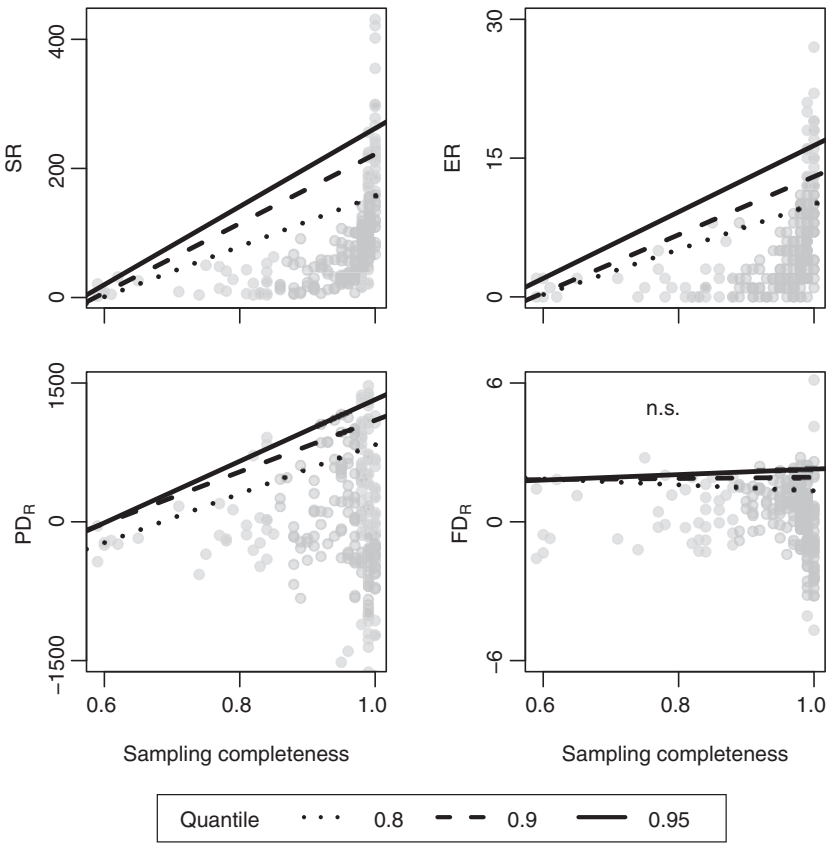

Figure 3 Quantile regression between values of sampling completeness and species richness (SR), endemism richness (ER) and richness-independent measures of phylogenetic and functional diversity (PD_R and FD_R, respectively). The effect of sampling completeness was significant ($P < 0.05$) across all diversity quantiles, except for FD_R. Grey dots show diversity records from species-occurrence data. See coefficients of regressions in Appendix S7.

(except those based on FD_R) from species-occurrence data, even though the OMPNP is one of the best prospected areas in the Iberian Peninsula (Font *et al.*, 2010). While such spatial biases have already been demonstrated in priority areas defined according to SR (Freitag & Jaarsveld, 1998; Guilhaumon *et al.*, 2008) and stressed elsewhere (Hortal *et al.*, 2015), this is the first empirical evidence showing that important areas for PD conservation may be misidentified too. Several alternatives have been suggested to cope with this kind of sampling bias**,** including the use of predictive models based on environmental variables to bridge existing gap in the diversity distribution (Hortal *et al.*, 2007). However, this approach might have been problematic, given that the difference regarding environmental variability (including habitat) was scarce across 1×1 km grid cells of the OMPNP (Elith & Leathwick, 2009). Another recurrent alternative is to restrict analyses to well-sampled units (Hortal *et al.*, 2015), although it is meaningless in the context of this study, where we demonstrated that even small differences in botanical sampling made the difference in terms of diversity between well-sampled grid cells.

