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Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks

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Summary

Fire alters ecosystems by changing the composition and community structure of soil microbes. The phylogenetic structure of a community provides clues about its main assembling mechanisms. While environmental filtering tends to reduce the community phylogenetic diversity by selecting for functionally (and hence phylogenetically) similar species, processes like competitive exclusion by limiting similarity tend to increase it by preventing the coexistence of functionally (and phylogenetically) similar species. We used co-occurrence networks to detect co-presence (bacteria that co-occur) or exclusion (bacteria that do not co-occur) links indicative of the ecological interactions structuring the community. We propose that inspecting the phylogenetic structure of co-presence or exclusion links allows to detect the main processes simultaneously assembling the community. We monitored a soil bacterial community after an experimental fire and found that fire altered its composition, richness and phylogenetic diversity. Both co-presence and exclusion links were more phylogenetically related than expected by chance. We interpret such a phylogenetic clustering in co-presence links as a result of environmental filtering, while that in exclusion links reflects competitive exclusion by limiting similarity. This suggests that environmental filtering and limiting similarity operate simultaneously to assemble soil bacterial communities, widening the traditional view that only environmental filtering structures bacterial communities.

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Introduction

Fires are important disturbances that affect forest ecosystems through the combination of effects that are initially triggered by heat (Certini, 2005; Bárcenas-Moreno and Bååth, 2009). The consequences of fire on the soil environment are complex, including the removal of plant cover and changes in physical and chemical parameters (Certini, 2005; Smith *et al.*, 2008; Goberna *et al.*, 2012; Xiang *et al.*, 2014). Fire affects soil microbial communities both directly by high temperatures inducing mortality or cell damage (Daniel and Cowan, 2000) and indirectly through the combustion of organic matter, increase in available nutrients, destruction of the soil physical structure and shifts in soil pH, humidity or electrical conductivity, among others (Certini, 2005), although the magnitude of these effects depends on fire intensity (Bárcenas-Moreno and Bååth, 2009). In turn, the composition and community structure of soil microbial communities is highly dependent on the environmental parameters that are altered by fire (Fierer and Jackson, 2006; Smith *et al.*, 2008; Goberna *et al.*, 2012; Xiang *et al.*, 2014). Some microbial groups can benefit from fire-altered conditions, while others are harmed. For example, fire increases the abundance of both endospore-forming *Firmicutes* in low to moderate fires following the peak temperature that triggers germination (Smith *et al.*, 2008; Ferrenberg *et al.*, 2013) and clades like *Betaproteobacteria* in response to changed environmental conditions (Ferrenberg *et al.*, 2013; Xiang *et al.*, 2014). Conversely, other taxa such as *Nitrobacter* seem to be more heat-sensitive and thus less abundant after a fire (Janzen and Tobin-Janzen, 2008). Fluctuations

in community composition induced by fire concomitantly change the phylogenetic structure of the community (e.g., Xiang *et al.*, 2014). This observation agrees with empirical and conceptual models of temporal changes in microbial community structure, which postulate that niche-based assembling processes like environmental filtering and competition increase its relative importance after a perturbation (Ferrenberg *et al.*, 2013; Dini-Andreote *et al.*, 2015).

The way a community is phylogenetically structured provides clues about its main assembling mechanisms (Webb *et al.*, 2002; HilleRisLambers *et al.*, 2012). Environmental filtering decreases functional and phylogenetic diversity, both through the existence of: (i) abiotic filters, which can be only surpassed by species sharing certain traits (Webb *et al.*, 2002), and (ii) biotic filters, by which one (or a few) clade of strong competitors outcompete distantly-related lineages (Mayfield and Levine, 2010). In contrast, processes like competitive exclusion by limiting similarity increase the phylogenetic diversity of the communities by preventing the coexistence of species that are too functionally (and phylogenetically) similar (Pausas and Verdú, 2010; Mayfield and Levine, 2010). This community phylogenetics framework relies on two assumptions. First, traits are phylogenetically conserved, that is, evolutionarily related species tend to be functionally similar, which has been recently demonstrated for microbes (Martiny *et al.*, 2013; 2015; Goberna and Verdú, 2016). Second, community patterns unequivocally reflect ecological processes, which is not straightforward in the traditional framework (Mayfield and Levine, 2010; Narwani *et al.*, 2015). Here, we try to overcome this limitation by (i) incorporating to the traditional framework the ideas by Mayfield and Levine (2010), that is, expanding the concept of environmental filtering to include biotic filters, and (ii) suggesting a new approach that incorporates network analysis to detect the contribution of assembly processes operating simultaneously. Specifically, we propose to evaluate the phylogenetic community structure in co-occurrence microbial networks, which allow separately investigating the patterns of co-presence (microbes that co-occur) and exclusion (microbes that do not co-occur).

The study of communities from a network-based approach has been dealt with for a long time, comprising numerous studies in food-webs, plant–animal interactions or host–parasite systems (e.g., Solé and Montoya, 2001; Bascompte *et al.*, 2003; Gómez *et al.*, 2013). Ecological networks show complex relationships between nodes (species) connected by links (interactions), which inform about the composition and ecological interactions taking place in biological communities. Improvements of sequencing techniques in environmental samples have made also possible the inference of microbial co-occurrence networks from sequence data (Faust and Raes, 2012). Co-occurrence networks may detect pairs of microbes that co-occur more (co-presence links) or less often (exclusion links) than expected by chance. Co-presence links may be reflecting shared niches while exclusion links suggest niche segregation (Barberán *et al.*, 2012; Faust and Raes, 2012). Applying the community phylogenetics framework described above to co-presence and exclusion links, we can test whether environmental filtering alone (scenario A in Fig. 1), competitive exclusion by limiting similarity alone (scenario B in Fig. 1) or both mechanisms simultaneously (scenario C in Fig. 1) are assembling the soil bacterial communities. Environmental filtering, by favouring the coexistence of functional (and phylogenetically) similar species, will reduce the phylogenetic diversity of co-presence links (dark grey boxes in scenarios A and C, Fig. 1 and Supporting Information Appendix S1). Following the same rationale, environmental filtering, by excluding distantly related species, will increase phylogenetic diversity of exclusion links (light grey box in scenario A, Fig. 1 and Supporting Information Appendix S1). The other main assembling mechanism – competition by limiting similarity – will prevent the coexistence of closely related species, resulting thus in high phylogenetic diversity of co-presence links (the dark grey box in scenario B, Fig. 1 and Supporting Information Appendix S1). For the same reason, non-coexisting species under limiting similarity will be those that are functional (and phylogenetically) similar and therefore, exclusion links will have low phylogenetic diversity (light grey boxes in scenarios B and C, Fig. 1 and Supporting Information Appendix S1). Simulations to validate this theoretical framework are provided in Supporting Information Appendix S1 (Figs. A1 and A2).

