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Agricultural matrix affects differently the alpha and beta structural and functional diversity of soil microbial communities in a fragmented Mediterranean holm oak forest

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Abstract

Given the increase in habitat fragmentation in the Mediterranean forests, understanding its impacts over the ecology of soil microbial communities, responsible for many ecosystem functions, and their capacity to metabolize different substrates from soil organic matter, is of upmost importance. We evaluated how the influence of the agricultural matrix, as one of the main consequences of forest fragmentation, may affect both the composition and the functioning of soil microbial communities in Mediterranean holm oak forests. We determined structural and functional alpha and beta-diversity of microbial communities, as well as microbial assemblages and metabolic profiles, by using a commonly used fingerprinting technique (Denaturing Gel Gradient Electrophoresis) and a community level physiological profiles (CLPP) technique (EcoPlate). Key drivers of soil microbial structure and metabolism were evaluated by using structural equation models (SEM) and multivariate ordination (envfit) approaches. Our results pointed out that forest fragmentation affects microbial community structure and functioning through a complex cascade of causal-effect interactions with the plant-soil system, which ultimately affects the nutrient cycling and functioning of forest soils. We also found a strong scale-dependency effect of forest fragmentation over the ecology of microbial communities: fragmentation increases the local (alpha) diversity, but affected negatively microbial diversity at the landscape scale (beta diversity). This homogenization of the microbial communities and their metabolism at landscape scale resulting from habitat fragmentation may have unknown potential consequences on the capacity of these communities, and hence these ecosystems, to respond to the climate change. Finally, we found a consistent relation between the structure and functional diversity of bacterial community, which further

showed the important role that the assemblage of microbial communities might have over their functioning.

Keywords: Forest fragmentation, alpha-diversity, beta-diversity, EcoPlates**,** DGGE

1. Introduction

In the Mediterranean basin, forest fragmentation, resource overexploitation, and poor management are the main drivers of forest degradation (FAO, 2011), which is likely to be magnified by the increasing intensity of summer drought induced by climate change (Valladares et al., 2014a). Little research has been conducted to understand the effects of forest fragmentation on ecosystem functioning (Turner, 2005), despite the fact that it has important implications for forest conservation and management strategies (Saunders et al., 1991), particularly taking into account its strong impact on the plant-soil-microbial system (Flores-Rentería et al., 2015). Within this framework, microbes are critical for driving ecosystem nutrient cycling, providing plants with the necessary nutrients to grow. Moreover, bacteria and fungi are responsible for about 90% of all organic matter decomposition (McGuire and Treseder, 2010; Ushio et al., 2013), and at least 50% of all CO_2 globally emitted from soils (Bond-Lamberty et al., 2004). However, very few studies have been designed to understand how forest fragmentation may affect the functioning of these microbial communities (Flores-Rentería et al., 2015).

Disturbance is generally detrimental to soil biodiversity, especially in agro-ecosystems (Walker, 2012). However, depending on the disturbance regime, changes in spatial environmental heterogeneity associated with fragmentation have been linked to

either increases or decreases in soil biodiversity (Flores-Rentería et al., 2015; Rantalainen et al., 2005). For example, studies on forest fragmentation effects on microbial community structure have shown modest changes (Flores-Rentería et al., 2015; Malmivaara-Lämsä et al., 2008) or no changes (Rantalainen et al., 2005) in species composition. On the contrary, forest fragmentation can affect the functioning of microbial communities, as previously showed in other studies (Flores-Rentería et al., 2015; Malmivaara-Lämsä et al., 2008; Riutta et al., 2012). Furthermore, while it is often hypothesized that diversity is important for the maintenance of soil processes, and that reductions in the richness of soil microbial communities will disrupt the functional capability of soils (Giller et al., 1997; Wagg et al., 2014), we are just beginning to address this question, and the results presented so far draw contradictory conclusions (Bell et al., 2005; Curiel Yuste et al., 2014; Griffiths et al., 2000; Langenheder et al., 2010; Levine et al., 2011; Mendes et al., 2015; O'Donnell et al., 2001; Tardy et al., 2014). More knowledge about microbial diversity and its function is therefore required for current and future predictions of ecosystem functioning in a changing world; much more empirical work is needed to define the functional consequences, at the ecosystem scale, of changes in microbial composition and their responses to disturbances and global change.

Diversity measurement is particularly challenging for microbial communities (Haegeman et al., 2013; Lozupone and Knight, 2008; Magurran, 2004). Commonly, microbial diversity has been characterized as the diversity within a given community (alpha- diversity) generally using the total number of operational taxonomic units (OTU's richness), their relative abundances (Shannon diversity), or indices that combine these two dimensions (evenness). Studies have generally used microbial alpha-diversity to explore the relationships between structure and functioning of microbial

communities (e.g. Curiel Yuste et al., 2011), whereas beta-diversity, which analyses the biological diversity among communities along environmental gradients (Anderson et al., 2006; Lozupone and Knight, 2008; Maaß et al., 2014), has been probably less studied for these communities. However, patterns of microbial community structure and diversity at the landscape scale and in perturbation gradients may also add info on co-occurrence -examining which organisms sometimes or never occur together-, that may help us understanding which conditions prefer or not (Fuhrman, 2009; Rincón et al., 2014). Several ecological processes potentially contribute to changes in co-occurrence patterns at the landscape scale, including competition, habitat filtering, historical effects and neutral processes (Horner-Devine et al., 2007; Maaß et al., 2014).

In this study, we used a molecular fingerprinting technique, Denaturing Gradient Gel Electrophoresis (DGGE), to characterize the structure of microbial communities (bacteria and fungi) coupled with the community level physiological profiles (CLPP), using Biolog^{TM} EcoPlates, as indicator of microbial functioning, in order to evaluate the influence of the agricultural matrix, as one of the main consequences of forest fragmentation, on soil microbial ecology (i.e. structure and functioning) in fragmented Mediterranean holm oak forests. More precisely, we evaluated if the impact of forest fragmentation on the capacity of soil microbial communities to metabolize different substrates (metabolic profile) could be explained through its effects on microbial structure (assemblage, alpha and beta diversity) and/or changes in microhabitat characteristics. Based on previous studies, we here hypothesized that the agricultural matrix will exert strong direct (via changes in nutrient availability) and indirect (via its influence over tree growth) effects over the microbial community structure, as well as over its capacity to metabolize different substrates (Fig. 1). Secondly, we hypothesized that the metabolic activity of soil microbial communities will be largely influenced by

100 the structure of these communities (Fig. 1). Specifically, our objectives were: (1) to
101 analyze the response of structural and functional diversity of soil microbial communities
102 to the agricultural matrix influence; (2) to understand which biotic and abiotic factors
103 associated with fragmentation (i.e. matrix influence) affect this diversity; and (3) to
104 analyze causal relations between microbial community structure and its capacity to
105 metabolize different substrates.
106
107 **2. Material and Methods**
108 *2.1 Study area*
109 The study area is located near Quintanar de la Orden (39º30'-39º35'N, 02º47'-02º59'W;
110 870 a.s.l.), in Toledo, southeastern Spain. This area has a Mesomediterranean climate
111 characterized by 434 mm of mean annual precipitation and 14 ºC of mean annual
112 temperature, respectively (Ninyerola et al., 2005), with a pronounced summer drought,
113 usually lasting from July to September. The landscape, a former predominant holm oak
114 Mediterranean forest, is currently highly fragmented and surrounded by active
115 croplands of cereals and legumes, with scattered grape crops that complete the mosaic.
116 The original forests are in a variety of patch sizes, covering only 28 % of their original
117 area (Díaz and Alonso, 2003). The dominant tree is the holm oak (*Quercus ilex* L. ssp.
118 ballota (Desf.) Samp; Fagaceae), with the understory mainly composed by shrubs of
119 Kermes oak (*Quercus coccifera* L.) and scattered *Genista*, *Asparagus*, and *Rhamnus*
120 species (for a full description of the study area see: (Díaz and Alonso, 2003; Santos and
121 Tellería, 1998).
122 *2.2 Experimental design and sampling*
123 A total of three large (> 10 ha) and five small (< 0.5 ha; with at least three trees) forest
124 fragments within an area of 1000 ha, separated of a minimum of 50 m (to avoid spatial

125 dependence) and a maximum of 8 km, were studied (Supplementary material, Fig. S1).
126 Prevalent soils were Cambisols (calcic) (WRB, 2007), with sandy loam texture (17-39-
127 44 % clayey).

