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## Does functional soil microbial diversity contribute to explain within-site plant beta-diversity in an alpine grassland and a *dehesa* meadow in Spain?

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**Title:** Does functional soil microbial diversity contribute to explain within-site plant beta-diversity in an alpine grassland and a *dehesa* meadow in Spain?

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### Summary

**Questions:** Once that the effects of hydrological and chemical soil properties have been accounted for, does soil microbial diversity contribute to explain change in plant community structure (i.e. within-site beta-diversity)? If so, at which spatial scale does microbial diversity operate?

**Location:** La Mina in Moscosa Farm, Salamanca, western Spain (*dehesa* community) and Laguna Larga in the Urbión Peaks, Soria, central-northern Spain (alpine grassland).

**Methods:** The abundance of vascular plant species, soil gram-negative microbial functional types and soil chemical properties (pH, available phosphorus, and extractable cations) were sampled at both sites, for which hydrological models were available. Redundancy analysis (RDA) was used to partition variation in plant community structure into hydrological, chemical and microbial components. Spatial filters, arranged in scalograms, were used to test for the spatial scales at which plant community structure change.

**Results:** In the case of the *dehesa* the diversity of soil gram-negative microbes, weakly driven by soil pH, contributed to a small extent ( $\text{adj-}R^2 = 2\%$ ) and at a relative medium spatial scale to explain change in plant community structure. The abundance of a few *dehesa* species, both annual (*Trifolium dubium*, *Vulpia bromoides*) and perennial (*Poa bulbosa*, *Festuca ampla*), was associated with either increasing or decreasing soil microbial diversity. In the alpine meadow the contribution was negligible.

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**Conclusions:** Microbial diversity can drive community structure, though in the hierarchy of environmental factors structuring communities it appears to rank lower than other soil factors. Still, microbial diversity appears to promote or restrain individual plant species. This paper aims to encourage future studies to use more comprehensive and insightful techniques to assess microbial diversity and to combine this with statistical approaches such as the one used here.

**Key words:** community structure; grassland; Iberian Peninsula; MEM (Moran's Eigenvector Maps) spatial variables; RDA (redundancy analysis); scalogram; soil properties.

**Abbreviations:** AWTD = average water-table depth; CLPP = Community Level Physiological Profiling; MEM = Moran's eigenvector maps; PSF = Plant-Soil Feedback; RDA = redundancy analysis.

**Nomenclature:** Castroviejo 1986-2015 or (when species are missing) Tutin et al. 1964-1980, except for three species otherwise specified in Appendix S1.

## ***Introduction***

Soil micro-organisms are of great importance for long-term sustainability of ecosystems, because they play key roles in organic matter decomposition, nutrient cycling and soil structure (Wagg et al. 2014). Plants are, in general terms, impacted upon by microbiota (Philippot et al. 2013). Positive interactions are well-known in this context (see Mendes et al. 2013 for an overview). For instance, the presence of N-fixing symbiotic bacteria can impact on plant community composition by fostering their plant symbionts (Van Der Heijden et al. 2006). Also, some bacterial strains can support plant growth under abiotic stress like flooding (Grichko & Glick 2001) or drought (Mayak et al. 2004). Likewise, negative interactions with bacteria are well known (Mansfield et al. 2012; Mendes et al. 2013). These have recently received increased attention in research on plant-soil feedback (PSF), portraying that plants, especially the dominant ones, impact on microbes (and other organisms) and are in turn affected by them (e.g. Kardol et al. 2007; Hodge & Fitter 2013). Accordingly, the role of microorganisms for plants in general is well recognized and microbes are acknowledged as a driving factor in mechanisms of plant community assembly.

The concept of environmental filtering, for instance, puts different environmental factors in a hierarchy according to their power of allowing or denying a plant species to establish in a community (Diaz et al. 1998; Kraft et al. 2015). Thus, microbes are seen as a filtering factor, though of minor importance to other factors like water availability, frost, or nutrients. More precise assessments on the position of microbes in such hierarchies of factors would be desirable.

In the context of environmental filtering, only few studies have explored microbial physiological response to environmental change as the driving mechanisms for vegetation composition and productivity. Such examples are Schimel et al. (2007), who studied extreme weather events and carbon dynamics, and also van der Heijden et al. (1998) who explored the role of mycorrhizal diversity in nutrient acquisition and vegetation diversity. This has set the precedent to study soil microbes as drivers of plant diversity, and it has received corroboration from microcosm experiments to field-based modelling studies (Van Der Heijden et al. 2008; van der Putten et al. 2013). As far as the diversity of microorganisms is concerned, we know that soil bacterial diversity can impact on plant performance (Weidner et al. 2015), making it likely that different plant species can differentially profit from microbial biodiversity. Beyond this, relatively little research work has been done on the relationship between the diversity of soil micro-organisms and the composition of plant communities (Bardgett & van der Putten 2014). Hence, there is a requirement to do further analysis on the significance of microbial diversity in the field, to determine where and how observed patterns of impacts on plants may be generalised (van der Putten et al. 2013). This would undoubtedly help improve understanding and prediction of responses to change and promote wise intervention in managing ecosystems. Developing a stronger connection between microbial and ecosystem ecology will also provide a contribution towards a better understanding of plant ecology, particularly in response to contemporary environmental challenges, such as invasiveness of exotic

species as well as response to climate change (Schimel et al. 2007; van der Putten et al. 2013).

