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Title: Plant litter mixture partly mitigates the negative effects of extended drought on soil biota and litter decomposition in a Mediterranean oak forest

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Summary
1. A major challenge of current ecological research is to determine the responses of plant and animal communities and ecosystem processes to future environmental conditions. Ecosystems respond to climate change in complex ways, and the outcome may significantly depend on biodiversity.
2. We studied the relative effects of enhanced drought and of plant-species mixture on soil biota and on litter decomposition in a Mediterranean oak forest. We experimentally reduced precipitation, accounting for seasonal precipitation variability, and created a single-species litter (Quercus pubescens), a two-species litter mixture (Q. pubescens + A. monspessulanum) and a three-species litter mixture (Q. pubescens + A. monspessulanum + Cotinus coggygria).
3. In general, drier conditions affected decomposers negatively, directly by reducing fungal biomass and detritivorous mesofauna, but also indirectly by increasing the predation pressure on detritivorous mesofauna by predatory mesofauna. This is reflected under drier conditions in that Collembola abundance decreased more strongly than Acari abundance. One Collembola group (i.e. Neelipleona) even disappeared completely.
4. Increased drought strongly decreased litter decomposition rates. Mixed litter with two and three plant species positively affected soil biota communities and led to a more efficient litter decomposition process, probably through a greater litter quality. Faster decomposition in mixed litter can thus compensate slower decomposition rates under drier condition.
5. **Synthesis.** Our results highlights that, within our study system, drier climate strongly impacts on soil biodiversity and hence litter decomposition. Species-rich litter may mitigate such a decline in decomposition rates. Diverse plant communities should hence be maintained to reduce shifts in ecosystem functioning under climate change.

**Key-words:** climate change; drought; ecosystem functioning; food web; Mediterranean forest; nutrient dynamics; plant-soil interaction; prey-predatory interaction; soil mesofauna

**Introduction**

Human activities are altering the diversity of many of the World’s ecosystems (Millennium Ecosystem Assessment 2005; Butchart 2010) through numerous processes, one of which is climate change (Kerr, Kharouba & Currie 2007; IPCC 2014). Unravelling the likely response of communities and ecosystem processes to future environmental conditions is a major challenge of current ecological research and may help in adaptive ecosystem management (Weltzin 2003). As climate is one of the major drivers of plant species distribution, changes in environmental conditions are expected to induce shifts and modification of plant community structure (Parmesan 2006; Lenoir et al. 2008). However, besides the identity of species that drive ecosystem functions (Bardgett 2005), plant diversity plays a key role for resilience and vulnerability of ecosystems to environmental changes (Hooper et al. 2005). According to the Insurance Hypothesis (Yachi & Loreau 1999), more diverse plant communities are more likely to resist to stressful environmental conditions such as drought. Moreover, according to the Diversity-Stability Hypothesis (Tilman, Reich & Knops 2006), species-rich communities could have greater interspecific variation in responses to perturbation than species-poor communities. In the context of climate change and sustainable forest management, increasing tree richness has been proposed as a possible
strategy to reach both ecological functioning and productivity goals (Cavard et al. 2011). Forest managers have been showing increased interest in mixed forest stands dominated by two or three tree species (San-Miguel-Ayanz et al. 2011) thanks to their higher productivity compared to single-species stands (Vallet & Perot 2011), their better resistance to biotic disturbances (Jactel & Brockerhoff 2007) and their suspected better resilience to drought (Merlin et al. 2015).

Water availability is the most important environmental constraint in Mediterranean ecosystems, due to the combination of high temperatures and low rainfall during summer (Larcher 2000). Regional climate models predict an increase in both temperature and drought conditions in the Mediterranean region in the next coming years (Gibelin & Déqué 2003; Kirtman et al. 2013; Polade et al. 2014). These changes are expected to result in increased frequency, intensity and duration of drought, especially during the warm season (Dubrovsky et al. 2014). However, the potential effects of increased drought on Mediterranean ecosystem biodiversity and functioning are still poorly studied (Sardans & Peñuelas 2013; Frank et al. 2015).

Roughly 90% of global terrestrial plant production enters the dead organic matter pool (Cebrian 1999), making decomposition of plant material the dominant driver of carbon and nutrient cycling in ecosystems (Hobbie 1996; Aerts 1997; Gessner et al. 2010). This process is governed by environmental conditions (e.g. humidity, temperature and UV radiation), litter quality (i.e. chemical and physical characteristics of litter) and the decomposer community (i.e. composition and activity) (Couteaux, Bottner & Berg 1995; Cornelissen 1996; Gholz et al. 2000; Hättenschwiler, Tiunov & Schau 2005). Since climate,
litter quality and the decomposer community are closely linked, their interactions are important for decomposition and nutrient release in litter mixtures (Gartner & Cardon 2004).

In Mediterranean terrestrial ecosystems, short-term effects of a drier climate on decomposition may lead to a reduction of soil microbial biomass (Curiel Yuste et al. 2011), of soil respiration (Emmett et al. 2004; Asensio et al. 2007; De Dato et al. 2010) and of soil enzyme activities (Sardans & Peñuelas 2005; Hueso, Hernández & García 2011). Mid-term (i.e. a few decades) effects may impact on litter quality by reducing nutrient content (Wessel et al. 2004; Sardans et al. 2008) or by increasing recalcitrant compounds (Munné-Bosch & Alegre 2000; Hernandez, Alegre & Munné-Bosch 2004), and altering the composition of decomposer communities by feedback processes. Eventually, long-term effects of drought may result in a shift in plant species composition (Hobbie 1996; Bakkenes et al. 2002; Thuiller et al. 2005; Aerts 2006; Fortunel et al. 2009; Osanai et al. 2012), and thereby in quality and quantity of plant litter entering the dead organic matter pool and the soil food web.