Here, we analyse the temporal changes of soil bacterial communities before and after (from 1 day to 1 year) an experimental fire by focusing on the phylogenetic structure of co-presence and exclusion links. Because fire may impose filters to some microbial lineages unable to survive high temperatures and, at the same time, favour other lineages that are able to take advantage of nutrient release, we hypothesise that both environmental filtering and competitive exclusion by limiting similarity are simultaneously assembling post-fire soil bacterial communities.

Results

Fire effects on the soil bacterial community

Fire altered most soil physical and chemical properties (Supporting Information Fig. S1). Some variables showed a significant increase as soon as 1 day after fire, for example, the inorganic forms of nitrogen (NO_3^--N and NH_4^+-N) and electrical conductivity (EC). Others exhibited a delayed response to fire, such as soil humidity, which started decreasing after 1 week. Total organic carbon (TOC) doubled its levels after 1 month with the associated decrease

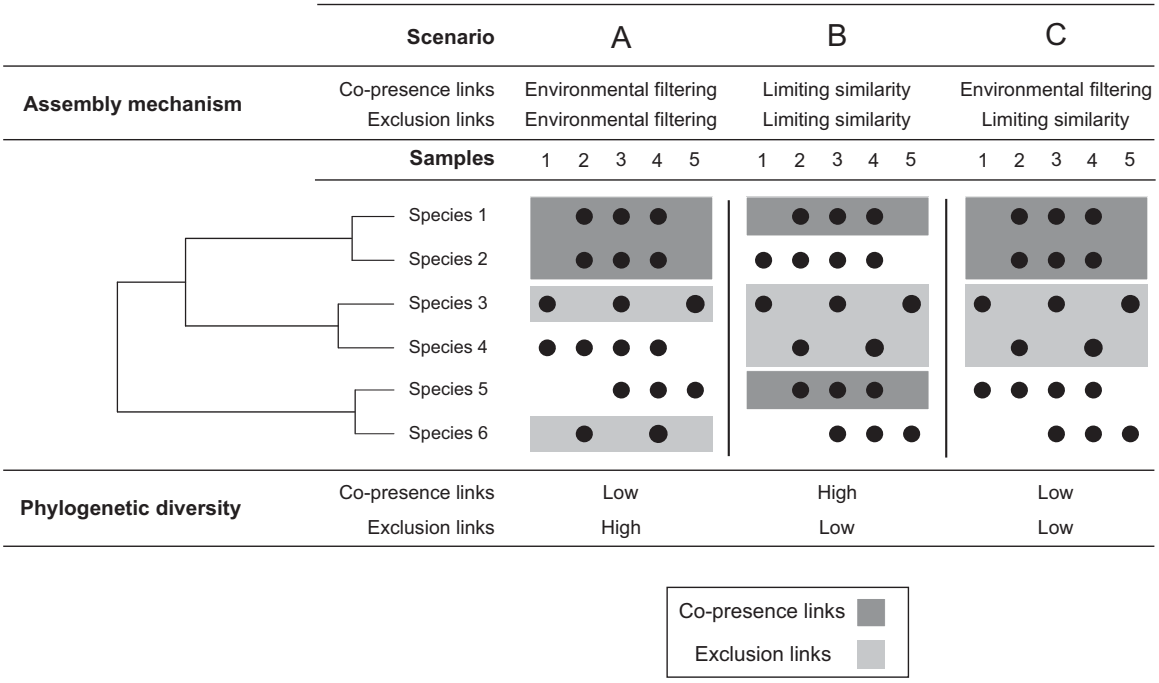

Fig. 1. Schematic representation of the phylogenetic structure of co-occurring species as a result of two assembly mechanisms operating simultaneously in the community. Species co-occurrence is represented as an incidence matrix (i.e., presence–absence) of six species in five plots, where • is drawn when a species is present in a sample. The species whose abundance patterns are positively-correlated (e.g., species 1 and 2 in scenario A) form co-presence links (shaded by a dark grey background) whereas those species whose abundance patterns are negatively-correlated (e.g., species 3 and 6 in scenario A) form exclusion links (shaded by a light grey background). Species with uncorrelated-abundance patterns are not shaded. Assuming trait conservatism (Goberna and Verdú, 2016), three different scenarios are possible (A–C), depending on how members of the co-presence and exclusion links are phylogenetically related: A and C correspond to scenarios in which two phylogenetically close species (species 1 and 2) in a co-presence link co-occur as the result of an environmental filter, while B corresponds to a scenario in which competitive exclusion by limiting similarity causes the coexistence of phylogenetically distant species (species 1 and 5). Simultaneously, not co-occurring species in exclusion links would be phylogenetically related (species 3 and 4, scenarios B and C) as the result of competitive exclusion by limiting similarity whereas they would be distantly related (species 3 and 6, scenario A) as a consequence of environmental filtering.

in pH and increase in the C:N ratio. Total nitrogen (TN) tended to increase in response to fire, but differences were not significant due to a high inter-plot variation (Supporting Information Fig. S1). Generally, soil parameters differed the most from the pre-fire levels after 1 and 4.5 months (Supporting Information Fig. S1). Pre-fire soil properties were recovered after 12 months except for soil humidity, TOC and the C:N ratio (Supporting Information Fig. S1). PCoA showed that bacterial community structure differed the most from pre-fire conditions after 1 and 4.5 months based on the separation of these plots along axis 1 (Supporting Information Fig. S2A). TOC, NH_4^+-N and EC were positively correlated with axis 1, while soil humidity and pH had a negative correlation with the same axis (Supporting Information Fig. S2A, Supporting Information Table S1). A similar temporal trajectory in the community composition space was observed across plots (Supporting Information Fig. S2B).

Bacterial richness before fire was 602 ± 13 OTUs (mean $\pm$ SE) and significantly decreased 1 month after fire but recovered 1 year later (Fig. 2). Fire reduction of bacterial richness was significant even when seasonal climatic variation was taken into account (Table 1). Fire also produced a high turnover of species (Table 2). Indeed, a substantial proportion of species at different time points after fire had not been present at the previous time point (Table 2). Fire also shifted the relative abundance of relevant taxonomic groups (Supporting Information Fig. S3). Specifically, fire immediately (1 day after burning) increased the relative abundances of candidate division KSB1 and *Bacilli* while decreasing those of *Alphaproteobacteria* and candidate division NC10 (Supporting Information Fig. S3). The relative abundance of *Bacilli*, whose initial increase was mainly due to that of the genus *Bacillus*, decreased along the year, while *Alphaproteobacteria* recovered its pre-fire levels after 9 months. Interestingly, *Betaproteobacteria* almost tripled its pre-fire values between 1 and 4.5 months since fire due to the increased abundance of the genus *Massilia* (Supporting Information Fig. S3). The analysis of bacterial community composition through OTU-based distance metrics revealed that soils harboured significantly different bacterial

