Running Head

Plant and microbes affect multifunctionality

Cascading effects from plants to soil microorganisms explain how plant species richness and simulated climate change affect soil multifunctionality

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Abstract

Despite their importance, how plant communities and soil microorganisms interact to determine the capacity of ecosystems to provide multiple functions simultaneously (multifunctionality) under climate change is poorly known. We conducted a common garden experiment using grassland species to evaluate how plant functional structure and soil microbial (bacteria and protists) diversity and abundance regulate soil multifunctionality responses to joint changes in plant species richness (1, 3 and 6 species) and simulated climate change (3°C warming and 35% rainfall reduction). The effects of species richness and climate on soil multifunctionality were indirectly driven via changes in plant functional structure and their relationships with the abundance and diversity of soil bacteria and protists. More specifically, warming selected for the larger and most productive plant species, increasing the average size within communities and leading to reductions in functional plant diversity. These changes increased the total abundance of bacteria that, in turn, increased that of protists, ultimately promoting soil multifunctionality. Our work suggests that cascading effects between plant functional traits and the abundance of multitrophic soil organisms largely regulate the response of soil multifunctionality to simulated climate change, and ultimately provides novel experimental insights into the mechanisms underlying the effects of biodiversity and climate change on ecosystem functioning.

Keywords: bacteria, biodiversity, climate change, ecosystem functioning, environmental filtering, nutrient cycles, protist, species richness.

Introduction

A large body of literature suggests that alterations in climate, such as warming and shifts in rainfall regimes, largely impact plant communities, and the multiple ecosystem functions that depend on them (Peñuelas et al., 2013). Similarly, most recent studies suggest that the diversity and abundance of soil microbes are also highly vulnerable to climate change (Castro, Classen, Austin, Norby, & Schadt, 2010; Maestre et al., 2015). Given the strong links between plant and microbial diversity and the provision of multiple ecosystem functions simultaneously (multifunctionality; Delgado-Baquerizo, Maestre, Reich, Jeffries, et al., 2016; Maestre et al., 2012), alterations in the diversity of multiple trophic levels (microbial and plant communities) could largely regulate the cascading effects of climate change on ecosystem functioning (Jing et al., 2015). However, the indirect role of multitrophic diversity in driving multifunctionality responses to climate change has not been deciphered yet. Previous studies have rarely considered the indirect effect of climate change on multifunctionality driven by changes in soil microbes and plant traits (Jing et al., 2015), nor the joint effects of simulated climate change and plant diversity on ecosystem functioning (but see Kardol, Cregger, Campany, & Classen, 2010). Furthermore, most studies exploring the impacts of global change drivers on ecosystem functioning have focused on a single trophic level (e.g. plants or particular soil microorganisms), ignoring the trophic interactions across multiple aboveground and belowground organisms taking place in natural ecosystems. These gaps in our knowledge limit our understanding of the direct and indirect impacts of changes in climate and biodiversity on terrestrial ecosystems, and thus our ability to accurately predict the ecological consequences of global change.

We know that the effects of plant diversity on ecosystem functioning are largely driven by variations in plant species composition and functional traits, such as height and specific leaf area (Díaz et al., 2016). Moreover, the functional structure of plant

communities can, either directly (e.g., via litter quality and inputs) or indirectly (e.g., via changes in abiotic factors), explain variation in soil microorganisms (de Vries et al., 2012). For instance, Maestre et al. (2009) showed how the increase in height associated with shrub encroachment in grasslands, and the modifications in environmental conditions associated to it (e.g. reductions in soil temperature under plant canopies), affected the biomass of fungi, actinomycetes and other bacteria. Plant functional traits are highly sensitive to climate, with important consequences for multiple ecosystem properties (Butler et al., 2017; Valencia et al., 2015). The traits of the dominant species and the functional diversity of plant communities may determine how plant species respond to climate change, possibly via intraspecific shifts in trait values, changes in species relative abundances, and/or species turnover. For example, climate change could promote species with traits that confer a better adaptation to warmer conditions, such as thicker leaves (more stress-tolerant plant strategies) and smaller plant size (Westoby, Falster, Moles, Vesk, & Wright, 2002), and these changes would cascade to affect soil communities and ecosystem functioning (Díaz et al., 2007; Grime, 1998).

Within the soil food web, protists occupy a key position, as they feed on bacteria and yeasts, linking the flow of energy and the cycling of nutrients to higher trophic levels (Bonkowski, 2004). These organisms also promote defence mechanisms and/or suppress some pathogens in bacterial communities (Jousset, 2012), and their activity could explain an important part of nitrogen mineralization (Trap, Bonkowski, Plassard, Villenave, & Blanchart, 2016), among other processes. Despite their importance for multiple ecosystem process and the control they exert on bacterial populations, protists are not routinely considered in either plant-soil or global change biology studies (Geisen et al., 2017). Therefore, to gain a more mechanistic understand of the ecological impacts of climate

106 change we must consider the 'indirect effects' of climate change on ecosystem functioning
107 via changes in plant functional traits and soil microorganisms, including protists.