This study investigates the relationship between plant community structure and soil microbial diversity (more specifically gram-negative bacteria), while simultaneously monitoring soil abiotic ecological factors such as water regime and topsoil chemistry (major nutrients and cations) in species-rich Iberian plant assemblages. The key questions to be asked are: (1) Once that the effects of hydrological and chemical soil properties have been accounted for, does soil microbial diversity contribute to explain within-site plant beta-diversity (multivariate variation in community structure)?; (2) If so, what is the spatial scale at which soil microbial diversity operates?

### ***Materials and methods***

This study is situated in the Iberian Peninsula, which is one of the main constituents to the Mediterranean flora (Médail & Quézel 1999). The Mediterranean area is one of the recognized twenty-five global Biodiversity Hotspots (Myers et al. 2000).

#### ***Field survey***

An Iberian *dehesa* meadow, which corresponds to Eunis habitat type 6310 (European Commission 2013), on acidic (granite) soils was sampled in spring at La Mina site in Moscosa Farm (41° 8' 21.88" N, 6° 6' 52.33" W; 780 m a.s.l.), Salamanca province, western Spain. This is a highly diverse (both annuals and perennials) seasonally dry (Mediterranean) grassland with scattered trees (*Quercus ilex* subsp. *ballota* (Desf.) Samp. = *Q. rotundifolia* Lam.). Likewise, an Iberian alpine meadow, also on acidic (sandstone/conglomerate) soils, was sampled in summer at Laguna Larga in the Urbión Peaks (42°0' 19.50" N, 2°52' 2.26" W; 2080 m a.s.l.), Soria province, central-northern Spain. This is a wet alpine pasture, rich in perennial herbs but not in annuals, close to a kettle pond. In both sites, chosen for study on the grounds of their contrasting plant diversity, the abundance of plant species was recorded (2007) in  $n = 38$  1-m<sup>2</sup> quadrats placed randomly on a 50 x 50 m study plot with a 1.8% gradient of slope (*dehesa*) and a 80 x 35 m study plot with a 2.1% gradient of slope (alpine meadow). Random sampling with quadrats of 1m<sup>2</sup> is a standard for the study of herbaceous vegetation. Two adjacent soil cores were collected from the centre of each quadrat at 10-cm depth, thereby obtaining similar soil samples of 0.34 l each. These samples were then analysed within 2 weeks of collection. The position of each quadrat in a Cartesian coordinate system was recorded using a Leica Geosystems TPS800 machine. Plant nomenclature followed standard Floras (Tutin et al. 1964-1980; Castroviejo 1986-2015), except for the species otherwise specified (Table S1 in Appendix S1).

#### ***Quantification of the hydrological gradient***

Field-scale hydrological models for both sites had been previously built (García-Baquero et al. 2016). These models quantified the average water-table depth (AWTD) in each quadrat during the growing season (from mid February to end of September, about 30 weeks, for La Mina site; from mid-May to mid-September, about 20 weeks, for Laguna Larga). Water-table depth was measured from an origin at ground level with a positive downwards direction. Hence high values of AWTD match high levels of water-deficit stress (soil drying) and low values of AWTD match high levels of oxygen-deficit stress (waterlogging). A more exhaustive description of the methods employed to construct the hydrological model as well as the model itself can be found in García-Baquero et al. (2016).

#### ***Soil properties***

One core per quadrat was air-dried and divided into three portions. One portion was used to measure soil pH. The second portion was analysed using Olsen's method of phosphorus extraction (Gilbert et al. 2009) and available soil phosphorus was determined by means of the molybdenum method (MAFF 1986). The third portion was used to determine soil content in cations calcium, magnesium, potassium, and sodium. Extractable soil cations were extracted with ten grams of air-

dry soil passed through 2 mm sieve and shaken with 50 ml of  $\text{NH}_4$ -acetate for 30 minutes. The slurry was then filtered using Whatman® No. 42 filter paper and the extract analysed using Gallenkamp® SGA\_330C (Gallenkamp, Loughborough, UK) flame photometer (MAFF 1986).

The second soil core was used to determine the abundance of soil microbe functional groups. Soil microbial community composition was monitored with the technique of Community Level Physiological Profiling (CLPP) using BIOLOG® microplates (Garland & Mills 1991) with the protocol outlined by Garland (1997). The Biolog® GN microplates (Biolog Inc., Hayward, CA, USA) are used to identify living Gram-negative bacteria. They contain 95 different carbon sources, together with exudate profile microplates, prepared using Biolog® MT plates, in which dilution of 10 g of rhizosphere soil in 100 ml of one-quarter strength Ringers solution (Oxoid) for 10 min are centrifuged and 150 microlitre aliquot of each sample was dispensed into each well of the GN and exudate plates. The microplates were incubated at 15°C for 5 days and colour development intensity (carbon utilisation) was measured as absorbance at 590 nm (A590) every 24 h using a microplate reader (Vmax, Molecular Devices, Oxford, UK). The full tables for the Biolog® profiles, two matrices of 38 rows  $\times$  95 columns, and are deposited at the Dryad Digital Repository (<http://datadryad.org/>).