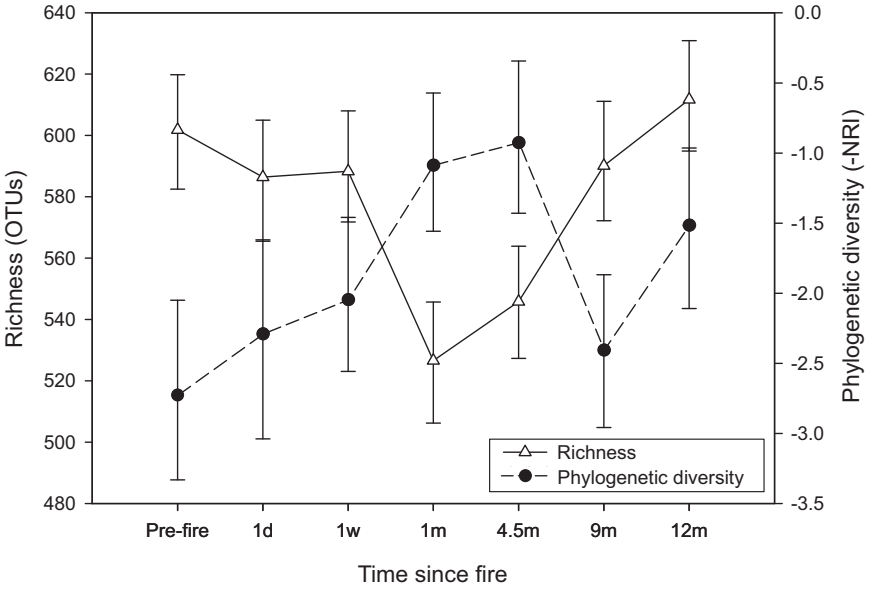

Fig. 2. Post-mean estimates and credible intervals (95%) of the OTU richness and phylogenetic diversity of the soil bacterial community regarding time since fire. Negative values of phylogenetic diversity indicate phylogenetic clustering.

communities immediately after the fire (PERMANOVA: $F_{6,61} = 2.1$, $P < 0.001$, $R^2 = 0.17$). Furthermore, pairwise comparisons showed that pre-fire composition had not been recovered at any time point after the fire (all $P < 0.01$, data not shown).

Changes in the composition of the bacterial community were translated into changes in the phylogenetic diversity of the bacterial community (Fig. 2). The high phylogenetic clustering showed by the pre-fire bacterial community was relaxed with time after fire, reaching values close to randomness at 1 and 4.5 months and fluctuating later (Fig. 2). Fire effects on phylogenetic diversity were significant after controlling for climatic conditions (Table 1).

Fire effects on the soil bacterial co-occurrence networks

The main topological parameters describing our study networks, including the number of nodes and the number and ratio of co-presence and exclusion links, were similar before and after the fire (Table 3). Networks were dominated by co-presence links, which accounted for approximately 60% of the links (Table 3; Supporting Information Table S2, Supporting Information Fig. S4).

OTUs belonging to the same link, either co-presence or exclusion, tended to be more evolutionarily related than expected by chance, as indicated by a phylogenetic diversity significantly lower than zero (Fig. 3). A significant interaction between time since fire and interaction type occurred ($F_{6,7636} = 2.5$, $P = 0.021$, Fig. 3) because the phylogenetic diversity of co-presence links was initially higher than that of exclusion links but the opposite trend occurred 1 month later, and both link types had similar values after 4.5 months.

Discussion

Our results show that fire did not alter general network parameters describing the soil bacterial co-occurrence patterns but changed the richness, composition and consequently the phylogenetic diversity of the community. Delving into the phylogenetic signature left in the network by species that co-occur and by those that do not co-occur

Table 1. Post-mean estimates and their expected credible intervals (95%) for the fire-driven effect on the part of richness and phylogenetic structure (residues) that were not explained when climatic variables (temperature) were taken into account.

	Richness residuals	Phylogenetic diversity (−NRI) residuals
Pre-fire (Intercept)	14.95 [−3.46, 34.61]	−0.68 [−1.19, −0.14]
1 d	−16.18 [−43.73, 9.90]	0.43 [−0.30, 1.17]
1 w	−13.83 [−39.76, 14.21]	0.67 [−0.05, 1.40]
1 m	**−44.57 [−75.02, −20.93]**	**0.78 [0.02, 1.48]**
4.5 m	−10.08 [−34.23, 22.08]	**0.77 [1×10^{-3}, 1.50]**
9 m	**−37.91 [−63.37, −6.91]**	**1.05 [0.02, 1.82]**
12 m	13.60 [−12.60, 40.57]	**1.05 [0.28, 1.89]**

Significant differences ($P < 0.05$, Bayesian GLM) with the pre-fire level are in bold.

Table 2. β-diversity analysis and number of shared and not shared (lost and new) species between pairs of samples at different time points. Lost (new) species are those present (absent) in the first time point and absent (present) in the second time point.

Time points		β-diversity			Species		
Initial	Final	Turnover	Nestedness	Total	Shared	Not shared (Lost)	Not shared (New)
Pre-fire	1 d	0.62 ± 0.03	0.02 ± 0.02	0.64 ± 0.02	213 ± 32	396 ± 28	349 ± 39
1 d	1 w	0.60 ± 0.03	0.02 ± 0.01	0.62 ± 0.02	221 ± 30	341 ± 39	383 ± 29
1 w	1 m	0.62 ± 0.03	0.02 ± 0.01	0.64 ± 0.02	211 ± 19	397 ± 39	344 ± 38
1 m	4.5 m	0.60 ± 0.02	0.03 ± 0.02	0.62 ± 0.03	217 ± 27	338 ± 35	376 ± 61
4.5 m	9 m	0.60 ± 0.02	0.04 ± 0.02	0.64 ± 0.03	192 ± 25	400 ± 73	296 ± 35
9 m	12 m	0.60 ± 0.05	0.06 ± 0.03	0.66 ± 0.03	182 ± 26	306 ± 52	403 ± 82

Average values (± SD) of 10 plots are provided.

helps us to discern the mechanisms assembling soil bacterial communities after fire.

Fire changed the soil abiotic environment as has been previously described (Certini, 2005). The combustion of organic matter provoked an immediate increase in the inorganic compounds of nitrogen and electrical conductivity whereas the complete depletion of the plant cover reduced the soil humidity. The massive input of burned debris into the soil, which doubled the TOC contents, cannot be attributed to plant recovery that was very slight 1 month after fire. Seasonality might have also altered the levels of several parameters, such as TOC, humidity or pH, but the magnitude of seasonal effects is lower than that detected here as we previously described in nearby Mediterranean ecosystems (Goberna *et al*., 2007). Even if the use of an unburned control in an adjacent area could have helped us to partly account for the influence of seasonal effects during this study, it would have not been without the presence of other confounding factors such as the environmental heterogeneity (e.g., presence of a natural plant cover, differences in soil properties) or the spatial distance which are a remarkable source of variation in microbes (Ramette and Tiedje, 2007). Instead, we have directly controlled for seasonal climatic variation in our statistical models to test fire effects on microbial community parameters.

