



HAL
open science

Phenolics of the understory shrub *Cotinus coggygia* influence Mediterranean oak forests diversity and dynamics

J. Gavinet, M. Santonja, Virginie Baldy, H. Hashoum, S. Peano, T. Tchong, Stephane Greff, R Gros, Catherine Fernandez, A. Bousquet-Mélou

► **To cite this version:**

J. Gavinet, M. Santonja, Virginie Baldy, H. Hashoum, S. Peano, et al.. Phenolics of the understory shrub *Cotinus coggygia* influence Mediterranean oak forests diversity and dynamics. *Forest Ecology and Management*, 2019, 441, pp.262-270. 10.1016/j.foreco.2019.03.049 . hal-02085975

HAL Id: hal-02085975

<https://amu.hal.science/hal-02085975>

Submitted on 1 Apr 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **TITLE:** Phenolics of the understory shrub *Cotinus coggygria* influence Mediterranean oak
2 forests diversity and dynamics

3

4 **AUTHORS**

5 Gavinet J.[†], Santonja M.[†], Baldy V., Hashoum H., Peano S., Tchong T., Gros R., Greff S.,
6 Fernandez C.^{*}, Bousquet-Mélou A.

7

8 Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France.

9 [†] Equal contribution to the work

10

11 ^{*}Corresponding author

12 catherine.fernandez@imbe.fr

13 Tel: +33 (0)4 13 55 12 22

14

15 **KEYWORDS:** Biotic interactions; oak seedlings; soil microorganisms; soil mesofauna;
16 regeneration; allelopathy

17

18 **ABSTRACT**

19 Chemical interactions in forested ecosystems play a role in driving biodiversity and ecosystem
20 dynamics. Plant phenolics released by leaching can influence surrounding plants and soil
21 organisms such as bacteria, fungi or arthropods. However, our knowledge about such
22 chemically-mediated biotic interactions in Mediterranean oak forests is still limited, in
23 particular whether they play a role in the limited forest regeneration. In this study, we analyzed
24 how phenolics of *Cotinus coggygia*, a dominant shrub of Mediterranean downy oak (*Quercus*
25 *pubescens*) forests, influence understory herbaceous plant species, downy oak regeneration and
26 soil organisms in order to obtain a more integrative view of possible direct and indirect
27 interactions triggered by this shrub species. We performed a series of experiments testing the
28 effect of aqueous extracts of *C. coggygia*, mimicking natural leachates, on these organisms.
29 *Cotinus coggygia* contained a high quantity of phenolics in green and senescent leaves but
30 much less in leaf litter. Extracts from *C. coggygia* leaves stimulated bacterial communities,
31 exhibited few effects on both saprophytic and symbiotic fungi, and negatively affected
32 Collembola. Herbaceous species growth was particularly impaired by extracts from green and
33 senescent leaves, although these effects were alleviated in the presence of soil microorganisms.
34 In both greenhouse and field experiments, *C. coggygia* affected early oak seedling
35 establishment in particular through a reduced root growth, but exhibited no effect on later
36 seedling and sapling growth. We discussed the implication of these results for the balance
37 between competition and facilitation in oak forests and concluded that *C. coggygia* has the
38 potential to strongly alter biotic interactions, understory plant diversity and oak forest dynamics.

39

40 1. INTRODUCTION

41 Forests are multi-layered and heterogeneous ecosystems in which canopy trees, understory
42 plants and soil organisms interact in complex networks (Wardle et al. 2004). Phenolics released
43 by woody plant species can play a key role in these interactions by influencing the structure and
44 diversity of plant and soil communities (Chou 1999; Souto et al. 2000a; Wardle et al. 1998; Das
45 and Joy 2009), with important feedback on forest community composition, richness or
46 dynamics (Mallik 2008). For instance, autotoxicity of canopy trees on their own seedlings
47 probably plays a role in forest species turnover along succession in Mediterranean forests
48 (Fernandez et al. 2008, 2016). However, understory shrub species can also profoundly affect
49 forest ecosystem functioning and dynamics. For example, phenolics released by the
50 understory dwarf shrub *Empetrum hermaphroditum* was reported to impair the regeneration of
51 the dominant tree *Pinus sylvestris* in boreal forests (Nilsson 1994; Nilsson et al. 2000).

52 Soil microorganisms can be directly affected by plant phenolics (Chomel et al. 2014;
53 Santonja et al. 2018) but they may also use these plant chemicals as carbon source and thus
54 modify the plant-plant chemical interactions (Fernandez et al. 2013; Souto et al. 2000a). Litter
55 phenolics can inhibit tree fungal symbionts (Rose et al. 1983; Souto et al. 2000b) which may
56 have important consequences for tree seedlings development. However, these effects on fungi
57 are species-specific and only few studies tested whether plant phenolics can decrease tree
58 mycorrhization in natural soil (Souto et al. 2000b). Phenolics released by plants may also affect
59 soil arthropods (Poinsot-balaguer et al. 1993; Das and Joy 2009; Asplund et al. 2015), which
60 play a key role on soil microbial community structure (Berg et al. 2004; Chahartaghi et al. 2005)
61 and litter decomposition process (Seastedt 1984; Filser 2002; Santonja et al. 2017). However,
62 to our knowledge, such plant-soil arthropod chemical interactions were poorly studied.