The few studies of climate change effects on decomposer biota in Mediterranean ecosystems so far only focussed on microbial communities. Most of them reported only minor effects of drier conditions on these communities (e.g. Bérard et al. 2011; Curiel Yuste et al. 2011; Sherman et al. 2012), as for example fungi appeared to be quite resistant to drought (Curiel Yuste et al. 2011; Barnard et al. 2013). To our knowledge, no study has attempted to evaluate the effect of climate change on decomposition through changes in soil mesofauna (except Morón-Rios et al. 2000), despite the crucial role this biological component plays in the soil food web (Coleman, Crossley & Hendrix 2004). Detritivorous mesofauna affects directly the decomposition process through litter fragmentation and transport, including distribution of chemical compounds released from litter, but also indirectly by controlling abundance, diversity, activity and dispersion of microbial communities (Seastedt 1984; Beare
et al. 1992; Rusek 1998; Sackett, Classen & Sanders 2010). Furthermore, predatory mesofauna can affect indirectly the decomposition process through the predation pressure exerted on detritivorous mesofauna (Coleman, Crossley & Hendrix 2004; Donoso et al. 2013). These trophic interactions have a profound impact on mass balance of essential elements such as carbon and nitrogen during decomposition, with feedback on plant abundance, diversity and productivity (Chapman, Whittaker & Heal 1988; Kardol et al. 2006; Bardgett & Van der Putten 2014). However, as observed in other ecosystems, drier conditions could reduce the abundance and the species richness of soil mesofauna (Lindberg, Bengtsson & Persson 2002; Kardol et al. 2011) with cascading effects on the decomposition process and the overall soil food web.

Besides climate, the composition of the multi-species litter mixture of an ecosystem could alter litter decomposition (De Vries et al. 2012; Jiang, Yin & Wang 2013), but the magnitude and strength of litter-mixing effects depend on the structure and the type of the considered ecosystem (Hoorens, Aerts & Stroetenga 2003; Gartner & Cardon 2004; Quested et al. 2005). However, despite their huge diversity and the essential role that soil fauna plays in ecosystem processes, very few studies have focused on litter-mixing effects on soil fauna (Korboulewsky, Perez & Chauvat 2016) and, to our knowledge, no study has attempted to evaluate these effects in Mediterranean ecosystem, and still less with the potential interrelated litter-mixing and drought factors.

In this study, we experimentally manipulated both precipitation patterns (by extending the summer drought period) and plant litter diversity (through single- or multi-species litter mixtures) in a deciduous Mediterranean oak forest in order to determine the relative effect of these two factors on soil biota, including both microbial and mesofauna components, and litter
decomposition. We hypothesised a negative effect of increased drought period on soil biota (i.e. decrease of abundance and diversity) and on litter decomposition (i.e. decrease of leaf mass loss, carbon and nitrogen releases), as soil moisture has been reported to be the most constraining environmental condition for soil fauna in Mediterranean ecosystems (Tsiafouli et al. 2005; Morón-Rios et al. 2010; Kardol et al. 2011). Further, we hypothesised a positive effect of multi-species litter mixtures on soil biota (i.e. increase of abundance and diversity) and on litter decomposition (i.e. increase of leaf mass loss, carbon and nitrogen releases). Finally, we hypothesized that mixing of plant species can mitigate the effects of increased drought periods, with lower negative effect in multi-species litter mixtures compared to single-species litter.

Material and methods

Study site

This study was conducted in the Oak Observatory at the OHP (O3HP) experimental field site located in the research center “Observatoire de Haute Provence” (OHP), 60 km north of Marseille, France (43°56’115” N, 05°42’642” E). The soil is pierric calcosol (with S horizon between limestone rocks) or calcarisol when limestone appears less than 25 cm deep. pH is between 6.5 and 7.5 for A horizon and 7.5 for S horizon (Baize & Girard 1998). According to the WRB system (IUSS Working group WRB 2006) the soil can be classified as mollic leptosol.

The site is covered by a French Mediterranean natural old-growth oak forest belonging to the site Natura 2000 “FR9302008 Vachères”. It has been forested at least since the late 18th century (Hilaire et al. 2012) and has been managed for centuries by coppicing. 53 different plant species have been identified on the experimental core plot, where Downy oak (Quercus
pubescens Willd.: 75% coverage) and Montpellier maple (Acer monspessulanum L.: 25% coverage) are the two dominant tree species, with understory vegetation often dominated by European smoke bush (Cotinus coggyria Scop.: 30% coverage).

A rainout-shelter above 15 m × 20 m of canopy dynamically excluded precipitations by deploying automated shutters during rainfall events of the vegetation period (i.e. from spring to autumn). An adjacent plot without this device served as control plot. A network of sensors at different levels of the forest (including soil) allowed regular recording of environmental parameters (e.g. wind speed and direction, atmospheric pressure, precipitation, air temperature, global radiation, soil water content, soil temperature, soil conductivity).

The experiment was conducted during the years 2012 and 2013. Temperature in both plots during the study period varied from -11°C to 36°C, with a mean annual temperature of 12.7°C and 11.9°C for 2012 and 2013, respectively, similar to the local climate data recorded between 1960 and 2010 (WMO standard, temperature and precipitation, 1960-2003 St Michel l’Observatoire / Meteo France 04192001; 2003-2010 Dauphine / Meteo France 04068001 - on and close to the OHP, respectively). Precipitation in the control plot were 783 mm in 2012 and 896 mm in 2013 (annual mean was 830 mm over the 1967-2000 period). The device excluded 35% of precipitation in 2012 and 33.5% of precipitation in 2013 (Supplementary Fig. S1). From the ombrothermic diagram (P = 2T) (Supplementary Fig. S1), we identified a dry period when the monthly precipitation (mm) drops below two-times the mean monthly temperature (°C). For 2012, the dry period was around 3 months in the control plot (from early June to late August) and 4.5 months in the rain exclusion plot (from mid May to late September). For 2013, the dry period was only 0.5 month in the control plot (from mid May to early June) and reached 4.5 months in the rain exclusion plot (from mid May to late
September; i.e. the same than in 2012). Thereby the experimental device excluded around 35% of precipitation and the summer drought period was increased up to 4.5 months, in line with a typical climate scenario for the Mediterranean region predicting a 20-30% decrease of annual precipitation (Giorgi & Lionello 2008) and the extension of summer drought periods (Guiot & Cramer 2016).