Fire dramatically altered the specific composition of the soil bacterial community, showing particular shifts in some groups with a range of potential strategies that respond differentially to fire. In particular, it has been found that *Firmicutes*, which contains species able to form spores whose germination is triggered by high temperatures (Dworkin, 2006), benefit from post-fire soil conditions in different environments (Yeager *et al*., 2005; Smith *et al*., 2008). In contrast, other groups (e.g., *Alphaproteobacteria*) decrease after fire (e.g., Smith *et al*., 2008; Xiang *et al*., 2014), suggesting that they could either be more sensitive to heating or harmed by the post-fire conditions. Temporal fluctuations in the community composition were not restricted to the immediate days following fire but continued to occur several weeks later. Notably, *Betaproteobacteria* experienced an important increase mainly caused by the rise of *Massilia*, a root-colonizing copiotrophic genus which is related to both early stages of microbial succession and plant development (Ofek *et al*., 2012).

Changes in the bacterial composition should be reflected in changes in the phylogenetic structure of the community if the traits allowing survival or competitive superiority are phylogenetically conserved (Pausas and Verdú, 2010). This seems to be the case of traits conferring either environmental tolerance or competitive abilities in soil bacterial communities (Goberna *et al*., 2014a). Our results show that the community phylogenetic structure was always clustered, which could indicate the prevalence of environmental filtering in the community assembly (Webb *et al*., 2002; Mayfield and Levine, 2010). However, fire reduced the richness while increasing the phylogenetic diversity at the community level as soon as 1 month after fire. These concomitant changes in richness and phylogenetic diversity could indicate that missing species after fire were phylogenetically related as a consequence of other mechanisms like competition by limiting similarity. Alternatively, it could also indicate that the communities are being

Table 3. Overall characteristics of the microbial networks regarding the fire event.

	Co-presence nodes	Exclusion nodes	Co-presence links	Exclusion links	Co-presence links/total links
Pre-fire	566	474	606	456	0.57
1 d	543	439	727	499	0.59
1 w	584	450	630	438	0.59
1 m	545	426	617	402	0.61
4.5 m	592	423	637	431	0.60
9 m	479	385	677	436	0.61
12 m	563	427	656	438	0.60

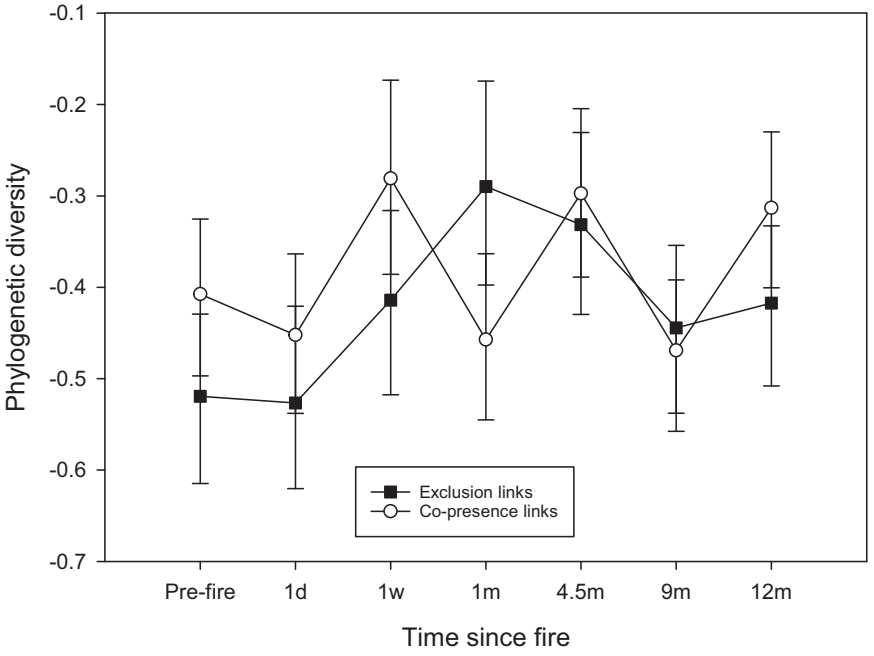

Fig. 3. Post-mean estimates and credible intervals (95%) of the average phylogenetic diversity of co-presence and exclusion links regarding time since fire. Negative values indicate phylogenetic clustering of links.

stochastically re-assembled through other mechanism like dispersal. This could be the case if (1) the contribution of turnover with respect to nestedness were high and (2) the phylogenetic patterns in the community structure were erased, as our simulations confirm (Supporting Information Appendix S1, Fig. A3). While we found a strong role of the species turnover after fire, this process did not erase the phylogenetically clustered pattern across communities, suggesting thus that dispersal was phylogenetically structured. This raises the possibility that other mechanisms like competition by limiting similarity are also acting.

Co-occurrence networks allow a deeper analysis of the ecological processes structuring microbial communities, identifying patterns that could be indicative of environmental filtering (e.g., Levy and Borenstein, 2013; Pascual-García *et al*., 2014) but also other processes (e.g., competitive exclusion by limiting similarity) that would be indistinguishable at the community level if environmental filtering dominates (e.g., Horner-Devine and Silver, 2007; Steele *et al*., 2011; Faust and Raes, 2012). Positively and negatively correlated co-occurrence patterns indicated by co-presence and exclusion links, respectively, could be interpreted in terms of either niche preferences or ecological interactions (Faust and Raes, 2012; Barberán *et al*., 2012; Pascual-García *et al*., 2014). For instance, co-presence links could be the result of species sharing niche (i.e., species that exhibit abiotic or biotic abilities allowing its growth in similar environments) and/or interacting through cross-feeding, co-aggregation or co-colonization whereas exclusion links could arise because species have different niche and/or are involved in interactions like amensalism, competition or predation (Faust and Raes, 2012). By phylogenetically informing the co-presence and exclusion links we have tried to shed light on the relative contribution of two types of processes (niche preference vs. competitive ecological interactions) after fire. The phylogenetic analysis of our network links supports the hypothesis that both processes are acting because co-presence and exclusion links were phylogenetically clustered, which agrees with environmental filtering determining co-presence and competition by limiting similarity favouring exclusion (see Fig. 1, scenario C).