63 The Mediterranean basin has an exceptionally high plant diversity and endemism (Myers
64 et al. 2000) shaped by a high diversity of human and ecological factors such as geology,

65 topography or perturbation regimes (Blondel 2006). Many Mediterranean plants synthesize a
66 wide variety of specialized metabolites, which help them withstanding the summer drought and
67 high radiative stress typical of Mediterranean-type ecosystems (Chaves and Escudero 1999),
68 and which can also influence ecosystem structure and functioning (Scognamiglio et al. 2013;
69 Vilà and Sardans 1999). The downy oak (*Quercus pubescens* Mill.) is a long-lived
70 submediterranean tree that occurs mainly in Southern Europe, from northern Spain to the
71 Caucasus (Quézel and Médail 2003). Downy oak forests cover about 400 000 ha in
72 Mediterranean France (IFN, 2014) and were traditionally managed as coppices, but the
73 abandonment of this practice during the second half of the 20th century resulted in ageing stands
74 with frequent signs of dieback. With the abandonment of vegetative reproduction through
75 coppices, the future of these stands should depend upon sexual regeneration, but local
76 observations underline a lack of seedlings and saplings (Prévosto et al. 2013). The role of plant-
77 plant chemical interactions during the germination or establishment phases have still been
78 poorly investigated. The understory of downy oak forests is frequently dominated by the shrub
79 *Cotinus coggygria* Scop. (Anacardiaceae), which has a wide distribution from southern Europe,
80 the Mediterranean, Moldova and the Caucasus to central China and the Himalayas (Matić et al.
81 2011). This species has been traditionally used as a dyestuff since antiquity (Valianou et al.
82 2009) but also widely used in ornamental horticulture. This shrub produces high diversities and
83 amounts of phenolics and terpenes (Novaković et al. 2007; Hashoum et al. 2017). This species
84 has consequently been studied for a source of bioactive substances such as those from extracts
85 or essential oils that present antibacterial, antifungal and antioxidant properties (Marčetić et al.
86 2013; Novaković et al. 2007; Matić et al. 2011). Considering these characteristics, this species
87 could play a major role in biotic interactions occurring in Mediterranean downy oak forests.

88 The aim of the present study was to analyze how phenolics of *C. coggygria* influence
89 understory herbaceous plant species, downy oak regeneration and soil organisms, including

90 both microorganisms and arthropods. More precisely, our objectives were to (i) quantify
91 phenolics present in *C. coggygia* leaf leachates; (ii) determine whether phenolics of *C.*
92 *coggygia* affect the germination and seedling growth of understory plant species and downy
93 oak; (iii) evaluate the impact of these phenolics on soil microorganisms and arthropods; and
94 finally (iv) assess the role of soil microorganisms for plant-plant chemical interactions.

95

96 **2. MATERIAL AND METHODS**

97

98 ***2.1. Experimental site and material collection***

99 Field experiment and biological material collection (*Cotinus coggygia* leaves, oak acorns
100 and soils) were performed at the Oak Observatory at the OHP (O₃HP) experimental site located
101 in the research center “Observatoire de Haute Provence”, 60 km north of Marseille (43°56'115”
102 N, 05°42'642” E; 680 m a.s.l.). The climate is Mediterranean with a mean annual temperature
103 of 11.9 °C and a mean annual precipitation 830 mm (1967–2000). The site is covered by an
104 old-growth oak forest belonging to the site Natura 2000 “FR9302008 Vachères”, which was
105 managed for centuries by coppicing. Downy oak (*Quercus pubescens*: 75% coverage) and
106 Montpellier maple (*Acer monspessulanum*: 25% coverage) are the two dominant tree species,
107 with understory vegetation dominated by smoke tree (*Cotinus coggygia*: 30% coverage). The
108 soil is a pierric calcosol (with S horizon between limestone rocks) or calcarisol when limestone
109 appears less than 25 cm deep.

110 Three types of *C. coggygia* leaves (according to leaf maturity) were collected to perform
111 chemical analyses and to prepare the aqueous extracts (mimicking *C. coggygia* leachates) for
112 bioassays: green and senescent leaves were collected directly on the shrub and leaf litter on the
113 forest floor. Oak acorns were collected on the ground in autumn, floated and visually screened
114 to eliminate non-viable acorns. Soil samples used as bioassay substrate were collected in zones

115 without *C. coggygia* (i.e. at least 10 m from shrubs), sieved at 2 mm, and then stored at room
116 temperature until the start of the experiments.

117 We selected four herbaceous target species naturally present in downy oak forests to
118 perform the bioassays: *Linum narbonense* L. (Linaceae), *Satureja montana* L. (Lamiaceae),
119 *Silene nutans* L. (Caryophyllaceae) and *Verbascum pulverulentum* Vill. (Scrophulariaceae).
120 Seeds were collected from wild populations on the study site, and then stored in a cold chamber
121 at 5 °C until the start of the experiment. We also selected *Lactuca sativa* L. (Asteraceae) as
122 target plant species because this species is known for its sensitivity to specialized metabolites
123 and frequently used for bioassays as a reference (e.g. Chou et al. 1998; Fernandez et al. 2006).
124 Seeds of *L. sativa* were purchased from a commercial company (Vilmorin ®).