128 Since the exposure of the edges of the fragmented forest causes changes in the
129 abiotic and biotic conditions in comparison with the forest interiors (Fischer and
130 Lindenmayer, 2007; Flores-Rentería et al., 2015; Murcia, 1995; Valladares et al.,
131 2014b), while the small forest effectively consist only in edge habitat (Young and
132 Mitchell, 1994), we defined the influence of the agricultural matrix on forest fragments
133 by the factor "matrix influence" with three levels: (1) low influence, at the interior of
134 large fragments (at least 30m from the forest edge; coded as "forest interior"); (2) mid
135 influence, at the edges of large fragments (coded as "forest edge"); and (3) high
136 influence, in small fragments (coded as "small fragments"), all fragments imbibed in an
137 active agricultural matrix. Additionally, the factor "tree cover" was evaluated at two
138 levels: (1) under holm oak canopy (halfway of the canopy, starting from the trunk;
139 coded as "under canopy"), and (2) outside the canopy (1.5 m outside any canopy
140 projection; coded as "open areas"). For each of the three large fragments, we selected
141 five holm oak trees in the forest interior and five trees at the forest edge, and three trees
142 at five small fragments (15 trees per matrix influence-fragmentation level), resulting in
143 a total of 45 selected trees. For each selected tree, two coverage-sampling points were
144 established: one under canopy and the other in open areas, resulting in a total of 90 soil
145 samples.

146 Height, basal area and canopy projection were measured for each of the 45 holm
147 oak multi-stem trees. A tree influence index *(Tii)* was calculated at each sampling point,
148 according to the formula: $Tii = \frac{Basal\ area}{Distance\ from\ the\ trunk}$. The basal area was selected to
149 calculate this tree influence index given its recognized direct relationship with soil

functioning (Barba et al., 2013). Soil moisture was determined by weight lost of samples oven-dried at 105 ºC for 48 h. Total C and N contents were measured on air-dried soil samples, using a C:N elemental analyzer (Flash EA 1112 Series, Thermo Fisher Scientific). Total concentrations of P, K, Ca, Na, S, Mg, Fe, Mn, Cu, Mo, and Zn were determined by digestion with HNO_3 + H_2O_2 (4:1, v:v), followed by inductively coupled plasma-optical emission spectrometry (ICAP-6500 Duo/Iris Intrepid II XDL, Thermo Fisher Scientific, Massachusetts, USA). Soil pH was determined on a 1:10 (w:v) aqueous suspension. Soil organic matter (SOM) was assessed by loss on ignition at 400 °C, during 4 hours.

2.3 Soil community structure

The structure of soil bacterial and fungal communities was assessed by the DNA community fingerprinting technique of denaturing gradient gel electrophoresis (DGGE). Soil DNA was extracted with the MoBio Power soil DNA isolation kit (Solana Beach, USA), and yields assessed by electrophoresis at 80 V on a 1.2 % agarose gel. The universal primers 338F/518R were used for amplification of the bacterial 16S rRNA gene (Muyzer et al., 1993). In the case of fungi, the internal transcribed spacer nrDNA region ITS-1 was PCR-amplified using the primer pair ITS1-F/ITS2 (Gardes and Bruns, 1993). A GC clamp was respectively added to the 5' end of forward bacterial (338F) and fungal (ITS1-F) primers to stabilize the melting behavior of the DNA fragments (Muyzer et al., 1993). PCRs were carried out on a Mastercycler® gradient Thermocycler (Eppendorf, Germany), with 50 µl final volume containing 10x NH_4 reaction buffer, 2 and 1.5 mM $MgCl_2$ (for fungi and bacteria, respectively), 0.2 mM total dNTPs, 2.5 U Taq (Bioline, London, UK), 1µM of each primer, 0.5 µl of 10 mg ml^{-1} bovine serum albumin (BSA) and 50 ng of template DNA, determined using a

NanoDrop 1000 (Thermo Scientific, USA). PCR cycling parameters were: 94 ºC for 5 min, followed by 35 cycles of 94 ºC for 30 s, 55 ºC for 30 or 45 s (fungi or bacteria, respectively), and 72 ºC for 30 or 45 s (fungi or bacteria), with a final extension at 72 ºC for 5 or 10 min (fungi or bacteria, respectively). Negative controls (containing no DNA) were included in each PCR run.

DGGE was carried out on a DCode universal mutation detection system (Bio-Rad, Hemel Hempstead, UK), using 10% polyacrilamide gels, with denaturant urea-formamide gradients of 10-50% for fungi (Anderson et al., 2003) and 30-60% for bacteria (Grossman et al., 2010), with the concentrations of 7 M urea and 40 % formamide (v/v) for the 100 % denaturant. Electrophoreses were run at 60 ºC 75 V for 16 h, loading equal volumes of amplified DNA. Gels were stained with SYBR Gold nucleic acid stain (Molecular Probes, The Netherlands). DGGE fingerprint profiles were digitized and analyzed using a Kodak DC290 zoom digital camera with KODAK 1D Image Analysis software (Kodak, NY, USA). Bands were adjusted with a Gaussian model with a profile width of 80%. Noise was eliminated by removing bands below a 10% band peak intensity threshold. Each band of the DGGE profile was hereafter referred to as an operational taxonomic unit (OTU). Gel bands were analyzed by using internal reference bands, and known reference markers loaded in lanes at either side of the gel. The number and pixel intensity of bands in a particular sample were considered comparative proxies of richness and relative abundance of fungal or bacterial OTUs, respectively (Cleary et al., 2012). From here, we define microbial community "assemblage" as the community composition with respect to other. Similar analysis of DGGE banding patterns have been previously used in other studies (Anderson et al., 2003; Cleary et al., 2012; Flores-Rentería et al., 2015; Gafan et al., 2005; Suzuki et al., 2012; Vaz-Moreira et al., 2013).

2.4 Microbial metabolic profile

Community level physiological profiles (CLPP) of cultivable microbial communities (both bacteria and fungi, those not inhibited by tetrazolium dye) were determined with Biolog™ EcoPlates (BIOLOG Inc., Hayward, CA). From here, we define "metabolic profile" as the identity and abundance of the substrates that microbial communities were able to metabolize, measured either by qualitative (presence/absence) or quantitative (abundance) approaches. We used the procedure adapted from (Garland and Mills, 1991). Briefly, 4 g (dry weight equivalent) of each soil sample was added to 36 ml of sterile 0.8% saline solution (NaCl). The mixture was then shaken on an orbital shaker for 20 min, and left to stand at room temperature, for 30 min. A volume of 250 µl supernatant was diluted into 24.75 ml of sterile saline solution. Only in the case of fungal plates, 25 µl streptomycin and 25 µl tetracycline (dilution 1:1000, w:v, in both cases) were added to 24.7 ml of sterile saline solution to limit the bacterial growth. Supernatant dilutions were mixed for 30s and left to stand for 10 min. A 100 µl aliquot of each diluted solution was added to each of 96 wells in a Biolog™ EcoPlates (arranged by triplicate for each substrate). Plates were incubated at 28 ºC in a humidity-saturated environment. Color formation in each well was monitored at monochromatic light (590 nm) absorbance using a Victor3 microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA). Measurements were performed once per day during 7 and 10 days for bacterial and fungal plates, respectively. A single time point absorbance was used in all posterior analyses at 96 and 168 h for bacterial and fungal plates, respectively, when the asymptote was reached (data not shown). Optical density (absorbance) value from each well, was corrected by subtracting the blank well (inoculated, but without a substrate), and then normalized by the color summation of the entire plate. Subsequently, we averaged the three values for each individual substrate

within a plate. The EcoPlates system has been recognized as a useful tool for comparing microbial communities (Classen et al., 2003; Frąc et al., 2012; Gomez et al., 2004; Weber et al., 2007; Weber and Legge, 2009), since it can detect functional changes in microbial communities as a result of differing carbon availability in soil, its physiological basis has been considerate to provide an ecologically relevant overview as long as results are interpreted as a profile of phenotypic potential and not in terms of *in situ* activity (Gomez et al., 2004).

2.5 Data analysis

A principal component analysis (PCA) was conducted to reduce the *n*-dimensional of soil nutrients data into two linear axes explaining the maximum amount of variance (Supplementary material, Fig. S2).

