Larger aboveground neighbourhood scales maximise similarity but not eliminate discrepancies with belowground plant diversity in a Mediterranean shrubland

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Abstract

Aims

An unresolved question in plant ecology is whether diversity of the aboveground and belowground compartments of a plant community is similar at different neighbourhood scales. We investigated how the similarity between both compartments varies with the aboveground sampling grain and if significant discrepancies exist between aboveground and belowground plant diversity at the maximum similarity scale.

Methods

We fully mapped the perennial plant community of a 64 m^2 plot in a Mediterranean shrubland and analysed the aboveground compartment by assessing diversity in 5 to 50 cm radii circles centred in soil cores. We sampled 2.5 cm radius root cores at two different depths and identified plant species by using DNA *metabarcoding* to characterise the belowground compartment. We quantified differences in species richness, composition and species' spatial distribution above- and belowground.

Results

The differences between aboveground and belowground were affected by the size of the aboveground sampling grain and were minimised when considering a circle of 20 cm radius in the aboveground. We found a significant dissimilarity in richness and composition between the two compartments, with larger differences when considering the deeper soil layer only.

Conclusions

Our results showed that the spatial grain selected to sample a plant community aboveground and belowground is critical to characterise them in a comparable manner. Although their composition is related, species distribution patterns strongly differ, suggesting the simultaneous action of different assembly mechanisms. Our results call for caution when studying community assembly considering only the standing vegetation, since total plant diversity can be underappreciated.

Abbreviations

ΔR = Richness dissimilarity

J = Jaccard dissimilarity index

AR = Aboveground Richness

AR_5 = Aboveground Richness at 5 cm radius grain

AR_{20} = Aboveground Richness at 20 cm radius grain (similarity peak)

BR = Belowground Richness

$BR_{0\text{-}30}$ = Belowground Richness at 0-30 cm of depth

$BR_{0\text{-}10}$ = Belowground Richness at 0-10 cm of depth

$BR_{10\text{-}30}$ = Belowground Richness at 10-30 cm of depth

OTU = Operational Taxonomic Unit

Introduction

Roots are an important fraction of total ecosystem biomass in all vegetation types (Mokany et al. 2006). This is especially evident in stressful habitats such as water-limited environments, where plant root:shoot ratios are significantly higher than in more benign conditions (Schenk

and Jackson 2002; Walter 1963). As early as in the 1960's, some authors tried to determine the relative weight of shoots and roots in plant communities (see *e.g.* Bray 1963; Davidson 1969). However, the lack of straightforward, feasible sampling techniques, strongly limited the integration of belowground information in the toolbox of plant community ecologists (Rewald et al. 2012). Recent advances in molecular techniques such as DNA metabarcoding, which allows the simultaneous identification of multiple taxa through next generation sequencing, are changing this scenario (Deiner et al. 2017; Hiiesalu et al. 2012). This powerful molecular tool has opened new venues to explore the hidden compartment of plant communities by identifying all the species present in root mixtures, and potentially, also their relative biomass partition (Matesanz et al. 2019). Incorporating a detailed characterization of the belowground compartment into the study of plant communities can help to unveil mechanisms controlling community assembly at fine spatial scales (Pärtel et al. 2012). In addition, it can be basic for the estimation of total plant diversity, which represents a priority in conservation ecology because of the known linkages between biodiversity and ecosystem functioning (Cardinale et al. 2012).

Only a few studies (*e.g.* Hiiesalu et al. 2012; Kesanakurti et al. 2011; Träger et al. 2019), have jointly assessed richness and composition of both the above- and belowground compartments of plant communities. A general pattern that emerged from these studies are the discrepancies in species richness, since the number of species is generally higher belowground than aboveground (*e.g.* Hiiesalu et al. 2012; Jones et al. 2011). This could be explained by several concomitant factors, including a higher prospective ability of roots in space and time, and a greater heterogeneity in the distribution of soil resources and conditions compared with those in the aboveground, which could in turn promote more opportunities for niche diversification (Pärtel et al. 2012). Although some studies (*e.g.* Kesanakurti et al. 2011; Li et al. 2017) observed similarity on the species distribution between the above- and

belowground compartments (both in terms of presence-absence and abundance), they also reported a general asymmetry in species frequencies between both compartments and a sharp segregation of species with soil depth. Consequently, the arising paradigm is that species diversity and distribution observed aboveground are different from those belowground, thus limiting its value as a robust and integrative proxy of the total diversity structure in the plant community.

The species distribution asymmetry often observed in plant communities could be related to different processes that structure diversity in the two compartments. Some authors (*e.g.* Casper and Jackson 1997) suggested that plant to plant interactions may be more important and frequent belowground than aboveground. Price et al. (2012), however, suggested that such interactions (*e.g.* competition) are possibly more important in the aboveground, while abiotic factors, such as soil heterogeneity, would affect more directly species' patterns belowground. In this sense, it has been hypothesised that the mechanisms underlying patterns of richness and composition aboveground and belowground may act at different spatial scales (Pärtel et al. 2012). If true, this would suggest that the observed similarity (or dissimilarity) in species patterns between both compartments could vary along both horizontal and vertical spatial scales.

Previous studies exploring similarity between both compartments performed their comparisons at the same spatial scale (*i.e.*, using the same sampling grain, Hiiesalu et al. 2012; Träger et al. 2019) or, alternatively, used different scales in the aboveground and belowground (Frank et al. 2010; Kesanakurti et al. 2011; Li et al. 2017), without any assessment of whether it was the most appropriate scale for comparison. Therefore, it cannot be excluded that the dissimilarities observed between the two compartments are simply a consequence of the sampling grain used. Furthermore, several studies (*e.g.* Hiiesalu et al. 2012; Träger et al. 2019) only sampled the most superficial soil layer (up to 10 cm), standing

on the fact that the greater portion of root biomass is usually found in the most superficial part of the soil (Kesanakurti et al. 2011). However, sampling only the top layer, particularly on habitats characterised by deep root systems (see Schenk and Jackson 2002) could limit our understanding of how belowground and aboveground communities are structured. Accordingly, to assess whether a robust characterization of the entire plant community may be done using only the information of the aboveground compartment (or alternatively, the belowground), it is critical to first determine whether these communities differ. In this sense, firstly identifying the spatial scales and soil depths that maximise the similarity between the two compartments could be crucial.

In this study, we compared species richness and composition in the aboveground and belowground compartments of a rich Mediterranean shrubland, considering different aboveground sampling grains and soil depths. We conducted a spatially-explicit approach on a fully mapped Mediterranean dwarf shrubland in combination with DNA metabarcoding of the root fraction, to provide a high resolution of both aboveground and belowground compartments. Fully mapping the aboveground community allowed the subsequent application of different aboveground sampling grains to identify the scale at which the similarity with the belowground compartment is maximised. Because soil heterogeneity may differentially affect plant distribution both aboveground and belowground, we also evaluated how soil composition affects diversity in both compartments. Specifically, we ask: (1) Do species richness, composition and distribution differ between the aboveground and belowground compartments of the plant community? (2) Which is the aboveground sampling grain maximising similarity between the aboveground and belowground compartments? And, finally (3), how does soil heterogeneity affect the two plant compartments?

Methods

Study area and sampling design

This study was conducted in a species-rich Mediterranean shrubland located in the south-easternmost part of Madrid province (Spain) (40°17'17.5" N 3°12'19.4" W, 760 m asl). The plant community is dominated by chamaephytes and hemicritophytes (mostly < 50 cm in maximum height), and it occurs in calcareous soils with a variable content of gypsum. This creates a patchy environment with many species, varying from gypsophiles, such as *Helianthemum squamatum* (L.) Dum. Cours, *Thymus lacaitae* Pau, *Centaurea hyssopifolia* Vahl, *Arenaria cavanillesiana* (Font Quer & Rivas Goday) Nieto Fel. and *Ononis tridentata* L., to gypsovags and calciphyllous plants (both on and off gypsum soils) as *Bupleurum fruticescens* L., *Thymus vulgaris* L., *Linum suffruticosum* L., *Helianthemum cinereum* Pers., *Stipa pennata* L., *Salvia lavandulifolia* Vahl and *Lithodora fruticosa* (L.) Griseb (Escudero et al. 2015).

In May 2016, we established an 8 × 8 m (64 m^2) plot (10% of slope) in a representative and well conserved area (Fig. 1), *i.e.*, without recent evidences of human impact, and all the aboveground perennial individuals were mapped (at their centroid or rooting point) with centimetric resolution using a Leica Viva GS15 system (Leica, Wetzlar, Germany) (see *e.g.* Chacón-Labella et al. 2017). We also measured their major perpendicular diameters (length and width) and the maximum height of each plant (excluding the reproductive shoots). The crown of each individual was represented by a circle with diameter equal to the average of its major diameters. In addition, 64 sampling points were located on the nodes of a regular 1 × 1 m grid. In order to incorporate a finer spatial scale, 30 additional points were sampled in a similar 1 × 1 m grid offset 0.5 m from the first grid (see Fig. 1). To account for different aboveground neighbourhood scales, we sampled the aboveground community with circles centred in the location of each sampling point considering eight different sampling grains, *i.e.*, with radii varying from 5 to 50 cm. For each point, we

recorded all aboveground plants (thereafter converted to presence-absence data) whose crown was included within or intersected with the sampling circle (see Fig. 1). We sampled the belowground plant community in the same plot during the first two weeks of June 2016, just after the aboveground sampling was finished. We collected 94 soil cores, each in every sampling point (5 cm of diameter, 30 cm of depth; root cores hereafter). Specifically, each root core was separated in two subsamples: the superficial fraction, between 0 and 10 cm, and the deeper fraction ranging from 10 to 30 cm, which is reported to include at least 50% of the total root biomass in most environments including Mediterranean shrublands (Schenk and Jackson 2002b), rendering a total of 188 root samples. In addition, to account for soil heterogeneity in the plot, in September 2016 we collected 84 soil cores reaching a depth of 10 cm, which were located adjacent to the root cores (see Fig. 1).