A more certain assessment of hotspots was achieved instead using data from plant inventories. In this case, results were regardless of the completeness of the data, thus indicating that it was not necessary to explore further alternatives to overcome spatial biases as in the species-occurrence data. Our results stress the importance of grasslands and some types of forest in terms of multifaceted diversity in the OMPNP. Hotspots of SR and ER were mostly found in phylogenetically poor grasslands, indicating a higher abundance of recent and species-rich lineages in this habitat (Forest *et al.*, 2007; Davies & Buckley, 2011). We suggest that this pattern may be related to historical events, such as the vicariance and allopatric speciation associated with glacial–interglacial episodes throughout the Pleistocene in the Alpine arc (Tribsch, 2004). In turn, the concentration of hotspots of PD_R in forests, which were not particularly rich in terms of species, pointed out the co-occurrence of ancient and modern lineages in these habitats. As some Tertiary taxa evolved under a more humid climate than today (Barrón *et al.*, 2010), it is plausible that they find more suitable microsite conditions for persistence in certain forests than in more open habitats (De Frenne *et al.*, 2013). The partial congruence between PD_R and FD_R was probably due to the strong phylogenetic signal of traits considered in this study. However, the concentration of FD_R in forests may still suggest that environmental filtering in these habitats was less severe than at high-elevation grasslands, where harsh environmental conditions and the long grazing history might have exerted a strong selection on life-history traits and plant propagation strategies (de Bello *et al.*, 2013).

Beyond the ecological significance, the spatial mismatch between diversity components has important practical implications for conservation. The utility of hotspots as a conservation tool has often been evaluated according to the degree of overlap between diversity components (Prendergast *et al.*,