125

126 **2.2. Chemical analysis**

127

128 **2.2.1. Total phenolics**

129 Extraction of phenolics was carried out based on the method described by Singleton and
130 Rossi (1965) and adapted to smaller amounts of plant material. Briefly, a dry mass (DM) of 250
131 mg of crushed leaf was extracted with 20 mL of deionized water. The mixture was left for 1 h
132 under constant shaking at ambient temperature shielded from light. The extract was then filtered
133 on Whatman GF/C paper filter. A volume of 25 µL of the extract were added to 1650 µL of
134 ultrapure water, 200 µL of saturated Na₂CO₃ aqueous solution and 100 µL of Folin-Ciocalteu's
135 reagent. After 30 min, the phenolic index was measured at 765 nm on a spectrophotometer
136 (Spectronic Biomate 3 Thermo Electron Scientific Instrument Corporation ®) and expressed as
137 equivalent of mg of gallic acid per g of plant material (DM).

138

139 **2.2.2. Flavonoids**

140 Among phenolic compounds, we focused on flavonoids which were quantified in terms of
141 proanthocyanidins and flavonoid index (total flavonoid). Extraction and quantification were
142 based on a previous work (Kaundun et al. 1998) adapted by our laboratory to smaller amounts
143 of plant material. A mass of 0.5 g DM of crushed leaf was suspended in 15 ml of HCl 2N
144 solution and heated to 90 °C in a water bath with reflux for 50 min with every 10 min of air
145 influxes. The acidic treatment generated anthocyanidins from homologous proanthocyanidins
146 and flavonol aglycones from corresponding flavonol glycosides. The solution was left to cool
147 approximately 30 min and filtered (filter porosity 3). Anthocyanidins were quantified
148 spectrophotometrically at 435 nm (Spectronic Biomate 3 Thermo Electron Scientific
149 Instrument Corporation ®) and expressed as mg per g of plant material (DM). Flavonol
150 aglycones were extracted three times with 9 mL of diethyl ether. The extracts were recombined
151 and evaporated to dryness. The residue was then dissolved and mixed with 1.5 mL of methanol.
152 An aliquot of 100 µL was added to 5 mL of 1 % AlCl₃ / MeOH solution and let 20 min to react.
153 The flavonoid index was measured at 530 nm and expressed as equivalent of mg of quercetin
154 per g of plant material (DM).

155

156 **2.3. Bioassays**

157 We chose to test the effects of natural leachates using leaf aqueous extracts because water-
158 soluble compounds have been shown to be most involved in allelopathy (Fernandez et al. 2013,
159 2016). These extracts were prepared by soaking entire leaves in deionized water for 24 h at
160 room temperature (20 ± 1°C) in darkness (Fernandez et al. 2013; Hashoum et al. 2017). After
161 24 h, extracts were filtered through #42 Whatman® paper filter and stored at 4 °C until use.
162 New extracts were prepared to prevent compound degradation once a week (Experiment 1,
163 stock solution at 5% dry weight further diluted at 2.5%) or once a month (Experiment 2, solution
164 at 5% dry weight) with fresh material.

165

166 2.3.1. Experiment 1: Response of understory plant species & microorganisms to *C. coggygia*
167 aqueous extracts

168 We compared natural and autoclaved soil bioassays to evaluate the impact of soil microbial
169 communities in shaping plant-plant chemical interactions (Kaur et al. 2009; Fernandez et al.
170 2013). Sterilization consisted in autoclaving soil for two cycles of 1 h (24 h apart) at 121 °C to
171 eliminate a fraction of the microbial community (Alef and Nannipieri 1995; Trevors 1996).

172 Bioassays were conducted in Petri dishes with 50.0 g (\pm 0.1 g) of soil, either natural or
173 autoclaved, corresponding to a thickness of 0.5 to 0.6 mm (Fernandez et al. 2013). Each Petri
174 dish was sown with 20 seeds of each target species that were watered every 2 days with 5 mL
175 of deionized water (control) or *C. coggygia* extracts (2.5% and 5%) from one of the three leaf
176 types (green leaf, senescent leaf and leaf litter). Five replicates were performed for each
177 treatment (target plant species \times leaf type \times concentration \times soil type). Bioassays were
178 conducted under natural photoperiod and controlled temperature (20.5 °C \pm 1 °C) for 40 days.

179 Seed germination was monitored every day and used to compute total germination rate and
180 germination speed using the velocity coefficient (Mazliak 1982): $C_v = 100 (\sum N_i / \sum N_i T_i)$, where
181 N_i is the number of seeds germinated at time i , and T_i is the number of days since the start of
182 the experiment. The higher the velocity coefficient, the faster the germination. A seed was
183 considered as germinated when the protruding radicle achieved the length of 1 mm beyond the
184 seed coat. Lengths of root and shoot were measured for each individual at the same age, i.e. 10
185 days after germination (accuracy: 1 mm).

186 A further set of Petri dishes containing either natural or autoclaved soils was used to test
187 the effects of *C. coggygia* extracts on biomass and basal respiration of soil microbial
188 communities. Microbial biomass (MB) was estimated using substrate-induced respiration (SIR)
189 rates (Anderson and Domsch 1978). Ten grams (dry weight equivalent) of subsamples were

190 placed in 117 mL glass jars and amended with powdered glucose (1000 $\mu\text{g C.g}^{-1}$ soil). After
191 incubation (1 h, 22 °C), a volume of 1 mL of air was sampled in the headspace with a syringe
192 and injected onto the gas chromatograph (ChrompackCHROM 3-CP 9001) to analyze CO₂
193 production. SIR rates were converted into MB using equations given by Beare et al. (1990).