Structural alpha-diversity of both bacterial and fungal communities was estimated from the number and intensity of bands (OTUs): richness (*S*), Shannon (*H'*) and evenness (E_H) diversity indexes were calculated as follows:

$$\text{Shannon } (H') = -\sum_{i=1}^{S}\left({}^{n_i}/_{N}\right)\cdot ln\cdot\left({}^{n_i}/_{N}\right) \text{ and evenness}(E_s) = \frac{H\prime}{ln\,S},$$

where n_i is the band intensity, *N* is the sum of all intensities of a sample and *S* is the number of bands of a sample (richness). Similarly, the functional alpha-diversity was evaluated as functional richness (*SS*, total number of C substrates catalyzed), functional Shannon (*SH'*; using the optical density as abundance), and functional evenness (SE_S, functional diversity divided by ln substrate richness) (Classen et al., 2003; Grizzle and Zak, 2006).

Environmental variables and structural and functional alpha-diversity were analyzed by two-way Analysis of Variance (ANOVA) considering the factors matrix influence and coverage. Subsequently, and due to the high effect of tree coverage factor,

fragmentation effects within each coverage level, as well as coverage effects within each fragmentation level were separately evaluated by one-way ANOVA. Tukey's HSD were used as post hoc test ($p < 0.05$). Linear correlations between all measured variables were tested using Pearson's r with $p < 0.05$ significance threshold.

Microbial community assemblages and metabolic profiles (both bacterial and fungal) were explored by Nonmetric multidimensional scaling (NMDS) analysis, which provided graphical ordination of the community grouping, using the functions *metaMDS* and *isoMDS* in vegan and MASS R packages, Oksanen et al. (2013). We used the NMDS analysis instead of other ordination technique (i.e. PCoA, PCA, CA) since its use its widely extended in microbial ecology to identify patterns among multiple samples that were subjected to molecular fingerprinting techniques, including denaturing gradient gel electrophoresis (DGGE), being the NMDS iterative procedure more computer intensive than the mentioned eigen-analyses (Ramette, 2007), and in order to be able of correlate it with the environmental variables (see below). Nonetheless, both techniques were tested with the data and the results obtained using PCoA and Hellinger transformed data (using *cmdscale* and *decostand* functions in vegan R package) were highly comparable to those obtained with NMDS (data not shown). For bacteria and fungi, these analyses were performed using both quantitative (abundance) and qualitative (presence/absence) data: we used the data of relative DGGE band intensity for microbial assemblage analyses, whereas for analyzing microbial metabolic profiles we used the normalized optical density data obtained in the EcoPlates. The dissimilarity matrices were built using the Bray-Curtis distance measure. Regarding NMDS, a measure of stress <5 provides an indication of an excellent fit of the model, hence suggesting that the structure of the community is well represented in reduced dimensions, a measure of stress between 20 and 30 provides a good fit, and

measures of stress above 30 provides a poor fit, and hence and indication o a poor representation in reduced dimensions. The preferred solution, based on the lowest stress and instability was three dimensional, although two dimension graphs were finally presented. To seek for differences among microbial assemblage and metabolic profile we applied a non-parametric multivariate analysis of variance (NPMANOVA), performed with Bray-Curtis distances as a measure of dissimilarity among treatments (Anderson, 2001), considering the factors matrix influence, coverage and their interaction. Significance was obtained from permutations of the raw data (*F* test based in 1000 sums of squares). The agricultural matrix influence effects within each coverage level, as well as the coverage effects within each matrix influence level, were separately evaluated by subsequent NPMANOVAs.

As a measure of beta-diversity we used the multivariate dispersion (as non-directional variation in species' identities), using the distance to the centroid (Anderson et al., 2006) calculating one centroid for each soil provenance (i.e. under canopy or open areas for each: forest interior, forest edge or small fragments), calculated using *betadisper* and *permutest* functions in the vegan R package (Oksanen et al., 2013), that calculate the average distance of group members to the group centroid or spatial median in multivariate space. Beta-diversity was determined by using both quantitative (abundance) and qualitative (presence/absence) data, which may provide complementary information on the structural and functional response of these communities to disturbances (Lozupone and Knight, 2008; Maaß et al., 2014). In both cases, we used the Bray-Curtis dissimilarity matrix (same used to calculate NMDS), considering the factors matrix influence and coverage. Subsequent multiple comparison of means was performed through Tukey's HSD test ($p < 0.05$). As proposed by Warton et al. (2012), we check for restrictions in the use of Bray–Curtis distance measure to

analyze dispersion effect (i.e. the mean variance plots in all cases followed approximately a line of slope two, and the within-group standard deviations were approximately equal for all groups). Additionally we confirm the trends of our results using *Beta.div* function (Legendre and De Cáceres, 2013)

2.6 Controlling factors

To determine which environmental variables explained most of the variation of the structure and function of the microbial communities, we used two approaches including the variables: tree influence index, SOM, pH, soil moisture, C:N ratio, nutrients and PC1 and PC2 of nutrients PCA's. In the first approach, the *envfit* function (vegan R package; Oksanen et al. (2013), was used to plot the vectors of variables that were significantly correlated ($p<0.05$) with the assemblage and metabolic profile of microbial communities on the NMDS ordination. The second approach consisted of structural equation modeling (SEM) to test not only the direct influence of biotic and abiotic factors on microbial functioning, but also their indirect effects, with an aprioristic model in which the causal relationships among measured variables were explicitly included (Iriondo et al., 2003; Milla et al., 2009; Shipley, 2002). SEM models were individually performed for each soil bacterial and fungal functional indicator (functional alpha and beta-diversity, and NMDS 1, NMDS 2 of the community assemblage), but only the best fitted ones are presented (quantitative functional Shannon and beta diversity). Beta-diversity presented in the SEM, coded as community assemblage, this was based in a quantitative multivariate dispersion to a unique centroid. Our models considered a complete set of hypotheses showed in Figure 4a and 4b for bacterial and fungal communities, respectively. These hypotheses were based on literature, previous exploratory analyses (ANOVA, correlations), and our own previous experience (Flores-Rentería et al., 2015). First, we hypothesized that microbial functioning will depend on

microbial community structure (Flores-Rentería et al., 2015; Giller et al., 1997; McGuire and Treseder, 2010; Ushio et al., 2013; Wagg et al., 2014), and both would be dependent on abiotic and biotic conditions, such as pH, (Fierer et al., 2009; Hamman et al., 2007), SOM (Curiel Yuste et al., 2007; Franklin and Mills, 2009), soil moisture (Curiel Yuste et al., 2007; Saul-Tcherkas et al., 2012), nutrients (Franklin and Mills, 2009; Laughlin et al., 2014; Legay et al., 2014; O'Donnell et al., 2001), and that all these variables would be on their turn, influenced by the tree (Classen et al., 2003; Legay et al., 2014; Pugnaire et al., 2004). Additionally, we included in our model causal relations among abiotic variables, i.e. SOM influence over soil moisture, pH and C:N (Abu-Hamdeh, 2001; Boix-Fayos et al., 2001; Pugnaire et al., 2004). Standardized path coefficients were estimated by using the maximum likelihood algorithm (Shipley, 2002).

To determine the possible links between microbial assemblage and metabolic profile (for both bacterial and fungal communities) independent Mantel Tests of correlation (*mantel* function on vegan package in R) were performed between the Bray–Curtis dissimilarity indices of each bacterial and fungal DGGE matrix and the corresponding Bray–Curtis dissimilarity indices of the bacterial and fungal EcoPlates. The Mantel Test uses the similarity of two dissimilarity matrices by permuting each of the elements in the dissimilarity matrix 999 times to derive a distribution of correlation values (Franklin and Mills, 2009). The resulting R-statistic is similar to the Pearson's Product Moment Correlation Coefficient; with increasingly similar dissimilarity matrices, the Mantel R-statistic will approach 1. Abundance proxies of microbial assemblage and metabolic profile matrixes were not transformed.

Prior to analyses, all variables were tested for normality, and log transformations were applied to meet variance homoscedasticity when required, except abundance

matrices of microbial assemblage and metabolic profiles. Additionally, we used the *Moran.I* function (ape library, (Gittleman and Kot, 1990) to find a possible spatial autocorrelation; none of the measured variables had a significant correlation with the sampling point, discarding, therefore a spatial dependence of the samples. SEMs were performed by using IBM®, SPSS® (IBM Corporation Software Group, Somers, NY) and IBM®, SPSS® AMOS 20.0 software (IBM Corporation Software Group, Somers, NY), the rest of analyses were performed using R 3.1.0 (The R Foundation for Statistical Computing, (2014).