**Experimental set-up and field procedures**

Freshly abscised leaves of *A. monspessulanum*, *C. coggygria* and *Q. pubescens* were collected from nearby the rain exclusion system over the whole period of maximum litter fall from October to November 2011 by using litter traps. Immediately after collection, the leaves were air-dried at room temperature and stored until the beginning of the experiment.

Leaf litter decomposition was studied over 24 months using the litterbag method (Swift, Heal & Anderson 1979). Ten grams (in equivalent dry weight) of fresh leaf litter were placed in a 4 mm-mesh litterbag (20 cm × 20 cm) with mesh size designed to allow colonization by microbes, soil mesofauna and macrofauna. The initial size of the different leaves was large enough to prevent litter loss through the mesh net, but some fragments could be lost after faunal activity. Three litter types, all containing *Q. pubescens*, were made with an equal partitioning on a dry mass basis among plant species: a single-species litter (*Q. pubescens* named *Quercus* thereafter), a two-species litter mixture (*Q. pubescens* + *A. monspessulanum* named *Quercus/Acer* thereafter) and a three-species litter mixture (*Q. pubescens* + *A. monspessulanum* + *C. coggygria* named *Quercus/Acer/Cotinus* thereafter). We used a nested experimental design with four blocks of 60 m² in each plot, control and rain exclusion, respectively. In each block, 18 pairs of adjacent replicate-litterbags (one bag being used for chemical and fungal biomass measurements and the other for mesofauna measurements) were randomly placed on soil (after removing the OL horizon) in February.
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After 4, 8, 12, 16, 20 and 24 months, 4 pairs of litterbags for each litter type were randomly collected. A total of 288 litterbags (3 litter types × 2 litterbags × 6 sampling dates × 4 replicates × 2 environmental conditions) were thus used for this experiment.

**Litter mass loss and moisture content**

Removed litterbags were sealed in plastic bags. In the lab, leaves were separated according to species, which was possible even with small fragments of leaves due to the marked morphological differences among species. The samples were then freeze-dried (Lyovac GT2®) and each leaf fragment was wiped clean individually before being weighed, ground and analysed, in order to prevent litter contamination by soil. Moisture content was calculated as ratio of dry mass to fresh mass, and litter mass loss was calculated as ratio of remaining dry mass to initial dry mass. We used dry mass data and not ash free dry mass data as we did not observe a significant effect of time of litter decomposition (three-way ANOVA; $F_{\text{time}} = 1.80, P = 0.17$) and environmental conditions (three-way ANOVA; $F_{\text{rain exclusion}} = 0.56, P = 0.46$) on the litter ash content.

**Chemical analysis**

Initial litter quality was determined for four samples of each litter type. Organic carbon (C) and total nitrogen (N) content of litter were determined by thermal combustion on a Flash EA 1112 series C/N elemental analyser (Thermo Scientific, USA). Lignin and cellulose concentrations were determined according to the van Soest extraction protocol (van Soest & Wine 1967) using a fiber analyser (Fibersac 24; Ankom, Macedon, NJ, USA) (Hättenschwiler et al. 2008). Total folin phenolics were measured colorimetrically by using gallic acid as a standard (Peñuelas et al. 1996). A 0.25 g litter sample was dissolved in 20 ml of a 70% aqueous methanol solution, shaken for 1 h and then filtered (0.45 μm filter); 0.25 ml
of filtered extract was mixed with 0.25 ml Folin-Ciocalteu reagent (Folin & Denis 1915), 0.5 ml of saturated aqueous Na₂CO₃ (to stabilize the color reaction) and 4 ml of distilled water. After 60 min, the reaction was completed, and concentration of phenolics was determined at 765 nm on a UV/Vis spectrophotometer (Thermo Scientific, USA). To determine the water holding capacity (WHC) of initial litter, 4 samples of each litter type were soaked in distilled water for 24 h, drained and weighed. The dry weight was determined after drying samples at 60 °C for 48 h. WHC was calculated as (moist weight / dry weight) × 100%.

**Fungal biomass**

Fungal biomass was determined by quantifying ergosterol, which is a fungal membrane constituent and thus a good indicator of living fungal biomass (Gessner & Chauvet 1993; Ruzicka et al. 2000). Ergosterol was extracted from 50 mg of litter with 5 mL of an alcohol base (KOH/methanol 8 g l⁻¹) for 30 min and purified by solid-phase extraction on a Waters® (Milford, MA, USA) Oasis HLB cartridge (Gessner & Schmitt 1996). The extract produced was purified and quantified by high-performance liquid chromatography (HPLC) on a Hewlett Packard series 1050 system running with HPLC-grade methanol at a flow rate of 1.5 ml min⁻¹. Detection was performed at 282 nm, and the ergosterol peak was identified based on the retention time of an ergosterol standard.

**Mesofauna extraction and identification**

Mesofauna was extracted from fresh litter using the Tullgren funnel method for ten days (Berlese 1905). Arthropods collected were stored in 95% ethanol, counted using a binocular scope and separated in 4 different morphological groups for Collembola (Entomobryomorpha, Neelipleona, Poduromorpha and Symphypleona) and in different
suborders for Acari (Oribatida, Mesostigmata and Prostigmata) (Gisin 1960; Hopkins 1997; Jansen 2010). Collembola and Acari Oribatida were regarded as detritivorous mesofauna, whereas Acari Mesostigmata and Prostigmata as predatory mesofauna (Coleman, Crossley & Hendrix 2004; Donoso et al. 2013).

**Data analysis**

Statistical analyses were performed using a combination of univariate and multivariate techniques with R software (R version 3.3.1 - 2016.06.21). Significance was evaluated in all cases at $P < 0.05$. Normality and homoscedasticity of the data were first checked using Ryan–Joiner and Levene tests, respectively. When conditions were not met, data were ln-transformed before performing statistical tests. The experimental design included three fixed factors: the decomposition time (6 sampling dates: 4, 8, 12, 16, 20 and 24 months), the litter type (3 types: *Quercus*, *Quercus/Acer* and *Quercus/Acer/Cotinus*) and the environmental conditions (2 conditions: control and rain exclusion plots). The experimental design also included the four blocks within each environmental condition (control or rain exclusion plot) as a random factor in order to take into account the site heterogeneity.