194

195 *2.3.2. Experiment 2: Response of oak seedling (2a) and sapling (2b) to C. coggygria aqueous*
196 *extracts in greenhouse and (2c) to C. coggygria presence in the field*

197 This experiment was conducted in greenhouse to determine if *C. coggygria* aqueous
198 extracts could alter (i) oak acorns germination and early development and (ii) oak saplings
199 development and associated mycorrhiza. Finally, a seeding experiment was performed *in situ*
200 to compare patterns obtained in greenhouse (only chemical interactions) and in the field (all
201 types of interaction).

202 In the experiment 2a, thirty oak acorns were sown in individual pots filled with natural
203 soil and vermiculite (2:1, for a total of 150 g of substrate per pot). Three treatments were applied
204 on each set of 10 pot replicates: i) control, ii) leachates, watered with *C. coggygria* aqueous
205 extracts at 2.5% DM of plant material, and iii) litter, where soil substrate was mixed with 10 g
206 of *C. coggygria* leaf litter. Pots were watered every 3 days with deionized water or aqueous
207 extracts and kept under a 12h-photoperiod for 2 months. At the end of this period, germinated
208 acorns in each treatment were counted. The seedlings were separated into leaves, stem and
209 roots, and weighed after drying them at 60 °C for 48 h.

210 The experiment 2b was conducted to assess *C. coggygria* impact on older saplings and their
211 mycorrhizae. A total of 70 forty-month old individuals of *Q. pubescens* certified mycorrhized
212 by *Tuber melanosporum* Vittad. were grown in 10 L plastic pots containing a substrate
213 consisting of mold (pH 6), perlite and vermiculite (1/3 of each). Three grams of magnesium
214 carbonate per L of substrate were added to obtain an alkaline pH of 7.6 that favors *T.*

215 *melanosporum* development. Half of the saplings were watered monthly with 200 mL of
216 aqueous extracts at 5% DM of plant material whereas control saplings received 200 mL of
217 deionized water. In order to mimic as close as possible the natural conditions, *C. coggygia*
218 extracts were prepared according to the shrub phenology: with senescent leaves sampled from
219 October to January, litter sampled from February to April or green leaves sampled from May
220 to September. After 4, 12 and 16 months, 10 saplings per treatment were harvested and divided
221 into leaf, stem and root after careful removing of soil. A subsample of 10 secondary root
222 segments (3 cm) per sapling were randomly selected for the analysis of the mycorrhizal
223 colonization (Garbaye 2013). Root segments were kept in 60 % ethanol to stop the mycelial
224 development until analysis. Segments were placed in Petri dishes and all root tips were observed
225 using a binocular scope and classified as mycorrhized or not. We then computed the
226 mycorrhization rate according to the formula number of mycorrhizal root tip / total root tips \times
227 100.

228 For the field experiment (2c), 50 sowing points were installed on the *Q. pubescens* forest
229 understory, either in the presence or absence of *C. coggygia* shrub (100 sowing points in total).
230 Each sowing point consisted of manually dug holes of about 2 cm in which 2 downy oak acorns
231 were laid flat, covered with soil and a wire mesh (10 cm x 10 cm, 0.6 cm mesh size) to prevent
232 predation by rodents. Acorns collection and sowing took place in November 2013. Acorns were
233 collected on several trees to encompass intraspecific variation and non-viable acorns were
234 eliminated by floating and visual screening. Sowing points were distributed in 5 blocks in each
235 treatment (with or without *C. coggygia*). Plots were fenced to limit predation by wild boar.
236 Emerged seedlings were counted in June 2014, and seedling number and dimensions (diameter
237 at 2 cm and length) were then recorded yearly in winter until 2017 (4-year-old seedlings).

238

239 2.3.3. Experiment 3: response of soil saprophytic fungi and mesofauna to *C. coggygia* aqueous

240 *extracts*

241 For this experiment, intact soil from the upper 10 cm were sampled, transported to the
242 laboratory and placed in aluminum mesocosm (20 × 15 cm). The mesocosms were placed in a
243 culture room with a natural photoperiod, a temperature of about 23 °C and an air humidity of
244 about 40%. Mesocosms were sprayed each 3 days using 50 mL of deionized water (control) or
245 aqueous extracts of *C. coggyria* senescent leaves at 2.5% and 5% DM of plant material.

246 After 15 days, soil arthropods were extracted using the Tullgren funnel method, and
247 stored in 95% ethanol to be counted and separated into Collembola and different suborders for
248 Acari (Oribatida, Mesostigmata and Prostigmata) (Hopkins 1997; Santonja et al. 2017) using a
249 binocular scope. Collembola and Acari Oribatida were regarded as microbi-detritivore
250 mesofauna, whereas Acari Mesostigmata and Prostigmata as predatory mesofauna (Coleman et
251 al. 2004; Santonja et al. 2017).

252 Fungal biomass was determined by quantifying ergosterol, which is a specific fungal
253 membrane constituent and thus a good indicator of living fungal biomass (Gessner and Chauvet
254 1993). Ergosterol extraction and quantification were performed following the method described
255 in Santonja et al. (2017).

256

257 **2.4. Data analysis**

258 Differences in concentration of phenolics, flavonols and proanthocyanidins according to
259 *C. coggyria* leaf type (green leaf, senescent leaf and leaf litter) were tested using Kruskal-
260 Wallis tests followed by post hoc Student-Newman-Keuls tests.