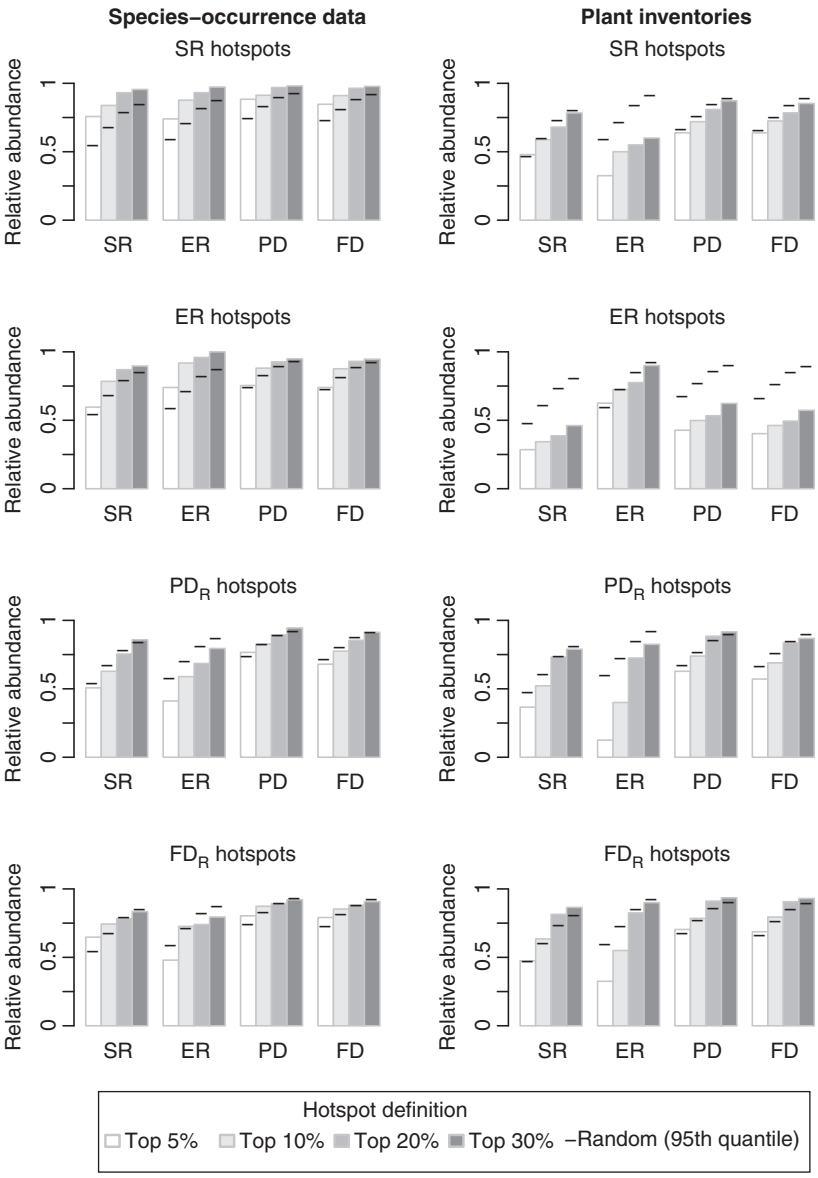

Figure 4 Percentage of each diversity component represented in hotspots based on based on species richness (SR), endemism richness (ER) and richness-independent measures of phylogenetic and functional diversity (PD_R and FD_R, respectively). Observed diversity values were contrasted with those expected at random with a permutation test (n = 1000) at the 0.05 significance level, to assess the efficiency of each type of hotspot to include other diversity components.

1993; Brooks *et al.*, 2006). However, our results from species-occurrence data demonstrate that a spatial mismatch between different hotspots does not necessarily translate into a poor representation of diversity components (see also Rodrigues & Gaston, 2002). This may be the case when the scale of analyses (i.e. the size of the sampling unit) is too coarse relative to the extent of the study area and involves a large topographic complexity too so that large amounts of diversity are captured. In this study, for example, more than 30% of the taxa and between 40 and 55% of existing endemisms, PD and FD were found in just a single grid cell of 1 × 1 km (< 1% of the study area). Under such scenario, prioritization efforts focused on diversity representation (e.g. hotspots) may be trivial, as almost virtually any selection of sites may capture diversity extremely well. In contrast, the amount of multiple diversity components captured by any type of hotspots inferred from plant inventories was much lower and not significantly higher than if we had selected priority areas at random. We are aware that the use of hotspots as a conservation tool should consider other socioeconomic and ecological aspects (e.g. threats and/or land-use conflicts) neglected in this study (Margules & Pressey, 2000; Cardawine *et al.* 2009). However, under the strong protection regime of a National Park, the representation of biodiversity often constitutes the ultimate goal (Schwartz, 1999 and references herein), and in this regard, the use of hotspots based on a single diversity component might be of limited use.

In summary, our results highlight the importance of the right diversity metrics and assessing the quality of distributional data for an accurate identification of hotspots of multiple diversity components. Previous studies may need some critical revision regarding the potential effects of these methodological aspects that may mask true diversity patterns, before making general predictions about the spatial

mismatch between diversity components. After accounting for the spurious correlation between metrics, and spatial sampling bias in data, our results show that multiple diversity components might not be efficiently captured in hotspots based on the richness of taxa or endemisms. Thus, small reserves designed to protect areas with elevated number of taxa or other target species (e.g. Gjerde *et al.*, 2004 see references herein; Laguna *et al.*, 2004) should be reviewed and ideally complemented with outstanding areas of other diversity components such as phylogenetic and functional ones. Otherwise, we would risk leaving out from protection meaningful components of diversity.

ACKNOWLEDGEMENTS

We are grateful to the many researcher and anonymous 'flower hunters' who have contributed to the knowledge of the Pyrenean flora and the staff of the Jaca Herbarium and SIVIM for their invaluable effort in making this information available. We also thank M.P. Pata, S. Pironon, J. Villellas, J. Arroyo, R. Molina, F. Rodríguez for discussion on early stages of this research and J. Bolliger, R.J. Ladle and several anonymous referees for providing constructive comments. This study was supported by two National Parks projects 018/2008 and 430/211 to M.B.G. I.P. was supported by a Doctoral Grant (FPU AP2008-01449), and J.M.O. was supported by a grant from the Danish Science Research Council. C.R. and S.L. received funding from the European Research Council within the European Community's Seven Framework Programme FP7/2007-2013 Grant Agreement no. 281422 (TEEMBIO).

