La réponse souterraine à une sécheresse accentuée dans un écosystème de garrigue méditerranéenne

Ammar Shihan

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Présentée par
Ammar SHIHAN

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Belowground responses to increased drought in a Mediterranean shrubland ecosystem

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M. **Alain BRAUMAN**, Dir. de recherche, INRA	**Président/Examinateur**
Mme. **Claire CHENU**, Professeur, AgroParisTech	Rapporteur
Mme. **Catherine PICON-COCHARD**, Chargée de Recherche, INRA	Rapporteur
Mme. **Annette BERARD**, Dir. de recherche, INRA	Examinateur
M. **Stephan HÄTTENSCHWILER**, Dir. de recherche,CNRS	Directeur de thèse
Mme. **Nathalie FROMIN**, Chargée de Recherche, CNRS	Co-encadrante de thèse

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Sous la direction de

Stephan Hättenschwiler
&
Nathalie FROMIN

« We made from water every living thing »

Coran (surat 21_verse 30)

Abstract

Longer drought periods and/or overall less precipitation are thought to be major consequences of ongoing climate change in the Mediterranean region. These changes in the water regime will likely affect community composition, biodiversity and ecosystem processes, but very little is known about how biodiversity and changes in precipitation interactively affect ecosystem functioning. In my PhD thesis, I aimed to quantify the role of plant diversity in the response of Mediterranean shrubland ecosystem to a decrease in water availability, with a particular interest in belowground processes, such as soil microbial community functioning and functional responses in plant roots.

I used a rhizotron approach under partially controlled conditions to study plant growth responses to repetitive severe droughts, with a particular focus on root growth. Two individuals of the same species or in all possible combinations of the three dominating species at our field site (*Quercus coccifera*, *Cistus albidus*, *Brachypodium retusum*) were grown together in a rhizotron. Repetitive severe droughts had a negative effect on survival of the two woody species (*Q. coccifera*, *C. albidus*), but not of the grass *B. retusum*. Interspecific competition generally increased survival of *C. albidus* and *B. retusum* compared to monospecific competition. Conversely, interspecific competition decreased the survival of *Q. coccifera*. Likewise, I found that root morphological traits were mostly affected by the neighbor species identity rather than by severe drought. The community level physiological profiles (CLPPs) of root associated soil microbial communities did not differ between drought treatments and were also not affected by plant species identity. However, CLPPs changed towards more total microbial activities but less diverse resource use at increasing soil depth. Collectively these results suggest that plant species composition of the studied Mediterranean shrubland has a stronger effect on growth, intraspecific variability in root traits and survival than repetitive severe droughts.

In the context of a larger collaborative project (CLIMED), I used a natural gradient of shrub species diversity in a Mediterranean shrubland ecosystem (garrigue) to which a permanent partial rain exclusion treatment (12% less precipitation) was added. This field experiment allowed me to study the responses of soil microbial community level physiological profiles (CLPPs) to reduced precipitation and to a change plant-produced leaf litter material decomposing on the ground as a key resource for heterotrophic soil microorganisms over two years. While rain exclusion had only a minor impact on the diversity of substrates metabolized by the microbial communities, litter species richness promoted global soil microbial activity by increased catabolic diversity of the soil microbial community. These results suggest that indirect climate change effects on plant species composition and richness might have more important consequences for soil microbial functioning than reduced precipitation in the studied Mediterranean shrubland ecosystem.

Both, the field study of soil microbial functioning and the rhizotron study of plant growth and survival clearly showed that plant species identity and diversity may be more important for the functioning of these Mediterranean shrublands than increased drought. I conclude that climate change induced shifts in plant species composition and diversity may have more important consequences for the functioning of Mediterranean shrublands than the direct effects of altered precipitation.

Keywords: Climate change, Mediterranean ecosystem, biodiversity, root system, soil microbial activity, functional diversity.

Résumé

Les modèles disponibles prévoient que le changement climatique entrainera de plus longues périodes de sécheresse et/ou une diminution globale des précipitations en région méditerranéenne. Ces changements affecteront probablement la composition des communautés, la biodiversité et les processus écosystémiques. Cependant, les effets de la biodiversité, des changements de précipitations et de leurs interactions sur le fonctionnement des écosystèmes restent mal connus. Dans cette thèse, j'ai cherché à quantifier le rôle de la diversité des plantes dans la réponse d'un écosystème de garrigue méditerranéenne à une diminution de la disponibilité en eau, avec un intérêt particulier pour les processus souterrains, tels que le fonctionnement de la communauté microbienne du sol et les réponses fonctionnelles des racines.

Afin d'étudier les conséquences d'une sécheresse sévère répétée sur la croissance végétale, et particulièrement racinaire, j'ai mis en place une expérience en rhizotrons, en conditions semi-contrôlées. Dans chaque rhizotron ont été plantées deux individus d'une même ou de différentes espèces des trois espèces dominantes de notre site d'étude (*Quercus coccifera*, *Cistus albidus*, *Brachypodium retusum*). Les sécheresses sévères répétées ont eu un effet négatif sur la survie des deux espèces ligneuses mais pas sur celle de l'herbacée. La compétition interspécifique a généralement augmenté la survie de C. albidus et B. retusum comparé à la compétition intraspécifique. A l'inverse, la compétition interspécifique a diminué la survie de Q. coccifera. De la même manière, les traits morphologiques racinaires ont été plus affectés par l'identité des espèces voisines que par le traitement de sécheresse. Les profils métaboliques des communautés microbiennes (CLPP) de sol associé aux racines n'ont pas été affectés par les traitements de sécheresse ni par l'identité des espèces végétales. Cependant, avec l'augmentation de la profondeur du sol, les CLPP ont augmentés mais avec une utilisation de ressources moins diversifiées. Ces résultats suggèrent que la composition d'espèces végétales de la garrigue méditerranéenne étudiée a un effet plus important sur la croissance, la variabilité intraspécifique des traits racinaires et la survie que les sécheresses sévères répétées.

Dans le contexte d'un projet collaboratif (CLIMED), j'ai utilisé un gradient naturel de diversité d'espèces d'arbustes d'un écosystème de garrigue méditerranéenne ayant reçu un traitement d'exclusion de pluie partielle (-12% de précipitation). Cette expérience m'a permis d'étudier les réponses des profils métaboliques des communautés microbiennes du sol CLPP à une diminution des précipitations ainsi qu'à un changement de diversité des litières végétales décomposant au sol comme ressource clé pour les microorganismes hétérotrophiques du sol, pendant deux ans. Alors que l'exclusion de pluie n'a eu qu'un impact mineur sur la diversité des substrats métabolisés par les communautés microbiennes, la richesse spécifique de la litière a favorisé l'activité microbienne du sol en augmentant la diversité catabolique de la communauté microbienne du sol. Ces résultats suggèrent que les effets indirects du changement climatique sur la composition et la richesse des espèces végétales pourraient avoir des conséquences plus importantes pour le fonctionnement microbien du sol que la réduction des précipitations prévue dans l'écosystème de garrigue méditerranéenne étudié.

Mes deux études, de terrain et en rhizotron, ont clairement montrées que l'identité et la diversité des espèces végétales peuvent être plus importantes pour le fonctionnement de ces garrigues méditerranéennes qu'une augmentation des sécheresses. Je conclus que les changements de composition et de diversité d'espèces végétales induits par le changement climatique peuvent avoir des conséquences plus importantes pour le fonctionnement des garrigues méditerranéennes que les effets directs d'une modification des précipitations.

Mots clés: Changement climatique, écosystème méditerranéen, biodiversité, système racinaire, activité microbienne du sol, diversité fonctionnelle.

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Que la paix règne le monde entier, y compris la Syrie…

à Mon premier encadrant depuis ma naissance…

à Papa

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Contributeurs

FROMIN, N.

Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), UMR 5175, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier – EPHE, 1919 route de Mende, 34293 Montpellier Cedex 5, France. Adresse actuelle: ROMES-CNRS, 7 rue du Four Solaire, 66120 Font Romeu – Odeillo, France.

e-mail: nathalie.fromin@cefe.cnrs.fr

HÄTTENSCHWILER, S.

Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), UMR 5175, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier – EPHE, 1919 route de Mende, 34293 Montpellier Cedex 5, France.

e-mail: stephan.hattenschwiler@cefe.cnrs.fr

MILCU, A.

Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), UMR 5175, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier – EPHE, 1919 route de Mende, 34293 Montpellier Cedex 5, France. CNRS, Ecotron - UPS 3248, Campus Baillarguet, FR-34980 Montferrier-sur-Lez, France.

e-mail: alex.milcu@cnrs.fr

VOLAIRE, F.

INRA USC 1338, Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), UMR 5175, CNRS, Université de Montpellier - Université Paul-Valéry Montpellier–EPHE, 1919 Route de Mende, 34293 Montpellier cedex, France.

e-mail: florence.volaire@cefe.cnrs.fr

Introduction générale

1. Changements climatiques et fonctionnement de l'écosystème

Il existe un consensus scientifique sur le fait que les émissions de gaz à effet de serre d'origine anthropique sont et vont être responsables de changements considérables dans le climat mondial au cours de ce siècle (Houghton et al. 2001). Une augmentation de la précipitation annuelle totale est ainsi prévue dans de nombreuses régions du monde (Dai et al. 1997), tandis que pour les régions méditerranéennes, on attend une diminution de l'ordre de 10 à 30% de la précipitation totale dans les 100 prochaines années (Giorgi and Lionello 2008), accompagnée d'une augmentation des événements extrêmes de précipitation (Alpert et al. 2002; Gao et al. 2006). Selon Weltzin and McPherson (2003), les changements de régimes de précipitation, surtout dans les milieux arides et semi-arides, peuvent avoir un impact encore plus important que les effets, singuliers ou combinés, de la hausse des émissions de CO_2 et de la température sur la dynamique des écosystèmes (IPCC 2013).

Ces changements régionaux des régimes des précipitations modifient la disponibilité en eau pour les communautés biologiques. En effet, l'humidité du sol est un facteur de contrôle majeur des processus biologiques en général, et des communauté végétales (Weltzin and McPherson 2003) et microbiennes du sol en particulier (Berg and Steinberger 2008; Clark et al. 2009). Il est probable que toute modification du régime des précipitations aura des conséquences sur la répartition, la structure, la composition et la diversité des communautés biologiques du sol et le fonctionnement des écosystèmes (Weltzin and McPherson 2003). La modification spatiale et temporelle de la disponibilité en eau peut affecter des processus tels que la décomposition de la litière, le recyclage des nutriments, la productivité primaire, et, en retour, la biodiversité (Robinson et al. 1998; Sternberg et al. 1999; Chapin et al. 2000).

Le bassin méditerranéen est considéré comme un *hotspot* de biodiversité à l'échelle mondiale (Myers et al. 2000). Malgré sa petite superficie (moins de 3 % des écosystèmes terrestres selon le Millennium Ecosystem Assessment (2005), le bassin méditerranéen compte environ 25000 espèces de plantes vasculaires dont la moitié est endémique (Cowling et al. 1996). Ces espèces pourraient être en danger d'extinction avec les changements climatiques prédits par les modèles.

Les écosystèmes méditerranéens subissent une forte pression humaine, soit directement sous les effets combinés d'un des taux de croissance démographique parmi les plus élevés d'Europe et de la pression des activités touristiques, soit indirectement via le changement climatique, avec une diminution sensible des précipitations et une augmentation des températures estivales prévues par les modèles climatiques dans les décennies à venir. Ces pressions pourraient se traduire par des pertes importantes de biodiversité (Sala et al. 2000) avec des conséquences sur les processus écosystémiques et les services associés. Cependant, ces conséquences sont en général mal appréhendées pour la zone méditerranéenne, et en particulier pour les communautés biologiques des sols et les processus qu'elles portent.

Il est donc urgent de comprendre comment les changements de régimes de précipitation prévus par les modèles climatiques vont affecter la biodiversité des communautés biologiques du sol et les processus qu'elles portent, afin de disposer d'une base scientifique solide pour la prise de décision dans le contexte des changements climatiques et l'augmentation de la demande de terres.

2. Particularités biologiques des écosystèmes méditerranéens

2.a La garrigues méditerranéenne

La végétation de la garrigue méditerranéenne est composée essentiellement d'arbrisseaux et d'arbustes qui se développent sur les sols calcaires, comme le *Quercus* spp., *Cistus* spp.,

Juniperus spp., *Thymus* spp., *Rosmarinus officinalis*, *Ulex* spp., *Artemisia* spp. et *Lavandula* spp., entre lesquels de nombreuses espèces graminées et herbacées, annuelles ou pérennes, sont fréquentes. Les feuilles des plantes sont souvent petites, charnues et odorantes en adaptation à une sécheresse estivale importante. En plus de la sécheresse estivale, la garrigue méditerranéenne est typiquement exposée à des incendies périodiques et est souvent surpâturée. Les végétaux méditerranéens sont bien adaptés au retour périodique des incendies. Ce caractère pyrophile de la végétation méditerranéenne est le résultat d'une longue évolution qui remonte au moins au Néolithique. Les principaux mécanismes de régénération des plantes méditerranéennes après le feu sont le « resprouting » et la regermination des graines (Keeley and Zedler 1978). La stratégie de « resprouter » se traduit par la résistance de la souche au feu et la forte capacité de remettre des rejets après un incendie (Canadell et al. 1991).

2.b *Une phénologie adaptée pour éviter la sécheresse*

La plupart des organismes méditerranéens évitent la sécheresse en concentrant leurs activités pendant les périodes de l'année où l'eau est disponible et où la température est clémente, c'est-à-dire au printemps et à l'automne. L'été est donc généralement marqué par une phase d'activité réduite : les plantes limitent leur assimilation/transpiration, et souvent leur surface foliaire (van der Molen et al. 2011). L'activité microbienne durant cette période est également diminuée (de Dato et al. 2010). Les plantes qui gardent leur feuillage pendant l'été présentent typiquement des adaptations morphologiques et physiologiques leur permettant de résister à la sécheresse (sclérophyllie, pubescence, présence de composés aromatiques, aphyllie,…). Ces adaptations se traduisent de manière générale par une litière plus résistante à la décomposition (Gallardo and Merino 1993; Pérez-Harguindeguy et al. 2000).

2.c *Les communautés microbiennes dominées par les champignons*

Contrairement à la plupart des bactéries, les champignons et les actinomycètes ont la capacité de rester actifs dans des sols à très faible disponibilité en eau (Salamanca et al. 2003). De plus, les champignons possèdent des enzymes adaptées à la dégradation des substrats complexes, tels que les litières produites par les plantes méditerranéennes qui sont souvent récalcitrantes. Par conséquent, les champignons occupent une place importante dans les communautés microbiennes des écosystèmes méditerranéens (Wilkinson et al. 2002) et y jouent un rôle fonctionnel prépondérant (Collins et al. 2008).

3. Stratégies des plantes pour survivre à la sécheresse

Même si les espèces méditerranéennes sont adaptées à de longues périodes de sécheresse estivale et peuvent faire face à un déficit hydrique saisonnier (Peñuelas 1996; Peñuelas et al. 2001; Micco and Aronne 2012), ces espèces peuvent se retrouver dans une phase critique de survie en cas d'évènements de sécheresse particulièrement intenses ou fréquents (Prévosto et al. 2011; Benigno et al. 2014; Louhaichi et al. 2015). Les seuils critiques de survie varient au sein des espèces co-occurrentes selon leur capacité à survivre à la sécheresse, capacité qui est associée à des traits morphologiques et physiologiques spécifiques (Grime 2001; Gallé and Feller 2007; Hernandez et al. 2010). Alors que les stratégies de 'résistance à la sécheresse' contribuant à maintenir une activité physiologique sous stress hydrique modérée ont été décrites pour la plupart des espèces (Levitt 1972; Ludlow 1989), il n'existe pas de cadre théorique unifié décrivant les stratégies de 'survie à la sécheresse' pour toutes les espèces végétales. Pour les espèces ligneuses, de nombreux travaux ont montré que les traits hydrauliques associés à la résistance à la cavitation étaient très corrélés à la mortalité inter-spécifique des arbres (Choat et al. 2012; Anderegg et al. 2016). Pour les espèces herbacées, des résultats récents montrent

également une forte variabilité du seuil de cavitation, associée au niveau d'aridité des sites des espèces et suggérant que leur vulnérabilité à l'embolisme n'est pas inférieure à celle des ligneux (Lens et al. 2016). Néanmoins, le cadre des stratégies végétales décrites de manière globale pour la 'résistance à la sécheresse' reste utile bien qu'imparfait lorsque différents groupes fonctionnels (ligneux/ herbacées) sont directement comparés.

Alors que les plantes annuelles évitent les périodes de sécheresse en terminant leur cycle de vie pendant la période la plus favorable et survivent à la période la plus sèche sous forme de semences, les plantes pérennes présentent généralement deux types de stratégies pour retarder et/ou résister aux effets de la sécheresse (Tardieu and Simonneau 1998; David et al. 2007; Quero et al. 2011):

i) La stratégie d'évitement de la déshydratation « dehydration avoidance » permet aux plantes, en condition de sécheresse modérées, de maintenir un niveau d'eau élevé dans les feuilles pendant une plus longue période grâce à une augmentation de leur capacité d'absorption de l'eau, par un système racinaire profond et efficace, ou par une diminution des pertes d'eau par régulation stomatique (Volaire et al. 1998; Ferrio et al. 2003). Cette stratégie permet le maintien de l'activité photosynthétique et limite la privation de carbone. Cependant, l'évitement de la déshydratation ne s'avère pas efficace pour faire face à une sécheresse sévère puisque les feuilles matures ne tolèrent en général pas la déshydratation.

ii) La stratégie de tolérance à la déshydratation « dehydration tolerance » assure la survie des plantes en conditions de sécheresse sévère malgré une faible teneur en eau des tissus en maintenant l'intégrité des cellules des tissus qui peuvent survivre (par exemple les méristèmes des pousses) grâce à la stabilisation de la membrane cellulaire et à l'accumulation de glucides solubles (Volaire et al. 1998; Verslues et al. 2006). Cette stratégie a été identifiée chez les espèces herbacées pérennes pour lesquelles les limbes matures sénescent alors que

seuls les méristèmes foliaires peuvent survivre à une faible disponibilité en eau et ce pendant plus longtemps que les tissus matures (Volaire et al. 2014). En ce qui concerne les espèces ligneuses, la tolérance des cellules à la sécheresse est également reconnue comme un facteur aussi important que la résistance à la cavitation dans la détermination des limites physiologiques de survie des arbres (McDowell et al. 2008).

D'un point de vue morphologique, on s'attend à ce que, dans des conditions de disponibilité en eau limitée, les plantes investissent proportionnellement plus dans les structures impliquées dans l'acquisition et le transport de l'eau, selon l'hypothèse d'équilibre fonctionnel, (Brouwer 1962). La stratégie d'évitement de la déshydratation « avoidance strategy » comprend des ajustements des patrons d'allocation de biomasse, par exemple en réduisant la superficie totale des feuilles (ce qui permet de diminuer la perte d'eau due à la transpiration DeLucia et al. 2000), ou en favorisant plus d'allocation dans la croissance des racines (Markesteijn and Poorter 2009). Sous stress hydrique modéré, cette stratégie permet d'assurer la croissance en maintenant une activité photosynthétique (Martínez-Ferri et al. 2000; McDowell et al. 2008). Par conséquent, les espèces employant la stratégie d'évitement de la déshydratation (avoider species) sont plus susceptibles de succomber à la privation de carbone pendant une sécheresse modérée prolongée, tandis que les espèces tolérantes à la déshydratation sont plus susceptibles de mourir suite à une défaillance hydraulique lors d'un épisode de sécheresses sévère. Les espèces 'évitantes' sont donc plus susceptibles si elles sont soumises à des épisodes de sécheresses sévères ou répétées. Au niveau de la communauté végétale, une stratégie d'évitement de la déshydratation semble être le principal levier pour améliorer la productivité et la stabilité dans des conditions de sécheresse (Skinner et al. 2006). On pourrait donc s'attendre à ce que les plantes mettant en œuvre une stratégie d'évitement de déshydratation soient plus

compétitives que celles à stratégie de tolérance sous des conditions climatiques caractérisées par des événements de sécheresse *ne conduisant pas à des risques de mortalité.*

La plupart des espèces méditerranéennes ont été classées comme adoptant soit une stratégie d'évitement de la déshydratation soit une stratégie de tolérance à la déshydratation (David et al. 2007; Galmés et al. 2007b; Galmés et al. 2007a; Quero et al. 2011). Alors que les espèces herbacées pérennes (par exemple *Brachypodium retusum*) peuvent résister et survivre à une sécheresse sévère grâce à des stratégies contribuant à la fois à l'évitement et à la tolérance à la déshydratation, selon l'intensité de la sécheresse (Blum 1996), les espèces ligneuses méditerranéennes (par exemple *Quercus coccifera* et *Cistus albidus*) sont plutôt caractérisés par une stratégie d'évitement de la déshydratation, qui se traduit par une augmentation de la transpiration et de la conductance stomatique, même lorsque le potentiel hydrique des feuilles est très faible (Damesin et al. 1998; Galmés et al. 2007b). En conséquence, la mortalité de ces espèces est particulièrement probable lors d'une sécheresse sévère répétée (McDowell et al. 2008). Dans ce cadre, il semble de tester les effets de ces stratégies contrastées en comparant la survie à la sécheresse des espèces ligneuses et herbacées provenant d'une même garrigue méditerranéenne.

4. Nécessité de prendre en compte la réponse souterraine

La compréhension de la réponse des écosystèmes terrestres aux changements climatiques, et notamment aux changements des régimes de précipitations, est un enjeu crucial de la recherche actuelle en écologie fonctionnelle (Sternberg et al. 2011). Alors que la plupart des études évaluent la réponse « aérienne » (e.g. production de la biomasse végétale) des écosystèmes aux changements climatiques, les réponses « souterraines » (systèmes racinaires, communautés

microbiennes du sol) restent encore peu considérées. La prise en compte des processus souterrains complètera l'évaluation des conséquences écosystémiques des changements climatiques dans un contexte de changement combiné du climat et de la biodiversité. Des scénarios réalistes de changement de la biodiversité en lien avec les changements climatiques prévus seront utiles pour évaluer les conséquences attendues sur les processus et les services de l'écosystème et pour mieux anticiper les risques pour les écosystèmes particulièrement exposés aux effets combinées d'une forte pression humaine et des changements climatiques, comme c'est le cas pour les écosystèmes méditerranéens. Evaluer les conséquences d'une diminution de la disponibilité en eau sur la communauté microbienne du sol pourrait fournir un outil important pour évaluer ces risques (Sherman et al. 2012). Cependant, on sait peu de choses sur la façon dont les acteurs principaux (les plantes, et en particulier leur système racinaire, et les microorganismes du sol) répondent à la diminution des précipitations dans les écosystèmes méditerranéens, alors même que les microorganismes du sol sont des acteurs critiques pour les processus clés de l'écosystème (recyclage du carbone et des nutriments, et leurs effets retours (feed-backs) sur les plantes).

4.a Sécheresse et croissance racinaire

La complexité des relations entre régime des précipitations et dynamique de l'eau dans les sols entraine une grande variabilité de la disponibilité de la ressource hydrique pour les plantes dans les différents horizons de sol (Fitter and Stickland 1991). En milieu méditerranéen, la saisonnalité des pluies combinée à une forte demande évaporative estivale contribue à l'irrégularité temporelle du déficit hydrique (Schwinning and Ehleringer 2001) entrainant chez certaines plantes soit des changements adaptatifs importants (notamment une modification de la distribution verticale des racines dans les différents horizons) soit des effets délétères sur le développement de la plante (Chaves et al. 2002).

Un déficit hydrique modéré peut entrainer une augmentation du taux d'élongation racinaire (Pritchard 1994). Mais l'augmentation de l'intensité du déficit hydrique peut ensuite ralentir ce taux d'élongation (Sharp and Davies 1985), ce qui génère alors à une situation de stress au cours de laquelle les tissus racinaires commencent à subir des dommages au début réversibles (Pritchard 1994), puis irréversible en-dessous du point de flétrissement, au point de provoquer la mort des tissus et de l'organisme. En-dessous du seuil létal, l'effet du stress sera fortement dépendant de sa durée et de sa périodicité (Lichtenthaler 1996).

4.b Sécheresse et activité microbiennes de sol

En ce qui concerne les communautés d'organismes du sol, une dégradation des conditions hydriques entraine une modification de la structure des communautés d'organismes (Wilkinson et al. 2002; Schimel et al. 2007; Bérard et al. 2011): on peut observer une perte de diversité et d'abondance des organismes, ou un changement dans la dominance de la communauté par certains groupes plus résistants et mieux adaptés à des conditions hydriques plus sèches. La respiration du sol (respiration microbienne hétérotrophe), qui contribue pour 40 à 60% à la respiration totale du sol, semble être relativement sensible à des changements des conditions environnementales (Janssens et al. 2001). Les changements de régimes de précipitations peuvent entraîner une diminution de la biomasse microbienne du sol et altérer la capacité de la communauté à utiliser différentes formes de carbone (Bell et al. 2008). De façon générale, une dégradation des conditions hydriques entraine à la fois une diminution de la respiration du sol (Schimel et al. 2007; Talmon et al. 2011) et des activités enzymatiques (Hueso et al. 2011).

Au-delà de leurs effets sur la disponibilité en eau, les changements climatiques pourraient altérer les communautés microbiennes du sol et les processus qu'elles portent indirectement, via des changements dans la composition de la communauté végétale ou dans la qualité des apports

organiques au sol (Kardol et al. 2010; Sardans and Peñuelas 2013) ou encore par une réduction de la production de biomasse et donc de la quantité de matière organique apportée au sol (Sardans and Peñuelas 2013). La chute de litière et la rhizodéposition assurent l'entrée régulière de matière organique dans le sol et sont à la base du métabolisme des microorganismes hétérotrophes, qui sont impliqués, par exemple, dans le processus de minéralisation de la matière organique et le recyclage des nutriments (Wardle 2002; Hättenschwiler et al. 2005; Bardgett et al. 2005). En réponse à une baisse de la disponibilité en eau, les changements attendus de composition de la communauté végétale ou de la physiologie des espèces présentes s'accompagnera de modifications (qualitatives ou quantitatives) des apports liés à la rhizodéposition et/ou aux litières (Sardans et al. 2008b; Matías et al. 2011), avec des conséquences sur les communautés microbiennes du sol et les processus qu'elles portent (Sardans et al. 2008a).

5. Traits fonctionnels

Les classifications fonctionnelles, reliant forme et fonction des espèces, et basées sur l'utilisation des traits de vie des plantes, sont aujourd'hui couramment utilisées en écologie (voir (Lavorel et al. 1997) pour une présentation détaillée du concept et des exemples). Il existe de nombreuses définitions des traits fonctionnels selon la discipline (e.g. évolution ou écologie (Ackerly et al. 2000; Lavorel et al. 2007)), le niveau d'organisation (e.g. individu, population, communauté ou écosystème (Violle et al. 2007)), ou selon que l'on considère leur rôle dans le fonctionnement de l'écosystème (e.g. (Diaz and Cabido 1997; Lavorel and Garnier 2002)). Nous utiliserons ici la définition suivante : les traits fonctionnels sont des paramètres morphologiques, physiologiques ou phénologiques mesurés au niveau de l'individu, qui

influencent sa performance individuelle (fitness) et ses effets sur le fonctionnement de l'écosystème (Diaz and Cabido 1997; Lavorel and Garnier 2002; Violle et al. 2007). Les traits fonctionnels peuvent donc être mis à profit pour comparer les espèces végétales mais également pour décrire, comprendre et prévoir l'assemblage des communautés végétales (Diaz et al. 1998). Le concept des filtres environnementaux stipule que les conditions abiotiques et biotiques de l'environnement effectuent un tri écologique en sélectionnant les espèces portant certains traits de réponse. On peut ainsi définir i) des groupes fonctionnels de réponse rassemblant des espèces qui présentent une réponse similaire à un facteur environnemental donné (Lavorel et al. 1997; Lavorel and Garnier 2002), et ii) des groupes fonctionnels d'effet, regroupant des espèces végétales qui présentent des traits d'effet particuliers influençant certains processus et fonctions de l'écosystème (Lavorel et al. 1997; Lavorel and Garnier 2002).

Actuellement, les traits fonctionnels des plantes sont considérés comme des indicateurs potentiellement puissants dans l'étude de l'écologie des espèces. L'étude des traits fonctionnels a contribué à la compréhension des facteurs qui déterminent la répartition des espèces (Grime 2002) et des mécanismes impliqués dans la réponse des plantes aux facteurs abiotiques et biotiques, ainsi que leur impact sur le fonctionnement des écosystèmes (Lavorel and Garnier 2002; Storkey et al. 2013; Garnier and Navas 2013). Deux approches, largement complémentaires fondées sur les traits fonctionnels sont actuellement utilisées. La première, qui vise à identifier les traits dominants qui contrôlent les processus écosystémiques, stipule que l'effet de chaque espèce sur un processus écosystémique donné est proportionnel à l'abondance relative de cette espèce dans la communauté («biomass–ratio hypothesis» , Grime 1998). En conséquence, les traits moyens de la communauté végétale (community weighted mean - CWM) devraient permettre de prédire l'effet de la communauté végétale sur les processus écosystémiques. La seconde approche vise à quantifier la diversité fonctionnelle des

communautés, stipule qu'une plus grande gamme de valeurs de traits garantit une meilleure complémentarité de niche entre les espèces, et donc une meilleure utilisation des ressources disponibles au niveau de la communauté (hypothèse de dissimilarité ou équitabilité, Petchey and Gaston 2006; Díaz et al. 2007).

5.a Impact des traits fonctionnels des plantes sur la communauté microbienne

Les plantes, via leur influence sur les conditions physico-chimiques du sol (pools de nutriments, conditions microclimatiques) et la composition de leurs apports organiques, contrôlent fortement les communautés microbiennes présentes dans le sol. En conséquence, les traits fonctionnels des plantes devraient constituer de bons indicateurs de la communauté microbienne et de ses paramètres fonctionnels. Je focaliserai ici ma présentation sur l'effet des traits racinaires (chapitre 1 et 2) et des traits des litières (chapitre 3).

5.a.1 Effet des traits des litières sur la communauté microbienne

Les litières végétales diffèrent selon les espèces en fonction des proportions relatives de composés carbonés, nutriments, composées secondaires, mais aussi des caractéristiques physique comme par example la dureté des limbes (Hättenschwiler et al. 2008; Cornwell et al. 2008; Makkonen et al. 2012). Ces différences de qualité expliquent largement les taux de dégradation des litières pendant la décomposition (Aerts 1997; Cornwell et al. 2008; Coq et al. 2010; Makkonen et al. 2012). En conditions non limitantes, les espèces tendent à avoir une croissance rapide en produisant des feuilles à courte période de vie mais riches en N, qui est souvent une ressource limitante pour les décomposeurs (Parton et al. 2007). Au contraire, dans des conditions limitantes (e.g. peu de nutriments ou faible disponibilité en eau), les espèces vont avoir plutôt une croissance lente, souvent associé à des feuilles avec une durée de vie plus longue et une concentration de nutriments plus faible (Wardle et al. 2004). Les communautés

biologiques du sol sont influencées par la qualité des litières déposés à la surface et donc des ressources disponibles (Ayres et al. 2009; Carrillo et al. 2011; Milcu et al. 2013; Fanin et al. 2014) : elles peuvent être modifiés en cas de changement dans la composition des espèces végétales présentes et de leurs litières. Malgré l'importance de l'identité des espèces (Cornwell et al. 2008; Makkonen et al. 2012) et de leur diversité fonctionnelle (Hättenschwiler et al. 2005; Handa et al. 2014) dans le processus de décomposition des litières, on sait peu de chose sur l'effet de ces paramètres sur les communautés microbiennes de sol qui sont l'un des principaux acteurs du processus de décomposition. L'identification de traits de réponse aux changements environnementaux permettra une évaluation prédictive des conséquences des changements environnementaux qui induisent un changement de la biodiversité de sol ainsi des processus de l'écosystème.

5.a.2 Effet des traits racinaires sur la communauté microbienne

Une grande partie des apports de litière dans le sol provient d'organes autres que les feuilles, en particulier en forme de racines mortes (Gill and Jackson 2000; Yuan and Chen 2010). A l'état vivant, les racines libèrent une grande variété de composés organiques labiles dans le sol tels que des sucres, polysaccharides, acides organiques et aminés, des peptides et des protéines sous forme d'exsudats racinaires (Jones et al. 2004; Carvalhais et al. 2011). Ces composés constituent, au même titre que les litières, une source importante de C et de l'énergie pour les microorganismes de la rhizosphère, le C étant rapidement minéralisée ou immobilisé dans la biomasse microbienne, ce qui conduit à une prolifération de micro-organismes, à la surface et à l'extérieur de la racine (Lynch and Whipps 1990). Toutefois, il existe peu de données sur les relations entre traits racinaires et communautés microbiennes du sol (Legay 2013).

5.a.3 Stratégie écologique des plantes et communauté microbienne du sol

Certains traits de feuilles et de racines peuvent être corrélés suite aux réponses identiques de ces deux organes à des pressions sélectives, lesquelles favorisent des stratégies plus conservatrices ou plus "exploitative" et qui entraînent les mêmes conséquences en termes de qualité de tissue dans les racines et les feuilles. Par exemple, il a été démontré que les concentrations en N dans les feuilles et dans les racines peuvent être fortement et positivement corrélées (Roumet et al. 2006; Westoby and Wright 2006). Dans une autre étude, les surfaces spécifiques des feuilles et des racines dans une communauté herbacée étaient également, bien que faiblement, corrélées (Craine et al. 2001). Étonnamment, les théories écologiques qui étudie l'effet de la diversité fonctionnelle des plantes sur les processus du sol considèrent les plantes comme des organismes homogènes qui peuvent être représentées par les traits foliaires seulement (Wardle et al. 2004). Il y a donc un besoin urgent de considérer les traits des racines afin d'améliorer notre compréhension des conséquences d'un changement des conditions environnementales sur ces traits, mais aussi pour mieux décrire l'effet des espèces végétales sur le fonctionnement du sol, aussi bien pour la contribution des litières végétales que pour leurs effets liés à la rhizodéposition.

5.b Traits fonctionnels des racines et réponse à la sécheresse

Lors d'évènements de sécheresse, une croissance rapide du système racinaire, et donc une augmentation de la capacité à explorer le volume du sol, constitue une adaptation importante de la plante pour éviter la déshydratation. Elle permet en particulier à la plante d'explorer des couches de sol plus profondes ayant souvent des taux d'humidité plus élevés (Lloret et al. 1999; Paula and Pausas 2011). Une autre adaptation à la sécheresse peut se faire au niveau de la structure des racines, qui joue un rôle important dans le statut hydrique de la plante (Hernández et al. 2010). Les traits fonctionnels des racines telles que la profondeur d'enracinement, l'allocation à la biomasse racinaire, la longueur spécifique des racines, leur diamètre et la

densité des tissus racinaires devraient être des paramètres clef de l'évitement de la déshydratation car ils jouent un rôle crucial dans l'acquisition de l'eau (Hernández et al. 2010; Pérez-Ramos et al. 2013). La longueur spécifique des racines (SRL, i.e. longueur des racines par unité de biomasse racinaire investie, qui dépend du diamètre des racines et/ou de la densité des tissues) est l'un des traits racinaires les plus importants parce qu'il est lié à la capacité d'exploration du sol et à l'absorption des ressources en eau et en nutriments (Wright and Westoby 1999; Nicotra et al. 2002). Une SRL plus élevée implique un rapport surface/volume également plus élevé, qui est habituellement négativement corrélé avec la quantité de carbone investie par unité de longueur de racine, et qui permet de maximiser l'interface racine-sol et donc le potentiel d'absorption des racines (Larcher 2003). De plus, un faible diamètre racinaire combiné à une SRL élevée permettent de diminuer la résistance au flux radial d'eau et d'augmenter la conductivité radiale (Huang and Eissenstat 2000). Par conséquent, les espèces ayant une SRL élevée présentent des taux élevés d'absorption d'eau (Eissenstat 1991), d'azote (Reich et al. 1998) et de phosphore (Comas et al. 2002). Les racines des plantes peuvent aussi réagir à la diminution des précipitations ou au déficit hydrique en augmentant la densité des racines et ou de leurs longueurs (Padilla et al. 2015), la taille et la productivité des racines fines (Hertel et al. 2013), ce qui se traduit par une augmentation de l'allocation de la biomasse à la partie racinaire (Markesteijn and Poorter 2009; Padilla et al. 2009).

6. Le rôle relatif de la complémentarité et de la compétition

Les plantes interagissent entre elles de deux manières principales pour l'utilisation des ressources disponibles dans le milieu : soit en compétition soit en complémentarité pour l'acquisition de ces ressources (Tilman et al. 1997). Ces deux types d'interactions ne sont pas

mutuellement exclusifs et leur importance relative dépend de la disponibilité des ressources en question et de l'identité des espèces végétales en interaction. Pour une densité d'individus donnée, on s'attend à une compétition pour les ressources limitantes plus importante pour une communauté monospécifiques (individus d'une seule espèce) que pour une communauté plurispécifiques (Tilman et al. 1997), parce que les individus d'une même espèce présentent entre eux les mêmes traits fonctionnels impliqués dans le prélèvement de la ressource. Au contraire, la compétition au sein d'une communauté composée de plusieurs espèces avec une diversité de traits fonctionnels, impliquant des prélèvements de ressource différents dans le temps et l'espace, est théoriquement plus faible, avec des indidividus potentiellement complémentaires (complémentarité de niche, Leibold 1995). Dans des prairies européennes, (Hector et al. 1999) ont montré qu'une augmentation de la richesse spécifique des plantes conduit à une augmentation de la production de la biomass aérienne probablement grâce à une complémentarité d'utilisation des ressources. En garrigue méditerranéenne (Montès et al. 2008) ont montré une augmentation de la croissance des plantes avec l'augmentation de la richesse spécifique, suggérant des mécanismes de facilitation entre les espèces, un autre mécanisme qui permer d'expliquer les interactions positives entre différentes espèces. La facilitation pourrait par ailleurs augmenter avec un déficit hydrique selon l'hypothèse de gradient de stress (Bertness and Callaway 1994), en raison de l'augmentation des interactions positives entre les espèces dans des conditions plus stressantes. Toutefois, selon une autre hypothèse de compétition spatiale pour des ressources limitantes (Tilman 1994), cette facilitation pourrait au contraire, disparaître en raison de l'augmentation des interactions compétitives pour une ressource limitante.

Dans la région méditerranéenne, la compétition souterraine pour l'eau est un facteur important contrôlant la croissance des plantes (Craine and Dybzinski 2013). Pendant les périodes de

sécheresse, la compétition pour l'eau entre individus voisins augmente (Gómez-Aparicio et al. 2011). Mais on comprend mal le rôle de la diversité dans cette compétition, à savoir si les mélanges d'espèces mixtes sont plus ou moins sensibles à la sécheresse comparés à des communautés monospécifiques (Jucker et al. 2014). Bien que certaines études aient signalé une diminution de la sensibilité à la sécheresse pour les espèces cultivées en mélange (Lebourgeois et al. 2013), d'autres ont suggéré que les interactions compétitives entre espèces différentes augmentent en condition de sécheresse (Grossiord et al. 2014). Les travaux théoriques de Craine and Dybzinski (2013) concilient ces résultats visiblement contradictoires en proposant que la compétition pour l'eau se traduit principalement par une réduction de la disponibilité de l'eau, favorisant les espèces les mieux adaptées aux conditions de sécheresse. On pourrait donc s'attendre à ce que les espèces tolérantes à la sécheresse bénéficient du mélange, tandis que les espèces exigeantes en eau seraient défavorisées quand elles poussent en mélanges dans des conditions de sécheresse (Jucker et al. 2014).

Par conséquent, le mélange d'espèces végétales avec des traits racinaires contrastés, c'est-à-dire l'augmentation de la diversité fonctionnelle des racines, devrait permettre d'améliorer la capacité d'absorption de l'eau sur tout le profil du sol, en particulier grâce à une meilleure utilisation de l'eau profonde du sol qui reste disponible plus longtemps dans des conditions de sécheresse.

7. Objectifs de la thèse et questions de recherche

L'objectif spécifique de la thèse est de caractériser/quantifier le rôle de la diversité végétale dans la réponse de l'écosystème de garrigue méditerranéenne à une diminution de la disponibilité en eau, avec un intérêt particulier pour le compartiment souterrain (communauté microbienne du sol, système racinaire).

Deux questions principales sont abordées:

1- *Comment les plantes de garrigue répondent-elles à une sécheresse accrue ? Cette réponse est-elle modifiée par l'identité d'espèce des individus en compétition?*

2- *Une diminution de la disponibilité en eau, influent-elle la communauté microbienne du sol ? Cette réponse dépend-elle de la diversité de la communauté végétale ?*

Pour répondre à ces questions, j'ai choisi deux approches expérimentales différentes pour manipuler la diversité végétale (ou l'identité de l'espèce végétale) et la disponibilité en eau: une expérience sur le terrain et une expérience, en conditions contrôlées, en rhizotron.

Sur le terrain, j'ai utilisé un dispositif d'exclusion de pluie le long d'un gradient de richesse d'espèces arbustives dans une garrigue sur le site du Massif de l'Etoile (Marseille), lequel a été mis en place en collaboration avec l'IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale, UMR 7263) dans le projet ANR-CEP09 « Climate change effects on Mediterranean biodiversity and consequences for ecosystem functioning (CLIMED) ». L'impact d'une diminution des précipitations sur la communauté microbienne du sol a été évalué au cours d'une expérimentation de décomposition de mélanges de litières lesquels reflétaient la composition en espèces d'arbustes.

Etant donnée la difficulté à étudier les racines des plantes *in situ* (sol avec 65.7% de cailloux à notre site d'étude), la réponse des systèmes racinaires des espèces de garrigue a été évaluée lors d'une expérimentation en rhizotron sur le terrain expérimental du CEFE (Centre d'Ecologie Fonctionnelle et Evolutive – CNRS de Montpellier). Trois espèces dominantes de la garrigue du 'Massif de l'Etoile' y ont été cultivées en mettant deux individus de plantes soit en compétition intraspécifique soit en compétition interspécifique, et en appliquant deux traitements de disponibilité en eau, afin d'évaluer l'impact d'une réduction des précipitations

sur la croissance et les traits des racines pendant un an. Les relations entre traits racinaires des plantes et activités microbiennes de sol y ont également été abordées.

Les résultats issus de ces deux expérimentations sont structurés en trois chapitres :

Chapitre I: Effet d'une sécheresse accentuée sur la survie et la croissance d'espèces méditerranéennes : compétition inter- et intra-spécifique

L'objectif de ce premier chapitre était de quantifier l'effet de la sécheresse sur la croissance des plantes de trois espèces (deux ligneuses : *Cistus albidus* et *Quercus coccifera*, et une graminée : *Brachypodium retusum*) dominantes dans la garrigue de notre site d'étude (Massif de l'Etoile à Marseille) et d'évaluer si ces plantes répondaient différemment lorsqu'elles étaient en présence d'un con-spécifique ou d'un individu d'une autre espèce. Plus particulièrement je me suis intéressé à quantifier l'effet d'une sécheresse accentuée sur la survie et la croissance de ces 3 espèces, et à évaluer l'importance des effets d'interaction entre individus de la même espèce ou de différentes espèces sur la croissance racinaire en condition de sécheresse accentuée.

Pour atteindre cet objectif, des plantules des trois espèces (*Quercus coccifera*, *Cistus albidus, Brachypodium retusum*) ont été cultivées en rhizotron, avec à chaque fois deux individus soit de la même espèce soit de deux différentes espèces et en appliquant deux traitements contrastés de sécheresse. Pour le traitement « sec », les rhizotrons étaient arrosés quand le niveau d'humidité gravimétrique du sol atteignait (11 ± 0.4) % durant 2 cycles (dessèchement/arrosage). Pendant cette période, les rhizotrons de traitement « control » étaient arrosés quand l'humidité gravimétrique du sol atteignait (17.5 ± 0.5) % (ce qui représente plusieurs événements d'arrosage selon les espèces présentes). A la fin de ces cycles d'arrosage, un troisième cycle de dessèchement (permettant d'atteindre 11 % d'humidité gravimétrique) a été appliqué à

l'ensemble des rhizotrons (traitements « sec » et « control ») et l'ensemble des plantes ont été récoltées trois semaines après la fin de ce troisième cycle de desséchement (c'est-à-dire 3 semaines après la reprise de l'arrosage). La capacité au champ du sol utilisé pour cette expérimentation était de 23% et le point de flétrissement de 5.1% (unité UR 272, INRA Orléans, France).

Connaissant les stratégies de ces trois espèces pour faire face à la sécheresse (stratégie d'évitement de la sécheresse pour les espèces ligneuses et stratégie de tolérance à la sécheresse pour la graminée), j'ai testé dans cette étude les hypothèses selon lesquelles i) les plantules d'espèces ligneuses à stratégie d'évitement de la sécheresse (*C. albidus* et *Q. coccifera*) montreraient un taux de survie plus faible comparé aux individus de la graminée à stratégie de tolérance (*B. retusum*) et ii) cet effet négatif de la sécheresse accrue sur la survie des plantules d'espèces ligneuses serait plus prononcé en condition de compétition intraspécifique comparé à une compétition interspécifique (complémentarité et/ou de facilitation).

Les résultats ont montré que des épisodes sévères et répétés de sécheresses ont diminué le taux de survie des deux espèces ligneuses, mais n'ont pas eu d'impact sur celui de *B. retusum* qui a résisté à des sécheresses plus nombreuses en diminuant son rythme de croissance. Selon l'identité de l'espèce, la compétition intra-spécifique a diminué (*Cistus albidus*) ou au contraire augmenté les taux de survies et de croissance (*Quercus coccifera*), alors que l'identité de l'espèce en co-culture n'a pas impacté la survie de *Brachypodium retusum*.

Cette étude montre que, dans nos conditions expérimentales, le brachypode était plus résistant aux conditions de sécheresse répétées que les deux espèces ligneuses (en termes de taux de survie), au moins à court terme. Cette étude suggère également que la survie de certaines espèces végétales de garrigue en réponse à une sécheresse dépend des autres espèces présentes

dans la communauté, et donc que la diversité végétale est un facteur de contrôle important de la réponse de l'écosystème à une augmentation des événements de sécheresse.