**Fungi and mesofauna**

Three-way analyses of variance (ANOVA), followed by Tukey test for post hoc pairwise comparisons, were used to test the effects of time (T), litter type (L) and rain exclusion treatment (E) on fungal biomass and mesofauna parameters (detritivore and predator abundances; detritivore/predator ratio; Shannon diversity index). We used nested ANOVAs with considering the four blocks as nested within corresponding environmental conditions (control or rain exclusion plot), and blocks were treated as a random factor in order to take into account the site heterogeneity. If any interaction occurred, we performed
separated one-way ANOVAs to test the effects of time, litter type and environmental condition on soil biota parameters. Relationships between fungal biomass, detritivore and predator abundances were tested using Ordinary Least Square regression (OLS).

In order to compare structure of mesofauna communities, in terms of both presence and abundance of the different groups of arthropods, among litter types and environmental conditions, for each sampling date, a data matrix of pairwise comparisons among samples was then calculated using the Bray-Curtis distance index, which ranges between 0 and 1 (Bray & Curtis 1957). Prior to the analysis, data were square-root transformed and then standardized using a Wisconsin double standardization. Non-metric multidimensional scaling (NMDS) was used to find the best low-dimensional representation of the distance matrix using the package vegan (Oksanen et al. 2013). The null hypothesis of no difference in patterns of mesofauna composition among litter types, between control and rain exclusion plots and among sampling dates was tested with a permutational multivariate analysis of variance (PERMANOVA) in the package vegan (Oksanen et al. 2013). PERMANOVA is a permutation-based version of the multivariate analysis of variance, nonparametric (in the case of one-factor models) and thus with no assumption regarding the distributions of the original variables (Anderson 2001). However, PERMANOVAs are very sensitive to heteroscedasticity (Warton, Wright & Wang 2012). Therefore prior to analysis, homoscedasticity was ensured using a multivariate analogue of Levene’s test for homogeneity of variance. PERMANOVAs were run on the Bray-Curtis distance index (Bray & Curtis 1957) with 1000 permutations per analysis, first for the global dataset and then for each sampling date.
Initial litter quality and decomposition process

Differences in initial litter characteristics of the three litter types were assessed using separated one-way ANOVAs, followed by Tukey tests for post hoc pairwise comparisons, for each litter parameter. Principal Component Analysis (PCA) involving all 8 litter characteristics was applied to explore the variability in litter quality within the three litter types.

Three-way ANOVAs, followed by Tukey test for post hoc pairwise comparisons, were used to test the effects of time (T), litter type (L) and environmental conditions (E) on litter mass loss, carbon, nitrogen and phenolic releases. We used nested ANOVAs with considering the four blocks as nested within corresponding environmental condition (control or rain exclusion plot), and blocks were treated as a random factor in order to take into account the site heterogeneity. If any interaction occurred, we performed separated one-way ANOVAs to test the effects of time, litter type and environmental condition on decomposition parameters.

Relationships between litter quality, rainfall regime, soil biota and decomposition process

Significant results from ANOVA were linked using a priori knowledge in a path analysis model in order to evaluate the impacts of litter type and rainfall exclusion on soil biota parameters and linkages with litter decomposition process. Path analysis represents a particular case of structural equation modeling (SEM) involving only measured variables, where SEMs are probabilistic models that can include multiple predictors and response variables in a single causal network. SEM provides a means of representing complex hypotheses about causal networks, accounting for factors that are both causes and effects, and testing for model data consistency while accounting for the unavoidable colinearities among the explanatory variables (Grace 2006). The path analyses were conducted in R using the
The *lavaan* package (Rosseel 2012). Based on an a priori knowledge, we developed an initial conceptual model that was both consistent with our data and which made biological sense (Supplementary Fig. S4). We first created four conceptual groups of measured variables, which were measured in the present study. These conceptual groups represented (i) litter quality, (ii) environmental condition, (iii) three variables related to soil biota and (iv) two variables related to litter decomposition process. The difference in litter quality was based on ordination axis derived from Principal Coordinates Analysis of litter characteristics data for each litter type (Supplementary Fig. S3; PCA axis 1 explaining 69.19% of variation). The difference in environmental conditions was based on mean annual summer drought period in both control and rain exclusion plots. The three variables related to soil biota included fungal biomass, abundance of detritivore mesofauna and abundance of predator mesofauna. The two variables related to litter decomposition process included carbon and nitrogen losses. The full model was simplified by stepwise exclusion of non-significant variables (either by weights or covariance) as estimated by AIC (Akaike Information Criterion), until a minimal adequate model was reached (Milcu et al. 2013). The adequacy of the model was determined by non-significant differences between the predicted and observed covariance matrices ($\chi^2$ tests, $p > 0.05$), low root mean squared error of approximation index (RMSEA < 0.1), high Tucker-Lewis index (TLI > 0.90) and high comparative fit index (CFI > 0.90) (Grace 2006; Rosseel 2012). The normal distribution and homogeneity of variances of the residuals of each component model of the global path models were checked.

Finally, we also performed Pearson correlations to link each initial litter characteristic of three litter types with soil biota and litter decomposition parameters after 24 months of litter decomposition.
Results

**Fungal biomass**

Fungal biomass was affected by time of decomposition, litter mixture and environmental conditions (Table 1; Fig. 1). Fungal biomass was on average 23% lower in drier condition compared to control condition (Table 1; Fig. 1), for all the sampling dates except for 12 months of decomposition (Table 1, time × rain exclusion interaction; Fig. 2). Fungal biomass was generally higher in the multi-species litter mixtures compared to *Quercus* litter alone (Table 1; Fig. 1). However, fungal biomass could increase with the number of plant species but this effect depends on the decomposition time (Table 1, time × litter type interaction; Fig. 2).

**Mesofauna abundance and diversity**

We collected a total of 38,024 arthropods in the litterbags during the experiment (Supplementary Table S1). Acari dominated the litter mesofauna, and composed 75% of the total number of individuals collected. Oribatida were the most abundant arthropods found across all the samples (46%), following by Mesostigmata (18%) and Entomobryomorpha (18%).