261 For the experiment n°1, differences in plant performance (germination rate and velocity,
262 shoot and root growths) in the control treatments between species and soil types (autoclaved
263 and natural) were first analyzed using a binomial GLM for germination rate and two-way
264 ANOVAs for other response variables, followed by post-hoc Tukey tests. Then, we computed

265 a Relative Allelopathic Effect (RAE) as the relative difference between plant performance in
266 the control (Pc) and leachates (Pt) treatments: $RAE (\%) = (Pc - Pt) / Pt$ and calculated a mean
267 and bootstrapped confidence interval at 95% (n=1000) for each combination of species, leaf
268 type (green, senescent and litter), concentration (2.5 or 5%) and soil type (autoclaved or
269 natural). RAE was considered significantly different from zero (i.e. treatment different from the
270 control) when zero was not included in the bootstrapped confidence interval. Differences in
271 microbial biomass according to the *C. coggygia* leaf type, concentration and soil type were
272 tested using a three-way ANOVA followed by post hoc Tukey tests.

273 Concerning the experiment n°2, differences in oak seedling root, stem and leaf biomasses
274 according to the treatments (control, leachates or litter) were tested using one-way ANOVAs
275 followed by post hoc Tukey tests. Differences in oak sapling biomass (root, stem and leaf) and
276 mycorrhization rate according to the treatment, sampling date and their interactions were tested
277 using two-way ANOVAs followed by post-hoc Tukey tests. For the field experiment, the effect
278 of *C. coggygia* presence on seedling emergence and survival was tested using binomial GLMs
279 and using a one-way ANOVA for seedling growth. For all ANOVAs, normality and
280 homoscedasticity of the residuals were assessed by Shapiro-Wilk and Bartlett tests,
281 respectively, and data were log or root-squared-transformed when necessary.

282 Finally, concerning the experiment n°3, differences in mesofauna abundance and fungal
283 biomass according to *C. coggygia* leachates were tested by Kruskal-Wallis tests, followed by
284 post-hoc Student-Newman-Keuls tests, due to heteroscedasticity.

285 All statistical analyses were performed with R software (R Development Core Team 2017).

286

287 **3. RESULTS**

288

289 **3.1. Chemical analysis**

290 Concentrations of total phenolics, flavonols and proanthocyanidins increased from green
291 to senescent leaves but strongly decreased in leaf litter (Fig. 1).

292

293 **3.2. Bioassays**

294

295 **3.2.1. Soil microorganisms and herbaceous plant responses to *C. coggygia* aqueous extracts**

296 The sterilization process reduced by two-fold microbial biomass on natural soil (*t*-test,
297 $P < 0.01$; Fig. 2). On natural soil, this biomass increased regularly from green leaf to leaf litter
298 extracts watering with a weak effect of dose (2.5 vs. 5% DM), except for leaf litter extract that
299 showed a higher dose effect. On autoclaved soil, stimulatory effects of all aqueous extracts were
300 observed on microbial biomass, especially with extracts at 5% DM with 2 to 3-fold increases
301 (Fig. 2).

302 Target plant species exhibited highly different germination rate and velocity, and growth
303 length values in the control treatments (Table 1). *Lactuca sativa* had the highest germination
304 rate (79-85 %) and velocity (43-44). *Verbascum pulverulentum* presented the lowest
305 germination rate (14-34 %) concomitant to the lowest growth (1.0-1.4 and 1.0-1.2 cm for root
306 and shoot, respectively). *Linum narbonense* demonstrated the highest growth (6.0-6.1 and 5.4-
307 5.6 cm for root and shoot, respectively) with the slowest germination velocity (8.2-8.3). The
308 effect of soil type on germination rate depended on species (Species \times Soil interaction,
309 likelihood ratio $\chi_2 = 221.8$, $P < 0.001$). *Silene nutans* and *V. pulverulentum* presented lower
310 germination rates on autoclaved soil compared to natural soil (47.7 ± 3.0 vs. 55.3 ± 3.9 for *S.*
311 *nutans*; 13.7 ± 2.7 vs. 34 ± 3.5 for *V. pulverulentum*). Higher germination rates were however
312 observed for *L. sativa* (85.3 ± 1.6 vs. 79.3 ± 1.6) and *Satureja montana* (82.0 ± 2.8 vs. $74.0 \pm$
313 3.2) on autoclaved soil compared to natural soil. *Linum narbonense* was the only species for

314 which germination rate was not affected by the soil treatment (74.3 ± 2.0 vs. 75.3 ± 7.2 for
315 autoclaved and natural soils, respectively). As a consequence, the presence/absence of
316 microorganisms in soils influenced species ranking for germination rate. In addition to lower
317 germination rate on autoclaved soil, *S. nutans* was the only species presenting a lower
318 germination velocity on autoclaved soil compared to natural soil ($F_{4,140} = 8.4$, $P < 0.001$; 15.8
319 ± 0.7 vs. 55.3 ± 3.9 , respectively), whereas this parameter seemed to be not affected by soil
320 treatment for the four other species. Shoot and root growth of all species were not affected by
321 soil treatment ($P > 0.05$, Table 1).

322 *Cotinus coggygia* aqueous extracts generally affected plant growth more than plant
323 germination, but extract effects depended on target plant species, leaf type, extract
324 concentration and soil type.