Root cores processing and soil properties analysis

Upon collection in the field, root samples were placed in a cooler, maintained at 4ºC and processed within 48 hours since collection, to avoid DNA degradation. We carefully washed all roots contained in each root sample, filtering them with a 1 mm mesh sieve. Then, we centrifuged roots at 3000 rpm for 30 seconds to remove excess water and weighed them to obtain fresh root biomass per root sample. Then, we thoroughly mixed the root fragments in each sample. From each sample, we took a subsample of 100 mg, snap-froze it with liquid nitrogen and stored it at -80ºC until DNA extraction.

Even though biochemical properties could be potentially altered on air-dried soil samples, Zornoza et al. (2009) showed that biochemical properties from Mediterranean semi-arid soils are stable in the medium-term in stored air-dried soil samples. Therefore, for practical reasons, the soil cores were air-dried for four weeks and stored for subsequent analysis. Then, they were sieved (2 mm mesh size) to determine both physical and chemical

soil properties of the finest fraction. Texture was estimated following the Kettler et al. (2001) method. Electrical conductivity and pH were measured in deionised water, in a proportion of 1:2.5 and 1:5 (mass/volume), respectively, by using a conductivity meter GLP 31 and a pH meter GLP 21 (Crison, Barcelona, Spain). Soil organic C (SOC) was estimated by a wet oxidation procedure according to Yeomans and Bremner (1988). Total N and extractable P were estimated by Kjeldahl digestion (Anderson and Ingram 1993), while total K was determined applying Radojević and Bashkin (1999) methodology. Moreover, we quantified key soil enzymatic activities as an estimation of the current microbiome soil dynamics, which are relevant for soil quality assessment and functioning (Adetunji et al. 2017), by applying the techniques described by Eivazi and Tabatabai (1988) and Tabatabai and Bremner (1969), for the measurement of β-glucosidase activity and acid phosphatase, respectively.

Root identification through DNA metabarcoding

To identify all the plant species in each root sample, we used DNA metabarcoding using the *rbcL* gene as barcode (see Matesanz et al. 2019). We built a complete in-house reference library for the identification of species in the study plant community, considering at least 95% of the perennial species rooting in the sampled plot. In addition, to account for either the occurrence of perennial organs belowground or roots from plants with their aerial part outside the plot, we also included other species which were not present in the plot but occurred in the surroundings. The final database contained the *rbcL* reference sequences of 45 species. A detailed description of the metabarcoding pipeline is provided in Methods S1 (Online Resource 1). Briefly, as a first step, DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, CA, USA) in the lab at Universidad Rey Juan Carlos, including negative controls for each extraction batch. Afterwards, DNA extractions were processed in the AllGenetics laboratories (AllGenetics & Biology SL, A Coruña, Spain). We amplified a fragment of the

rbcL chloroplast gene using primers rbcLa-F (5' ATG TCA CCA CAA ACA GAG ACT AAA GC 3'; Levin et al. 2003) and rbcLa-R (5' GTA AA ATC AAG TCC ACC RCG 3'; Kress et al. 2009). A first PCR was performed to amplify the selected fragment of the *rbcL* chloroplast gene. A second PCR was required to attach the Illumina index sequences for multiplexing distinct libraries in the same sequencing pool. Four negative controls that contained no DNA were included to check for contamination during library preparation. The pool was sequenced in a run of the MiSeq PE300 (Illumina). Then, samples were demultiplexed, removing indexes and sequencing primers. Sequences were then dereplicated, clustered at a similarity threshold of 100% and sorted. The taxonomical assignment was performed by querying the clustered sequences against the in-house reference library in VSEARCH (usearch global option) with a 99% similarity threshold. The output was a table listing the number of sequences from each OTU found in each sample. We removed the OTUs with a number of sequences lower than 0.005% of the total number of sequences (Bokulich et al. 2013) to apply a quality filtering. Finally, we removed those OTUs that did not match any reference sequence in the database at a similarity of 99% and remained unidentified ('No hit'). These OTUs accounted for an average of 9.4% of the total reads before filtering. We corroborated by blasting them in GenBank that at least the 70% of them corresponded to bryophytes or *Thymus* sp. sequences of lower quality. Finally, we converted the OTUs abundance table into a species presence-absence table for subsequent analysis.

Statistical analysis

Similarities between the two plant community compartments were quantified as differences in terms of species richness and composition. In each sampling point (*i.e.*, around each root core), we calculated the richness and composition dissimilarities (ΔR and *J* hereafter, respectively) between the aboveground and belowground compartments, for each horizontal

(aboveground sampling grain) or vertical (soil depth) scale considered in the study. ΔR was calculated as the absolute difference between aboveground and belowground richness (hereafter AR and BR, respectively), *i.e.* $\Delta R = |AR - BR|$. Species composition similarity between aboveground and belowground compartments was calculated the with the Jaccard dissimilarity index as:

$$J = (b + c) / (a + b + c),$$

where *a* is the number of species present in both compartments, *b*, the number of species present only aboveground and *c*, the number of species present only belowground. To assess for significant differences in Jaccard (J index) and richness differences (ΔR) between aboveground grains, for the three possible depths (0-10, 10-30 and 0-30 cm), we performed Tukey tests, when the variables had a normal distribution, and Dunn's tests when this was not feasible.

Once identifying the aboveground sampling grain with maximum similarity, we carried out the comparison between aboveground and belowground compartments considering this spatial scale. We performed a Mann-Whitney-Wilcoxon test to evaluate differences between aboveground richness and belowground richness estimated at different depths: i) 0-10 cm ($BR_{0\text{-}10}$), ii) 10-30 cm ($BR_{10\text{-}30}$) and iii) 0-30 cm ($BR_{0\text{-}30}$). We tested the correlation between aboveground and belowground richness, *i.e.*, $BR_{0\text{-}10}$, $BR_{10\text{-}30}$ and $BR_{0\text{-}30}$, using Kendall rank correlation coefficients (Kendall 1976). Differences in species composition between the aboveground and belowground compartments were also evaluated at the three different depths, 0-10, 10-30 and 0-30 cm. For this, we carried out a PERMANOVA analysis (Anderson 2001). Moreover, we assessed the number of species shared between the two compartments (shared richness), those appearing only aboveground (additional aboveground richness), and, finally, the species found only belowground (additional belowground richness), considering all three different depths. For this estimation, we also

considered the 5 cm radius grain and the 0-10 cm layer belowground, as it is the most commonly used in previous studies (*e.g.* Hiiesalu et al. 2012; Träger et al. 2019).

To assess the existence of a spatial concordance of individual species between the aboveground and belowground compartments, we implemented two complementary tests. First, for each species, we quantified its frequency in the 94 aboveground and belowground samples, for all the soil layers (0-10, 10-30 and 0-30 cm) and tested for differences in species' frequencies with Pearson's *Chi*-squared tests. Second, we explored spatial correspondence for each species between aboveground and belowground (again for the 0-10, 10-30 and 0-30 cm layers) with the McNemar's *Chi*-squared test (Agresti 1990). We controlled the false discovery rate for multiple testing using the approach of Benjamini and Hochberg (1995).

Finally, we analysed the effect of soil heterogeneity on aboveground and belowground richness. Since the effect of soil heterogeneity could change with the sampling scale, we again chose two different aboveground sampling grains: i) 5 cm, the most similar scale to that belowground, ii) and 20 cm radius circles, the scale where we observed the maximum similarity between the two compartments (see Results), and all different depths (0-10, 10-30 and 0-30 cm). After checking for collinearity, the soil variables considered were sand (%), C, N, K contents, glucosidase, phosphatase, conductivity and pH. First, we fitted Poisson GLMs, then we tested models residuals for spatial autocorrelation with Moran's tests and applied a simulation-based approach for other residual diagnostics (Hartig 2020). In the case of a significant spatial autocorrelation, we included a distance-weighted autocovariate into the model (F. Dormann et al. 2007). As in most cases (with the exception of AR at the 5 cm scale) we found a significant under-dispersion in the GLM, we fitted a VGLM following Hilbe (2014).

All statistical analyses were conducted in R (R Core Team 2020). Wilcoxon tests, correlation analysis, Pearson's and McNemar's *Chi*-squared tests, false discovery rate

correction and GLMs were respectively performed with functions *wilcox.test*, *cor.test*, *chisq.test*, *mcnemar.test*, *p.adjust* and *glm* of the stats package (R Core Team 2020). Jaccard dissimilarity and PERMANOVA analysis were performed, respectively, with functions *vegdist* and *adonis* in the *vegan* package (Oksanen et al. 2019). Moran's tests and distance-weighted autocovariates were computed, respectively, with functions *moran.test and autocov_dist* in the *spdep* package (Bivand and Wong 2018). Residual diagnostics were computed with *simulateResiduals* and *testDispersion* functions in the DHARMa package (Hartig 2020). VGLMs were fitted with the *vglm* function in the *VGAM* package (Yee 2010).