3. Results

3.1 Cover and forest fragmentation effect on soil microbial communities

As expected, soils in the holm oak forest fragments studied were strongly influenced by the canopy cover. Under canopy, significantly higher values of nutrients, SOM, soil moisture, and lower Ca and pH values were found compared with open areas (Supplementary material, Fig. S2; Table S1).

Structural alpha-diversity of the fungal community was neither affected by coverage nor agricultural matrix, only fungal community evenness *(E_S)* was sensitive to the influence of the agricultural matrix (Table 1); whereas bacterial community structure was mainly influenced by the agricultural matrix, with higher values of bacterial richness *(S*) and Shannon (*H'*) at small fragments and lower values at forest interior (Table 1). On the contrary, the metabolism of both bacterial and fungal communities was strongly influenced by the coverage, showing higher functional alpha-diversity under the influence of the tree canopy in all measured parameters: functional richness *(SS)*, Shannon *(SH')*, and evenness *(SE_S)* (Table 1). Additionally, the agricultural matrix positively influenced, although to a lesser extent, the functional

alpha-diversity of both bacterial and fungal communities. Specifically, bacterial functional Shannon *(SH')*, and richness (*SS*) were higher at forest edge and small fragments, and those of fungi in small fragments (Table 1). An interactive effect between coverage and matrix influence was found for bacterial *SH'* and SE_S, which were higher in soils from small fragments and under the tree canopy (Table 1). Substrate consumption in both bacterial (Table S2) and fungal (Table S3) communities was mainly dependent on the tree canopy, affecting 24 and 19 substrates for each microbial community, respectively. The agricultural matrix also affected the consumption of some substrates, more evidently in the case of bacteria (15 substrates) than fungi (4 substrates; Tables S2 and S3).

Structural beta-diversity of microbial communities based in both quantitative (abundance; Fig. 2a-b) and qualitative data (presence/absence; Fig. 2c-d) pointed out to a negative influence of the agricultural matrix in small fragments and edges, in comparison to the forest interior, which generally showed higher beta-diversity. When qualitative data were analyzed (Fig. 2c-d), only the bacterial community was also influenced by coverage, with higher beta-diversity observed in open areas (Fig. 2c). In the case of quantitative data, the higher beta-diversity of the fungal community was observed in the forest interior in comparison with forest edges and small fragments (Fig. 2b); whereas qualitative data analysis revealed an interaction between coverage and matrix influence, with the highest beta-diversity of fungal communities under canopy and open areas in forest interior (Fig. 2d).

On the contrary, when functional beta-diversity was analyzed (quantitative data Fig. 3a-b), both bacterial and fungal communities were significantly influenced by coverage and not by the matrix influence, showing in both cases higher values in open areas than under canopy (Fig. 3a-b). By contrast, for both bacteria and fungi, functional

beta-diversity (qualitative data Fig. 3c-d) was affected by the interaction between coverage and matrix influence with the highest beta-diversity usually observed for forest interiors, in both open areas and under canopy (Fig. 3c-d).

3.2 Controlling factors of structure and metabolism of soil microbial communities

Bacterial and fungal communities were significantly correlated with some environmental variables (Table S4): e.g. PC1 of nutrients-PCA was strongly correlated with functional alpha-diversity in all cases (Table S4). Bacterial and fungal metabolism was strongly correlated with all the environmental variables measured, e.g. SOM and soil moisture were positive correlated with functional alpha-diversity of both bacterial and fungal communities (Table S4).

The assemblage of bacterial and fungal communities (NMDS), based on quantitative data, was strongly influenced by tree coverage and agricultural matrix (Fig. S3), although the NPMANOVA indicated that the matrix influence exerted the strongest effect, in both cases (Table S5). The respective assemblage of OTUs within bacterial and fungal communities (NMDS) showed a good fit (stress value of bacteria=19.98, Fig. S3a, and fungi=20.76, Fig. S3b). Similar results were obtained when qualitative (presence/absence) matrices were analyzed, with no substantial changes concerning the factors controlling the grouping of OTUs with respect to results obtained with quantitative (abundance) matrices (Fig. S3c-d; Table S5). According to the *envfit* permutation test, soil physicochemical properties were highly correlated suggesting them as contributing factors influencing the grouping of bacteria and fungi (Fig. S3). Specifically, all nutrients, except organic carbon and Mo in the case of fungi, affected the assemblage of both bacterial and fungal communities (Fig. S3a-b; Table S6). Additionally, tree influence index (*Tii*), soil organic matter (SOM), soil moisture and pH also influenced the grouping of bacteria (Fig. S3a-b; Table S6).

Regarding the metabolic profile of both bacteria and fungi (quantitative data; Fig. S4a-b) the NMDSs analysis showed very good fit (stress values of 10.04 and 16.74, respectively), and accordingly to the NPMANOVA both microbial communities were affected by the tree influence (Table S5). Additionally, the metabolic profile of bacterial community in open areas was significantly influenced by the agricultural matrix (Table S5). Contrary to the assemblage of the bacterial community, its metabolic profile was only affected by total and organic C, N, P, SOM, soil moisture, tree influence and pH (Fig. S4a; Table S7), being, in consequence strongly segregated by the influence of the tree canopy. By contrast, the fungal metabolic profile was not significantly influenced by any variable (Fig. S4b; Table S7). Unlike results obtained from quantitative data analyses of metabolic profile, qualitative analyses (i.e. just testing the capability of substrate utilization, not its relative use) showed that the bacterial and fungal metabolic profiles were clustered with almost no influence of the environmental variables (Fig. S4c-d, Table S5). Almost the same variables influenced the ordination of qualitative (presence/absence) bacterial metabolic profile, in comparison with quantitative analysis, except pH (Fig. S4c); whereas fungal metabolic profile by tree influence and total carbon (Fig. S4d).

The structural-equation models (SEM) proposed for bacterial and fungal communities (Fig. 4a-b) and based on the correlations observed above, provided a good general fit, as indicated by the non-significant *f* value and by the goodness-of-fit indices (RMSEA, NFI and GFI). Squared multiple correlations for SEMs showed that the variance of the bacterial functional Shannon (*SH'*) was highly explained (R^2= 0.60) in comparison with the community assemblage variance (R^2= 0.42; Table S8). Both bacterial functional Shannon (*SH'*) and metabolic profile were affected by soil moisture and bacterial structure (Shannon *H'* and assemblage, respectively). Agricultural matrix

indirectly affected bacterial Shannon (*H'*) and assemblage, mainly through its effect over the size of the trees (tree influence), which on the other hand, exerted a strong positive effect over pH and SOM quantity (Fig. 4c,e). Additionally, the agricultural matrix influenced the bacterial community assemblage and the quantity of nutrients (i.e. PC1; Fig. 4c), which in turn influenced bacterial structural Shannon (*H'*) (Fig. 4e). Soil pH affected both bacterial Shannon (*H'*) and community assemblage, but with opposite influence, negatively to the Shannon (*H'*; Fig. 4c) and positively to the community assemblage (Fig. 4e).

The agricultural matrix exerted both a direct and indirect (through its effect on nutrients) influence over fungal Shannon (*H'*) and community assemblage, as well as over tree influence (Fig. 4d,f). Fungal functional Shannon (*SH'*) and community assemblage were drove by SOM, tree influence and soil pH, showing opposite patterns to those observed in each the functional Shannon (*SH'*) or community assemblage models (Fig. 4d,f; Table 2).

3.3 Relations between assemblage and function in soil microbial communities

Relationships among indicators of structural and functional alpha-diversity (richness, diversity and evenness) showed significant but weak correlations; i.e. bacterial richness (*S*) and bacterial functional richness (*SS'*) showed a R^2=0.26 (Table S4), whereas fungal community structure and function (richness, diversity and evenness) were uncorrelated (Table S4).

In the case of the bacterial community, SEMs revealed a direct effect of structure (Shannon and assemblage) over functioning (functional Shannon and metabolic profile; Fig. 4c,e; Table 2). In the case of fungal communities, SEMs revealed that neither fungal Shannon (*H'*) nor community assemblage exerted a significant effect on fungal functioning, neither on Shannon *SH'* nor on metabolic profile (Fig. 4d, f;

Table 2). When the effect of the bacterial community structure (i.e. the effect of Shannon *H'* over functional Shannon *SH'*; or assemblage over metabolic profile) over its functioning was removed (data not show), the explained variance dropped almost 8% in the case of Shannon (*SH'*) model (R^2= 0.55), and a 12% in the case of the metabolic profile model (R^2= 0.37). Indeed, Mantel test showed that the dissimilarity matrices of fungal community assemblage and metabolic profile were not significantly correlated (R=0.04; p=0.26); while, dissimilarity matrices of bacterial assemblage and metabolic profile exhibit a significant correlation (R=0.12; p=0.006).