325 The effects of *C. coggygia* extracts on growth ranged from - 57% to no effect, with no
326 positive effect detected (Fig. 3). Generally, we observed higher inhibitory effects on root growth
327 (up to -57%) than shoot growth (up to -29%). Inhibitory effects were higher with extracts
328 prepared with green leaf, followed by those based on senescent leaf. Inhibitory effects were
329 strongly reduced or totally disappeared with litter extracts and were generally stronger on
330 autoclaved soil than on natural soil. *Linum narbonense* was the most sensitive species,
331 especially on autoclaved soil where growth was reduced by more than 50% for several leaf
332 types and extract concentrations (Fig. 3). *Satureja montana* and *V. pulverulentum* were also very
333 sensitive to green leaf extracts on autoclaved soil, with almost 40% of root length reduction,
334 whereas *S. nutans* reached similar reductions with senescent leaf extracts on autoclaved soil.
335 On natural soil, plant growths were also reduced by about 20% for *L. narbonense*, *S. nutans*
336 and *V. pulverulentum* in presence of leaf extracts while *S. montana* maintained a similar plant
337 growth than in control treatment.

338 *Cotinus coggygia* extracts effects on germination rate and velocity ranged from -38% to

339 +26% and from -24% to +17%, respectively (Supplementary Fig. S1). *Lactuca sativa* had more
340 stable germination rate and velocity across all treatments than the other species. Extracts
341 reduced the germination rate and velocity of *L. narbonense* to about 38%, but this reduction
342 was overall lower on natural soil where 2.5% extracts slightly stimulated germination.
343 Germination rate of *S. montana* was reduced on autoclaved soil, while on natural soil its
344 germination velocity was stimulated by senescent leaves. Germination rate of *V. pulverulentum*
345 was inhibited by green leaf extracts on natural soil. No clear hierarchy of extract effects was
346 evidenced regarding leaf type (Supplementary Fig. S1).

347

348 3.2.2. Oak seedling and sapling responses to *C. coggygria* aqueous extracts

349 In the greenhouse, leaf extracts did not influence acorn germination rate as all sowed acorns
350 germinated (Exp. 2a). Both *C. coggygria* extracts and litter presence caused a 39% reduction
351 of seedling root biomass ($F_{2,27} = 6.4$, $P < 0.01$) but did not affect stem ($F_{2,27} = 0.2$, $P = 0.8$) or
352 leaf biomass ($F_{2,27} = 0.3$, $P = 0.8$) of the 2 month-old oak seedlings (Exp. 2a, Fig. 4). However,
353 aqueous extracts did not affect the 40-months-old oak sapling leaf, stem and root biomasses,
354 although leaf biomass slightly increased for saplings watered with *C. coggygria* extracts at the
355 end of the experiment (Exp. 2b, Table 2, Fig. 4). Mycorrhization rates, rather low at 4 months
356 ($18.8 \pm 2.6\%$), increased after 12 and 16 months of experiment (68-82%) but were not
357 influenced by *C. coggygria* extracts whatever the sampling date (Table 2).

358 In the field, oak seedling emergence was lower under *C. coggygria* shrubs (LR $\chi^2 = 8.7$, P
359 $= 0.003$), but *C. coggygria* presence did not affect seedling survival (LR $\chi^2 = 4.1$, $P = 0.5$, data
360 not shown), diameter ($F_{1,70} = 0.1$, $P = 0.7$) or length ($F_{1,70} = 0.4$, $P = 0.5$) growth over the
361 following 3 years (Exp. 2c, Fig. 4).

362

363 3.2.3. Saprophytic fungi and mesofauna responses to *C. coggygria* aqueous extracts

364 Saprophytic fungal biomass as well as Oribatida and predatory Acari abundances were
365 not affected by *C. coggygria* extracts (KW = 1.3, P = 0.5; KW = 1.5, P = 0.5; KW = 0.4, P =
366 0.8, for saprophytic fungi, Oribatida and predatory Acari, respectively; Table 3). Collembola
367 abundance was 3.5 times lower in mesocosms watered with *C. coggygria* extracts at 5% than
368 in mesocosms watered with deionized water (KW = 7.0, P = 0.03; Table 3).

369

370 4. DISCUSSION

371 *C. coggygria* contained a particularly high quantity of phenolics in green and senescent
372 leaves. For instance, total phenolic and flavonoid contents were at least 10 times higher than
373 values reported for *Pinus halepensis* (Fernandez et al. 2009; Santonja et al. 2015). Phenolic
374 quantities were however strongly reduced in litter. Phenolics are water-soluble compounds that
375 are rapidly leached during the initial phases of decomposition (Chomel et al. 2014; Santonja et
376 al. 2015). For example, Santonja et al. (2015) reported that *C. coggygria* lost 73% of initial
377 phenolic content after 100 days of litter decomposition.