Chapitre II: La réponse des systèmes racinaires de plantes méditerranéennes et des communautés microbiennes du sol à une sécheresse accentuée

Ce chapitre est basé sur la même expérimentation en rhizotron que celle décrite plus haut pour le chapitre I. Ici je m'intéresse spécifiquement aux traits racinaires en lien avec les activités microbiennes du sol et leur réponse à une sécheresse sévère répétée.

Les racines fines (> 2 mm) ont été analysées pour leurs traits morphologiques en utilisant un scanner et le logiciel d'analyse d'image WinRHIZO. Les profils métaboliques des communautés microbiennes du sol (community level physiological profiles, CLPP) ont été caractérisées en utilisant la technique MicroResp™ : des paramètres d'activité métabolique globale (somme des taux de respiration pour les 15 substrats), de diversité (Shannon H') et de dominance métabolique (Simpson D) de la communauté microbienne du sol ont ensuite été calculés à partir des CLPP et leurs relations avec les traits racinaires ont été évaluées.

Du fait des stratégies contrastées des trois espèces en condition de sécheresse, j'ai testé dans ce chapitre les hypothèses selon lesquelles, dans des conditions de sécheresse accrue,: i) une sécheresse sévère répétées affecterait les traits morphologiques des racines de *B. retusum* plus fortement que ceux des espèces ligneuses, et par conséquences les communautés microbiennes du sol seraient plus affectées dans le sol associé à l'herbacée par rapport au sol associé aux espèces ligneuses, et ii) ces effets seraient plus prononcé en condition de compétition intraspécifique comparé à la compétition interspécifique.

Les résultats de cette étude montrent que les traits morphologiques des racines ont été plus affectés par l'identité de l'espèce voisine que par le traitement de sécheresse. Les profils métaboliques (CLPP) des communautés microbiennes de sol associé aux racines ne répondaient ni au traitement de sécheresses répétées ni à l'identité des espèces végétales voisines. L'activité métabolique de la communauté microbienne du sol a augmenté en profondeur (comparé à celle du sol de surface), mais avec l'utilisation de ressources carbonées moins diversifiées.

Ces résultats suggèrent que la composition d'espèces végétales de la garrigue méditerranéenne étudiée a un effet plus important sur la variabilité intraspécifique des traits racinaires que les sécheresses sévères répétées.

Chapitre III : Effet d'une baisse des précipitations sur l'utilisation des ressources de la communauté microbienne du sol le long d'un gradient de diversité végétale

Dans ce chapitre j'ai évalué l'activité et la diversité catabolique de la communauté microbienne du sol en fonction d'un gradient de diversité végétale, sans ou avec exclusion partielle de pluie dans la garrigue méditerranéenne du Massif de l'Etoile à Marseille. Cette expérimentation a été réalisée sur le site expérimental du projet CLIMED qui comprend 92 placettes expérimentales présentant toutes les combinaisons possibles de quatre espèces ligneuses dominantes. Les dispositifs d'exclusion de pluie permettaient d'exclure 12% des précipitations, ce qui se traduit par une diminution de 6.5% de la teneur en eau du sol en moyenne sur l'année, avec des effets plus marqués pour de faibles évènements pluvieux.

Plus spécifiquement, j'ai étudié les potentiels cataboliques des communautés microbiennes de sols prélevés sous différents mélanges de litières des quatre espèces ligneuses (*Quercus coccifera, Cistus albidus, Rosmarinus officinalis, Ulex parviflorus*) dominantes, après 1 et 2

années de décomposition. Cette approche a permis de contrôler la quantité et la composition de la litière présente au-dessus des sols analysés en s'affranchissant de l'hétérogénéité spatiale et temporelle de la couche de litière. Les activités microbiennes étudiés étaient le potentiel de décomposition de la cellulose (DCP) et les profils métaboliques de la communauté microbienne du sol pour 15 substrats carbonés (community level physiological profiles, CLPP) en utilisant la technique MicroResp™ (voir chapitre II).

Les activités microbiennes ont été mesurées sur les échantillons de sol prélevés sur le site d'étude après un et deux ans de décomposition des litières, ce qui m'a permis de quantifier l'effet des différents facteurs testés (diversité végétale, identité et dissimilarité fonctionnelle des mélanges de litière, et baisse des précipitations) sur l'activité et la diversité métabolique des communautés microbiennes. Pour cette étude j'ai testé les hypothèses suivantes i) une diversité végétale plus importante (richesse spécifique et dissimilarité fonctionnelle des mélanges de litière) est associées à des communautés microbiennes plus complémentaires qui sont capables de dégrader plus efficacement une large gamme de substrats carbonés et ii) les communautés microbiennes du sol associés à des mélanges de litière plus diversifiés sont également plus résistants à une baisse des précipitations.

Les résultats ont montré que l'exclusion de pluie n'a pas eu d'impact direct sur les paramètres microbiens du sol après un et deux ans de décomposition des litières. Après un an de décomposition, c'est l'identité du mélange de litière (composition, présence de certaines espèces) qui expliquait le mieux l'activité et la diversité métabolique de la communauté microbienne. En revanche, au bout de 2 ans de décomposition, la richesse spécifique (nombre d'espèces présentes dans le mélange de litière) et la dissimilarité de la teneur en composés phénoliques (évaluée par l'entropie quadratique de Rao) ont montré un effet positif sur la

diversité des substrats métabolisés par les microorganismes de sol. En outre, l'activité métabolique globale de la communauté microbienne était plus élevée sous des mélanges de litière plus diversifiés. Une analyse de pistes a permis de montrer que cet effet positif se faisait indirectement via une stimulation de la diversité métabolique (H') des communautés microbiennes associées à des mélanges de litière plus diversifiés.

Cette étude suggère qu'une légère baisse de précipitation annuelle n'affectera pas directement les potentiels cataboliques des microorganismes du sol. Par contre, une altération des ressources en matière organique, par exemple consécutive à un changement de la communauté végétale, va affecter les activités des microorganismes.

Ce troisième chapitre, qui fait l'objet d'un article accepté (Shihan et al. 2017, Biology and Fertility of Soils), ainsi que les deux premiers chapitres seront présenté en anglais sous forme d'article.

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Chapter I

Neighbor effects on growth and survival of Mediterranean plants under severe drought

Ammar Shihan, Florence Volaire, Nathalie Fromin, Stephan Hättenschwiler

Abstract

The predicted decrease in annual rainfall and duration of summer drought in the Mediterranean region may affect plant growth increased and survival. These drought effects are likely modified by the identity and drought strategies of neighbor plants but such interactions and their potential consequences for community composition are not well understood. Here we quantified the effect of a recurrent severe drought on the growth and survival of three common and abundant species of the Mediterranean shrubland depending on neighbor identity. The study compared two drought avoiders and dehydration sensitive woody species *Quercus coccifera* and *Cistus albidus*, and the dehydration tolerant perennial grass species *Brachypodium retusum*. Two individuals of the same species or in all possible combinations of the three species were grown together in rhizotrons in the open but under fully controlled watering regimes for two years. A treatment with three repetitive severe droughts was imposed, and compared to a treatment with only one drought cycle at the end of the experiment. Relative growth rates of shoots and roots in *B. retusum*, but not those of woody species, decreased under drought. Relative growth rates of *C. albidus* also differed according to neighbor plant identity, showing higher growth rates when grown together with *Q. coccifera* than with *B. retusum* or conspecific. Repetitive severe droughts had a negative effect on the survival of *Q. coccifera* and even more so on that of *C. albidus*, while the mortality of *B. retusum* remained unaffected. The survival of *C. albidus* and of *B. retusum*, but not of *Q. coccifera* increased when the neighbor individual was a different species compared to a conspecific neighbor. In comparison with a single drought event, a stronger negative effect of repetitive severe droughts was found on the woody species survival, but not on the grass species survival. Moreover, drought survival of the woody species was significantly affected by the identity of the associated other species that are co-existing in the Mediterranean shrubland.

KEYWORDS:

Climate change; repetitive severe drought; survival; mortality; biomass allocation; inter- vs. intra-specific competition.

1. Introduction

Rising temperatures and changes in precipitation patterns are the two major components of climate change (Gao et al. 2006; Giorgi and Lionello 2008; Field et al. 2014). Climate warming is expected to induce changes in large-scale species distribution patterns with poleward or upward movements of species and also species extinctions (Walther et al. 2002; Parmesan 2006). Specific changes in precipitation can strongly vary at relatively small spatial scales, and are difficult to predict with the current climate models (IPCC 2013). In the Mediterranean area, Hasanean (2004) showed a general decrease in precipitation in the south, a decrease in winter, autumn and in total annual precipitation in the northeast, a slight decrease in annual precipitation in the central north, and a decreased winter precipitation in the central west. Such important regional differences are also supported by studies at lower spatial scales (Alpert et al. 2004; Tošić 2004; Sarris et al. 2007). It is, however, generally acknowledged that changes in precipitation will create more extreme events such as prolonged drought or flooding rather than changes in average annual precipitation (IPCC 2013). For the Mediterranean basin, for example, climate change projections foresee prolonged drought periods during the summer dry season (Gao et al. 2006; Giorgi (2006) Lionello and Giorgi 2007) with an expected increase in the frequency and intensity of drought episodes (IPCC 2013, Fig. 1.1). In fact, compared to the last few centuries, the Mediterranean region appears to have gone through a particularly dry period over the last two decades (Luterbacher et al. 2006; Nicault et al. 2008; García-Ruiz et al. 2011). For example, annual precipitation has already declined over 90.1% of the surface area of the Mediterranean Iberian Peninsula in the last 50 years, in particular during summer and spring (-22.5 and -19.3%, respectively, de Luis et al. 2009). In addition, major ecosystem shifts are predicted for the Mediterranean basin with notably the replacement of most of the deciduous forests by shrubland vegetation under climate change (Guiot and Cramer 2016). As a result,

increasing tree mortality has been reported worldwide for the last decades and may significantly increase (McDowell et al. 2015).

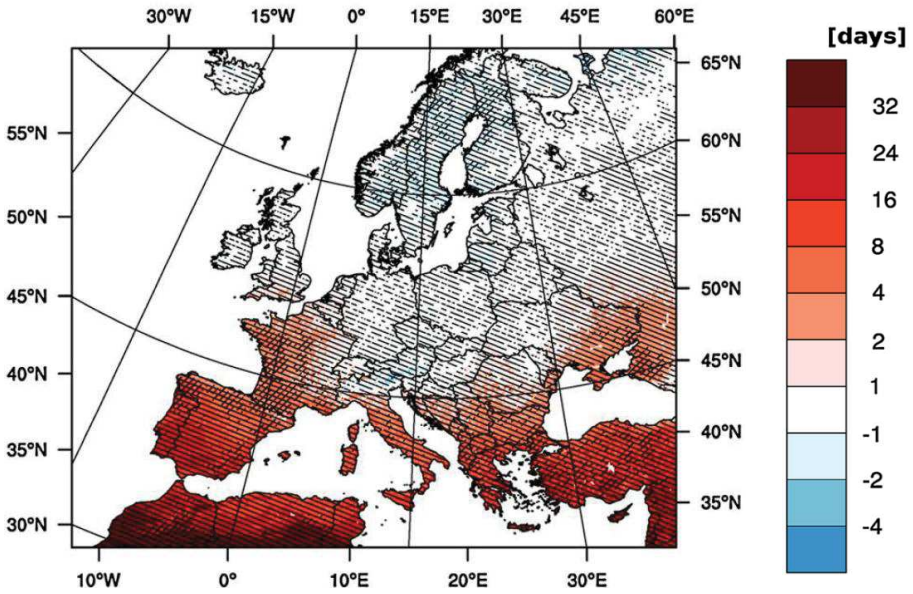

Fig. 1.1. Increased of mean length of dry spells by the end of the century in Europe. Projected changes in the 95th percentile of the length of dry spells (days) for 2071 – 2100 compared to 1971 – 2000. Mean of 20 regional EUROCODEX climate models (extracted from (Jacob et al. 2014).

Even though Mediterranean plants are adapted to extended periods of summer drought and can cope with low soil water availability (Peñuelas 1996; Peñuelas et al. 2001; Micco and Aronne 2012), they may run into a critical stage for survival under excessive drought (Prévosto et al. 2011; Benigno et al. 2014; Louhaichi et al. 2015). Critical thresholds vary among co-occurring plant species with their capacity to survive drought being associated with specific morphological and physiological traits (Grime 2001; Gallé and Feller 2007; Hernandez et al. 2010). While annual plants avoid drought periods by completing their life cycle during the more favorable period of the year and by surviving drought as seeds, most perennial plants may exhibit two types of strategies for delaying or withstanding dehydration under drought conditions (Ludlow, 1989; Tardieu and Simonneau 1998; David et al. 2007; Quero et al. 2011):

i) Dehydration avoidance allows plants to maintain a high water status in leaves for a longer period of time under moderate drought through increased water uptake through a deep and efficient root system or reduced water loss (Volaire et al. 1998; Ferrio et al. 2003). It may allow the maintenance of the photosynthetic activity and limit carbon starvation. However, dehydration avoidance may not be efficient to cope with severe drought since mature leaves are not dehydration tolerant.

ii) Dehydration tolerance under severe drought ensures plant survival at low tissue water content by maintaining cell integrity in the tissues that can survive (for instance shoot meristems) through cell membrane stabilization and accumulation of water-soluble carbohydrates (Volaire et al. 1998; Verslues et al. 2006). This strategy has been well identified in perennial herbaceous species for which lamina senesce and only the meristems at the leaf bases can survive at low hydration for longer than mature tissues (Volaire et al. 2014). In woody species, cellular drought tolerance is also recognized as collimating as hydraulics in determining plant physiological limits (McDowell et al. 2008).

While perennial herbaceous species combine both strategies according to the stress intensity, they survive severe drought through a specific high dehydration tolerance strategy in meristematic tissues, the Mediterranean shrub species such as *Q. coccifera* and *C. albidus* were shown to be mainly dehydration avoiders, with a strategy associated with increased transpiration and stomatal conductance even when the leaf water potential is very low (Damesin et al. 1998; Galmés et al. 2007b). As a result, the mortality of these species is particularly likely during intense droughts (McDowell et al. 2008). It is then interesting to test the effects of these contrasting strategies by comparing drought survival of woody and herbaceous species originating from a similar dry environment. According to the "functional equilibrium hypothesis" (Brouwer 1962) plants in drought-stressed environments are expected to invest

proportionately more in structures involved in water acquisition and transport. The avoidance strategy includes adjustments in biomass allocation patterns by, for example, reducing the total leaf area, which decreases water loss due to transpiration (DeLucia et al. 2000), or by enhancing allocation to root growth, which improves access to limiting soil water (Markesteijn and Poorter 2009). At the community level, enhancing dehydration avoidance appears to be the prime lever to enhancing productivity and stability under drought (Skinner et al. 2006).

Plant individuals in monocultures compete for resources in the same way (Tilman et al. 1997), but co-occurring plants with different strategies to cope with limiting resources lead to higher resource use, and generally enhanced total community biomass (Stevens and Carson 2001). According to the stress gradient hypothesis (Bertness and Callaway 1994), facilitation between species may increase with a water deficit caused by reduced precipitation, due to an increase in positive interactions between species under more stressful conditions. Complementarity is the main mechanism which has been proposed to explain why diversity increases productivity (Hooper et al. 2005). In fact, complementarity influences ecosystem processes in two ways: by favorably shaping species interactions, and by increasing resource use efficiency as a result of niche partitioning (Loreau and Hector 2001). However, according to another hypothesis of spatial competition (Tilman 1994), facilitation may disappear due to increased competitive interactions when resources are limited. In the Mediterranean area, belowground competition for water is an important driver of plant growth (Craine and Dybzinski 2013). During drought events, competition for water among neighboring individuals increases (Gómez-Aparicio et al. 2011), but it remains unclear whether mixed-species are more or less susceptible to drought compared to monocultures (Jucker et al. 2014). While some studies have reported decreased sensitivity to drought for species grown in mixtures (Lebourgeois et al. 2013), others suggested that drought triggers a proportionally greater

increase in competition for water in mixed-species (Grossiord et al. 2014). Theoretical work by Craine and Dybzinski (2013) reconciles these seemingly contrasting results by suggesting that competition for water occurs primarily through a reduction of water availability, favoring species that are best able to deal with high soil water deficits. It follows that drought tolerant species will profit from mixing with other species, in particular with species that are less drought tolerant, and thus compete for a limiting resource over a shorter period of time, while water demanding species will rather suffer in mixtures under dry conditions because they are getting increasingly outcompeted by the species that tolerate lower water availability (Jucker et al. 2014).

Previous research has mainly focused on the consequences of single drought events on plant performance and recovery (Gallé et al. 2007; Gallé and Feller 2007; Galmés et al. 2007a; Xu et al. 2009). From these short-term species-specific responses, predictions were drawn about longer term impacts on the community structure (McDowell et al. 2008). However, multiple recurrent drought events that are likely to increase in frequency with ongoing climate change, are expected to have stronger consequences than single events, with potentially more negative effects on ecosystem resilience and performance (Scheffer et al. 2001). In particular, Mediterranean woody plants with the 'drought avoidant' strategy may be strongly affected by recurrent drought periods (Vilagrosa et al. 2003; Lo Gullo and Salleo 1988).

The understanding of plant responses to repetitive drought events is critical for the assessment of future ecosystem resilience (Watkinson et al. 2003) and for decision making on restoration and conservation programs (Benigno et al. 2014), in particular with the projections of an increase in the duration and recurrence of drought events in Mediterranean ecosystems (Schar et al. 2004; Gao et al. 2006). In this study, we compared three co-existing dominant perennial species from a Mediterranean shrubland, two woody species (*Q. coccifera* and *C.*

albidus), both characterized by a strategy of dehydration avoidance in leaves, and one perennial grass species (*Brachypodium retusum)* with a dehydration tolerance strategy in meristematic tissues. Two individuals of these three species were growing together in custom-made rhizotrons in all possible combinations (i.e. two individuals of the same species or with an individual of the other two species). The rhizotrons were subjected to three repeated severe drought events or to only one drought event in order to quantify the effects of repetitive drought and the impact of intra- and interspecific competition on the growth and survival of these species. Based on their contrasting growth forms and distinct strategies to face drought, we hypothesized that (1) the dehydration avoiders woody species are more affected by repeated drought than the dehydration tolerant grass species, and (2) any drought effect is more pronounced under intra-specific competition than under inter-specific competition, because of complementary water use when associated species strategies differ.

2. Material and Methods

2.1. Plant species selection and rhizotron design

The study was conducted at the experimental station of the Centre of Evolutionary and Functional Ecology (CEFE, CNRS) in Montpellier, France (43°38’19’’N, 3°51’43’’E, 56 m above sea level), from the 5th of December 2013 to the 15th of December 2015. We chose the three plant species *Cistus albidus* L., *Quercus coccifera* L., and *Brachypodium retusum* (Pers.) P.Beauv. All three species are highly abundant and often locally dominant in garrigue-type shrublands in the Mediterranean South of France. *Q. coccifera* is an evergreen woody shrub to small tree, *C. albidus* is a semi-deciduous woody shrub, and *B. retusum* is a rhizomatous perennial grass species of strong vegetative reproduction, but which also reproduces sexually.

All species are fire adapted, *Q. coccifera* and *B. retusum* are typical "resprouters" that replace burnt above-ground biomass with new shoots from belowground structures, and *C. albidus* is a classical "seeder" that recolonizes burnt areas through germinating seeds. With regard to drought tolerance, the two woody shrub species are dehydration avoiders, while *B. retusum* is dehydration tolerant. Individuals of *C. albidus* were obtained from seeds collected in a garrigue in Loupian-Southern France (43°27'52''N, 3°38'35''E, and 59 m above sea level). *Q. coccifera* individuals were obtained from acorns collected in a garrigue of the Massif de l'Étoile, near Marseille, France (43°22' N; 5°25' E, and 275 m above sea level) at the end of November 2013. Only acorns of homogeneous size were selected to limit potential effects of variable seed size on seedling growth. *C. albidus* seeds were grown in tall (0.25 m) 1 L-pots and *Q. coccifera* seeds were grown in tall (0.10 m) 1 L-pots filled with a soil (soil from the experimental station at CEFE):compost (Huminsubstrat N2, Neuhaus, Belgium) mixture (in a 50:50 volume ratio). Pots were kept outside and were regularly watered. One to three *C. albidus* individuals and one *Q. coccifera* individual grew per pot. At time of planting into the rhizotrons, *C. albidus* seedlings were 1-year-old with a mean total biomass of 1.6 ± 0.1 g (n = 10) per seedling, and *Q. coccifera* seedlings were 5 months old with an average total biomass of 1.2 ± 0.08 g per seedling (n = 10). Only visibly healthy individuals of similar size were used for planting. Individuals of *B. retusum* were collected from the same site as *Q. coccifera* directly from well-established *B. retusum* patches by digging out several interconnected ramets. Ramets from the same genets were individually marked and kept in pots filled with soil from the field until planting in the rhizotrons. At planting we cut individual ramets that consisted of a 3 to 5 cm long piece of rhizome with roots and one single shoot with a total average biomass of 0.12 ± 0.02 g (n = 10). Three of these ramets from the same genet were planted per rhizotron, but each rhizotron contained a different genet. For planting, seedlings (*C. albidus* and *Q. coccifera*) and

ramets (*B. retusum*) were carefully separated from the soil and all adhering soil was removed by soaking the root system of each individual plant in a tray filled with water, and brushing off all substrate particles. Each individual seedling and ramet was weighed before planting.

Fig. 1.2. Custom-made rhizotrons used to test the impact of inter- and intra-specific competition under repeated drought.

In order to test the impact of inter- and intra-specific competition under repeated drought we used custom-made rhizotrons. They measured 85 x 34 x 2.3 cm and were made of white PVC plates with a pane of glass on one side (Fig 1.2). The windowpane was light protected with a 1.5 cm thick mobile polystyrene plate. As substrate, we used a mix of sand and the same soil from the experimental station at CEFE used for the multiplication of individuals (see above) in a 50:50 volume ratio. Because the calcareous soil is rich in clay, we added sand to improve watering and infiltration in the only 2.3 cm-wide rhizotrons of an overall small soil surface area, and also to facilitate root growth measurements and root harvest at the end of the experiment. Also we added a layer of 3 cm of small gravel in the bottom of the rhizotron to ensure good water drainage during the experiment. The soil-sand mix was then sterilized at 120

°C for 48 hours three days before planting to avoid emergence of other plants from the soil seed bank. We took three random samples to characterize texture, C, N, P, Ca, Mg, Na, K, Fe, Mn, and Al concentrations, cation exchange capacity (CEC) and pH of the soil-sand mix (Laboratoire d'Analyse des Sols, INRA Arras, France, see Table S1.1). Field capacity of the soil-sand mix was at 23% gravimetric water content, and the wilting point was at 5.1% (unité UR 272, INRA Orléans, France). Each rhizotron was weighed individually and then filled with an equal amount of substrate (8711 ± 59 g).

2.2. Experimental design and drought treatment

Seedlings and ramets were planted at the same two spots within each rhizotron, which have been defined at equal distances from the border and from each other (0.11 m) between the 3rd and 5th of December 2013 (for *B. retusum* and *C. albidus*) and between the 23rd and 25th of April 2014 (for *Q. coccifera*). *Q. coccifera* was planted later because we initially used *Ulex parviflorus* as third species, which however, had too high mortality upon transplanting into the rhizotrons and had to be replaced. We allocated an individual plant (or three ramets in the case of B. retusum) of either the same species or of a different species in all possible combinations (a total of six different plant treatments) to each of the two predetermined spots determined for planting in each rhizotron. Each of the six plant combinations was replicated 12 times for a total of 72 rhizotrons. Four additional rhizotrons without plants were used to quantify water loss through evaporation. The rhizotrons were installed in six blocks (6 plant treatments x 2 drought treatments = 12 rhizotrons each) and placed at an 18° inclination to favor root growth on the windowpane. After each weighing of the rhizotrons (when they were watered – see below), their position was randomized within each block. Rhizotrons were kept at the experimental site of CEFE in Montpellier under a transparent plastic rain shelter (3 m above the ground, resulting

in a reduction of 27% of the photosynthetically active radiation), which was open at the sides to allow aeration. Air temperature and relative humidity under the rain shelter were measured on one hour of frequency for the harvest period (data not shown). To measure changes in soil moisture, one rhizotron by treatment was equipped with soil humidity sensor (WATERSCOUT SM 100, SDEC, France) measuring volumetric soil moisture installed horizontally at two depths (10 and 50 cm from the soil surface), with a measured frequency of one measured per hour (data not shown). At planting, all rhizotrons were watered to reach field capacity. To allow establishment of freshly planted seedlings and ramets, all rhizotrons were kept close to field capacity during 363 days (224 days for *Q. coccifera*). On 3rd December 2014, half of the rhizotrons were assigned to a drought treatment with the other half continuously watered as soon as soil volumetric water content reached 17.5 ± 0.5 % (predefined threshold corresponding to 75 ± 3 % of soil field capacity, Fig. 1.3a).

The drought treatment consisted in two drying cycles reaching 11 ± 0.4% volumetric soil water content (Fig. 1.3b), which is the threshold of all leaves senescence in *B. retusum* under our experimental condition (preliminary test – data not shown). The soil water content in the rhizotrons was determined gravimetrically once per week by weighing each individual rhizotron. Watering was resumed as soon as a particular individual rhizotron reached 17.5% (control treatment) or 11% (drought treatment) soil water content. Each watering event was adjusted to reach field capacity at 23%. After the second drying cycle of the drought treatment a third drying cycle was applied to both control and drought rhizotrons from the respective plant treatment within the same block. After the final drying cycle applied to all rhizotrons (including all controls), each rhizotron was again watered to reach field capacity when the threshold of 11 % of soil water content was reached and maintained at soil water contents higher than 17.5% for three weeks. After these three weeks, the plants were harvested.

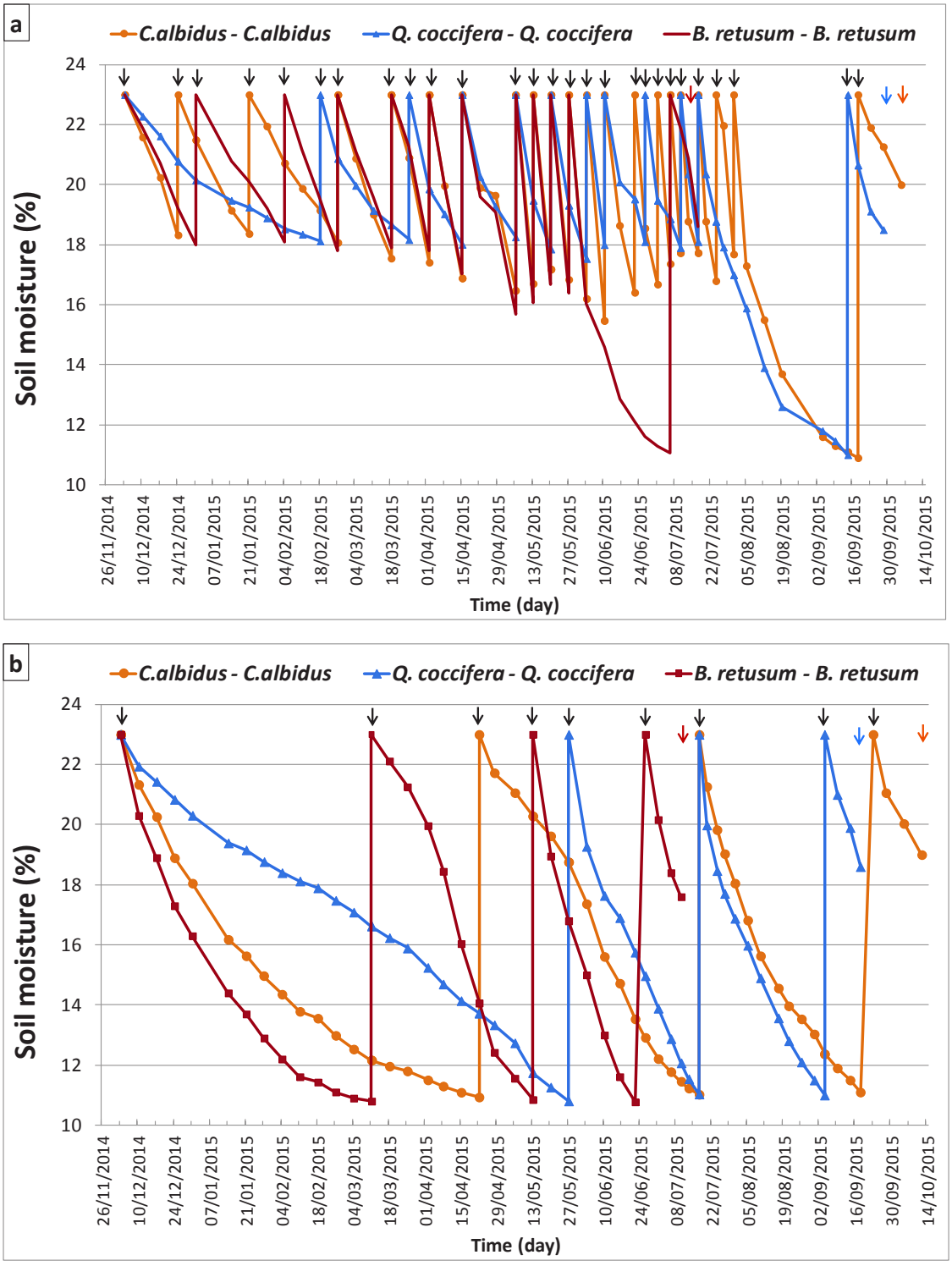

Fig. 1.3. The evolution of soil moisture (%) measured gravimetrically for each individual rhizotron over the entire experimental duration. Data present soil moisture values for three individual rhizotrons, each with a different plant treatment (two individuals of the same of the three plant species) under control (a) and repetitive drought conditions (b). Black arrows indicate individual irrigation events, and colored arrows indicate harvesting date of corresponding rhizotron.

Because individual rhizotrons passed through the three drying cycles at different rates, the final harvest varied between 211 and 377 days after the beginning of the drought treatment.

2.3. Plant harvest

All the rhizotrons were harvested exactly three weeks after the end of the third drying cycle (see above). We cut stems of *C. albidus* and *Q. coccifera* at the level of the soil surface, and separated their shoots into dead leaves, living leaves and stems (including main stems and all branches). The above-ground part of *B. retusum* plants was divided into dead and living stems and the leaves were separated from living stems, but not from dead ones. After shoot harvest, the rhizotrons were placed horizontally and the glass pane was removed. After the soil cores have been taken (see chapter II), roots were separated from the soil by washing under tap water and were separated for the two individuals (with some difficulties when *B. retusum* and *C. albidus* grew together).

2.4. Plant measurements

We measured the total initial biomass (fresh weight) for each individual plant before planting, and we also determined the total dry biomass for a subset of 10 individuals that were dried at 65°C for fresh to dry mass conversion of planted individuals. These same 10 individuals per species were also used to determine leaf, stem, and root biomasses, specific leaf area (SLA, defined as the ratio of leaf area to their dry mass), and leaf area ratio (LAR, defined as the ratio of total leaf area to total plant dry mass) for a characterization of plant biomass allocation before the experiment started. Throughout the entire experiment, plant status was evaluated every two weeks in order to monitor survival of each individual plant. Mortality was

determined visually, when the plants had completely brown and brittle stems and leaves. At the end of every drought cycle, and at the end of the experiment, we measured on surviving plants the height and diameter of stems and the maximal height of ramets (data not presented). Surviving plants were separated into coarse roots, fine roots, stems and leaves upon final harvest (see above). The leaf mass fraction (LMF) and the root mass fraction (RMF) were calculated as a ratio between leaf dry mass or total root dry mass, respectively, and the total plant dry mass. Relative growth rates (RGR, mg g^{-1} day^{-1}) for whole plants, shoots (stems + leaves + floral buds) and roots (main root + lateral roots) were calculated using plant biomass data at planting (*M1)* and at the end of the experiment at harvest (*M2*):

$$RGR = \frac{(M2-M1)/M1}{t2-t1} \times 1000$$

Where (*M2*) stands for the whole plant, shoot or root mass at harvest time ($t2$), and (*M1*) stands for the average of whole plant, shoot or root mass at plantation time ($t1$), and with (*t2-t1)* varying according to harvest date. We measured leaf area and dry mass (at 65 °C) for 15 leaves per plant to calculate specific leaf area (SLA, as the ratio of leaf area to dry mass) and leaf area ratio (LAR, as the ratio of total leaf area to total plant dry mass).

2.5. Data analysis

Plant survival rates were analyzed separately for each target species for the effects of repeated drought (control *vs* drought) and neighbor plant identity using the *survdiff* function of the survival *R* package and the *survfit* function of the same package to plot survival distribution (Kaplan-Meier estimator). Plant growth (RGRs) and biomass allocation (LMF, RMF and LAR) were analyzed separately for each target species to test for the effects of repeated drought and of neighbor plant identity, using multiple linear models, with SS II - ANOVA outputs (unbalanced models and drought x neighbor identity interaction was never significant). As a

result of mortality, the period of presence of individuals that died during the experiment was integrated as the percentage of their total presence as living individuals, and used in model fits as a co-variable. Likewise, the time during which each rhizotron was kept in the experiment (from plantation to harvest, depending on the duration of the drying cycles) was also fitted as a co-variable in the model. Differences between plant parameters were tested using Tukey HSD post hoc comparison tests. Statistical analyses were performed with the R software (version 3.2.4, The R Foundation for Statistical Computing 2016) with significance levels indicated as ‘ for $p < 0.10$, * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. Data are presented as means ± Standard Error.

3. Results

3.1. Plant survival

The three species showed different survival rates irrespective of drought treatment and neighbor plant identity, with the lowest rates in *C. albidus* and the highest in *B. retusum* (likelihood ratio test, Fig. 1.4a). Across all treatments a total of 14 *C. albidus* individuals, 27 *Q. coccifera* individuals and 40 *B. retusum* individuals survived until final harvest. This corresponds to average survival rates of 30% (*C. albidus),* 58% (*Q. coccifera),* and 80% (*B. retusum)* across all treatments. Repeated drought negatively affected *C. albidus* survival ($p = 0.043$, Table 1.1), and, to a lower extent, that of *Q. coccifera* ($p = 0.064$), while *B. retusum* survival did not differ between control and drought treatments (Table 1.1, Fig. 1.4, Fig. 1.5). The identity of the neighbor plant in the rhizotrons also had significant effects on the survival rates for *C. albidus* and *Q. coccifera*, but such an effect was independent of the drought treatment (no significant effect of the drought x neighbor species identity interaction, Table 1.1, Fig. 1.5). However, neighbor plant identity did not affect the survival of *B. retusum* (Fig. 1.5).

Table 1.1. Output of Cox's proportional hazards models (coxph model of survival package) to test for the effect of drought treatment (**Drought**) and neighbor species identity (**Id**) on survival rates of *B. retusum*, *C. albidus* and *Q. coccifera* individuals. We reported Chi-squared and associated statistical significance (**'** $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$), and $\mathbf{R^2}$ and the likelihood ratio test of Cox's proportional hazards models (in bold for significant model). Interactions (Drought X Id) were never significant and are not reported.

	Effect	Df	loglik	Chisq	*p* (>|Chi|)	Likelihood ratio test	R2
C. albidus	Drought	**1**	**-109.567**	**4.09**	**0.043 * ↓**	**26.7**	**0.43**
	Id	**2**	**-98.277**	**22.581**	**<0.001 *** **		
Q. coccifera	Drought	1	-70.530	3.43	0.064 ' ↓	**23.6**	**0.40**
	Id	**2**	**-60.440**	**20.18**	**<0.001 *** **		
B. retusum	Drought	1	-37.912	0.49	0.484	1.94	0.04
	Id	2	-37.187	1.451	0.484		

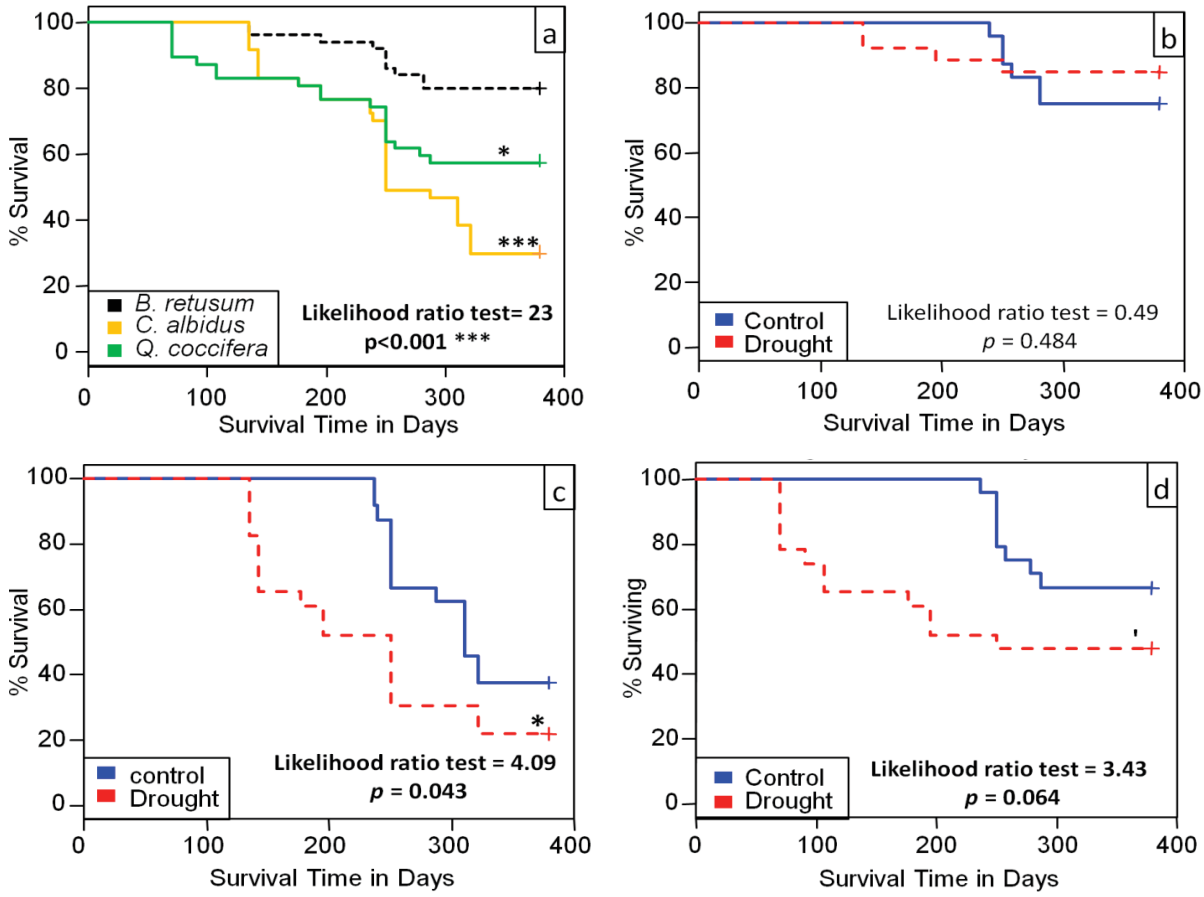

Fig. 1.4. Survival rate distributions of *C. albidus* and *Q. coccifera* (continued lines) compared to that of *B. retusum* (dashed black line) across the two drought treatments and all species identity treatments **(a)**, and survival rate distributions of each of the three species (*B. retusum* **(b)**, *C. albidus* **(c)** and *Q. coccifera* **(d)**) under repeated drought (dashed red lines) compared to the control (continued blue lines) conditions. Asterisks indicate significant statistical differences (**'** p < 0.01, * p < 0.05 and *** p < 0.001 - Likelihood ratio test).

In fact, irrespective of drought treatment, *C. albidus* survival rates were higher when it grew together with a *Q. coccifera* individual compared to when it grew with a conspecific individual (21 vs. 73% survival, respectively, Likelihood ratio test; $p = 0.029$) or with a *B. retusum* individual (8% vs. 73% survival, respectively, Likelihood ratio test; $p = 0.001$) (Fig. 1.5). Irrespective of drought treatment, *Q. coccifera* survival was highest when it grew together with a conspecific compared to when it grew with a *C. albidus* individual (88 vs. 27%, respectively, $p = 0.001$, Likelihood ratio test) or when it grew with a *B. retusum* individual (88 vs. 25%, respectively, $p = 0.001$, Likelihood ratio test, Fig. 1.5).

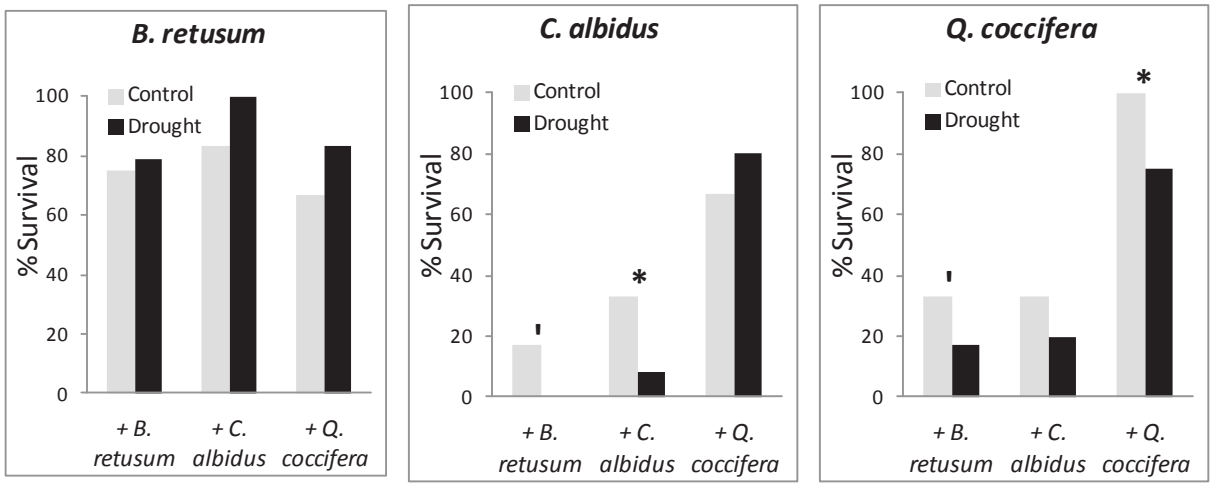

Fig. 1.5. Survival rates of the three studied species at the end of the third drought cycle under repetitive, drought (black bars) and control conditions (grey bars) in presence of conspecific or the other two species. Asterisks indicate significant statistical differences (' $p < 0.1$, * $p < 0.05$ - Likelihood ratio test).

3.2. Plant growth and biomass allocation

On average (± SE) across both drought treatments and all neighbor identity treatments, final plant biomass differed among the three species with the highest biomass measured in *C. albidus* (15.6 ± 2.2 g) followed by *B. retusum* (14.6 ± 1 g) and the lowest biomass in *Q. coccifera* (6.1 ± 0.5 g). Species also differed strongly in total plant RGR ($p < 0.0001$, F-test), mostly driven by the substantially larger RGR measured in *B. retusum* compared to the other two species.

Actually whole plant RGR did not differ between C. *albidus* and *Q. coccifera*. Overall, the relative growth rates of the root system (RGR-R) were greater than those of the shoot (RGR-S, Fig. 1.6), particularly in *C. albidus* with an average 7-fold higher RGR-R compared to RGR-S. In contrast, *Q. coccifera* showed only a 2-fold higher RGR-R compared to RGR-S. However, the differences in RGR between roots and shoots in *Q. coccifera* depended on the drought treatment, with similar root RGR under both treatments, but significantly higher shoot RGR in the drought compared to the control treatment (Fig. 1.6). On the other hand, relative growth rates (RGR-S and RGR-R) were overall higher in the control compared to the drought treatment in *B. retusum* and *C. albidus,* with stronger negative drought effects observed in *B. retusum* (Fig. 1.6).

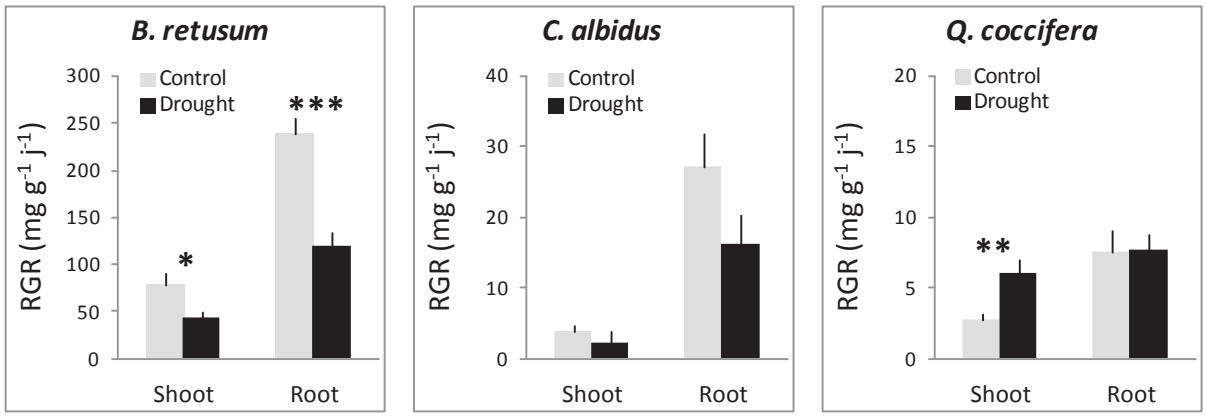

Fig. 1.6. Relative growth rates (**RGR**) of shoots and of roots (mean ±SE) measured for the three studied species grown under control and drought conditions (across all neighbor species identity treatments). Levels of significance from Student *t*-test to test for the effect of drought within species are shown (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

The duration of the presence of individuals (because of mortality during the experiment and replacements of individuals during the establishment phase at the beginning of the experiment) and the age of individual plants (because of the differences in harvest dates) had no impact on measured RGRs of any species, except for a significant negative effect of the age of individual plants on RGR-S for *B. retusum* ($p = 0.047$, Table 1.3): RGR-S was lower in older plants of *B.*

retusum in the drought but not in the control treatment. RGRs of both shoots and roots also sometimes varied according to neighbor species identity. In *C. albidus* both root and shoot RGRs increased when it grew together with *Q. coccifera* compared to when it grew with a conspecific neighbor (Table 1.3, $p = 0.058$ and $p = 0.050$, respectively, Tukey HSD test).