108 To the best of our knowledge, no previous study has explicitly evaluated, using an
109 experimental approach, whether the simultaneous impacts of biodiversity and climate
110 change on soil multifunctionality are mediated by trophic level interactions across plants
111 and soil microorganisms. We aimed to do so with a full factorial mesocosm experiment
112 involving the use of open top chambers (OTCs) to increase temperature, rainfall shelters to
113 alter precipitation, and manipulated plant species richness to evaluate its direct and indirect
114 (via changes in plant functional structure and soil microbes) effects on soil
115 multifunctionality (Figure S1). First, we assessed how climate change (warming and
116 rainfall reduction) and initial plant species richness affect plant functional structure and soil
117 microorganisms. Second, we used a variance partitioning analysis to quantify how plants,
118 bacteria and bacterivorous protists alter the impacts of simulated climate change and initial
119 species richness on soil multifunctionality. Finally, we used a multi-scale path analysis (d-
120 sep, Shipley, 2013) to quantify the direct and indirect effects of these different trophic levels
121 on soil multifunctionality. We expect that: (1) warming and rainfall reduction will increase
122 plant mortality, reducing its species richness (Klein, Harte, & Zhao, 2004) and promoting
123 the dominance of plants with more stress-tolerant strategies, ultimately altering the
124 diversity and abundance of soil microbes (e.g., via rizosphere interactions); (2) altered
125 microbial abundance and/or diversity linked to climate change and shifted plant attributes
126 will regulate soil multifunctionality; (3) mesocosms with the highest diversity will be
127 positively correlated with the highest soil multifunctionality (Maestre et al., 2012); (4) the
128 proportion of soil multifunctionality variance explained will substantially increase when
129 trophic level complexity is considered (Jing et al., 2015); and (5) protists –rarely studied in
130 this context- are expected to be a key intermediary of the impacts of global change on soil

multifunctionality via their role in regulating bacterial populations (Trap et al., 2016; Weidner, Latz, Agaras, Valverde, & Jousset, 2017).

Materials and Methods

Experimental design

We conducted a mesocosm experiment in the Climate Change Outdoor Laboratory (CCOL), established at the facilities of Rey Juan Carlos University (URJC, Móstoles, Spain: 40°20'37''N, 3°52'00''W, 650 m a.s.l.; Figure S2), between April 2011 and September 2013. Mean annual temperature and precipitation for this site are 16.6°C and 362 mm.

The experiment was designed as a fully factorial design, with three treatments: initial plant richness (one, three and six species), warming (ambient and 3ºC increase) and rainfall reduction (control and ~35% reduction in total annual rainfall). We selected 27 herbaceous species typical of grasslands from semi-arid areas in central Spain (García-Palacios et al., 2010) for our experiment. In each mesocosm, the species were randomly selected (García-Palacios, Maestre, & Gallardo, 2011). The selected species (Table S1) belong to three main functional groups (grasses, nitrogen-fixing legumes and forbs) differing in traits that, such as specific leaf area and maximum plant height (Díaz et al., 2016), are potentially relevant to ecosystem functioning, such as biomass production, resource use and N-fixation ability (Table S1; McLaren & Turkington, 2010). Because of these differences, the selected species are expected to show contrasted strategies regarding their responses to climate change and their impacts on ecosystem functioning (Suding et al., 2008).

Monocultures and mixtures were established in plastic pots (depth 38 cm, internal diameter 28 cm, volume 0.023 m^3). The base of the pots was filled with 3 cm of expanded

156 clay for drainage, and then we added 32 cm of natural soil (sand content: 73.5 %, silt
157 content: 18.5 %, clay content: 8.0 %). In April 2011, we randomly sowed seeds of each
158 species within each mesocosm obtained from a commercial supplier (Intersemillas Ltd,
159 Valencia, Spain), reaching a final density of 194 individuals m^{-2}. After seeding, all the pots
160 were buried and levelled to the ground (Figure S2b). After this burial, we added 500 mL of
161 a soil microbial inoculum to all mesocosms to recreate realistic soil microbial communities,
162 as described in Maestre et al. (2005). We irrigated all the mesocosms with 1000 mL of
163 water three times per week during the first 6 weeks of the experiment to improve seed
164 establishment. We also watered once a week in July and August 2011 to ensure the survival
165 of the plants during summer before the installation of the warming and rainfall exclusion
166 treatments, which took place in December 2011 (once all the mesocosms had an established
167 population). Individuals that failed to survive in our mesocosms once these treatments were
168 installed were not replaced. We regularly removed weeds throughout the experiment.
169 The warming treatment aimed to simulate climatic predictions for central Spain by
170 the end of the 21st century, *i.e.*, an increase in annual temperature ranging between 2.6 -
171 4.8°C (de Castro, Martín-Vide, & Alonso, 2005; RCP8.5 scenario, IPPC, 2013). To achieve
172 this temperature increase, we used open top chambers (OTCs), described in detail in
173 Appendix 1. The rainfall exclusion treatment consisted on passive rainfall shelters, which
174 did not modify the frequency of rainfall events but reduced the total amount of rainfall
175 reaching the soil surface (35.4% ± 2.2 on average; means ± SE; n = 25 rain events).
176 Additional details about these shelters can be found in Appendix 1. Rainfall reduction
177 values obtained with them are consistent with predictions from climatic models in central
178 Spain, which forecast reductions between 10% and 50% in the total amount of rainfall
179 received during spring and fall (de Castro, Martín-Vide, & Alonso, 2005).