4. Discussion

4.1 Tree coverage and forest fragmentation effect on soil microbial community

Our results show strong differences in the mechanisms of control of the variability of both structure and function of the microbial communities, which also exhibited dissimilar susceptibility to the proximity to the agricultural matrix. The structure of the microbial communities was directly influenced by changes in nutrient availability associated with the presence of the agricultural matrix, while the effect of the matrix over the functioning of these communities was mainly indirect, through its stimulation over plant growth and plant-soil interactions (i.e. increasing plant productivity and, in turn, the amount of soil organic matter). This different response to the agricultural matrix and the tree influence of the studied microbial community structure (DGGE) and functional (EcoPlates) indicators suggests that the environmental factors controlling the composition/assemblage of species and the functioning of microbial communities differed, at least partially. Indeed, bacterial richness (*S*) and Shannon (*H'*) were positively affected by the influence of the agricultural matrix, whereas the functional alpha-diversity of bacterial and fungal communities were positively influenced by both

500 factors (matrix influence and coverage), and particularly by the tree (canopy cover). The
501 presence of the tree, therefore, exerted a strong positive influence over the relative
502 amount of consumed substrates, corroborating the findings of studies that have
503 previously showed a higher microbial functional diversity (Classen et al., 2003) under
504 canopy in comparison with open areas. The generally higher bacterial alpha-diversity
505 (i.e. richness and Shannon) in areas more influenced by the agricultural matrix (i.e.
506 small forest fragments), can be also explained by the formation of new available niches
507 after disturbance (Curiel Yuste et al., 2012). On the other hand, the differences observed
508 in the degree at which the factors controlling the structure and the functioning of
509 microbial communities differed might be an indication of the strong differences in the
510 composition of the active microbial community from the total community at local scales
511 (Jones and Lennon, 2010; O'Donnell et al., 2001). Indeed, it might be that the
512 unfavorable environmental conditions of the open areas can be associated with
513 dormancy of most bacterial and fungal lineages present in the community, as it has been
514 previously observed (Jones and Lennon, 2010). However, it has to be pointed out that
515 the used techniques may have some limitations in their capacity to detect changes in the
516 taxonomic composition and the overall functioning of soil microbial communities, since
517 DGGE explores only the most abundant, still representative OTUs of the microbial
518 community (Vaz-Moreira et al., 2013), whereas EcoPlates represents only functions
519 associated with the C cycling (Classen et al., 2003).

520 This general tendency of positive influence of both tree coverage and
521 agricultural matrix over local diversity of the microbial communities (i.e. structural
522 richness and Shannon and all functional indicators) contrasted, moreover, with the
523 results obtained when analyzing their beta-diversity, as a measure of the spatial
524 structural and functional heterogeneity of these communities at the landscape scale.

Indeed, areas highly influenced by the agricultural matrix (i.e. small fragments and forest edges), or by the tree, promoted more spatially homogeneous (less beta-diverse) microbial communities, suggesting a clear scale-dependent response of microbial communities to environmental perturbations. Hence, forest fragmentation with high agricultural matrix and tree influences would enhance bacterial alpha-diversity, both structural (*S* and *H*') and functional (*SS*, *SH* and SE_s), while decreasing both the structural and functional spatial heterogeneity (less beta-diversity) of these communities. This landscape convergence of microbial communities under the tree canopies and at areas highly influenced by the agricultural matrix (small fragments and forest edges) could be attributable to the environmental filtering of these communities by more uniform soil properties in these areas, i.e. abiotic homogenization, in comparison with open areas and forest interiors, since a beta-diversity decrease indicates community similarity increase over space, i.e. biotic homogenization (Olden et al., 2004). These results would finally indicate that despite the fact that forest fragmentation might be associated with species enrichment at the local scale, it might cause a general species and functional impoverishment of soils at the landscape scale, with likely negative consequences for the capacity of these soils to respond to different engines of global change (Curiel Yuste et al., 2011; Flores-Rentería et al., 2015). Further studies should, therefore, take into account this scale-dependency effect to fully understand the implications of environmental perturbations over the ecology of microbial communities.

4.2 Controlling factors of soil microbial communities

Our results suggest that, in general, the strong effect of the agricultural matrix over the proxies for microbial community structure (i.e. alpha-diversity and assemblage of both bacteria and fungi) could be mainly attributable to the soil nutrient enrichment

associated with fragmentation. Variability in soil nutrient contents was mainly explained by the matrix influence (44%) even more than by the tree cover (26%). Indeed, as it has been previously reported, nutrient availability is a major factor controlling the variability in microbial structure (Bowen et al., 2011; Fierer and Jackson, 2006; Franklin and Mills, 2009; O'Donnell et al., 2001; Ramirez et al., 2012; Tardy et al., 2014; Wardle, 1998). While almost all nutrients influenced bacterial and fungal assemblages, only macronutrients (C, N, P), which were strongly associated with plant cover, influenced their metabolism, or at least the rates at which the different C sources were metabolized.

Indeed, the use of both quantitative and qualitative analyses allowed us to unveil the controls of both forest fragmentation and canopy cover (as well as their interaction) over both the genetic structure and the metabolic profile of microbial communities highlighting the importance of using both approaches to explore, for example, functional redundancy and complementarity within these microbial communities (Lozupone and Knight, 2008; Miki et al., 2010). According to our results, microbial communities with different structure (i.e. under canopy vs. open areas) are able to metabolize the same diversity of C substrates, only differing in the amount of substrate metabolized (more under trees). This functional convergence, at least qualitatively, of microbial communities differing in structure and diversity is a clear indication of functional redundancy of these microbial communities (i.e. the ability of one microbial taxon to carry out a process as another; Allison and Martiny, 2008).

Structural equation models further allowed us to disentangle the complexity of the direct and indirect effects of the agricultural matrix over the microbial ecology and potential roles of these communities within the plant-soil-microbial system. The magnitude of the agricultural matrix effect, and hence the effect of forest fragmentation,

575 over both functional Shannon and metabolic profile, was the result of a complex
576 cascade of causal-effect relations involving changes in plant growth and modifications
577 of nutrient quantity (and probably quality and/or availability). The influence of
578 agricultural matrix over tree size modified the micro-environmental conditions
579 (nutrients, SOM, pH and moisture), which, in turn, strongly influenced the relative
580 amount of consumed substrates and the ability of microbial communities to metabolize
581 different substrates. In a causal-effect cascade, trees exert a strong direct effect over
582 SOM, soil nutrients (PC1) and pH, which, in turn exert a strong influence over variables
583 directly related to microbial functioning. In particular, and according to SEMs results,
584 the quantity of SOM was strongly influenced by the tree and is usually strongly
585 correlated with higher microbial metabolism (Frąc et al., 2012; Gomez et al., 2004),
586 appeared to be indirectly related with bacterial metabolism through increasing moisture
587 availability, modifying de pH, among other variables.

588 On the other hand, the direct effect of agricultural matrix over the soil microbial
589 communities suggests that there might be other factors not measured in this study, such
590 as the quality and composition of SOM, influencing species composition in soil
591 microbial communities . One possibility is that the agricultural matrix is influencing the
592 quality of soil substrates (e.g. configuration of humic molecules, presence of secondary
593 metabolites) inducing changes in the microbial community (Asensio et al., 2012), as
594 suggested in our study by the influence of the agricultural matrix on the microbial
595 preference for the consumption of determinate substrates.

596 SEMs also showed that in this causal-effect cascade the paths controlling the
597 metabolic capacity of both bacterial and fungal communities markedly differed. For
598 instance, the capacity of fungi to metabolize different substrates was strongly and
599 directly influenced by the tree, with no apparent relation with the structure of the fungal

community. These results point out to the strong ecological co-dependence of fungi and vegetation, which are organisms that have historically co-evolved in the colonization of terrestrial ecosystems (Boer et al., 2005), and able to establish strong mutualistic relations (e.g. mycorrhiza). The fact that in this co-evolution fungi have developed the enzymatic machinery able to degrade the complex vegetal molecules (Kohler et al., 2015) further reinforces the strong control of tree influence over the functioning of these fungal communities observed. However, it is important to consider limitation issues when using EcoPlates, particularly in the case of fungi, although it is still a largely accepted and useful tool to explore the potential activity of microbial communities (Gomez et al., 2004).