### **Data preparation**

A four-matrix dataset was constructed for each site: a plant species matrix of  $n = 38$  quadrats  $\times$   $p$  plant species, where each matrix element represented the abundance of species ( $p = 91$  for La Mina;  $p = 39$  for Laguna Larga; see Tables S2-S3); a microbe functional groups matrix of 38 quadrats  $\times$  95 microbe functional groups, where each matrix element represented the abundance of microbial functional groups; a geographic matrix of 38 quadrats  $\times$  2 Cartesian coordinates ( $X, Y$ ), where matrix elements specified the position of the quadrats in the Cartesian coordinate system; and an environment matrix of 38 quadrats  $\times$  8 hydrological and soil chemical descriptors, where each matrix element specified the value of the environmental variables for each quadrat. These environmental descriptors were: AWTD in m (as given by our hydrological models; see García-Baquero et al. 2016), soil available phosphorus ( $\text{mg kg}^{-1}$ ), pH, and soil content ( $\text{meq kg}^{-1}$ ) in cations calcium, potassium, magnesium and sodium. The microbial matrix was used to derive the Shannon diversity index (Magurran 2004), which was subsequently used as the eighth environmental variable (describing soil microbial diversity). Summaries of these environmental variables are available in Tables S4-S5 and Figs. S3-S4.

### **Data analysis**

To answer our questions, we developed a two-step analysis strategy. As the first step, to find out whether soil microbial diversity contributes to explain within-site plant beta-diversity, we used a constrained ordination technique: Redundancy Analysis (Legendre & Legendre 2012). As the second step, to identify the spatial scale at which soil properties and soil microbial diversity operate, we used MEM (Moran's eigenvector maps) spatial variables (Dray et al. 2006) arranged in scalograms as proposed by Dray et al. (2012). The data analysis was carried out using R software v.3.2.2 (R Core Team 2015). Supporting information provides explained R coding (Appendix S2) to replicate the analysis and the full Laguna Larga dataset (Appendix S3). These appendices are also intended to complement, with all necessary detail, the data analysis strategy summarized below.

In the first step, and prior to any analysis, the above plant species matrix was Hellinger-transformed (Legendre & Gallagher 2001) to create the response matrix  $\mathbf{Y}$ : the matrix elements are standardized by row and then square-root transformed, making species data amenable to Principal Component Analysis and RDA modeling (Legendre & Legendre 2012). Once created, matrix  $\mathbf{Y}$  was submitted to both Principal Component Analysis and RDA modeling, for which we used function `rda()` of "vegan" (Oksanen et al. 2013). RDA is a statistical technique for constrained ordination based in multivariate (multi-response) multiple linear regression (Legendre & Legendre 2012). Hence the Hellinger-transformed plant species matrix (i.e.,  $\mathbf{Y}$ ) was constrained through the above environment

matrix using RDA, thus creating matrix **F** (“fitted”). To select a parsimonious explanatory RDA model, all environmental variables were submitted to forward selection with the Blanchet et al. (2008) double stopping criterion. Many of the species in the field sites show nonlinear relationships with AWTD (García-Baquero et al. 2016; see also Figs. S1-S2), so we modelled these relationships using a quadratic model (Borcard et al. 2011), i.e. through orthogonal first- and second-degree AWTD polynomial terms. Finally, we partitioned variation (Borcard et al. 1992) in plant abundance into hydrological, chemical and microbial components –for which function `varpart()` of “vegan” was used. A similar procedure was applied to fit RDA models explaining microbial community structure in terms of soil properties.

In the mentioned second step, to determine the spatial scales at which the canonical axes of raw (matrix **Y** after Principal Component Analysis) and environmentally constrained (matrix **F**) multivariate plant species abundance change (the residual matrix **R** can also be tested), we constructed  $n - 1 = 37$  MEM (Moran’s eigenvector maps) spatial variables (Dray et al. 2006) as follows. Initially, for each field site, we defined a spatial weighting matrix **W**, which is the result of the Hadamard product of a connectivity matrix **B** and an edge weighting matrix **A** ( $\mathbf{W} = \mathbf{B} * \mathbf{A}$ , where  $*$  indicates the Hadamard product). Matrix **B** is binary and specifies whether two sample units (quadrats) in the geographic space specified by our Cartesian coordinate systems are connected (1) or not (0). Then two connected sample units are assumed to be linked by an edge, specified by matrix **A**. In this application the connectivity matrix **B** was based on the Gabriel graph (Legendre & Legendre 2012). This provides a sufficiently dense, but not crowded, connectivity matrix **B**, apt for ecological modeling (Borcard et al. 2011; Chapter 7; Dray et al. 2012); for this purpose, we used the function `gabrielneigh()` the package `spdep` (Bivand 2010) and the geographic matrix above described. Finally, the elements in matrix **B** were weighted (edge weighting matrix **A**) by a function of inverse distance (Dray et al. 2006), so that nearby sample units are more intensely connected than far-away sample units.

Having defined matrix **W**, we created the MEM spatial variables through `scores.listw()` of `spacemaker` (Dray 2013). The procedure simply consists of an eigenvector decomposition of matrix **W**, where the resulting  $n - 1 = 37$  eigenvectors (Moran’s eigenvectors) are the desired spatial variables (Dray et al. 2006; Legendre & Legendre 2012). These eigenvectors are spatial templates (“filters” sensu Sojininen 2016) that may capture spatial patterns (Legendre & Legendre 2012). Once obtained (Figs. S5-S6), the first 36 MEMs (for consistency, the last one was not used) were arranged in scalograms as proposed by Dray et al. (2012), for which these 36 MEMs were organised in six groups of six spatial variables each. The scalograms were built by adapting the relevant part of the R code published in Dray et al. (2012). These scalograms display (vertical axis) the fraction of variance ( $R^2$ ) in the canonical axes of matrices **Y** and **F** that is explained by the MEM spatial variables, which are ordered along the horizontal axis by decreasing eigenvalues (Legendre & Legendre 2012) in such a way that bars 1 and 2 correspond with variation at a relative broad scale, 3 and 4 with variation at a relative medium scale, and 5 and 6 with variation at a relative fine scale. In our application, bars 1 and 2 correspond with variation over a spatial extent of tens of meters, 3 and 4 with variation over a spatial extent of about ten-fifteen meters, and 5 and 6 with variation over a spatial extent of less than ten meters; only the scale that corresponds to the highest observed  $R^2$  was tested statistically.