The effects of the OTCs and rainfall shelters on air temperature and humidity were monitored using automated sensors (HOBO U23 Pro v.2 Temp/RH, Onset Corporation, Bourne, MA, USA). Ten replicates per richness level (one, three or six species) were maintained in all possible warming × rainfall exclusion combination (plant assemblages), resulting in a total of 120 mesocosms. We eliminated a monoculture of *Lotus corniculatus* from all climate change drivers (four mesocosms), because that species experienced a severe mortality just after the end of irrigation.

Experimental measurements and harvest

The experiment was harvested in September 2013. We measured plant functional traits, following standard protocols (Cornelissen et al., 2003) at the end of the second growing season (June 2013), just after the peak of vegetation growth and before summer drought to sample mature leaves and to avoid damage of leaf materials. The following traits were measured on one individual per species and mesocosm: vegetative height (VH, cm), lateral spread (LS, cm^2), leaf area (LA, cm^2), leaf length (LL, cm), leaf width (LW, cm) and leaf thickness (LT, mm), phenology, measured using a phenology index (1 = no reproductive stem; 2 = reproductive stem starting to grow; 3 = flowering; 4 = flower fading; 5 = fruit present; and 6 = fruit absent and senescence of the reproductive stem); and specific leaf area (SLA, $cm^2\ g^{-1}$). These traits were selected because they are related to water use efficiency, competitive ability, light interception, water stress tolerance, leaf-level carbon gain strategies, plant relative growth rate and resource capture and utilization (Westoby et al., 2002; Wright et al., 2004) and are important traits in determining plant community responses to climate in drylands (Gross et al., 2013). Additionally, we visually estimated the total cover (cm^2) per species in each mesocosm. For species with compound leaves, foliar traits were measured in one leaflet per individual.

205 In September 2013, we collected three soil cores (0–7.5 cm depth) from each
206 mesocosm, which were bulked and homogenized to obtain a composite sample per
207 mesocosm. Soil samples were sieved (2 mm mesh) in the laboratory and separated into
208 three fractions. The first fraction was air-dried for one month for measurement of soil
209 variables related to nutrient cycles, the second fraction was stored at 4°C for estimations of
210 protist diversity and total abundance, and the last fraction was frozen at -20º C for
211 quantification of the abundance and diversity of bacterial communities.
212
213 *Molecular analyses*
214 Soil DNA was extracted from defrosted soil using the Powersoil DNA Isolation Kit (Mo
215 Bio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The
216 abundance of bacteria was estimated using quantitative PCRs on an ABI 7300 Real-Time
217 PCR (Applied Biosystems, Foster City, CA, USA), and the bacterial diversity (Shannon)
218 was obtained from 16s amplicon sequencing using the Illumina MiSeq platform, as
219 described in detail in Appendix 1. The abundance (total number of protists$\cdot g^{-1}$ of soil) and
220 diversity (Shannon´s index) of bacterivorous protists was determined using a modified
221 version of the liquid aliquot method, as described in detail in Appendix 1. Both protist
222 variables represent the feeding pressure on bacteria, since the method estimates the viable
223 encysted protists that accumulated in soil over time due to high bacterial production rates.
224
225 *Assessing soil multifunctionality*
226 We measured seven variables related to carbon (organic C, β-glucosidase activity), nitrogen
227 (total N, ammonium, nitrate) and phosphorus (available inorganic P and phosphatase
228 activity) storage and cycling. These variables constitute a good proxy for nutrient cycling,
229 biological productivity, and buildup of nutrient pools, and are important determinants of

ecosystem functioning. They also have been extensively used as proxies of ecosystem functioning in many different ecosystem types (Jax, 2010). These variables were measured in the laboratory as described in detail in Appendix 1. We estimated soil multifunctionality from all soil variables measured using the multifunctionality index of Maestre et al. (2012). Raw data for each soil variable were first normalized using a $\log_{10}$-transformation. Following this, the Z scores of the seven soil variables were averaged to obtain soil multifunctionality. This index is statistically robust (Maestre et al., 2012) and provides an intuitive way and easily interpretable measure to assess changes in multifunctionality, as the higher the values for the different ecosystem functions we measured, the higher the multifunctionality (Byrnes et al., 2014). We acknowledge that using an *a priori* standardized average may not allow to discriminate when all functions are performing at similar levels from situations when one function could be strongly outperforming the others. However, all correlations between the soil variables measured were positive (Table S2), suggesting that there are not potential trade-offs between the surrogates of ecosystem functioning evaluated. We also acknowledge that if two variables are highly correlated, the inclusion of both provides redundancy, albeit the interpretation of soil multifunctionality values is much more simple. However, among our soil variables only one out of the 25 correlations had had a r value higher than 0.7, suggesting that our dataset did not contain high redundancy among the variables used to estimate soil multifunctionality (Table S2). Finally, the comparison between our estimates of soil multifunctionality and other alternative approaches revealed that all indices were strongly related, even when a multiple-threshold soil multifunctionality approach (Byrnes et al., 2014) was used (Figure S3). Indeed the relationships between soil multifunctionality and their predictors were similar when averaging soil multifunctionality (Figure S4) or multiple-threshold soil multifunctionality (Figure S5) were used, hence only the former are presented in the main

text. For instance, CW-VH, CW-SLA, bacterial abundance and protist abundance all had positive relationships with averaging soil multifunctionality, but the opposite was found for bacterial diversity (Figure S4). The same relationships were found across all thresholds when using the multiple-threshold approach (Figure S5). Additionally, we calculated functions related to carbon, nitrogen and phosphorus cycles by averaging the Z scores of the variables involved in each of these cycles.