378

379 4.1. Impacts of *C. coggygria* on herbaceous species and oak regeneration

380 Bioassays conducted on several target plant species generally highlight species-specific
381 response to allelochemicals. For instance, Fernandez et al. (2013) found that 40% of 15 target
382 plant species tested were inhibited by aqueous extracts of *P. halepensis* green needles, while
383 20% were insensitive and 40% were even stimulated. In the present study, although sensitivity
384 to *C. coggygria* aqueous extracts varied depending on target species, effects were generally
385 negative and only a slight positive effect on germination was detected for one species,
386 highlighting a strong phytotoxic potential of *C. coggygria*. Contrary to our expectations, the
387 commonly used *L. sativa* was not the most sensitive target species. The high sensitivity of *L.*
388 *narbonense* is concordant with previous studies that also outlined a high sensitivity of *Linum*

389 *strictum*, a species from the same genus (Bousquet-Mélou et al. 2005; Fernandez et al. 2006,
390 2013). Interestingly, this finding is contradictory with the general view that specialized
391 metabolites are less efficient against co-occurring species (Callaway and Ridenour 2004).
392 Litter, which had by far the lowest phenolic content, also presented the lowest effects. However,
393 green leaves showed generally a higher effect than senescent leaves despite a lower phenolic
394 content. Different chemical compositions between these two phenological stages may probably
395 explain the particularly high effect of green leaves (Hashoum et al. 2017).

396 The negative influence of *C. coggygia* on early oak seedling root development may
397 explain the lower emergence below shrubs recorded on the field. Later sapling development in
398 contrast was poorly affected by extracts, both in the field and greenhouse experiments. Older
399 saplings may be less sensitive to this type of interaction because of a lower quantity of absorbed
400 phenolics relative to seedling biomass (dilution effect). Alternatively, older seedlings may be
401 better protected against phytotoxic compounds thanks to their mycorrhizal associations (Mallik
402 and Zhu 1995; Zeng and Mallik 2006).

403

404 **4.2. Phenolics influence on soil organisms**

405 In the present study, *C. coggygia* extracts generally increased microbial biomass. Hortal
406 et al. (2015) also found that specialized metabolites of the shrub *Thymus hyemalis* promotes
407 microbial activity and biomass. Microbial stimulation may be due to the use of these
408 compounds as energy source by microorganisms (Inderjit 1996; Blum and Shafer 1998), which
409 could explain the lower inhibition of herbaceous species in natural soils containing
410 microorganisms. An alternative explanation is that aqueous extracts also contain nutrients and
411 sugars that stimulate microorganisms, or that microorganisms may have been present in extracts
412 according to leaf types, adding more microbes to the soil. Increase in microbial biomass was
413 stronger at higher concentration of extracts, probably because of a higher quantity of

414 compounds or microorganisms. This was particularly the case with high increase of
415 microorganisms on autoclaved soils, containing less initial microbial biomass, which may be
416 linked to a higher colonizing capacity of remaining microorganisms and/or extract
417 microorganisms favored by a lower microbial competition.

418 Saprophytic and symbiotic fungi were not affected by *C. coggygia* extracts. *Ex situ* studies
419 found that phenolics may inhibit, have neutral effect or even stimulate fungal development and
420 respiration depending on the source species, on extracts concentration and on fungal target
421 species (Rose et al. 1983; Souto et al. 2000a,b). Rose et al. (1983) also showed that root
422 colonization of Douglas fir (*Pseudotsuga menziesii* Mirb.) seedlings by *Rhizopogon sp.* was
423 inhibited by the mere application of litter on soil surface. In our study, no effect on total
424 mycorrhization rate was detected but we did not investigate such potential species-specific
425 effects by examining mycorrhizal species present on oak roots. Even if qualitative changes in
426 mycorrhizal communities occurred, this did not seem to affect oak seedling development during
427 the 16 months of our experimentation. In addition, it is worth noting that our experiment was
428 designed to test chemical effects on already mycorrhized saplings, but it would be interesting
429 to further study whether initial root colonization by mycorrhizae could be affected.

430 Among soil arthropods, Collembola abundance was negatively affected while Acari
431 appeared as insensitive to extracts of *C. coggygia* senescent leaves. Previous experiments also
432 reported that phenolic compounds, including phenolic acids, flavonoids and tannins, can limit
433 litter colonization, growth and activity of soil Collembola (Poinsot-balaguer et al. 1993; Das
434 and Joy 2009; Chomel et al. 2014; Asplund et al. 2015). For example, Asplund et al. (2015)
435 reported a negative effect of lichen phenolics on Collembola abundance and species richness in
436 boreal *P. sylvestris* forest, while Das and Joy (2009) showed a decrease in litter colonization by
437 *Cyphoderus javanus* Börner (Collembola: Hexapoda) according to the increase in phenolic
438 concentration in tropical forest. In the present study, by negatively affecting Collembola,

439 phenolics present in *C. coggygria* senescent leaves could favor microbial and Acari
440 colonization of leaf litter during the initial stages of litter decomposition. However, in later
441 decomposition stages positive effects of *C. coggygria* litter on Collembola have been observed
442 (Santonja et al. 2017), suggesting that the leaching of phenolics remove the inhibitory effect
443 found here with senescent leaves. In support to this hypothesis, Chomel et al. (2014) also
444 reported than phenolics present in pine needles delayed the litter colonization by Collembola in
445 Mediterranean *P. halepensis* forest.

446

447 ***4.3. Soil organisms modulate plant-plant chemical interactions***

448 Aqueous extracts of *C. coggygria* leaves exhibited more negative effects on plant target
449 species on autoclaved than on natural soils. Microbial activities can influence the persistence,
450 availability and biological activity of phenolics in soil (Inderjit 2005; Kaur et al. 2009; Meiners
451 et al. 2012). Here, microbial community present in natural soil released the toxicity of *C.*
452 *coggygria* extracts, despite a similar microbial biomass than in autoclaved soil. We can
453 hypothesize that this alleviation of negative effects is due to a qualitative difference in soil
454 microbial community, i.e. sterilization removed a part of soil microbial community able to
455 degrade phytotoxic compounds. This highlights the possible importance of microbial
456 community composition in determining the intensity or direction of plant-plant chemical
457 interactions.