Table 1.2. Final total biomass, biomass fractions and relative growth rates for *B. retusum*, *C. albidus, and Q. coccifera* individuals grown under control conditions or under repetitive drought from December 2014 to December 2015. Mean values (± SE) across all neighbor treatments are shown with *t*-values of Student Test to test for the effect of drought. Values that were statistically different between control and drought treatment ($p<0.05$) are reported in bold.

	B. retusum			***C. albidus***			***Q. coccifera***		
Variable	Control (n=18)	Drought (n=22)	***t*-value**	Control (n=8)	Drought (n=5)	***t*-value**	Control (n=16)	Drought (n=11)	***t*-value**
Total plant mass (g)	**19,7 ±1,2**	**10,5 ±0,9**	**<0,001**	**19,2 ±2,6**	**10,0 ±2,1**	**0,019**	5,4 ±0,5	7,2 ±0,7	0,053
Leaf mass (g)	**3.3 ±0.5**	**1.9 ±0.4**	**0.033**	2.3 ±0.6	1.8 ±0.6	0.588	**0.7 ±0.1**	**1.2 ±0.2**	**0.023**
Stem mass (g)	**2.7 ±0.3**	**1.6 ±0.2**	**0.005**	**6.2 ±0.9**	**2.4 ±0.7**	**0.008**	**0.4 ±0.05**	**0.8 ±0.1**	**0.007**
Root mass (g)	**13,7 ±0,9**	**7,0 ±0,5**	**<0,001**	**10,7 ±1,4**	**5,7 ±1,0**	**0,016**	4,3 ±0,4	5,1 ±0,6	0,229
Leaf mass fraction (g g^{-1})	0.17 ±0.02	0.17 ±0.02	0.788	0.11 ±0.02	0.16 ±0.03	0.213	0.13 ±0.01	0.17 ±0.02	0.066
Root mass fraction (g g^{-1})	0.7 ±0.03	0.69 ±0.03	0.8	0.56 ±0.02	0.59 ±0.04	0.523	**0.78 ±0.02**	**0.71 ±0.03**	**0.048**
Leaf area ratio (cm^2 g^{-1})	15,1 ±2,0	14,9 ±2,0	0,963	6,0 ±1,2	10,1 ±1,9	0,113	6,8 ±0,7	8,4 ±0,8	0,152
Specific leaf area (cm^2 g^{-1})	85.3 ±7.6	98 ±7.9	0.252	69.1 ±11.9	642 ±4.1	0.71	52.1 ±1.4	50.5 ±1.9	0.5
RGR-Total (mg g^{-1} j^{-1})	145 ±10.7	74.9 ±9.3	**<0,001**	8.6 ±1.6	5.5 ±2	0.261	5.5 ±0.7	7.2 ±0.9	0.163

When the neighbor plant was *B. retusum*, only one individual of *C. albidus* survived, which did not permit any statistical analysis. There was also a marginally significant effect of neighbor species identity on shoot RGR of *B. retusum* (Table 1.3), driven by a trend for increased RGR-S of *B. retusum* in the presence of *Q. coccifera* ($p = 0.066$) and of *C. albidus* ($p = 0.097$, Tukey HSD test)). Root and shoot RGRs of *Q. coccifera* were not influenced by the identity of the neighbor plants.

Table 1.3. Output of multiple linear models testing for the effects of drought treatment (**Drought**), neighbor species identity (**Id**) and their interactions (**Drought : Id**) on relative growth rates of shoots (**RGR-S**), relative growth rates of roots (**RGR-R**), leaf mass fraction (**LMF**), root mass fraction (**RMF**) and leaf area ratio (**LAR**). The duration of the presence of seedlings that died during the experiment (**Presence**) and the age of seedlings (determined by harvest date) (**Age**) were fitted as co-variables in the models. *F*-values and the corresponding *p*-values (superscripts) are reported (in bold when *p*-values < 0.05). Arrows indicate the direction of the effect (↓ lower values and ↑ higher values under drought condition as compared to control condition.). (ns = not significant terms, na = not available data).

***B. retusum* (n=40)**	***F*-values (*p*-values)**				
Effect (df)	**RGR-S**	**RGR-R**	**LMF**	**RMF**	**LAR**
Presence (1)	ns	ns	ns	ns	ns
Age (1)	**4.262** **(0.047)** ↓	ns	**5.926** **(0.021)** ↓	**4.471** **(0.042)** ↑	ns
Drought (1)	**6.093** **(0.019)** ↓	**30.424** **(<0.001)** ↓	0.047 (0.829)	0.524 (0.474)	0.001 (0.997)
Id (2)	3.109 (0.058)	0.171 (0.492)	**4.741** **(0.016)**	**6.838** **(0.003)**	1.134 (0.334)
Drought:Id (2)	1.354 (0.272)	0.555 (0.70)	2.043 (0.146)	2.394 (0.107)	1.142 (0.331)
***C. albidus* (n=13)**					
Presence (1)	ns	ns	ns	ns	ns
Age (1)	ns	ns	ns	ns	ns
Drought (1)	**5.509** **(0.044)** ↓	**6.390** **(0.032)** ↓	1.042 (0.334)	0.939 (0.358)	2.345 (0.160)
Id (2)	**6.275** **(0.034)**	**5.606** **(0.042)**	0.327 (0.582)	0.733 (0.414)	2.807 (0.128)
Drought:Id (2)	na	0.229 (0.644)	0.362 (0.582)	0.228 (0.645)	1.770 (0.216)
***Q. coccifera* (n=27)**					
Presence (1)	ns	ns	ns	ns	ns
Age (1)	ns	ns	ns	ns	ns
Drought (1)	**13.431** **(0.002)** ↑	0.009 (0.924)	3.963 (0.060) ↑	**4.404** **(0.048)** ↓	2.409 (0.136)
Id (2)	1.213 (0.319)	1.451 (0.257)	0.015 (0.985)	0.577 (0.57)	0.241 (0.788)
Drought:Id (2)	1.238 (0.312)	2.255 (0.130)	1.614 (0.223)	0.264 (0.771)	1.975 (0.164)

The leaf mass fraction (LMF) was higher, and the root mass fraction (RMF) was lower in *Q. coccifera* after repeated drought compared to control condition (Table 1.2 and 1.3), but the repetitive drought did not affect LMF nor RMF in the two other species (Table 1.2 and 1.3). Longer presence of *B. retusum* individuals decreased LMF and increased RMF (Table 1.3).

LMF and RMF of *B. retusum* also differed depending on the identity of the neighbor plant (Table 1.3), with the presence of *Q. coccifera* and *C. albidus* associated to increased LMF and decreased RMF of *B. retusum* as compared to when the neighbor plant was a conspecific (p = 0.024 and 0.029, respectively for LMF, and p = 0.019 and 0.004 for RMF, Tukey HSD test, Fig. 1.7). LMF and RMF of *Q. coccifera* and *C. albidus* were not influenced by the identity of the neighbor plants. Regarding the morphological parameters, the LAR did not vary according to the drought treatment or to the neighbor species identity.

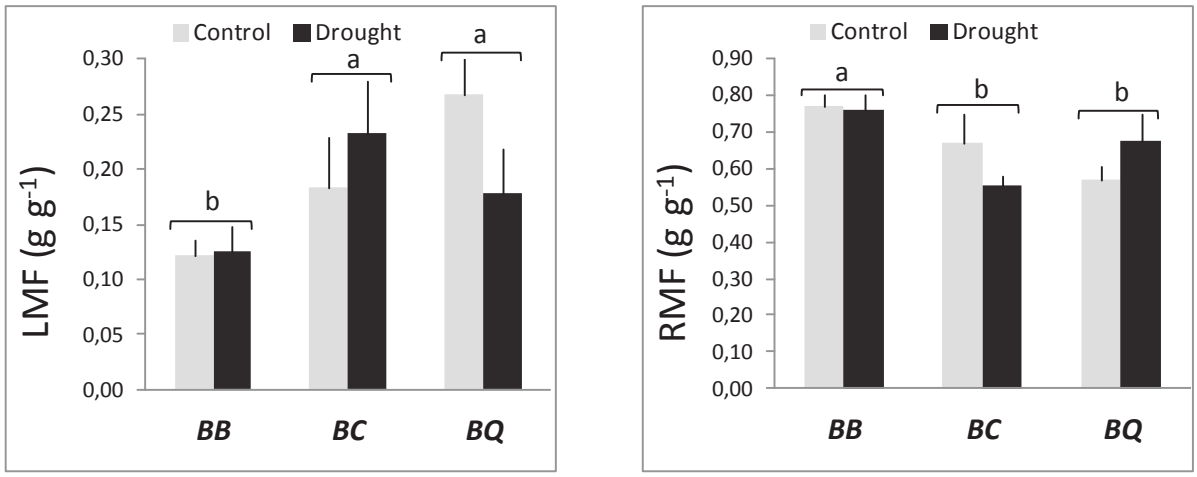

Fig. 1.7. Leaf mass fraction (**LMF**) and root mass fraction (**RMF**) (mean ±SE) measured for *B. retusum* studied grown under control (grey bars) and drought (black bars) conditions in presence of conspecific or the other two species. Small letters indicate significant statistical differences from Tukey HSD test.

4. *Discussion*

We addressed the question of how three abundant co-occurring plant species, which are typical for Mediterranean shrublands, respond to repeated severe drought events. In contrast to most previous studies that applied drought events for a defined period of time (e.g. Miyashita et al. 2005; Zang et al. 2014), we chose here to control drought based on individual rhizotron-specific soil water content in order to achieve identical soil water conditions across all rhizotrons. This allowed us to expose plants to the same level of water stress while accepting that the time it

takes to reach this level may differ among rhizotrons. We set the end of each drought cycle at a soil water content of 11% (Fig. 1.3b) beyond which *C. albidus* was not recovering anymore, with a field capacity of 23% and a wilting point of 5.1% volumetric soil water content. The control treatment was maintained at soil water contents higher than 17.5%, which was also followed for each rhizotron individually by weekly measuring individual rhizotron weight and adding the respective amount of water to reach field capacity (Fig. 1.3a).

The three studied species strongly differed in their overall survival rates during the whole experiment. According to our initial hypothesis, the dehydration tolerant grass *B. retusum* showed the highest survival rates with 80% of initially planted individuals alive at the end of the experiment. Moreover, *B. retusum* survival did not differ in rhizotrons exposed to three repetitive drought cycles compared to the control rhizotrons with only one drought cycle towards the end of the experiment. These results suggest that *B. retusum* can cope well with severe drought even if it occurs repeatedly within a comparatively short period of time.

Mediterranean grass species commonly delay dehydration by increasing water uptake, but they can also survive drought conditions by diminishing water loss (Volaire et al. 2009; Volaire et al. 2014). A previous study showed that *B. retusum* minimized water loss and survived drought with a low baseline stomatal conductance rate when growing together with the Mediterranean shrub *Rosmarinus officinalis* (Clary et al. 2004). The perennial grass, *B. retusum*, responded very quickly to water addition after drought, recovering its water potential at a rate four times higher than that of *R. officinalis*, what may contribute to its competitive success (Clary et al. 2004). In another study, Caturla et al. (2000) showed that *B. retusum* had a high capacity to recover after disturbances (e.g. fire). Both studies support our results of high survival despite of repeated severe drought and of high competitiveness under drought of the grass *B. retusum*. In our study, we observed a decrease of relative growth rates of both shoot (RGR-S) and root

(RGR-R) in *B. retusum* in response to repeated drought, but with no effects on survival (Table 1.3). This suggests that through an adjustment in growth rate, and in particular leaf senescence (personal observations), *B. retusum* adapts to severe drought events. The survival of meristematic tissues under severe drought, while a large part of leaves dry out, followed by rapid growth when conditions are improved, ensures the survival of most tillers as shown in many Mediterranean ecotypes of perennial species (Volaire et al. 2009; Poirier et al. 2012; Pérez-Ramos et al. 2013).

In contrast to the grass *B. retusum*, the two woody shrub species showed lower survival, with *C. albidus* showing the lowest survival rates (30%) of all three species. According to their dehydration avoidance strategy, the two woody species keep their leaves green and photosynthetically active under moderate drought. However, an adjustment in their leaf area by dropping leaves under high water limitation was found, as often observed in Mediterranean woody plants during summer drought (Hoff and Rambal 2003; Baldocchi and Xu 2007; Limousin et al. 2009). Leaf shedding may protect from hydraulic stress although this effect depends on species and environments (Wolfe et al. 2016). In addition, a deeper growing root system, and thus higher relative allocation to root biomass, may allow maintaining their water uptake, and hence plant water status under water limiting conditions while keeping the stomata open (Dodd et al. 1984; Veneklaas and Poot 2003). Root mass fraction (RMF) was slightly higher in only one of the two woody species (*Q. coccifera*) compared to the grass *B. retusum* in our study (Table 1.2). RMF decreased in response to repeated drought in *Q. coccifera*. Surprisingly, *Q. coccifera* was rather showing lower RMF and higher LMF in the drought treatment compared to the control treatment (Table 1.2 and 1.3). Leaf area ratio (LAR) was distinctively lower in the two woody species compared to *B. retusum* as expected. Neither of the two woody species showed a decrease in LAR in response to repeated drought as could

have been expected. Dehydration avoidance can be achieved by increasing the below ground biomass allocation and by decreasing the shoot evaporating surface (Niinemets 2006). Under drought, among seven Mediterranean woody species, *Quercus* species (*Q. coccifera*, *Q. faginea* and *Q. ilex* subsp. *ballota*) exhibited higher RMF and lower LMF and LAR than the rest of species. But these *Quercus* species did not exhibit the best performance under drought (Sánchez-Gómez et al. 2008). In agreement with these results, *Q. coccifera*, although displaying the highest RMF and the lowest LAR among the three tested species, survived poorly, especially compared to *B. retusum*. In fact, *Q. coccifera* individuals in our study had the lowest total dry mass (Table 1.2). The total dry mass has been linked positively to drought tolerance (Coomes and Grubb 2000). Large seedlings may offer some advantages under drought (e.g. a larger RGR and a larger root system which could allowed a preferential access to limiting water) (Coomes and Grubb 2003). Neighbor plant size had been shown to be more important than neighbor species identity to affect growth performance of some woody species grown in mixtures (Lübbe et al. 2015). In contrast to our results, LMF of eight Mediterranean woody species had been positively correlated with drought resistance in a community field experiment with 35% of rain exclusion (Matías et al. 2012). In the same study, LAR had been showed to negatively correlate with drought resistance, whereas there was no correlation between RMF and drought resistance (Matías et al. 2012). In another study, low total dry mass and high LMF, which together result in a water inefficient allocation pattern, were associated with low performance under drought in the woody species *Pistacia terebinthus* (Sánchez-Gómez et al. 2008). In line, *C. albidus* with low LMF and LAR, but with a total dry mass four times higher than *Q. coccifera* and comparable to that of *B. retusum,* had the lowest survival rates in both control and drought conditions. In our study *C. albidus* also had the lowest RMF which could to be more important in drought condition than high total biomass.

The species identity of the second individual within the rhizotron affected some of the plant growth, biomass and allocation parameters. *B. retusum* was the only species that showed no difference in survival rate as a function of its neighbor species identity. However, the relative growth rates of its shoot and biomass allocation to roots and leaves were influenced by the identity of the neighbor plant species (Table 1.3). Benefiting from his neighbor, *B. retusum* increased its shoot growth rates when grown together with either *C. albidus* or *Q. coccifera*, independently of drought treatments, and this effect was mainly driven by the presence of *Q. coccifera*. Such an effect was supported by an increase in both root and leaf biomass allocations (RMF and LMF). Globally, *B. retusum* survived drought well irrespective to neighbor species identity. *Q. coccifera* survival was affected by species identity of its neighbor with lower survival rates when grown with another species compared to with a conspecific neighbor (Fig. 1.5). A most likely explanation is competitive exclusion by the overall larger individuals of *B. retusum* and *C. albidus*, with larger root systems and higher RGR of their roots as compared to *Q. coccifera*. Larger plants and a larger root system may have allowed a preferential access to limiting water by *B. retusum* and *C. albidus* growing with *Q. coccifera*. In line we observed that soil moisture decreased at higher rates in the mono-specific rhizotrons containing *B. retusum or C. albidus* compared to those with *Q. coccifera* (Fig. 1.3), especially during the first drought cycle that coincided with the highest *Q. coccifera* mortality in the drought treatment (Fig. 1.4a, d). In mixed rhizotrons, the grass probably consumed available soil water faster than the two woody species could, recovering high water potential rate (Clary et al. 2004). *C. albidus* survival was affected by neighbor species identity, with lowest rate when grown with *B. retusum* (0% and 17% in drought and control conditions, respectively), and highest survival rates when growing with *Q. coccifera*, especially in drought conditions (Fig. 1.5). *C. albidus* RGR was affected by plant neighbor identity, and this effect was mainly driven by the presence

of *Q. coccifera,* which increased both root and shoot RGRs as compared to C-C treatment. These results refer, again, to a lower competition and facilitation effects of *Q. coccifera* on *C. albidus* survival and growth. As mentioned previously, small individuals of *Q. coccifera* open the opportunity to larger ones of *C. albidus* to benefit, especially in drought conditions, because a larger total mass (Coomes and Grubb 2003; Lübbe et al. 2015). Finally, having a highest RGRs rates, the grass species excluded *C. albidus* individuals, especially in drought condition where any *C. albidus* individual succeeded to survive until the end of the experiment (Fig. 1.5).

In another study analyzing the competition interaction between *B. retusum* and the shrub *R. officinalis*, Clary et al. (2004) underlined the capacity of the perennial grass to consume available soil water before the shrub. Furthermore, *B. retusum* was also able to respond very quickly to water addition after drought, thus reducing the availability of water to slower-responding plants growing in its competitive sphere (Clary et al. 2004). Mediterranean grass species were shown to successfully compete with tree seedlings (Harrington 1991; Rey et al. 2003; Rey et al. 2005). In a diversification forests experience, *B. retusum* was identified as playing a detrimental role on *Q. ilex* and *Q. pubescens* seedling survival (Prévosto et al. 2011). In another study Caturla et al. (2000) showed that *B. retusum* has a high capacity to recover after disturbances. Studying the effect of fire severity on seedling establishment in *Pinus halepensis*, Pausas et al. (2003) observed a positive relationship between pine seedling mortality and *B. retusum* cover at small scale after 8 and 13 months of three class fire severity. These studies support our results that showed the grass *B. retusum* as a good competitor when growing with woody species. Survival rates of the two woody species (*C. albidus* and *Q. coccifera*) decreased significantly with *B. retusum* presence, especially in drought conditions (Fig. 1.5) showing a strong competition capacity in limited conditions.

In line with our results, in control conditions experiment, Benigno et al. (2014) identified biphasic drought as a significant factor limiting the establishment of trees species in south-west Australia. Other studies have shown that, compared to one drought event, repetitive drought events have equal impacts on plant functional traits (Liu et al. 2010). This difference is likely to be the result of the high intensity of the drought imposed in our study, which reflects the harsh field conditions experienced by the species, compared with more moderate stresses imposed in other studies.

Finally, our results suggest that both drought treatments and plant species composition impact the response of plant growth and survival in the studied shrubland, as the dominant species are not equally susceptible to repetitive severe droughts. Species composition could even have a stronger effect on growth and survival than repetitive severe droughts. Threat on biodiversity in the Mediterranean shrubland ecosystem could then have significant implications for the future evolution of the biodiversity in such plant communities under a drying climate.

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Chapter II

Belowground responses of three Mediterranean plant species to severe drought

Ammar Shihan, Nathalie Fromin, Stephan Hättenschwiler

Abstract

Plant responses to increasing water limitation as a result of the predicted changes in precipitation patterns in the Mediterranean region differ among species and depend also on competitive interactions. While the plant root system plays a particularly important role in how plants are affected by drought, growth and functional characteristics of roots in response to changes in water availability are poorly documented in the existing literature. In this study we evaluated the consequences of repeated severe drought on root morphological traits of three common Mediterranean shrubland species exposed to inter- and intraspecific competition. We also analyzed the metabolic diversity of microorganisms in root associated soil. The study compared two drought avoiders woody species *Quercus coccifera* and *Cistus albidus*, and the dehydration tolerant perennial grass species *Brachypodium retusum*. Two individuals of the same species or in all possible combinations of the three species were grown together in rhizotrons under controlled watering regimes for two years in a rainout shelter. A treatment with three repetitive droughts was imposed, and compared to a treatment with only one drought cycle at the end of the experiment. Interestingly, root morphological traits of all three species were more affected by the species identity of the neighbor individual than by repeated drought. Root traits of the grass *B. retusum* differed more as a result of repeated drought and neighbor plant than those of the anisohydric woody species. Specific root length (SRL) of *B. retusum*, but not of the other species, correlated positively with survival rates. The community level physiological profiles (CLPPs) of root associated soil microbial communities did not differ between drought treatments and were also not affected by plant species identity. Our results suggest that the identity of neighbor plants have a stronger effect on species-specific root traits than repeated severe drought, and that only the dehydration tolerant species may modify root traits with concomitant increasing survival. Plant species composition of Mediterranean shrublands may thus determine how particular species respond to drought even more than the direct drought effects.

Keywords:

Climate change; repetitive severe drought; root functional traits; soil microbial activity; inter- vs. intra-specific competition.

1. Introduction

The Mediterranean climate gets drier as suggested in two studies comparing recent climatic conditions with those over the last four centuries (e.g. (Luterbacher and et al. 2006; García-Ruiz et al. 2011)). This overall trend towards a drier climate leading to more severe summer drought and/or less precipitation during the wetter parts of the year (Gao et al. 2006; Giorgi and Lionello 2008; Field et al. 2014) may have important consequences for the structure and function of Mediterranean ecosystems already characterized by a relatively long summer.

The capacity of plant species to survive drought is associated with specific morphological and physiological traits conferring drought resistance, such as lower surface area, deeper root system, higher root allocation and higher specific root length (Grime 2001; Gallé and Feller 2007; Hernandez et al. 2010). These traits are associated to three major ways of how plants adapt to drought. First, the annual life form allows to escape drought by completing the life cycle during the wetter part of the year and surviving drought as seeds in a state of dormancy (Kreeb et al. 1989). Second, plants that avoid drought are characterized by physiologically adjust to occurring drought by increasing their water-use efficiency by reducing water loss or by increasing their water uptake (Volaire et al. 1998; Ferrio et al. 2003) which could be achieved by reducing transpiration, reduced vegetative growth, or increased root growth to deeper, moisture soil layers. It may allow the maintenance of the photosynthetic activity and limit carbon starvation. However, dehydration avoidance may not be efficient to cope with severe drought since mature leaves are not dehydration tolerant and most woody species cannot survive when leaves are entirely senescent. Finally, tolerating drought is a third type of adaptation to withstand drought. Plants that are tolerating dehydration maintain growth during moderate drought and reduce damage in meristematic tissues under severe drought through cell membrane stabilization and accumulation of water-soluble carbohydrates (Volaire

et al. 1998; Verslues et al. 2006). This strategy has been well identified in perennial herbaceous species for which lamina senesce and only the meristems at the leaf bases can survive at low hydration for longer time than mature tissues (Volaire et al. 2014). In woody species, cellular drought tolerance is also recognized as colimiting as hydraulics in determining plant physiological limits (McDowell et al. 2008).

However, despite a relatively clear understanding of these major strategies to survive drought, some key features like specific responses of root activity and growth root to drought events are comparatively poorly studied. For example, while traits of aboveground plant organs, especially those related to leaf physiology and phenology, are well documented in the literature (Diaz and Cabido 1997; Garnier et al. 2001; Reich et al. 2003; Wright et al. 2005), root traits are were much less assessed, also because of the obvious reasons of difficult access to the root system (e.g. (Poorter and Markesteijn 2008; Hernández et al. 2010)). A powerful way to avoid dehydration during drought is mediated by a rapidly growing root system, and thus a high soil exploration capacity, allowing the plant to grow roots to deeper soil layers with higher moisture levels (Lloret et al. 1999; Paula and Pausas 2011). In addition to root growth rate, root functional traits such as rooting depth, root mass fraction, root diameter, specific root length, and root tissue density are expected to be the cornerstones of dehydration avoidance because they all play a critical role in water acquisition (Hernández et al. 2010; Pérez-Ramos et al. 2013). Rooting depth and the root mass fraction in deep soil layers provide a general characteristic of how well deep soil layers can be exploited by a plant, while root morphological traits provide more precise information on how plants adapt for the uptake of limiting water at a given location within the soil profile (Kembel and Cahill 2005). Plant roots can respond to reduced rainfall or water shortage by increasing both root length density (Padilla et al. 2015) and the overall length of fine roots (Hertel et al. 2013), which both

ultimately translates into more biomass allocation to the root system (Markesteijn and Poorter 2009; Padilla et al. 2009). However, independently of the capacity of a given species to grow roots, dry soils make it physically difficult for roots to penetrate the soil (Clark et al. 2003; Whitmore and Whalley 2009), a situation that is typically encountered in Mediterranean ecosystems during the dry season (Martínez-Vilalta et al. 2003; Padilla and Pugnaire 2007). Soil desiccation increases resistance to soil penetration by roots, which renders more difficult the soil exploration by fine roots, and thus, water absorption (Whitmore and Whalley 2009; Bengough et al. 2011). One of the most relevant root traits related with soil exploration and resource uptake is the specific root length (SRL; the root length achieved per unit of root biomass invested), which in turn depends on root diameter and/or tissue density (Wright and Westoby 1999; Nicotra et al. 2002). A high SRL implies a high surface:volume ratio, which is usually negatively correlated with the amount of carbon invested per unit root length, and which maximizes the root–soil interface and hence the root absorption potential (Larcher 2003). Moreover, thin roots with high SRL offer less resistance to the radial flow of water, thus increasing radial conductivity (Huang and Eissenstat 2000). In addition, plants with high SRL tend to show higher root hydraulic conductance per unit leaf surface area (Peman et al. 2006) or per stem cross-section area (Hernández et al. 2010). Consequently, plants with high SRL show high uptake rates of water (Eissenstat 1991), nitrogen (Reich et al. 1998) and phosphorus (Comas et al. 2002).

Plant individuals in monocultures compete for resources in the same way (Tilman et al. 1997), but co-occurring plants with different strategies to cope with limiting resources lead to higher resource use (Stevens and Carson 2001). According to the stress gradient hypothesis (Bertness and Callaway 1994), facilitation between species may increase with a water deficit caused by reduced precipitation, due to an increase in positive interactions between species under more

stressful conditions. Mixing plant species with contrasting root traits, i.e increasing root functional diversity, could improve water uptake capacity over the entire soil profile, especially through a better use of deep soil water which remains available longer under drought. However, according to another hypothesis of spatial competition (Tilman 1994), facilitation may disappear due to increased competitive interactions when resources are limited.

Most often, root growth patterns and structural traits are quantified without distinguishing between different soil depths. However, root biomass and density generally decrease with soil depth (Vanguelova et al. 2005; Makita et al. 2010) leading also to lower fine root density, that is often associated to lower SRL as well (Zhou and Shangguan 2007; Makita et al. 2010). Gaining knowledge on deep root systems and testing whether root functional traits are similar for shallow and deep roots globally would help us to understand how plants cope with reduced water availability (Prieto et al. 2015). There are theoretical reasons to expect different trait expressions in roots in the top soil compared to roots in deeper soil layers (Schenk 2008). In surface soil layers, different factors, including comparatively higher nutrient concentrations (Jobbágy and Jackson 2001; Arndt et al. 2004), and higher water availability during small precipitation events may favor the proliferation of roots with higher SRL, and thus a higher capacity of resource uptake. In contrast, roots in deeper soil layers ensure plant anchorage and provide often a safety investment for water uptake during dry spells because of a generally higher and more long term water availability in deeper layers (Laclau et al. 2013). Roots in deeper soil layers are exposed to oxygen deficiency and less fluctuating soil temperatures (Pregitzer et al. 2000). In consequence the traits of roots growing in deep soil layers are expected to be associated with higher persistence through time and conservation of resources (i.e. higher root tissue density and lower root diameter). These differences in abiotic

conditions between surface and deep soil layers may lead to contrasting suites of traits with depth since root traits are highly plastic and respond to heterogeneous resource distributions (Hodge 2004; Ostonen et al. 2007).

Through their roots, but also through the modification of microclimatic conditions and the production of dead organic matter deposited on and in the soil, plant communities affect soil microorganisms and the processes they drive (Grayston et al. 1998; Eviner and Chapin 2003). Soil processes may be relatively well predicted with plant functional traits characterizing plant growth strategies in terms of resource acquisition or conservation. In fact, plant species with fast growth rates, and high leaf-level N concentrations and SLA tend to favor fast growing microorganisms, *i.e.* bacteria rather than fungi (Orwin et al. 2010). In contrast, slow growing plant species that tend to have a resource conservation strategy with for example rather low leaf N concentrations, small SLAs and high dry matter contents rather promote microbial food webs with slow and conservative nutrient cycling that are more dominated by fungi (Grigulis et al. 2013). Despite their important role in soil functioning and in water uptake, belowground plant functional traits remain poorly quantified compared to leaf functional traits (Legay et al. 2014; Reich 2014). The few existing studies that compared leaf and root traits across a number of species, however, reported that traits characteristic for resource conservation or acquisition correlated well between leaves and roots (Birouste et al. 2012). We thus would expect that species with high SRL are favoring a metabolically active microbial community, while species with low SRL are favoring a microbial community of lower activity

In addition to these plant species driven impacts on soil microbial communities via their roots, microbial communities are susceptible to change in response to changing climate either directly through, for instance, reduced water availability (Bérard et al. 2015), or indirectly through alteration of the vegetation. These modifications in the soil microbial community may

then affect soil processes that are relevant for the cycling of carbon and nitrogen (Hooper et al. 2005; Bardgett et al. 2008; De Deyn et al. 2009). It is thus of paramount importance to understand how changes in vegetation structure impact soil microbial communities and soil processes under ongoing climate change, such as reduced precipitation in the Mediterranean area.

In this study, we compared three co-existing abundant perennial plant species from a Mediterranean shrubland, the two woody species *Q. coccifera* and *C. albidus* known for their dehydration avoidance strategy, and the perennial grass species *Brachypodium retusum*, that rather has dehydration tolerant behaviour. We aimed to quantify the effect of severe repetitive drought events on plant root traits and on the community level physiological profiles (CLPPs) of root associated soil microbial communities in two soil depths. We grew these species in custom-made rhizotrons allowing to monitor root growth dynamics [{1}]. In each rhizotron we planted two individual plants either of the same species or in all possible combinations of the three species. This allowed us to test the relative importance of intra- versus inter-specific competition for root growth and root traits and their consequences for soil microbial substrate utilization pattern (MicroResp™). Based on the species-specific strategies to cope with drought (dehydration tolerance strategy for the grass species compared to dehydration avoidance for the woody species), we hypothesized that (1) repetitive drought affects root morphological traits of the grass species more than those of the woody species, and that in consequence soil microbial communities are more affected in the soil with grass roots than in the soil with woody shrub roots, and (2) this effects will be more pronounced under more intense intraspecific than interspecific competition for water.

{1} ***I have chosen to use rhizotrons for different reasons (see chapter IV: General Discussion): the possibility to follow the growth of roots and their responses to drought and plant neighbor identity was the most important one. However, root dynamics data is not presented here but is proposed as a short term perspective before submitting the chapter to a peer-reviewed journal.***

2. Material and Methods

2.1. Plant species and rhizotron construction

We chose the two woody shrub species *Cistus albidus* L. and *Quercus coccifera* L., and the perennial grass species *Brachypodium retusum* (Pers.) P. Beauv., three common and abundant plant species in the Mediterranean shrubland ecosystem of Southern France. These three species represent different growth forms and strategies to cope with drought. *Q. coccifera* and *C. albidus* are woody shrubs common in the Mediterranean garrigue of usually short stature of about 1 m, but *Q. coccifera* can reach 3 to 4 meters in height without regular disturbance (e.g. fire). *Q. coccifera* and *C. albidus* are evergreen species, but *C. albidus* sheds its leaves earlier during summer drought than *Q. coccifera*, and may thus be considered as semi-deciduous. *B. retusum* is a perennial, mostly evergreen (because new leaves grow constantly) grass that is very abundant in the Mediterranean garrigue. It is of short stature with a height of about 20 to 50 cm, and has highly branched ramets that form tussock-like patches. *B. retusum* combines dehydration avoidance under moderate drought and dehydration tolerance under severe drought whereas the two woody species are mainly dehydration avoidant. Individuals of *C. albidus* were obtained from seeds collected in a garrigue in Loupian - Southern France (43°27’52’’N, 3°38’35’’E, 59 m a.s.l.) at the end of summer in 2012. *Q. coccifera* individuals were obtained from acorns collected in a garrigue of the Massif de l’Étoile, near Marseille, France (43°22’ N; 5°25’ E, 275 m a.s.l.) at the end of November 2013. Only acorns of homogeneous size were selected to limit potential effects of variable seed size on seedling growth. *C. albidus* seeds were grown in 0.25 m tall 1L-pots and *Q. coccifera* seeds were grown in 0.10 m tall 1 L-pots filled with a soil:compost (Huminsubstrat N2, Neuhaus, Belgium) mixture (in a 50:50 volume ratio). The soil, collected at the CEFE experimental field, is clay loam (chromic cambisol, FAO classification) with low organic matter content

(1.8-2.5 %) and a pH of 8.2. Pots were kept outside under natural climatic conditions and were regularly watered. One to three *C. albidus* individuals and one *Q. coccifera* individual grew per pot. At time of planting into the rhizotrons, *C. albidus* seedlings were 1-year-old with a mean total biomass of 1.6 ± 0.1 g (n = 10) per seedling, and *Q. coccifera* seedlings were 5 months old with an average total biomass of 1.2 ± 0.08 g per seedling (n = 10). Only visibly healthy individuals of similar size (each individual was weighed before planting) were used. Individuals of *B. retusum* were collected from the same site as *Q. coccifera* directly from well-established *B. retusum* patches by excavating several interconnected ramets. Ramets from the same genets were individually marked and kept in pots filled with soil from the field until planting in the rhizotrons. At planting we cut individual ramets that consisted of a 3 to 5 cm long rhizome with roots and one single shoot with a total average biomass of 0.12 ± 0.02 g (n = 10). Three of these ramets from the same genet were planted per rhizotron, but each rhizotron contained a different genet. For planting, seedlings (*C. albidus* and *Q. coccifera*) and ramets (*B. retusum*) were carefully separated from the soil, and all adhering soil was removed by soaking the root system of each individual plant in a tray filled with water and brushing off all substrate particles. Each individual seedling and ramet was weighed before planting.

We used custom-made rhizotrons for planting, which measured 85 x 34 x 2.3 cm and were made of white PVC plates with a pane of glass on one side to facilitate visual assessment of root growth. The windowpane was kept dark with a 1.5 cm thick polystyrene plate that could be removed for root observations. As substrate we used a mix of the same calcareous Mediterranean soil as for pot culture (see above) and calcareous sand (in a 50:50 volume ratio). We added sand to improve water infiltration and to facilitate root growth measurements and root harvest at the end of the experiment since the natural soil has a high clay and loam content, and a layer of 3 cm of small gravel in the bottom of the rhizotron to ensure good

water drainage during the experiment. The soil-sand mix was sterilized at 120 °C for 48 hours three days before planting to avoid emergence of other plants from the soil seed bank. In consequence this also removed the largest part (but not all) of the soil microorganisms. We took three random samples to characterize texture, C, N, P, Ca, Mg, Na, K, Fe, Mn, and Al concentrations, cation exchange capacity (CEC) and pH of the soil-sand mix (Laboratoire d'Analyse des Sols, INRA Arras, France, see Table S1.1). The field capacity and wilting point were determined at the UR 272, INRA Orléans, France, and were at 23% and 5.1% of total soil volume, respectively (Table S1.1). Each rhizotron was weighed empty individually and then filled with an equal amount of substrate (8711 ± 59 g), and weighed again individually.

2.2. Experimental design and drought treatment

Seedlings or ramets were planted in the rhizotrons at the same two spots, which have been defined at equal distances from the border and from each other (0.11 m). *B. retusum* and *C. albidus* were planted between the 3rd and 5th of December 2013, and *Q. coccifera* was planted between the 23rd and 25th of April 2014. Two planting periods were not ideal, but were unavoidable due to high mortality of *Ulex parviflorus* seedlings that have initially been planned as the third species for our experiment. The two planting spots within each rhizotron were occupied by two individuals of the same species or by one individual of two different species in all possible combinations, yielding a total of six different plant treatments (three mono-specific treatments and three different mixtures). Each of the six plant combinations was replicated 12 times for a total of 72 rhizotrons. Four additional rhizotrons without any plants were used to quantify water loss through evaporation. Just before planting, soil rhizotrons were humidified, and then watered at field capacity immediately after planting. During the establishment period of the freshly planted seedlings and ramets, all rhizotrons were kept close to field capacity during 363 days (224 days for *Q. coccifera*). The root

systems of all individuals of the three species reached the bottom of the rhizotrons towards the end of this establishment period. On the 3rd December 2014, half of the rhizotrons were assigned to a drought treatment with the other half continuously watered as soon as soil water content reached 17.5 ± 0.5 % (predefined threshold corresponding to 75 % soil field capacity), (Fig. 1.2a, Chapter I). The drought treatment consisted in two drying cycles, during which the rhizotrons were not watered until the soil water content reached 11 ± 0.4% (Fig. 1.2b, Chapter I). This soil water content was determined as the threshold of all leaves senescence in *B. retusum* under our experimental condition (preliminary test – data not shown). The soil water content in the rhizotrons was determined gravimetrically once per week by weighing each individual rhizotron. Watering was resumed to field capacity as soon as any given rhizotron reached 17.5 % soil water content (control treatment), or 11 % (drought treatment). After the second drying cycle of the drought treatment a third drying cycle was applied to both control and drought rhizotrons from the respective plant treatment within the same block. After the final drying cycle applied to all rhizotrons (including all controls), each rhizotron was again watered to reach field capacity when the threshold of 11 % of soil water content was reached and maintained at soil water contents higher than 17.5% for three weeks. After these three weeks, individual rhizotrons were harvested. Because watering treatments were applied at the level of individual rhizotrons, time of harvest varied between 211 and 377 days after the beginning of the drought treatment.

During the experiment, the rhizotrons were installed in six blocks (6 plant treatments x 2 drought treatments = 12 rhizotrons each) and placed at an 18° inclination to favor root growth towards the windowpane. After each weighing of the rhizotrons, their position was randomized within each block. Rhizotrons were kept at the experimental field of the Centre of Evolution and Funtional Ecology (CEFE) in Montpellier, France, (43°38’19’’N, 3°51’43’’E,

56 m a.s.l.), under a transparent plastic rain shelter. The rain shelter was installed at 3 m above the ground and was open on the sides to allow aeration. The rain shelter resulted in a total reduction of 27 % of photosynthetically active radiation. We measured air temperature and relative humidity at hourly intervals during the harvest period. One rhizotron of each plant composition and drought treatment was equipped with a soil humidity sensor (WATERSCOUT SM 100, SDEC, France) installed horizontally at two depths (10 and 50 cm from the soil surface) to measure volumetric soil moisture (one measurement per hour, data not shown).

Additionally, to these two-individual rhizotrons, 5 individuals of *C. albidus* and of *B. retusum* and 3 of *Q. coccifera* were cultivated in rhizotrons following the same protocol as for the other plants for the establishment period but with one single individual per rhizotrons, i.e. without intra- or interspecific competition. These individuals were harvested at the end of establishment period and before the start of drought treatment.

2.3. Plant harvest and measurements on roots and soil

All rhizotrons were harvested exactly three weeks after the end of the third drying cycle that was common for the drought and the control treatment (see above). We placed the rhizotrons horizontally and removed the glass pane. Three soil cores (44 mm in diameter) were then at exactly the same soil depth within the 15 cm-center zone of the rhizotron at each of two different depths (at 17.5 and at 57.5 cm below the soil surface, Fig. 2.1). Roots were immediately recovered from soil cores, washed to remove adhering soil particles, and separated by species (for the 2-species rhizotrons). Fine roots (< 2 mm in diameter) were selected for the morphological analysis and the remaining roots (> 2 mm in diameter) were kept for total biomass determination. Fine roots from two of the three soil cores per soil depth were put in water and kept for morphological analyses (within the next days). The third core

was use only to harvest the soil, and roots were put together with the remaining roots of the same soil depth for biomass determination. The soil recovered from all three soil cores per depth was mixed and homogenized to produce representative samples for microbial analyses. For the microbial analyses, the soil samples were air-dried in the dark at 25°C for 10 days, sieved at 2 mm and stored in a dry and dark storage room until analysis. After the soil cores have been taken, the remaining root systems were separated into three horizons (A (0-25 cm), B (25-50 cm) and C (50-80 cm), Fig. 2.1. The roots were separated from the soil gently by washing under tap water for each horizon. For the rhizotrons containing two different plant species, the roots were additionally separated by species. For the rhizotrons containing two individuals from the same species we could separate the roots that were still attached to the shoot in the first horizon (A), but this became impossible for the two deeper horizons, (see Chapter I for more details).

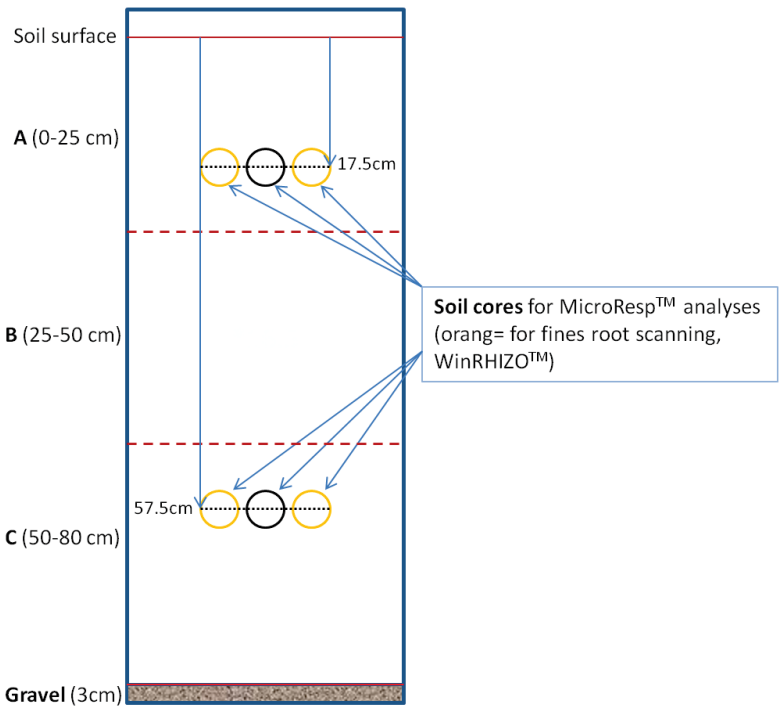

Fig. 2.1. A schematic representation of soil and roots sampling in rhizotron at the end of the experiment.

2.3.a. Root morphological traits

Before root morphology could be assessed, we stained *B. retusum* fine roots with methylene blue (1 g l^{-1}) for five minutes to increase the contrast for the scanning. The darker roots of *C. albidus* and *Q. coccifera* did not require staining. The fine roots were then rinsed in distilled water, spread out on a fine mesh while immerged in water to avoid overlapping roots, and transferred to a transparent acetate sheet (Roumet et al. 2008; Birouste et al. 2012). Samples were scanned as grayscale images at a resolution of 600 dpi using a scanner equipped with a transmitting light source that avoids shadows (EPSON Expression 1680). All roots were then recovered from the acetate sheet, oven-dried at 65 °C for 48 h and weighed to determine root dry mass (RDM). A digital image analyzing software (Winrhizo, version 2003b, Regent Instrument, Québec, Canada) was used to determine root length (L), root mean diameter (Diam), and root volume (V) from the sum of all volumes calculated on the basis of 41 diameter classes (from 0 to 2 mm, with a class width of 0.05 mm). From these measurements we calculated specific root length (SRL, m g^{-1}, as the ratio L/RDM), root tissue density (RTD, g cm^{-3}, as the ratio of RDM/V), root thickness (RTh, cm^3 m^{-1}, as the ratio V/L), and finally, root length per soil volume (L/Volume, cm cm^{-3}, as the ratio L/soil volume).

The community-weighted mean traits (CWM) of fine roots (SRL_{CWM}, RTD_{CWM}, RTh_{CWM}, $L/volume_{CWM}$, and $Diam_{CWM}$) were calculated as the average trait values of morphological traits following (Garnier et al. 2004): $Trait_{CWM} = \sum_{i=1}^{n} B_i \times trait_i$, where B_i is the relative abundance for species i (50% for each species in rhizotrons with two species), and $trait_i$ is the trait value for species *i*. The functional dissimilarity of traits in rhizotrons with two species was calculated according to Rao's quadratic entropy (Epps et al. 2007) for each rhizotron (SRL_{FD}, RTD_{FD}, RTh_{FD}, $L/Volume_{FD}$, $Diam_{FD}$ and the global trait dissimilarity All_{FD}) as: $Trait_{FD} = \sum_{i=1}^{n} \sum_{j=1}^{n} pipj * dij$, where pi and pj are 50% each in the bi-specific rhizotrons,

and dij the Euclidian distance between species i and j for the trait considered (TraitFD values are 0 in mono-specific rhizotrons). Because the measured traits differ in their numerical value ranges, standard normal deviates (so as to get an expected value of zero and a variance of one for traits values) were used to calculate functional dissimilarity.