Closely-related species co-occurring more often than expected by chance is a common result that has been mainly attributed to environmental filtering in bacterial communities across a wide range of environments (Chaffron *et al*., 2010; Faust *et al*., 2012; Stegen *et al*., 2012; Levy and Borenstein, 2013; Pascual-García *et al*., 2014). Levy and Borenstein (2013) found that metabolic competition was positively correlated to microbial co-presence in the human microbiome, suggesting that despite closely-related species being more likely to share nutritional profiles and therefore to compete more, they tended to co-occur frequently probably because they also share other traits allowing them to survive a strong environmental filter. In agreement with the predominant evidence of environmental filtering determining bacterial co-occurrence, our co-presence links were populated with closely related species suggesting environmental filtering once more. This is not to say that ecological interactions like competition are not operating in bacterial communities (Levy and Borenstein, 2013). In fact, our exclusion links also showed a phylogenetically clustered structure. We interpret this as the result of competitive exclusion by limiting similarity,

where non-coexisting species belonging to an exclusion link were closely related species competing and excluding each other. In brief, both assembly processes occur at the same time and do not necessarily involve the same bacterial taxa. For example, immediately after fire, the co-presence links involving the most closely related taxa occurred between *Bacilli* species, suggesting that fire filtered the sporulation character. However, the exclusion links involving closely related taxa occurred between *Alphaproteobacteria*, indicating their role in competitive interactions (Goberna *et al*., 2014b). Other assembly processes (e.g., priority effects) could be relevant to the community after a disturbance (Nemergut *et al*., 2013). However, the fact that temporal trajectories in community composition after fire were similar across plots in addition to the phylogenetic patterns not being erased after the fire suggests that initial taxonomic composition, and therefore priority effects, were not determinant.

Fire changed the relative importance of niche-based assembling mechanisms over time, as postulated by empirical and conceptual models of microbial community succession (Ferrenberg *et al*., 2013; Dini-Andreote *et al*., 2015). This was suggested by the temporal variation in the phylogenetic diversity of both co-presence and exclusion links after fire indicating that this perturbation alters the contribution of environmental filtering and competition by limiting similarity. Ferrenberg *et al*. (2013) showed that soil bacterial community assembly in burned sites 1 month after fire was significantly more stochastic compared with the control, the reverse trend appearing several weeks later. We detected a very similar trend in our community, with phylogenetic diversity values approaching randomness 1 month after fire and the low phylodiversity values indicative of environmental filtering (*sensu* Mayfield and Levine, 2010) recovering later. By carefully inspecting the phylogenetic diversity of co-presence and exclusion links, we interpret this temporal fluctuation at the community level as the result of the balance between environmental filtering and competition by limiting similarity pushing toward low or high phylogenetic diversities. Species sharing a link might represent common life strategies to cope with the environmental conditions imposed by the great diversity of microhabitats contained in the soil (Raynaud and Nunan, 2014; Koeppel and Wu, 2014; Pascual-García *et al*., 2014). Examples of these strategies could include the ability to sporulate, the early colonization of the environment (e.g., by fast-growing copiotrophic organisms), or the use of the newly available forms of mineral nitrogen by denitrifiers, able to thrive in low-oxygen microniches that can be found in any aerobic soil. Those strategies, which involve traits related to either environmental tolerance (e.g., endospore formation) or competitive abilities (e.g., denitrification), are phylogenetically conserved with a varying strength (Goberna *et al*., 2014a). Ultimately, the phylogenetic signatures at the community level will be the result of both the evolutionary conservatism and the importance of these traits to survive post-fire conditions. Thus, combining phylogenetic and functional analyses will provide a better understanding of the post-fire community assembly mechanisms.

In conclusion, we suggest that despite the weak changes showed in the general parameters of the co-occurrence networks, fire altered community assembly mechanisms by changing species richness and composition. By phylogenetically informing co-presence and exclusion links of co-occurrence networks, we detected that fire altered the relative importance of environmental filtering and competitive exclusion by limiting similarity.

Experimental procedures

Study site and experimental fire

An experimental fire was ignited on 22 April 2009 in a 500 m^2 area of a dense shrubland dominated by *Rosmarinus officinalis* L. in eastern Spain (Teresa de Cofrentes, Valencia). Fire completely burned the plant cover that started slightly recovering 4 months later (Supporting Information Fig. S1). Soils are Haplic Leptosols (Calcaric, Humic) (FAO–ISRIC–IUSS, 2006) developed on limestones. The mean annual rainfall in the study site is 446 mm and mean annual temperature 13.7°C (Supporting Information Fig. S5). Surface soil samples (0–2 cm) were taken from about, 1 × 1 m georeferenced plots ($n = 10$), which were randomly located at 1–3 m apart from each other within a 150 m^2 area. A total of 70 topsoil samples (i.e., 10 plots × 7 time points) were collected immediately before fire, and 1 day, 1 week, 1 month, 4.5 months, 9 and 12 months after the fire. To reduce the spatial heterogeneity that results from sampling an adjacent unburned area, the pre-fire samples were considered as the unburned control. Soils were transported to the laboratory on ice, immediately sieved (<2 mm) and stored at 4°C. Soil samples (~300 g) were analysed for their physical and chemical properties, including pH, gravimetric humidity, total organic carbon (TOC), electrical conductivity (EC), total nitrogen (TN), nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) using standard procedures as described by Goberna *et al*. (2012).

Soil DNA extraction and pyrosequencing

Soil DNA was extracted within 24 h after sampling from about 0.25 g soil with the PowerSoil® DNA isolation kit (MO BIO Laboratories, Carlsbad, California), which directly extracts the DNA after the physical and chemical lysis of cells. After a quality check of DNA extracts, the bacterial 16S rRNA gene was amplified using primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′; Turner *et al*., 1999) and 534R (5′-ATTACCGCGGCTGCTGGC-3′; Muyzer *et al*., 1993), including each sample a 454 sequencing adaptor (5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3′) and a barcode in its 5′-end randomly selected from those published by Hamady *et al*. (2008). Pyrosequencing was performed by GATC Biotech (Konstanz,

Germany) with the 454 GS-FLX platform (Roche). Further details of PCR conditions and purification can be found in Pérez-Valera *et al.* (2015).