REFERENCES

Barrón, E., Rivas-Carballo, R., Postigo-Mijarra, J.M., Alcalde-Olivares, C., Vieira, M., Castro, L., Pais, J. & Valle-Hernández, M. (2010) The Cenozoic vegetation of the Iberian Peninsula: a synthesis. *Review of Palaeobotany and Palynology*, **162**, 382–402.

Bell, C.D., Soltis, D.E. & Soltis, P.S. (2010) The age and diversification of the angiosperms re-revisited. *American Journal of Botany*, **97**, 1296–1303.

de Bello, F., Lavore, l.S., Lavergne, S., Albert, C.H., Boulangeat, I., Mazel, F. & Thuiller, W. (2013) Hierarchical effects of environmental filters on the functional structure of plant communities: a case study in the French Alps. *Ecography*, **36**, 393–402.

Boakes, E.H., McGowan, P.J.K., Fuller, R.A., Chang-qing, D., Clark, N.E., O'Connor, K. & Mace, G.M. (2010) Distorted views of biodiversity: spatial and temporal bias in species occurrence data. *PLoS Biology*, **8**, e1000385.

Brooks, T.M., Mittermeier, R.A., da Fonseca, G.A., Gerlach, J., Hoffmann, M., Lamoreux, J.F., Mittermeier, C.G., Pilgrim, J.D. & Rodrigues, A.S.L. (2006) Global biodiversity conservation priorities. *Science*, **313**, 58–61.

Cade, B.S. & Noon, B.R. (2003) A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*, **1**, 412.

Cadotte, M.W., Carscadden, K. & Mirotchnick, N. (2011) Beyond species: functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology*, **48**, 1079–1087.

Cadotte, M., Albert, C.H. & Walker, S.C. (2013) The ecology of differences: assessing community assembly with trait and evolutionary distances. *Ecology Letters*, **16**, 1234–1244.

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, **25**, 1972–1973.

Carwardine, J., Klein, C.J., Wilson, K.A., Pressey, R.L. & Possingham, H.P. (2009) Hitting the target and missing the point: target-based conservation planning in context. *Conservation Letters*, **2**, 4–11.

Ceballos, G. & Ehrlich, P.R. (2006) Global mammal distributions, biodiversity hotspots, and conservation. *Proceedings of the National Academy of Sciences USA*, **103**, 19374–19379.

Curnutt, J., Lockwood, J., Lue, H.K., Nott, P. & Russell, G. (1994) Hotspots and species diversity. *Nature*, **367**, 326–327.

Davies, T.J. & Buckley, L.B. (2011) Phylogenetic diversity as a window into the evolutionary and biogeographic histories of present-day richness gradients for mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**, 2414–2425.

Davies, T.J., Barraclough, T.G., Chase, M.W., Soltis, P.S., Soltis, D.E. & Savolainen, V. (2004) Darwin's abominable mystery: insights from a supertree of the angiosperms. *Proceedings of the National Academy of Sciences USA*, **101**, 1904–1909.

Davies, T.J., Fritz, S.A., Grenyer, R., Orme, C.D.L., Bielby, J., Bininda-Emonds, O.R., Cardillo, M., Jones, K.E., Gittleman, J.L. & Mace, G.M. (2008) Phylogenetic trees and the future of mammalian biodiversity. *Proceedings of the National Academy of Sciences USA*, **105**, 11556–11563.

De Frenne, P., Rodriguez-Sanchez, F., Coomes, D.A. *et al.* (2013) Microclimate moderates plant responses to macroclimate warming. *Proceedings of the National Academy of Sciences USA*, **110**, 18561–18565.

Devictor, V., Mouillot, D., Meynard, C., Jiguet, F., Thuiller, W. & Mouquet, N. (2010) Spatial mismatch and congruence between taxonomic, phylogenetic and functional diversity: the need for integrative conservation strategies in a changing world. *Ecology Letters*, **13**, 1030–1040.