## Results

### ***Does soil microbial diversity contribute to explain within-site plant beta-diversity?***

For the *dehesa* meadow (La Mina in Moscosa Farm), where 91 plant species were recorded (Table S2 in Appendix S1), AWTD, AWTD<sup>2</sup>, soil content in cation calcium and microbial diversity explained  $\text{adj.-}R^2 = 24.5\%$  of multivariate variation in plant community structure (Table 1). In the corresponding constrained ordination (Fig. 1), the first axis (RDA1) is strongly related with AWTD and cation calcium, whereas the second RDA axis (not shown in Fig. 1) is related with the second-degree term AWTD<sup>2</sup>. The third axis (RDA3), however, is related with microbial diversity: the abundance of a few species such as *Festuca ampla* or *Trifolium dubium* is associated with high values of soil

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microbial diversity (positive association), whereas the abundance of a few species such as *Poa bulbosa* or *Vulpia bromoides* is associated with low values of microbial diversity (negative association). Partitioning of variation (Fig. 2) shows that within-site plant beta-diversity is primarily driven by the hydrological and soil chemical components; by contrast, the contribution of the pure soil microbial component is just  $\text{adj-}R^2 = 2\%$ .

For the alpine meadow (Laguna Larga in Urbion Peaks), where 39 plant species were recorded (Table S3), AWTD and AWTD<sup>2</sup>, as well as soil calcium, potassium and available phosphorus, but not microbial diversity, explained  $\text{adj-}R^2 = 41.6\%$  of multivariate variation in plant community structure (Table 1). Hence the contribution of soil microbial diversity in Laguna Larga to the explanation of within-site plant beta-diversity is null (Fig. S8).

#### **What is the spatial scale at which soil microbial diversity operates?**

For the *dehesa* meadow, the first two axes are not related with microbial diversity. For this reason, although these axes are explained in Fig. S7a-b (including residual variation for completeness), they are not further explained here. The third canonical axis of the community structure matrix, **Y** (Fig. 3a.1-a.2), shows a significant accumulation of spatial variance at a broad spatial scale ( $R^2 = 45\%$ ;  $p = 0.0048$ ). However, the third axis of the fitted community structure matrix **F** (Fig. 3b.1-b.2), which shows how within-site plant beta-diversity is constrained by microbial diversity (Table 1 and Fig. 1), displays a significant medium-scale component ( $R^2 = 33\%$ ;  $p = 0.0466$ ). This indicates that although the plant species variance described by the third axis tends to accumulate at a relatively broad scale (i.e. over a spatial extent of tens of meters), soil microbial diversity constrains plant beta-diversity at a medium scale (i.e. over a spatial extent of about ten-fifteen meters).

For the alpine meadow, none of the axes are related with microbial diversity. For this reason, although these axes are explained in Figs. S9a-c (including residual variation for completeness), they are not further commented on here.

On a side note, we also examined to what extent soil microbial structure is driven by soil chemical and hydrological properties. For the *dehesa* meadow, soil pH and soil content in cation potassium explained  $\text{adj-}R^2 = 7.4\%$  of multivariate variation in microbial community structure (Table S6). For the alpine meadow, soil pH and soil content in cation sodium, together with AWTD, explained  $\text{adj-}R^2 = 8.7\%$  of multivariate variation in microbial community structure (Table S6). Soil pH was the most important environmental factor in both cases.

#### **Discussion**

To date we have a fairly good picture of possible mechanisms how microbial diversity can impact on plant occurrence (Bardgett & van der Putten 2014; Wagg et al. 2014), but not on how strong this impact may be. The latter is where we put the focus of our study. Striving to quantify how strong the impact of microbial diversity on plant community structure may be, we found that for the *dehesa* plant community two percent of variance in plant community structure was explained by microbial diversity. This association was operational at a relative medium scale. For the alpine meadow, we found no such association.

#### **Fraction of variance in plant species occurrence explained by microbial diversity**

Two percent of explained variance may sound like a very modest value, but this should not be underestimated. After all, this explained variance is the unique contribution of the microbial component, once that soil and hydrological heterogeneity has been accounted for (Fig. 2). We also have to be aware that plant occurrence and microbial diversity is a complex network with feedbacks in both directions (Philippot et al. 2013). Hence, the results from our observational study can be seen as complementary to results from manipulative studies dealing with plant-soil feedbacks (PSF). Studies on PSF have identified the accumulation of species-specific pathogens as a factor restricting otherwise abundant plant species (e.g. Klironomos 2002; Kardol et al. 2007; Hodge & Fitter 2013).

Although we found a significant relationship in only one site, the above notion is compatible with our result that composition of vegetation is in fact responsive to microbial (in our case specifically bacterial) diversity. Possible reasons why microbial diversity impacts on species composition in the dehesa site, but not in the alpine site might include vegetation dynamics. Arguably, there is likely a higher year-to-year dynamic in the dehesa, where high proportions of annual species are found (46 out of 77 species as compared to 1 out of 23 species at the alpine site, compare Table S2, S3). The reasoning here is that annuals face a number of additional challenges that are likely impacted upon by bacteria, like germination, seedling survival, and establishment.