2.3.b. Soil microbial analysis

We used the MicroResp™ procedure (Macaulay Scientific Consulting, Aberdeen, UK) to characterize community level physiological profiles (CLPP) of the soil microbial community (Campbell et al. 2008). MicroResp™ offers a convenient, rapid and sensitive method for the determination of microbial community functional diversity. Its application in a number of studies has demonstrated its utility in a wide range of soils and land-cover types (Chapman et al. 2007; Creamer et al. 2016). About 0.49 g of soil were incubated in triplicate with 1.5 mg C g^{-1} soil (except for the low-solubility phenolic acids and cellulose for which 0.75 mg C g^{-1} soil were added) of 15 different carbon substrates, plus one control with deionized water, so as to reach 80% of the field capacity in 96-DeepWell microplates (Fisher Scientific E39199). C substrates included three carbohydrates (D-glucose, xylan, cellulose, by order of complexity), one amine (N-acetyl-glucosamine), five amino acids (L-asparagine, L-glutamine, L-lysine, L-serine, L-glycine), three carboxylic acids (malic acid, oxalic acid, uric acid), and three phenolic acids (caffeic acid, which is an intermediate in lignin biosynthesis, syringic acid, and vanillic acid which is a by-product during the degradation of polyphenols by fungi). Cresol red gel detection plates were prepared as recommended by the manufacturer. After an initial two-hour pre-incubation at 25°C in the dark, each deepwell microplate was covered with a detection plate using a silicone gasket (MicroResp™, Aberdeen, UK). The assembly was secured with a clamp, and incubated for four additional hours. Optical Density at 590 nm (OD_{590}) was measured for each detection well before and after incubation using a Victor 1420

Multilabel Counter (Perkin Elmer, Massachusetts, USA). Final OD_{590} were normalized using pre-incubation OD_{590}, and converted as substrate induced respiration rates expressed in µg C-CO_2 respired per g of soil per hour.

The respiration rates for the different C substrates were summed across all 15 substrates (sum15) as a proxy of the global metabolic activity, and standardized rate for each substrate was computed as the rate measured for this substrate divided by sum15, as proposed by (Leff et al. 2012). For each plot, a Shannon metabolic diversity index was computed as: $H' = -\sum_{i=1}^{15} pi * \log(pi)$, and a Simpson metabolic dominance index was computed as: $D = \sum_{i}^{j} pi^2$, where (*pi)* is the standardized respiration rate for substrate (*i)*.

2.4. Data analysis

Species root traits (SRL, RTD, RTh, L/Volume and Diam) were summarized in a common principal component analysis (PCA) to explore the variability of root traits before and after drought manipulation. Plant parameters were analyzed separately for each species, but soil microbial parameters were analyzed at the rhizotron level because they cannot be associated to a particular plant species or individual, for the effects of drought, neighbor plant identity, soil depth sampling and their interactions. We used mixed linear models (*lme* function of *R* package "nlme" and *r.squaredGLMM* function of *R* package "MuMIn" to calculate R square values) to assess whether average root diameter (Diam), root tissue density (RTD), root thickness (Rth), root length by soil volume (L/volume), specific root length (SRL) and soil microbial parameters (sum15, H' and D) differed according to the drought treatment (control *vs* drought) and neighbor plant identity and their interactions. We also included soil depth (17.5 and 57.5 cm) according to the two sampling zones within the soil profile) as an additional factor in the model. Soil depth was nested within rhizotron identity and fitted as

random variable in the models. As a result of mortality during the experiment, the period of presence of individuals that died during the experiment was integrated as the percentage of total presence period of living individual, and used in model fits as a co-variable. Likewise, the time during which each rhizotron was kept in the experiment (from plantation to harvest, depending on the duration of the drying cycles) was also fitted as a co-variable in the model. Differences between drought treatments, composition and horizon were tested using *Tukey HSD* post hoc comparison tests (*lsmeans* function of the eponymous *R* package). The relationship between soil microbial parameters (sum15, H', D and individual substrate of CLPPs analyses) and root traits (either community weighed mean traits ($Trait_{CWM}$) or functional dissimilarity ($Trait_{FD}$)) were explored using Pearson's correlations. Heteroscedastic variables were transformed to meet the assumption of normal distribution of residuals. Statistical analyses were performed with the R software (version 3.2.4, The R Foundation for Statistical Computing 2016) with significance levels indicated as ' for $p < 0.10$, * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. Data are presented as means ± one standard error.

3. Results

3.1. Root traits responses

After the one-year establishment phase and before we started the drought treatment, root traits of the three studied species spread across the first two axes of a PCA to explain a total of 93.8% of the species trait variation (Fig. 2.2a). PC1 accounting for 70% of the variation was determined mostly by SRL and root thickness (RTh) and diameter (Diam), and it significantly discriminated species ($p < 0.0001$, Tukey HSD test). The highest SRL, but lowest RTD, RTh and Diam was measured in *B. retusum* and lowest SRL, but highest RTD, RTh, and Diam was measured in *Q. coccifera* (Table 2.1). While these two species differed significantly in all

these traits, *C. albidus* root traits were either similar to those of *Q. coccifera* (RTD) or to those of *B. retusum* (RTh and D), or intermediate (SRL) (Table 2.1).

At final harvest, after we applied repetitive droughts, root trait variation across the three species was still well explained by the two first PCA axes accounting for a total of 79.2% of variation within the single species rhizotrons and across both treatments of control and repetitive drought (Fig. 2.2b). The two axes were determined by the same traits as before the experiment started. The first PCA axis (PC1), accounting for 61.6% of the total variation, had a positive loading for root thickness (RTh) and negative loadings for both specific root length (SRL) and the root length by soil volume (L/Volume). PC1 also had a positive loading for *Q. coccifera* and a negative loading for *B. retusum* when added as supplementary variables similar to what was observed before the drought treatment started. The second axis (PC2), accounting for 17.6% of the total variation (Fig. 2.2b) had a positive loading for average root diameter (Diam) and a negative loading for root tissue density. PC2 also had a positive loading for soil depth and a negative loading for *C. albidus* when added as supplementary variables. Overall, PC1 discriminated *Q. coccifera* to two other species ($p < 0.001$ and $p < 0.0001$, respectively, Tukey HSD test), without difference between *B. retusum* and *C. albidus*, while PC2 distinguish between *C. albidus* and *Q. coccifera* only ($p = 0.026$, Tukey HSD test).

Table 2.1. Mean (± SE) fine root traits* of the grass species *B. retusum* and the two woody shrub species *C. albidus* and *Q. coccifera* determined from a subset of rhizotrons (where only one individual was cultivated) immediately before the drought treatment started, across soil depth. *p*-values from one-way ANOVAs along with the significance levels (** $p < 0.01$; *** $p < 0.001$) are shown. Different letters indicate significant differences among species (Tukey HSD test).

	B. retusum (n=19)		*C. albidus (n=20)*		*Q. coccifera (n=6)*		*p*-value
SRL (m^{-1} g^{-1})	117,897 ±6,765	**a**	71,490 ±7,144	**b**	13,466 ±3,926	**c**	**<0.001*** **
RTD (g^{-1} cm^{-3})	0,156 ±0,014	**b**	0,247 ±0,022	**a**	0,317 ±0,089	**a**	**<0.01*** **
RTh (cm^{3} m^{-1})	0,063 ±0,005	**b**	0,078 ±0,010	**b**	0,397 ±0,023	**a**	**<0.001** **
Diam (mm)	0,279 ±0,011	**b**	0,303 ±0,019	**b**	0,709 ±0,020	**a**	**<0.001*** **

* Specific root length (**SRL**), root tissue density (**RTD**), root thikness (**RTh**) and average root diameter (**Diam**).

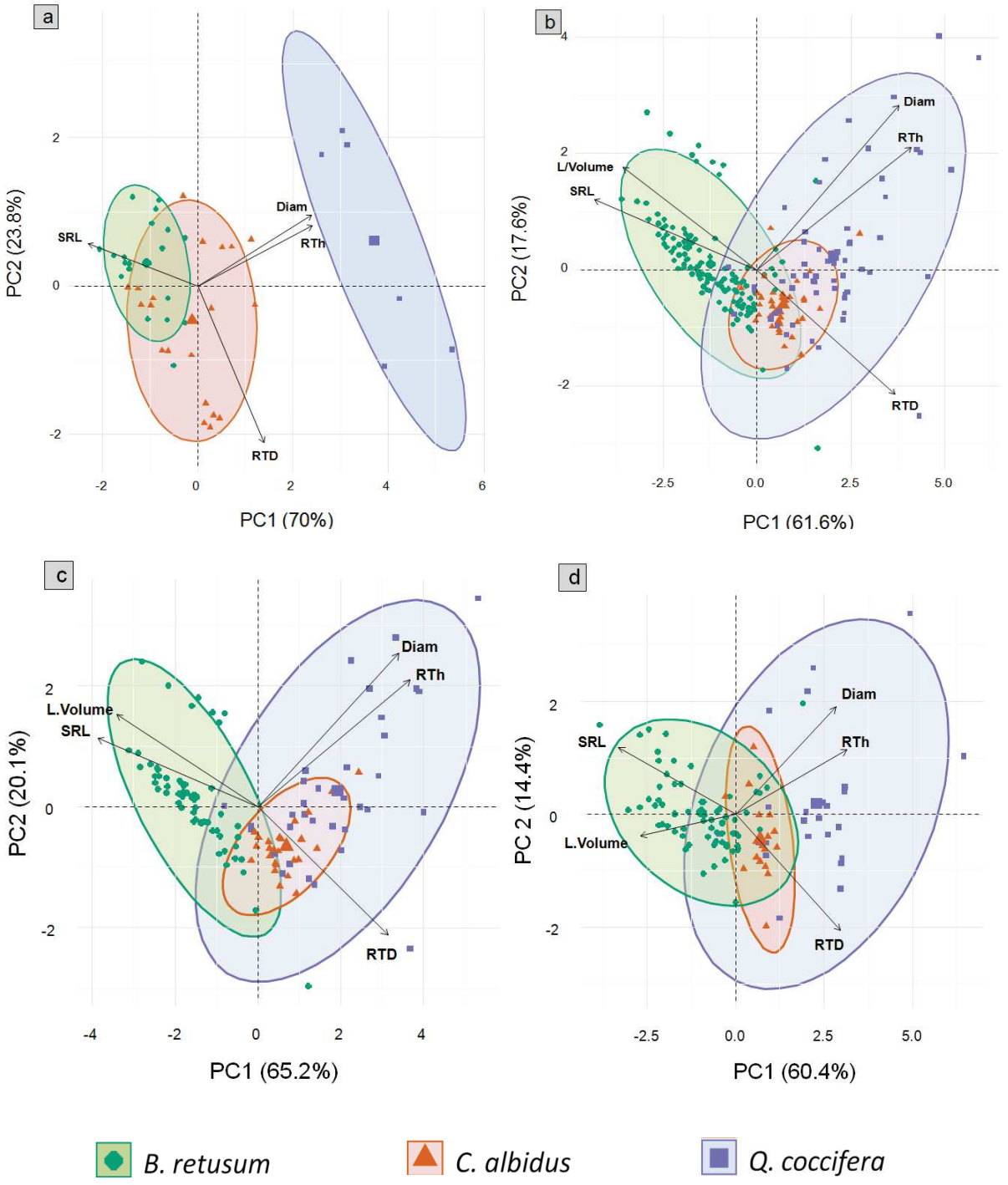

Fig. 2.2. Principal component analysis based on five root functional traits related to root morphology (specific root length (**SRL**), root tissue density (**RTD**), root thikness (**RTh**), average root diameter (**Diam**) and root length per soil volume (**L/Volume**)) as measured in the three studied species one year after transplantation and before drought manipulation (a), at the end of the experiment (across drought and control treatment (b)), at the end of the experiment in control conditions only (c) and at the end of the experiment in drought conditions only (d), across all neighbor plant species treatments and the two soil depths.

Compared to the root traits measured before the drought treatment started, the tissue density of roots (RTD) increased, and Diam and RTh decreased in all three species at the end of the experiment, irrespective of the drought treatment (Table 2.1 and 2.2). The SRL was higher in *B. retusum* and *Q. coccifera* and lower in *C. albidus* at final harvest compared to the pre-drought values. However, the species-specific differences remained mostly the same for all root traits before and after the drought treatment.

Table 2.2. Mean (± SE) fine root traits* of the grass species *B. retusum* and the two woody shrub species *C. albidus* and *Q. coccifera* under repetitive drought and control conditions at final harvest. One-way ANOVAs were performed for differences between treatments (*p*-values are reported).

	B. retusum (n=123)	*C. albidus (n=42)*	*Q. coccifera (n=59)*	*p*-value
SRL (m^{-1} g^{-1})	132,262 ± 64,60 a	42,024 ± 16,81 b	28,066 ± 25,86 b	<0.001***
RTD (g^{-1} cm^{-3})	0,339 ± 0,149 b	0,559 ± 0,14 a	0,618 ± 0,284 a	<0.001***
RTh (cm^{3} m^{-1})	0,028 ± 0,012 b	0,050 ± 0,019 b	0,104 ± 0,06 a	<0.001***
L/Volume (cm cm^{-3})	20,054 ± 12,57 a	5,560 ± 3,673 b	0,866 ± 0,941 c	<0.001***
Diam (mm)	0,199 ± 0,056 c	0,250 ± 0,041 b	0,364 ± 0,144 a	<0.001***

* Specific root length (**SRL**), root tissue density (**RTD**), root thikness (**RTh**), root length per soil volume (L/Volume) and average root diameter (**Diam**).

Root traits were affected by several treatment factors for *B. retusum,* but not for *Q. coccifera* and *C. albidus* for which traits remained similar across the different treatments, with the exception of a soil depth effect on *C. albidus* (Table 2.3.). The duration of the presence of individual plants within the rhizotrons had generally little effects on root traits, except for a positive effect on L/Volume in *B. retusum* and on RTD in *C. albidus*. In contrast, the age of individual plants (due to replacements of dead individuals and differences in harvest dates) had a significant effect on all root traits in *B. retusum,* but not in the other two species. SRL and L/Volume were lower, and RTD, RTh and D were higher in older compared to younger *B. retusum* plants (Table 2.3).

B. retusum neighbor identity affected four (SRL, RTh, L/Volume, Diam) of the five root traits across the two horizons (Table 2.3). Specific root length in *B. retusum* was higher when it

grew together with an individual of *C. albidus* than when it grew together with a conspecific individual (p = 0.019, Tukey HSD test, Fig. 2.3a), and without difference when it grew with *Q. coccifera*. RTh and L/Volume in *B. retusum* were also affected by the presence of *C. albidus,* with lower values compared to when it grew with a conspecific individual (p = 0.003 and p = 0.01, respectively, Fig. 2.3b and c) with intermediate values when it grew with a *Q. coccifera*. Finally, the average root diameter (Diam) of *B. retusum* was higher when it grew with a conspecific rather than with *C. albidus* (p < 0.001, Tukey HSD test, Fig. 2.3d), and, to a lower extent, with *Q. coccifera* (p = 0.081).

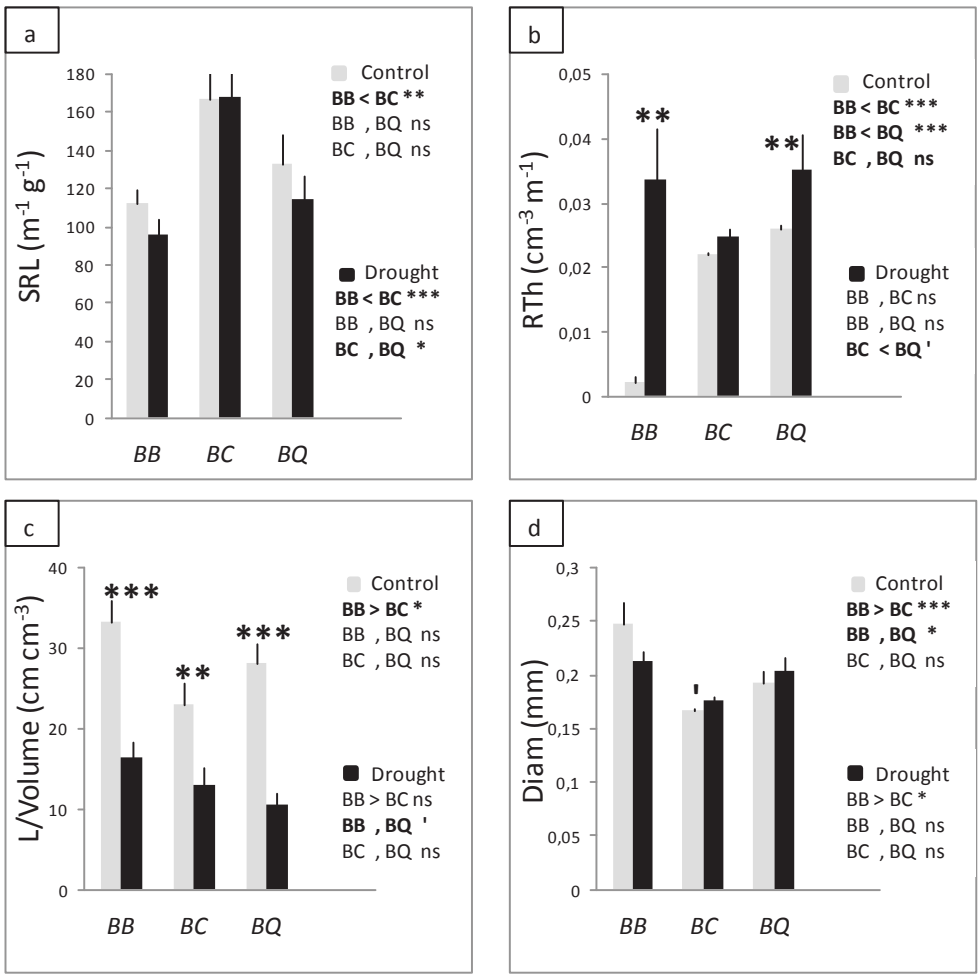

Fig. 2.3. Root morphological traits (specific root length (**SRL**), root tissue density (**RTD**), root length per soil volume (**L/Volume**) and mean root diameter (**Diam**)) of ***B. retusum*** as a function of neighbor species identity (**B** for *B. retusum*, **C** for *C. albidus*, and **Q** for *Q. coccifera*) in control and drought conditions. Mean values (±SE) are shown. Levels of significance from Tukey HSD post hoc test to test for the effect of drought within species are shown (**ns** for not significant difference, ' p < 0.1, * p < 0.05, ** p < 0.01 and *** p < 0.001).

Table 2.3. Output of multiple linear mixed models testing for the effects of drought treatment (**Drought**), community composition (**Id**), horizon sampling (**Depth**) and their interactions (**Drought:Id** and **Drought:Id:Depth**) on specific root length (**SRL**), root tissue density (**RTD**), root thickness (**RTh**), root length per soil volume (**L/Volume**) and average root diameter (**Diam**). The period of presence of died seedling (**Presence**) and the age of seedling (from plantation to harvest date) (**Age**) was fitted as co-variables in the models. Marginal and conditional R squared ($\mathbf{R^2}$) of the mixed model are reported (in bold when p-values < 0.05). *F*-values and the corresponding *p*-values (superscripts) are reported (in bold when *p*-values < 0.05). Arrows indicate the direction of the effect.

***B. retusum* (n=123)**	***F*-values (*p*-values)**				
Effect (df)	**SRL**	**RTD**	**RTh**	**L/Volume**	**Diam**
Presence (1)	ns	ns	ns	**28.3650** **(<.001) ↑**	ns
Age (1)	**52.559** **(<.001) ↓**	**7.726** **(0.010) ↑**	**33.625** **(<.001) ↑**	**13.4179** **0.001) ↓**	**14.596** **(<.001) ↑**
Drought (1)	0.024 (0.878)	0.462 (0.503)	0.523 (0.476)	**11.445** **(0.003) ↓**	1.190 (0.286)
Id (2)	**6.483** **(0.006)**	0.983 (0.389)	**7.423** **(0.003)**	**3.959** **(0.033)**	**9.010** **(<.001)**
Depth (1)	**24.861** **(<.001) ↑**	**31.886** **(<.001) ↓**	0.479 (0.496)	**15.502** **(<.001) ↑**	3.244 (0.084) ↑
Drought:Id (2)	0.546 (0.586)	0.216 (0.807)	0.038 (0.963)	0.722 (0.496)	1.753 (0.195)
Drought:Depth (1)	**6.272** **(0.019)**	**8.913** **(0.006)**	1.671 (0.208)	**9.842** **(0.004)**	2.403 (0.134)
Id:Depth (2)	0.887 (0.424)	1.260 (0.301)	0.931 (0.408)	1.028 (0.372)	2.504 (0.102)
Drought:Id:Depth (2)	1.537 (0.235)	2.192 (0.133)	0.045 (0.957)	0.311 (0.736)	0.932 (0.407)
$\mathbf{R^2}$ Marginal	**0.50**	**0.34**	**0.46**	**0.58**	**0.37**
Conditional	**0.56**	**0.53**	**0.66**	**0.86**	**0.61**
***C. albidus* (n=42)**					
Presence (1)	ns	**11.506** **(0.015)↑**	ns	ns	ns
Age (1)	ns	ns	ns	ns	ns
Drought (1)	2.293 (0.174)	0.038 (0.852)	0.745 (0.417)	0.3 (0.6)	0.758 (0.413)
Id (2)	0.05 (0.83)	1.432 (0.277)	0.417 (0.54)	4.6 (0.069)	0.366 (0.565)
Depth (1)	**6.963** **(0.033)↑**	**6.160** **(0.042) ↓**	2.97 (0.129)	**7.88** **(0.026)↓**	2.435 (0.163)
Drought:Id (2)	0.078 (0.788)	0.104 (0.758)	0.005 (0.945)	0.58 (0.471)	0.002 (0.967)
Drought:Depth (1)	0.0003 (0.987)	0.001 (0.998)	0.049 (0.83)	1.239 (0.302)	0.01 (0.922)
Id:Depth (2)	3.637 (0.098)	3.531 (0.102)	1.17 (0.315)	0.44 (0.529)	1.625 (0.243)
Drought:Id:Depth (2)	0.395 (0.55)	0.253 (0.631)	1.215 (0.307)	0.829 (0.393)	1.483 (0.263)
$\mathbf{R^2}$ Marginal	**0.22**	**0.46**	**0.10**	**0.33**	**0.10**
Conditional	0.78	0.64	0.86	0.82	0.82
***Q. coccifera* (n=59)**					
Presence (1)	ns	ns	ns	ns	ns
Age (1)	ns	ns	ns	ns	ns
Drought (1)	0.238 (0.635)	0.002 (0.968)	1.379 (0.263)	2.886 (0.115)	0.246 (0.629)
Id (2)	0.138 (0.873)	0.029 (0.971)	0.558 (0.586)	2.064 (0.17)	0.539 (0.597)
Depth (1)	2.654 (0.129)	0.219 (0.648)	0.398 (0.540)	1.517 (0.242)	3.661 (0.08)↓
Drought:Id (2)	0.672 (0.529)	0.127 (0.882)	0.703 (0.515)	0.314 (0.737)	1.02 (0.39)
Drought:Depth (1)	3.437 (0.089)	3.683 (0.079)	0.551 (0.472)	0.172 (0.686)	1.322 (0.272)
Id:Depth (2)	0.895 (0.434)	2.583 (0.117)	2.489 (0.125)	0.697 (0.517)	0.851 (0.451)
Drought:Id:Depth (2)	0.215 (0.81)	0.117 (0.890)	0.531 (0.602)	1.943 (0.186)	0.14 (0.871)
$\mathbf{R^2}$ Marginal	0.13	0.11	0.16	0.24	0.15
Conditional	0.43	0.43	0.22	0.75	0.26

The specific root length (SRL) was generally higher in the deeper soil layer compared to the top soil (regardless of the neighbor identity, no identity x soil depth interaction, Table 2.3). However, SRL was distinctly affected by drought in the two soil depths (significant Drought X Depth interaction, Table 2.3), with lower SRL under drought than in the control treatment in deeper soil, but no difference between control and drought treatments in the top soil (Fig. 2.4a). Root tissue density (RTD) in *B. retusum* did not respond to drought treatments nor to the identity of the neighbor plant (Table 2.3), but was lower in the deeper soil layer compared to the top soil, a response that was particularly pronounced in the control treatment (significant drought x soil depth interaction, Table 2.3, Fig. 2.4b). Finally, L/Volume in *B. retusum* increased in the deeper soil layer compared to the top soil, and decreased in drought compared to control treatment, with a more pronounced drought effect at higher soil depth (significant drought X soil depth interaction Table 2.3, Fig. 2.4c).

C. albidus root traits were mostly affected by soil depth (SRL was higher and RTD and L/Volume were lower in the deeper soil layer compared to the top soil, Table 2.3), with no interaction with drought treatment and neighbor identity, and with statistical models explaining 10 to 46% of trait variability (marginal R^2 values)

Q. coccifera root traits were overall much less affected by any of the treatment factors compared to the other two species, with no effect of drought, neighbor species identity or depth, and no interactions among the treatment factors on any of the root traits (Table 2.3, $p > 0.05$ for global models).

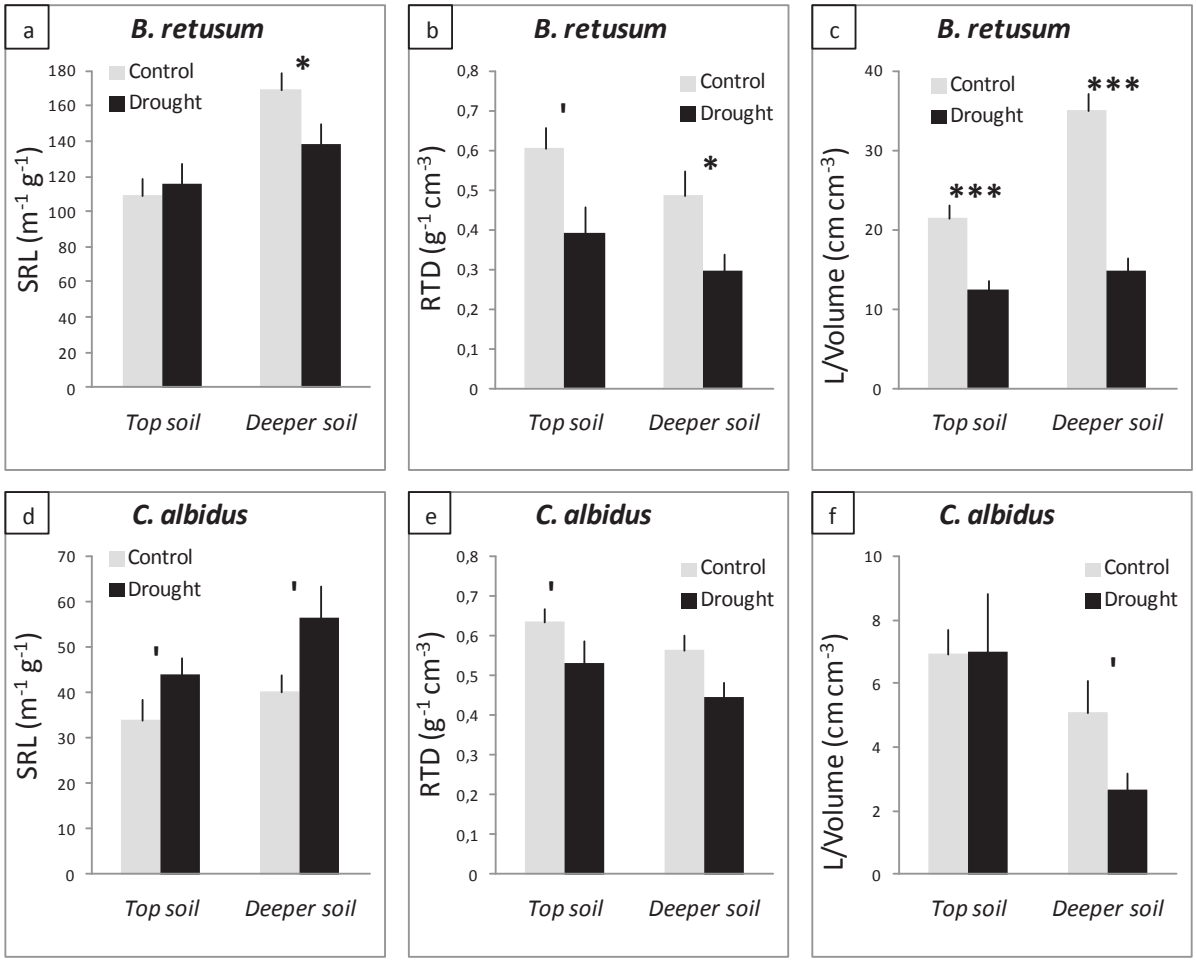

Fig. 2.4. Root morphological traits (specific root length (**SRL**), root tissue density (**RTD**) and root length per soil volume (**L/Volume**)) of ***B. retusum*** (a, b and c) and ***C. albidus*** (d, e and f) as a function of soil depth (Top soil=17.5 cm and Deeper soil=57.5 cm), in control and drought conditions (across all neighbor species identity treatments). Mean values (±SE) are shown. Levels of significance from Tukey HSD post hoc test to test for the effect of drought within species are shown (‘ $p < 0.1$, * $p < 0.05$ and *** $p < 0.001$).

Soil microbial substrate utilization

Contrary to root traits, soil microbial properties could not be easily associated to individual plants, instead they were evaluated at the level of individual rhizotrons at two different soil depths. The average global metabolic activity (sum15), the Shannon metabolic diversity index (H’) and the Simpson metabolic dominance index (D) are shown in (Table 2.4) for all six plant species combinations. Except for respiration rate of syringic acid, plant species composition

did not affect soil microbial parameters and they were also not affected by repeated drought (Table 2.5). Syringic acid respiration was higher in soil when *C. albidus* was the only plant species compared to soil from rhizotrons in which *C. albidus* grew together with *Q. coccifera* or when *Q. coccifera* grew together with *B. retusum* ($p < 0.025$ and $p = 0.007$, respectively, Tukey HSD test), (no interaction with soil depth). Soil depth was the main factor influencing the soil microbial properties, with higher total microbial activity (sum15) and microbial metabolic diversity (H') and lower microbial metabolic dominance (D) in deep soil layers compared to top soil across all plant species composition and both drought treatments. Respiration rates were higher for cellulose and malic acid, but lower for lysine and glutamic acid in deep compared to top soil (Table 2.5).

Table 2.4. Means ± SE (pooled across drought treatments) of global metabolic activity (**sum15**) expressed in µg $C\text{-}CO_2$ g^{-1} soil h^{-1}), Shannon microbial metabolic diversity index (**H'**) and Simpson microbial metabolic dominance index (**D**) at two soil depths (top = 17.5 cm and bottom = 52.5 cm) for each of the six different species combinations (**B** for *B. retusum*, **C** for *C. albidus*, and **Q** for *Q. coccifera*).

Species composition	sum15 ± SE		H' ± SE		D ± SE	
	Top	Bottom	Top	Bottom	Top	Bottom
BB	8,0 ± 0,7	7,7 ± 0,8	1,141 ± 0,008	1,145 ± 0,005	0,081 ± 0,004	0,078 ± 0,002
CC	9,8 ± 0,9	10,2 ± 0,7	1,139 ± 0,005	1,126 ± 0,026	0,080 ± 0,002	0,087 ± 0,012
QQ	8,0 ± 0,8	8,2 ± 0,6	1,146 ± 0,005	1,150 ± 0,004	0,078 ± 0,002	0,076 ± 0,002
BC	6,7 ± 0,4	8,8 ± 0,9	1,153 ± 0,003	1,131 ± 0,007	0,075 ± 0,001	0,084 ± 0,003
BQ	7,6 ± 0,7	8,3 ± 0,7	1,149 ± 0,007	1,141 ± 0,003	0,077 ± 0,004	0,080 ± 0,001
CQ	7,4 ± 0,5	7,8 ± 0,5	1,142 ± 0,006	1,126 ± 0,008	0,080 ± 0,003	0,086 ± 0,004

Table 2.5. Output of multiple linear mixed models testing for the effects of drought (**Drought**), plant composition (**Id**), soil depth (**Depth**) and their interactions on soil microbial parameters (global metabolic activity (**sum15**), microbial metabolic diversity (**H'**) and microbial metabolic dominance (**D**)) and on the community level respiration rates of 15 carbon substrates. Marginal and conditional R squared ($\mathbf{R^2}$) of the mixed model are reported (in bold when *p*-values < 0.05). *F*-values and the corresponding *p*-values (superscripts) are reported (in bold when *p*-values < 0.05). Arrows indicate the direction of the effect.

		***F*-values** (*p*-values)								
Effect (df)		**sum15**	**H'**	**D**	**GLU**	**XYL**	**CELL**	**ASP**	**SER**	**LYS**
Drought (1)		0.0004 (0.983)	0.43 (0.514)	0.6 (0.443)	0,116 (0,735)	0,622 (0,435)	1,974 (0,167)	0,001 (0,997)	0,030 (0,875)	1,717 (0,197)
Id (2)		0.661 (0.655)	0.99 (0.435)	0.9 (0.492)	1,356 (0,260)	1,051 (0,401)	1,482 (0,216)	1,557 (0,193)	0,700 (0,627)	1,199 (0,326)
Depth (1)		**10.847 (<.001)↑**	**5.2 (<.028)↓**	**4.32 (0.044) ↑**	0,059 (0,810)	1,213 (0,278)	**8,096 (0,007) ↑**	3,264 (0,079)	2,190 (0,147)	**11,783 (0,002) ↓**
Drought:Id (2)		0.083 (0.995)	0.31 (0.903)	0.28 (0.920)	1,339 (0,267)	**2,773 (0,030)**	1,160 (0,345)	1,992 (0,100)	0,880 (0,502)	1,033 (0,411)
Drought:Depth (1)		0.060 (0.808)	1.42 (0.240)	1.89 (0.178)	0,312 (0,580)	0,004 (0,951)	0,787 (0,381)	1,804 (0,187)	3,580 (0,066)	0,965 (0,332)
Id:Depth (2)		1.811 (0.134)	1.91 (0.115)	1.88 (0.121)	0,442 (0,816)	**4,093 (0,005)**	1,127 (0,363)	0,086 (0,994)	2,070 (0,090)	1,454 (0,228)
Drought:Id:Depth (2)		0.679 (0.642)	0.24 (0.941)	0.25 (0.936)	0,237 (0,944)	0,418 (0,833)	0,372 (0,865)	0,569 (0,723)	1,380 (0,254)	2,350 (0,059)
R^2	Marginal	0.11	0.17	0.17	**0.13**	**0.29**	**0.23**	**0.20**	**0.23**	**0.28**
	Conditional	0.86	0.93	0.96	**0.61**	**0.98**	**0.99**	**0.99**	**0.29**	**0.46**
Effect (df)		**GLY**	**GLUT**	**N-AC**	**OX**	**UR**	**MAL**	**CAF**	**SYR**	**VAN**
Drought (1)		0,222 (0,640)	0,390 (0,536)	0,167 (0,685)	0,167 (0,685)	0,212 (0,647)	2,686 (0,109)	0,428 (0,517)	1,063 (0,308)	0,662 (0,420)
Id (2)		0,824 (0,540)	1,407 (0,241)	0,592 (0,706)	0,592 (0,706)	0,272 (0,926)	1,083 (0,384)	0,771 (0,576)	**2,720 (0,032)**	0,606 (0,696)
Depth (1)		1,113 (0,298)	**8,618 (0,006) ↓**	0,183 (0,672)	0,183 (0,672)	1,827 (0,185)	**4,238 (0,046) ↑**	3,498 (0,069)	0,312 (0,580)	2,349 (0,134)
Drought:Id (2)		0,173 (0,971)	0,587 (0,710)	1,402 (0,243)	1,402 (0,243)	1,407 (0,241)	0,309 (0,905)	1,477 (0,218)	2,304 (0,062)	0,401 (0,846)
Drought:Depth (1)		0,051 (0,823)	2,445 (0,126)	3,151 (0,084)	3,151 (0,084)	1,617 (0,211)	**4,175 (0,048)**	0,322 (0,574)	0,045 (0,833)	2,319 (0,136)
Id:Depth (2)		0,958 (0,455)	0,894 (0,495)	1,103 (0,375)	1,103 (0,375)	0,690 (0,634)	2,368 (0,058)	2,379 (0,057)	1,518 (0,207)	1,471 (0,222)
Drought:Id:Depth (2)		0,950 (0,460)	1,951 (0,109)	1,749 (0,147)	1,749 (0,147)	2,222 (0,072)	0,531 (0,751)	2,939 (0,024)	0,474 (0,793)	0,948 (0,461)
R^2	Marginal	**0.12**	**0.25**	**0.21**	**0.19**	**0.22**	**0.19**	**0.28**	**0.26**	**0.15**
	Conditional	**0.96**	**0.34**	**0.95**	**0.91**	**0.78**	**0.93**	**0.97**	**0.99**	**0.99**

Relationship between root trait-based metrics and soil microbial properties

We analyzed potential correlations between mean root traits ($Trait_{CWM}$) or functional dissimilarity ($Trait_{FD}$) and soil microbial properties. Pearson's correlations showed no relationship between sum15, H' or D of the soil microbial community and any of the mean or functional dissimilarity root traits. On the other hand, there were some significant correlations between $Trait_{CWM}$ or $Trait_{FD}$ and respiration rates of individual substrates (Table 2.6). The ability of the soil microbial community to utilize lysine increased with increasing RTD and RTh and with decreasing SRL and L/Volume. The ability of the microbial community to utilize i) malic acid, ii) caffeic acid and iii) syringic acid decreased with increasing i) RTh_{FD}, ii) $L/Volume_{FD}$ and iii) RTh_{FD}, $L/Volume_{FD}$, and global trait dissimilarity All_{FD}, respectively.

Table 2.6. Pearson's correlation matrix between mean fine root traits (**SRL_{CWM}**, **RTD_{CWM}**, **RTh_{CWM}**, L/**$Volume_{CWM}$** and **D_{CWM}**) or trait dissimilarity (**SRL_{FD}**, **RTD_{FD}**, **RTh_{FD}**, L/**$Volume_{FD}$**, **$Diam_{FD}$** and **All_{FD}**) and soil microbial parameters (total metabolic activity (**sum15**), microbial metabolic diversity (**H'**), microbial metabolic dominance (**D**) and the community level respiration rates of 15 carbon substrates (only those that correlated significantly with at least one trait are mentioned). **R^2** value (* < 0.05) are shown and arrows indicate the direction of the effect.

	sum15	*H'*	*D*	*LYS*	*MAL*	*CAF*	*SYR*
SRL_{CWM}	ns	ns	ns	**0.05*↓**	ns	ns	ns
RTD_{CWM}	ns	ns	ns	**0.07*↑**	ns	ns	ns
RTh_{CWM}	ns	ns	ns	**0.06*↑**	ns	ns	ns
$L/Volume_{CWM}$	ns	ns	ns	**0.06*↓**	ns	ns	ns
$Diam_{CWM}$	ns	ns	ns	ns	ns	ns	ns
SRL_{FD}	ns	ns	ns	ns	ns	ns	ns
RTD_{FD}	ns	ns	ns	ns	ns	ns	ns
RTh_{FD}	ns	ns	ns	ns	**0.04*↓**	ns	**0.04*↓**
$L/Volume_{FD}$	ns	ns	ns	ns	ns	**0.04*↓**	**0.06*↓**
$Diam_{FD}$	ns	ns	ns	ns	ns	ns	ns
All_{FD}	ns	ns	ns	ns	ns	ns	**0.06*↓**

4. Discussion

The properties of plant root systems are critical for understanding how plants respond to changes in water availability in time and space (Grime et al. 1986; Davies and Zhang 1991; Eissenstat and Yanai 1997). There are, however, relatively few root data available, and assessments of induced differences in soil water content on root traits are particularly rare. Moreover, data from controlled experiments are often difficult to extrapolate, especially across different plant species because of the complex dynamics and interactions of root growth and soil water content. In particular, most previous studies applied drought treatments on the basis of a fixed time span, which means that individual plants may experience vastly different soil water conditions because of differences in the size and growth rate of root systems and different response dynamics to changing soil water content. Here we chose to simulate an increased frequency of drought events and to control the drought intensity at the level of individual rhizotrons in order to expose each individual plant to exactly the same drought intensity. With this approach we assured the comparison of root properties of different species and individual plants of different sizes at the same drought exposure, while we had to accept a rhizotron-specific duration of the experiment.

4.1. Root traits responses

We reported clear differences in how the three Mediterranean plant species, the grass *B. retusum* and the woody *C. albidus* and *Q. coccifera*, that frequently co-occur in Mediterranean shrubland, responded in terms of root traits to repeated drought, and how their responses were affected by the identity of the neighbor plant. In line with our initial hypothesis of a higher drought sensitivity of the species that tolerate dehydration by protecting some shoot meristematic tissues, we showed that the root traits of *B. retusum*

were globally more affected by repetitive drought (in interaction with soil depth) and by the identity of the neighbor species, than those of the drought-avoiding woody species. *B. retusum* responded to drought or neighbor species identity or their interactions with soil depth in all of the five studied root traits. In contrast, *C. albidus* root traits were not affected by drought or neighbor species identity, but it showed some variation in root traits related to soil depth. *Q. coccifera* root traits were the same regardless of treatment factors or soil depth. Under moderate drought, Mediterranean grass species commonly combine different strategies and may first delay dehydration increasing water uptake (Volaire et al. 2009). This could be translated in physiological and morphological root adaptations towards a more efficient acquisition of available soil water (i.e. an increase in SRL and root density and a decrease in RTD and Diam). Grass species can also tolerate dehydration by diminishing water loss (Volaire et al. 2009) with physiological regulations, such as stomatal conductance, or by morphological adjustments, such as advanced leaf senescence, and thus a substantial reduction of transpiring leaf area. On the other hand, plants with dehydration avoidance strategy operate with narrower hydraulic safety margins during drought to ensure growth maintenance (West et al. 1990; Volaire et al. 1998). Consequently they are predisposed to a greater risk of hydraulic failure by maintaining cell turgor in their shoots while it decreases in the roots. This might be what happened in the two woody species, as they did not show any indication of root trait adjustments.

Our data indicated that specific root length was the most responsive of the traits we measured. SRL reflects well the potential of the plant to explore the soil and acquire resources (Eissenstat 1992; Larcher 2003; Chapman et al. 2012) and is thought to play an important role in the strategy of plant water-use facing drought (Comas et al. 2013). The strategy of high resource acquisition requires fine roots with high SRL, as it was documented for a large range of herbaceous species (Freschet et al. 2015; Prieto et al.

2015). In boreal forest, (Bauhus and Messier 1999) showed the importance of SRL in soil exploitation strategies of the fine roots. Compared to the two woody species, *B. retusum* had the highest rates of SRL indicating a higher capacity of competition, since species with high SRL invest their root biomass more efficiently than species with low SRL because of an increased potential to explore the soil and acquire resources with the same amount of biomass (Eissenstat 1992). This is in line with our results, in chapter 1, showing higher survival rates for *B. retusum* compared with those of the two woody species when exposed to repetitive severe drought. In *B. retusum,* SRL was positively correlated with survival rates ($r^2 = 0.26^{**}$, Fig. 2.5a), with a similar, although marginally significant correlation in *C. albidus* ($r^2 = 0.24$', Fig. 2.5b), but not in *Q. coccifera*. The high SRL in *B. retusum* (Table 2.2) compared to the two woody species and its adjustment in response to repetitive drought may be a key characteristic of this species for its persistence despite repetitive drought. SRL in *B. retusum* also responded to competition (it was higher when it grew together with *C. albidus* compared to when it grew with a conspecific individual), while *C. albidus* showed no changes in SRL in response to neighbor plant identity. Although the particular above-ground responses of maintaining only the meristems but basically none of the aboveground structures is an important strategy of *B. retusum* to survive drought, the observed differences in root trait responses to repetitive drought may also have contributed to the much higher survival rates in *B. retusum* compared to *C. albidus* when they grew together (see chapter 1). Opposite to our results of either an increased SRL or no change in response to drought, a previous study with the tree *Fagus sylvatica* reported a decrease in SRL when it grew under drier conditions, a response that was driven by an increase in the fine root diameter with no change in total fine root length (van Hees 1997). In Australia, woody species from dry areas were reported to have lower SRL than species from wet

areas (Wright and Westoby 1999; Nicotra et al. 2002) due to higher RTD, which was interpreted as a greater capacity of roots to physically penetrate dry soil.