Results

We mapped a total of 8551 perennial individuals aboveground, belonging to 45 species. In the belowground compartment, we retrieved a total of 1701120 sequence reads and assigned taxonomically 90.6% of them to species in our reference database. We identified a total of 30 taxa belowground, 26 at the species level and four at the genus level (*Thymus* sp., *Stipa* sp., *Teucrium* sp. and *Quercus* sp.), which were represented by two different species in the aboveground. The species that were mapped aboveground but not detected belowground had, in all cases, very low abundances, accounting together for 3.94% of the total number of individuals in the aboveground community (see Table S1, Online Resource 2).

Aboveground and belowground diversity across different spatial scales

The total number of species characterizing the aboveground compartment of the plant community ranged from 22 to 41, respectively, for the 5 and 50 cm sampling grains. Average aboveground and belowground richness varied consistently with the aboveground grain and depth (see Fig. S1, Online Resource 3). The average aboveground richness ranged from 1.61 ± 1.59 (mean ± SD) to 16.37 ± 3.49 species per sample, from the 5 to the 50 cm sampling

grains, respectively. Average belowground richness was similar in the 0-10 (BR_{0-10}) and 10-30 cm (BR_{10-30}) layers, with 6.02 (± 1.82) and 6.05 (± 2.14) species per sample respectively, while BR in the complete 0-30 cm (BR_{0-30}) core was higher, with 7.90 (± 2.08) species per root core.

Richness and composition dissimilarities (ΔR and Jaccard index, *J*, respectively) between the above- and belowground (0-30 cm) compartments (see Fig. 2) changed across aboveground sampling grains but followed a similar pattern. They both were higher at the smaller and larger sampling grains (*i.e.*, ΔR = 6.29 and *J* = 0.84 at 5 cm scale; ΔR = 8.53 and *J* = 0.64 at 50 cm scale) while reaching a minimum at 20 cm radius (ΔR = 2.15 and *J* = 0.53). When we separately accounted for the 0-10 and 10-30 belowground depths (see Fig. 2), results were similar, and the 20 cm radius had again the highest match. Even though there were not significant differences between the 15, 20 and 25 cm aboveground grains for any of the soil depths considered (see *P*-values of Tukey and Dunn's tests in Tables S3 and S4 in Online Resource 2), we selected the 20 cm radius grain for subsequent analyses, as it was the grain where the mean similarity was maximised. At the 20 cm radius aboveground and 10 cm of depth, 42.50% of species were found in both compartments, while 38.94% of them were found only in the aboveground and 18.55% in the belowground (Fig. 3). Similar results were obtained for the 10-30 cm layer and the complete 0-30 cm layer (Fig. 3), but species composition similarity (*i.e.*, shared species) reached a maximum (47.04%) when considering the 0-30 cm layer, while it was minimised in the 10-30 (38.09%) cm layer. At the 5 cm grain, the shared species between aboveground and belowground (0-10 cm of depth) were only 18.35% (see Fig. 3), while most of the species were found only belowground, *i.e.*, additional belowground richness, (75.54%).

Similarity between aboveground and belowground compartments

Aboveground richness at the sampling grain with the largest similarity (*i.e.*, 20 cm, AR_{20}, hereafter) did not differ significantly from $BR_{0\text{-}30}$ (Mann-Whitney-Wilcoxon's test $P = 0.21$). However, when comparing AR_{20} with both $BR_{0\text{-}10}$ and $BR_{10\text{-}30}$, we found significant differences (Mann-Whitney-Wilcoxon's test $P < 0.0001$ in both cases). In parallel, Kendall tests (Fig. S2, Online Resource 3) showed that AR_{20} was significantly correlated with both $BR_{0\text{-}10}$ and $BR_{0\text{-}30}$ ($BR_{0\text{-}10}$ $R = 0.32$, $P = 0.0008$; $BR_{0\text{-}30}$ $R = 0.25$, $P = 0.007$), while $BR_{10\text{-}30}$ was not correlated neither with AR_{20} ($P = 0.18$) nor $BR_{0\text{-}10}$ ($P = 0.32$). In addition, results from PERMANOVA showed that species composition differed significantly between the aboveground (20 cm grain) and all three root depths ($F = 23.07$, 18.87 and 24.02, for 0-10, 10-30 and 0-30 respectively; $P < 0.001$ in all cases).

Species frequencies, *i.e.*, the number of occurrences, were significantly different (Pearson's *Chi*-squared tests, $P < 0.0001$) between above- and belowground (0-10, 10-30 and 0-30 layers). However, these results were mostly driven by a few species (Fig. S3, Online Resource 3), such as *Lithodora fruticosa* and *Quercus* sp., which were significantly more frequent in the belowground, or *Koeleria vallesiana*, which instead, was more frequent in the aboveground. Indeed, most of the species had similar frequencies in both compartments (see Table S1, Online Resource 2). Spatial tests for each species (McNemar's *Chi*-squared tests) showed differences in the spatial distribution between aboveground and belowground for the 26.67% and 33.33% of species, respectively, considering the 0-30 cm layer and both the 0-10 and 10-30 cm layers (see Table S2, Online Resource 2). The species with a different distribution, regardless of the soil layer considered, were *Helianthemum cinereum*, *Arenaria cavanillesiana*, *Koeleria vallesiana*, *Sideritis incana* and *Quercus* sp.

Effects of soil heterogeneity on aboveground and belowground richness

We found only marginal effects of the soil heterogeneity on richness. Aboveground richness was significantly affected by the phosphatase activity at the two aboveground grains selected for the analyses (at 5 and 20 cm, $P = 0.047$ and $P = 0.017$, respectively) (Table 1). On the other hand, belowground richness was affected differently according to the soil layer considered. In the case of $BR_{0\text{-}30}$, the organic carbon content was the only significant and positive predictor. However, when we separately considered the two belowground layers (0-10 and 10-30), results were different: $BR_{0\text{-}10}$ was affected by carbon content and phosphatase, whereas $BR_{10\text{-}30}$ was significantly affected only by the potassium content.

Discussion

In semi-arid environments, such as Mediterranean shrublands characterised by higher biomass root allocation (Schenk and Jackson 2002) and sparse distribution of aboveground vegetation (Martens et al. 1997), we expected aboveground and belowground diversity patterns to be different. Indeed, our findings showed important diversity discrepancies, in terms of species richness, composition and spatial distribution, between the aboveground and belowground compartments. To understand how plant diversity is structured within aboveground and belowground fractions, and whether the aboveground robustly informs on the whole community diversity, pioneer studies (*e.g.* Hiiesalu et al. 2012) considered equivalent and unique scales of comparison, usually sampling units of 10 × 10 centimetres, both above- and belowground. Their results, specifically those related to the lack of congruence in the corresponding species-area curves (Hiiesalu et al. 2012), induced other authors (*e.g.* Pärtel et al. 2012) to suggest that different spatial scales of comparison should be considered. Some studies adopted different aboveground sampling grains (*e.g.* Kesanakurti et al. 2011), but did not justify what scale was the most appropriate to compare both plant compartments. Given that the drivers of community structure are likely different above- and belowground, a

previous and necessary step for an accurate description of a plant community should be identifying the scale at which the similarity between both components is maximised. Indeed, our study shows that the neighbourhood scale adopted to sample the aboveground community strongly affects the similarity between the aboveground and belowground compartments (Fig. 2), which represent different facets of the same plant community. Importantly, this result conditions the ability to robustly answer whether the aboveground can be a good surrogate of the whole plant community composition and structure.

In our community, the highest similarity in species richness and composition between aboveground and belowground compartments was registered with a 20 cm radius grain in the aboveground (and belowground root cores of 2.5 cm radius). It is also noteworthy that this scale was not affected by the different sampling depths. It is likely that the spatial scale at which the similarity reaches a maximum would vary with the plant community considered, as it may vary with the lateral spread of different species, which in turn depends on both their growth form and climatic conditions (*e.g.* Schenk and Jackson 2002). In our study case, this sampling grain roughly matched the maximum height of most individuals, which might be pointing to an allometric relationship between maximum plant height and the lateral root spread in these species. Interestingly, in a mesophytic grassland the best match between the aboveground and belowground richness was obtained when considering an aboveground cumulative sampling area three times larger than belowground (Hiiesalu et al. 2012; Pärtel et al. 2012). In other words, in another plant community, where dominating species are shorter than in our shrubland, maximum richness similarity was also encountered at a smaller aboveground scale. This hypothesis, however, needs further research and testing in other plant communities to be confirmed.