In PSF research, recent studies have shown that plant species respond differentially to whether the soil has been sterilized or not (Maron et al. 2016), indicating that soil microorganisms are beneficial for some plant species, but detrimental to others. This matches well with our finding that some species are promoted by high microbial diversity, while some are not. Some species (Fig. 1) were identified as positively associated with microbial diversity (*Festuca ampla*, *Trifolium dubium*), whereas other (*Vulpia bromoides*, *Poa bulbosa*) were identified as negatively associated with microbial diversity. Since our RDA analysis (Figs. 1-2; Table 1) examines the relationship between microbial diversity and community structure after taking into account the effects of soil and hydrological heterogeneity, we are not looking at any indirect effects like, for instance, available water impacting both on plant occurrence and microbial diversity. We are in fact looking at the relationship between the unique contribution of soil microbial diversity and plant community structure, suggesting that two percent of plant variance explained by microbial diversity is not at all negligible.

The (moderate) importance of microbial diversity for plant community structure can also be discussed in the context of species assembly. These include schemes of environmental filtering (see Kraft et al. 2015 for review). Following the thread of environmental filtering, to be able to occur at a certain site, a species has to be adapted to the various abiotic filters like frost, drought, soil chemical properties, etc. Species also need to be adapted to biotic factors like predation, competition and facilitation, and of course interactions with microbiota. Even if the effect size of two percent is not large, our results underpin that microbial diversity as a potential filter can be relevant for plant community structure. At the same time, in accordance with the environmental filtering approach, our results agree with the notion that many of the aforementioned factors rank higher in the hierarchy of filters. This might apply not only to our examined *dehesa* and alpine meadows, but possibly to other habitats as well. Despite the modest reach of our findings and the fact that the CLPP method is limited to gram-negative bacteria, ours is a keen analytical attempt of using observational field data to disentangle the complex relationships between microorganisms, soil characteristics and plant community structure. We believe that future research aimed at disentangling such complex relationships, will benefit from the script-documented (Appendices S2-S3) statistical methods suggested here. We also suggest utilising more comprehensive microbial community profiling approach such Phospholipid Fatty Acid (PLFA), which assesses both bacteria and fungi (Grayston et al. 2004) and high-throughput sequencing approaches.

### ***Spatial scale***

Our analysis also indicated that, when present, the effect of microbial diversity on plant community structure is found mainly at relatively medium spatial scales (i.e. over an extent of about 10-15 meters) but not at larger scales (Fig. 3b.1-b.2). We cannot exclude the possibility that this is an outcome specifically related to La Mina in Moscosa Farm field site. Here, microbial diversity is dependent on soil pH and, secondarily, other soil chemical properties (Table S6), the variance of which is possibly dependent on spatial scales. Yet, a second, more general explanation is also conceivable: along larger spatial scales, other gradients like hydrological gradients impact quite strongly on plant species distribution (Araya et al. 2011; García-Baquero et al. 2016). Modest effects of microbial diversity would possibly be undetectable due to marked plant species turnover. The point that microbial diversity can affect species abundance at such spatial scale is relevant, because



it may be related back to the concept of environmental filtering (Kraft et al. 2015). This considers a hierarchy of factors with some operating at continental scales (like humidity of macroclimate), some at rather regional scales (like disturbance), and some on a more local scale (including most biotic factors) (Diaz et al. 1998; Kraft et al. 2015). Hence the spatial scale at which microbial diversity has been found to be operational somewhat reflects its small, yet noteworthy contribution to species assembly. Very fine scales (e.g. over an extent of 1 or 2 meters) were not accessed in this study due to the lower scale-limits of the sampling design undertaken. However, it is conceivable that microbes operate even on such small scales to impact on plant species composition (e.g. Ettema & Wardle 2002).

### ***Interrelation between vegetation and microbiota is bidirectional and complex***

Not only do microbes impact on plant community structure, but plants also impact on the occurrence of microbes. A number of studies have focused on the latter. The composition of vegetation impacts on, for instance, the nitrifying community (Hawkes et al. 2005) or the community of specific bacterial genera (Bakker et al. 2013). More specifically, it is known that the microbial community in a plant's rhizosphere is dependent on plant species and even on genetic identity (Lundberg et al. 2012; Philippot et al. 2013). Hence, the interplay between plant species occurrence and microbial diversity is clearly bidirectional and complex. Our results can be put into this context by pointing out that our observation study is by its nature looking for associations between variables rather than for demonstrating causal connections. There is a requirement to do further research on the consequences of PSF to determine where and how observed patterns may be generalised (van der Putten et al. 2013), ideally combining observational and manipulative studies.

### ***Conclusions***

Microbial diversity can be a driver of plant community structure, at least in some habitats. In the hierarchy of ecological factors structuring plant communities, microbial diversity appears to rank lower than a number of other soil factors in our study, but it still has a potential to promote or restrain individual plant species. This finding accommodates well with the concept of environmental filtering. It is plausible that studies using more powerful and selective methods, like high throughput sequencing, will be able to come to even more differentiated results. This paper aims to encourage such studies, and we propose to combine them with statistical procedures such as those carried out in our analysis.