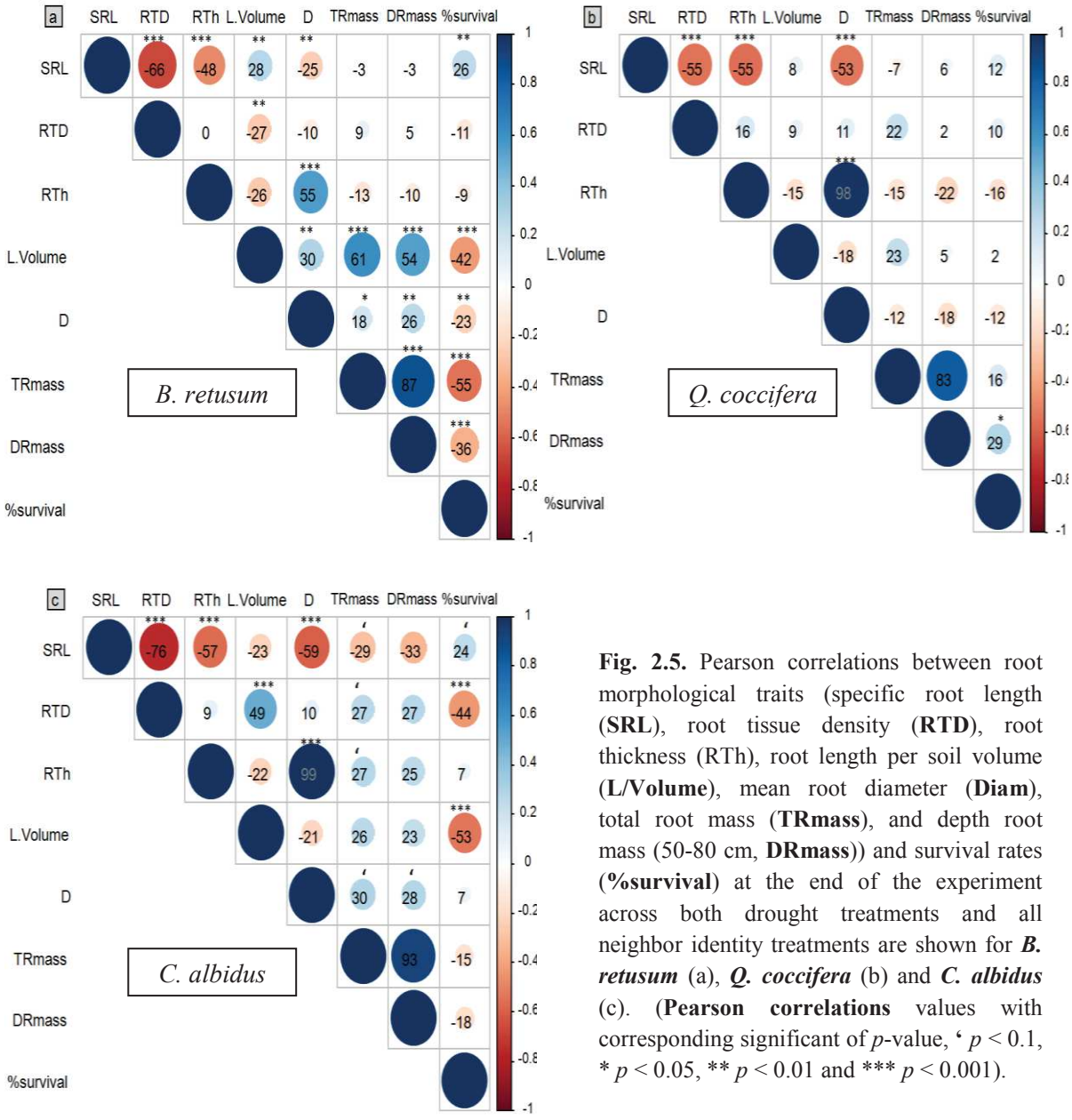

Fig. 2.5. Pearson correlations between root morphological traits (specific root length (**SRL**), root tissue density (**RTD**), root thickness (RTh), root length per soil volume (**L/Volume**), mean root diameter (**Diam**), total root mass (**TRmass**), and depth root mass (50-80 cm, **DRmass**)) and survival rates (**%survival**) at the end of the experiment across both drought treatments and all neighbor identity treatments are shown for ***B. retusum*** (a), ***Q. coccifera*** (b) and ***C. albidus*** (c). (**Pearson correlations** values with corresponding significant of *p*-value, ‘ $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

High SRL is correlated with high surface:volume ratio for the same carbon investment, which is maximizing the root–soil interface and hence the root absorption potential (Larcher 2003). Comparatively high SRLs might therefore be particularly important in deeper soil layers, where water content is usually less or at least later affected by drought compared to the top soil. In fact, drought stress often develops from the top down as the

soil surface dries, while adequate soil moisture can remain deep in the soil profile after surface soils have dried. (Skinner and Comas 2010). Indeed, in the two species *B. retusum* and *C. albidus* SRLs were higher at greater soil depth compared to the top soil layer (Fig. 2.4a and d), with a more pronounced effect of soil depth in control compared to drought conditions. Perhaps greater physical resistance of drier soils with repetitive drought even in deep soil layers led to somewhat denser root tissues compared to the control treatment as suggested elsewhere (Whitmore and Whalley 2009; Bengough et al. 2011).

The total root length per soil volume (L/Volume) of *B. retusum* increased with soil depth, but it was lower when *B. retusum* grew with *C. albidus* than when it grew with a conspecific. Under severe water deficit, limited root growth may occur because of low soil water availability and high soil resistance (Cornissh et al. 1984; Comas et al. 2005). Accordingly, root proliferation in deep soil, where moisture remains favorable for root growth over a longer period of time, would result in a higher proportion of deep to shallow roots (Skinner et al. 1998; Skinner 2008). Such higher root proliferation in the deep soil layer relative to that in top soil occurred in *B. retusum*, but not in *C. albidus* (lower L/Volume in deep soil compared to top soil).

As discussed above, the SRL is not only influenced by root length, but also by its diameter and tissue density (RTD) (Fitter 2002; Picon-Cochard et al. 2012). In addition to its importance for the penetration of dense or dry (and hard) soils, RTD also reflects anatomical differences between species (Ryser 1998; Wahl and Ryser 2000). High RTD implies high biomass investment per unit length of roots which is not favoring a high spatial spread of roots, and thus the exploitation of limiting water as argued above. From allometric rules, RTD is expected to be negatively correlated with SRL and positively with root diameter (Ryser and Lambers 1995). In our study, RTD was negatively correlated with SRL but showed no distinct pattern with average root diameter, across all three

studied species (Fig. 2.5). Other studies showed no correlation between root tissue density and SRL or root diameter in woody species (Comas et al. 2002; Comas and Eissenstat 2004; Comas and Eissenstat 2009) or in grass species (Picon-Cochard et al. 2012). However, high SRL could be achieved either through small root diameter or through low root tissue density. Here we observed negative correlations between SRL and root diameter for all studied species. For woody plants, root diameter predominately controls differences in SRL among species, with root tissue density affecting plasticity within species due to plant responses to edaphic factors such as soil water (Comas et al. 2002; Comas and Eissenstat 2009). Decrease in root diameter has been proposed as a trait for increasing plant water acquisition and productivity under drought condition (Wasson et al. 2012). In this study, root diameter of *B. retusum* was not affected by drought treatment, but it responded to neighbor species identity, indicating that the intra-specific competition had a more pronounced effects on this parameter than repetitive severe droughts did. *B. retusum* adjusted its mean root diameter as a function of neighbor species identity, with thinner roots when it grew together with individuals from a different species than when it grew together with a conspecific individual. Small root diameters together with high SRL, *i.e.* high surface area in contact with soil water, would translate into an increased hydraulic conductance by decreasing the apoplastic barrier of water entering the xylem (Eissenstat and Achor 1999; Hernández et al. 2010; Comas et al. 2012). Accordingly, a decrease in root diameter has been proposed as an efficient strategy to improve plant acquisition of water and productivity under drought (Wasson et al. 2012). Both woody and herbaceous plants adapted to dry conditions were found to have smaller fine root diameter and greater SRL as compared to plants that were not drought-adapted (Hernández et al. 2010; Henry et al. 2012). Our study confirms these responses for *B. retusum* but not for the two woody

species, suggesting that *B. retusum* is more competitive for soil water resources under repetitive drought.

4.2. Soil microbial responses and correlation with morphological root traits

In our study, the global soil metabolic parameters (sum15, H' and D) were affected neither by plant species composition nor by repeated drought, while several root traits in *B. retusum* were affected by identity of the neighbor species, and by drought or its interaction with depth. Respiration rates on xylan and malic acids were the only parameters responding to drought in interaction with species composition and depth, respectively. Such a discrepancy between drought impact on root traits (especially in *B. retusum*) and on soil microbial parameters was rather surprising, as the strategies of plants in acquiring resources, that translate in plant functional traits, generally influence the soil microbial communities and their functioning (Orwin et al. 2010; de Vries et al. 2012; Legay et al. 2014). One possible explanation can be related to our experimental design that did not allow the characterization of soil microbial parameters associated to each plant species. The substrate utilization patterns were analyzed on soil cores influenced by both plant individuals present in each rhizotrons: any potential impact of changing root traits in *B. retusum* on soil microbial parameters could be masked by the absence of response in root traits in other species. In our field study with leaf litter decomposing in single or multi-species mixtures (Shihan et al. 2017, see Chapter 3), the experimentally applied moderate rain-exclusion did not impact soil microbial parameters while litter species composition and richness did. Under drought condition, soil microbial respiration generally decreases (Schimel et al. 2007; Talmon et al. 2011; Berard et al. 2015 for review). Once again, our experimental design can be allocated as we used repeated compared to single drought events, that could not have contrasted effects on soil microbial properties, also with a three

week-period under non-stressing soil water content after last drought event, during which soil microbial communities could have recovered from drought more rapidly than root systems.

Soil depth affected significantly several microbial parameters. Total microbial activity was higher, but less diverse substrates were used in deep soil layers. Theses could be explicated first by wetter soil layers in depth (data about soil water availability are available and have to be analyzed) soil which enhanced soil microbial activity, and second by changes in several root traits in deep compared to shallow soil in two of the three species (Table 2.3). In fact, drought stress often develops from the top down as the soil surface dries, while adequate soil moisture can remain deep in the soil profile after surface soils have dried (Skinner and Comas 2010). The lower microbial metabolic diversity observed in deep soil is rather unexpected as soil water condition is less stress, and less selective, for microorganisms. Indeed, previous studies have been demonstrated that degradation of water conditions leads to changes in the microbial community structure (Wilkinson et al. 2002; Schimel et al. 2007; Bérard et al. 2011), for example, a decrease in microbial diversity and abundance, or a change in a dominance toward a microbial community dominated by certain groups more resistance and better adapted with drought conditions.

Shift in root traits in the deep compared to shallow soil horizon can be advocated to explain changes in the microbial parameters. Indeed, root functional identity ($Trait_{CWM}$) and functional dissimilarity ($Trait_{FD}$) explained changes in some substrate utilization. Four of the five root traits CWM were weakly but significantly correlated with respiration rate on lysine. Despite their critical role in the response of plants to drought, the relationships between soil microbial communities and root functional traits remain poorly studied as compared to those with leaf functional traits (Reich 2014). Only a few studies addressed the issue of how resource (mainly nitrogen) acquisition/conservation strategies of plants

are related to soil microbial functioning (Grigulis et al. 2016, Legay et al. 2014), but this was rarely addressed in a context of water shortage and for root traits. For example, Lõhmus et al. (2006a, 2006b) showed that the specific root area of short roots was positively correlated with the functional diversity and activity of microbial communities in the soil–root interface and in the bulk soil. In our study, we observed negative correlations between the functional dissimilarity of RTD and L/Volume and respiration rate on some carbon substrates. This in line with our field study (Shihan et al. 2017, see Chapter 3) in which we reported significant effects of functional dissimilarity in traits of litter mixture decomposing at the soil surface on soil microbial parameters. For its importance in controlling the length and surface area of root systems for a given biomass allocated to the roots (Fitter 2002), root tissue density (RTD) and root diameter control the amount of surface directly interacting with soil, such as the root surface colonized by mycorrhizal fungi (Smith and Read 2002). Also, roots with contrasting functional traits might produce exudates of contrasted chemical composition, favoring different microbial groups that differ in their metabolic capabilities.

As a whole, our results showed that both repeated drought treatment and plant species composition impacted the response of root morphological traits in the studied shrubland. In line with our first hypothesis of dehydration tolerant strategy for the grass species, we measured stronger of the grass species responses to drought and competition compared to the two woody species. Soil microbial substrate utilization patterns were generally not directly affected by drought or species composition (although our experimental design did not allow us to conclude about such effects) but differed between deep and top soil layers. A thorough investigation of relationship between root (including root biomass distribution in the deep and top soil layers) and soil parameters (including dynamics of soil water content in the profile, data that we did not have the time to include in our analysis yet) will

be helpful for understanding the response of the soil microbial parameters in the two soil layers.

Overall, our results suggest that plant species composition of Mediterranean shrublands may thus determine how particular species respond to drought even more than the direct drought effects.

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Chapter III

Changes in soil microbial substrate utilization in response to altered litter diversity and precipitation in a Mediterranean shrubland

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Ammar Shihan [1, 2, *]; Stephan Hättenschwiler [1]; Alexandru Milcu [1, 3]; François-Xavier Joly [1]; Mathieu Santonja [4] † and Nathalie Fromin [1] ‡

[1] : Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), UMR 5175, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier – EPHE, 1919 route de Mende, 34293 Montpellier Cedex 5, France.
[2] : Department of Renewable Natural Resources and Environment, Faculty of Agricultural, Aleppo University, Syria.
[3] : Ecotron Européen de Montpellier, Unité Propre de Service 3248, Centre National de la Recherche Scientifique (CNRS), Campus Baillarguet, F-34980 Montferrier-sur-Lez, France.
[4] : Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE), Aix Marseille Université, CNRS, IRD, Avignon Université, CS 80249, Case 4, 13331 Marseille Cedex 03, France.

† Present address: Université Rennes 1 - UMR CNRS 6553 ECOBIO, Avenue du Général Leclerc, Campus de Beaulieu, 35042 Rennes, France.
‡ Present address : PROMES-CNRS, 7 rue du Four Solaire, F- 66120 Odeillo (France).

* *Corresponding author*: Tel: (+33) 467 61 32 37
Fax: (+33) 467 61 33 36

E-mail addresses: ammarsh80@hotmail.com (A. Shihan),
stephan.hattenschwiler@cefe.cnrs.fr (S. Hättenschwiler),
alex.milcu@cnrs.fr (A. Milcu),
joly.fx@gmail.com (F.-X. Joly),
mathieu.santonja@gmail.com (M. Santonja),
nathalie.fromin@cefe.cnrs.fr (N. Fromin).

Abstract

This study aimed at quantifying the consequences of reduced precipitation and plant diversity on soil microbial community functioning in a Mediterranean shrubland of southern France. Across a natural gradient of shrub species diversity, we established a total of 92 plots (4 × 4 m) with and without a moderate rain exclusion treatment of about 12% of total precipitation. Shrub diversity included all possible combinations of the four dominant species (*Cistus albidus*, *Quercus coccifera*, *Rosmarinus officinalis* and *Ulex parviflorus*). Respective leaf litter mixtures of these species combinations were exposed in all plots over two years. We quantified how litter species richness and the reduction in precipitation affected the soil microbial substrate utilization (measured by CO_2 evolution using the MicroResp method) on soil samples collected underneath each individual litter mixture after one and two years of decomposition. Moderate precipitation reduction had a minor impact, but litter species richness and the dissimilarity in phenolic concentrations (estimated usingRao's quadratic entropy) showed a positive effect on the diversity of substrates metabolized by the microbial communities. Moreover, litter species richness increased soil microbial activity by increasing the catabolic diversity of the soil microbial community. These effects were mostly driven by the presence of *Quercus* and *Ulex* leaf litter, which at the same time reduced microbial metabolic dominance. The presence of *Rosmarinus* had opposite effects. Our data suggest that plant species loss can have stronger effects on the functioning of soil microbial communities than moderate drought, with potentially important feedbacks on biogeochemical cycling in Mediterranean shrubland ecosystems.

KEYWORDS:

Climate change; Mediterranean ecosystem; litter decomposition; functional diversity; soil microbial activity; MicroResp$^{(TM)}$.

1. Introduction

Anthropogenically induced changes in biodiversity and precipitation patterns are mutually impacting the functioning of a wide range of ecosystem processes (Field et al. 2015). However, the understanding of how these two drivers of global change interact and affect ecosystem functioning is limited. In particular, the consequences for the functioning of the soil microbial communities, known to exhibit a strong control over the carbon (C) and nutrient cycles have been little explored (Rodríguez-Loinaz et al. 2008; Chapman and Newman 2009).

There is increasing evidence that plant diversity underpins the functioning of ecosystems (Cardinale et al. 2012; van der Plas et al. 2016). Different plant species produce litter with a wide range of distinct morphological and chemical characteristics (Cornwell et al. 2008). The emerging complex interactions between direct effects of plant community composition on the quality and quantity of litter entering the soil system, and indirect effects mediated by the responses of the decomposer communities (Wardle 2002) make the identification of general patterns for the interaction between litter characteristics and soil microbial functioning a considerable challenge. Moreover, plant diversity impacts on microclimatic conditions (Lorentzen et al. 2008) are known to be major driving factors of microbial processes (Freiberg 1998) affecting C and nitrogen (N) cycling. In general, it appears that plant functional diversity is more important than species richness for the understanding of how plants and soil biota interact (Johnson et al. 2003; Porazinska et al. 2003; De Deyn et al. 2004; Salamon et al. 2004). The majority of studies assessing the role of litter diversity for processes related to decomposition demonstrated that biodiversity effects are mostly driven by the functional traits represented in litter mixtures, independently of how many species are contributing to the mixtures (e.g. Wardle et al. 1997; Ball et al. 2008; Barantal et al. 2014; Handa et al. 2014; but see Chen et al. 2015).

Two broadly complementary functional trait-based approaches are currently being used. One approach aims at identifying the dominant traits driving the ecosystem processes, and can be related to the "biomass–ratio hypothesis" (Grime 1998) stating that the effect of any species on a given ecosystem process is proportional to the relative abundance of that species in the community (or in the litter mixture). Accordingly, plant community weighted mean traits (CWM) have been found to be relevant predictors of plant community effects on certain ecosystem processes. The second approach aims at quantifying the functional diversity of communities by assessing the variety, range and evenness of the traits present in communities (or in the litter mixture), making reference to the niche complementarity hypothesis, since a greater range of trait-values is generally considered to indicate less niche overlap (Petchey and Gaston 2006; Díaz et al. 2007). However, functional identity and functional diversity are not mutually exclusive in affecting ecosystem processes (Violle et al. 2007) such as primary productivity (Schumacher and Roscher 2009; Roscher et al. 2012) or litter decomposition (Mokany et al. 2008; Barantal et al. 2011; Handa et al. 2014). Although these functional trait-based approaches have primarily been developed at the level of the plant community, several studies showed that they can be also useful in the understanding of diversity effects on litter decomposition (Epps et al. 2007; Meier and Bowman 2008, 2010) or other soil processes (Meier and Bowman 2008, 2010).

Soil communities have been shown to vary as a function of the litter resources deposited at the soil surface (e.g. Ayres et al. 2009; Carrillo et al. 2011; Milcu et al. 2013), and thus, they may be modified with alterations in the species composition of the litter layer. There is compelling evidence that litter functional identity and diversity are important drivers of litter decomposition (Cornwell et al. 2008; Handa et al. 2014), with species-rich plant communities supporting more complementary microbial communities

(Eisenhauer et al. 2010). A higher microbial diversity could translate in a more efficient use of organic substrates because of a greater functional niche exploitation (Loreau 2001). As a further consequence of more complementary niche exploitation, microbial biomass may increase in species rich litter mixtures offering higher resource diversity (Chapman and Newman 2009). Indeed, results from plant diversity experiments documented higher soil microbial biomass and activity with increasing plant species and functional diversity (Eisenhauer et al. 2010; Milcu et al. 2013; Vogel et al. 2013; Steinauer et al. 2015).

The predictability of how plant diversity loss is affecting C processing in the soil is difficult also because concomitant changes in environmental factors such as temperature or precipitation may interact with changes in plant diversity. Mediterranean ecosystems are considered to be particularly sensitive to changes in precipitation (Lionello and Giorgi 2007), and altered frequency of rainfall events and altered frequency of rainfall events (Gao et al. 2006). For example, the indirect drought effects on litter decomposition via changes in litter quality may be substantial even without a change in plant diversity, and should be considered along with direct soil moisture driven effects for accurate predictions of litter decomposition in Mediterranean ecosystems under climate change (García-Palacios et al. 2016). Several studies evaluated the response of soil microbial properties to plant diversity (e.g. Eisenhauer et al. 2010; Milcu et al. 2013) or to climate change factors (in the Mediterranean context see: Berard et al. 2012; Pailler et al. 2014; Yuste et al. 2014). However, very few studies addressed the combined effects of plant diversity and climate change factors (Niklaus et al. 2007; Bloor and Bardgett 2012; Eisenhauer et al. 2013; Vogel et al. 2013). A synthesis of 12 studies with orthogonal manipulations of global environmental change factors and plant diversity (Takhur et al. (2015) showed that effects of environmental change factors such as drought or nutrient enrichment and of plant diversity effects on the soil microbial biomass appeared to be independent with no

evidence for interactions. However, very little experimental evidence exists for Mediterranean ecosystems, where both decreasing precipitation and biodiversity loss are expected to occur simultaneously in the forthcoming decades. Lloret et al. (2015) showed that the quite drastic effects of drought via plant mortality increased the richness and changed the composition in soil microbial community of Mediterranean woodlands. Despite the recent emergence of methodologies characterizing the functional differences between microbial communities (e.g. MicroResp™, Chapman et al. 2007; Campbell et al. 2008), a functional assessment of the impact of the interaction between diversity and reduced precipitation is currently lacking.

Here, we assessed how experimentally reduced precipitation in a Mediterranean shrubland ecosystem affects the functioning of soil microbial community along a natural gradient of plant diversity (one to four woody plant species). We hypothesized that i) an increase in litter species richness and functional dissimilarity (Rao's quadratic entropy; Botta-Dukát 2005; Epps et al. 2007) will lead to more complementary microbial communities that are able to degrade multiple substrates more efficiently, and ii) the soil microbial communities associated to more diverse litter mixtures are also more resistant to reduced precipitation. To test our hypotheses, we characterized the ability of soil microbial communities to respire on 15 different C substrates and to decompose cellulose paper in the soil associated to all possible combinations of litter mixtures of four woody shrub species (*Cistus albidus* L., *Quercus coccifera* L., *Rosmarinus officinalis* L. and *Ulex parviflorus* Pourr.) dominating at our study site, in a fully factorial rainfall exclusion experiment, and after one and two years of *in situ* decomposition. Furthermore, we explored how the soil microbial substrate utilization was affected by litter species richness, litter species composition and identity as well as functional dissimilarity of the litter traits using Rao's diversity metric and community weighted mean traits (CWM).

2. Materials and Methods

Study site

The study site was located in the Massif de l'Etoile near Marseille, France (43°22' N; 5°25' E) at 275 m above sea level (see Montès et al. 2008)) for a detailed description of the site). The mean annual precipitation is 552 mm and the mean annual temperature is 14.6°C. The soil is classified as a shallow rendzina developed over limestone bedrock with 65.7% of stones in the top 50 cm. Soil texture, C, N, P, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Fe^{3+}, Mn^{2+}, Al^{3+} and Pb^{2+} concentrations, CEC and pH were analyzed for each of the 92 individual plots by the Laboratoire d'Analyse des Sols (INRA Arras) and are presented in Supplementary Table S3.1.

The vegetation is a woody shrub dominated "garrigue" with individual shrub heights ranging from 0.2 to 1.4 m and a cover between 25 and 95%, see (Montès et al. 2008) for further details). Four woody shrub species dominate the community: *Quercus coccifera* L. (*Quercus*, with an average cover across all plots of 36%), *Cistus albidus* L. (*Cistus*, 18%), *Ulex parviflorus* Pourr. (*Ulex*, 10%) and *Rosmarinus officinalis* L. (*Rosmarinus*, 9%).

Experimental design

Ninety-two 4 × 4 m plots were selected based on plant community composition in order to include all 11 possible combinations of the four dominant shrub species and their single species patches (a total of 15 treatments). Each of the 15 different plant combinations was replicated six times, except for the 4-species mixture that was replicated eight times. Half of the plots (four replicates for the 4-species mixtures and three replicates for all other treatments) were equipped with a rain exclusion device that consisted in an aluminum frame holding stainless steel gutters at 2 m above the ground and covering 40% of the plot

surface. A supplementary PVC gutter and a pipe mounted at the border of the frame allowed to evacuate the rainwater away from the plots. The remaining plots were used as control plots that were also equipped with gutters, but mounted inversely in order to diminish interception of rainfall. The experimental rain exclusion was set up in October 2011. The exact amount of precipitation excluded was estimated using i) rain gauges installed at ground level underneath the gutters in both control and rain-excluded plots, and ii) TDR100 probes (Campbell Scientific Inc., Logan Utah) installed in 7 control and 8 rain-excluded plots at 10 cm soil depth, and by iii) determining the gravimetric humidity in the soil sampled in control and excluded plots. Compared to control plots, the rain-exclusion plots on average received less rainfall 12 ± 2% s.e.m.). This exclusion resulted in an average lower soil humidity of -6.5% (that could reach between -13 to -24% during rain events) at 10 cm soil depth between control and rain exclusion plots (determined by permanently installed TDR probes; Supplementary Fig. S3.1), (Santonja et al. submitted).

Litter decomposition and soil sampling

Because the relative contribution of the different species is not even at our field site, and in order to standardize the litter material decomposing on the soil surface as well as the stage of decomposition across all plots, the microbial substrate utilization was estimated from the soil (down to 5 cm depth) under which the litter mixtures decomposed in field mesocosms. Freshly shed leaves from *Cistus*, *Quercus*, *Rosmarinus*, and the smallest distal twigs from *Ulex* (this species has photosynthesizing stems rather than leaves) were collected during peak leaf litterfall from June to July in 2011 using litter traps suspended under the shrubs that were emptied regularly. This material was air-dried and pooled to prepare single batches of leaf litter for the four species. A common leaf litter material from *Pinus halepensis* (a species that does not occur at our study site but is typical for the Mediterranean area) was collected like for the other species in an adjacent forest site and

used in the incubation experiment in an attempt to separate the direct effects of decomposing leaf litter from the overall effects of the living plants (for example through root-driven processes).

The litters used in the decomposition experiment (4 single shrub species and 11 possible combinations of these four species) were composed of equal amounts of each contributing species with a total of six grams of dry leaf litter (or also six grams for the single species treatments) per mesocosm. These litters were incubated in the plots with the corresponding shrub species composition (for instance, the *Quercus*/*Cistus* mixtures were incubated in the plots where these two species, but not *Rosmarinus* or *Ulex* occurred). Additionally, mesocosms with 6 g of *Pinus* litter were incubated in all the 92 plots. Mesocosms consisted in "open-bottom" plastic cylinders (8 cm in diameter and 5 cm tall) that were randomly placed and fixed on the bare soil surface (naturally occurring litter was removed previously) within the central 4 m^2 of each plot. Two openings (12 × 3 cm) were cut on the side of the plastic cylinders and covered with 10 mm mesh. These side openings and the open bottom of the cylinders allowed full access to the litter for the naturally occurring soil and litter-dwelling fauna. The top of the cylinders was covered with 1 mm mesh to avoid canopy litter mixing with the target litter while allowing the passage of rain water. Seven mesocosms of plot-specific leaf litter and two mesocosms of *Pinus* litter were prepared and installed in the field for each plot in December 2011.

We retrieved four replicates in December 2012 and three replicates in December 2013 of plot-specific leaf litter. The two mesocosms filled with *Pinus* litter were both collected after two years in December 2013. The remaining leaf litter was collected from the plastic cylinders after one and two years of field exposure (see Santonja et al. submitted for results). After litter removal, we collected the underlying top soil to about 5 cm depth, yielding a total of 828 soil samples (368 samples after one year and 276 samples after two

years for plot-specific litter, and 184 samples after two years for *Pinus* litter). The soil samples for the microbial analyses were air-dried in the dark at 25°C for 10 days and sieved through a 2 mm sieve. Equal amounts of soil of the three or four replicate samples (plot-specific litter) or two replicate samples (*Pinus* litter) from each plot and at each sampling date were mixed and homogenized to produce representative composite samples. These composite samples were stored in a dry and dark storage room until analysis.

Leaf litter traits

Eight leaf litter functional characteristics (traits) of the shrub species, known from the literature to correlate with litter decomposition rates were determined: the concentrations of total C (C), N (N), phosphorus (P), lignin, total phenolics (Phenolics), dissolved organic C (DOC), and total dissolved N (TDN) (Joly et al. (2016) underlined the critical role of soluble litter compounds to explain litter mixture effects on soil microbial properties), as well as the water holding capacity (WHC) (Supplementary Table S3.2). The community-weighted mean traits of litter mixtures (C_{CWM}, N_{CWM}, P_{CWM}, $Lignin_{CWM}$, $Phenolics_{CWM}$, $Lignin{:}N_{CWM}$, DOC_{CWM} TDN_{CWM} and WHC_{CWM}) were calculated as the average trait values of litter mixtures according to the following equation (Garnier et al. 2004): $Trait_{CWM} = \sum_{i=1}^{n} B_i \times trait_i$, where B_i is the relative abundance for species i and $trait_i$ is the trait value for species i. The functional dissimilarity was calculated according to the Rao index (Epps et al. 2007) for each litter mixture (C_{FD}, N_{FD}, P_{FD}, $Phenolics_{FD}$, $Lignin_{FD}$, $Lignin{:}N_{FD}$, DOC_{FD}, TDN_{FD} and WHC_{FD}) as: $Trait_{FD} = \sum_{i=1}^{n} \sum_{j=1}^{n} pipj * dij$ (using the dbFD function of the R package "FD") where pi and pj are the relative abundance for shrub species i and j in the litter mixture, and dij the Euclidian distance between species i and j for the trait considered. Because the measured traits differ in their numerical value ranges, standard normal deviates (so as to get an expected value of zero and a variance of one for traits values) were used to calculate functional dissimilarity.

Soil microbial substrate utilization by MicroResp

MicroResp™ offers a convenient, rapid and sensitive method for the determination of soil microbial substrate utilization which allowed us to estimate the microbial community functional diversity (Campbell et al. 2008). Its application in a number of case studies has demonstrated its utility in a wide range of soils and land-cover types (Chapman et al. 2007; Creamer et al. 2016). About 0.49 g dry weight of soil were incubated in triplicate with 1.5 mg C g^{-1} soil (except for the low-soluble phenolic acids and cellulose for which 0.75 mg C g^{-1} soil were added) of 15 different C substrates, plus one control with deionized water, so as to reach 80% of the field capacity in 96-DeepWell Microplates (Fisher Scientific E39199). Carbon substrates included three carbohydrates (D-glucose, xylan, cellulose, by order of complexity), one amine (N-acetyl-glucosamine), five amino acids (L-asparagine, L-glutamine, L-lysine, L-serine, L-glycine), three carboxylic acids (malic acid, oxalic acid, uric acid), and three phenolic acids (caffeic acid, which is an intermediate in lignin biosynthesis, syringic acid, and vanillic acid which is a by-product during the degradation of polyphenols by fungi). Cresol red gel detection plates were prepared as recommended by the manufacturer. After an initial two-hour pre-incubation at 25°C in the dark, each deepwell microplate was covered with a detection plate using a silicone gasket (MicroResp™, Aberdeen, UK). The assembly was secured with a clamp, and incubated for four additional hours. Optical Density at 590 nm (OD_{590}) was measured for each detection well before and after incubation using a Victor 1420 Multilabel Counter (Perkin Elmer, Massachusetts, USA). Final OD_{590} were normalized using pre-incubation OD_{590}, and converted as substrate induced respiration rates expressed in µg C-CO_2 respired per g of soil per hour.

The respiration rates for the different C substrates were summed across all 15 substrates (sum15) as a proxy of the global metabolic activity, and standardized rate for

each substrate was computed as the rate measured for this substrate divided by sum15, as proposed by (Leff et al. 2012). For each plot, a Shannon metabolic diversity index was computed as: $H' = -\sum_{i=1}^{15} pi * \log(pi)$, and a Simpson metabolic dominance index was computed as: $D = \sum_{i}^{j} pi^2$, where (*pi)* is the standardized respiration rate for substrate (*i)*.

Decomposition of Cellulose Paper (DCP)

Cellulose paper disks (0.20 ± 0.009 g) with a diameter of 55 mm (filter paper qualitative 388, 84 g/m^2, Sartorius) were weighted, enclosed between two nylon nets (0.2 mm mesh size) and incubated with 20 g of soil in 90 mm-diameter Petri dishes. The soil was wetted with deionized water so as to reach 80% of field capacity, and then placed in the dark for three weeks at a constant temperature of 25°C (Wardle et al. 1999). After 21 days of incubation, the remaining cellulose was recovered, cleaned gently with a brush in deionized water, and then oven dried to constant mass (65°C) to determine remaining cellulose dry mass and cellulose mass loss. The cellulose disks were then burned in a muffle oven (550°C) to determine ash-free dry mass to correct for adhering mineral soil. DCP was measured from soil samples underneath plot-specific litter only, but not from soil samples underneath *Pinus* litter.

Data analysis

Statistical analyses were performed with the R software (version 3.1.0, The R Foundation for Statistical Computing 2014) with significance levels indicated as ' for $p < 0.10$, * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$). We used a multiple linear model approach ("*lm"* function from the "stats" package) to determine the most parsimonious models predicting the microbial parameters (sum15, H', D and DCP). For each microbial response variable, we carried out four distinct statistical models aiming to assess the importance of

experimental treatments, species identity, functional diversity of litter traits (Rao's quadratic entropy) and CWM of litter traits, respectively. The first model tested the impact of precipitation treatments (control *vs.* precipitation reduction), species richness, species composition and their interactions. The second model tested the impact of species identity (i.e. the presence/absence of a particular species), precipitation treatment and their interactions. The third and fourth models tested the impact of functional diversity ($traits_{FD}$) and the CWM ($traits_{CWM}$) of individual traits, respectively. Given the high number of litter traits measured, for the third and fourth models we preselected four traits using the "*randomForest*" function of the eponymous package, which classifies predictor variables by importance (Cutler et al. 2007). To take into account the effects of soil heterogeneity between plots, we used the scores of the first axis of a PCA analysis of plot-specific soil characteristics [soil texture, pH, cation exchange capacity (CEC), and the concentrations of C, N, P, calcium, magnesium, sodium, potassium, iron, lead, manganese and aluminium (see Supplementary Table S3.1)], named *Soil-PCA* (accounting for 40.3% of the total variability, see Supplementary Fig. S3.2) as covariable in all models. The full models were then simplified to determine the most parsimonious models using the "*stepAIC*" function of the "MASS" package, an established model selection procedure with both forward and backward selection algorithms, which ranks all candidate models (all possible combinations of initial explanatory variables included in the full model) based on lowest AICs (Crawley 2013). We present the r^2 and AIC-values for both the full model (with all initial explanatory variables) and the most parsimonious model. Relationships between soil microbial parameters (sum15, H', D and DCP) and individual litter mixture parameters (litter species richness, $trait_{CWM}$ or of $trait_{FD}$) were plotted using linear regressions across all plots. To meet the assumption of normal distribution of residuals, we applied a log transformation for sum15 values and a square root transformation for DCP values

As sum15, H' and litter species richness turned out to be correlated, and in order to test our first hypotheses we also performed a path analysis using the "lavaan" package (Rosseel 2012) to assess whether the effect of species richness on sum15 was mediated by a richness to H' pathway. Good model fit was indicated by non-significant differences between the predicted and observed covariance matrices (χ^2 tests with $p > 0.05$), lower Root Mean Squared Error Approximation (RMSEA < 0.1), higher Comparative Fit Index (CFI > 0.90) (Grace 2006; Rosseel 2012) and an $AIC_{model} < AIC_{unrestricted\_model}$.

3. Results

Effects of litter species richness and composition on microbial substrate utilization

The global metabolic activity that included respiration rates of all 15 substrates (sum15) in the soil from single species litter treatments varied only little after one year of decomposition (between 47.0 ± 8.6 and 51.1 ± 7.0 µg $C\text{-}CO_2$ g^{-1} of soil h^{-1}), but considerably more after two years of decomposition (between 37.0 ± 6.0 and 50.1 ± 10.2 µg $C\text{-}CO_2$ g^{-1} of soil h^{-1}) across control and rain-excluded plots (Supplementary Table S3.3). When all litter mixture treatments were included, the overall range in sum15 values was wider with the highest rates measured under the *Cistus*/*Ulex* mixture (66.6 ± 4.6 µg $C\text{-}CO_2$ g^{-1} soil h^{-1}) and the lowest rates measured under the *Quercus*/*Ulex*/*Rosmarinus* mixture (37.6 ± 4.3 µg $C\text{-}CO_2$ g^{-1} soil h^{-1}) after one year of decomposition across both precipitation treatments (Supplementary Table S3.3). Neither species richness nor species composition affected total microbial activity after one year (Table 3.1a).

Table 3.1. Output of multiple linear models testing for the effects of rain exclusion treatment (RE), litter mixture species richness (richness), litter composition (COMP) and their interactions on the soil microbial parameters (global metabolic activity (sum15), microbial metabolic diversity (H'), microbial metabolic dominance (D), and rate of decomposition of cellulose paper (DCP)). Plot-specific soil characteristics (Soil-PCA) were included as co-variable. We report the R^2 and AIC weight of the general model including all factors (All) and of the most parsimonious model (MPM**,** in bold). Only the variables retained in the most parsimonious models are reported (intercept = none of variable was retained). n = 92 plots in 2012 (a), 2013 (b) and for pine litters (c), respectively. (F-value and associated significance of p-value (' $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Arrows indicate the direction of the effect.

(a) 2012		D.f	S.sq	M.sq	*F*-value	*p*-value	All R^2 (AIC)	**MPM R^2 (AIC)**
sum15	Soil-PCA	1	0.085	0.085	3.261	0.074'	0.17 (-317.4)	**0.04 (-333.8)**
	Residuals	90	2.341	0.026				
H'	RE	1	1e-08	1e-08	2e-05	0.997	0.49 (-637.5)	**0.48 (-638.44)**
	COMP	**14**	**0.022**	**0.002**	**2.102**	**0.024***		
	RE x COMP	**14**	**0.021**	**0.002**	**2.026**	**0.030***		
	Residuals	62	0.046	0.001				
D	RE	1	2e-05	1.6e-06	0.011	0.915	0.47 (-793)	**0.46 (-793.6)**
	COMP	**14**	**0.004**	**2.7e-04**	**1.967**	**0.036***		
	RE x COMP	14	0.003	2.5e-04	1.785	0.061'		
	Residuals	62	0.009	1.4e-04				
DCP	**Soil-PCA**	**1**	**34.16**	**34.16**	**16.175**	**<0.001*****	0.44 (85.2)	**0.44 (83.3)**
	COMP	**14**	**91.01**	**6.50**	**3.078**	**<0.001*****		
	Residuals	76	160.52	2.11				
(b) 2013								
sum15	**Soil-PCA**	**1**	**0.260**	**0.260**	**14.467**	**<0.001*****	0.36 (-353.3)	**0.36 (-355.3)**
	COMP	**14**	**0.508**	**0.036**	**2.022**	**0.027***		
	Residuals	76	1.364	0.018				
H'	**Soil-PCA**	**1**	**0.001**	**0.001**	**7.800**	**0.006****	0.29 (-786.9)	**0.14 (-797.2)**
	richness	**1**	**0.001**	**0.001**	**6.864**	**0.01* ↑**		
	Residuals	89	0.015	0.0002				
D	**Soil-PCA**	**1**	**0.0001**	**1.5e-04**	**6.152**	**0.015***	0.26 (-960.3)	**0.13 (-972.5)**
	richness	**1**	**0.0002**	**1.7e-04**	**6.808**	**0.01* ↓**		
	Residuals	89	0.002	2.5e-05				
DCP	Intercept						0.16	
(c) Pinus litters								
sum15	**Soil-PCA**	**1**	**0.227**	**0.227**	**10.34**	**0.002 ****	0.36 (-322.7)	**0.10 (-349.6)**
	Residuals	90	1.971	0.022				
H'	Soil-PCA	1	0.0009	0.0009	2.34	0.13	0.37 (-696.7)	**0.03 (-714.1)**
	Residuals	90	0.038	0.0004				
D	Intercept						0.35	

After two years of decomposition, the range in sum15 values covered was comparable to that after one year of decomposition, with the highest values measured under the *Quercus*/*Ulex* mixture (64.0 ± 9.4 µg C-CO_2 g^{-1} soil h^{-1}) and the lowest under the *Quercus*/*Rosmarinus* mixture (31.1 ± 3.0 µg C-CO_2 g^{-1} soil h^{-1}) across both precipitation

treatments. In year two, species richness still did not affect sum15, but there was a significant litter composition effect (Table 3.1b). The plot-specific soil characteristics (*Soil-PCA*) accounted for most of the observed variation in sum15 in both years of sampling, although only marginally significantly after one year of decomposition (Tables 3.1a and 3.1b). The mean sum15 values measured under *Pinus* litter after two years of decomposition ranged between 43.1 ± 5 and 79.8 ± 12.5 µg C-CO_2 g^{-1} soil h^{-1}. There was a similar strong effect of plot-specific soil characteristics (*Soil-PCA*) on sum15 under *Pinus* compared to plot-specific leaf litter, but species richness or composition had no effect (Table 3.1c).

Microbial metabolic diversity indices were not affected by rain exclusion (Tables 3.1a and 3.1b), but they varied among shrub litter treatments in both years (Supplementary Table S3.3). These differences were largely explained by species composition and its interaction with rain exclusion that affected both H' and D after one year of decomposition (Table 3.1a). After two years of decomposition, litter diversity effects were driven by species richness rather than composition (Table 3.1b): H' increased (Fig. 3.1a), and D decreased (Fig. 3.1b) with increasing species richness in the litter mixtures. The plot-specific soil characteristics (*Soil-PCA*) also had a significant effect on H' and D after two years of decomposition, which was of similar importance overall compared to litter species richness (Table 3.1b). Finally, soil microbial indices from *Pinus* mesocosms showed no effect of rain exclusion, species composition or species richness, nor of *Soil-PCA* (Table 3.1c).

There was substantial variation in DCP, with the lowest rates measured in soils covered with pure *Rosmarinus* litter (2.8 ± 1.7% mass loss) and the highest rates measured in soils covered with pure *Cistus* litter (27.9 ± 8.0%) after one year of decomposition. Rates of DCP varied somewhat less after two years, with the lowest rates measured in soils

covered with the *Rosmarinus/Ulex* litter mixture (25.6 ± 7.3%) and the highest rates measured in soils covered with the four species mixture *Cistus/Quercus/Rosmarinus/Ulex* (46.8 ± 7.5%, Supplementary Table S3.3).

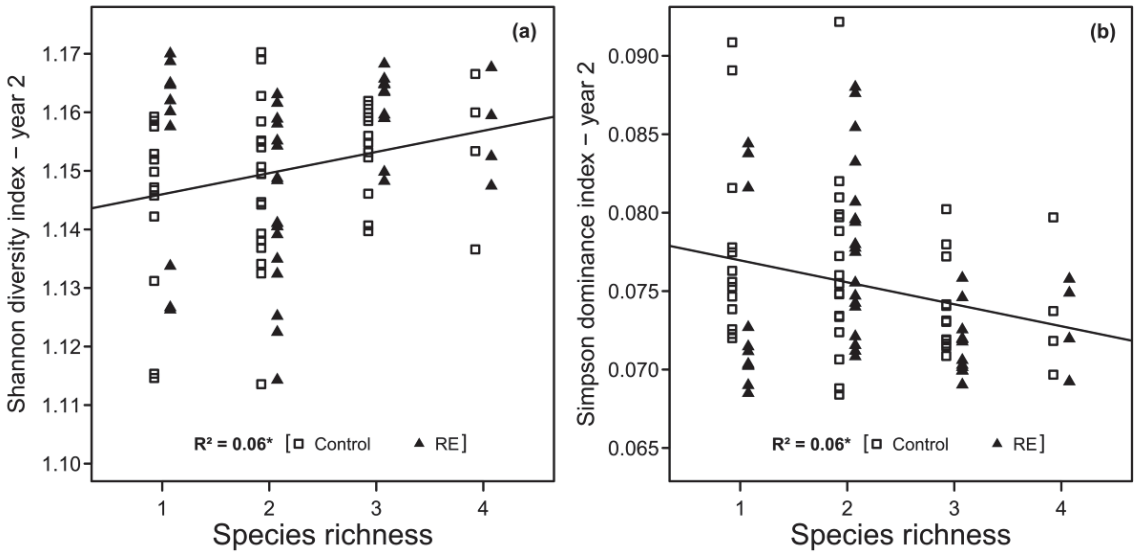

Fig. 3.1. Relationships between species richness in the litter mixtures and Shannon soil microbial metabolic diversity index H' (a) or Simpson soil microbial metabolic dominance index (b) after two years. Open symbols are for control plots and close symbols are for rain excluded plots. R^2 (with associated significance of p-value * $p < 0.05$) and solid lines are the linear regressions across all plots (n=92).

After one year of decomposition, DCP rates were mainly explained by soil physico-chemical parameters, and to a lower extent by litter species composition (Table 3.1a). None of the factors we evaluated had any significant effect on rates of DCP after two years of decomposition. The observed litter composition effects on soil microbial parameters were in part related to the presence of particular litter species alone or in a mixture. For example, in the presence of *Ulex* litter, sum15 after two years of decomposition was higher than when this species was absent (Table 3.2).

Similarly, when *Quercus* litter was present, H' tended to be higher and D to be lower after two years compared to when this species was absent (Table 3.2). The rate of DCP was constantly higher in the presence of *Quercus* leaf litter after one and two years of

decomposition (Table 3.2) compared to litter treatments without any *Quercus* leaf litter. The presence of *Rosmarinus* litter had a negative effect both on sum15, but only after one year, and on DCP in both years (Table 3.2). The presence of *Cistus* litter had no effect on any of the microbial parameters in either year (Table 3.2). There were no interactions between the presence of a given species and the rain exclusion treatment.

Table 3.2. Output of multiple linear models testing for the effect of rain exclusion treatment (RE) and presence of the different individual litter species (*Cistus*, *Quercus*, *Rosmarinus*, and *Ulex*) in the litter mixture and their interactions with RE on microbial parameters (global metabolic activity (sum15), microbial metabolic diversity (H'), microbial metabolic dominance (D), and rate of decomposition of cellulose paper (DCP)). Plot-specific soil characteristics (Soil-PCA) were included as co-variable. Only the variables retained in the most parsimonious models are reported. (*F*-value and associated significance of *p*-value (NS = not significant effect; ' $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). n = 92 plots in 2012 (a) and 2013 (b), respectively. Arrows indicate the direction of the effect. Interactions between individual litter species and RE were never significant and are not reported.

	sum15		H'		D		DCP	
	2012	2013	2012	2013	2012	2013	2012	2013
Soil-PCA	*NS*	**12.902*** **	**4.388***	**7.515****	**5.106***	**5.945***	**13.648*** **	*NS*
RE	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Quercus	*NS*	*NS*	*NS*	3.366' ↑	*NS*	3.586' ↓	**5.437*** ↑	2.763' ↑
Cistus	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Ulex	*NS*	**4.026*** ↑	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Rosmarinus	**4.965*** ↓	*NS*	*NS*	*NS*	*NS*	*NS*	**7.041**** ↓	2.881' ↓

Effects of functional trait-based metrics on microbial substrate utilization

For a more detailed understanding of how the identity and diversity of leaf litter decomposing at the soil surface affected the soil microbial parameters, we evaluated the relationships between litter mixture aggregated functional traits ($Trait_{CWM}$) or functional dissimilarity based on the Rao index ($Trait_{FD}$). We found no relationship between sum15, H' or D of the soil microbial community with any of the aggregated traits of the litter mixtures in either year of sampling (Table 3.3). The only weak effect of aggregated litter traits was observed in the first year on DCP rates, which increased with increasing

DOC_{CWM} and with increasing N_{CWM} (Table 3.3, Fig. 3.2). Together, DOC_{CWM} and N_{CWM} accounted for 23% of the variance in DCP rates after one year of decomposition across all plots. However, these effects disappeared after two years of decomposition (Table 3.3) while the variation observed in the soil microbial parameters was more regularly associated to variation in soil characteristics (*Soil-PCA*, Table 3.3).