Detritivore abundance was 55% lower and predator abundance was 37% lower under rain exclusion condition compared to control condition (Table 1; Fig. 1). While mesofauna abundances increased gradually during time of decomposition, these increases were less pronounced in drier condition, with sharp decreases after 16 months corresponding to summer drought period (Table 1, time × rain exclusion interaction; Fig. 2). Mesofauna abundances were generally higher in the multi-species litter mixtures compared to *Quercus* litter alone (Table 1; Fig. 1). However, mesofauna abundances could increase with the number of plant species but this effect depends on the decomposition time and environmental condition (Table
1, time × litter type interaction and litter type × rain exclusion interaction; Fig. 2). Detritivores to predators ratio was not influenced by the number of plant species in the litter mixture (Table 1) but was negatively affected by the rain exclusion treatment, from 3.2 ± 0.2 under control condition to 2.2 ± 0.1 under rain exclusion condition.

Shannon diversity index of mesofauna community varied according to time of decomposition (Table 1) with generally an increase of diversity after 8 (H’ = 1.49 ± 0.03) and 20 (H’ = 1.43 ± 0.03) months of litter decomposition (corresponding to Autumn, Supplementary Fig. S1) and a decrease of diversity after 12 (H’ = 1.26 ± 0.02) and 24 (H’ = 1.23 ± 0.02) months of litter decomposition (corresponding to Spring, Supplementary Fig. S1). Shannon diversity index was higher in the two multi-species litter mixtures (H’ = 1.32 ± 0.02) compared to *Quercus* litter alone (H’ = 1.24 ± 0.04) (Table 1), and decreased in drier condition compared to control condition only after 16 (H’ = 1.34 ± 0.03 and H’ = 1.41 ± 0.04, respectively) and 20 (H’ = 1.37 ± 0.02 and H’ = 1.49 ± 0.04, respectively) months of litter decomposition (Table 1, time × rain exclusion interaction).

Mesofauna community structure was also strongly affected by time of decomposition (Pseudo-*F* = 76.39, *P* < 0.001), litter type (Pseudo-*F* = 9.34, *P* < 0.001) and rain exclusion treatment (Pseudo-*F* = 2.17, *P* < 0.05). Concerning rain exclusion, community structure was clearly different in control and rain exclusion plots at all sampling dates (Supplementary Fig. S2). This difference was particularly acute during rain exclusion period (summers 2012 and 2013 but also autumn 2013 when rain exclusion period was extended until the end of October, Supplementary Fig. S1; Supplementary Fig. S2). In addition, community structure differed significantly among litter types in both plots after 4, 8 and 24 months (time × litter type interaction, Pseudo-*F* = 3.23, *P* < 0.001; Supplementary Fig. S2). Finally, after 20 months,
litter mixture effect observed in control plot disappeared under rain exclusion and mesofauna community in rain exclusion plot, whatever the type of litter, was similar to mesofauna community associated to *Quercus* litter alone in control plot (time × rain exclusion interaction, Pseudo-$F = 15.54$, $P < 0.001$; Supplementary Fig. S2).

A positive relationship was observed between the abundance of detritivorous mesofauna and the fungal biomass under both control (Fig. 3a) and rain exclusion conditions (Fig. 3b). In control plots, we observed higher potential grazing pressure (i.e. higher detritivore abundance for a same fungal biomass) in multi-species litter mixtures compared to *Quercus* alone (Multiple-slopes comparison, $P < 0.0001$; Fig. 3a). In rain exclusion plots, we observed higher potential grazing pressure in *Quercus/Acer/Cotinus* litter-mixture compared to *Quercus* alone and *Quercus/Acer* litter-mixture (Multiple-slopes comparison, $P < 0.0001$; Fig. 3b). There was also a positive relationship between abundances of detritivorous and predatory mesofauna under both control (Fig. 3c) and rain exclusion conditions (Fig. 3d). We observed higher predation pressure (i.e. higher predator abundance for a same detritivore abundance) in *Quercus* alone compared to the multi-species litter mixtures in control plots (Multiple-slopes comparison, $P < 0.0001$; Fig. 3c) and a similar predation pressure across the three litter types in rain exclusion plots (multiple-slopes comparison, $P > 0.05$; Fig. 3d).

**Initial litter quality and decomposition process**

Except for organic carbon, initial litter characteristics varied among the three litter types (Table 2). The first axis of the PCA (Supplementary Fig. S3), explaining 69.19% of variation in litter quality throughout the gradient *Quercus/Acer/Cotinus* > *Quercus/Acer* > *Quercus*, was strongly negatively correlated with WHC (Pearson’s $r = -0.89$; $P < 0.001$), phenolics (Pearson’s $r = -0.86$; $P < 0.001$) and N (Pearson’s $r = -0.72$; $P < 0.001$), but
positively correlated with lignin (Pearson’s $r = 0.97$; $P < 0.001$), cellulose (Pearson’s $r = 0.98$; $P < 0.001$), lignin/N ratio (Pearson’s $r = 0.99$; $P < 0.001$) and C/N ratio (Pearson’s $r = 0.75$; $P < 0.001$).

During the decomposition process, litter mass loss increased through time and with the number of plant species and decreased with rain exclusion (Table 3; Fig. 4). After 24 months, *Quercus* litter mass loss was 13% higher in *Quercus/Acer* litter mixture and 18% higher in *Quercus/Acer/Cotinus* litter mixture in control condition compared to the *Quercus* litter alone (Table 3; Fig. 4). This positive litter mixture effect was only preserved in *Quercus/Acer/Cotinus* litter mixture in the rain exclusion condition with 16% higher litter mass loss compared to the singly *Quercus* litter (Table 3, mixture × rain exclusion interaction; Fig. 4).