The comparison of the aboveground and belowground richness showed contrasting results according to sampling depth. We detected a high and positive correlation between

richness of the two compartments when considering both the 0-10 and the 0-30 cm soil layers. This correlation was stronger in case of the 0-10 cm layer only, while it was not significant for the 10-30 cm layer. This result concurs with Li et al. (2017), who reported that aboveground richness was more correlated with richness in the most superficial soil layer (first 5 cm) than in the deepest one (10 -15 cm of depth). When comparing average above- and belowground richness values, our results also varied with the soil layer. Above- and belowground richness were statistically similar only when the entire sampled soil profile (0-30 cm of depth) was considered, while a significant difference emerged when considering the shallow or the deeper layer in the soil separately. These results suggest that, in environments dominated by a greater root allocation, we cannot reduce our consideration of the plant diversity only to the shallowest part of the soil (10 cm), as plant community dynamics invest even deeper layers and possibly vary consistently with soil depth. This idea is supported by the fact that, even though there was an obvious decrease in root biomass (Fig. S4, Online Resource 3), we did not observe any reduction in species richness with depth. Results of the composition similarity analysis did not differ considerably with the soil layer. Indeed, our results evidenced significant differences in species composition between aboveground and belowground compartments, regardless of the soil layer considered. However, the strength of dissimilarities changed according with the layer considered, due to the fact that the 0-10 cm and 10-30 cm layers had a consistent portion of unshared species (a mean value of 45.44%), showing a certain species turnover between the shallow and the deeper (10-30 cm) layers. Altogether, our results show that, even at the scale of comparison in which the maximum similarity is reached, the aboveground and belowground communities present significant dissimilarities in richness and composition.

This discrepancy may be due to different factors, including different species' frequencies and/or a different spatial distribution in the two compartments. Our results

showed a generalised concordance in species frequencies between aboveground and belowground, with only a very few exceptions, such as, for instance, *Quercus* sp. This tree species was by far much more common in the belowground than in the aboveground compartment in our fully-mapped plot. It is worth noting that this tree is almost absent in the plot, with only a few seedlings, but it is relatively common in the vicinity, which informs on the strong ability of this species to spread its roots far beyond their canopies. The generalised symmetry in species frequencies observed for the majority of the species contrasts with other studies reporting clear asymmetries between the aboveground and belowground in grasslands (*e.g.* Kesanakurti et al. 2011; Hiiesalu et al. 2012). Interestingly, tests carried out to compare the distribution of individual species in the two compartments at the sampling point level evidenced that an important portion (ranging from 26.67% to 33.33%, according to the soil layer) of species presented a significantly different spatial distribution between the aboveground and belowground. In other words, our results indicate that although many of the species in our plant community have similar frequencies above- and belowground, several of them are differentially distributed in space in both compartments. This suggests that most of the composition dissimilarities observed are caused by species differentially prospecting the two compartments. This may be also the reason why we identified a significant amount (more than 50%), of unshared species per sampling point (circle/core) between the two compartments, for all the belowground layers.

A significant fraction of the diversity in each sampling point was only present in the belowground (*i.e.*, additional belowground richness, see Fig. 3), regardless of the aboveground sampling grain considered. The detection of certain species only in the belowground is in agreement with previous studies (*e.g.* Hiiesalu et al. 2012; Träger et al. 2019), and reinforces the idea that the soil contains a very relevant fraction of the total diversity (*i.e.*, hidden diversity; Pärtel 2014) that is systematically ignored when sampling is

only conducted aboveground. The additional aboveground richness was also a relevant fraction of diversity (Fig. 3), suggesting that neither the aboveground nor the belowground community include all species present at small spatial scales. This could be related to the fact that not all the species can be easily detected in the belowground, contrarily to those in the aboveground, as molecular techniques still have some limitations (see *e.g.* Hiiesalu et al. 2012).

Richness variation in the two compartments showed contrasting responses to soil heterogeneity even at the fine scale of our study. This result differed from Kesanakurti et al. (2011) who observed that soil heterogeneity was able to structure species diversity only in the belowground, but not in the aboveground. In our case, although the number of significant predictors was low, phosphatase activity in the soil, a surrogate of microbial activity (Nannipieri et al. 2011), explained a small fraction of the aboveground richness, at both 5 and 20 cm radius scale, while the organic carbon content affected the root diversity at 0-30 cm of depth. In the case of the belowground richness at the shallow layer, 0-10 cm, the response was similar to the aboveground richness (*i.e.*, both were positively affected by the phosphatase activity). Our findings disagree with those of Hiiesalu et al. (2012) who analysed richness variation at a local scale, very different from our very fine spatial scale, in a 2-ha diverse mesophytic grassland, and pointed out that both aboveground and belowground richness responded to the nitrogen content in the soil, but in different ways. Moreover, richness in the deepest layer, 10-30 cm, was positively and exclusively related to the level of potassium in the soil. The fact that richness was differently affected by soil heterogeneity with the layer considered suggests that the dynamics regulating belowground diversity patterns vary with depth. The results of our analysis, including the aboveground *vs*. belowground comparison as well as the richness variation with soil heterogeneity, shed light on another important issue, the huge complexity of belowground plant communities.

Our results challenge the current views of plant community assembly in Mediterranean arid and semi-arid shrubby vegetation. Indeed, the fact that we observed that species are strongly intermingled in very small spaces in the soil questions several hypotheses. First, species territoriality, *i.e.*, defending soil spaces to avoid competitors to achieve resources, has been hypothesised to represent a possible strategy to avoid competition for water and nutrients in the soil, mainly in environments where these are limited (Schenk et al. 1999). Species segregation along soil depth is also missing, contrary to what we could expect according to the niche differentiation theory, as we observed similar species frequencies in the two different soil layers (see Fig. S3 in Online Resource 3). However, we cannot rule out the possibility that, in our study, environment soil segregation with depth occurs at higher depths. The high belowground species richness detected also contrasts the idea that the sparse distribution of the aboveground vegetation in environments where light is abundant and soil resources (including water) are scarce responds to belowground competition (Cipriotti and Aguiar 2015; Deng et al. 2006; Martens et al. 1997). Contrary to this view, the coexistence of a high number of species in very small pockets in the soil (*i.e.*, within 5 cm soil cores) suggests that belowground competition does not determine the plant diversity patterns observed in the aboveground, while competition for aboveground resources (*e.g.* light) could actually have a more important contribution. This idea could be further supported by studies (*e.g.* Price et al. 2012) showing that aboveground competition is an important driver of community assembly at small spatial scales, while abiotic processes could be more important in the belowground. Our results, however, do not completely support this hypothesis, since we found only a small effect of soil heterogeneity on species richness distribution both aboveground and belowground.

Conclusions

Identifying the spatial scale at which the similarity between aboveground and belowground compartments reaches a maximum is critical not only to understand if they differ or not, but also to properly assess the processes determining the diversity patterns of the plant community as a whole. In this work, we show the importance for sampling a larger aboveground scale than in the belowground to maximise the similarity in species richness and composition between these two compartments. However, our findings show that, even at the scale of maximum similarity, there are relevant discrepancies between above- and belowground richness (except when a complete profile of 0-30 cm of depth was considered) and composition (for any of the three soil layers). This result confirms that, although the above- and belowground compartments are clearly related, the processes operating in each compartment differ, limiting their reciprocity and their ability to characterise the plant community individually. This is further reinforced by the fact that soil heterogeneity exerts a different effect on richness patterns in the two compartments. We show that to identify the scales maximising the similarity between aboveground and belowground compartments is a necessary step to obtain a complete perspective of the diversity structure in a plant community. This is critical to infer the mechanisms controlling plant coexistence in natural communities, which represent one of the most important challenges of plant community ecology.

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Author contribution

Silvia Matesanz and Adrián Escudero conceived and designed the study. All authors conducted field and laboratory work. Angela Illuminati analysed the data with extensive input by Jesús López-Angulo and Marcelino de la Cruz. Angela Illuminati wrote the manuscript, with input from all other authors.

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Table 1 Differential effects of soil heterogeneity on aboveground and belowground richness: Estimates (Est.) and *P*-values from GLMs and VGLMs for aboveground richness at 5 and 20 cm sampling grains (AR_5 and AR_{20}) and belowground richness at different depths (BR_{0-30}, BR_{0-10}, BR_{10-30}, 0-30 cm, 0-10 cm and 10-30 cm, respectively).