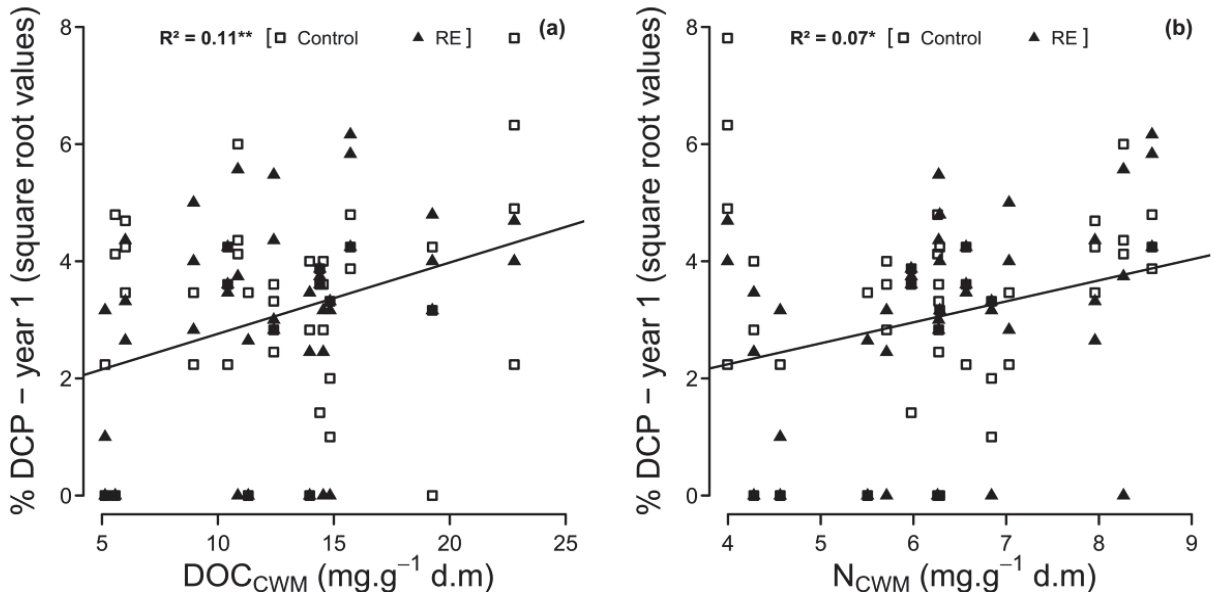

Fig. 3.2. **Relationship between DCP rates and community weighted mean traits of litter mixtures for dissolved organic C (DOC_{CWM}) (a) or for total N (N_{CWM}) (b) after one year.** Open symbols are for control plots and close symbols are for rain excluded plots. R^2 (with associated significance of *p*-value * $p < 0.05$; ** $p < 0.01$) and solid lines are the linear regressions across all plots (n=92).

Soil-PCA accounted also for most of the variation in soil microbial parameters when assessing the impact of functional dissimilarity of traits in litter mixtures. However, functional trait dissimilarity appeared to be generally more important for soil microbial parameters compared to CWM traits, in particular after two years of decomposition (Table 3.4b).

After two years, sum15 increased with increasing $Lignin_{FD}$ (Table 3.4b, Fig. 3.3a), and H' increased and D decreased with increasing $Phenolics_{FD}$ in the litter mixtures (Table 3.4, Fig. 3.3c and 3.3d). Rates of DCP showed a marginally significant increase with

increasing $Phenolics_{FD}$ (Table 3.4). After one year there was an additional negative effect of increasing WHC_{FD} on rates of DCP (Table 3.4, Fig. 3.3b).

Finally, interactions between individual $Trait_{CWM}$ or $Trait_{FD}$ and rain exclusion were never significant, indicating that the reported effects did not depend on the amount of rainfall received.

Table 3.3. Outputs of multiple linear models testing for the effects of aggregated traits ($trait_{CWM}$) in litter mixture, rain exclusion treatment (RE) and their interactions on soil microbial parameters (global metabolic activity (sum15), microbial metabolic diversity (H'), microbial metabolic dominance (D), and rate of decomposition of cellulose paper (DCP)). Plot-specific soil characteristics (Soil-PCA) were included as co-variable. Interactions between $trait_{CWM}$ and RE treatment were never significant. Only the variables retained in the most parsimonious models are reported (intercept = none of variable was retained). We report the R^2 and AIC weight of the general model including all factors (All) and of the most parsimonious model (MPM**,** in bold). n = 92 plots in 2012 (a) and 2013 (b), respectively. (F-value and associated significance of p-value (' $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Arrows indicate the direction of the effect.

(a) 2012		D.f	S.sq	M.sq	F-value	p-value	All R^2 (AIC)	**MPM R^2 (AIC)**
sum15	Soil-PCA	1	0.085	0.085	3.261	0.074'	0.05 (-327.4)	**0.04 (-333.8)**
	Residuals	90	2.341	0.026				
H'	**Soil-PCA**	**1**	**0.004**	**0.004**	**4.388**	**0.039***	0.05 (-630.6)	**0.05 (-638.4)**
	Residuals	90	0.086	9e-04				
D	**Soil-PCA**	**1**	**9e-04**	**9e-04**	**5.106**	**0.026***	0.06 (-790.3)	**0.05 (-798.4)**
	Residuals	90	0.015	2e-04				
DCP	**Soil-PCA**	**1**	**34.16**	**34.16**	**14.328**	**<0.001*****	0.27 (87.2)	**0.27 (84.0)**
	DOC_{CWM}	**1**	**19.32**	**19.32**	**8.104 ↑**	**0.006****		
	N_{CWM}	**1**	**22.37**	**22.37**	**9.380 ↑**	**0.003****		
	Residuals	88	209.84	2.38				
(b) 2013								
sum15	**Soil-PCA**	**1**	**0.260**	**0.260**	**12.48**	**<0.001*****	0.15 (-349.6)	**0.14 (-354.5)**
	Residuals	90	1.872	0.021				
H'	**Soil-PCA**	**1**	**0.001**	**0.001**	**7.323**	**0.008****	0.09 (-785.7)	**0.08 (-792.3)**
	Residuals	90	0.016	2e-04				
D	**Soil-PCA**	**1**	**2e-04**	**1.5e-04**	**5.779**	**0.018***	0.07 (-961.0)	**0.06 (-967.8)**
	Residuals	90	0.002	2.6e-05				
DCP	Intercept						0.06	

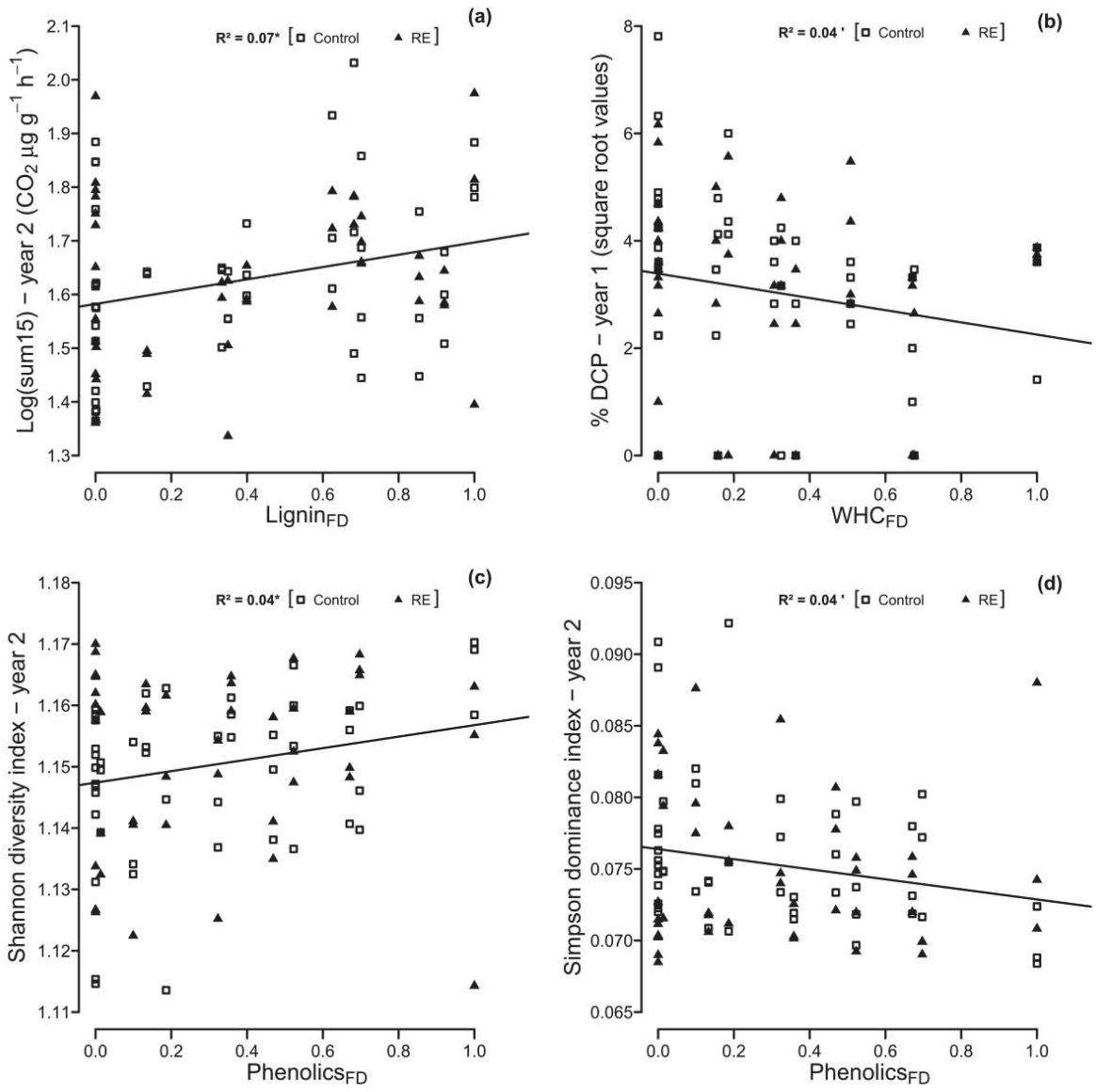

Fig. 3.3. Relationships between soil microbial metabolic parameters and functional dissimilarity of litter traits in the litter mixtures (a) between sum15 and $Lignin_{FD}$ after two years, (b) between cellulose decomposition rates (DCP) and WHC_{FD} after one year (c, d) between H' or D index, respectively, and $Phenolics_{FD}$ after two years. Open symbols are for control plots and closed symbols are for rain excluded plots. R^2 (with associated significance of p-value ' $p < 0.10$; * $p < 0.05$) and solid lines are the linear regressions across all plots (n=92).

Path analysis testing links between species richness, H' and sum15

As H' correlated with sum15 (R^2 = 0.36) (Fig. 3.4a) and species richness significantly affected H' after two years (Table 3.1), we used path analysis to test the emerging conjecture that species richness affects sum15 via an indirect pathway from H' to sum15. The model converged ($\chi^2 = 0.03$, p = 0.83) and showed a very good model fit (RMSEA =

0, CFI = 1, AIC_{model} = 14.97, $AIC_{unrestricted\_model}$ = 16.94). The path diagram of the model (Fig. 3.4b) displays a significant pathway between litter species richness and H' (standardized regression weight = 0.26, $p < 0.01$).

Table 3.4. Output of multiple linear models testing for the effects of functional diversity of litter mixtures ($trait_{FD}$), rain exclusion treatment (RE), and their interactions on soil microbial parameters (global metabolic activity (sum15), microbial metabolic diversity (H'), microbial metabolic dominance (D), and rate of decomposition of cellulose paper (DCP)). Plot-specific soil characteristics (Soil-PCA) were included as co-variable. Interactions between $trait_{FD}$ and RE treatment were never significant. We report the R^2 and AIC weight of the general model including all factors (All) and of the most parsimonious model (MPM**,** in bold). Only the variables retained in the most parsimonious models are reported. n = 92 plots in 2012 (a) and 2013 (b), respectively. (F-value and associated significance of p-value (' $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Arrows indicate the direction of the effect.

(a) 2012		D.f	S.sq	M.sq	*F*-value	*p*-value	All R^2 (AIC)	**MPM R^2 (AIC)**
sum15	Soil-PCA	1	0.085	0.085	3.261	0.074'	0.10 (-329.9)	**0.04 (-333.8)**
	Residuals	90	2.341	0.026				
H'	**Soil-PCA**	**1**	**0.004**	**0.004**	**4.388**	**0.039***	0.07 (-630.2)	**0.05 (-638.2)**
	Residuals	90	0.086	9e-04				
D	**Soil-PCA**	**1**	**9e-04**	**9e-04**	**5.106**	**0.026***	0.08 (-790)	**0.05 (-798.2)**
	Residuals	90	0.015	2e-04				
DCP	**Soil-PCA**	**1**	**34.16**	**34.16**	**12.867**	**<0.001*****	0.20 (97.7)	**0.17 (92.8)**
	WHC_{FD}	**1**	**15.22**	**15.22**	**5.731 ↓**	**0.019***		
	Residuals	89	236.31	2.66				
(b) 2013								
sum15	**Soil-PCA**	**1**	**0.260**	**0.260**	**13.99**	**0.0003*****	0.28 (-362.7)	**0.23 (-366.7)**
	$Lignin_{FD}$	**1**	**0.220**	**0.220**	**11.84 ↑**	**<0.001*****		
	Residuals	89	1.65	0.019				
H'	**Soil-PCA**	**1**	**0.001**	**0.001**	**7.679**	**0.0068****	0.18 (-792.9)	**0.13 (-795.8)**
	$Phenolics_{FD}$	**1**	**9e-04**	**9e-04**	**5.376 ↑**	**0.0227***		
	Residuals	89	0.015	2e-04				
D	**Soil-PCA**	**1**	**2e-04**	**1.5e-04**	**6.035**	**0.0160***		
	$Phenolics_{FD}$	**1**	**1e-04**	**1.3e-04**	**4.980 ↓**	**0.0281***	0.16 (-967.8)	**0.11 (-970.9)**
	Residuals	89	0.002	2.5e-05				
DCP	$Phenolics_{FD}$	1	5.06	5.061	2.945 ↑	0.0896'	0.09 (56.2)	**0.03 (52.5)**
	Residuals	90	154.66	1.718				

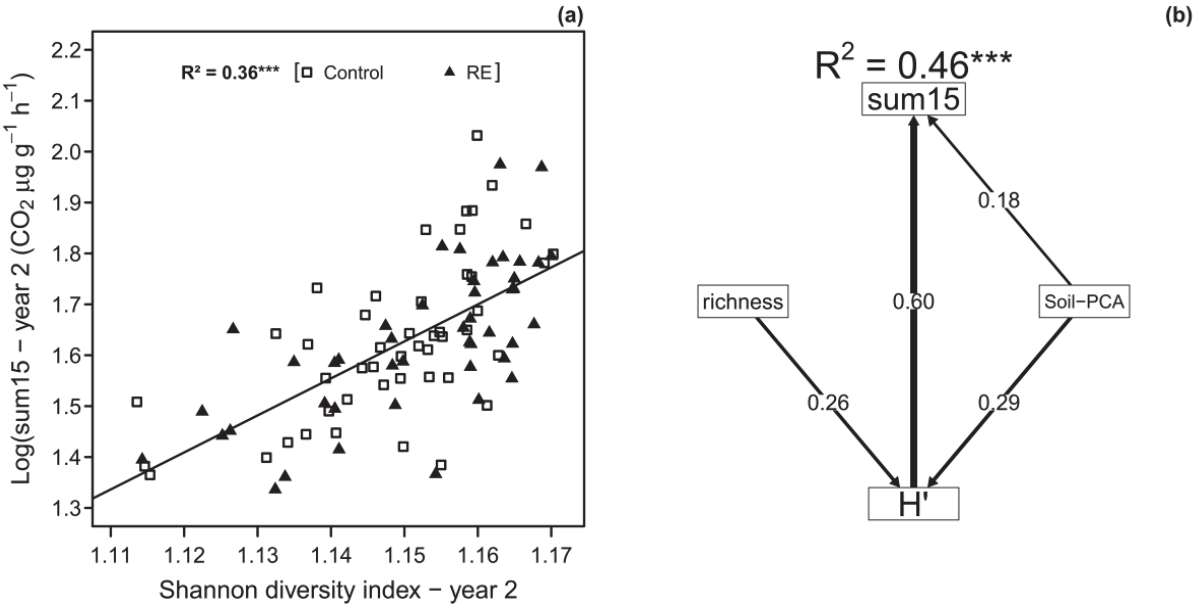

Fig. 3.4. (a) Relationships between the global metabolic activity (sum15) and the Shannon soil microbial metabolic diversity index (H') after two years. The solid line is the linear regression across all plots (n=92). **(b) Path analysis showing the supported causal links between leaf litter species richness, the soil microbial metabolic diversity (H'), the global soil microbial metabolic activity (sum15) and the soil characteristics (Soil-PCA) after two years.** Arrows indicate significant relationships. Numbers on the arrows show standardized regression weights. All paths are significant at $p \leq 0.01$ except the Soil-PCA to sum15 pathway ($p = 0.022$).

4. Discussion

In this study, we used a natural diversity gradient with four Mediterranean shrub species to address the question how a combined change in diversity of plants litter input and a reduction in precipitation affect the functioning of the soil microbial community.

Our results showed that the measured soil microbial parameters that were based on how microbial communities utilize a range of different C substrates, depended on physico-chemical properties, with a particular strong effect after two years. Such effects of soil characteristics have been reported previously (see for instance Delmont et al. 2014). On the other hand, soil microbial parameters were generally not affected by the moderate rain exclusion we applied (see Fig. S3.3 and associated comment for more details). Accordingly, but in contrast to our initial hypothesis stating that microbial communities underneath higher diverse litter mixtures are less affected by reduced precipitation, there

were no interactive effects between rain exclusion and litter diversity measures on microbial parameters. The sole exception was that litter species composition distinctly affected the microbial H' and D in control compared to rain-excluded plots after one year of field exposure of litter. This comparatively short-term response, which did not persist into the second year, suggests that the soil microbial communities associated to different litter mixtures responded differently to rain exclusion during the initial stage of decomposition. Because litter leaching is an important process early in the decomposition dynamics (Swift et al. 1979; Berg and Laskowski 2005), the interaction between litter composition and the amount of precipitation in the first year of the experiment may result from less leachate transfer to the soil with partial rain exclusion in some litter combinations. However, in our further tests evaluating the effects of species presence or of mean trait values of the litter mixtures we could not relate this response to the presence of a particular shrub species or to a particular litter trait (Table 3.2 and 3.3). The relatively moderate rain exclusion applied in our study that resulted in a 6.5 % lower soil water content on average in rain-excluded plots compared to the control plots, might not have been strong enough to affect the functioning of the soil microbial communities more clearly and more consistently over the two years of our experiment. On the other hand, previous studies applying more severe treatments of rain exclusion and evaluating the effect on soil processes in Mediterranean ecosystems also reported weak or no effects (Sardans et al. 2006; de Dato et al. 2010; Pailler et al. 2014). For example, de Dato et al. (2010) applied a total rain exclusion during spring and autumn in a semi-arid Mediterranean shrubland and reported decreased soil CO_2 emissions for only three of ten occasions in rain-excluded plots compared to control plots after five years of climate manipulation. Pailler et al. (2014) showed that a severe heat-drought stress applied on soil samples from a Mediterranean forest (ten days at 50°C) decreased the activity of the soil

microbial community but did not affect their catabolic profiles, compared to control conditions. No effect of summer drought on soil community catabolic profiles was reported by Sardans et al. (2006) and de Dato et al. (2010). In another experiment assessing the effect of multiple drying-rewetting cycles on surface and subsurface soils from a California annual grassland soil, Xiang et al. (2008) reported that microbial biomass and activity were not or only moderately affected by wetting and drying cycles. Collectively, these results suggest that because microbial communities in drought-affected ecosystems evolved and adapted to periodic drought, they may represent a considerable resistance to inter-annual variations in soil water availability. However, consistently lower precipitation over a longer time period may affect the adaptive trajectory of microbial communities that has not yet been detected in the relatively short term studies cited above, including our own. In fact, in a long-term experiment excluding 29% of rainfall over 11 years, García-Palacios et al. (2016) reported negative effects on soil microbial biomass and altered substrate utilization patterns in a soil under Mediterranean holm oak forest, indicating that soil microbial functioning may well change in response to long-term changes in soil water availability.

We predicted with our first hypothesis that increasing diversity of leaf litter decomposing at the soil surface would increase the capacity of soil microbial communities to metabolize diverse substrates and therefore lead to an increased overall microbial activity. The different litter treatments representing the plot-specific woody shrub composition indeed led to altered microbial metabolic diversity, dominance indices and DCP rates after the litter had been decomposed for one year. This litter composition effect, however, was overall relatively weak (Table 3.1). Species composition effects on ecosystem processes could be driven by the presence of specific species and the physical and chemical traits they represent. In our study, DCP rates consistently increased with the

presence of *Quercus* litter and decreased with that of *Rosmarinus*. Also, the presence of *Ulex* litter increased sum15 and H', while that of *Rosmarinus* decreased H' (Table 3.2). The negative effect of *Rosmarinus* may be related to inhibiting compounds produced by this species (Gómez-Aparicio et al. 2004), and the positive effects of *Quercus* and *Ulex* may be associated to their relatively high N concentrations (in comparison to *Cistus* and *Rosmarinus*). In fact, DCP rates measured after one year increased with increasing N concentrations in the litter mixtures (N_{CWM}, Table 3.3). This N effect and a DOC effect on DCP were the only significant effects of community-weighted mean traits we observed, but they disappeared in the second year, probably once DOC and soluble N are leached from decomposing litter. This transient effect of DOC on soil microorganisms is in line with a recent study that reported that leaf litter leachates induced rapid increase in soil microbial respiration and shift in community composition, that were positively associated with soluble C and N concentrations (Joly et al. 2016). In contrast, soil microbial metabolic parameters were better explained by functional dissimilarity of litter traits, especially after two years. This finding supports our initial hypothesis that increasingly dissimilar litter substrates provide a greater spectrum of resources fostering complementary resource use by soil microorganisms. The soil microbial metabolic activity increased with increasing $Lignin_{FD,}$ while H', and DCP (only marginally significant) rates increased, and D decreased with increasing $Phenolics_{FD}$. These results indicate that a high dissimilarity in C compounds within litter mixtures has a particularly important role for microbial metabolic diversity and substrate use efficiency. Higher soil microbial activity with increasing litter lignin content is rather unexpected since this compound is recalcitrant, but this could result from the stimulation of lignin-degrading enzymes in the soil following long-term inputs of lignin-rich litter substrates in the corresponding plots. The relatively important role of $Phenolics_{FD}$ was not unexpected since phenolic compounds can directly affect the

composition and activity of soil organisms (Schimel et al. 1998; Hättenschwiler and Vitousek 2000; Coq et al. 2010; Chen et al. 2015). The observed decrease in D and the concomitant increase in H' with increased dissimilarity of phenolics in litter mixtures indicate that litter mixtures containing species with distinct phenolics composition promote a metabolically more diverse microbial community because of more diverse C substrates.

In contrast to the litter species composition effect that disappeared with ongoing decomposition, but in line with increasing relative importance of trait dissimilarity vs. community-weighted mean traits discussed above, the species richness effect apparently got stronger and was positively related to H' and negatively related to D after two years of decomposition in the field (Fig. 3.1). Field studies explicitly addressing the question how surface litter influence the functioning of microbial communities in the underlying soil are rare in general, and in particular for the evaluation of litter diversity effects. A previous study in a tree species-rich tropical rainforest showed that up to 50% of the variability in soil microbial respiration was accounted for by the quality of decomposing leaf litter at the soil surface (Fanin et al. 2011). Also, in an incubation experiment using mixed litter from alpine steppe species, Chen et al. (2015) found that plant species richness affected soil N and C dynamics (in particular the cumulative soil CO_2 emission), but to a lower extent compared to the chemical traits of litter. Here, we showed that an increasing number of plant litter species increased the metabolic diversity of soil microbial communities. According to our path analysis, the plant richness related increase in microbial metabolic diversity was then the major driver of the higher global soil microbial activity. In fact, H' accounted for a higher amount of variability observed in sum15 than the soil parameters (Fig. 3.4b) that varied greatly among plots. These findings strongly suggest that higher plant species richness increases metabolic diversity of soil microbial communities via an increased resource diversity that ultimately stimulates microbial activity. Total activity and

metabolic diversity of soil microbial communities therefore would not solely be a characteristic of microbial diversity, but would depend also on the diversity of substrates provided by the plants in contrast to the theoretical model proposed by Loreau (2001). Similarly, Fonte et al. (2013) reported a higher biomass production and growth efficiency of aquatic bacterial communities provided with a mixture of different C compounds compared to single C compounds, with only a minor role of bacterial community composition. Plant species richness effects on soil microbial communities and the processes they drive have been reported in grassland diversity experiments before (Reich et al. 2001; Chung et al. 2007; Eisenhauer et al. 2010, 2013). For instance, in the Jena Biodiversity experiment, Lange et al. (2015) showed that soil microbial activity in temperate grassland increased underneath plant communities with higher species richness, which they interpreted as a result of higher rhizosphere C input in more diverse compared to less diverse plant communities. However, the underlying mechanisms of how higher plant species richness is affecting the various components of the soil microbial communities (e.g. biomass, activity, functional diversity) is yet to be demonstrated. It is difficult to separate living plant effects from plant litter effects, especially in a field experiment with continuous long-term inputs of litter material and well-developed root systems. In our study, the exposed litter mixtures represented the plot-specific plant composition, and the reported effects may be a combination of root and decomposing litter effects. There are two lines of evidence suggesting that the species richness effects we observed are primarily a consequence of litter decomposing at the soil surface. First, species richness had no influence in the first year. If root processes would be the dominant factor, the relationship between plant richness and microbial parameters should have been similar in both years. However, our results showed that the relationship was only detectable in the second year, suggesting that with ongoing decomposition and progressive

transfer of decomposition products to the underlying soil, decomposing surface litter was probably a more determinant plant richness effect than root processes. Second, there were no plant diversity effects on microbial parameters in soils underneath the common leaf litter from *Pinus halepensis*, which we exposed in all plots in addition to the plot-specific litter. Again, if root processes would dominate the responses of soil microbial communities, the effect of plant richness after two years should have been similar independently of the identity of litter decomposing at the soil surface. Hence, our results suggest that plant diversity can exhibit an important control over soil microbial functioning through the input of surface litter and the associated diversity of organic resources.

We conclude that the microbial activity and their capacity to use a range of different substrates were little affected by the moderate rain exclusion we applied to the studied Mediterranean shrubland. Such weak effects are probably the result of an only small change in soil humidity. Also, the soil microbial community in the studied Mediterranean ecosystem is adapted to recurrent drought stress and may only change after several years of modifications in the precipitation regime. In contrast, the composition, species richness, and functional diversity of the shrub community had a clear effect on the microbial parameters we assessed here. There was some striking evidence that the reported plant diversity effects on soil microbial communities are driven by leaf litter decomposing on the soil surface rather than mediated by plant root systems. In line with a previous study (Lloret et al 2015), our results suggest that climate change effects on plant species composition and richness might have more important consequences for soil microbial functioning than reduced precipitation in the studied Mediterranean shrubland ecosystems. However, the relative impact of direct and indirect climate change driven consequences will depend on the magnitude of change and the temporal scale considered.

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Chapter IV

General Discussion

The specific objective of my thesis was to assess the role of plant diversity in the response of belowground processes (soil microbial community and root system) of a Mediterranean shrubland (garrigue), a typical ecosystem type in the Mediterranean climate zone, to reduced water availability. Changes in plant diversity and reduction in annual rainfall and its frequency are among the most important drivers of global environmental change predicted to affect ecosystem structure and functioning in the future (Field et al. 2014; Guiot and Cramer 2016). My research was primarily motivated by the following general questions:

1) *How do abundant plants of Mediterranean shrublands respond to increased drought? Are these responses species specific and does the identity of neighbor individuals competing for the same limiting resource modifies these responses?*
2) *How do the soil microbial community and the processes it drives respond to increased / more frequent drought? Does the response of the soil microbial community depend on the diversity of the plant community?*

To address these questions, I have chosen two different experimental approaches. First, I constructed rhizotrons to manipulate the identity of plant species growing together as well as water availability by creating repetitive drought events under controlled conditions (rhizotron experiment, chapters 1 and 2). Second, I used a field experiment where I took advantage of a naturally established plant diversity gradient combined with partial rain exclusion, where I manipulated litter mixtures decomposing in mesocosms along the diversity gradient.

I structure this discussion in three main parts, first I will discuss the experimental approaches I used, second I will focus on the main results and conclusions from the two experiments, and finally, I will highlight some potential research perspectives.

1. Experimental approaches

1.1.Rhizotron approach

With the use of rhizotrons I was addressing the question of how three co-occurring plant species that are abundant in the studied Mediterranean shrubland, respond to repeated severe drought events, as compared to a single drought event. Two individuals of these three species were grown together in custom-made rhizotrons in all possible combinations, with a pane of glass on one side facilitated visual assessment of root growth. I have chosen to use rhizotrons for different reasons: the possibility to follow the growth of roots and their responses to drought and plant neighbor identity was the most important one. The rhizotrons also allowed me to accurately control water availability through time and to apply the same degree of drought for each individual rhizotron. Finally, the rhizotrons allowed me to control for inter- and intraspecific competition by determining plant species combinations and by keeping distances between individuals constant.

The originality of my approach was the application of repeated drought events at exactly the same intensity for each individual rhizotrons, irrespective of rhizotron-specific evapotranspiration. A second novelty was that I could investigate the impact of intra- and interspecific competition on plant survival and growth for specific plant combinations including interactions between woody shrubs and a grass species that is rarely done. In fact, the studied woody species (*Cistus albidus* L., *Quercus coccifera* L.) and the perennial grass (*Brachypodium retusum* Pers.) are among the most abundant species in the type of shrubland we studied (Fig. S3.2), but their responses to drought have never been studied together.

Previous research focused mainly on the consequences of single drought events on plant performance and recovery rarely pushing the drought impact to mortality of the studied plants. From these short-term species-specific responses, predictions were drawn about

longer term impacts on the community structure (McDowell et al. 2008), which could be problematic. The likely scenario of multiple recurrent drought events predicted to increase in frequency with ongoing climate change, are very rarely taken into account, but are expected to have stronger consequences than single events, with potentially more negative effects on ecosystem resilience and performance (Scheffer et al. 2001). In particular, Mediterranean shrublands are commonly dominated by woody plants that generally withstand drought by maintaining their leaf tissues active. Such a strategy may render them more susceptible to damage when drought periods are getting more intense and/or more frequent (Vilagrosa et al. 2003).

Most of previous studies applied drought over a fixed time span (Miyashita et al. 2005; Zang et al. 2014) which seems reasonable in view of the predictions of changes in the duration of drought. However, this means that individual plants may experience vastly different soil water conditions because of differences in the size and growth rate of root systems and different response dynamics to changing soil water content. While this may reflect the reality under field conditions very well, it makes the interpretation of results difficult and the identification of underlying mechanisms for differences in drought responses basically impossible. Here we chose to simulate an increased frequency of drought events and to control the drought intensity at the level of individual rhizotrons in order to expose each individual plant to exactly the same drought intensity. With this approach we assured that we compare root properties of different species and individual plants that had experienced the same drought exposure, while we had to accept differences in the duration of the experiment among individual rhizotrons.

1.2. Field approach

In the second part, I took advantage of a large partial rain exclusion field experiment in a typical Mediterranean shrubland ecosystem to study the functioning of soil microbial

communities along a natural gradient in woody plant species richness. For this purpose I chose to characterize the soil microbial communities functionally, by measuring their ability to respire 15 different C substrates and to decompose cellulose paper in the soil associated to all possible combinations of litter mixtures of the four woody shrub species dominating at our study site. I explored how the soil microbial substrate utilization was affected by partial rain exclusion, litter species richness, litter species composition and identity as well as litter traits. Litter traits were incorporated in the models in two different ways, by considering average trait values (community weighted mean traits - CWM), and by considering functional trait dissimilarity (Rao's quadratic entropy). Because the relative contribution of the different species is not even at our field site, and in order to standardize the litter material decomposing on the soil surface as well as the stage of decomposition across all plots, the microbial substrate utilization was estimated from the soil (down to 5 cm depth) under which the litter mixtures decomposed in field mesocosms. A common leaf litter material from *Pinus halepensis* (as typical Mediterranean species) allowed to separate the direct effects of decomposing leaf litter from the overall effects of the living plants (see Chapter 3).

Because of the extraordinary diversity of soil microorganisms and their supposed high functional redundancy (Bardgett et al. 2005), the functional diversity of soil microbial communities is likely more relevant for the understanding of microbial processes and how they might be affected by litter diversity than the number of species (Zak et al. 1994; Rodríguez-Loinaz et al. 2008). Moreover, a species approach can be difficult for microorganisms because the traditional species concept used for other groups of organisms does not readily apply for microorganisms (Hooper et al. 2000). While molecular (genetic) or biochemical (phenotypic) diversity measurements have their place and generally measure the diversity of the numerically dominant members of the community (Loisel et

al. 2006), functional diversity is popular in that it relates to the activity of the soil and gives information on the functioning of those members of the soil microflora involved in carbon cycling. Soil functional diversity is generally simpler to assess without the recourse to the specialized laboratory requirements for, e.g., DNA or PLFA (Phospholipid fatty acid) analysis (Chapman et al. 2007). The functional diversity of microbial communities can be described by different processes such as CO_2 production, nitrification or enzyme activities (1982; Beare et al. 1990; Gianfreda et al. 1994; Pinay et al. 2007). Alternatively, instead of measuring the global CO_2 production, the community level physiological profiles (CLPPs) of microbial communities using the MicroResp™ method provides a relatively fine resolution of functional differences among communities (Campbell et al. 2008). The CLPPs are assessed by the utilization of different carbon substrates, as an indicator of the capacity of soil microbial communities to metabolise a range of different organic compounds (Chapman et al. 2007). Consequently, MicroResp™ offers a convenient, rapid and sensitive method for the determination of soil microbial substrate utilization which allowed us to estimate the microbial community functional diversity (Campbell et al. 2008). The application of this technique in a number of case studies has demonstrated its utility in a wide range of soils and land-cover types (Chapman et al. 2007; Creamer et al. 2016).

2. Main results

2.1. Neighbor identity affects growth and survival of Mediterranean plants more than repeated severe drought

Our results showed that the three studied species strongly differed in their overall survival rates during the experiment, at least in young individuals. According to our initial hypothesis, the grass *B. retusum* showed the highest survival rates that did not change in

response to repetitive severe drought and did also not depend on neighbor species identity. These results suggest that *B. retusum* can cope well with severe drought even if it occurs repeatedly within a comparatively short period of time. In contrast, survival of the two woody species was clearly negatively affected by repetitive drought. Moreover, neighbor species identity affected survival rates of the two woody species: when *C. albidus* grew with a different species its survival improved compared to when it grew with a conspecific individual; in contrast, when *Q. coccifera* grew with a different species its survival was lower compared to when it grew with a conspecific individual. Such species-specific differences in surviving repetitive drought suggest important consequences on community composition in this type of Mediterranean ecosystem, where drought events should get more frequent or more severe in the near future. However, it should be noted that we investigated relatively young individuals and drought effects on older and possibly deeper rooting individuals may differ.

With its strategy to first reduce stomatal conductance, and to then limit survival to a few meristematic tissues when drought persists and gets more intense, the perennial grass *B. retusum* appears to be well adapted to more frequent drought events and to be a relatively good competitor. This is in line with a previous study that showed that *B. retusum* minimized water loss and survived drought with a low baseline stomatal conductance rate when growing together with the Mediterranean shrub *Rosmarinus officinalis* (Clary et al. 2004). Moreover, *B. retusum*, responded very quickly to water addition after drought, recovering its water potential at a rate four times higher than that of *R. officinalis*, what may contribute to its competitive success (Clary et al. 2004). The survival of meristematic tissues under severe drought, while a large part of leaves die off, followed by rapid new growth when conditions are improved, ensures the survival of most tillers as shown in many Mediterranean ecotypes of perennial species (Volaire et al. 2009; Poirier et al. 2012;

Pérez-Ramos et al. 2013). The two woody species *Q. coccifera* and *C. albidus,* on the other hand, keep transpiration and stomatal conductance relatively high even when the leaf water potential is low (Damesin et al. 1998; Galmés et al. 2007). This may become critical when drought events last longer, causing higher rates of mortality in species with such water regulation (McDowell et al. 2008). However, we observed a reduction in total leaf area by dropping leaves under high water limitation, in particular in *C. albidus*, which is a commonly observed response in Mediterranean woody plants during summer drought (Hoff and Rambal 2003; Baldocchi and Xu 2007; Limousin et al. 2009).

Root morphological traits of all three species were more affected by the species identity of the neighbor individual than by repeated drought. In general, the root traits of *B. retusum* were more affected by repetitive drought and also by neighbor plant identity than those of the woody species. Compared to the two woody species, *B. retusum* had the higher SRL which may be interpreted as a higher competitive ability, as high SRL translates into more space exploration per unit root biomass (Eissenstat 1992). This is in line with our results, in chapter 1, showing higher survival rates for *B. retusum* compared with those of the two woody species when exposed to repetitive severe droughts. In fact, specific root length (SRL) of *B. retusum*, but not of the other species, correlated positively with survival rates.

The community level physiological profiles (CLPPs) of root associated soil microbial communities did not differ between drought treatments and were also not affected by plant species identity. CLPPs changed towards higher total microbial activity but less diverse resource use with increasing soil depth. Such contrasted effects between deep and top soil layers may reflect differences in the temporal dynamics in soil water content that tend to be higher in deeper soil layers, which may lead to differences in soil microbial community structure (Laclau et al. 2013).

A quite unexpected key result was that, beyond the identity of the plant species (*B. retusum* was the only species that adjusted its root traits to the occurrence of repetitive drought), the identity of neighbor plants impacted the response of root traits, and even had stronger effects on species-specific root traits than repetitive severe drought. Additionally, root trait (SRL) in *B. retusum* was positively related to its survival rates. All together, such features strongly suggest that the response of the shrubland plant community to increasing drought frequency will highly depending on the structure of the plant community (relative abundance of the different species with different strategies and with different interactions among species).

2.2. Plant community composition affects microbial activity more than a change in soil water availability

In both rhizotrons and the in situ experiment, I found that the activity and the capacity of the soil microbial community to use a range of different substrates were little affected by the repeated drought event or by the moderate rain exclusion, respectively. In the *in-situ* experiment, such a weak effect could result of an only small change in soil humidity. We can also argue that the soil microbial communities in the Mediterranean garrigue are adapted to recurrent drought stress and may only change after several years of alteration in the precipitation regime. In the rhizotrons experiment, severe droughts that were repeatedly applied impacted the root traits in *B. retusum*, but this did not translate into the soil microbial properties.

In contrast, the composition, species richness, and functional diversity of the shrub community had a clear effect on the microbial parameters during the in situ experiment. Litter species richness increased soil microbial activity by increasing the catabolic diversity of the soil microbial community. These effects were mostly driven by the

presence of *Q. coccifera* and *U. parviflorus* leaf litter that both reduced microbial metabolic dominance, and that of *Rosmarinus* that increased the microbial metabolic dominance. Plant species richness effects on soil microbial communities and the processes they drive have been reported in grassland diversity experiments (Reich et al. 2001; Chung et al. 2007; Eisenhauer et al. 2013)). In our study, there was some striking evidence that the reported plant diversity effect on soil microbial communities was driven by leaf litter decomposing on the soil surface rather than by the long term presence of the plants along the diversity gradient. Our results suggest that climate change effects on plant species composition and diversity might have more important consequences for soil microbial functioning than the direct effects of reduced water availability in the studied Mediterranean shrubland ecosystems, which is in line with a previous study in Mediterranean woodland (Lloret et al. 2015) showed that the quite drastic effects of drought via plant mortality increased the richness and changed the composition in soil microbial community of Mediterranean woodlands.

3. Perspectives

3.1. Short term perspectives

Because of the long time needed to settle this experiment (the establishment period lasted one year and the contrasted drought treatment up to 377 additional days) and of the numerous soil and plant parameters that were determined, I did not have the time to make use of all the results. However, additional data such as root dynamics in the rhizotrons or distribution of root biomass along the soil profiles will be useful for a better interpretation of our results on root and microbial responses to drought.

Soil water content in the rhizotrons, that was determined gravimetrically once per week for each individual rhizotron, will allows determining the dynamics of water demand in each

rhizotrons according to plant composition and drought treatment. Moreover, 4 rhizotrons without plant allowed calculating the water loss due to evaporation. These data would allow calculating the water-use efficiency (WUE, i.e. the amount of biomass produced per unit water used), as an often used variable to interpret drought tolerance and to compare among studies. This would be an important addition to the data presented here. As a short-term perspective I plan to develop this aspect before submitting the chapters about the rhizotron study to a peer-reviewed journal.

Another aspect that might be worth to explore in more detail is to compare plant survival rates exclusively during the third drying cycle that was common to both watering treatments. Such a comparison would allow to evaluate the consequences of previous drought events for plant survival in addition to what I did here across the entire period of the experiment.

At the beginning of the drought treatment and at the end of every drying cycle, I recorded the root systems by successive drawings through the glass pane at two different soil depths (corresponding to the soil depths of final root harvest). These data would allow to have a better understanding of root dynamics in response to the treatment factors, which may improve our understanding of root responses to repetitive drought and changing neighbor species identity, especially in combination with root biomass and root trait data.

Finally, in the rhizotron experiment, I analyzed potential correlations between root functional identity ($Trait_{CWM}$) or functional dissimilarity ($Trait_{FD}$) and soil microbial properties. In order to improve the understanding or these relationships, I propose to calculate the functional root traits ($Trait_{CWM}$ or $Trait_{FD}$) using the root biomass for each individual/species (that was estimated in sampled soil cores) rather than the relative abundance of present species in a given rhizotrons (50% in bi-specific rhizotrons).

3.2. Longer-term perspectives

A general conclusion of my experiments was that the effects of aggravated drought on soil microbial parameters were rather weak. This could be because plants as well as microbial communities in the Mediterranean shrubland are accustomed to repeated, sometimes intense drought event. However, their response to drought may change after several years of alteration in the precipitation regime. For example, in a long-term experiment excluding 29% of rainfall over 11 years, (García-Palacios et al. 2016) reported negative effects on soil microbial biomass and altered substrate utilization patterns in a soil from a Mediterranean holm oak forest, indicating that soil microbial functioning may well change in response to long-term changes in soil water availability. It would certainly be interesting to continue the measurements of soil microbial activity on the rain exclusion site over the long term. As a quite general remark I believe that long term studies in Mediterranean ecosystems are absolutely crucial for the understanding of climate change effects on these systems.

Data about plant communities in the experimental plots (CLIMED site) and their responses to the rain-exclusion treatment are collected by another PhD student (Natalia Rodrigez) since 2012. Combining this data to our results obtained in rhizotron experiment could improve our understanding of the response of Mediterranean shrubland to predicted drought.

Although seedling establishment is of major importance in Mediterranean ecosystems, further studies on saplings, juveniles and adults are also required to get a better understanding of the mechanisms underlying interspecific differences in performance with predicted drought in the Mediterranean region.

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Annexe 1 : Site d'étude

Fig. S1: Photo aérienne du site expérimental du massif de l'Etoile. Les structures carrées correspondent aux dispositifs d'exclusion de pluie.

Mon projet de thèse s'inscrit dans le cadre du projet CLIMED (Climate change effects on Mediterranean biodiversity and consequences for ecosystem functioning), projet financé par le programme Changements Environnementaux et Planétaires de l'ANR et réalisé en collaboration avec l'IMBE sous la coordination de Stephan Hättenschwiler. Ce projet vise à comprendre l'impact des changements climatiques sur la biodiversité et le fonctionnement d'un écosystème méditerranéen.

Notre système modèle est l'écosystème de garrigue méditerranéenne. La diminution des précipitations prévue par les modèles climatique pour les décennies à venir constitue une menace majeure pour la biodiversité dans cet écosystème. Depuis décembre 2011, nous étudions les conséquences d'une baisse des précipitations sur les communautés végétales et biologiques du sol (communautés microbiennes et macrofaune) avec une expérience

d'exclusion de pluie à grande échelle mise en place sur le Massif de l'Etoile, au nord de Marseille. Le site Massif de l'Etoile (43°22' N; 5°25' E) offre un gradient naturel de la diversité de quatre espèces dominantes ligneuses dans un environnement homogène (Montès et al. 2008). Cette situation assez unique permet de tester l'impact d'une baisse des précipitations sur un gradient de diversité végétal, avec des placettes expérimentales comprenant toutes les combinaisons possibles des 4 espèces ligneuses dominantes, avec pour objectif d'améliorer notre compréhension du rôle de la diversité des espèces ligneuses dans la réponse aux changements climatiques. Sur ce site, nous avons réalisé l'expérience de terrain du chapitre 1. Les caractéristiques de cet écosystème ont servi de référence pour préparer l'expérience en rhizotron utilisée dans les deux autres chapitres.

- **La garrigue du massif de l'Etoile**

Notre site d'étude fait partie du massif de l'Etoile, un petit massif montagneux qui culmine à 779 m et une surface de 10 000 ha. Ce massif est dépourvu d'habitations mais est entouré du tissu urbain dense de l'agglomération d'Aix-Marseille. Les roches sont issues de sédiments calcaires déposés pendant la période mésozoïque (de -270 à -80 Ma). C'est ensuite pendant le Paléocène et l'Eocène (de -65 à -35 Ma) que des mouvements de compression ont provoqué les plis et les failles à l'origine des reliefs actuels. Le site d'étude du projet CLIMED est situé dans la partie sud du massif, sur un petit plateau de calcaire dur à 275 m d'altitude. Il est situé sur la commune de Marseille.