Díaz, S. & Cabido, M. (2001) Vive la différence: plant functional diversity matters to ecosystem processes. *Trends in Ecology and Evolution*, **16**, 646–655.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792–1797.

Elith, J. & Leathwick, J.R. (2009) Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 677–697.

Faith, D.P. (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*, **61**, 1–10.

Flynn, D.F.B., Mirotchnick, N., Jain, M., Palmer, M.I. & Naeem, S. (2011) Functional and phylogenetic diversity as predictors of biodiversity – ecosystem-function relationships. *Ecology*, **92**, 1573–1581.

Font, X., Rodríguez-Rojo, M.P., Acedo, C., Biurrun, I., Fernández-González, F., Lence, C., Loidi, J., & Ninot, J.M. (2010) SIVIM: An on-line database of Iberian and Macaronesian vegetation. Forest Ecology. *Landscape Research and Nature Conservation*, **9**, 15–22.

Forest, F., Grenyer, R., Rouget, M., Davies, T.J., Cowling, R.M., Faith, D.P., Balmford, A., Manning, J.C., Proches, S., van der Bank, M., Reeves, G., Hedderson, T.A.J. & Savolainen, V. (2007) Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature*, **445**, 757–760.

Freitag, S. & Jaarsveld, A.S.V. (1998) Sensitivity of selection procedures for priority conservation areas to survey extent, survey intensity and taxonomic knowledge. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 1475–1482.

Fritz, S.A. & Rahbek, C. (2012) Global patterns of amphibian phylogenetic diversity. *Journal of Biogeography*, **39**, 1373–1382.

Gjerde, I., Sætersdal, M., Rolstad, J., Blom, H.H. & Storaunet, K.O. (2004) Fine-scale diversity and rarity hotspots in northern forests. *Conservation Biology*, **18**, 1032–1042.

Gouy, M., Guindon, S. & Gascuel, O. (2010) SeaView Version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, **27**, 221–224.

Gower, J.C. (1971) A general coefficient of similarity and some of its properties. *Biometrics*, **27**, 857–874.

Guilhaumon, F., Gimenez, O., Gaston, K.J. & Mouillot, D. (2008) Taxonomic and regional uncertainty in species-area relationships and the identification of richness hotspots. *Proceedings of the National Academy of Sciences USA*, **105**, 15458–15463.

Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. & Challenger, W. (2008) GEIGER: investigating evolutionary radiations. *Bioinformatics*, **24**, 129–131.

Hortal, J., Lobo, J.M. & Jiménez-Valverde, A. (2007) Limitations of biodiversity databases: case study on seed-plant diversity in Tenerife, Canary Islands. *Conservation Biology*, **21**, 853–863.

Hortal, J., de Bello, F., Diniz-Filho, J.A.F., Lewinsohn, T.M., Lobo, J.M. & Ladle, R.J. (2015) Seven shortfalls that beset large-scale knowledge of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, **46**, 523–549.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, **33**, 511–518.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P. & Webb, C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.

Kleyer, M., Bekker, R.M., Knevel, I.C. *et al.* (2008) The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology*, **96**, 1266–1274.

Klimeš, L., Klimešová, J., Hendriks, R. & Van Groenendael, J. (1997) Clonal plant architectures: a comparative analysis of form and function. *The ecology and evolution of clonal plants* (ed. by de Kroon H. and J. Van Groenendael), pp. 1–29. Backhuys Publishers, Leiden, the Netherlands.

Koenker, R. (2016) *quantreg: Quantile regression*. Version 5.21. Available at: https://cran.r-project.org/web/packages/quantreg/index.html. (accessed 31 March 2016).

Koenker, R. & Bassett, G. (1978) Regression quantiles. *Econometrica*, **46**, 33–50.

Kück, P. & Meusemann, K. (2010) FASconCAT: convenient handling of data matrices. *Molecular Phylogenetics and Evolution*, **56**, 1115–1118.