Evaluating functional traits variation among plant species

We considered the species pool sampled in the experiment to obtain an ordination of functional traits. The trait variables obtained per species and mesocosm were normalized using a log transformation to reduce the impact of species with extreme trait values. We then performed a principal component analysis (PCA) with Varimax rotation on trait data to identify the main axes of specialization and covariation among traits. We used a PCA for these analyses because different traits are associated with different niche axes (Butterfield & Suding, 2013), and to avoid selecting highly correlated traits for further analyses (Table S3). We identified two independent PCA components, which together explained around 59% of the total variance in the data (Table S4). To represent each axis of variation, we selected one trait for each PCA component as a functional marker of specialization (Gross et al., 2013). The selected traits (VH and SLA) are related with two main plant ecological strategies (Díaz et al., 2016). VH reflects water use efficiency and/or competitive ability, whereas SLA is related with light interception and water stress tolerance (Westoby et al., 2002).

To assess the effect of simulated climate change drivers and initial species richness on plant community structure we quantified community-weighed functional traits (CWT hereafter, Violle et al., 2007) and functional diversity (FD, called functional dispersion in

Laliberté & Legendre, 2010) using the trait values measured at the end of the experiment. CWT indicates the "average trait value" for the selected traits, and was calculated using the traits measured in each mesocosm weighted by the relative abundance (total cover) of each species within the mesocosm. FD quantifies the variance of the community trait distribution weighted by the relative abundance of each species within the mesocosm, and reflects the functional differences between the species present in a given community. We focused on VH and SLA for CWT and in all of the traits measured for FD. At the community level, changes in CWT reflect a change in the trait values of the dominant species. Higher FD values suggest higher niche complementarity among the species forming the community (Maire et al., 2012), enhancing the use of resources, and in turn ecosystem functioning (Petchey & Gaston, 2006). This index is one of the few indices that can be used when there are monocultures, and it is widely used (e.g., Castro-Díez, Pauchard, Traveset, & Vilà, 2016; Valencia et al., 2015).

Statistical analyses

We first evaluated how the functional structure of plant assemblages (CWT of selected traits and FD) and both the abundance and diversity of soil microorganisms (bacteria and protists) responded to climate change drivers (as a combination of warming and rainfall reduction treatments, resulting in 4 levels: control, warming, rainfall reduction, and warming + rainfall reduction) and initial plant species richness using Linear Mixed Models (LMM). In all models, we used climate change drivers, plant species richness and their interaction as fixed factors, while plant assemblages (30, ten per species richness level) were considered as a random factor (Kuebbing, Maynard, & Bradford, 2018). We log-transformed bacterial and protist abundance, and Box–Cox transformed community-weighed vegetative height (CW-VH), FD, bacterial diversity and protist diversity data prior

to LMM (and subsequent) analyses to improve their normality. A Tukey post-hoc analysis was used to evaluate the differences within levels of the factors evaluated (climate change drivers and plant species richness).

We tested the direct effects of climate change drivers and initial plant species richness on soil multifunctionality and C, P and N nutrient cycles, using a LMM that considered plant assemblages as a random factor. Note that we removed the interaction between climate change drivers and initial plant species richness as a predictor in our models because it did not explain additional variation. We conducted a Tukey post-hoc analysis to evaluate the differences among levels of climate change drivers or plant species richness, when significant responses were found. We then performed a variance partitioning analysis with all predictors (full model, without model simplification), climate change drivers, initial plant species richness, the functional structure of plant assemblages (CWT of selected traits and FD) and the attributes of microbial communities (abundance and diversity of bacteria and protists and interactions between the abundance and diversity of both bacteria and protists). We conducted this analysis to quantify the relative importance (unique portion of the variance) of each factor for soil multifunctionality and C, P and N nutrient cycles. Finally, we assessed different hypotheses to evaluate the responses of soil multifunctionality (and nutrient cycles) to different trophic levels. These included: model 1) climate change drivers and initial richness; model 2) previous variables and plant functional structure; model 3) previous variables and abundance and diversity of bacteria; model 4) previous variables and abundance and diversity of protists; and model 5) previous variables and interactions between the abundance and diversity of bacteria and protists. Then, we fitted all five models, and dropped terms that did not improve model fit, based on the Akaike information criterion (AIC) (Akaike, 1973), until the best model remained.