La végétation est typiquement méditerranéenne : environ 60% du massif est recouvert par la garrigue. La présence humaine, qui est attestée de longue date, est marquée par de petites industries du Moyen Age (carrières et four à chaux) ainsi que des communautés monastiques. Le massif a longtemps été utilisé par ses habitants pour le pâturage et le prélèvement de bois, l'homme a donc joué un rôle important dans la structuration de la

végétation. Le dernier grand incendie, en 1997, a couvert les 3500 hectares correspondant au versant sud du massif où se trouve le site expérimental. Le feu est maintenant le seul élément qui maintient une végétation ouverte sur le massif car le pâturage n'est plus pratiqué. Si l'action combinée du feu et des activités humaines a permis l'évolution et le maintien d'une flore diversifiée, c'est probablement au détriment de la qualité des sols et de leur fertilité. En effet, le sol est aujourd'hui peu profond et comporte une grande proportion de cailloux ce qui est caractéristique d'un sol érodé.

- **Le site expérimental CLIMED**

- **La végétation**

Sur l'ensemble des 2,5 hectares du site expérimental, 64 espèces végétales ont été identifiées en 2011 par le botaniste Daniel Pavon (annexe 2). Les relevés d'abondance de la végétation sur les 92 placettes équipées de dispositifs montrent que 5 espèces dominent la communauté végétale, dont 4 espèces arbustives (N. Rodriguez, comm. pers., Fig. 2). J'ai analysé les activités microbiennes de sol associé aux microcosmes incubant des litières (Fig. 3) de toutes les combinaisons possibles de ces 4 espèces arbustives, (Chapitre 3):

***Quercus coccifera* L.**

Le chêne kermès est un arbuste de 50 à 200 cm de haut, sempervirent, pyrophyte, de la famille des Fagaceae. C'est une espèce méditerranéenne typique de garrigue, que l'on retrouve dans l'étage mésoméditerranéen jusqu'à 400 m d'altitude (600 m en adret). C'est une espèce thermophile, héliophile et xérophile à amplitude moyenne (Rameau et al. 2008). Le chêne kermès a une stratégie de « resprouter » ; sa souche résiste au feu et il émet des rejets rapidement après un incendie. Bien que leur âge n'ait pas été estimé, les chênes kermès présents sur notre site sont probablement plus vieux que l'incendie de 1997 (>29ans).

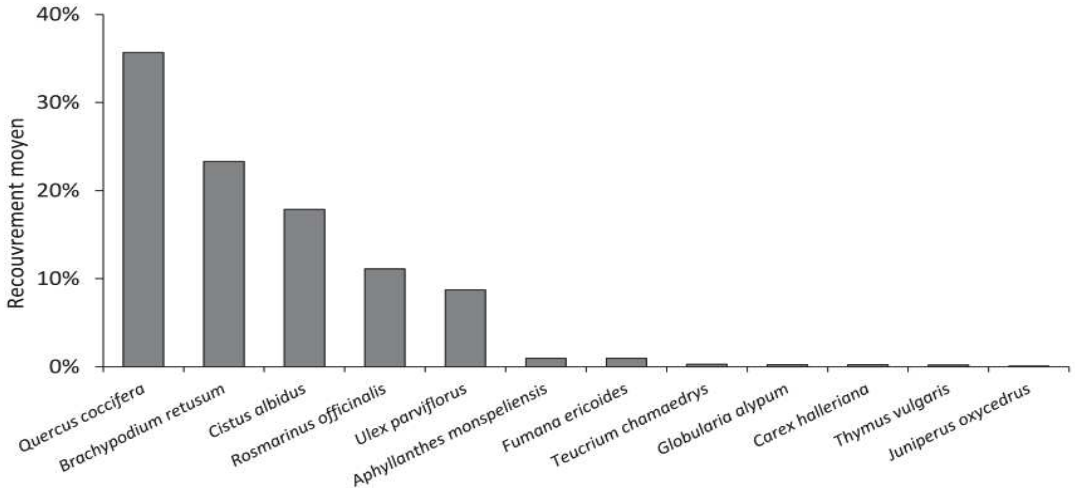


Fig. S2: Recouvrements relatifs des 12 principales espèces végétales présentes sous les dispositifs d'exclusion de pluie (Données Natalia Rodrigez, Juin 2012).

***Cistus albidus* L.**

Le ciste blanc est un arbrisseau de 30 à 100 cm de haut, semi-decidu, chaméphyte, pyrophyte, de la famille des Cistaceae. C'est une espèce ouest-méditerranéenne typique de garrigue, que l'on retrouve principalement dans l'étage mésoméditerranéen (jusqu'à 1200 m d'altitude dans les Alpes Maritimes). C'est une espèce relativement thermophile, héliophile et xérophile à large amplitude (Rameau et al. 2008).

***Rosmarinus officinalis* L.**

Le romarin officinal est un arbrisseau de 50 à 100 cm de haut, sempervirent, nanophanérophyte, pyrophyte, de la famille des Lamiaceae. C'est une espèce circum-méditerranéenne typique de garrigue, que l'on trouve dans l'étage mésoméditerranéen jusqu'à 650 m d'altitude (et au-delà à l'état subspontané). C'est une espèce thermophile supportant des climats assez variés, héliophile et xérophile (Rameau et al. 2008).

***Ulex parviflorus* Pourr.**

L'ajonc de Provence est un arbrisseau épineux de 30 à 100 cm de haut, sempervirent (avec des feuilles transformées en épines), nanophanérophyte, pyrophyte, de la famille des

Fabaceae. C'est une espèce ibéro-provençale typique de garrigue, que l'on trouve dans l'étage mésoméditerranéen (à supraméditerranéen) jusqu'à 600 m d'altitude. C'est une espèce thermophile supportant des climats assez variés, héliophile et neutrocline à large amplitude (Rameau et al. 2008).

Ces trois espèces arbustives (*Cistus albidus*, *Rosmarinus officinalis* et *Ulex parviflorus*) sont des « seeders », c'est-à-dire que le feu provoque la mort des individus mais favorise la germination des graines. Les individus de ces trois espèces sont donc forcément plus jeunes que l'incendie (<29ans).

Fig. S3: Récolte d'un microcosme après 1 an de décomposition des litières, (Santonja thesis 2014)

Enfin, j'ai utilisé la cinquième espèce *B. retusum* (avec le ciste et le chêne kermès) pour l'expérimentation de rhizotron (Chapitre 1 et chapitre 2):

***Brachypodium retusum* (Pers.) P.Beauv.**

Le brachypode rameux est une herbe pérenne de 15 à 20 cm, sempervirent, pyrophyte, hémicryptophyte, de la famille des Poaceae. Les tiges sont très rameuses et très feuillées avec des rhizomes étendus et profond dans le sol (5-10 cm) ce qui lui permet de repousser après les incendies. C'est une espèce typique de garrigue, que l'on trouve au centre et à l'ouest du bassin mediterranean et qui occupe une large gamme de sols et de climats. Le brachypode rameux sur le site d'étude est caractéristique des pâturages ovins abandonnés.

Les 5 espèces dominantes du site traduisent bien les éléments structurants des communautés végétales que sont le feu et les pratiques anthropiques sylvo-pastorales.

Les dispositifs d'exclusion de pluie

Pour simuler une sécheresse expérimentale, des dispositifs de 4 x 4 m surmontés de gouttières en acier inoxydable et couvrant 40 % de la surface des placettes expérimentales ont été installés sur le site (Fig. 4). Ces dispositifs sont couplés à un gradient de diversité végétale. Des placettes comprenant les 4 espèces arbustives dominantes du site dans toutes les combinaisons de diversité possibles (une, deux, trois ou quatre espèces) ont été équipées. Pour chacune des 15 combinaisons, 3 placettes ont été installées (avec 1 placette de plus pour la combinaison à quatre espèces afin d'augmenter la puissance des estimations de la variabilité). Comme les dispositifs perturbent le rayonnement car ils recouvrent en partie la végétation, autant de placettes ont été équipées de dispositifs témoins dont les gouttières retournées n'excluent pas la pluie. Au total de 92 placettes réparties sur une superficie de 2.5 ha environ.

Fig. S4: Photos des dispositifs expérimentaux du site CLIMED (partie gauche : dispositif témoin avec des gouttières inversées; partie droite : dispositif d'exclusion de pluie), (Santonja thesis 2014)

***Quercus coccifera.* L**

***Cistus albidus* L.**

***Rosmarinus officinalis* L.**

***Ulex parviflorus* Pourr.**

***Brachypodium retusum* (Pers.) P.Beauv.**

Fig. S4: Les 5 espèces utilisées pour les deux expérimentations de présent manuscrit.

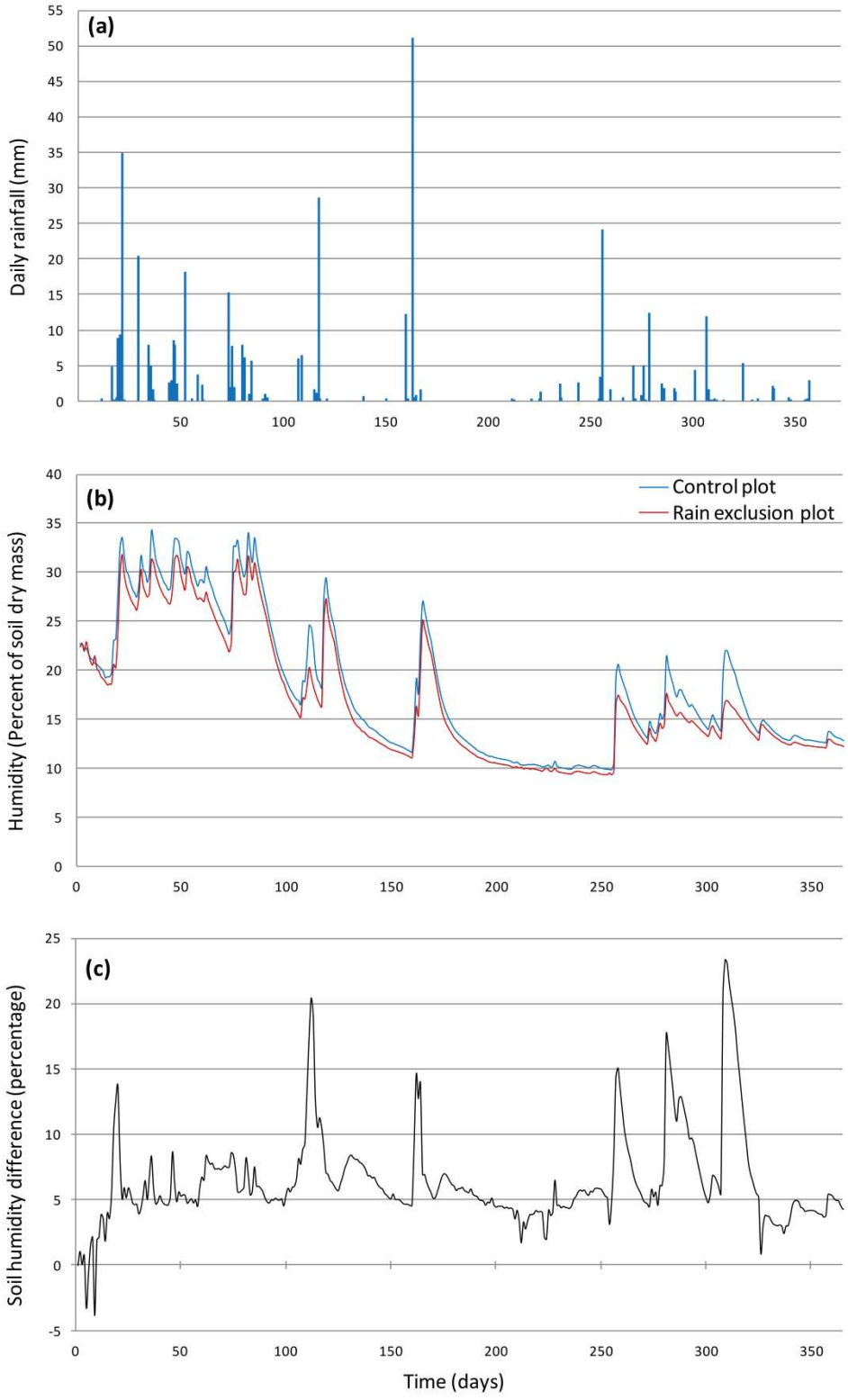
Annexe 2 : Liste des espèces végétales du site de l'Etoile

Nom latin	Famille
Allium sphaerocephalon L. subsp. sphaerocephalon	Alliaceae
Alyssum alyssoides (L.) L.	Brassicaceae
Anthyllis vulneraria L. subsp. praepropera (A. Kern.) Bornm.	Fabaceae
Aphyllanthes monspeliensis L.	Aphyllanthaceae
Arenaria serpyllifolia L. subsp. leptoclados (Rchb.) Nyman	Caryophyllaceae
Argyrolobium zanonii (Turra) P.W. Ball subsp. zanonii	Fabaceae
Asterolinon linum-stellatum (L.) Duby	Duby Primulaceae
Avenula bromoides (Gouan) H. Scholz subsp. bromoides	Poaceae
Bituminaria bituminosa (L.) C.H. Stirt.	Fabaceae
Bombycilaena erecta (L.) Smoljan.	Asteraceae
Brachypodium retusum (Pers.) P. Beauv.	Poaceae
Carex halleriana Asso subsp. halleriana	Cyperaceae
Cistus albidus L.	Cistaceae
Clypeola jonthlaspi L.	Brassicaceae
Dianthus sylvestris Wulfen subsp. longicaulis (Ten.) Greuter & Burdet	Caryophyllaceae
Dorycnium pentaphyllum Scop. subsp. pentaphyllum	Fabaceae
Erodium cicutarium (L.) L'Hér. subsp. cicutarium	Geraniaceae
Fumana ericoides (Cav.) Gand. subsp. montana (Pomel) Güemes & Muñoz Garm.	Cistaceae
Fumana thymifolia (L.) Spach ex Webb	Cistaceae
Galium corrudifolium Vill.	Rubiaceae
Globularia alypum L. subsp. alypum	Globulariaceae
Hornungia petraea (L.) Rchb.	Brassicaceae
Iris lutescens Lam. subsp. lutescens	Iridaceae
Juniperus oxycedrus L. subsp. oxycedrus	Cupressaceae
Leuzea conifera (L.) DC.	Asteraceae
Linaria simplex (Willd.) DC.	Scrophulariaceae
Phillyrea angustifolia L.	Oleaceae
Pinus halepensis Mill. subsp. halepensis	Pinaceae
Poa bulbosa L.	Poaceae
Quercus coccifera L.	Fagaceae
Quercus ilex L. subsp. ilex	Fagaceae
Reseda phyteuma L. subsp. phyteuma	Resedaceae
Rosmarinus officinalis L. subsp. officinalis	Lamiaceae
Rubia peregrina L. subsp. peregrina	Rubiaceae
Rumex intermedius DC.	Polygonaceae
Sanguisorba minor Scop.	Rosaceae
Scandix australis L.	Apiaceae
Scilla autumnalis L.	Hyacinthaceae
Teucrium chamaedrys L.	Lamiaceae
Teucrium flavum L. subsp. flavum	Lamiaceae
Teucrium polium L. subsp. polium	Lamiaceae
Thapsia villosa L. Apiaceae	Apiaceae
Thymus vulgaris L. subsp. vulgaris	Lamiaceae
Trifolium campestre Schreb. subsp. campestre	Fabaceae
Ulex parviflorus Pourr. subsp. parviflorus	Fabaceae
Valantia muralis L.	Rubiaceae

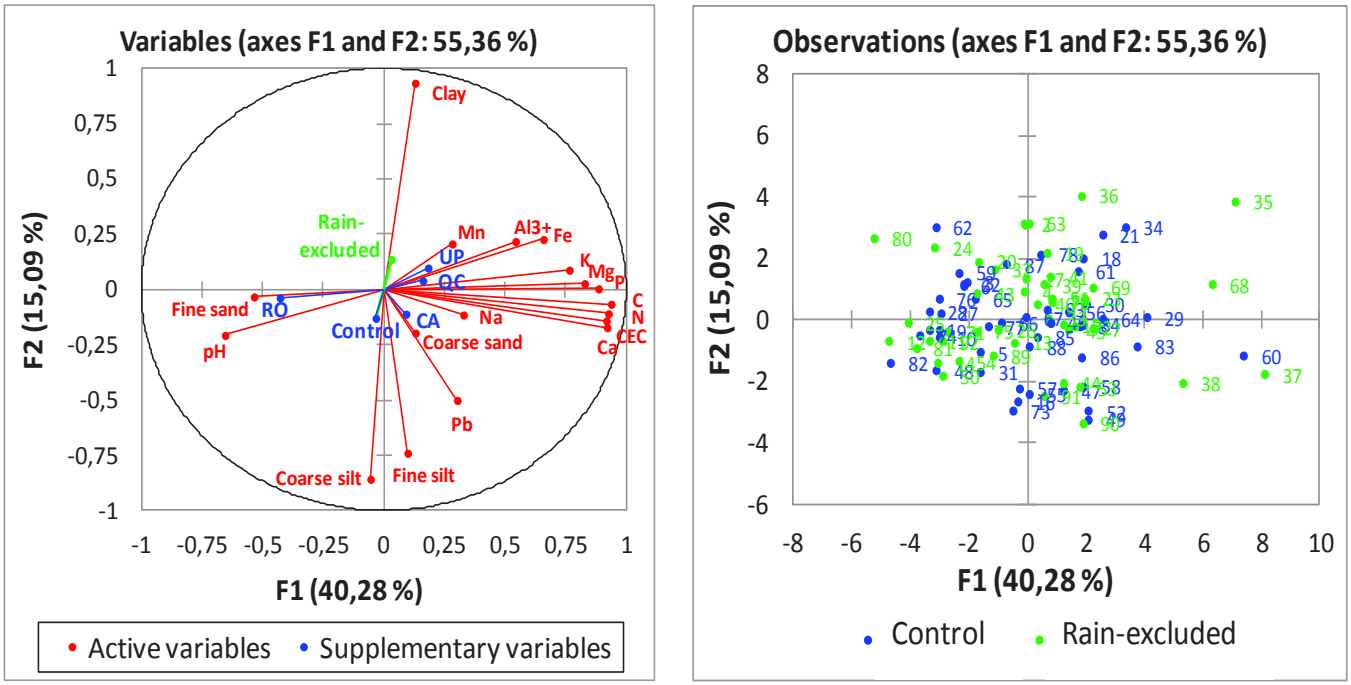
Supplementary Material

Table S1.1- Physico-chemical parameters of soil substrate used in the rhizotrons

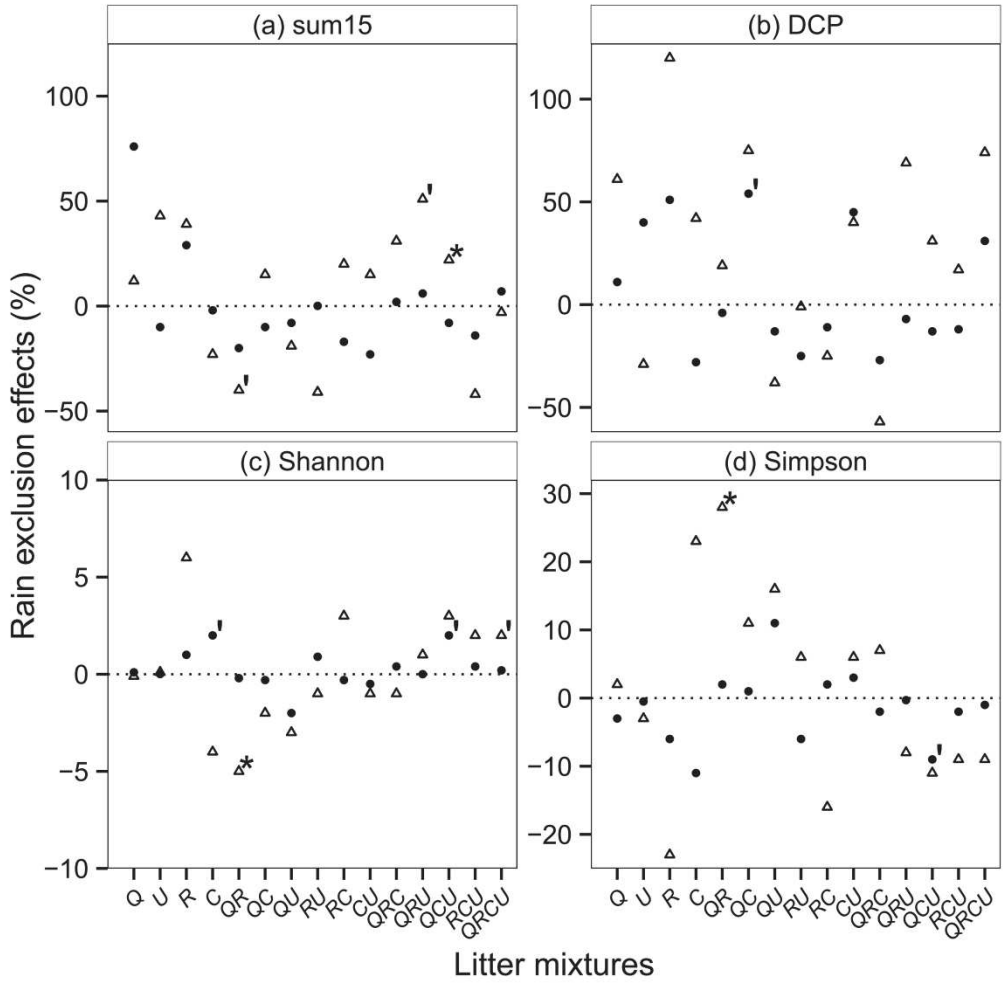
Soil parameters	Unit	Mean values
Clay	g/kg	200,67
Fine silt	g/kg	123,33
Coarse silt	g/kg	133,33
Fine sand	g/kg	159,67
Coarse sand	g/kg	383,00
pH	-	8,63
CEC Metson	$cmol^{+}/kg$	7,72
Organic carbon (C)	g/kg	6,90
Total azot (N)	g/kg	0,52
C/N	-	13,33
Organic material	g/kg	11,93
P (P2O5)	g/kg	0,02
Al	cmol+/kg	0,10
Mn	cmol+/kg	<0,005
Fe	cmol+/kg	0,02
K	cmol+/kg	0,16
Na	cmol+/kg	0,12
Mg	cmol+/kg	0,35
Ca	cmol+/kg	11,57
Soil field capacity		23%
Soil wilting point		5.1%



Supplementary Material- Fig. S3.1: (a) Daily rainfall expressed as mm of precipitation, (b) daily soil humidity expressed as percent of soil dry mass and (c) percentage of soil humidity difference between control and rain exclusion plots during 2015. Rainfall was determined by using rain gauges and soil humidity was determined by using TDR probes installed in control and rain exclusion plots at 10 cm soil depth. Soil humidity data were not available for 2012 and 2013. From (Santonja et al. submitted).



Supplementary Material- Fig. S3.2: PCA analysis on soil characteristics including texture, pH, cation exchange capacity (CEC), and the concentrations of carbon, nitrogen, phosphor, calcium, magnesium, sodium, potassium, iron, lead, manganese and aluminum. Vectors corresponding to abundance of the four species (QC for *Q. coccifera*, UP for *U. parviflorus,* RO for *R. officinalis*, and CA for *C. albidus*) in the litter mixture and to the rain exclusion treatment (control plots in blue and rain-excluded plots in green) were plotted as additional variables in the PCA. The two first PCA axes explained 40.28% and 15.09%, respectively, of the variance in soil characteristics patterns.



Supplementary Material- Fig. S3.3: Relative rain exclusion effect (REE, expressed as %) on soil microbial parameters (sum15, H', D, DCP) for the monospecific and the eleven litter mixture combinations after one (triangle) and two (circle) years of decomposition. Significant dissimilarity between control and drought plots for each plant litter mixture are indicated with the respective symbols (' for <0.1 and * for $p < 0.05$, t-test). C = *C. albidus*, Q = *Q. coccifera*, R = *R. officinalis*, U = *U. parviflorus*. Combinations of capital letters correspond to combinations of plant species in litter mixtures.

Comment on Fig. S3, Relative rain exclusion effects

The relative rain exclusion effect (REE) on soil microbial parameters was calculated for each litter mixture combination as: $REE = \frac{Exclusion - Control}{Control} \times 100$ (expressed in %) where *Exclusion* is the microbial parameter (sum15, H', D or DCP) determined in rain-excluded plots (mean value for the three or four replicate plots), and *Control* is the same microbial parameter in control plots (mean value for the three or four replicate plots) for the same litter mixture. Student's t-tests were used to determine if the difference between

each mean response variable for each litter mixture were significantly different in rain-excluded plot compared to control ones.

Apart of significant interactions between rain exclusion (RE) and litter composition on H' and D after one year of decomposition, rain exclusion had no main effect on any of the measured microbial parameters (Tables 3.1, 3.3, 3.4). In order to explore these interactions in some more detail, we calculated REE for each individual litter treatment. REE was rarely significantly different from zero on any of the measured soil microbial parameters (Fig. S3.3). There was also no indication that REE were more important with increasing litter diversity, but it is interesting to note that *Q. coccifera* was present in all of the overall ten cases of significant REE underneath litter mixtures (Fig. S3.3). Also quite interesting were the few marginally significant correlations were found, between litter mixture $Trait_{FD}$ and REE on sum15 after two years of decomposition, with more negative REE on sum15 underneath litter mixtures with increasing C_{FD}, DOC_{FD} or P_{FD} (R^2= 0.22, 0.23 and 0.22, respectively with $p < 0.10$, Table S3.5). The community weighted mean traits explained a part of REE on sum15 after two years of decomposition with positive correlation between $Phenolics_{CWM}$ and REE on sum15 (F=6.882*, R^2=0.35) and negative correlations between $Lignin_{CWM}$ or $Lignin/N_{CWM}$ and REE on sum15 (F=5.183*, R^2=0.29 and F=4.29', R^2=0.25).

Supplementary Material- Table S3.1- Soil parameters in the 92 experimental plots

Plot	Treatment	Clay	Fine silt	Coarse silt	Fine sand	Coarse sand	Carbon	Azot	P	pH	CEC	Ca	Mg	Na	K	Fe	Mn	Al	Pb
(unit)		g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg		cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg	mg/kg
p46	Control	275,00	359,00	231,00	114,00	21,00	90,70	4,41	0,02	7,58	35,60	36,40	1,64	0,06	1,53	0,04	0,14	0,10	102,00
p21	Control	405,00	273,00	193,00	121,00	8,00	112,00	6,01	0,03	7,53	40,00	37,60	2,66	0,08	1,79	0,05	0,06	0,10	86,50
p58	Control	258,00	377,00	242,00	100,00	23,00	116,00	6,13	0,03	7,61	41,90	43,50	1,76	0,07	1,41	0,03	<0,005	0,11	119,00
p34	Control	446,00	308,00	140,00	97,00	9,00	115,00	5,86	0,04	7,56	42,20	42,80	2,41	0,10	1,72	0,05	0,03	0,13	108,00
p59	Control	387,00	278,00	201,00	129,00	5,00	71,80	4,02	0,02	7,67	32,60	30,10	1,72	0,07	1,00	0,01	0,02	<0,02	90,70
p78	Control	416,00	260,00	203,00	113,00	8,00	104,00	5,88	0,03	7,57	37,90	39,20	1,76	0,07	1,28	0,03	<0,005	0,07	90,20
p48	Control	263,00	399,00	213,00	109,00	16,00	64,00	3,52	0,02	7,94	32,70	30,30	1,55	0,06	1,16	<0,005	<0,005	<0,02	89,20
p82	Control	260,00	340,00	232,00	149,00	19,00	54,60	3,44	0,02	8,14	27,50	26,40	0,88	0,06	0,90	0,01	<0,005	<0,02	92,40
p88	Control	291,00	368,00	216,00	111,00	14,00	84,60	5,18	0,02	7,82	37,10	37,10	2,29	0,07	1,67	0,02	<0,005	0,06	94,60
p60	Control	344,00	352,00	207,00	87,00	10,00	162,00	9,69	0,05	7,46	67,40	65,80	3,98	0,11	1,37	0,03	<0,005	0,06	110,00
p93	Control	251,00	330,00	201,00	105,00	9,00	98,80	4,86	0,02	7,91	38,70	41,50	2,09	0,08	1,28	0,01	<0,005	<0,02	94,90
p73	Control	355,00	378,00	257,00	93,00	21,00	96,60	5,36	0,02	7,75	41,60	42,10	2,59	0,08	1,30	0,02	<0,005	0,06	114,00
p87	Control	410,00	284,00	200,00	97,00	9,00	90,30	5,08	0,02	7,77	34,30	35,70	2,06	0,08	1,09	0,03	<0,005	0,07	90,30
p52	Control	227,00	393,00	273,00	98,00	9,00	101,00	6,36	0,02	7,96	43,70	43,90	2,04	0,09	1,64	0,05	<0,005	0,16	119,00
p76	Control	355,00	282,00	225,00	131,00	7,00	72,10	3,88	0,02	7,68	30,60	28,10	1,50	0,06	1,00	0,01	<0,005	<0,02	94,10
p10	Control	267,00	296,00	235,00	197,00	5,00	73,50	5,20	0,02	7,65	32,50	29,70	1,56	0,06	1,02	0,01	0,01	<0,02	93,20
p9	Control	292,00	290,00	241,00	168,00	9,00	73,70	4,21	0,02	7,77	32,70	30,40	1,66	0,06	0,99	0,01	0,01	<0,02	89,90
p28	Control	352,00	275,00	240,00	125,00	8,00	65,90	4,15	0,02	7,86	32,20	30,40	1,36	0,06	0,82	<0,005	<0,005	<0,02	93,10
p47	Control	231,00	380,00	245,00	125,00	19,00	103,00	5,66	0,03	7,63	42,40	44,30	2,43	0,06	1,34	0,03	<0,005	0,06	98,30
p29	Control	357,00	295,00	227,00	112,00	9,00	139,00	7,66	0,04	7,53	47,10	46,60	2,63	0,11	1,62	0,04	0,04	0,04	118,00
p27	Control	346,00	279,00	232,00	133,00	10,00	76,20	4,65	0,02	7,91	32,10	30,10	1,64	0,09	0,86	0,01	0,01	<0,02	94,50
p67	Control	324,00	337,00	212,00	117,00	10,00	86,10	4,71	0,02	7,68	35,60	36,90	1,62	0,06	1,34	0,04	<0,005	0,12	110,00
p66	Control	337,00	334,00	205,00	113,00	11,00	81,70	5,01	0,02	7,97	36,20	37,50	1,85	0,11	1,16	0,03	<0,005	0,07	104,00
p18	Control	345,00	256,00	160,00	134,00	105,00	134,00	6,57	0,04	7,77	43,80	44,00	2,26	0,07	1,49	0,04	<0,005	0,05	50,60
p30	Control	351,00	310,00	211,00	118,00	10,00	130,00	6,93	0,03	7,60	42,30	43,40	1,94	0,07	1,22	0,05	0,03	0,08	106,00
p31	Control	247,00	325,00	263,00	136,00	29,00	85,30	5,01	0,02	7,63	33,30	31,40	2,29	0,08	1,21	0,01	0,01	<0,02	88,10
p6	Control	364,00	309,00	185,00	131,00	11,00	63,70	4,19	0,01	8,02	37,20	37,40	1,28	0,07	1,07	0,04	<0,005	0,05	89,40
p1	Control	286,00	319,00	207,00	170,00	18,00	65,30	4,42	0,02	7,93	33,20	30,90	1,61	0,07	0,96	0,01	<0,005	<0,02	85,40
p5	Control	296,00	346,00	220,00	128,00	10,00	81,80	4,81	0,02	8,06	37,60	38,40	1,41	0,07	1,03	0,04	<0,005	0,04	108,00
p57	Control	250,00	405,00	230,00	102,00	13,00	86,40	4,83	0,02	7,85	39,90	41,90	1,59	0,10	1,13	0,03	<0,005	0,08	100,00
p64	Control	355,00	322,00	198,00	115,00	10,00	129,00	6,62	0,03	7,54	45,20	45,80	2,86	0,08	1,26	0,03	<0,005	0,08	122,00
p62	Control	444,00	247,00	184,00	118,00	7,00	58,80	3,87	0,02	7,83	33,10	30,30	1,52	0,09	0,97	0,01	<0,005	<0,02	67,10
p65	Control	361,00	307,00	196,00	124,00	12,00	76,70	4,84	0,02	7,70	34,30	31,80	1,95	0,07	1,01	0,01	0,01	<0,02	96,20
p49	Control	246,00	350,00	276,00	116,00	12,00	108,00	6,31	0,03	7,66	49,60	50,80	2,35	0,06	1,07	0,03	<0,005	0,08	140,00
p16	Control	223,00	289,00	223,00	122,00	143,00	119,00	5,95	0,02	8,01	42,00	42,90	1,22	0,06	0,90	0,03	<0,005	<0,02	123,00
p77	Control	353,00	306,00	217,00	113,00	11,00	93,20	5,78	0,02	7,70	34,50	32,20	1,67	0,06	1,06	<0,005	0,01	<0,02	118,00
p22	Control	351,00	273,00	219,00	151,00	6,00	84,90	4,57	0,02	7,56	31,00	29,20	1,52	0,06	1,17	<0,005	0,05	<0,02	91,70
p84	Control	336,00	331,00	234,00	82,00	17,00	110,00	5,37	0,03	7,48	39,90	41,30	1,74	0,08	1,58	0,03	0,02	0,09	106,00
p86	Control	314,00	346,00	232,00	96,00	12,00	104,00	5,69	0,03	7,58	42,80	43,50	2,21	0,08	1,46	0,03	<0,005	0,08	129,00
p56	Control	344,00	310,00	223,00	110,00	13,00	130,00	6,52	0,04	7,45	39,60	40,70	2,29	0,10	1,23	0,02	0,01	0,02	82,90
p85	Control	327,00	356,00	216,00	90,00	11,00	94,30	5,46	0,02	7,91	40,60	42,40	2,05	0,07	1,26	0,03	<0,005	0,08	98,40
p55	Control	215,00	379,00	252,00	138,00	16,00	88,30	4,96	0,03	7,81	38,20	39,20	2,22	0,08	1,74	0,02	<0,005	0,06	93,00
p3	Control	389,00	302,00	173,00	127,00	9,00	65,40	4,21	0,01	8,05	39,50	39,30	1,67	0,06	0,91	0,03	<0,005	0,03	102,00
p83	Control	283,00	307,00	243,00	143,00	24,00	125,00	7,51	0,04	7,61	48,80	47,40	3,20	0,10	1,89	0,03	<0,005	0,07	91,30
p8	Control	305,00	306,00	238,00	142,00	9,00	119,00	6,31	0,03	7,39	42,10	41,00	2,37	0,09	1,48	0,03	0,09	<0,02	111,00
p74	Control	289,00	337,00	226,00	142,00	6,00	50,90	3,34	0,02	8,01	27,30	24,40	1,56	0,07	1,47	0,01	<0,005	<0,02	84,60
p61	Control	414,00	298,00	180,00	98,00	10,00	121,00	6,19	0,04	7,59	43,50	44,00	1,91	0,08	1,35	0,02	0,01	0,04	93,90
p2	Rain-excluded	409,00	260,00	173,00	149,00	9,00	78,30	4,54	0,03	7,89	36,60	36,50	1,65	0,08	1,35	0,06	<0,005	0,14	85,30
p35	Rain-excluded	469,00	303,00	130,00	86,00	12,00	172,00	8,55	0,04	7,22	52,40	51,40	3,52	0,11	1,94	0,04	0,06	0,12	80,50
p37	Rain-excluded	303,00	320,00	276,00	63,00	38,00	190,00	9,33	0,05	7,33	57,20	56,70	3,62	0,12	1,79	0,04	0,04	0,10	119,00
p38	Rain-excluded	316,00	336,00	252,00	84,00	12,00	154,00	7,70	0,04	7,50	48,10	49,90	2,37	0,86	1,50	0,03	0,01	0,05	110,00
p7	Rain-excluded	323,00	315,00	206,00	128,00	28,00	127,00	6,82	0,03	7,68	44,50	44,00	2,43	0,09	1,41	0,04	0,02	0,05	111,00
p15	Rain-excluded	337,00	309,00	218,00	122,00	14,00	116,00	6,26	0,03	7,63	41,30	41,00	1,74	0,07	1,39	0,04	0,01	0,04	113,00
p4	Rain-excluded	352,00	308,00	193,00	136,00	11,00	85,80	4,79	0,02	8,08	42,20	41,80	2,28	0,08	1,52	0,04	<0,005	0,08	88,40
p24	Rain-excluded	425,00	290,00	170,00	107,00	8,00	57,80	3,70	0,01	7,91	30,70	27,80	1,84	0,07	1,10	0,01	<0,005	<0,02	81,50
p71	Rain-excluded	317,00	327,00	219,00	123,00	14,00	81,30	4,42	0,02	7,90	32,60	30,80	1,58	0,06	1,03	0,01	<0,005	<0,02	90,20
p63	Rain-excluded	448,00	262,00	175,00	106,00	9,00	78,20	4,74	0,02	7,84	39,60	40,20	2,28	0,07	1,32	0,04	<0,005	0,10	78,90
p44	Rain-excluded	240,00	377,00	252,00	100,00	31,00	84,20	5,21	0,03	7,76	43,40	41,70	3,42	0,09	1,60	0,03	<0,005	0,05	80,20
p12	Rain-excluded	277,00	301,00	248,00	167,00	7,00	56,30	3,65	0,01	8,06	28,20	25,90	1,23	0,07	1,01	0,01	<0,005	<0,02	85,70
p53	Rain-excluded	271,00	342,00	270,00	107,00	10,00	119,00	6,62	0,03	7,95	48,10	50,00	2,50	0,12	1,27	0,03	<0,005	0,05	91,70
p92	Rain-excluded	307,00	353,00	230,00	100,00	10,00	68,20	3,83	0,02	7,80	30,80	28,50	1,51	0,05	1,06	0,01	<0,005	<0,02	86,00
p11	Rain-excluded	291,00	294,00	252,00	157,00	6,00	71,70	3,88	0,02	7,94	34,10	31,60	1,91	0,07	0,88	0,01	<0,005	<0,02	86,90
p13	Rain-excluded	292,00	295,00	252,00	152,00	9,00	95,50	4,99	0,03	7,62	41,10	40,20	1,99	0,08	1,14	0,03	<0,005	<0,02	96,80
p43	Rain-excluded	336,00	317,00	218,00	123,00	6,00	71,40	3,57	0,02	7,33	33,50	31,00	2,17	0,07	1,05	<0,005	0,05	<0,02	79,90
p40	Rain-excluded	387,00	330,00	208,00	66,00	9,00	86,90	4,63	0,03	7,75	39,20	40,90	2,11	0,08	1,23	0,03	<0,005	0,05	100,00
p33	Rain-excluded	364,00	297,00	201,00	127,00	11,00	98,60	5,34	0,03	7,73	38,40	40,30	1,78	0,07	1,26	0,04	<0,005	0,09	150,00
p14	Rain-excluded	271,00	345,00	240,00	124,00	20,00	69,40	4,17	0,02	7,80	30,20	28,00	1,38	0,07	1,02	0,01	0,01	<0,02	97,90
p80	Rain-excluded	418,00	276,00	188,00	112,00	6,00	39,80	2,62	0,01	7,88	26,40	24,60	1,19	0,07	0,66	0,01	<0,005	<0,02	58,60
p25	Rain-excluded	338,00	308,00	219,00	130,00	5,00	55,90	3,68	0,01	8,05	31,90	30,30	1,34	0,06	0,75	<0,005	<0,005	<0,02	97,60
p81	Rain-excluded	300,00	346,00	227,00	110,00	17,00	55,80	3,54	0,01	7,83	29,10	27,50	1,26	0,06	0,86	0,01	<0,005	<0,02	98,10
p17	Rain-excluded	369,00	333,00	160,00	114,00	24,00	82,20	4,81	0,02	7,90	41,80	40,40	2,36	0,06	1,13	0,04	<0,005	0,07	87,90
p32	Rain-excluded	389,00	288,00	197,00	117,00	9,00	73,20	4,11	0,02	7,81	36,50	37,10	1,90	0,07	1,10	0,04	<0,005	0,09	94,90
p54	Rain-excluded	275,00	364,00	236,00	112,00	13,00	67,40	3,97	0,02	7,92	34,00	30,60	2,00	0,08	1,34	0,01	<0,005	<0,02	85,00
p72	Rain-excluded	331,00	342,00	198,00	123,00	6,00	116,00	6,43	0,03	7,52	43,20	44,40	2,45	0,08	1,24	0,02	<0,005	0,04	105,00
p90	Rain-excluded	249,00	335,00	286,00	110,00	20,00	112,00	6,85	0,03	7,72	46,30	48,20	2,30	0,09	1,12	0,03	<0,005	0,05	137,00
p68	Rain-excluded	389,00	314,00	197,00	89,00	11,00	166,00	7,53	0,05	7,52	55,70	55,70	3,94	0,12	1,58	0,03	0,04	0,07	82,70
p45	Rain-excluded	266,00	342,00	246,00	131,00	15,00	113,00	5,41	0,03	7,54	39,40	39,70	2,24	0,09	1,53	0,04	0,04	0,11	72,30
p50	Rain-excluded	273,00	368,00	234,00	117,00	8,00	69,90	4,53	0,02	7,87	34,50	32,30	1,39	0,04	0,79	<0,005	<0,005	<0,02	102,00
p51	Rain-excluded	363,00	315,00	216,00	101,00	5,00	103,00	5,53	0,03	7,54	37,50	38,10	2,14	0,07	1,14	0,03	0,01	0,07	96,50
p75	Rain-excluded	340,00	298,00	230,00	120,00	12,00	86,10	4,90	0,03	7,80	34,70	33,10	1,68	0,08	1,02	<0,005	<0,005	<0,02	109,00
p20	Rain-excluded	381,00	287,00	194,00	129,00	9,00	79,20	4,78	0,03	7,43	30,40	28,30	1,56	0,08	1,02	0,01	0,07	<0,02	92,10
p19	Rain-excluded	402,00	297,00	165,00	110,00	26,00	106,00	5,52	0,02	7,44	42,30	40,10	2,38	0,09	1,34	0,02	<0,005	<0,02	73,20
p26	Rain-excluded	338,00	287,00	248,00	121,00	6,00	86,90	4,81	0,02	7,79	38,90	39,20	1,80	0,06	0,92	0,03	<0,005	0,04	110,00
p69	Rain-excluded	398,00	301,00	190,00	99,00	12,00	118,00	6,83	0,03	7,58	43,60	44,10	2,68	0,08	1,46	0,02	0,03	<0,02	102,00
p39	Rain-excluded	379,00	337,00	190,00	84,00	10,00	85,40	4,75	0,03	7,73	35,50	37,70	1,76	0,07	1,16	0,05	<0,005	0,14	106,00
p89	Rain-excluded	292,00	362,00	225,00	113,00	8,00	86,10	4,94	0,02	7,71	36,20	37,30	1,87	0,06	1,09	0,01	<0,005	<0,02	95,10
p41	Rain-excluded	391,00	290,00	211,00	93,00	15,00	127,00	6,34	0,03	7,61	38,20	39,90	1,84	0,07	1,25	0,02	<0,005	0,03	69,20
p23	Rain-excluded	351,00	307,00	207,00	130,00	5,00	113,00	6,02	0,04	7,66	41,90	39,50	2,95	0,12	1,80	0,03	0,01	0,03	89,90
p36	Rain-excluded	457,00	257,00	168,00	111,00	7,00	84,80	5,24	0,03	7,54	38,20	37,00	2,47	0,07	1,76	0,05	0,01	0,17	86,20
p91	Rain-excluded	227,00	343,00	279,00	134,00	17,00	107,00	5,80	0,03	7,56	39,60	41,80	2,13	0,07	1,34	0,02	<0,005	0,05	98,90
p70	Rain-excluded	362,00	322,00	200,00	109,00	7,00	103,00	5,64	0,03	7,56	37,40	39,20	1,71	0,07	1,54	0,03	<0,005	0,07	108,00
p79	Rain-excluded	360,00	308,00	214,00	110,00	8,00	92,30	6,01	0,04	7,72	35,80	37,80	1,53	0,08	1,47	0,03	<0,005	0,09	106,00

Supplementary Material- Table S3.2- Leaf litter traits for the four dominant shrub species (*Cistus albudus, Quercus coccifera, Rosmarinus officinalis* and *Ulex parviflorus*): total C (C) total N (N) and total P (P) concentrations, phenolics (Phenols), Lignin, water holding capacity (WHC), dissolved organic Carbon (DOC) and total dissolved Nitrogen (TDN), lignin to N ratio (Lignin:N). Litter traits were determined according to standard methods (Santonja et al. *in prep*). These values were used for the computing of community-weighted mean traits and of functional divergence of traits in litter mixtures. One-way ANOVAs were performed for differences among species. *F*-Ratio are indicated and P-values with the respective symbols *** for p <0.001. Different letters denote significant differences among species.

	C. albidus	Q. coccifera	R. officinalis	U. parviflorus	F-Ratio
C (mg g-1 dw)	421,57 ± 6,03 **c**	447,36 ± 7,50 **b**	467,49 ± 4,34 **ab**	479,95 ± 3,03 **a**	**21,58*** **
N (mg g-1 dw)	3,99 ± 0,08 **c**	8,57 ± 0,17 **a**	4,56 ± 0,15 **c**	7,96 ± 0,16 **b**	**264,13*** **
P (mg g-1 dw)	0,41 ± 0,01 **a**	0,33 ± 0,01 **b**	0,24 ± 0,02 **c**	0,18 ± 0,02 **d**	**48,77*** **
Phenols (mg g-1 dw)	39,44 ± 0,92 **b**	91,97 ± 4,13 **a**	48,37 ± 1,29 **b**	15,26 ± 0,23 **c**	**209,16*** **
Lignin (mg g-1 dw)	180,08 ± 12,84 **b**	130,09 ± 3,82 **c**	133,30 ± 9,74 **c**	209,23 ± 3,13 **a**	**22,74*** **
WHC %	201,36 ± 3,92 **a**	131,87 ± 4,88 **b**	127,85 ± 1,90 **b**	79,40 ± 2,95 **c**	**195,62*** **
DOC (mg g-1 dw)	22,78 ± 0,57 **a**	15,71 ± 0,63 **ab**	5,13 ± 0,12 **c**	6,01 ± 0,28 **bc**	**11,64*** **
TDN (mg g-1 dw)	0,28 ± 0,01 **b**	0,23 ± 0,01 **b**	0,11 ± 0,01 **b**	0,52 ± 0,02 **a**	**11,40*** **
Lignin:N ratio	45,05 ± 3,25 **a**	15,18 ± 0,42 **c**	29,44 ± 2,74 **ab**	26,31 ± 0,39 **bc**	**14,09*** **

Supplementary Material- Table S3.3- Soil microbial parameters: global metabolic activity (sum15) expressed in µg C-CO_2 respired g^{-1} of soil h^{-1}), Shannon microbial metabolic diversity index (H'), Simpson microbial metabolic dominance index (D), and rate of decomposition of cellulose paper (DCP) for 2012 and 2013 sampling (mean values by litter mixture ± Standard Error (SE)). (QC for *Q. coccifera*, UP for *U. parviflorus,* RO for *R. officinalis*, and CA for *C. albidus*).