Sequence analysis and phylogeny reconstruction

The initial sequence processing was performed by MR DNA (Shallowater, TX, USA) where short sequences (<200 bp) were removed, primers and barcodes trimmed, and chimeric sequences excluded. After the initial processing, a total of 69,143 sequences were obtained, with $1{,}016.81 \pm 198$ (Mean $\pm$ SD) sequences per sample (Supporting Information Table S3). Two samples (belonging to 1d and 9m time points) were discarded because they failed to amplify. Operational taxonomic units (OTUs) were defined at an identity level of 97% and, after removing singletons, 3,464 OTUs were aligned with PyNAST 1.2.2 in QIIME 1.8.0 (Caporaso *et al.*, 2010a,b). After manually checking the alignments and removing the hypervariable regions in QIIME, maximum likelihood phylogenetic trees were built with the GTRGAMMA substitution model using RAxML 7.3.0 (Stamatakis, 2006). We constructed three independent trees to account for the uncertainty of the phylogenetic reconstruction. The topology of the basal relationships in the trees was constrained to match that of the megatree built from the Silva database (Release 108; Quast *et al.*, 2013). Then, we constructed an OTU $\times$ plot abundance matrix showing the abundance of the total 3,464 OTUs in each of the 68 samples. In order to reduce the potential bias caused by the differential sequencing depth between samples, rarefied richness was calculated (at 1,023 sequences per sample) through an individual-based multinomial model which uses ten randomized samplings without replacement to estimate richness as in Colwell *et al.* (2012). The relative abundance of each OTU was corrected by the estimated number of 16S rRNA gene copies (Kembel *et al.*, 2012). Further details about the sequence analysis along with sequences from the pre-fire conditions are available in Pérez-Valera *et al.* (2015). Post-fire sequences were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena/data/view/PRJEB9090).

Network analysis

OTUs co-occurring more (co-presence) or less (exclusion) often than expected by chance were detected through co-occurrence network analysis. Co-presence and exclusion interactions were identified using an ensemble-based network approach, which captures links from two measures of correlation (Pearson and Spearman) and dissimilarity (Bray–Curtis and Kullback–Leibler) to cover a wide range of relationships (e.g., linear or non-linear), to deal with noise and outliers and thus, to reduce the impact of choosing a single measure (Faust and Raes, 2012). Links detected by several correlation/dissimilarity measures in the same pair of OTUs were considered as a single link. The interaction sign was used to distinguish between co-presence and exclusion links. The analyses were run with the help of CoNet 1.0b6 (Faust *et al.*, 2012; Faust and Raes, 2012) and the script available at http://psbweb05.psb.ugent.be/conet/cmdline.php. Seven networks, one per time point, were constructed from the OTU $\times$ plot relative abundance matrix. Before network construction, samples were filtered such that OTUs present in less than 1/3 of the samples, that is, low-abundant OTUs which could cause artefactual associations (Faust and Raes, 2012), were removed. The sum of the filtered OTUs was kept to preserve taxon proportions. Next, samples were normalized by calculating the relative abundance of each OTU. Then, networks were computed with the 1,000 initial top- and bottom-scoring links for each measure. Statistical significance was tested by obtaining the link- and measure-specific *P*-value as the mean of the permutation distribution under the bootstrap distribution, using 1,000 iterations for each distribution. In order to deal with the compositionality bias caused by the data normalization, that is, an increase in the absolute abundance of an organism implies a decrease in the relative abundance of all other, we re-normalized the data in each permutation (Faust *et al.*, 2012). Thus, the null model captures the effect of data normalization (Faust *et al.*, 2012). Dissimilarity measures (i.e., Bray–Curtis and KullBack–Leibler) were not re-normalized because they are not affected by this bias (Lovell *et al.*, 2010; Faust *et al.*, 2012; Weiss *et al.*, 2016,). Prior to computation, each row was divided by its sum for Bray–Curtis calculations. Unstable links with scores not within the 95% confidence interval of the bootstrap distribution (e.g., outliers) or those with an opposite interaction sign were removed. *P*-values of different correlation/dissimilarity measures supporting the same link were merged using Brown's method and corrected for multiple testing using Benjamini–Hochberg's procedure (Brown, 1975; Benjamini and Hochberg, 1995). Finally, networks were filtered to keep only links with an adjusted merged *P*-value below 0.05. In order to reduce the number of spurious and artefactual relationships, only those links supported by at least two correlation and/or dissimilarity measures were kept. We run sensitivity analyses to different parameters involved in network construction. Specifically, we modified data normalization (yes/no), number of correlation/dissimilarity measures (1/2), initial top- and bottom-scoring links (1,000/2,000) and minimal species occurrence (2/6) and results were not altered (data not shown).

Phylogenetic diversity

Phylogenetic diversity (PD) of the whole bacterial community was calculated as the mean pairwise distances between OTUs standardized by the expectation of a null model. This is equivalent to -1 times the abundance-weighted Net Relatedness Index (NRI):

$$PD = -NRI = (MPD_{obs} - MPD_{rand})/sd\_MPD_{rand}$$

where MPD_{obs} is the mean pairwise phylogenetic distances between the OTUs coexisting in a sampled plot, MPD_{rand} is the average of MPD calculated in *n* randomly constructed communities after shuffling the distance matrix labels of all the OTUs in the community, and sd_MPD_{rand} is the standard deviation of MPD_{rand} (Webb *et al.*, 2002). Phylogenetic diversity of the links was calculated as the phylogenetic distance of each species pair against the phylogenetic distance of two randomly selected species (999 iterations). This procedure allows examining whether OTUs belonging to co-presence or exclusion links are more (negative values) or less (positive values)

closely related than expected by chance. Thus, negative values of phylogenetic diversity indicate phylogenetic clustering while positive values indicate phylogenetic overdispersion. Calculations were run with the picante package for R (Kembel *et al.*, 2010; R Core Team, 2014). Significance was tested by an across-sample (link) analysis (Hardy, 2008). That is, we tested if the sets of communities (links) within a time point (link type) were significantly different from zero by calculating a Bayesian mean over sites with the help of the MCMMglmm package for R (Hadfield, 2010). Phylogenetic (i.e., patristic) distances were computed using the *cophenetic* function for R.

β-diversity analyses

Nestedness and turnover components of temporal β-diversity (i.e., through time) were computed in order to test whether species after fire were a subset of the previously present species or, conversely, the loss and gain of species were more relevant after fire. The β-diversity analysis was performed between pairs of samples of adjacent time points using incidence matrices and the *beta.temp* function (with the Sorensen dissimilarity index) of the betapart package for R (Baselga *et al.*, 2013). We also calculated the number of shared and not shared (lost and new) species between such samples using the *betapart.core* function of betapart.

Statistical analyses

Changes in the OTU composition of the bacterial communities after the fire were tested by permutational multivariate analysis of variance (PERMANOVA) using Bray Curtis dissimilarity matrices. This analysis was carried out with the *adonis* function using pairwise orthogonal contrasts comparing the pre-fire OTU × plot abundance matrix with all the post-fire matrices in the vegan package for R (Oksanen *et al.*, 2015). Principal coordinates analysis (PCoA) of the Bray Curtis dissimilarity matrix was used to analyse and visualize the spatial differences in the community structure among plots over time in R. Physical and chemical parameters were fitted onto the ordination with the *envfit* function in the vegan package for R, showing only the variables that were significantly ($P < 0.05$) correlated to either axis.