On the other hand, regarding bacterial communities, SEMs and Mantel test clearly showed that bacterial functioning was related to their community structure (alpha-diversity and community assemblage). The relationship between microbial structure and their metabolism is leastways highly complex (Mendes et al., 2015; O'Donnell et al., 2001), hence it has so far presented conflicting results (Bell et al., 2005; Curiel Yuste et al., 2014; Griffiths et al., 2000; Langenheder et al., 2010; Levine et al., 2011; Mendes et al., 2015; O'Donnell et al., 2001; Tardy et al., 2014). However, the relationship between bacterial structure and their metabolism found here and in others studies (Bell et al., 2005; Tardy et al., 2014), suggest that changes in bacterial community structure induced by environmental alterations, such as those derived from forest fragmentation, could lead to strong change on their overall functioning.

5. Conclusions

Collectively, our results suggest that forest fragmentation has a deep effect on microbial diversity and function through direct and indirect ways, affecting the functioning of the

plant-soil-microbial system and the cycling of nutrients. We also observed a strong scale-dependency on the controls of both the genetic structure and the functioning of soil microbial communities. Indeed, forest fragmentation (agricultural matrix influence) and tree canopy cover had an opposite effect over the local diversity (alpha) and the landscape diversity (beta). Forest fragmentation positively affects soil fertility and tree growth and productivity (SOM accumulation), inducing changes in both abiotic (moisture and pH) and biotic (higher microbial alpha-diversity) factors that ultimately improve the conditions for microbial metabolism at local scale. However, forest fragmentation and tree cover tend to homogenize the microbial community structure and their metabolism (lower microbial beta-diversity) at landscape scale with potential negative consequences on the capacity of these soils to respond to the climate change. Our study, therefore, reinforces our knowledge on how the complex alterations on the tree-soil-microbial system resulting from forest fragmentation may affect the capacity of terrestrial ecosystem to respond to environmental perturbations.

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Figure captions

Fig. 1 Hypothesized direct (1) and indirect (2) effects of forest fragmentation over soil microbial communities and functioning and between this last (3).

Fig. 2 Structural β-diversity of bacterial (a,c) and fungal (b,d) communities, determined by the distance to the centroid of multivariate dispersion using quantitative (abundance) (a-b) or qualitative (presence/absence) (c-d) datasets, of soils collected at three agricultural matrix influence levels, under canopy or in open areas, in fragmented holm oak forests in Spain. Note that higher distance to centroid (over-dispersion) means higher β-diversity. Different capital letters represent differences among coverage treatments, while different lowercase letters represent differences among matrix influence levels; Tukey HSD multiple comparison of the mean ($p<0.05$). The line within the box is the median value indicating the interquartile range (25th to 75th percentiles), and the whiskers extend to the most extreme value within the 1.5 interquartile range.

Fig. 3 Functional β-diversity of bacterial (a,c) and fungal (b,d) communities, determined by the distance to the centroid of multivariate dispersion using quantitative (abundance) (a-b) or qualitative (presence/absence) (c-d) dataset, of soils collected at three agricultural matrix influence levels, under canopy or in open areas, in fragmented holm oak forests in Spain. Note that higher distance to centroid (over-dispersion) means higher β-diversity. Different capital letters represent differences among coverage treatments, while different lowercase letters represent the interaction between coverage and matrix influence; Tukey HSD multiple comparison of the mean ($p<0.05$). The line within the box is the median value indicating the interquartile range (25th to 75th

percentiles), and the whiskers extend to the most extreme value within the 1.5 interquartile range.

Fig. 4 Structural equation models: general (a-b), and fitted to bacterial (c) and fungal (d) Shannon functional diversity (*H'*) (c-d, respectively), and bacterial (e) and fungal (f) metabolic profile (e-f, respectively), representing hypothesized causal relationships among matrix influence, biotic and abiotic predictors. Arrows depict casual relationships: positive effects are indicated by solid lines, and negative effects by dashed lines, with standardized estimated regression weights (SRW) indicated. SRW of each microbial diversity indicator are showed in Table 2, and squared multiple correlations for the structural equation models in Table S8. Arrow widths are proportional to p values. Paths with coefficients non-significant different from 0 ($p > 0.1$) are presented on gray.

Table

Table 1 Structural and functional alpha-diversity of soil microbial communities in holm oak forest fragments in Spain. Data are mean ± standard error. Two-way ANOVA results are presented (left columns), for factors C = coverage and MI = Matrix influence.

	Under canopy			Open areas			Factorial ANOVA		
Structural alpha-diversity	**Forest interior**	**Forest edge**	**Small fragments**	**Forest interior**	**Forest edge**	**Small fragments**	***C***	***MI***	***C x MI***
Bacterial community									
Richness *(S)*	34±0.52	36.93±0.38	37.53±0.52	32.6±0.6	35.27±0.5	36.8±0.47	n.s.	$F_{2,84}$=8.1 p<0.001	n.s.
Shannon diversity *(H)*	3.3±0.09	3.38±0.08	3.39±0.09	3.25±0.11	3.32±0.10	3.35±0.07	n.s.	$F_{2,84}$=3.8 p=0.027	n.s.
Evenness (E_S)	0.94±0.03	0.94±0.03	0.94±0.04	0.94±0.03	0.93±0.04	0.93±0.04	n.s.	n.s.	n.s.
Fungal community									
Richness *(S)*	29.4±0.47	28.8±0.32	27.93±0.37	29.73±0.45	29.27±0.37	28.73±0.31	n.s.	n.s.	n.s.
Shannon diversity *(H)*	3.06±0.10	3.10±0.09	3.14±0.09	3.12±0.09	3.14±0.09	3.17±0.08	n.s.	n.s.	n.s.
Evenness (E_S)	0.91±0.04	0.92±0.04	0.94±0.04	0.92±0.03	0.93±0.04	0.94±0.03	n.s.	$F_{2,84}$=12.9 p<0.001	n.s.
Functional alpha-diversity									
Bacterial community									
Functional richness *(SS)*	28.53±0.37	29.07±0.26	29.07±0.27	27±0.35	28.8±0.28	27.07±0.37	$F_{1,84}$=*14.11* p<0.001	$F_{2,84}$=4.30 p=0.017	n.s.
Functional Shannon diversity *(SH)*	3.05±0.08	3.07±0.11	3.13±0.07	2.73±0.09	2.99±0.08	2.81±0.1	$F_{1,84}$=85.09 p<0.001	$F_{2,84}$=9.87 p<0.001	$F_{2,84}$=10.04 p<0.001
Functional Evenness (SE_S)	0.91±0.04	0.91±0.06	0.93±0.03	0.83±0.05	0.89±0.04	0.85±0.06	$F_{1,84}$=62.65 p<0.001	$F_{2,84}$=5.93 p=0.002	$F_{2,84}$=7.17 p<0.001
Fungal community									
Functional richness *(SS)*	19.33±0.52	22±0.43	23.2±0.39	12±0.52	14.87±0.45	19.07±0.50	$F_{1,84}$=74.41 p<0.001	$F_{2,84}$=19.28 p<0.001	n.s.
Functional Shannon diversity *(SH)*	2.64±0.13	2.79±0.11	2.85±0.1	2.1±0.17	2.27±0.13	2.56±0.14	$F_{1,84}$=58.25 p<0.001	$F_{2,84}$=10.78 p<0.001	n.s.
Functional Evenness (SE_S)	0.90±0.04	0.90±0.05	0.91±0.05	0.88±0.06	0.85±0.07	0.87±0.05	$F_{1,84}$=16.28 p<0.001	n.s.	n.s.

Table 2 Microbial community metabolism and direct (D), indirect (I) and total (T) effects of tested variables, based on standardized regression weights (SRW), for each structural equation model. Significant direct effects are noted in bold. Tii= Tree influence index; PC1= Principal component 1 of the nutrients PCA.