Litter mixture	sum15 ± SE		H' ± SE		D ± SE		DCP ± SE	
	2012	2013	2012	2013	2012	2013	2012	2013
QC	47,0 ± 8,6	50,1 ± 10,2	1,114 ± 0,009	1,150 ± 0,007	0,089 ± 0,004	0,075 ± 0,002	24,3 ± 3,9	31,2 ± 1,5
UP	50,3 ± 7,6	49,3 ± 7,8	1,130 ± 0,008	1,153 ± 0,005	0,083 ± 0,003	0,075 ± 0,002	14,8 ± 2,3	39,6 ± 5,7
RO	51,1 ± 7,0	37,0 ± 6,0	1,111 ± 0,018	1,141 ± 0,007	0,090 ± 0,008	0,079 ± 0,003	2,8 ± 1,7	29,0 ± 6,1
CA	48,8 ± 4,4	49,6 ± 8,3	1,116 ± 0,014	1,151 ± 0,008	0,088 ± 0,006	0,075 ± 0,003	27,9 ± 8,0	38,5 ± 7,4
QC , RO	47,5 ± 7,1	31,1 ± 3,0	1,110 ± 0,014	1,144 ± 0,005	0,091 ± 0,006	0,077 ± 0,002	13,1 ± 1,9	36,7 ± 6,0
QC , CA	56,3 ± 5,7	43,3 ± 2,4	1,091 ± 0,010	1,146 ± 0,004	0,099 ± 0,005	0,076 ± 0,001	12,7 ± 3,3	40,9 ± 5,0
QC , UP	53,8 ± 12,8	64,0 ± 9,4	1,119 ± 0,012	1,155 ± 0,008	0,089 ± 0,005	0,074 ± 0,003	19,5 ± 5,3	40,8 ± 3,6
RO , UP	44,2 ± 11,3	40,1 ± 2,2	1,078 ± 0,021	1,145 ± 0,007	0,105 ± 0,009	0,077 ± 0,003	6,6 ± 4,2	25,6 ± 7,3
RO , CA	41,2 ± 6,8	35,3 ± 3,3	1,124 ± 0,015	1,145 ± 0,004	0,086 ± 0,006	0,077 ± 0,002	6,9 ± 2,6	30,3 ± 1,5
CA , UP	66,6 ± 4,6	33,7 ± 3,3	1,131 ± 0,006	1,137 ± 0,004	0,082 ± 0,002	0,080 ± 0,002	12,1 ± 2,0	36,2 ± 6,0
QC , RO , CA	39,8 ± 5,6	40,6 ± 1,9	1,131 ± 0,011	1,160 ± 0,001	0,082 ± 0,004	0,072 ± 0,0005	8,8 ± 2,3	35,9 ± 9,5
QC , RO , UP	37,6 ± 4,3	41,6 ± 4,0	1,124 ± 0,007	1,152 ± 0,003	0,085 ± 0,003	0,074 ± 0,001	13,0 ± 2,9	31,6 ± 6,3
QC , CA , UP	46,1 ± 2,5	60,9 ± 10,3	1,116 ± 0,009	1,157 ± 0,005	0,088 ± 0,003	0,073 ± 0,002	6,2 ± 2,1	41,8 ± 4,4
RO , CA , UP	49,4 ± 10,2	55,0 ± 7,1	1,098 ± 0,011	1,158 ± 0,002	0,096 ± 0,005	0,072 ± 0,001	4,5 ± 2,1	33,8 ± 3,3
QC , RO , CA , UP	48,7 ± 6,0	47,7 ± 4,6	1,133 ± 0,007	1,155 ± 0,004	0,082 ± 0,003	0,073 ± 0,001	13,1 ± 2,8	46,8 ± 7,5

Supplementary Material- Table S3.4- Community level physiological profiles for the soil microbial community in 2012 and 2013: global metabolic activity (sum15, computed as the sum of substrate induced respiration rates summed across the 15 C compounds), standardized catabolic rates for the 15 C compounds (in % of the global catabolic activity computed for a given plot), microbial metabolic diversity (H'), microbial metabolic dominance (D), and rate of decomposition of cellulose paper (DCP- square root values).

S3.4 (a) CLPP parameters in 2012

Plot	GLU	XYL	CELL	ASP	SER	LYS	GLY	GLUT	N-AC	OX	UR	MAL	CAF	SYR	VAN	H2O	Log sum15	Shannon	Simpson	√DCP
p46	0,033	0,078	0,019	0,082	0,071	0,039	0,081	0,081	0,064	0,165	0,055	0,076	0,048	0,059	0,050	1,402	1,565	1,130	0,082	4,796
p21	0,047	0,035	0,040	0,068	0,081	0,028	0,052	0,096	0,034	0,177	0,047	0,128	0,022	0,047	0,099	1,344	1,812	1,102	0,092	4,243
p58	0,062	0,049	0,051	0,042	0,062	0,034	0,045	0,063	0,065	0,179	0,050	0,141	0,047	0,053	0,057	1,765	1,495	1,120	0,089	3,873
p34	0,034	0,041	0,036	0,082	0,059	0,029	0,087	0,090	0,066	0,179	0,046	0,105	0,041	0,047	0,056	1,768	1,504	1,118	0,088	3,464
p59	0,073	0,060	0,051	0,090	0,081	0,044	0,084	0,085	0,068	0,115	0,042	0,084	0,035	0,038	0,050	1,981	1,637	1,151	0,074	4,243
p78	0,055	0,067	0,041	0,122	0,061	0,012	0,053	0,049	0,065	0,188	0,055	0,079	0,038	0,074	0,042	2,244	1,691	1,110	0,091	4,690
p48	0,023	0,050	0,017	0,070	0,070	0,014	0,038	0,066	0,030	0,258	0,040	0,170	0,043	0,065	0,048	0,841	1,318	1,034	0,125	0,000
p82	0,100	0,038	0,026	0,105	0,049	0,025	0,045	0,068	0,060	0,186	0,031	0,124	0,022	0,061	0,058	1,246	1,628	1,094	0,095	2,236
p88	0,107	0,025	0,017	0,071	0,058	0,030	0,089	0,071	0,085	0,150	0,069	0,098	0,025	0,063	0,042	3,717	1,813	1,115	0,085	0,000
p60	0,097	0,053	0,030	0,090	0,060	0,049	0,074	0,121	0,075	0,128	0,028	0,101	0,028	0,050	0,015	2,505	1,730	1,117	0,084	7,810
p93	0,066	0,064	0,043	0,092	0,067	0,041	0,082	0,096	0,062	0,120	0,044	0,093	0,040	0,050	0,039	2,336	1,764	1,148	0,075	6,325
p73	0,074	0,065	0,036	0,076	0,093	0,033	0,064	0,079	0,075	0,136	0,048	0,077	0,060	0,056	0,029	3,094	1,634	1,112	0,091	2,236
p87	0,080	0,049	0,050	0,080	0,053	0,025	0,073	0,073	0,078	0,112	0,053	0,115	0,047	0,065	0,048	1,453	1,751	1,145	0,077	4,899
p52	0,043	0,033	0,044	0,068	0,094	0,025	0,057	0,062	0,056	0,179	0,058	0,135	0,052	0,047	0,049	2,912	1,716	1,149	0,075	2,236
p76	0,119	0,060	0,073	0,102	0,085	0,041	0,078	0,074	0,053	0,066	0,057	0,068	0,047	0,042	0,037	2,618	1,879	1,114	0,090	4,243
p10	0,046	0,065	0,038	0,089	0,023	0,042	0,052	0,092	0,031	0,152	0,029	0,142	0,034	0,083	0,081	1,804	1,705	1,153	0,074	3,606
p9	0,059	0,034	0,053	0,082	0,060	0,037	0,070	0,058	0,063	0,232	0,034	0,089	0,031	0,050	0,048	2,348	1,794	1,108	0,089	0,000
p28	0,040	0,017	0,041	0,039	0,075	0,028	0,075	0,038	0,083	0,183	0,049	0,125	0,041	0,079	0,086	0,762	1,706	1,099	0,100	4,243
p47	0,043	0,063	0,042	0,105	0,066	0,039	0,043	0,083	0,064	0,126	0,039	0,122	0,048	0,077	0,041	0,631	1,644	1,102	0,093	3,162
p29	0,054	0,050	0,050	0,066	0,063	0,051	0,061	0,087	0,054	0,148	0,046	0,115	0,050	0,054	0,048	3,487	1,483	1,138	0,079	6,000
p27	0,041	0,065	0,055	0,053	0,051	0,048	0,047	0,057	0,040	0,163	0,048	0,156	0,045	0,050	0,081	4,438	1,830	1,144	0,078	4,359
p67	0,060	0,059	0,055	0,085	0,069	0,031	0,060	0,081	0,052	0,177	0,043	0,122	0,035	0,033	0,039	6,084	1,907	1,121	0,088	4,123
p66	0,022	0,026	0,035	0,083	0,046	0,044	0,033	0,109	0,027	0,187	0,021	0,225	0,022	0,081	0,041	1,249	1,995	1,119	0,088	0,000
p18	0,027	0,047	0,042	0,057	0,070	0,034	0,049	0,073	0,052	0,191	0,055	0,158	0,042	0,050	0,054	1,575	1,426	1,033	0,121	4,796
p30	0,031	0,032	0,028	0,088	0,050	0,031	0,063	0,071	0,064	0,217	0,041	0,194	0,019	0,043	0,026	2,636	1,618	1,102	0,096	4,123
p31	0,088	0,049	0,037	0,107	0,033	0,049	0,056	0,061	0,035	0,153	0,065	0,113	0,048	0,059	0,046	1,900	1,366	1,050	0,117	2,828
p6	0,095	0,026	0,049	0,073	0,046	0,029	0,089	0,073	0,083	0,130	0,044	0,104	0,060	0,044	0,054	0,831	1,812	1,129	0,083	0,000
p1	0,084	0,042	0,049	0,094	0,050	0,034	0,051	0,092	0,067	0,156	0,023	0,121	0,027	0,065	0,043	1,059	1,381	1,137	0,079	4,000
p5	0,059	0,048	0,043	0,083	0,046	0,048	0,078	0,077	0,071	0,139	0,061	0,105	0,045	0,043	0,054	4,266	1,719	1,118	0,086	3,873
p57	0,046	0,044	0,051	0,067	0,062	0,025	0,062	0,096	0,071	0,134	0,061	0,108	0,052	0,058	0,062	1,925	1,893	1,146	0,077	1,414
p64	0,107	0,077	0,042	0,086	0,066	0,022	0,055	0,082	0,047	0,159	0,052	0,089	0,026	0,050	0,039	1,414	1,742	1,144	0,077	3,606
p62	0,077	0,083	0,061	0,056	0,062	0,029	0,075	0,048	0,067	0,159	0,059	0,079	0,044	0,052	0,051	1,621	1,520	1,123	0,084	2,828
p65	0,080	0,038	0,034	0,080	0,094	0,048	0,056	0,081	0,074	0,109	0,058	0,101	0,043	0,055	0,052	1,145	1,526	1,143	0,079	4,000
p49	0,032	0,068	0,033	0,101	0,070	0,040	0,068	0,091	0,040	0,133	0,048	0,139	0,031	0,061	0,045	0,632	1,568	1,151	0,074	3,606
p16	0,062	0,046	0,050	0,060	0,087	0,027	0,069	0,062	0,069	0,156	0,064	0,129	0,033	0,051	0,036	0,675	1,390	1,124	0,084	3,464
p77	0,030	0,036	0,043	0,075	0,067	0,037	0,094	0,044	0,052	0,227	0,066	0,087	0,038	0,046	0,056	1,169	1,402	1,127	0,084	3,464
p22	0,064	0,039	0,030	0,134	0,021	0,047	0,023	0,112	0,034	0,181	0,032	0,143	0,030	0,048	0,060	1,647	1,605	1,099	0,100	2,236
p84	0,049	0,080	0,061	0,039	0,019	0,084	0,072	0,071	0,176	0,067	0,117	0,044	0,050	0,036	0,035	1,715	1,605	1,073	0,103	2,000
p86	0,036	0,061	0,045	0,068	0,097	0,032	0,069	0,076	0,061	0,165	0,036	0,140	0,043	0,027	0,043	1,900	1,660	1,116	0,088	3,317
p56	0,034	0,041	0,064	0,041	0,060	0,022	0,072	0,080	0,022	0,231	0,042	0,151	0,044	0,053	0,042	4,261	1,585	1,113	0,089	1,000
p85	0,047	0,017	0,042	0,022	0,047	0,023	0,069	0,045	0,059	0,234	0,082	0,139	0,032	0,074	0,066	2,610	1,975	1,071	0,109	0,000
p55	0,081	0,052	0,040	0,084	0,065	0,039	0,071	0,077	0,052	0,174	0,058	0,094	0,022	0,054	0,040	1,804	1,755	1,067	0,110	3,464
p3	0,078	0,046	0,034	0,106	0,068	0,033	0,066	0,093	0,058	0,143	0,028	0,122	0,043	0,049	0,034	2,177	1,565	1,126	0,084	0,000
p83	0,041	0,043	0,039	0,067	0,090	0,026	0,053	0,074	0,082	0,135	0,060	0,051	0,097	0,074	0,070	1,326	1,666	1,123	0,084	2,828
p8	0,045	0,043	0,025	0,133	0,043	0,030	0,056	0,059	0,048	0,169	0,069	0,042	0,131	0,046	0,061	1,507	1,668	1,143	0,077	3,606
p74	0,045	0,063	0,045	0,091	0,052	0,027	0,070	0,059	0,042	0,175	0,038	0,044	0,130	0,055	0,064	3,258	1,544	1,106	0,092	2,449
p61	0,051	0,038	0,027	0,045	0,059	0,026	0,051	0,131	0,055	0,216	0,030	0,127	0,026	0,075	0,041	1,507	1,844	1,119	0,088	3,317
p2	0,044	0,044	0,032	0,098	0,073	0,034	0,082	0,086	0,060	0,153	0,048	0,109	0,026	0,051	0,061	0,961	1,597	1,075	0,106	5,831
p35	0,100	0,040	0,045	0,075	0,091	0,010	0,069	0,064	0,058	0,120	0,055	0,124	0,044	0,063	0,041	5,175	1,460	1,126	0,083	6,164
p37	0,077	0,052	0,038	0,073	0,071	0,050	0,074	0,080	0,045	0,170	0,042	0,104	0,043	0,029	0,051	3,486	1,908	1,129	0,080	4,243
p38	0,052	0,046	0,041	0,087	0,026	0,040	0,069	0,082	0,078	0,175	0,035	0,122	0,042	0,069	0,036	2,439	1,926	1,129	0,084	3,317
p7	0,056	0,040	0,051	0,072	0,094	0,048	0,060	0,073	0,089	0,120	0,061	0,087	0,045	0,045	0,059	1,888	1,742	1,115	0,088	4,359
p15	0,070	0,048	0,058	0,049	0,067	0,031	0,052	0,059	0,088	0,129	0,072	0,101	0,063	0,040	0,072	2,950	1,579	1,154	0,074	2,646
p4	0,067	0,041	0,026	0,059	0,073	0,025	0,060	0,085	0,058	0,143	0,060	0,158	0,032	0,057	0,056	2,075	1,769	1,150	0,075	0,000
p24	0,089	0,046	0,052	0,069	0,110	0,024	0,068	0,083	0,072	0,063	0,065	0,076	0,055	0,058	0,067	2,771	1,727	1,118	0,087	1,000
p71	0,038	0,056	0,028	0,089	0,068	0,037	0,060	0,072	0,086	0,160	0,061	0,129	0,026	0,042	0,049	2,006	1,820	1,157	0,072	3,162
p63	0,054	0,043	0,029	0,078	0,039	0,036	0,074	0,084	0,054	0,143	0,048	0,178	0,047	0,050	0,043	2,627	1,718	1,119	0,086	4,000
p44	0,034	0,054	0,027	0,103	0,031	0,035	0,037	0,087	0,028	0,242	0,039	0,145	0,025	0,062	0,052	0,756	1,468	1,055	0,115	4,690
p12	0,039	0,046	0,050	0,036	0,050	0,032	0,056	0,093	0,071	0,189	0,023	0,187	0,025	0,062	0,042	0,724	1,403	1,077	0,105	3,464
p53	0,025	0,037	0,040	0,049	0,078	0,035	0,073	0,059	0,056	0,241	0,044	0,108	0,052	0,047	0,055	1,457	1,682	1,089	0,105	3,606
p92	0,068	0,078	0,021	0,103	0,030	0,032	0,085	0,079	0,026	0,181	0,023	0,132	0,016	0,050	0,076	2,075	1,525	1,082	0,097	4,243
p11	0,086	0,031	0,027	0,067	0,060	0,033	0,059	0,073	0,042	0,198	0,033	0,140	0,033	0,065	0,055	3,866	1,906	1,096	0,097	3,162
p13	0,056	0,045	0,039	0,089	0,036	0,021	0,052	0,083	0,055	0,179	0,025	0,149	0,030	0,084	0,057	2,908	1,760	1,096	0,095	4,000
p43	0,031	0,040	0,031	0,074	0,057	0,008	0,030	0,056	0,025	0,253	0,054	0,158	0,065	0,068	0,052	1,444	1,632	1,044	0,120	4,796
p40	0,080	0,038	0,033	0,060	0,067	0,030	0,069	0,069	0,049	0,203	0,044	0,111	0,043	0,045	0,060	4,397	1,978	1,111	0,093	0,000
p33	0,061	0,049	0,055	0,059	0,073	0,044	0,074	0,075	0,045	0,158	0,058	0,115	0,037	0,057	0,040	0,873	1,391	1,138	0,081	5,568
p14	0,036	0,032	0,027	0,042	0,084	0,016	0,065	0,090	0,054	0,242	0,061	0,132	0,027	0,037	0,056	0,557	1,386	1,063	0,112	3,742
p80	0,083	0,099	0,053	0,120	0,032	0,027	0,019	0,087	0,037	0,300	0,017	0,078	0,014	0,026	0,007	1,154	1,502	0,992	0,142	0,000
p25	0, 093	0,042	0,047	0,093	0,046	0,042	0,031	0,089	0,030	0,117	0,033	0,164	0,050	0,077	0,046	0,643	1,399	1,116	0,087	0,000
p81	0,062	0,023	0,051	0,067	0,088	0,023	0,070	0,046	0,050	0,157	0,050	0,174	0,043	0,058	0,039	1,651	1,620	1,104	0,093	0,000
p17	0,052	0,057	0,028	0,053	0,050	0,041	0,043	0,099	0,065	0,159	0,065	0,107	0,047	0,058	0,076	1,680	1,612	1,133	0,082	2,449
p32	0,072	0,044	0,058	0,086	0,070	0,038	0,048	0,068	0,082	0,109	0,054	0,112	0,039	0,070	0,051	1,391	1,578	1,153	0,074	0,000
p54	0,088	0,049	0,034	0,067	0,069	0,026	0,083	0,058	0,048	0,149	0,064	0,091	0,064	0,044	0,065	2,635	1,749	1,140	0,079	3,464
p72	0,031	0,036	0,034	0,057	0,035	0,065	0,073	0,081	0,068	0,118	0,040	0,181	0,045	0,091	0,046	3,801	1,802	1,114	0,089	3,742
p90	0,070	0,027	0,057	0,046	0,079	0,015	0,060	0,072	0,055	0,156	0,064	0,117	0,063	0,075	0,043	3,438	1,890	1,126	0,083	3,606
p68	0,055	0,054	0,044	0,071	0,058	0,040	0,076	0,072	0,044	0,151	0,046	0,131	0,047	0,065	0,047	3,961	1,864	1,136	0,082	3,873
p45	0,055	0,054	0,056	0,085	0,083	0,025	0,023	0,107	0,046	0,207	0,044	0,122	0,017	0,037	0,039	1,658	1,825	1,082	0,101	0,000
p50	0,049	0,049	0,029	0,072	0,084	0,043	0,079	0,088	0,080	0,132	0,054	0,102	0,052	0,042	0,045	1,658	1,456	1,142	0,077	3,162
p51	0,037	0,037	0,078	0,038	0,103	0,028	0,080	0,083	0,065	0,113	0,062	0,092	0,062	0,067	0,055	1,328	1,601	1,146	0,076	2,449
p75	0,043	0,029	0,052	0,061	0,080	0,018	0,083	0,048	0,085	0,174	0,055	0,120	0,053	0,039	0,060	1,026	1,644	1,115	0,088	4,000
p20	0,074	0,054	0,041	0,059	0,049	0,022	0,064	0,070	0,074	0,153	0,053	0,108	0,058	0,046	0,074	1,316	1,619	1,137	0,080	2,828
p19	0,040	0,032	0,044	0,076	0,088	0,041	0,088	0,062	0,074	0,139	0,049	0,087	0,053	0,080	0,047	3,181	1,700	1,143	0,078	5,000
p26	0,039	0,047	0,043	0,080	0,084	0,045	0,051	0,074	0,080	0,149	0,055	0,124	0,055	0,036	0,039	2,117	1,686	1,133	0,082	3,162
p69	0,055	0,067	0,048	0,059	0,094	0,028	0,059	0,080	0,063	0,154	0,060	0,118	0,037	0,040	0,040	1,240	1,683	1,131	0,082	3,317
p39	0,059	0,081	0,047	0,078	0,069	0,043	0,058	0,066	0,052	0,189	0,042	0,074	0,037	0,051	0,056	1,557	1,744	1,131	0,085	0,000
p89	0,042	0,041	0,045	0,092	0,059	0,016	0,066	0,095	0,031	0,213	0,028	0,139	0,039	0,048	0,046	1,648	1,620	1,080	0,103	2,646
p41	0,055	0,025	0,031	0,069	0,072	0,029	0,069	0,092	0,052	0,158	0,047	0,144	0,041	0,065	0,052	3,075	1,662	1,116	0,088	2,646
p23	0,046	0,057	0,030	0,063	0,098	0,035	0,065	0,092	0,057	0,148	0,035	0,129	0,051	0,060	0,036	0,799	1,318	1,125	0,084	0,000
p36	0,068	0,061	0,058	0,076	0,038	0,045	0,052	0,074	0,083	0,123	0,043	0,094	0,060	0,073	0,052	1,218	1,487	1,156	0,073	3,000
p91	0,060	0,046	0,029	0,102	0,055	0,045	0,042	0,067	0,046	0,185	0,065	0,064	0,090	0,061	0,042	4,591	1,896	1,124	0,087	2,828
p70	0,071	0,080	0,065	0,111	0,078	0,053	0,087	0,067	0,054	0,137	0,047	0,054	0,037	0,026	0,033	1,320	1,572	1,138	0,079	4,359
p79	0,066	0,036	0,052	0,069	0,077	0,038	0,068	0,072	0,070	0,099	0,060	0,059	0,112	0,059	0,063	2,353	1,653	1,159	0,072	5,477

S3.4 (b) CLPP parameters in 2013

Plot	GLU	XYL	CELL	ASP	SER	LYS	GLY	GLUT	N-AC	OX	UR	MAL	CAF	SYR	VAN	H2O	Log sum15	Shannon	Simpson	√DCP
p46	0,077	0,053	0,065	0,043	0,090	0,038	0,059	0,064	0,070	0,104	0,066	0,106	0,059	0,050	0,057	2,963	1,759	1,159	0,072	5,477
p21	0,081	0,084	0,039	0,083	0,072	0,031	0,034	0,083	0,036	0,154	0,041	0,094	0,041	0,061	0,066	1,264	1,399	1,131	0,082	5,568
p58	0,037	0,079	0,033	0,081	0,059	0,053	0,058	0,073	0,052	0,125	0,069	0,111	0,052	0,064	0,055	1,500	1,420	1,150	0,075	5,292
p34	0,043	0,043	0,065	0,082	0,075	0,047	0,072	0,062	0,073	0,106	0,074	0,102	0,055	0,054	0,048	4,734	1,884	1,159	0,072	5,385
p59	0,063	0,064	0,063	0,082	0,056	0,026	0,071	0,075	0,054	0,153	0,049	0,081	0,049	0,056	0,058	2,151	1,577	1,146	0,077	6,557
p78	0,057	0,081	0,068	0,089	0,053	0,041	0,043	0,063	0,056	0,107	0,053	0,122	0,040	0,063	0,065	1,779	1,618	1,152	0,075	5,196
p48	0,081	0,053	0,039	0,079	0,064	0,031	0,076	0,042	0,055	0,121	0,040	0,128	0,059	0,077	0,056	1,897	1,513	1,142	0,078	4,359
p82	0,051	0,050	0,045	0,069	0,065	0,026	0,057	0,052	0,056	0,197	0,040	0,120	0,041	0,067	0,063	1,240	1,365	1,115	0,091	5,477
p88	0,088	0,066	0,065	0,076	0,057	0,032	0,049	0,081	0,069	0,111	0,064	0,115	0,030	0,063	0,035	2,619	1,615	1,147	0,076	4,472
p60	0,071	0,077	0,053	0,061	0,088	0,039	0,031	0,071	0,073	0,115	0,042	0,101	0,057	0,059	0,064	3,378	1,847	1,153	0,074	7,937
p93	0,063	0,063	0,052	0,078	0,076	0,027	0,066	0,069	0,058	0,139	0,054	0,099	0,037	0,054	0,063	1,921	1,542	1,147	0,076	7,348
p73	0,083	0,063	0,046	0,077	0,082	0,034	0,069	0,062	0,063	0,117	0,057	0,090	0,049	0,053	0,054	2,787	1,382	1,115	0,089	5,745
p87	0,062	0,058	0,051	0,084	0,047	0,074	0,055	0,099	0,049	0,131	0,047	0,107	0,040	0,062	0,033	1,606	1,847	1,158	0,073	4,359
p52	0,039	0,073	0,054	0,101	0,071	0,038	0,055	0,051	0,080	0,108	0,060	0,099	0,052	0,055	0,065	2,008	1,575	1,144	0,077	5,568
p76	0,061	0,066	0,040	0,064	0,059	0,039	0,057	0,096	0,063	0,127	0,038	0,135	0,030	0,071	0,054	2,548	1,384	1,155	0,073	4,899
p10	0,043	0,064	0,058	0,101	0,052	0,043	0,039	0,070	0,074	0,126	0,062	0,123	0,022	0,065	0,058	2,611	1,622	1,137	0,080	7,550
p9	0,061	0,044	0,055	0,090	0,087	0,044	0,048	0,085	0,070	0,106	0,046	0,103	0,057	0,050	0,053	2,924	1,732	1,138	0,079	6,481
p28	0,079	0,064	0,063	0,079	0,059	0,034	0,065	0,071	0,070	0,146	0,050	0,071	0,044	0,040	0,064	1,291	1,637	1,155	0,073	5,657
p47	0,077	0,075	0,067	0,077	0,075	0,054	0,066	0,072	0,060	0,078	0,058	0,082	0,056	0,044	0,058	3,845	1,598	1,150	0,076	4,796
p29	0,058	0,048	0,067	0,064	0,075	0,048	0,071	0,064	0,069	0,093	0,071	0,081	0,070	0,050	0,072	4,091	1,799	1,170	0,068	6,403
p27	0,054	0,045	0,078	0,085	0,094	0,048	0,055	0,066	0,077	0,114	0,071	0,048	0,048	0,050	0,068	2,634	1,781	1,169	0,069	6,245
p67	0,048	0,063	0,052	0,057	0,089	0,034	0,048	0,071	0,040	0,205	0,046	0,098	0,034	0,070	0,046	1,876	1,883	1,158	0,072	7,141
p66	0,093	0,041	0,061	0,089	0,094	0,016	0,078	0,056	0,069	0,086	0,057	0,110	0,043	0,052	0,055	2,501	1,508	1,114	0,092	6,928
p18	0,070	0,064	0,035	0,070	0,081	0,042	0,069	0,079	0,062	0,095	0,056	0,096	0,053	0,061	0,068	1,694	1,679	1,145	0,075	1,000
p30	0,069	0,048	0,042	0,091	0,082	0,039	0,078	0,059	0,074	0,095	0,063	0,123	0,050	0,043	0,044	1,910	1,600	1,163	0,071	6,245
p31	0,073	0,065	0,044	0,095	0,070	0,026	0,070	0,065	0,062	0,115	0,054	0,104	0,044	0,042	0,070	1,873	1,643	1,151	0,075	5,745
p6	0,050	0,066	0,050	0,045	0,115	0,045	0,149	0,060	0,073	0,059	0,034	0,087	0,070	0,054	0,042	2,943	1,554	1,149	0,075	5,477
p1	0,052	0,076	0,048	0,094	0,073	0,027	0,074	0,069	0,034	0,153	0,048	0,105	0,055	0,046	0,046	1,189	1,555	1,139	0,080	5,745
p5	0,056	0,071	0,051	0,076	0,074	0,034	0,076	0,090	0,057	0,165	0,043	0,086	0,025	0,042	0,053	1,328	1,429	1,134	0,081	4,899
p57	0,084	0,054	0,053	0,081	0,071	0,045	0,069	0,074	0,067	0,115	0,049	0,096	0,028	0,068	0,047	2,655	1,642	1,132	0,082	5,831
p64	0,080	0,067	0,036	0,079	0,083	0,044	0,074	0,078	0,061	0,098	0,052	0,095	0,042	0,048	0,063	2,394	1,638	1,154	0,073	5,568
p62	0,049	0,066	0,053	0,066	0,062	0,028	0,090	0,055	0,093	0,099	0,079	0,098	0,064	0,042	0,054	2,497	1,650	1,159	0,072	8,124
p65	0,066	0,052	0,083	0,046	0,063	0,044	0,064	0,056	0,048	0,067	0,086	0,114	0,059	0,073	0,080	1,994	1,645	1,155	0,073	6,000
p49	0,061	0,052	0,057	0,063	0,080	0,046	0,079	0,085	0,075	0,105	0,065	0,093	0,034	0,052	0,052	3,632	1,502	1,161	0,071	4,690
p16	0,066	0,059	0,061	0,076	0,062	0,031	0,066	0,078	0,063	0,115	0,055	0,108	0,047	0,049	0,064	1,465	1,755	1,159	0,072	6,000
p77	0,078	0,062	0,037	0,064	0,061	0,021	0,064	0,080	0,068	0,136	0,055	0,113	0,050	0,071	0,041	1,430	1,556	1,156	0,073	5,477
p22	0,066	0,066	0,041	0,084	0,059	0,028	0,070	0,069	0,067	0,151	0,061	0,079	0,046	0,048	0,063	2,281	1,448	1,141	0,078	5,657
p84	0,080	0,067	0,058	0,079	0,064	0,050	0,058	0,086	0,037	0,103	0,047	0,093	0,045	0,073	0,061	6,271	1,716	1,146	0,077	6,782
p86	0,051	0,060	0,053	0,080	0,081	0,034	0,063	0,075	0,072	0,165	0,041	0,077	0,043	0,046	0,059	1,594	2,032	1,160	0,072	7,483
p56	0,065	0,067	0,059	0,070	0,092	0,034	0,068	0,062	0,075	0,079	0,066	0,102	0,038	0,062	0,062	4,837	1,490	1,140	0,080	5,657
p85	0,041	0,067	0,068	0,079	0,067	0,030	0,074	0,059	0,071	0,129	0,069	0,089	0,054	0,056	0,046	3,168	1,934	1,162	0,071	7,000
p55	0,073	0,047	0,042	0,071	0,085	0,036	0,075	0,078	0,067	0,112	0,039	0,109	0,051	0,056	0,059	2,777	1,611	1,153	0,074	5,292
p3	0,075	0,047	0,064	0,062	0,079	0,039	0,059	0,074	0,071	0,113	0,070	0,093	0,049	0,050	0,054	2,448	1,705	1,152	0,074	5,568
p83	0,081	0,083	0,055	0,066	0,080	0,041	0,062	0,061	0,060	0,115	0,044	0,098	0,042	0,035	0,078	1,365	1,687	1,160	0,072	5,099
p8	0,055	0,056	0,053	0,097	0,041	0,029	0,039	0,063	0,037	0,135	0,084	0,117	0,081	0,056	0,055	1,753	1,557	1,153	0,074	6,245
p74	0,053	0,047	0,062	0,072	0,079	0,048	0,071	0,069	0,071	0,103	0,071	0,078	0,048	0,064	0,063	4,215	1,445	1,137	0,080	6,164
p61	0,033	0,064	0,060	0,048	0,126	0,050	0,157	0,059	0,080	0,066	0,028	0,077	0,074	0,031	0,046	2,779	1,858	1,167	0,070	7,681
p2	0,083	0,052	0,058	0,061	0,066	0,061	0,084	0,075	0,069	0,106	0,053	0,080	0,051	0,047	0,054	3,075	1,651	1,127	0,084	5,292
p35	0,070	0,050	0,064	0,060	0,075	0,057	0,071	0,077	0,062	0,092	0,062	0,090	0,055	0,066	0,049	6,499	1,729	1,165	0,070	5,831
p37	0,049	0,054	0,050	0,076	0,076	0,038	0,075	0,080	0,066	0,113	0,076	0,059	0,071	0,053	0,064	2,878	1,969	1,169	0,069	6,083
p38	0,057	0,049	0,048	0,108	0,083	0,033	0,052	0,040	0,060	0,153	0,056	0,107	0,056	0,038	0,060	1,036	1,782	1,162	0,071	8,000
p7	0,052	0,084	0,061	0,085	0,064	0,054	0,059	0,081	0,052	0,100	0,052	0,083	0,066	0,056	0,050	4,100	1,361	1,134	0,082	5,568
p15	0,064	0,057	0,061	0,064	0,082	0,029	0,070	0,054	0,071	0,124	0,057	0,088	0,061	0,063	0,054	2,440	1,751	1,165	0,070	6,633
p4	0,058	0,051	0,062	0,086	0,095	0,043	0,093	0,080	0,074	0,084	0,061	0,072	0,039	0,042	0,061	1,354	1,808	1,158	0,073	5,000
p24	0,043	0,036	0,065	0,061	0,114	0,032	0,077	0,068	0,075	0,166	0,054	0,088	0,036	0,045	0,041	1,721	1,512	1,160	0,071	4,583
p71	0,064	0,064	0,071	0,064	0,075	0,041	0,074	0,058	0,074	0,090	0,066	0,081	0,057	0,060	0,061	4,035	1,451	1,126	0,084	7,616
p63	0,042	0,043	0,063	0,078	0,061	0,019	0,049	0,076	0,047	0,157	0,044	0,160	0,051	0,051	0,059	1,697	1,794	1,170	0,068	6,481
p44	0,077	0,059	0,072	0,074	0,083	0,049	0,070	0,062	0,049	0,096	0,057	0,095	0,061	0,050	0,048	1,719	1,554	1,165	0,070	4,359
p12	0,096	0,047	0,058	0,092	0,080	0,026	0,070	0,082	0,052	0,101	0,056	0,098	0,043	0,036	0,063	1,346	1,502	1,149	0,075	5,000
p53	0,044	0,055	0,051	0,071	0,072	0,029	0,071	0,090	0,063	0,177	0,044	0,102	0,031	0,054	0,046	1,067	1,442	1,125	0,085	5,385
p92	0,062	0,063	0,042	0,083	0,058	0,050	0,041	0,073	0,064	0,108	0,051	0,122	0,050	0,067	0,066	0,861	1,366	1,154	0,074	7,348
p11	0,094	0,067	0,054	0,063	0,088	0,032	0,048	0,067	0,032	0,149	0,042	0,107	0,046	0,048	0,061	1,458	1,586	1,135	0,081	7,681
p13	0,065	0,048	0,049	0,079	0,094	0,031	0,058	0,070	0,057	0,128	0,024	0,114	0,058	0,066	0,058	1,943	1,591	1,141	0,078	6,633
p43	0,037	0,068	0,071	0,084	0,080	0,039	0,041	0,068	0,067	0,100	0,063	0,101	0,050	0,059	0,072	2,978	1,654	1,158	0,072	6,782
p40	0,093	0,061	0,061	0,081	0,074	0,058	0,057	0,084	0,047	0,103	0,054	0,076	0,047	0,054	0,051	5,981	1,975	1,163	0,071	7,000
p33	0,058	0,055	0,052	0,081	0,072	0,045	0,072	0,050	0,058	0,139	0,052	0,087	0,066	0,061	0,052	3,622	1,814	1,155	0,074	6,164
p14	0,063	0,043	0,047	0,073	0,079	0,025	0,056	0,088	0,027	0,163	0,029	0,137	0,045	0,062	0,063	1,026	1,395	1,114	0,088	5,196
p80	0,067	0,064	0,028	0,082	0,080	0,028	0,046	0,071	0,050	0,116	0,063	0,131	0,046	0,072	0,057	1,478	1,585	1,140	0,078	5,196
p25	0,055	0,062	0,054	0,094	0,086	0,044	0,072	0,044	0,073	0,092	0,070	0,093	0,048	0,055	0,056	2,021	1,644	1,162	0,071	5,568
p81	0,044	0,069	0,067	0,042	0,067	0,032	0,090	0,060	0,083	0,106	0,039	0,123	0,058	0,064	0,055	2,091	1,580	1,148	0,076	2,828
p17	0,079	0,043	0,058	0,082	0,087	0,046	0,046	0,062	0,056	0,131	0,048	0,130	0,037	0,047	0,046	1,182	1,505	1,139	0,079	5,657
p32	0,088	0,058	0,063	0,085	0,089	0,032	0,089	0,066	0,079	0,067	0,075	0,053	0,033	0,051	0,071	1,994	1,625	1,159	0,072	5,477
p54	0,050	0,055	0,047	0,074	0,048	0,041	0,063	0,079	0,054	0,165	0,046	0,124	0,045	0,062	0,046	1,207	1,336	1,132	0,083	4,796
p72	0,081	0,103	0,047	0,092	0,091	0,037	0,060	0,061	0,035	0,122	0,046	0,076	0,033	0,039	0,077	0,692	1,415	1,141	0,077	4,690
p90	0,047	0,045	0,059	0,071	0,085	0,030	0,046	0,072	0,035	0,191	0,044	0,093	0,049	0,068	0,066	1,557	1,489	1,122	0,088	6,856
p68	0,044	0,049	0,067	0,060	0,080	0,036	0,045	0,078	0,059	0,157	0,063	0,100	0,041	0,067	0,052	1,226	1,495	1,141	0,080	7,746
p45	0,061	0,060	0,076	0,062	0,082	0,050	0,068	0,080	0,055	0,127	0,052	0,076	0,047	0,045	0,059	1,930	1,622	1,159	0,073	7,416
p50	0,072	0,053	0,063	0,066	0,077	0,044	0,076	0,069	0,080	0,092	0,035	0,087	0,047	0,076	0,063	2,304	1,594	1,164	0,070	5,916
p51	0,077	0,050	0,077	0,084	0,062	0,050	0,074	0,085	0,064	0,081	0,055	0,093	0,049	0,045	0,053	2,204	1,623	1,165	0,070	1,000
p75	0,077	0,065	0,065	0,061	0,065	0,031	0,080	0,044	0,064	0,144	0,055	0,085	0,055	0,047	0,061	2,386	1,587	1,150	0,076	3,742
p20	0,064	0,044	0,065	0,082	0,076	0,014	0,073	0,061	0,057	0,124	0,063	0,093	0,059	0,065	0,059	2,557	1,632	1,148	0,075	7,616
p19	0,049	0,067	0,063	0,082	0,085	0,049	0,072	0,067	0,037	0,107	0,073	0,094	0,043	0,061	0,052	2,536	1,672	1,159	0,072	4,359
p26	0,081	0,068	0,070	0,085	0,083	0,052	0,059	0,077	0,056	0,064	0,066	0,082	0,054	0,043	0,059	1,899	1,782	1,168	0,069	5,477
p69	0,072	0,051	0,071	0,091	0,074	0,034	0,064	0,078	0,063	0,088	0,081	0,068	0,058	0,058	0,049	2,455	1,730	1,165	0,070	5,916
p39	0,085	0,076	0,060	0,051	0,082	0,045	0,064	0,080	0,058	0,097	0,055	0,077	0,055	0,062	0,052	2,651	1,784	1,166	0,070	7,211
p89	0,070	0,071	0,065	0,091	0,068	0,031	0,066	0,057	0,063	0,108	0,056	0,094	0,051	0,058	0,050	2,945	1,723	1,160	0,072	5,292
p41	0,067	0,065	0,064	0,083	0,076	0,050	0,077	0,077	0,061	0,101	0,055	0,088	0,044	0,048	0,046	3,014	1,792	1,163	0,071	6,083
p23	0,069	0,070	0,068	0,082	0,096	0,053	0,062	0,076	0,042	0,089	0,060	0,101	0,043	0,048	0,041	1,819	1,577	1,159	0,072	5,477
p36	0,073	0,054	0,076	0,066	0,082	0,055	0,085	0,063	0,068	0,062	0,092	0,056	0,057	0,041	0,070	2,382	1,661	1,168	0,069	9,539
p91	0,050	0,062	0,050	0,049	0,081	0,041	0,078	0,060	0,084	0,112	0,057	0,087	0,047	0,065	0,076	3,447	1,745	1,159	0,072	7,416
p70	0,064	0,065	0,049	0,087	0,059	0,043	0,079	0,067	0,044	0,136	0,047	0,093	0,060	0,051	0,057	3,635	1,698	1,152	0,075	6,164
p79	0,078	0,065	0,050	0,052	0,080	0,026	0,082	0,072	0,059	0,120	0,055	0,115	0,043	0,057	0,046	1,884	1,658	1,147	0,076	5,292

Supplementary Material- Table S3.5– Pearson's correlations (R^2 value and associated significance of P-value (' $P < 0.10$)) between litter diversity/dissimilarity (specific richness and divergence litter traits) and relative rain exclusion effect (REE) on soil microbial parameters (sum15, H', D, and DCP) in the different litter mixture after one (2012) and two (2013) years of litter decomposition.

	REE (%) on sum15		REE (%) on *H'*		REE (%) on *D*		REE (%) on *DCP*	
	2012	*2013*	*2012*	*2013*	*2012*	*2013*	*2012*	*2013*
Specific richness	0	-0.09	0.04	0	-0.05	0.01	0	-0.05
C_{FD}	0	**-0.22**'	0.06	-0.02	-0.08	0.06	-0.02	0
N_{FD}	-0.01	-0.15	-0.02	0	0.02	0	0.01	0.01
P_{FD}	0	**-0.22**'	0.05	-0.03	-0.06	0.08	-0.02	0
$Phenolics_{FD}$	0	-0.05	-0.03	-0.12	0.02	0.17	-0.01	-0.04
$Lignin_{FD}$	-0.04	-0.05	0.01	-0.04	-0.02	0.06	-0.03	-0.14
WHC_{FD}	0	-0.19	0.06	0	-0.06	0.02	0	0.01
DOC_{FD}	0	**-0.23**'	0.06	-0.03	-0.07	0.09	-0.08	-0.02
TDN_{FD}	-0.13	-0.05	0.01	0	-0.02	0.01	-0.01	-0.15
$Lignin/N_{FD}$	-0.2	-0.11	0	0	0	0.01	0	0.03

Abstract

Longer drought periods and/or overall less precipitation are thought to be major consequences of ongoing climate change in the Mediterranean region. These changes in the water regime will likely affect community composition, biodiversity and ecosystem processes, but very little is known about how biodiversity and changes in precipitation interactively affect ecosystem functioning. In my PhD thesis, I aimed to quantify the role of plant diversity in the response of Mediterranean shrubland ecosystem to a decrease in water availability, with a particular interest in belowground processes, such as soil microbial community functioning and functional responses in plant roots.

I used a rhizotron approach under partially controlled conditions to study plant growth responses to repetitive severe droughts, with a particular focus on root growth. Two individuals of the same species or in all possible combinations of the three dominating species at our field site (*Quercus coccifera*, *Cistus albidus*, *Brachypodium retusum*) were grown together in a rhizotron. Repetitive severe droughts had a negative effect on survival of the two woody species (*Q. coccifera*, *C. albidus*), but not of the grass *B. retusum*. Interspecific competition generally increased survival of *C. albidus* and *B. retusum* compared to monospecific competition. Conversely, interspecific competition decreased the survival of *Q. coccifera*. Likewise, I found that root morphological traits were mostly affected by the neighbor species identity rather than by severe drought. The community level physiological profiles (CLPPs) of root associated soil microbial communities did not differ between drought treatments and were also not affected by plant species identity. However, CLPPs changed towards more total microbial activities but less diverse resource use at increasing soil depth. Collectively these results suggest that plant species composition of the studied Mediterranean shrubland has a stronger effect on growth, intraspecific variability in root traits and survival than repetitive severe droughts.

In the context of a larger collaborative project (CLIMED), I used a natural gradient of shrub species diversity in a Mediterranean shrubland ecosystem (garrigue) to which a permanent partial rain exclusion treatment (12% less precipitation) was added. This field experiment allowed me to study the responses of soil microbial community level physiological profiles (CLPPs) to reduced precipitation and to a change plant-produced leaf litter material decomposing on the ground as a key resource for heterotrophic soil microorganisms over two years. While rain exclusion had only a minor impact on the diversity of substrates metabolized by the microbial communities, litter species richness promoted global soil microbial activity by increased catabolic diversity of the soil microbial community. These results suggest that indirect climate change effects on plant species composition and richness might have more important consequences for soil microbial functioning than reduced precipitation in the studied Mediterranean shrubland ecosystem.

Both, the field study of soil microbial functioning and the rhizotron study of plant growth and survival clearly showed that plant species identity and diversity may be more important for the functioning of these Mediterranean shrublands than increased drought. I conclude that climate change induced shifts in plant species composition and diversity may have more important consequences for the functioning of Mediterranean shrublands than the direct effects of altered precipitation.

Keywords: Climate change, Mediterranean ecosystem, biodiversity, root system, soil microbial activity, functional diversity.