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## Supporting Information

Additional supporting information may be found in the online version of this article:

**Appendix S1.** Supplementary Tables S1-S6 and supplementary Figures S1-S9.

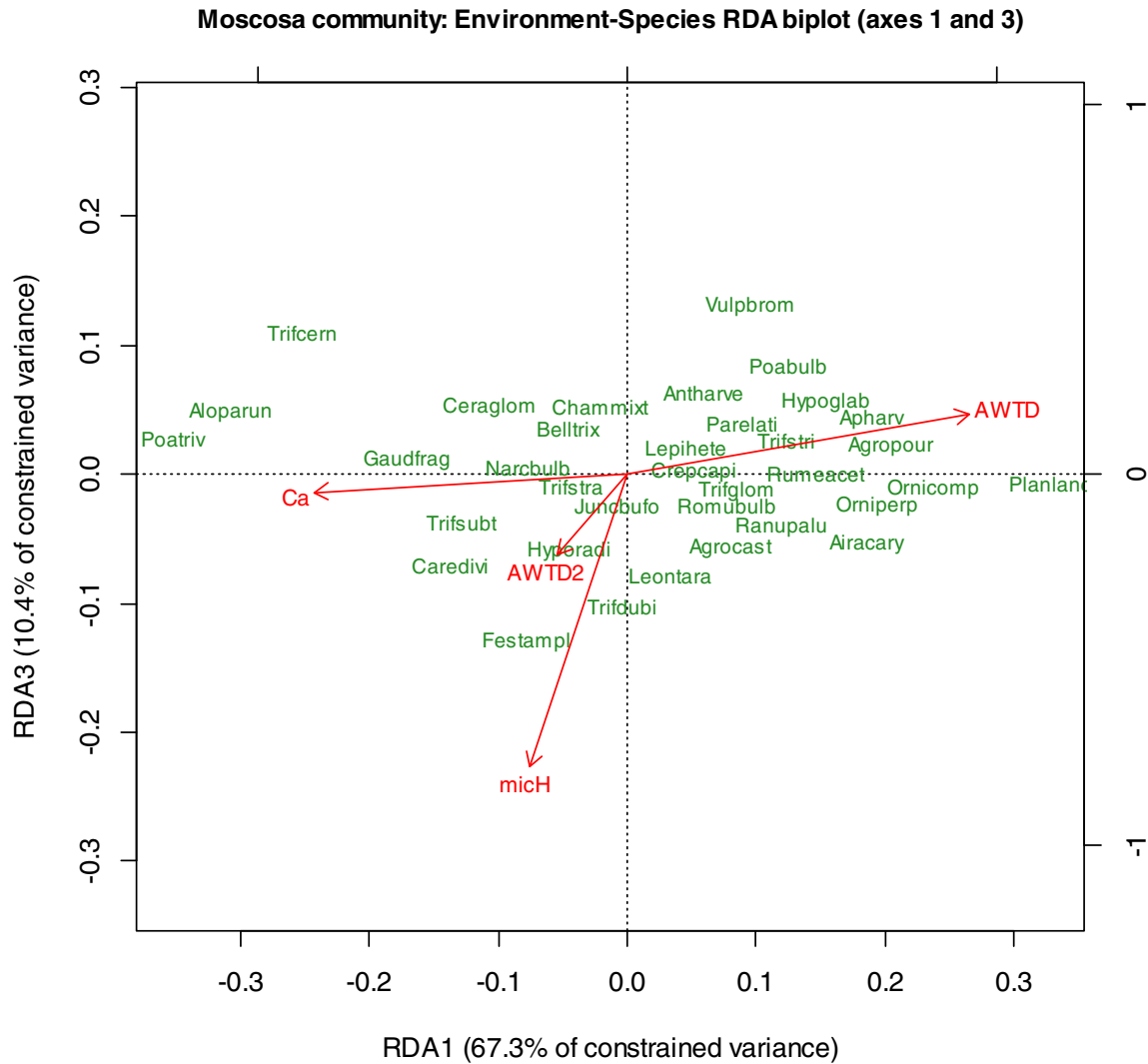
**Appendix S2.** R code.

**Appendix S3.** Urbión Peaks (Laguna Larga field site) data set.

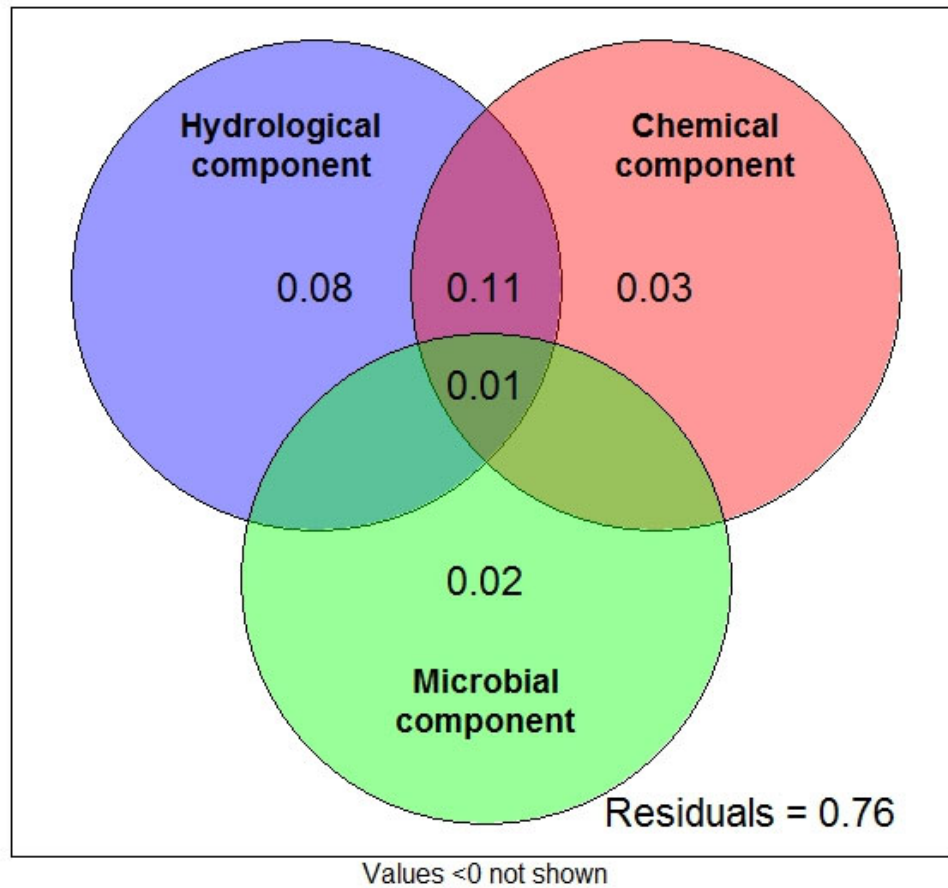
Data available from the Dryad Digital Repository: <http://dx.doi.org/00.0000/dryad.xx000>.