Bacterial functional alpha-diversity				*Fungal functional alpha-diversity*			
	D	**I**	**T**		**D**	**I**	**T**
Matrix influence	-0.02	0.16	0.13	Matrix influence	0.09	0.23	0.32
Tii	0.13	0.34	0.47	*Tii*	**0.23**	0.21	0.45
SOM	0.23	0.36	0.60	SOM	**0.41**	0.09	0.50
Soil moisture	**0.42**	0.00	0.42	Soil moisture	-0.12	0.00	-0.11
pH	-0.02	-0.03	-0.05	pH	**-0.20**	0.00	-0.20
PC1 nutrients	0.00	0.10	0.10	PC1 nutrients	**0.20**	0.00	0.20
Bacterial alpha-diversity	**0.21**	0.00	0.21	Fungal alpha-diversity	0.01	0.00	0.01
Bacterial metabolic profile				*Fungal metabolic profile*			
	D	**I**	**T**		**D**	**I**	**T**
Matrix influence	0.00	0.00	0.00	Matrix influence	-0.08	-0.12	-0.20
Tii	-0.15	-0.25	-0.40	*Tii*	**-0.25**	-0.22	-0.47
SOM	-0.13	-0.36	-0.49	SOM	**-0.43**	-0.15	-0.57
Soil moisture	**-0.38**	0.00	-0.38	Soil moisture	0.10	0.02	0.12
pH	0.05	0.03	0.08	pH	**0.27**	0.00	0.27
PC1 nutrients	0.00	-0.04	-0.04	PC1 nutrients	-0.08	-0.01	-0.10
Bacterial assemblage	0.21	0.00	0.21	Fungal assemblage	-0.10	0.00	-0.10

Figure 1

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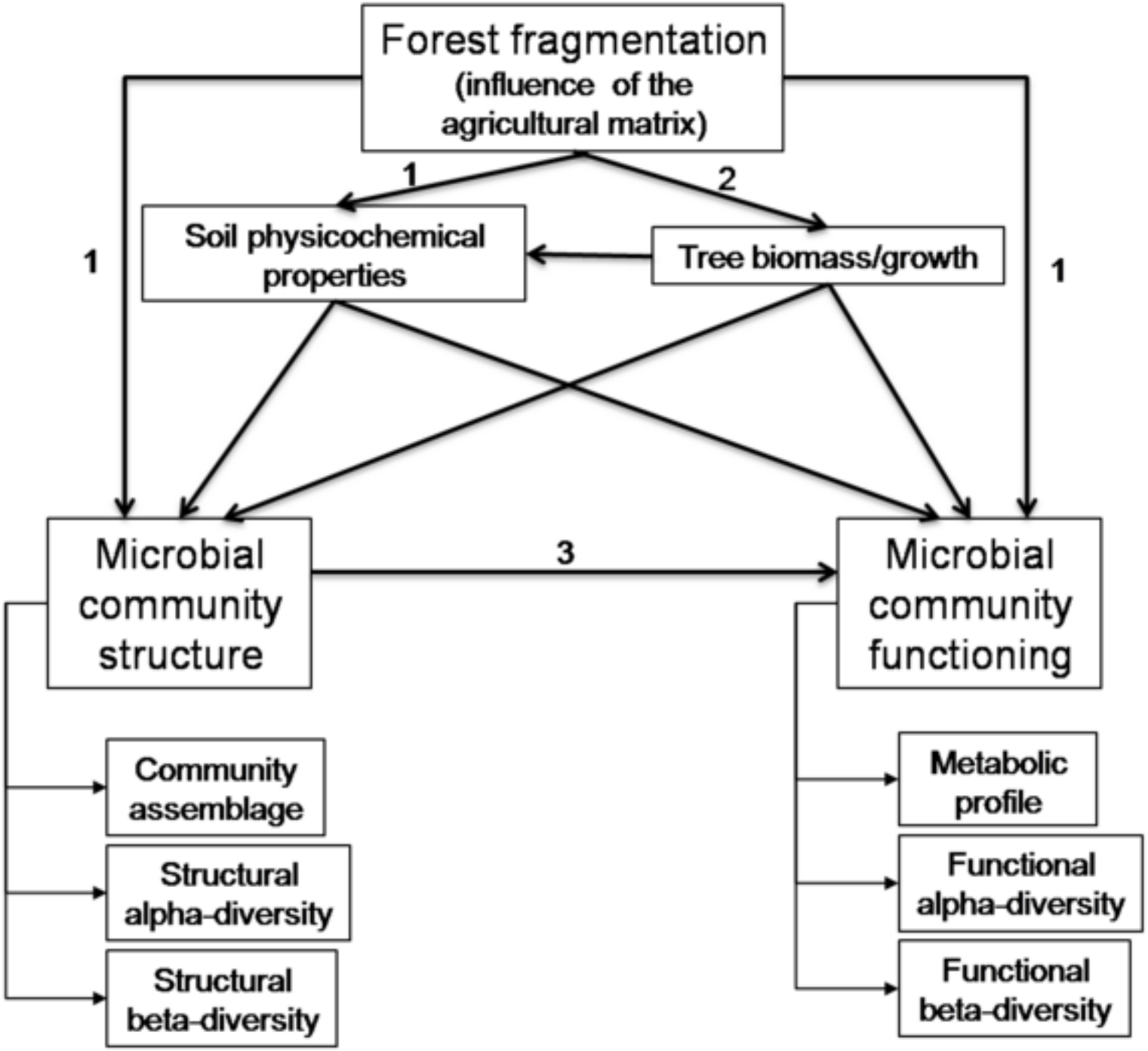

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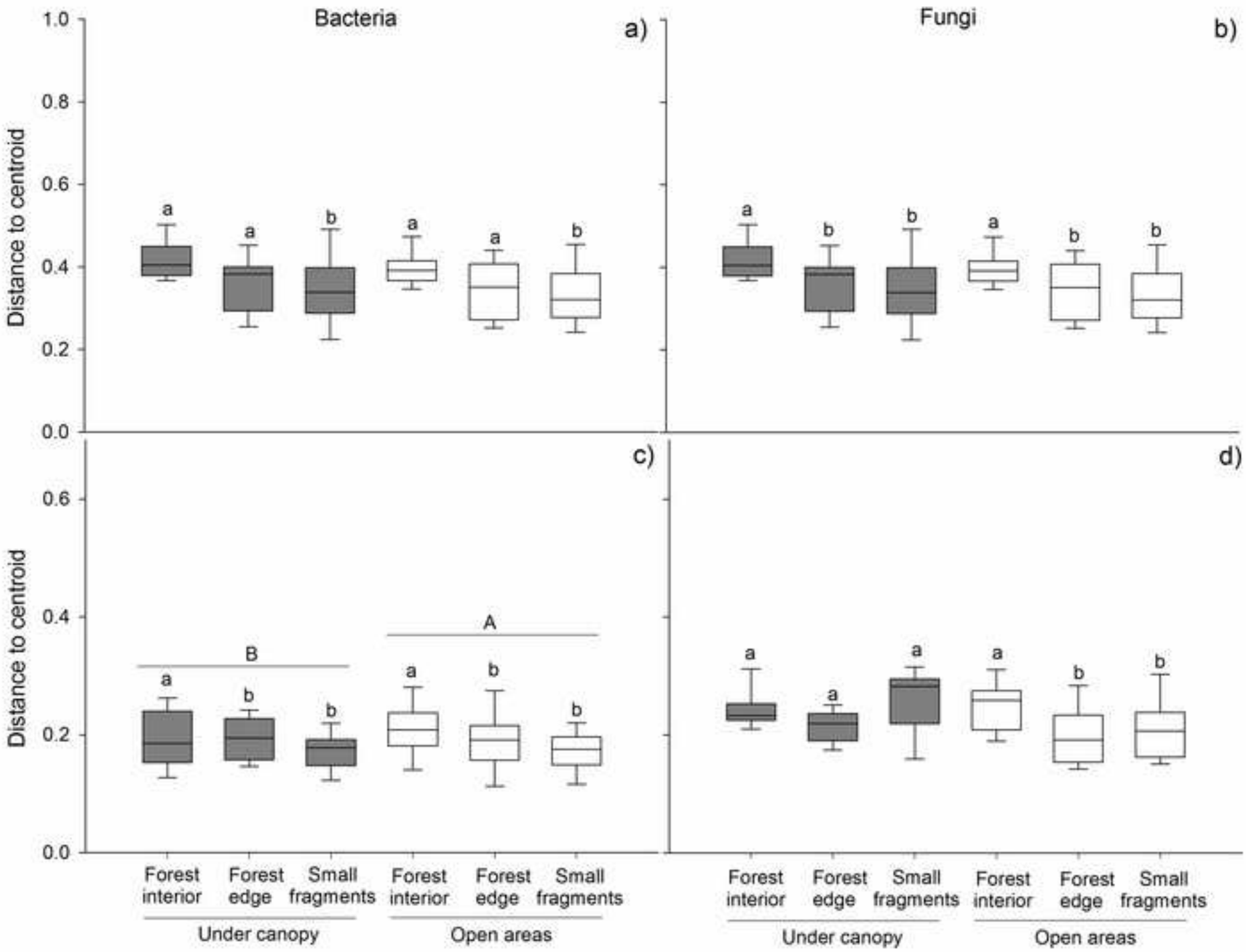