In addition to LMM analyses, we conducted a confirmatory path analysis using a d-sep approach (Shipley, 2013) to test direct and indirect causal relationships between climate change drivers, plant species richness, the functional structure of plant assemblages, the attributes of microbial communities and soil multifunctionality (Figure S1, Appendix 1, Table S6). This approach offers a flexible way to test causal relationships between variables in path analyses by relaxing some important limitations of standard structural equation models, including non-normal data distribution, non-linear relationships between variables and small sample sizes (Grace, 2006). This method is based on an acyclic graph that summarizes the hypothetical relationships between variables to be tested using the C statistic (Shipley, 2013). We selected the most appropriate predictors for soil multifunctionality using the stepAIC procedure described above. Plant assemblages were considered as random factors in all the models, except on those including soil multifunctionality as response variable. Finally, we used standardised path coefficients to measure the direct and indirect effects of predictors (Grace & Bollen, 2005). We also evaluated the effect of all these predictors on C, P and N nutrient cycles, instead of soil multifunctionality. All statistical analyses were conducted with R (R Core Team, 2016), using different R packages (see Appendix 1). Data are available on Figshare (Valencia et al., 2018).

Results

Our warming treatment increased air temperatures and reduced air relative humidity by 2.9 °C and 0.9% on average, respectively (Figure S6). Rainfall shelters decreased the amount of rainfall reaching the soil by an average 35%, varying between a minimum of 9% and maximum of 52% depending on the event (Figure S6). This treatment promoted a very slight increase in air temperatures of 0.1 ºC compared to the control, and a reduction in the

355 air relative humidity of 1.2% (Figure S6). Finally, the combination of rainfall shelters and
356 warming treatment promoted an average increase of 3.2 ºC in air temperature and an
357 average reduction of 2.2% in air relative humidity (Figure S6).
358
359 *Effects of climate change and plant species richness on ecosystem structure and functioning*
360 Climate change drivers and plant species richness largely affected the functional structure
361 of plant assemblages (Figure 1, Table S7). We found a significant interaction between plant
362 species richness and climate change drivers when evaluating CW-VH data (Table S7). We
363 observed that plant species richness was positively correlated with high CW-VH and high
364 FD in each of the climate change drivers evaluated (Figure 1). The analyses of the effects
365 of climate change drivers at each richness level showed that warming promoted a
366 significant increase of CW-VH, but only in mesocosms with high richness ($\chi^2 = 14.85$; $P =$
367 0.002). However, warming tended to decrease of CW-VH in mesocosms with low and
368 medium species richness (not significant relationship). Warming decreased FD regardless
369 of rainfall reduction, but this response was only significant in mesocosms with intermediate
370 richness ($\chi^2 = 11.10$; $P = 0.011$). CW-SLA did not respond to the experimental treatments
371 evaluated (Figure 1).
372 Rainfall reduction decreased the abundance of protists (Table S7). A significant
373 plant species richness × climate change drivers interaction was found when analysing
374 protist diversity data (Table S7). Thus, the combination of warming and rainfall reduction
375 increased protist diversity in mesocosms with low richness ($\chi^2 = 11.17$; $P = 0.011$), but
376 decreased protist diversity in mesocosms with high richness ($\chi^2 = 10.06$; $P = 0.018$). There
377 were no significant differences between bacterial abundance and diversity in any of the
378 treatments evaluated (Figure 1, Table S7).

Mesocoms with more plant species had increased soil multifunctionality and C cycling values ($\chi^2 = 7.43$; $P = 0.024$ and $\chi^2 = 12.08$; $P = 0.002$, respectively, see model 1 in Tables 1 and S8, Figure 2). Climate change drivers significantly affected P cycling ($\chi^2 = 7.83$; $P = 0.050$, Figure 2); a positive direct effect of warming plus rainfall reduction compared to control was observed when analysing this variable (Figure 2, Tukey post-hoc, $P = 0.057$).

Direct and indirect effects of climate change drivers, plants and soil microorganisms on soil multifunctionality

The quantification of the unique portion of the variance, using all predictors, showed that CW-VH, the abundance and diversity of bacteria and plant species richness were the variables with higher effects on soil multifunctionality (Figure 3). The sum of their unique effects explained the 37% of total variation in soil multifunctionality. Results obtained for variables from the C, N and P nutrient cycles were similar, but a higher contribution of CW-SLA and climate change drivers on the P cycle and protist abundance on the C cycle was observed (Figure 3).

We evaluated the responses of soil multifunctionality to the addition of different trophic levels. Remarkably, the best model included the combined effects of all trophic levels studied but without the interaction between bacterial and protist communities (model 4; Table 1). Additionally, for the nutrient cycles the best models included the combined effects of all trophic levels studied (model 4; Table S8a and S8c), except for the N cycle where the best model did not include protists (model 3; Table S8b). In the case of C and N cycling, models with the interaction between bacterial and protist abundances and diversity had almost the same AIC and higher R^2 than models without interaction (Table S8a and S8b).