Kuhn, I., Durka, W. & Klotz, S. (2004) BiolFlor – a new plant-trait database as a tool for plant invasion ecology. *Diversity and Distributions*, **10**, 363–365.

Laguna, E., Deltoro, V., Pérez-Botella, J., Pérez-Rovira, P., Serra, L., Olivares, A. & Fabregat, C. (2004) The role of small reserves in plant conservation in a region of high diversity in eastern Spain. *Biological Conservation*, **119**, 421–426.

Lassmann, T. & Sonnhammer, E.L.L. (2005) Kalign – an accurate and fast multiple sequence alignment algorithm. *BMC Bioinformatics*, **6**, 298.

Lassmann, T. & Sonnhammer, E.L.L. (2006) Kalign, Kalignvu and Mumsa: web servers for multiple sequence alignment. *Nucleic Acids Research*, **34**, W596–W599.

López-Osorio, F. & Miranda-Esquivel, D.R. (2010) A phylogenetic approach to conserving Amazonian biodiversity. *Conservation Biology*, **24**, 1359–1366.

Mace, G.M., Gittleman, J.L. & Purvis, A. (2003) Preserving the tree of life. *Science*, **300**, 1707–1709.

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M. & Hornik, K. (2013) *cluster: Cluster Analysis Basics and Extensions*. Version 2.0.3. (accessed 31 March 2016).

Margules, C.R. & Pressey, R.L. (2000) Systematic conservation planning. *Nature*, **405**, 243–253.

Moore, M.J., Soltis, P.S., Bell, C.D., Burleigh, J.G. & Soltis, D.E. (2010) Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. *Proceedings of the National Academy of Sciences USA*, **107**, 4623–4628.

Mouillot, D., Albouy, C., Guilhaumon, F., Ben Rais Lasram, F., Coll, M., Devictor, V., Meynard, C.N., Pauly, D., Tomasini, J.A., Troussellier, M., Velez, L., Watson, R., Douzery, E.J.P. & Mouquet, N. (2011) Protected and threatened components of fish biodiversity in the Mediterranean Sea. *Current Biology*, **21**, 1044–1050.

Myers, N. (1988) Threatened biotas: "Hot spots" in tropical forests. *The Environmentalist*, **8**, 187–208.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2016) *vegan: Community ecology package*. Available at https://CRAN.R-project.org/package=vegan. (accessed 31 March 2016).

Orme, C.D.L., Davies, R.G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V.A., Webster, A.J., Ding, T.-S., Rasmussen, P.C., Ridgely, R.S., Stattersfield, A.J., Bennett, P.M., Blackburn, T.M., Gaston, K.J. & Owens, I.P.F. (2005) Global hotspots of species richness are not congruent with endemism or threat. *Nature*, **436**, 1016–1019.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.

Pardo, I., Pata, M.P., Gómez, D. & García, M.B. (2013) A novel method to handle the effect of uneven sampling effort in biodiversity databases. *PLoS One*, **8**, e52786.

Pavoine, S., Gasc, A., Bonsall, M.B. & Mason, N.W.H. (2013) Correlations between phylogenetic and functional diversity: mathematical artefacts or true ecological and evolutionary processes? *Journal of Vegetation Science*, **24**, 781–793.

Petchey, O.L. & Gaston, K.J. (2002) Functional diversity (FD), species richness and community composition. *Ecology Letters*, **5**, 402–411.

Pio, D.V., Broennimann, O., Barraclough, T.G., Reeves, G., Rebelo, A.G., Thuiller, W., Guisan, A. & Salamin, N. (2011) Spatial predictions of phylogenetic diversity in conservation decision making. *Conservation Biology*, **25**, 1229–1239.

Poschlod, P., Kleyer, M., Jackel, A., Dannemann, A. & Tackenberg, O. (2003) BIOPOP – a database of plant traits and internet application for nature conservation. *Folia Geobotanica*, **38**, 263–271.

Prendergast, J.R., Quinn, R.M., Lawton, J.H., Eversham, B.C. & Gibbons, D.W. (1993) Rare species, the coincidence of diversity hotspots and conservation strategies. *Nature*, **365**, 335–337.