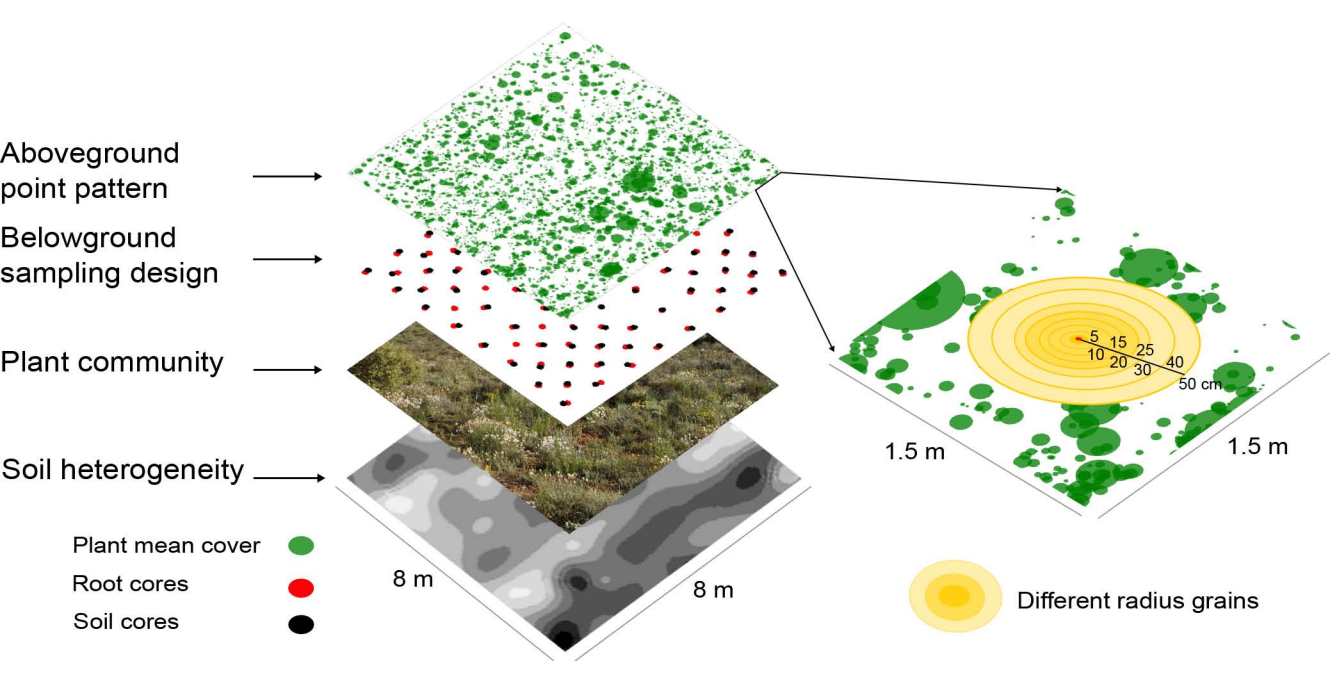
	AR_{20}		AR_5		BR_{0-30}		BR_{0-10}		BR_{10-30}	
	Est.	***P*-values**	**Est.**	***P*-values**	**Est.**	***P*-values**	**Est.**	***P*-values**	**Est.**	***P*-values**
(Intercept):1	-0.65	**0.003**	0.51	**0**	-0.76	**0.001**	-1.07	**0**	-0.32	0.078
(Intercept):2	2.39	**< 2e-16**	-		2.36	**< 2e-16**	2.19	**< 2e-16**	1.93	**<2e-16**
Sand	-0.06	0.06	-0.15	0.105	-0.05	0.125	-0.04	0.184	-0.07	0.06
C	-0.03	0.479	-0.07	0.548	-0.09	**0.019**	-0.11	**0.007**	-0.05	0.315
N	0.02	0.543	0	0.998	0.04	0.193	0	0.937	0	0.992
K	-0.01	0.872	-0.04	0.654	0.03	0.299	-0.06	0.09	0.09	**0.015**
Glucosidase	0.03	0.391	0.02	0.88	0.04	0.257	0.03	0.502	0.09	0.074
Phosphatase	0.09	**0.017**	0.23	**0.047**	0.06	0.144	0.13	**0.001**	-0.02	0.655
Conductivity	-0.06	0.194	0.1	0.328	-0.05	0.203	0.02	0.584	-0.05	0.298
pH	-0.04	0.362	0.04	0.741	0.01	0.797	0.05	0.225	-0.02	0.746
Autocovariate	0.05	**0**	0.06	0.069	-	-	-	-	-	-

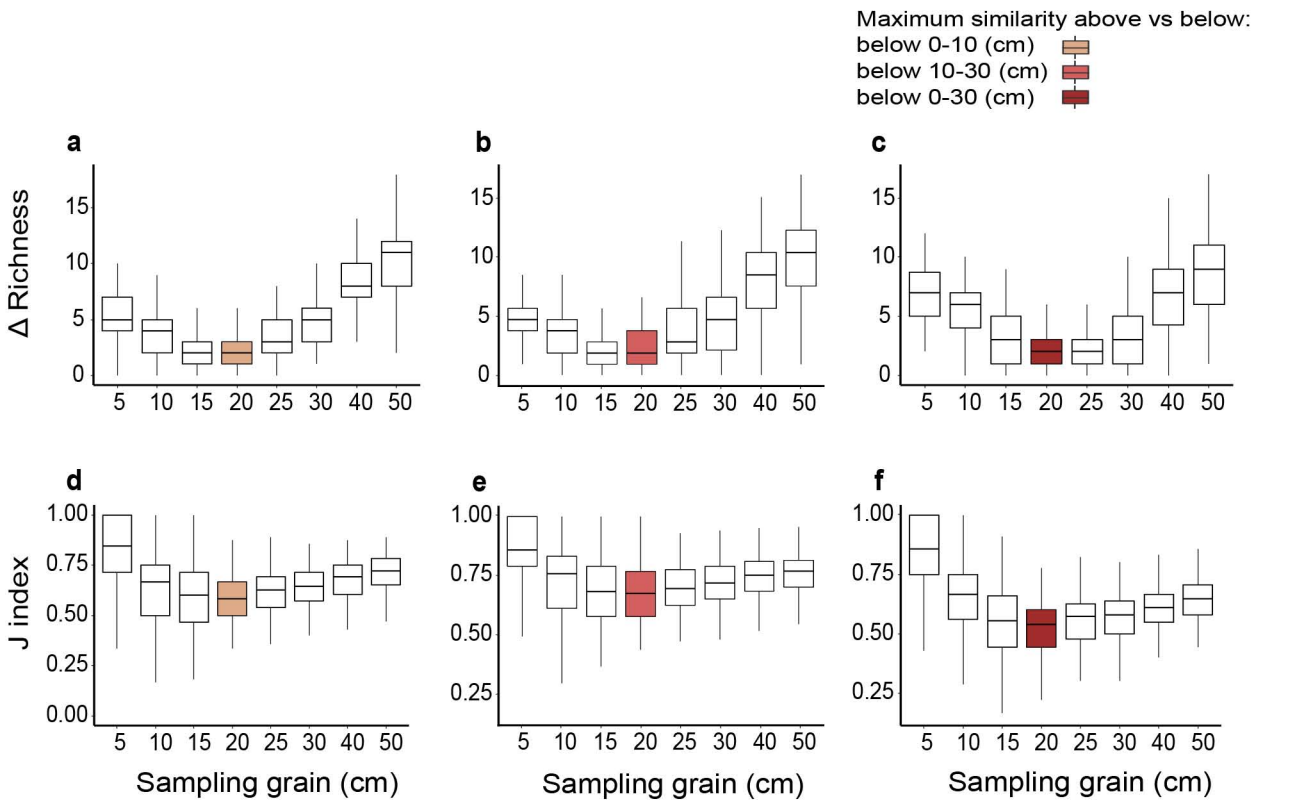
Figure captions.

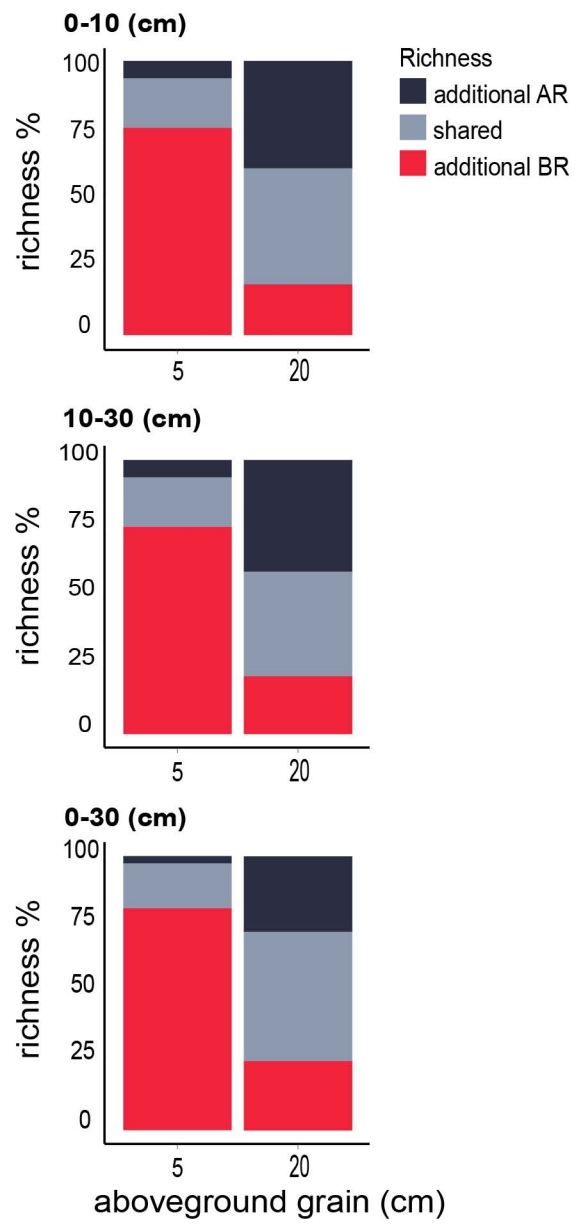
Fig. 1 Sampling design: From bottom to top, layers represent the soil heterogeneity, the sampled plant community (64 m^2), the grid of root and soil cores and the point pattern of the aboveground plant community (each point represents an individual, with size proportional to plant mean cover). In the right, a zoom of the aboveground point pattern is representing different radius circles, corresponding to different aboveground sampling grains, departing from the centre of a root core.

Fig. 2 Similarity between aboveground and belowground species richness and composition: a and **d** boxplots represent the median and the 1^{st} and 3^{rd} quartiles of both richness differences (ΔR) and Jaccard dissimilarity index (J), respectively, considering different aboveground sampling grains (circles with different radius size) aboveground vs the 0-10 cm depth layer belowground. **b** and **e** represent the same indices calculated with the belowground community at 10-30 cm, while **c** and **f** at 0-30 cm.