Post-fire changes in OTU richness and phylogenetic diversity were calculated through a Bayesian generalized linear model using time since fire as a categorical independent factor. To account for temporal variation in diversity parameters due to seasonal climatic conditions (i.e., air temperature and precipitation, Supporting Information Fig. S5), we used as the dependent variable of the model the residuals of a previous model including climatic conditions as independent factors. Both OTU richness and phylogenetic diversity were significantly correlated with air temperature (Richness post-mean estimate [95% credible interval]: −5.34 [−8.14, −2.89]; PD: 0.12 [0.06, 0.19]) but not with precipitation (Richness post-mean estimate: −0.08 [−0.33, 0.19]; PD: 3×10^{-3} [-3×10^{-3}, 1×10^{-2}]). Thus, temperature was the only climatic variable taken into account to obtain the statistical residuals. To accommodate the topological and chronological uncertainty of the trees in the phylogenetic diversity model, we ran three models with three independent trees and integrated over the posterior samples by drawing 1,000 random samples across models.

Post-fire changes in the phylogenetic diversity of co-presence and exclusion links were analysed following the same steps described above. In this case, the GLM had phylogenetic diversity as dependent variable and time since fire and link type (i.e., co-presence vs. exclusion links) as crossed independent factors. Neither temperature nor precipitation explained the phylogenetic diversity of co-presence links (temperature post-mean estimate [95% credible interval]: 5×10^{-3} [-5×10^{-3}, 0.01]; precipitation: 5×10^{-4} [-6×10^{-4}, 2×10^{-3}]). The phylogenetic diversity of exclusion links was correlated with air temperature (post-mean estimate: 0.01 [1×10^{-3}, 0.02]) but not with precipitation (post-mean estimate: 4×10^{-4} [-8×10^{-4}, 0.01]). Therefore, in this case we used the residuals from the climatic model.

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References

Baselga, A., Orme, D., Villeger, S., Bortoli, J.D., and Leprieur, F. (2013) betapart: Partitioning beta diversity into turnover and nestedness components. http://cran.r-project.org/package=betapart

Barberán, A., Bates, S.T., Casamayor, E.O., and Fierer, N. (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *Isme J* **6:** 343–351.

Bárcenas-Moreno, G., and Bååth, E. (2009) Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. *Soil Biol Biochem* **41:** 2517–2526.

Bascompte, J., Jordano, P., Melián, C.J., and Olesen, J.M. (2003) The nested assembly of plant-animal mutualistic networks. *Proc Natl Acad Sci U S A* **100:** 9383–9387.

Benjamini, Y., and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc* **57:** 289–300.

Brown, M.B. (1975) A method for combining non-independent, one-sided tests of significance. *Biometrics* **31:** 987–992. p

Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., and Knight, R. (2010a) PyNAST: a flexible

tool for aligning sequences to a template alignment. *Bioinformatics* **26:** 266–267.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010b) QIIME allows analysis of high-throughput community sequencing data. *Nat Meth* **7:** 335–336.
CEAM-UMH (2009) Resumen climático mensual en la Comunitat Valenciana: April-December. http://www.ceam.es/ceamet/observaciones/observaciones.html (Accessed October 21, 2016).
CEAM-UMH (2010) Resumen climático mensual en la Comunitat Valenciana: January-April. http://www.ceam.es/ceamet/observaciones/observaciones.html (Accessed October 21, 2016).
Certini, G. (2005) Effects of fire on properties of forest soils: a review. *Oecologia* **143:** 1–10.
Chaffron, S., Rehrauer, H., Pernthaler, J., and von Mering, C. (2010) A global network of coexisting microbes from environmental and whole-genome sequence data. *Genome Res* **20:** 947–959.
Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.Y., Mao, C.X., Chazdon, R.L., and Longino, J.T. (2012) Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *J Plant Ecol* **5:** 3–21.
Daniel, R.M., and Cowan, D.A. (2000) Biomolecular stability and life at high temperatures. *Cell Mol Life Sci* **57:** 250–264.
Dini-Andreote, F., Stegen, J.C., van Elsas, J.D., and Salles, J.F. (2015) Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proc Natl Acad Sci U S A* **112:** E1326–E1332.
Dworkin, M. (2006) Prokaryotic life cycles. In *The Prokaryotes. A Handbook on the Biology of Bacteria. Ecophysiological and Biochemical Aspects Vol 2*. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., and Stackebrandt, E. (eds). New York, USA: Springer, pp. 140–160.
FAO–ISRIC–IUSS (2006) *World Reference Base for Soil Resources*. Rome: FAO.
Faust, K., and Raes, J. (2012) Microbial interactions: from networks to models. *Nat Rev Microbiol* **10:** 538–550.
Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J., and Huttenhower, C. (2012) Microbial co-occurrence relationships in the Human Microbiome. *PLoS Comput Biol* **8:** e1002606.
Ferrenberg, S., O'neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., *et al.* (2013) Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *Isme J* **7:** 1102–1111.
Fierer, N., and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci U S A* **103:** 626–631.
Goberna, M., and Verdú, M. (2016) Predicting microbial traits with phylogenies. *Isme J* **10:** 959–967.
Goberna, M., Pascual, J.A., García, C., and Sánchez, J. (2007) Do plant clumps constitute microbial hotspots in semiarid Mediterranean patchy landscapes?. *Soil Biol Biochem* **39:** 1047–1054.
Goberna, M., García, C., Insam, H., Hernández, M.T., and Verdú, M. (2012) Burning fire-prone Mediterranean shrublands: immediate changes in soil microbial community structure and ecosystem functions. *Microb Ecol* **64:** 242–255.
Goberna, M., Navarro-Cano, J.A., Valiente-Banuet, A., García, C., and Verdú, M. (2014a) Abiotic stress tolerance and competition-related traits underlie phylogenetic clustering in soil bacterial communities. *Ecol Lett* **17:** 1191–1201.
Goberna, M., García, C., and Verdú, M. (2014b) A role for biotic filtering in driving phylogenetic clustering in soil bacterial communities. *Glob Ecol Biogeogr* **23:** 1346–1355.
Gómez, J.M., Nunn, C.L., and Verdú, M. (2013) Centrality in primate-parasite networks reveals the potential for the transmission of emerging infectious diseases to humans. *Proc Natl Acad Sci U S A* **110:** 7738–7741.
Hadfield, J.D. (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J Stat Softw* **33:** 1–22.
Hamady, M., Walker, J.J., Harris, J.K., Gold, N.J., and Knight, R. (2008) Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nat Meth* **5:** 235–237.
Hardy, O.J. (2008) Testing the spatial phylogenetic structure of local communities: statistical performances of different null models and test statistics on a locally neutral community. *J Ecol* **96:** 914–926.
HilleRisLambers, J., Adler, P.B., Harpole, W.S., Levine, J.M., and Mayfield, M.M. (2012) Rethinking community assembly through the lens of coexistence theory. *Annu Rev Ecol Evol Syst* **43:** 227–248.
Horner-Devine, M., and Silver, J. (2007) A comparison of taxon co-occurrence patterns for macro- and microorganisms. *Ecology* **88:** 1345–1353.
Janzen, C., and Tobin-Janzen, T. (2008) Microbial communities in fire-affected soils. In *Microbiology of Extreme Soils*. Dion, P. and Nautiyal, C.S. (eds). Berlin, Heidelberg: Springer, pp. 299–316.
Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., *et al.* (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26:** 1463–1464.
Kembel, S.W., Wu, M., Eisen, J.A., and Green, J.L. (2012) Incorporating 16s gene copy number information improves estimates of microbial diversity and abundance. *PLoS Comput Biol* **8:** e1002743.
Koeppel, A.F., and Wu, M. (2014) Species matter: the role of competition in the assembly of congeneric bacteria. *Isme J* **8:** 531–540.
Levy, R., and Borenstein, E. (2013) Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. *Proc Natl Acad Sci U S A* **110:** 12804–12809.
Lovell, D., Warren, M., Taylor, J., Zwart, A., and Helliwell, C. (2010) Caution! Compositions! Can constraints on omics data lead analyses astray?. Report Number: EP10994. CSIRO Mathematical and Information Sciences.
Martiny, A.C., Treseder, K., and Pusch, G. (2013) Phylogenetic conservatism of functional traits in microorganisms. *Isme J* **7:** 830–838.
Martiny, J.B.H., Jones, S.E., Lennon, J.T., and Martiny, A.C. (2015) Microbiomes in light of traits: a phylogenetic perspective. *Science* **350:** aac9323.