In general, carbon and phenolics release increased with time and the number of plant species in litter mixture (Table 3; Fig. 4). Rain exclusion treatment induced a delay in phenolics release during the first 8 months of decomposition in the 3 litter types, and this delay persisted after 20 and 24 months only for the singly *Quercus* litter (Table 3, time × rain exclusion interaction; Fig. 4). N release increased with the number of plant species in litter mixture and was lower on drier condition compared to control condition (Table 3; Fig. 4). N immobilization in control plot was reduced with the increase of the number of plant species in the litter mixtures with 8, 4 and 0 months of immobilization in *Quercus* litter, *Quercus/Acer* and *Quercus/Acer/Cotinus* litter mixtures, respectively (Fig. 4). Rain exclusion treatment increased the time of N immobilization in *Quercus* litter and *Quercus/Acer* litter mixture reaching 16 and 8 months, respectively (Fig. 4).
We investigated the effects of litter quality and rainfall on fungal biomass and mesofauna (detritivores and predators) abundance and their impacts on C and N releases after 24 months of litter decomposition. The fitting parameters of the minimal adequate path analysis model (SEM) were excellent ($\chi^2 = 0.45$, RMSEA = 0.00, TLI = 1.03, CFI = 1.00; Fig. 5), and the model explained 88% and 82% of the variance in C and N releases, respectively. We identified the increase of litter quality as important driver affecting positively fungal biomass and detritivore abundance (Supplementary Table S2 for more details). On the opposite, the decrease of rainfall was an important driver affecting negatively fungal biomass and detritivore abundance. Detritivore abundance appeared to be more sensitive (i.e. higher path coefficient) to change in litter quality and rainfall regime than fungal biomass. Greater fungal biomass was associated with increased detritivore abundance. Interestingly, predator abundance was not directly affected by the increase of litter quality or the rainfall decrease, but was strongly associated to the detritivore abundance. C and N releases increased with increasing fungal biomass and detritivore abundance. Detritivore abundance showed a higher contribution on C and N releases than fungal biomass. The model indicated strong indirect effects of litter quality and rainfall decrease on C and N releases via changes in fungal biomass and detritivore abundance, but also a weak direct negative effect of rainfall decrease on C and N releases.

Discussion

Our main findings are that (i) increasing the drought period decreased strongly soil biota abundance and diversity, increased potential predation pressure on detritivorous mesofauna and reduced the decomposition efficiency; (ii) the multi-species litter mixtures improved decomposer community structure and decomposition efficiency; (iii) the negative
effects of increased drought period on soil biota and decomposition were mitigated by the mixing of plant species in the litter.

**Increased drought period negatively affects the soil food web and litter decomposition**

In the present study, increasing the drought period reduced fungal biomass and detritivorous mesofauna abundance, but also altered the community structure of mesofauna associated with decomposing leaves. Our result of reduced fungal biomass in rain exclusion plot contrasts with most other previous studies in Mediterranean ecosystems that observed an absence or only minor effects of drier conditions on soil fungal communities (Wilkinson *et al.* 2002; Curiel Yuste *et al.* 2011; Sherman *et al.* 2012; Curiel Yuste *et al.* 2014). As suggested by Curiel Yuste *et al.* (2014), Mediterranean fungal communities are probably well adapted to the rather dry conditions that regularly occur during the summer hot season, compared to fungal communities in non-Mediterranean ecosystems without this drastic climatic conditions. This discrepancy is probably related to the Mediterranean ecosystem type that could respond distinctly to precipitation change. Indeed, the fungal communities from our downy oak forest could be more sensitive to drier conditions than fungal communities from Mediterranean shrubland (Curiel Yuste *et al.* 2011; Sherman *et al.* 2012), holm oak forest (Curiel Yuste *et al.* 2011, 2014) or pine forest (Wilkinson *et al.* 2002) as rainfall in our downy oak forest in the bioclimatic supramediterranean stage can be naturally higher compared to bioclimatic zones usually observed of shrublands of pine forests (Quézel & Médail 2003). However, we could not exclude that this discrepancy of results could be also related to different experimental setups, taking into account rain exclusion treatment (our experiment and Curiel Yuste *et al.* 2011, 2014), irrigation treatment (Wilkinson *et al.* 2002) or climatic gradient (Sherman *et al.* 2012), but also distinct experimental duration (ranging from 4 months (Wilkinson *et al.* 2002) to 10 years (Curiel Yuste *et al.* 2011, 2014)).
Previous studies on different ecosystems reported that drought treatment reduced abundance and species richness of soil arthropods (Lindberg, Bengtsson & Persson 2002; Hagvar & Klanderud 2009; Kardol et al. 2011; Xu et al. 2012). In our study, the effect of drier condition on soil mesofauna was more pronounced compared to the effect on fungal biomass and varied over time and according to the mesofauna group. However, Acari seem more adapted to increased summer drought period in this Mediterranean ecosystem than Collembola. These shifts leaded to an increase of Acari dominance through the mesofauna communities under drier conditions. As previously observed by Lindberg, Bengtsson & Persson (2002) in a temperate coniferous forest subjected to a decrease in summer precipitation, soil mesofauna communities could change towards a dominance of more drought-resistant species, and drier climate could induce the local extinction of some species. For example, Neelipleona completely disappeared in the rain exclusion plot. This group of Collembola is known to be highly sensitive to desiccation, partly due to their morphology facilitating water exchanges with the external environment (Jansen 2010). Loss of Neelipleona may induce the loss of associated functions in decomposition. Indeed, the strong sensitivity of detritivorous to drought can explained the decrease of C and N releases during the decomposition in the rain exclusion plot, as detritivores appeared to be important drivers of C and N losses in the present study.

Predator abundance was not directly affected by the rain exclusion treatment but was strongly dependent to the detritivore abundance that was directly affected by the rain exclusion. Drought also altered the relative importance of predatory and detritivorous mesofauna, modifying the trophic structure of the soil food web. Indeed, Mesostigmata and Prostigmata are recognized as predators of Collembola and Oribatida (Coleman, Crossley & Hendrix 2004; Donoso et al. 2013). Acari were more resistant compared to Collembola, but
Acari predators were also more resistant compared to Acari detritivores. The abundance of detritivores was reduced by 48% under rain exclusion condition whereas the abundance of predators was only reduced by 26%, leading to a change in the detritivore/predator ratio from 3.2 to 2.2. This indicates that predation pressure was potentially increased in drier conditions.