Fig. 3 Species composition similarities between aboveground and belowground: bar plot showing the species shared between the two compartments, the species found only aboveground (additional AR) and only belowground (additional BR), for the 5 and 20 cm sampling grains and the 0-10 , 10-30 and 0-30 cm of depth layers belowground.


Aboveground point pattern
Belowground sampling design
Plant community
Soil heterogeneity
Plant mean cover
Root cores
Soil cores
8 m
8 m
5
10
15
20
25
30
40
50 cm
1.5 m
1.5 m
Different radius grains


Maximum similarity above vs below:
below 0-10 (cm)
below 10-30 (cm)
below 0-30 (cm)
a
b
c
d
e
f
Δ Richness
J index
0
5
10
15
0.00
0.25
0.50
0.75
1.00
5 10 15 20 25 30 40 50
Sampling grain (cm)


0-10 (cm)
10-30 (cm)
0-30 (cm)
richness %
100
75
50
25
0
5
20
aboveground grain (cm)
Richness
additional AR
shared
additional BR

Larger aboveground neighbourhood scales maximise similarity but not eliminate discrepancies with belowground plant diversity in a Mediterranean shrubland

Plant and Soil

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Methods S1 Root identification through DNA metabarcoding

As a first step of the analysis, DNA extraction was carried out employing the DNeasy Plant Mini Kit (Qiagen, CA, USA) in the lab at Universidad Rey Juan Carlos, including negative controls for each extraction batch. Afterwards, DNA extractions were shipped to the AllGenetics laboratories (AllGenetics & Biology SL, A Coruña, Spain).

We amplified a fragment of the rbcL chloroplast gene (550 bp) using the primers rbcLa-F (5' ATG TCA CCA CAA ACA GAG ACT AAA GC 3'; Levin et al. 2003) and rbcLa-R (5' GTA AA ATC AAG TCC ACC RCG 3'; Kress et al. 2009). The Illumina sequencing primer sequences were attached at the 5' ends of primers. A first series of PCRs was performed to amplify the selected fragment of the rbcL chloroplast gene. It was carried out in a total volume of 25 µL, containing 2.5 µL of template DNA, 0.5 µM of the primers, 12.5 µL of Supreme NZYTaq 2x Green Master Mix (NZYTech), and ultrapure water up to 25 µL. The reactions were run as follows: the mixture was incubated at 95 °C for 5 min, than it was subjected to 30 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, and finally to 72 °C for 10 minutes. A latter series of PCRs was required to attach the Illumina index sequences for multiplexing distinct libraries in the same sequencing pool. Thermocycling conditions were identical to first PCRs series but with only 5 cycles and an annealing temperature of 60°C. During library preparation, four negative controls, with no DNA, were included to the check for contamination. The obtained libraries were run on 2 % agarose gels, which were stained with GreenSafe (NZYTech), and then observed under UV light to verify their size. After that, they were purified using Mag-Bind RXNPure Plus magnetic beads (Omega Biotek) and pooled in equimolar amounts. The pool was sequenced in a run of the MiSeq PE300 (Illumina). Then we proceeded with the demultiplexing step, which consists in removing indexes and sequencing primers. We carried out quality control on FASTQ files using the software FastQC and we filtered raw-reads in Geneious 11.1.2. PCR primers were

eliminated and a region at the 3´ end of each file was trimmed considering a minimum Phred score of 20. After, the R1 and R2 reads were concatenated (fuse.sh script, BBmap package, Bushnell 2014) and the sequences labelled (multiple split libraries.py) in Qiime (Caporaso et al., 2010). Labelling was crucial for the subsequent sample identification because sequences were combined later to perform downstream analysis. Next processing steps were carried out with the VSEARCH bioinformatics tool. Sequences were dereplicated, clustered at a similarity threshold of 100 %, and sorted. Furthermore, the bioinformatic pipeline included filters intended to reduce those artefacts, which normally generate during PCR and sequencing, and that can overestimate the number of OTUs. De novo chimera detection was implemented with the UCHIME algorithm (Edgar et al., 2011).

The taxonomical assignment was performed by querying the clustered sequences against the reference library in VSEARCH (usearch global option) with a 99% similarity threshold. As the query sequences mapped only to the 5´and 3´ends of the references sequences, their central region was previously removed, resulting in a final length of 517 bp. An OTU table resulted from the application of the script mesas-uc2clust.py. The new table listed the number of sequences from each OTU found in each sample. We removed the OTUs with a number of sequences lower than 0.005% of the total number of sequences (Bokulick et al., 2013) to apply a quality filtering. Moreover, the low abundance OTUs of each sample (0.1% threshold) were removed in order to contrast the phenomenon, which is normally referred to as mistagging, index jumping, tag jumping, etc. Indeed, a low percentage of the reads of a library can be misassigned to another library, during library preparation, sequencing and/or demultiplexing steps (Esling et al., 2015; Bartram et al., 2016; Guardiola et al., 2016). Finally, we removed those OTUs that did not match any reference sequence in the database at a similarity of 99% and remained unidentified (‘No hit’). These OTUs accounted for an average of 9.4% of the total reads before filtering.

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Table S1 Aboveground abundance in the entire 64 m^2 plot and aboveground (at 5 cm and 20 cm radius grains) and belowground (at three different

Species	Aboveground abundance		Frequency									
	64 m2 plot		aboveground 20		aboveground 5		belowground 0-30		belowground 0-10		belowground 10-30	
	n° individuals	%	0-94	%	0-94	%	0-94	%	0-94	%	0-94	%
Stipa sp	1758	20.56	88	93.62	20	21.28	85	90.43	67	62.98	72	67.68
Linum suffruticosum	1154	13.5	69	73.4	18	19.15	63	67.02	49	46.06	48	45.12
Helianthemum cinereum	1095	12.81	76	80.85	11	11.7	56	59.57	46	43.24	38	35.72
Bupleurum fruticescens	899	10.51	55	58.51	16	17.02	55	58.51	46	43.24	48	45.12
Thymus sp	683	7.99	77	81.91	14	14.89	84	89.36	81	76.14	67	62.98
Koeleria vallesiana	545	6.37	57	60.64	8	8.51	10	10.64	6	5.64	5	4.7
Sideritis incana	343	4.01	44	46.81	5	5.32	25	26.6	19	17.86	16	15.04
Fumana ericoides	339	3.96	56	59.57	8	8.51	43	45.74	34	31.96	23	21.62
Coronilla minima	270	3.16	41	43.62	12	12.77	42	44.68	23	21.62	33	31.02
Arenaria cavanillesiana	260	3.04	26	27.66	6	6.38	13	13.83	8	7.52	7	6.58
Avenula bromoides	169	1.98	18	19.15	2	2.13	0	0	0	0	0	0
Cephalaria leucantha	169	1.98	21	22.34	3	3.19	30	31.91	24	22.56	25	23.5
Helianthemum hirtum	154	1.8	22	23.4	7	7.45	13	13.83	11	10.34	6	5.64
Teucrium sp	118	1.38	19	20.21	6	6.38	30	31.91	23	21.62	21	19.74
Matthiola fruticulosa	81	0.95	10	10.64	4	4.26	7	7.45	4	3.76	6	5.64
Thesium divaricatum	77	0.9	14	14.89	0	0	6	6.38	4	3.76	3	2.82
Asperula aristata	67	0.78	9	9.57	0	0	0	0	0	0	0	0
Hippocrepis commutata	55	0.64	8	8.51	0	0	10	10.64	5	4.7	7	6.58
Coris monspeliensis	49	0.57	6	6.38	1	1.06	0	0	0	0	0	0
Lavandula latifolia	30	0.35	6	6.38	3	3.19	16	17.02	11	10.34	12	11.28
Ononis tridentata	30	0.35	7	7.45	3	3.19	2	2.13	1	0.94	1	0.94
Quercus sp	25	0.29	4	4.26	1	1.06	90	95.74	74	69.56	82	77.08
Euphorbia nicaeensis	24	0.28	6	6.38	0	0	4	4.26	2	1.88	3	2.82
Thymelaea pubescens	19	0.22	6	6.38	2	2.13	7	7.45	3	2.82	6	5.64
Linum narbonense	18	0.21	2	2.13	0	0	2	2.13	1	0.94	2	1.88
Salvia lavandulifolia	16	0.19	1	1.06	0	0	5	5.32	3	2.82	3	2.82
Helychrisum serotinum	14	0.16	2	2.13	0	0	0	0	0	0	0	0
Leuzea conifera	14	0.16	2	2.13	0	0	0	0	0	0	0	0
Phlomis lychinitis	13	0.15	1	1.06	1	1.06	5	5.32	1	0.94	4	3.76
Eryngium campestre	12	0.14	7	7.45	1	1.06	5	5.32	0	0	5	4.7
Helianthemum syriacum	9	0.11	2	2.13	0	0	0	0	0	0	0	0
Santolina chamaecyparissus	9	0.11	2	2.13	0	0	1	1.06	1	0.94	1	0.94
Sanguisorba minor	8	0.09	2	2.13	0	0	0	0	0	0	0	0
Staehelina dubia	8	0.09	3	3.19	0	0	13	13.83	10	9.4	9	8.46
Fumana thymifolia	6	0.07	1	1.06	0	0	4	4.26	3	2.82	4	3.76
Astragalus incanus	4	0.05	0	0	0	0	0	0	0	0	0	0
Jurinea humilis	2	0.02	1	1.06	0	0	4	4.26	0	0	4	3.76
Lithodora fruticosa	2	0.02	1	1.06	0	0	13	13.83	6	5.64	8	7.52
Aristolochia paucinervis	1	0.01	0	0	0	0	0	0	0	0	0	0
Centaurea hyssopifolia	1	0.01	0	0	0	0	0	0	0	0	0	0
Sideritis hirsuta	1	0.01	1	1.06	0	0	0	0	0	0	0	0

depths) species frequencies, calculated as the sum of presence-absence values in the 94 circles/cores (i.e. values from 0 to 94).