Mayfield, M.M., and Levine, J.M. (2010) Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecol Lett* **13:** 1085–1093.
Muyzer, G., de Waal, E.C., and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59:** 695–700.
Narwani, A., Matthews, B., Fox, J., and Venail, P. (2015) Using phylogenetics in community assembly and ecosystem functioning research. *Funct Ecol* **29:** 589–591.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'neill, S.P., Bilinski, T.M., Stanish, L.F., *et al*. (2013) Patterns and processes of microbial community assembly. *Microbiol Mol Biol Rev* **77:** 342–356.
Ofek, M., Hadar, Y., and Minz, D. (2012) Ecology of root colonizing *Massilia* (Oxalobacteraceae). *PLoS One* **7:** e40117.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., *et al*. (2015) Vegan: community ecology package. R Package Version 2.0-2.
Pascual-García, A., Tamames, J., and Bastolla, U. (2014) Bacteria dialog with Santa Rosalia: are aggregations of cosmopolitan bacteria mainly explained by habitat filtering or by ecological interactions?. *BMC Microbiol* **14:** 284.
Pausas, J.G., and Verdú, M. (2010) The jungle of methods for evaluating phenotypic and phylogenetic structure of communities. *BioScience* **60:** 614–625.
Pérez-Valera, E., Goberna, M., and Verdú, M. (2015) Phylogenetic structure of soil bacterial communities predicts ecosystem functioning. *FEMS Microbiol Ecol* **91:** fiv031.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., *et al*. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41:** D590–D596.
R Core Team (2014) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
Ramette, A., and Tiedje, J.M. (2007) Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. *Proc Natl Acad Sci U S A* **104:** 2761–2766.
Raynaud, X., and Nunan, N. (2014) Spatial ecology of bacteria at the microscale in soil. *PLoS One* **9:** e87217.
Smith, N.R., Kishchuk, B.E., and Mohn, W.W. (2008) Effects of wildfire and harvest disturbances on forest soil bacterial communities. *Appl Environ Microbiol* **74:** 216–224.
Solé, R.V., and Montoya, J.M. (2001) Complexity and fragility in ecological networks. *Proc R Soc Lond B Biol Sci* **268:** 2039–2045.
Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.
Steele, J.A., Countway, P.D., Xia, L., Vigil, P.D., Beman, J.M., Kim, D.Y., *et al*. (2011) Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *Isme J* **5:** 1414–1425.
Stegen, J.C., Lin, X., Konopka, A.E., and Fredrickson, J.K. (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. *Isme J* **6:** 1653–1664.
Turner, S., Pryer, K.M., Miao, V.P.W., and Palmer, J.D. (1999) Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J Eukaryot Microbiol* **46:** 327–338.
Webb, C.O., Ackerly, D.D., McPeek, M.A., and Donoghue, M.J. (2002) Phylogenies and community ecology. *Annu Rev Ecol Syst* **33:** 475–505.
Weiss, S., Van Treuren, W., Lozupone, C., Faust, K., Friedman, J., Deng, Y., *et al*. (2016) Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. *Isme J* **10:** 1669–1681.
Xiang, X., Shi, Y., Yang, J., Kong, J., Lin, X., Zhang, H., *et al*. (2014) Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. *Sci Rep* **4:** 3829.
Yeager, C.M., Northup, D.E., Grow, C.C., Barns, S.M., and Kuske, C.R. (2005) Changes in nitrogen-fixing and ammonia-oxidizing bacterial communities in soil of a mixed conifer forest after wildfire. *Appl Environ Microbiol* **71:** 2713–2722.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Post-mean estimates and credible intervals (95%) of several soil physical and chemical properties regarding time since fire. Significant differences ($P < 0.05$, Bayesian GLM) with the pre-fire level are indicated with *.
Fig. S2. PCoA based on Bray Curtis distances of the bacterial community showing differences in the OTU composition among samples and time since fire. Soil environmental parameters that were significantly correlated with changes in the community composition (Axis 1 and/or Axis 2) are shown in A). Individual trajectories of each plot over time after fire are linked by solid lines in B), where arrows indicate the final time point. Dashed lines indicate an indirect trajectory due to a missing intermediate sampling point. Abbreviations: *TOC* total organic C, *EC* electrical conductivity, NH_4^+-N ammonium-N.
Fig. S3. Relative abundance (post-mean and credible intervals [95%]) of the ten most abundant classes before and after the experimental fire. Significant differences ($P < 0.05$, Bayesian GLM) with the pre-fire level are indicated with *.
Fig. S4. Co-occurrence networks supported by positively (A) and negatively (B) correlated abundance patterns at the OTU level for the pre-fire time point. Each node belongs to a phylum following the colour code shown in the phylogenetic tree in such a way that phylogenetically related OTUs share similar colours.
Fig. S5. Monthly accumulated precipitation (expressed in mm), mean monthly temperature (in °C) and plant cover (in %) over the study period (CEAM-UMH, 2009; 2010). The arrows indicate the experimental fire and time since fire.
Table S1. Soil variables and their correlations with the axis 1 of the PCoA.
Table S2. Edge lists of the pre- and post-fire networks.
Table S3. Number of sequences and OTUs *per* sample and time since fire.
Appendix S1. Simulations to validate scenarios from Fig. 1. It includes Figs. A1, A2 and A3.