Prinzing, A., Reiffers, R., Braakhekke, W.G., Hennekens, S.M., Tackenberg, O., Ozinga, W.A., Schaminée, J.H.J. & Van Groenendael, J.M. (2008) Less lineages – more trait variation: phylogenetically clustered plant communities are functionally more diverse. *Ecology Letters*, **11**, 809–819.

Purvis, A., Agapow, P.M., Gittleman, J.L. & Mace, G.M. (2000) Nonrandom extinction and the loss of evolutionary history. *Science*, **288**, 328–330.

R Development Core Team (2016) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rahbek, C. (1995) The elevational gradient of species richness: a uniform pattern? *Ecography*, **18**, 200–205.

Reid, W.V. (1998) Biodiversity hotspots. *Trends in Ecology and Evolution*, **13**, 275–280.

Rodrigues, A.S.L. & Gaston, K.J. (2002) Maximising phylogenetic diversity in the selection of networks of conservation areas. *Biological Conservation*, **105**, 103–111.

Rodrigues, A.S.L., Grenyer, R., Baillie, J.E.M., Bininda-Emonds, O.R.P., Gittlemann, J.L., Hoffmann, M., Safi, K., Schipper, J., Stuart, S.N. & Brook, T. (2011) Complete, accurate, mammalian phylogenies aid conservation planning, but not much. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**, 2652–2660.

Roquet, C., Thuiller, W. & Lavergne, S. (2013) Building megaphylogenies for macroecology: taking up the Challenge. *Ecography*, **36**, 13–026.

Sanderson, M.J. (2003) r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics*, **19**, 301–302.

Schwartz, M.W. (1999) Choosing the appropriate scale of reserves for conservation. *Annual Review of Ecology and Systematics*, **30**, 83–108.

Sechrest, W., Brooks, T.M., da Fonseca, G.A.B., Konstant, W.R., Mittermeier, R.A., Purvis, A., Rylands, A.B. & Gittleman, J.L. (2002) Hotspots and the conservation of evolutionary history. *Proceedings of the National Academy of Sciences USA*, **99**, 2067–2071.

Smith, S.A., Beaulieu, J.M. & Donoghue, M.J. (2010) An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences USA*, **13**, 5897–5902.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, **57**, 758–771.

Tilman, D. (2001). Functional diversity. *Encyclopedia of biodiversity* (ed. by S.A. Levin), pp. 109–120. Academic Press, San Diego, CA.

Tribsch, A. (2004) Areas of endemism of vascular plants in the Eastern Alps in relation to Pleistocene glaciation. *Journal of Biogeography*, **31**, 747–760.

Winter, M., Devictor, V. & Schweiger, O. (2013) Phylogenetic diversity and nature conservation: where are we? *Trends in Ecology and Evolution*, **28**, 199–204.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Vegetation and diversity maps of the Ordesa and Monte Perdido National Park.

Appendix S2 Representation of habitats in plant inventories.

Appendix S3 A model based approach to estimate richness-independent measures of phylogenetic and functional diversity.

Appendix S4 Results based on subdatasets of plant inventories.

Appendix S5 Phylogenetic tree and functional dendrogram.

Appendix S6 Results based on raw phylogenetic and functional diversity.

Appendix S7 Results of quantile regressions between sampling completeness and diversity.

BIOSKETCH

Iker Pardo is an ecologist with primary interest in the spatial distribution and temporal dynamics of multiple components of plant diversity, particularly in mountain areas. His research is based on biodiversity databases and long-term data from communities and applies to understanding the response of biodiversity to global change. He is also interested in developing approaches to account for uncertainty in spatio-temporal analyses of biodiversity data.

Author contributions: I.P. and M.B.G. conceived of the ideas and designed the study; I.P., M.B.G. and D.G. collected and prepared data; I.P., C.R. and S.L. analysed data; I.P. and M.B.G. wrote the manuscript with the help of all authors.

Editor: Janine Bolliger