Table S2 Adjusted P-values of McNemar Chi-squared test comparing spatial distribution of each species between aboveground and belowground. We considered 30 species, which are the species found both aboveground and belowground.

	Species	McNemar Chi-squared test adj. *P*-value Aboveground (20 cm) versus Belowground		
		0-30 cm	**0-10 cm**	**10-30 cm**
1	*Arenaria cavanillesiana*	**0.017445**	**0.000672**	**0.000414**
2	*Bupleurum fruticescens*	1	0.097711	0.21992
3	*Cephalaria leucantha*	0.242176	0.757856	0.633379
4	*Coronilla minima*	1	**0.005945**	0.343296
5	*Eryngium campestre*	0.778051	NA	0.715807
6	*Euphorbia nicaeensis*	0.825402	0.207845	0.592776
7	*Fumana ericoides*	0.144114	**0.001628**	**6.41E-05**
8	*Fumana thymifolia*	0.399899	0.614764	0.359909
9	*Helianthemum cinereum*	**0.002391**	**1.25E-05**	**2.35E-07**
10	*Helianthemum hirtum*	0.125238	**0.038929**	**0.000733**
11	*Hippocrepis commutata*	0.896483	0.614764	1
12	*Jurinea humilis*	0.512462	NA	0.512462
13	*Koeleria vallesiana*	**7.23E-10**	**9.11E-11**	**2.21E-11**
14	*Lavandula latifolia*	**0.033984**	0.207845	0.159707
15	*Linum narbonense*	NA	1	NA
16	*Linum suffruticosum*	0.402264	**0.000778**	**0.001807**
17	*Lithodora fruticosa*	**0.008678**	0.128867	0.119955
18	*Matthiola fruticulosa*	0.512462	0.088796	0.355526
19	*Ononis tridentata*	0.242176	0.088796	0.159707
20	*Phlomis lychinitis*	0.242176	1	0.359909
21	*Quercus* sp	**1.43E-18**	**4.54E-15**	**8.17E-17**
22	*Salvia lavandulifolia*	0.376439	0.719921	0.715807
23	*Santolina chamaecyparissus*	1	1	1
24	*Sideritis incana*	**0.002391**	**5.83E-05**	**2.65E-05**
25	*Staehelina dubia*	**0.033984**	0.091001	0.21992
26	*Stipa* sp	0.665662	**0.00017**	**0.004459**
27	*Teucrium* sp	0.131951	0.614764	0.907548
28	*Thesium divaricatum*	0.148308	**0.026249**	**0.028488**
29	*Thymelaea pubescens*	1	0.546874	1
30	*Thymus* sp	0.242176	0.614764	0.159707

Table S3 Adjusted *p*-values of Tukey and Nemenyi tests (i.e. only significant results are reported) carried out to compare results obtained considering different aboveground scales in the calculation of the Jaccard dissimilarity index (J index) and the richness differences (ΔR), i.e. the two measures considered to quantify dissimilarities between aboveground and belowground compartments (at three different depths).

Aboveground scale (cm)		**Adj p-values J index**						**Adj P-values ΔR**					
		Belowground depth (cm)						**Belowground depth (cm)**					
		0-10		10-30		0-30		0-10		10-30		0-30	
5	**vs. 10**	<0.0001	***	<0.0001	***	<0.0001	***	<0.01	*	0.01	*	0.01	*
	vs. 15	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***
	vs. 20	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***
	vs. 25	<0.0001	***	<0.0001	***	<0.0001	***	0.0001	***	<0.01	*	<0.0001	***
	vs. 30	<0.0001	***	<0.0001	***	<0.0001	***	1.00		1.00		<0.0001	***
	vs. 40	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	0.001	**	0.48	
	vs. 50	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	0.48	
10	**vs. 15**	0.36		0.18		0.0001	***	<0.01	*	0.02	·	<0.0001	***
	vs. 20	0.06		0.12		<0.0001	***	0.01	*	0.04	·	<0.0001	***
	vs. 25	0.89		0.77		<0.0001	***	1.00		1.00		<0.0001	***
	vs. 30	1.00		0.99		<0.0001	***	0.03	·	0.04	·	<0.0001	***
	vs. 40	0.57		1.00		0.31		<0.0001	***	<0.0001	***	0.42	

	vs. 50	0.03	·	0.71		0.99		<0.0001	***	<0.0001	***	<0.0001	***
15	**vs. 30**	0.51		0.71		1.00		<0.0001	***	<0.0001	***	1.00	
	vs. 40	0.001	**	0.03	·	0.28		<0.0001	***	<0.0001	***	<0.0001	***
	vs. 50	0.001	**	0.01	*	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***
20	**vs. 30**	0.11		0.58		0.43		<0.0001	***	<0.0001	***	0.20	
	vs. 40	0.001	**	0.01	*	<0.01	*	<0.0001	***	<0.0001	***	<0.0001	***
	vs. 50	0.001	**	0.001	**	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***
25	**vs. 30**	0.96		1.00		0.99		<0.01	*	0.02	·	0.19	
	vs. 40	0.03	·	0.31		0.10		<0.0001	***	<0.0001	***	<0.0001	***
	vs. 50	0.0001	***	0.03	·	0.001	**	<0.0001	***	<0.0001	***	<0.0001	***
30	**vs. 40**	0.42		0.77		0.53		<0.0001	***	<0.0001	***	<0.0001	***
	vs. 50	0.01	*	0.19		0.02	·	<0.0001	***	<0.0001	***	<0.0001	***

Table S4 Adjusted *p*-values of Tukey and Nemenyi tests comparing Jaccard dissimilarity index (J index) and richness differences (ΔR) between the 20 cm aboveground scale (i.e. the scale considered to maximise similarity between aboveground and belowground compartments) and the other aboveground scales.

Aboveground scale (cm)		**Adj p-values J index**						**Adj P-values ΔR**					
		Belowground depth (cm)						**Belowground depth (cm)**					
		0-10		**10-30**		**0-30**		**0-10**		**10-30**		**0-30**	
20	**vs. 5**	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***
	vs. 10	0.06		0.12		<0.0001	***	0.01	*	0.04	·	<0.0001	***
	vs. 15	0.99		1.00		0.70		1.00		1.00		0.28	
	vs. 25	0.71		0.94		0.92		0.07		0.09		1.00	
	vs. 30	0.11		0.58		0.43		<0.0001	***	<0.0001	***	0.20	
	vs. 40	0.01	*	<0.01	*	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***
	vs. 50	0.001	**	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***	<0.0001	***

Larger aboveground neighbourhood scales maximise similarity but not eliminate discrepancies with belowground plant diversity in a Mediterranean shrubland

Plant and Soil

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Fig. S1 Boxplots representing aboveground richness at different scales (**a**) and belowground richness at different depths (**b**).