The model accepted in the d-sep regarding direct and indirect effects on soil multifunctionality explained 68% of the total variation (Figure 4 and Table 1). Climate change drivers did not have a direct effect on soil multifunctionality (Figure 4 and Table 1). Our path analysis revealed that initial plant species richness had a positive direct effect on soil multifunctionality (Figure 4 and Table 1). However, these initial treatments had multiple indirect effects on soil multifunctionality via altering plant functional structure and soil microorganism community (Figure 4). For instance, climate change drivers and plant species richness affect CW-VH and FD, but higher CW-VH values also had a negative effect on FD. High CW-VH and low FD values were associated with higher soil multifunctionality. By contrast, both climate change drivers and plant species richness did not affect CW-SLA values, but increasing CW-SLA values increased soil multifunctionality (Figure 4). The effect of plant species richness and climate change drivers on bacterial communities was mediated by plant functional structure (Figure 4). Increases in bacterial abundance were linked to high CW-VH and low FD (plant functional structure), which in turn increased soil multifunctionality. Bacterial diversity had an opposite response to CW-VH and FD, and was associated with a negative effect on soil multifunctionality. Increases in protist abundance and diversity were mainly favoured by increases in bacterial abundance and diversity, respectively, and had a positive impact on soil multifunctionality. Results from C, N and P nutrient cycles were similar, but it is interesting highlight the significant positive effect of protist abundance on variables from the P cycle (Figure S7).

Discussion

Our study provides experimental evidence that the effects of diversity and simulated climate change on soil multifunctionality are largely mediated by changes in the attributes

429 of plant, bacteria and protist communities. We found that warming impacted soil
430 multifunctionility through a complex multitrophic cascading effect. For instance, in plant
431 species-rich communities, warming selected for larger, and more productive, plants that
432 increased the abundance of soil microorganisms, ultimately enhancing soil
433 multifunctionality. The joint consideration of a wide variety of factors beyond the climate
434 and biodiversity treatments employed, including functional structure of plant assemblages
435 and the abundance and diversity of soil bacteria and protists, improved significantly the
436 proportion of explained variance on soil multifunctionality (Figure 4), and provided novel
437 insights into the cascading mechanisms underlying the effects of key global change drivers
438 on this variable.
439
440 *Climate change and plant species richness alter the functional structure of plant*
441 *assemblages*
442 Higher initial plant species richness resulted in communities with higher community-
443 weighed vegetative height (CW-VH) and FD (Figures 1 and 2). This response could be
444 related to initial "sampling effect" (Tilman, Lehman, & Thomson, 1997), *i.e.* treatments
445 with higher species richness had higher probabilities of having keystone species.
446 Alternatively, this response may have occurs during the experiment and may reflect an
447 effect of biotic interactions (Gross et al., 2013). Since tall species are better competitors
448 than short species for space, the latter can be outcompeted. Both mechanisms are related
449 with CWT and are not exclusive. The relationship between CW-VH and richness at the
450 beginning of the experiment (equal abundances) was not significant ($F = 1.57$; $P = 0.225$),
451 suggesting that initial sampling effect is not the mechanism underlying the results observed.
452 The decrease in FD observed with warming (Figures 1 and 4) agrees with our first
453 hypothesis: climate change reduces plant diversity (Klein et al., 2004). Such result is not

surprising, but provides empirical evidence that some species could be wiped out under climate change scenarios (see also Reich, 2009; Isbell et al., 2015). Also, warming had contrasting effects depending on the species richness level considered. Warming promoted a decrease, although not significant, of CW-VH in mesocosms with one and three species. This result agrees with our expectations, since we hypothesized that temperature increase and rainfall reduction would promote conservative plant strategies and smaller plant size because plants with these traits are better adapted to arid or semiarid environments (Westoby et al., 2002), as we had in the experimental area. However, the positive significant relationship between CW-VH and warming in high plant species richness was contrary to our expectations. In our study, warming favored the large and most productive species, and also reduced FD, driven by the disappearance of the smaller species from the mesocosms. Overall, warming resulted in higher average height and lower variability in trait values at the community level. This finding indicates that warming may first shift plant communities by selecting larger species, that are able to better exploit resources when available and outcompete more tolerant species in mixtures. In natural communities, this process may leave available niches that could be occupied by other species, but our weeding treatment avoided this possibility.

These results highlight the importance of environmental filtering (Maire et al., 2012), as warming select species with a similar set of traits, suggesting that this mechanism was the main driver of plant functional structure. Our findings advance our undestanding on how grassland species might respond to ongoing warming. Future studies should also consider the role of warming in regulating these large species in earlier developmental stages (*i.e.* germination and seedling stage), as well as responses of other functional groups that, such as non-herbaceous perennial species or annual species, may differentially respond to climate change.

Cascading effects of plant species richness and climate change on soil microorganisms via functional structure of plant assemblages

Warming and rainfall reduction are known to affect the abundance of soil microorganisms through changes in soil temperature and moisture conditions (Castro, Classen, Austin, Norby, & Schadt, 2010; Maestre et al., 2015). However, these treatments did not alter bacterial abundance or diversity, and only slightly affected (*i.e.* explained a low amount of variance) that of protists (Figures 1 and 2). Similarly, the abundance and diversity of soil bacteria and protists did not respond to initial plant species richness (Figure 1). However, we observed that plant species richness and climate change drivers affected bacterial and protist communities via changes in the functional structure of plant communities (Figure 4). Mesocosms with higher plant community size (CW-VH) had higher bacterial abundance, but lower bacterial diversity. Increases in CW-VH are correlated with increases in aboveground plant biomass (Valencia, Méndez, Saavedra, & Maestre, 2016), which in turn increases root biomass and root exudation (Eisenhauer et al., 2017; Mellado-Vázquez et al., 2016). The bacterial communities on plant roots are mainly composed of copiotrophic taxa with reduced diversity compared to bulk soil (Bulgarelli, Schlaeppi, Spaepen, van Themaat, & Schulze-Lefert, 2013). Also, CW-VH had a positive significant relationship with organic carbon ($\chi^2 = 32.61$; $P = <0.001$), possibly because of increased amounts of litter material. High ammounts of organic matter are related with high total bacterial abundance and diversity (Delgado-Baquerizo, Maestre, Reich, Trivedi, et al., 2016; Maestre et al., 2015). However, other studies showed a positive relationship with total abundance of bacterial communities, but negative with diversity of soil bacteria, at least in soils with enough carbon content (Delgado-Baquerizo et al., 2017). This agrees with our results and, together with the negative relationship between bacterial abundance and