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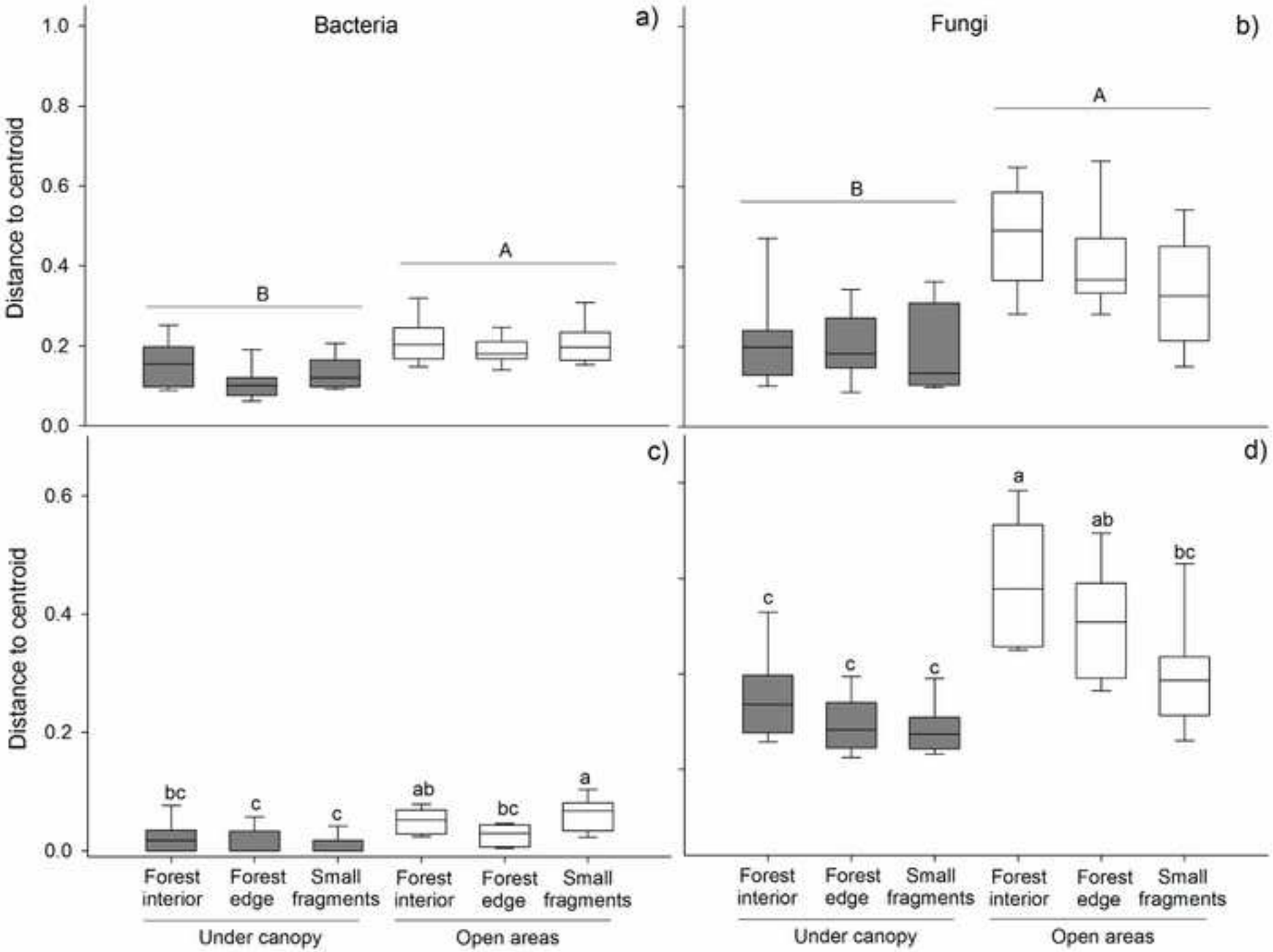

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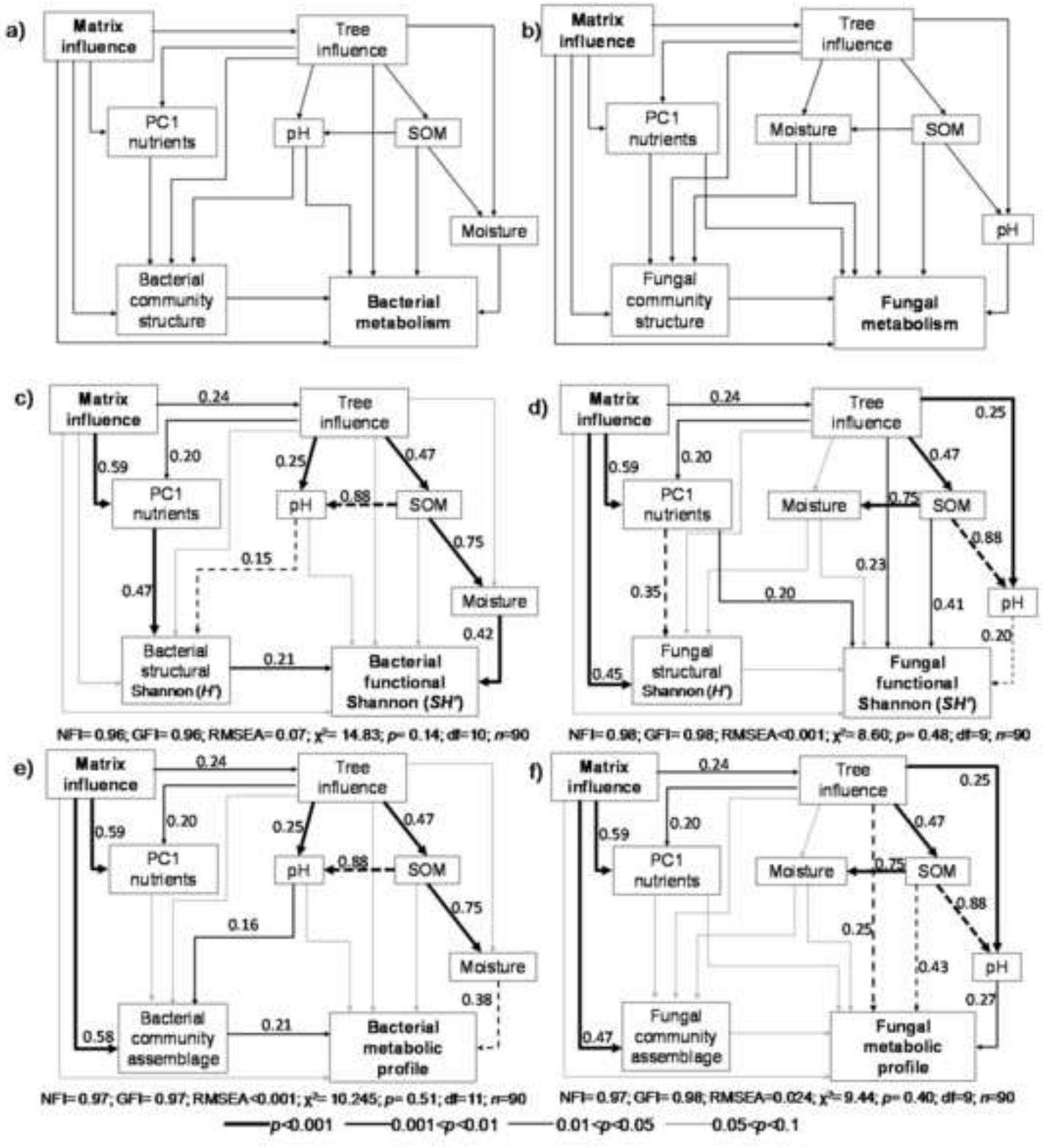

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