**Table 1.** Plant community structure (species abundance) explained by water table depth, soil chemical properties and soil microbial diversity (Shannon index). Redundancy analysis (RDA) results: (i) model summary, (ii) marginal effects of terms and (iii) variation explained by the individual axes. The response is a Hellinger-transformed matrix computed on the abundance of plants at both La Mina ( $n = 38$ ) in Moscosa Farm and Laguna Larga ( $n = 38$ ) in Urbión Peaks. For La Mina, variance inflation factors are 1.8 (AWTD), 1.2 (AWTD<sup>2</sup>), 2.5 (cation calcium), and 1.4 (microbial diversity); for Laguna Larga, 1.9 (AWTD), 1.2 (AWTD<sup>2</sup>), 3.6 (cation calcium), 3.1 (cation potassium), and 1.3 (phosphorus). AWTD = Average Water Table Depth.

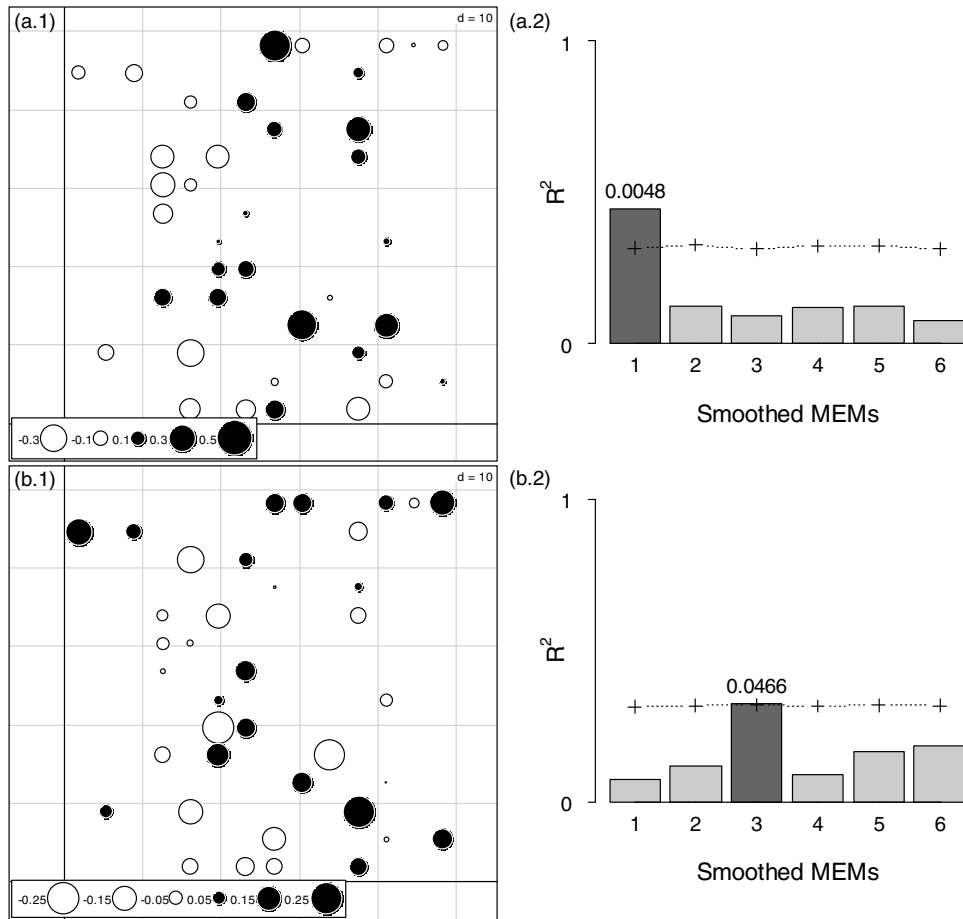
	Moscosa (Adj.-R <sup>2</sup> = 24.5%)				Urbión Peaks (Adj.-R <sup>2</sup> = 41.6)			
	df	var.	F-value	p-value	df	var.	F-value	p-value
<b>(i)</b>								
Model	4	0.127	3.99	0.001	5	0.301	6.28	0.001
Residual	33	0.262			32	0.307		
<b>(ii)</b>								
AWTD	1	0.076	9.50	0.000	1	0.150	15.67	0.001
AWTD <sup>2</sup>	1	0.021	2.58	0.007	1	0.049	5.08	0.002
Cation calcium	1	0.016	2.05	0.021	1	0.039	4.08	0.006
Microbial diversity	1	0.015	1.85	0.035	-	-	-	-
Cation potassium	-	-	-	-	1	0.041	4.24	0.003
Available phosphorus	-	-	-	-	1	0.022	2.31	0.046
Residual	33	0.262			32	0.307		
<b>(iii)</b>								
RDA1	1	0.086	10.75	0.001	1	0.190	19.77	0.001
RDA2	1	0.019	2.44	0.001	1	0.058	6.06	0.001
RDA3	1	0.013	1.67	0.016	1	0.030	3.09	0.003
RDA4	1	0.009	1.11	0.289	1	0.013	1.40	0.188
RDA5	-	-	-	-	1	0.010	1.06	0.369
Residual	33	0.262			32	0.307		