504 bacterial diversity observed, suggests an increase in the dominance of some bacterial
505 species/groups when CW-VH is higher, which overcompensates the decrease in the
506 abundance of other species. We propose that this process could be related to the competitive
507 exclusion principle, *i.e.* increases in the abundance of some species results in decreases or
508 even extinction of others (Eldridge et al., 2017). Additionally, increases in FD augmented
509 bacterial diversity, as found in other studies (Carney & Matson, 2005; Hendriks et al.,
510 2013). Higher plant FD may promote new niche spaces, increasing microclimate and
511 habitat variability, allowing the survival of more types of species. Finally, all these
512 cascading effects of plant community on bacterial communities also affected protists, as
513 increases in bacterial abundance and diversity were related to increases in protist abundance
514 and diversity, respectively (Figure 3). This result supports the tight coupling of protists to
515 their bacterial food source (Bonkowski, 2004).
516
517 *Direct and indirect effects of plant species richness and climate change on soil*
518 *multifunctionality*
519 We found a direct effect of initial plant species richness, but not of climate change drivers,
520 on soil multifunctionality (Table 1, Figure 3). Our results agree with studies finding
521 enhanced soil functioning with increasing plant species richness in drylands (Maestre et al.,
522 2012), and provide insights on the mechanisms underlying the interactive effects of
523 multiple trophic levels on soil multifunctionality. We found that the impact of plant species
524 richness and climate change drivers on soil multifunctionality were mostly indirect and
525 mediated by their effects on plant functional traits and soil microorganisms (Figure 4). As
526 found previously by field studies (Valencia et al., 2015), soil multifunctionality was driven
527 by changes in functional traits in our experiment. More specifically, plant communities
528 with larger plants, higher SLA, early flowering phenology and short leaves enhanced soil

multifunctionality (Figure 4, Table S3 and S4). These traits are associated to a fast-growing strategy (Reich, 2014, and also CSR scheme, Grime, 1977), characterized by rapid acquisition of water and other resources, as well as by the production of a dense litter layer (Quero, Gómez-Aparicio, Zamora, & Maestre, 2008), which in turn reduces losses in soil moisture due to evaporation (Holmgren, Gómez-Aparicio, Quero, & Valladares, 2012). These large species have higher water demands, but also obtain more resources than their neighbours, as they have extensive root systems, and under warming conditions they may outcompete smaller plant species. This process enhances the biomass and productivity of the mesocosms, but as we previously commented, decreases FD, especially under warming conditions. This result is contrary to our expectations because plant FD should enhance resource distribution between species and efficiency of resource use. Our results highlight the importance of the functional identity of dominant species over niche complementarity (Mokany, Ash, & Roxburgh, 2008), and how they affect the whole community among the different trophic levels.

Together, our results suggest that communities dominated by fast-growing plants, characterized by high SLA, and indirect cascading effects on soil microbial trophic networks might result in a higher soil multifunctionality. For instance, fast-growing plants promote bacterial production, via root exudation. This increases the release of N of bacteria consumed by protist grazers (microbial loop; Clarholm, 1985) and potentially the release of nutrients through priming of soil organic matter (Bonkowski & Clarholm, 2012), improving uptake of N by mycorrhiza (Koller, Rodriguez, Robin, Scheu, & Bonkowski, 2013) and further increasing plant growth. Also, soil drying could periodically kill significant amounts of microbial biomass, leading to larger nutrient pulses after rewetting. In this context, large and fast-growing plants could take faster advantage of these nutrients (*e.g.* dead microbial biomass).