Predatory arthropods could impact indirectly the decomposition process, but contradictory results are recorded in the literature depending on the ecosystem considered. Previous studies reported that predators increased decomposition in temperate forest (Lawrence & Wise 2004) and decreased decomposition in temperate grassland ecosystem (Kajak 1991). In our Mediterranean forest, we did not observe a direct effect of predators on decomposition, but the high dependence of the predators on detritivores whatever the conditions and a higher predation pressure on detritivorous mesofauna under drier conditions could explain the reduced litter decomposition by detritivorous mesofauna (e.g. fungal grazing and litter fragmentation; Bengtsson, Hedlund & Rundgren 1993; Hasegawa & Takeda 1995; Cragg & Bardgett 2001). This result is in accordance with findings from Lensing & Wise (2006) in temperate forest subjected to reduced precipitation, and points out the consequences of change in soil food web under drier condition for the litter decomposition process.

Drier climatic conditions directly negatively affected litter mass loss and carbon release. These findings are in line with previous studies demonstrating lower decomposition rates under drier conditions (Lensing & Wise 2007; Wieder, Cleveland & Townsend 2009; Saura-Mas et al. 2012; Vogel et al. 2013; Walter et al. 2013; Santonja et al. 2015a). We also observed lower N release and higher N immobilization duration under drier climatic conditions. Finally, phenolics release was delayed in rain exclusion condition, which could
delay litter colonization by mesofauna (Chomel et al. 2014) and then decomposition (Asplund, Bokhorst & Wardle 2013).

**Litter mixture positively affects soil biota and litter decomposition and mitigates negative drought effects**

Our results demonstrated a strong link between litter type and soil biota, since abundance, biomass, and diversity of decomposers changed with the presence of *Acer* and *Cotinus*. Here, presence of *Acer* and *Cotinus* in the litter mixtures increased the fungal biomass and both mesofauna abundance and diversity, suggesting an important role of these two plant species in structuring the soil food web of the downy oak forest. We could hypothesize that litter mixtures with diverse plant species increased the variety of substrates and microhabitats and thus enhanced the complexity of decomposers community (Hansen & Coleman 1998; Milcu et al. 2006; Wardle 2006). Moreover, the presence of *Acer* and *Cotinus* in the litter mixture with *Quercus* decreased the C/N and the lignin/N ratios and increased water-holding capacity. This increase of litter quality had a significant positive effect on decomposers, especially abundance and diversity of detritivores, in accordance with previous studies pointing out the increase of the abundance and diversity of soil organisms with higher litter quality (Hansen & Coleman 1998; Berg & McClougherty 2003; Hättenschwiler, Tiunov & Scheu 2005). We found a positive correlation with initial phenolic content and soil biota, contrary to what we observed for other studies (Hättenschwiler et al. 2005; Chomel et al. 2014). The presence of *Acer* and *Cotinus* increased the amount and the diversity of phenolics and could have acted as carbon source for soil organisms (Blum & Shafer 1988; Souto, Chiapusio & Pellissier 2000). We found a positive relationship between fungal biomass and detritivorous mesofauna, as most detritivorous Collembola and Oribatida are fungivorous and may feed selectively on different fungal species (Kaneko, McLean & Parkinson 1995). In
addition, as the litter mixing positive effect is higher on the detritivore abundance than on the fungal biomass, the grazing pressure on fungi might have increased from mono- to tri-specific litter mixtures. The predation pressure was likely highest in pure *Quercus* litters, given the low abundance of detritivores and high abundance of predators. Overall, mixing multiple litter species could then influence the relative abundance of different trophic levels and might hence affect the soil food web (Chapman & Newman 2010). In addition, the observed positive relationships between the number of plant species in the mixture and the diversity of mesofauna supports the diversity cascade hypothesis (Wardle *et al.* 2006; Chapman & Newman 2010) in Mediterranean forest. Previous studies carried out in boreal, temperate or tropical forest ecosystems found positive, negative or no plant mixture effect on soil mesofauna (Cavard *et al.* 2011; Korboulewsky, Perez & Chauvat 2016). Our study, the first concerning a Mediterranean forest ecosystem, reveals a positive effect of plant mixture on the diversity and the abundance of soil mesofauna.

The positive litter mixing effect on decomposers affected in turn positively the decomposition, with a greater litter mass loss and nutrient release from the multi-species litter mixtures containing *Acer* and *Cotinus*. This finding is in line with previous studies that observed these relationships between litter characteristics and litter decomposition rate (Wardle, Bonner & Barker 2002; Makkonen *et al.* 2013; Santonja *et al.* 2015b), but we evidenced that the control of litter decomposition rate through litter mixture can be mediated by decomposers, especially detritivorous mesofauna, in the downy oak Mediterranean forest. The improvement of the nitrogen release in multi-species litter mixtures is of special concern in Mediterranean ecosystems where nitrogen is recognized to be a frequent limiting nutrient (Sardans & Peñuelas 2013).
In accordance with our third hypothesis, positive effects of multi-species litter mixture persisted under rain exclusion condition, especially for the Quercus/Acer/Cotinus mixture. In fact, fungal biomass was two times less affected by rain exclusion in multi-species litter mixtures compared to Quercus litter decomposing alone. We observed a higher negative rain exclusion effect on predator abundance in multi-species litter mixtures compared to Quercus litter decomposing alone. Also, our results suggest that the presence of Acer and Cotinus allowed maintaining a greater nitrogen release compared to Quercus single-species litter under drier condition: we observed a nitrogen release reduction of 43% for Quercus singly contrary to 34% for the two- and 22% for the tri-species litter mixtures. Moreover, N immobilization in the litter mixtures is reduced by a factor two or totally disappeared with the presence of Acer or Acer and Cotinus, respectively, ensuring a continuous N release during the decomposition process. Contrary to Vogel et al. (2013) who did not observe any buffer effect of plant diversity on decomposition process under experimental rain exclusion treatment in temperate grassland, we observed a drought effect mitigation in litter mixtures in Mediterranean forest. In fact, positive litter mixing effects on soil biota and litter decomposition efficiency were maintained under drier conditions and negative drought effect were also reduced in litter mixtures contrary to Quercus litter alone. Thus, diverse plant communities should hence be maintained to reduce shifts in Mediterranean forest functioning under climate change.