**Figure 1.** Plot of the redundancy analysis (RDA) model summarized in Table 1: axes 1 and 3. AWTD = Average Water Table Depth; Ca = cation calcium; micH = microbial diversity (Shannon index); RDA = canonical axes. **Key to species** (to avoid crowding, only frequent species are shown): Agrocast = *Agrostis castellana*; Agropour = *Agrostis pourretii*; Airacary = *Aira caryophyllea*; Alopapun = *Alopecurus arundinaceus*; Antharve = *Anthemis arvensis*; Apharv = *Aphanes arvensis*; Belltrix = *Bellardia trixago*; Caredivi = *Carex divisa*; Ceraglom = *Cerastium glomeratum*; Chammixt = *Chamaemelum mixtum*; Crepcapi = *Crepis capillaris*; Festampl = *Festuca ampla*; Gaudfrag = *Gaudinia fragilis*; Hypoglab = *Hypochoeris glabra*; Hyporadi = *Hypochoeris radicata*; Juncbufo = *Juncus bufonius*; Leontara = *Leontodon taraxacoides*; Lepihete = *Lepidium heterophyllum*; Narcbulb = *Narcissus bulbocodium*; Ornicomp = *Ornithopus compressus*; Orniperp = *Ornithopus perpusillus*; Parelati = *Parentucellia latifolia*; Planlanc = *Plantago lanceolata*; Poabulb = *Poa bulbosa*; Poatriv = *Poa trivialis*; Ranupalu = *Ranunculus paludosus*; Romubulb = *Romulea bulbocodium*; Rumeacet = *Rumex acetosella*; Trifcern = *Trifolium cernuum*; Trifdubi = *Trifolium dubium*; Trifglom = *Trifolium glomeratum*; Trifstra = *Trifolium striatum*; Trifstri = *Trifolium strictum*; Trifsubt = *Trifolium subterraneum*; Vulpbrom = *Vulpia bromoides*.



**Figure 2.** Partitioning of variation in community structure of the *dehesa* meadow at La Mina field site in Moscosa Farm (Salamanca, western Spain) into hydrological, soil chemical and soil microbial (Shannon index) components after redundancy analysis (see Table 1 and Fig. 1). The values indicated within the circles in this Venn diagram correspond to adjusted- $R^2$  (proportion of variance explained by each component). The hydrological component includes orthogonal first- and second-degree terms for average water table depth (AWTD), i.e. consists of a quadratic model. The chemical component includes only soil content in cation calcium (all other soil chemical properties were not significant).



**Figure 3.** Maps (a.1-b.1) of La Mina field site in Moscosa Farm (50 x 50 m) depicting in Cartesian space the quadrat ( $n = 38$ ) scores on the third axis of the principal component analysis of matrix **Y** (a.1), which represents raw (unconstrained) variation in community structure, and the RDA-fitted (constrained) matrix **F** (b.1; see also Fig. 1 and Table 1). The scalograms (a.2-b.2), which test for the spatial scales at which raw (b.1) and fitted (b.2) variation in community structure change, show the fraction of variance ( $R^2$ ) explained by each spatial scale. Bars 1-2, 3-4, and 5-6 correspond with variation at a relative broad (over an extent of tens of meters), medium (over an extent of about ten-fifteen meters), and fine (over an extent of less than ten meters) spatial scales, respectively. The line of plus signs indicates the 95% confidence bound, although only the spatial scale that corresponds to the highest  $R^2$  is statistically tested.  $d$  = distance (in m).

The map in b.1 shows how community structure (on the third canonical axis) is constrained by microbial diversity: whereas positive (black) bubbles indicate high abundance of species such as *Festuca ampla* or *Trifolium dubium* that is explained by high values of soil microbial diversity (positive association), negative (white) bubbles indicate high abundance of species such as *Poa bulbosa* or *Vulpia bromoides* that is explained by low values of microbial diversity (negative association). The scalograms indicate that whereas unconstrained variation (on the third canonical axis) in community structure (as depicted in a.1) shows a non-random spatial pattern with accumulation of variance at a broad-scale (a.2:  $R^2 = 44.6\%$ ,  $p = 0.0048$ ), variation in community structure constrained by microbial diversity (b.1) shows a significant (b.2:  $R^2 = 32.5\%$ ;  $p = 0.0466$ ) medium-scale spatial pattern. This indicates that microbial diversity varying at a relative medium-scale contributes to explain plant community structure.