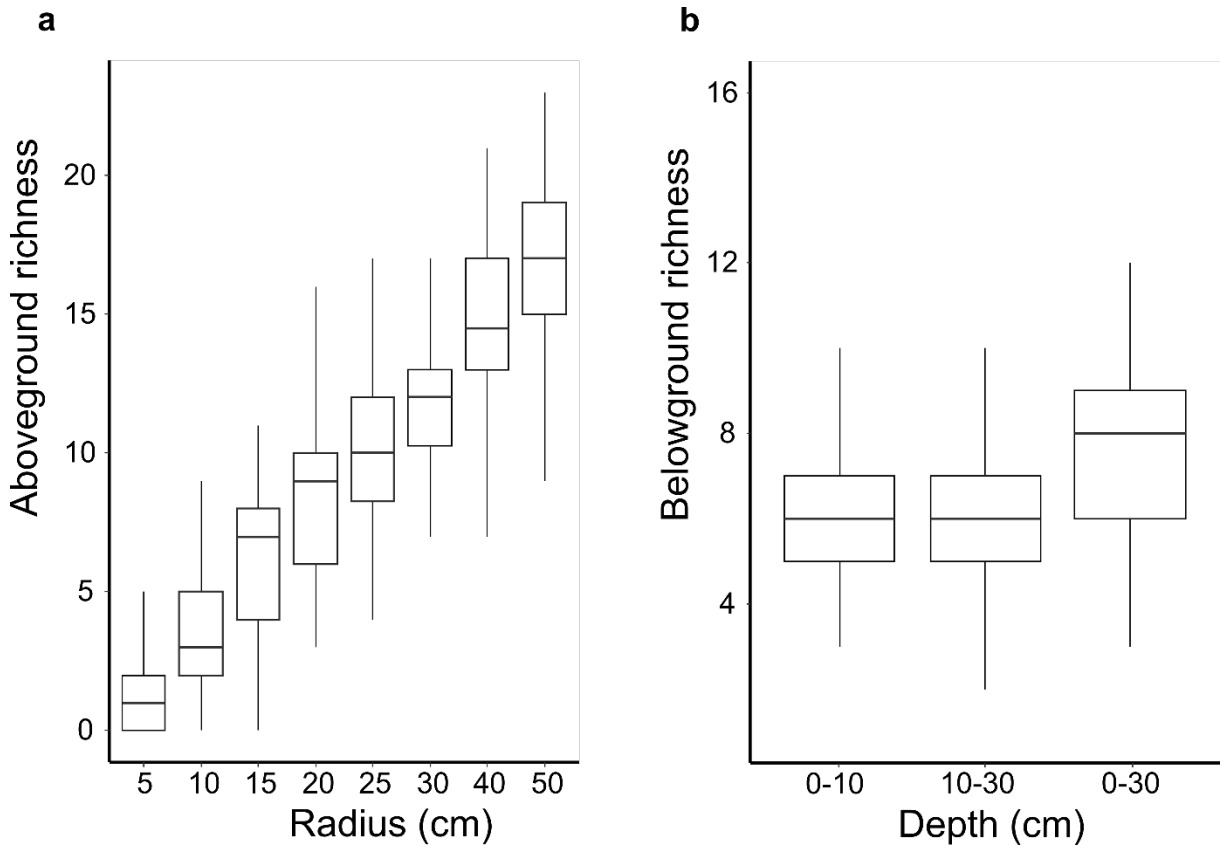

Fig. S2 Kendall correlation between aboveground richness at 20 cm of radius scale and belowground richness at different depths. **a**: belowground richness (0-10 cm); **b**: belowground richness (0-30 cm).

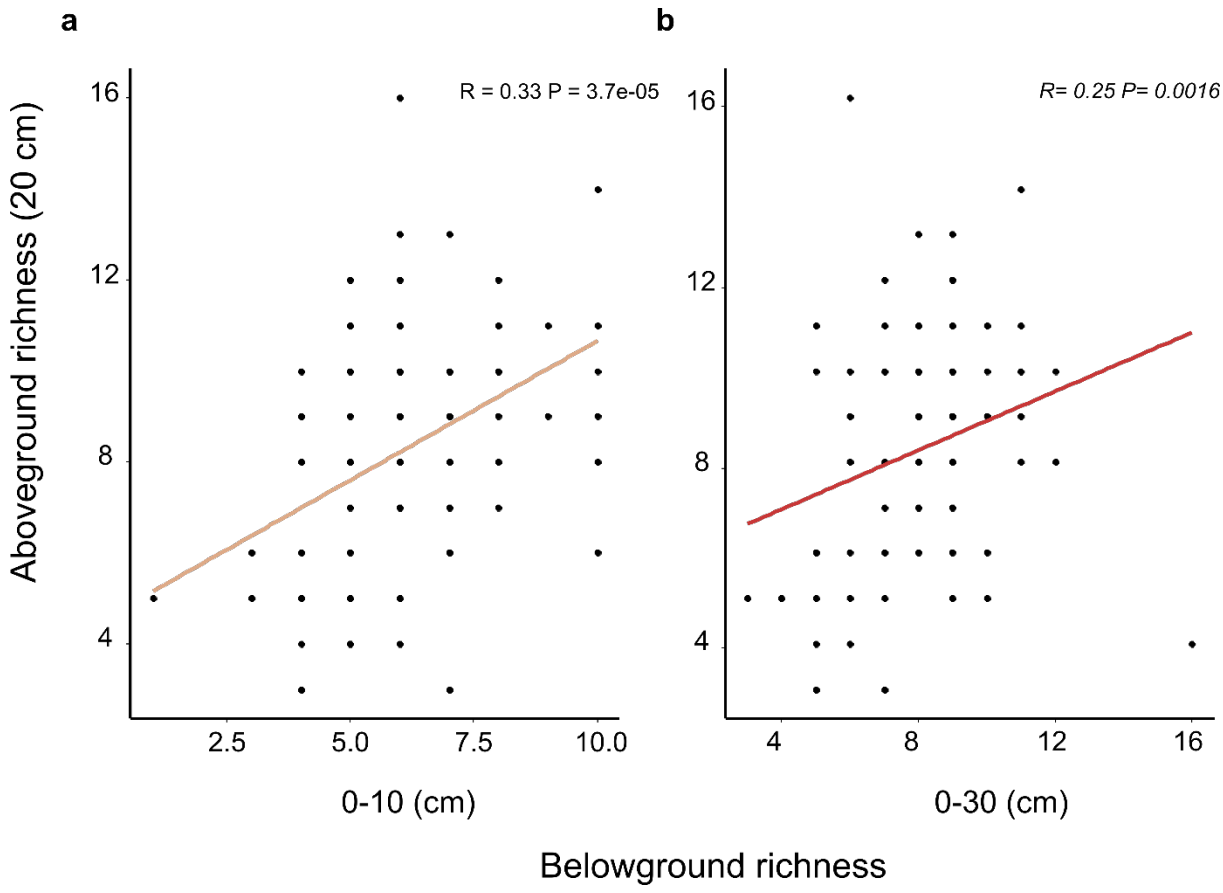

Fig. S3 Mosaic plots representing standardized residuals from the chi-squared test comparing species frequencies aboveground at 20 cm sampling grain vs belowground at different depths, 0-30, 0-10 and 10-30 cm (**a**, **b**, **c**, respectively), and belowground 0-10 vs belowground 10-30 (**d).** The size of the boxes is proportional to each species frequency. As shown by the figure, most of the species frequencies are similar between compared layers, indeed only the coloured boxes correspond to significant differences from the chi-squared test.

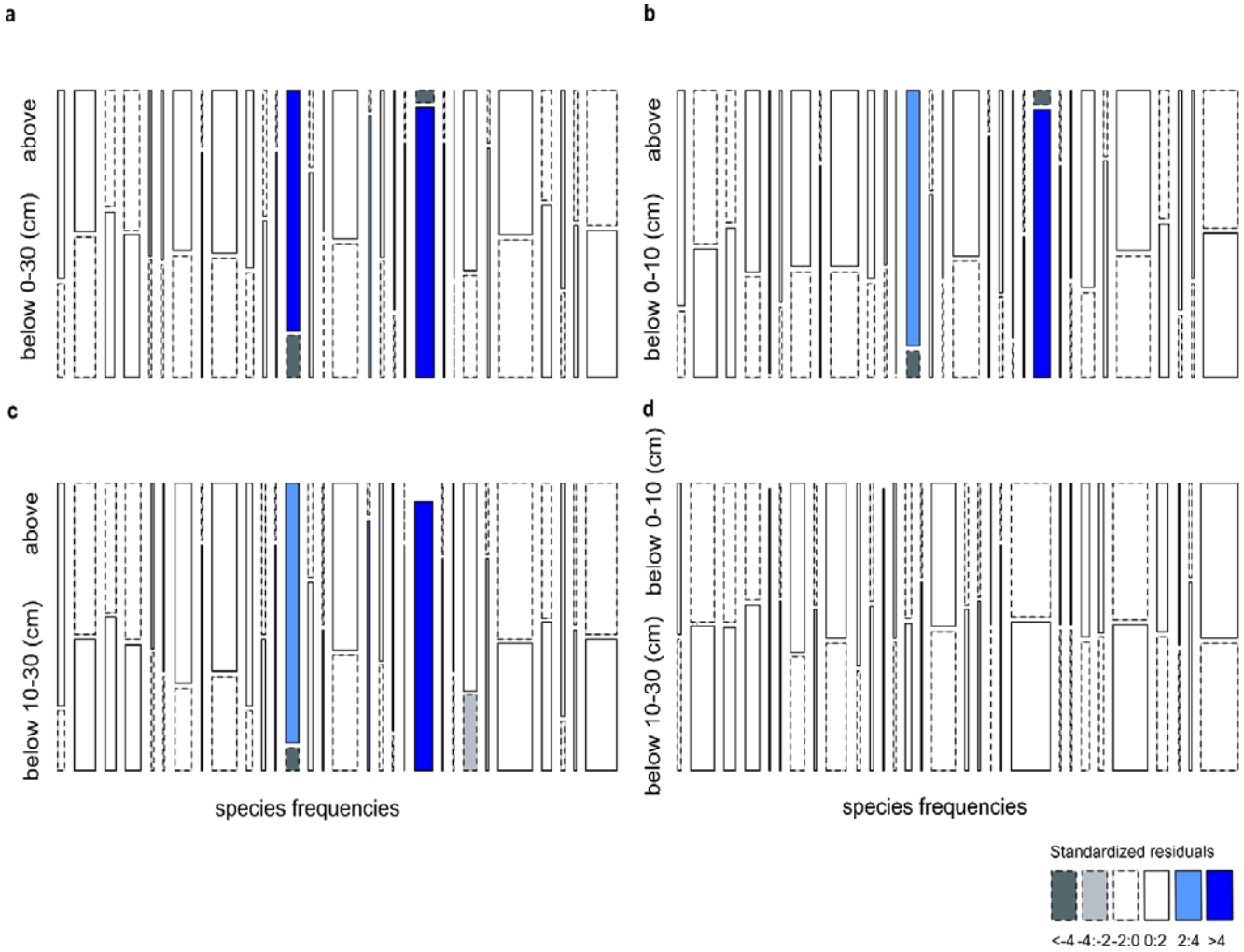

Fig. S4 Belowground richness and root biomass at three depths: 0-10, 10-30 cm and 0-30 cm.