554 Increases in plant FD and bacterial diversity were negatively related to soil
555 multifunctionality. Also, a higher abundance of soil bacteria and protists lead to higher soil
556 multifunctionality, regardless of their diversity. Then, the cascading effect between plant
557 and soil microorganisms and their feedbacks may promote higher sizes (biomass or
558 abundance) for the entire plant and soil microorganism community, ultimately promoting
559 soil multifunctionality. Finally, interactions between bacterial and protist communities did
560 not affect soil multifunctionality, but their inclusion increased the explained variance of N
561 cycle variables (even when the best selected model did not include them, Table S8b).
562 Despite these effects, we also detected a positive direct impact of warming and rainfall
563 reductions on P cycling variables (Figures 2 and S7). This positive effect can be related to
564 an increase in the activity of decomposers and microbes (Wolters et al., 2000).
565 Our results provide a strong evidence that climate change impacts on soil
566 multifunctionality are indirectly driven via changes in biotic attributes such as plant growth
567 rates and microbial abundance. Changes in plant functional structure promoted by warming
568 affected the microbial community and finally soil multifunctionality. Hence, grasslands
569 dominated by fast-growing plants (tall species with high SLA) favor a few groups of
570 bacteria, but these explode in numbers and have high turnover due to exudation and
571 constant protist predation, and finally could show higher levels of soil multifunctionality.
572 Higher bacterial and protist abundance positively impacted soil multifunctionality,
573 accelerating the decomposition of litter materials and increasing nutrient contents.
574 However, increases in plant and bacterial diversity promoted decreases in soil
575 multifunctionality. Overall, our results suggest that the trait values of the dominant plant
576 species, through their influence on other trophic groups, were the main driver of soil
577 multifunctionality in our experiment, and highlight the interest of evaluating multiple

trophic levels to increase our ability to explain complex ecosystem responses to climate change.

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Table 1. Results of the stepwise procedure to evaluate the effects of our experimental treatments, the functional structure of plant assemblages and microbial abundance and diversity on soil multifunctionality. We tested the responses of soil multifunctionality to the following models: 1) climate change drivers (warming and rainfall reduction, CCD) and initial richness, 2) plant functional structure, 3) bacterial abundance and diversity, 4) protist diversity and abundance, and 5) interactions between the abundance and diversity of bacteria and protists. Grey-filled cells indicate the variables that were not included in the models. CW-VH: Community Weighted mean of vegetative size, CW-SLA: Community Weighted mean of specific leaf area, Est: direction of relationship; DF: degrees of freedom.

		model 1			**model 2**			**model 3**			**model 4**			**model 5**		
AIC			-10.11			-75.37			-108.05			-112.12			-112.12	
Adjusted R^2			0.14			0.528			0.657			0.676			0.676	
	DF	Est	Fvalue	Pvalue	Est	Fvalue	Pvalue	Est	Fvalue	Pvalue	Est	Fvalue	Pvalue	Est	Fvalue	Pvalue
CCD	3		-	-		-	-		-	-		-	-		-	-
Richness	2		7.9	0.001		2.7	0.072		5.5	0.005		5.8	0.004		5.8	0.004
CW-VH	1				0.6	59.7	<0.001	0.3	10	0.002	0.3	11.5	0.001	0.3	11.5	0.001
CW-SLA	1				0.1	4.4	0.039	0.2	8.1	0.005	0.2	13.8	<0.001	0.2	13.8	<0.001
Functional diversity	1				-0.3	8.7	0.004	-0.2	5.6	0.02	-0.2	7.2	0.008	-0.2	7.2	0.008
Bacterial abundance (Ba)	1							0.3	15.7	<0.001	0.3	12.9	0.001	0.3	12.9	0.001
Bacterial diversity (Bd)	1							-0.3	16.4	<0.001	-0.3	16.8	<0.001	-0.3	16.8	<0.001
Protist abundance (Pa)	1										0.1	3	0.085	0.1	3	0.085
Protist diversity (Pd)	1										0.1	2.1	0.146	0.1	2.1	0.146
Ba:Pa	1													-	-	-
Bd:Pd	1													-	-	-

847 **List of Figures:**

848 **FIGURE 1.** Box plot showing the effects of climate change drivers (warming and rainfall
849 reduction) and plant species richness (one, three, and six plant species) on plant functional
850 structure (a, b, c), soil bacterial abundance (d) and diversity (e), and soil protist abundance
851 (f) and diversity (g). Significant differences among each treatment combination are
852 indicated by lowercase letters (Tukey post-hoc, $P < 0.05$). $n = 10$. CW-VH: Community
853 Weighted mean of vegetative size, and CW-SLA: Community Weighted mean of specific
854 leaf area.

855

856 **FIGURE 2.** Box plot showing the effects of climate change drivers (warming and rainfall
857 reduction) and plant species richness (one, three, and six plant species) on soil
858 multifunctionality (a) and similar indices calculated with nitrogen (b), phosphorus (c), and
859 carbon (d) cycling variables ($n = 10$).

860

861 **FIGURE 3.** Variance components showing the unique portion of variation (percentage of
862 total R^2) explained by each predictor on soil multifunctionality and variables from carbon,
863 nitrogen and phosphorus cycling. The predictors used are CCD (climate change drivers:
864 warming and rainfall reduction), plant species richness, community weighted mean of
865 vegetative size (CW-VH) and specific leaf area (CW-SLA), plant functional diversity,
866 abundance and diversity of bacterial and protist communities and the interactions between
867 them.

868

869 **FIGURE 4.** Relationships between climate change drivers, plant species richness, the
870 functional structure of plants, abundance and diversity of soil microorganisms and soil
871 multifunctionality. The width of each arrow is proportional to the standardized path

872 coefficients. Solid and dashed lines represent positive and negative effects, respectively.
873 CW-VH: Community Weighted Mean of vegetative size, CW-SLA: Community Weighted
874 Mean of specific leaf area, and FD: Functional diversity. Significance levels are as follows:
875 * $p < 0.05$ and ** $p < 0.01$.