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**Author’s contributions**

MS, CF, TG and VB designed the research; MS, CF and VB conducted the research; MS, CF, MP, CG, TG and VB collected and analyzed the data; MS, CF, MP, CG, TG, IR, WC and VB wrote the manuscript.

**Data accessibility**

All data are included in the manuscript and supporting information.

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### Tables

**Table 1.** Results of analysis of variance for the effects of time (T), litter type (L) and rain exclusion treatment (E) on soil biota parameters.

<table>
<thead>
<tr>
<th></th>
<th>Fungal biomass</th>
<th>Detritivore abundance</th>
<th>Predator abundance</th>
<th>Detrit./Pred. ratio</th>
<th>Shannon H'</th>
<th>Index</th>
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<tbody>
<tr>
<td></td>
<td>df</td>
<td>F-ratio</td>
<td>P-value</td>
<td>F-ratio</td>
<td>P-value</td>
<td>F-ratio</td>
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<td>0.64</td>
<td>0.7012</td>
<td>1.83</td>
<td>0.1010</td>
<td>0.60</td>
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<tr>
<td>Time (T)</td>
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<td>201.65</td>
<td>&lt;0.0001</td>
<td>46.54</td>
<td>&lt;0.0001</td>
<td>57.87</td>
</tr>
<tr>
<td>Litter type (L)</td>
<td>2</td>
<td>42.64</td>
<td>&lt;0.0001</td>
<td>25.04</td>
<td>&lt;0.0001</td>
<td>16.18</td>
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<td>Rain exclusion (E)</td>
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<td>138.43</td>
<td>&lt;0.0001</td>
<td>118.22</td>
<td>&lt;0.0001</td>
<td>38.37</td>
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<tr>
<td>T x L</td>
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<td>2.13</td>
<td>0.0287</td>
<td>4.44</td>
<td>&lt;0.0001</td>
<td>2.79</td>
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<tr>
<td>T x E</td>
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<td>2.58</td>
<td>0.0304</td>
<td>11.49</td>
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<tr>
<td>L x E</td>
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<td>0.8679</td>
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<td>0.59</td>
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Table 2. Main initial leaf litter characteristics of the three litter types. Values are mean ± standard error (SE). C = Carbon content, N = Nitrogen content, WHC = Water holding capacity. Separated one-way Anovas were performed to test the effects of litter type on initial litter characteristics. Different letters denote significant differences among litter types with a < b < c.

Table 3. Results of the analysis of variance for the effects of time of decomposition (T), litter type (L) and rain exclusion treatment (E) on litter decomposition parameters.
Figures

**Fig. 1.** Effects of (a, d, g) time of decomposition, (b, e, h) litter type and (c, f, i) environmental condition on (a, b, c) fungal biomass, (d, e, f) detritivore abundance and (g, h, i) predator abundance. Values are mean ± SE. Separated one-way ANOVAs were performed to test the effects of time, litter type and environmental condition on fungal biomass and detritivore and predator abundances. Different letters denote significant differences among time of decomposition, litter types or environmental conditions with a < b < c < d < e. DM = Dry mass of litter mixture. Q = *Quercus pubescens*, A = *Acer monspessulanum* and C = *Cotinus coggygria*.

**Fig. 2.** Dynamics of (a) fungal biomass, (b) detritivore abundance and (c) predator abundance associated with decomposing leaves in the three litter types and in the control and the rain exclusion plots. Values are mean ± SE per date time of litter decomposition. Separated one-way ANOVAs were performed for each sampling period in both control and rain exclusion plots to test the effects of litter type on fungal biomass and mesofauna abundances. Different letters denote significant differences among litter types with a < b < c. DM = Dry mass of litter mixture. Q = *Quercus pubescens*, A = *Acer monspessulanum* and C = *Cotinus coggygria*.

**Fig. 3.** Relationships between fungal biomass and detritivore abundance for (a) control and (b) rain exclusion plots. Relationships between detritivore and predator abundances for (c) control and (d) rain exclusion plots. Linear regressions are represented with their respective $R^2$ and slope. The data include all sampling periods. Q = *Quercus pubescens*, A = *Acer monspessulanum* and C = *Cotinus coggygria*. 
**Fig. 4.** Mean percentage of (a) *Q. pubescens* litter mass loss, (b) carbon, (c) nitrogen and (d) phenolic releases during the decomposition process through time, the two environmental conditions and the three litter types. Separated *t*-tests were performed for each sampling period and for each litter type to test the effect of the rain exclusion treatment on the decomposition parameters. * denotes significant differences between control and rain exclusion plots.

**Fig. 5.** Path analysis model for the effect of increased litter quality and decreased rainfall on fungal biomass and mesofauna (detritivores and predators) abundance and their effects on carbon and nitrogen releases after 24 months of litter decomposition. Solid arrows show significant positive relationships (i.e. pathways) between variables, dotted arrows indicate significant negative relationships, and numbers next to arrows show standardized parameter estimates (i.e. standardized regression weights). Significant values are indicated by * for $P < 0.05$ and *** for $P < 0.001$. $r^2$ values are indicated on the box of the dependent variables.
Fig. 1.
Fig. 2.

(a) Fungal biomass (μg g⁻¹ DM) for different treatments in CONTROL and RAIN EXCLUSION plots. Bars with different letters indicate significant differences.

(b) Detritivore abundance (ind g⁻¹ DM) over time in both plots. Letters above bars indicate significant differences.

(c) Predator abundance (ind g⁻¹ DM) over time in both plots. Letters above bars indicate significant differences.
Fig. 3.

(a) CONTROL PLOT

(b) RAIN EXCLUSION PLOT

(c)

(d)
Fig. 4.
Fig. 5.

Increased litter quality

Fungi
$r^2 = 0.79$

Detritivore
$r^2 = 0.87$

Predator
$r^2 = 0.88$

C loss
$r^2 = 0.88$

N loss
$r^2 = 0.82$

Decreased Rainfall

$r^2 = 0.79$

$r^2 = 0.87$

$r^2 = 0.88$

$r^2 = 0.82$

-0.19*

-0.13*

0.57***

0.94***

0.37***

0.68***

0.61***

0.66***