458

459 ***4.4. Synthesis: potential impacts of C. coggygria on forest regeneration***

460 Our study shows that *C. coggygria* leachates can inhibit both herbaceous species and
461 early oak seedling development. Herbaceous species are strong competitor for oak seedling
462 establishment (Rey Benayas et al. 2005; Gavinet et al. 2016) because they form a dense
463 superficial network of roots that strongly compete with tree seedlings for water in the upper

464 soil layers (Balandier et al. 2006). The inhibitory effect of *C. coggygria* on herbaceous
465 species could mediate indirect interactions favoring oak establishment. Although we did
466 not observe such indirect positive interactions in the field, they may take place in systems
467 with a higher herbaceous cover. In the USA, Petranka and McPherson (1979) found that
468 *Rhus copallina*, a shrub closely related to *C. coggygria* with high phenolics content, plays
469 a key role in the prairie – forest transitions as this shrub allows for tree seedling
470 establishment by inhibiting herbaceous species development. In addition, by stimulating
471 microbial biomass and reducing the abundance of microbivorous mesofauna, *C. coggygria*
472 may favor microbial community development and associated functions such as nutrient
473 mineralization. However, it is difficult to predict feedback effects on plant species without
474 more knowledge of the type of microbes being stimulated (neutral, mutualistic or
475 pathogens, Hortal et al. 2015) and our in-situ experiment suggests that modifications of
476 soil microbes may not play an important role for oak seedling establishment, as also shown
477 in a pot experiment (Gavinet et al., 2018).

478 Changes in seedling response to neighbor presence with ontogeny have been highlighted
479 in several studies (e.g. Le Roux et al. 2013) and generally attributed to a change in resource
480 requirement or availability (Soliveres et al. 2010). The results of our study suggest that
481 ontogenetic changes in plant-plant interaction outcomes may also result from a change in plant-
482 plant chemical interactions.

483

484 **ACKNOWLEDGEMENTS**

485 This study was funded by ECCOREV Research Federation (FR3098) and the French
486 National Research Agency (ANR) through the SecPriMe² project (ANR-12-BSV7-0016-01).
487 We also gratefully acknowledge the program MISTRALS (Mediterranean Integrated Studies
488 at Regional And Local Scales), particularly the axe BioDivMeX, and the LabEx OT-Med (no

489 ANR-11-LABX-0061) funded by the «Investissements d’Avenir» program of the French
490 National Research Agency through the A*MIDEX project (no ANR-11-IDEX-0001-02). The
491 field experiment and material sampling was carried out at the Oak Observatory at “Observatoire
492 de Haute-Provence” (O₃HP) platform with the help of Jean-Philippe Orts. The authors also
493 thank Sylvie Dupouyet for her help with the bioassays.

494

495 REFERENCES

496 Alef, K., Nannipieri, P., 1995. *Methods in applied soil microbiology and biochemistry*.
497 Academic, London.

498 Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative
499 measurement of microbial biomass in soil. *Soil Biol. Biochem.* 10, 215-221.

500 Asplund, J., Bokhorst, S., Kardol, P., Wardle, D.A., 2015. Removal of secondary compounds
501 increases invertebrate abundance in lichens. *Fungal Ecol.* 18, 18-25. DOI:
502 10.1016/j.funeco.2015.07.009

503 Balandier, P., Collet, C., Miller, J.H., Reynolds, P.E., Zedaker, S.M., 2006. Designing forest
504 vegetation management strategies based on the mechanisms and dynamics of crop tree
505 competition by neighbouring vegetation. *Forestry* 79, 3-27. DOI: 10.1093/forestry/cpi056

506 Beare, M.H., Neely, C.L., Coleman, D.C., Hargrove, W.L., 1990. A substrate-induced
507 respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues.
508 *Soil Biol. Biochem.* 22, 585-594. DOI: 10.1016/0038-0717(90)90002-H

509 Berg, M.P., Stoffer, M., Heuvel van den, H.H., 2004. Feeding guilds in Collembola based on
510 digestive enzymes. *Pedobiologia* 48, 589-601. DOI: 10.1016/j.pedobi.2004.07.006

511 Blondel, J., 2006. The “design” of Mediterranean landscapes: A millennial story of humans and
512 ecological systems during the historic period. *Hum. Ecol.* 34, 713-729. DOI:
513 10.1007/s10745-006-9030-4

514 Blum, U., Shafer, S.R., 1988. Microbial populations and phenolic acids in soils. *Soil Biol.*
515 *Biochem.* 20, 793-800.

516 Bousquet-Mélou, A., Louis, S., Robles, C., Greff, S., Dupouyet, S., Fernandez, C. 2005.
517 Allelopathic potential of *Medicago arborea*, a Mediterranean invasive shrub. *Chemoecology*
518 15, 193-198. DOI: 10.1007/s00049-005-0311-y

519 Callaway, R.M., Ridenour, W.M., 2004. Novel weapons: invasive success and the evolution of
520 increased competitive ability. *Front. Ecol. Environ.* 2, 436-443. DOI: 10.1890/1540-
521 9295(2004)002

522 Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based
523 on nitrogen stable isotope ratios. *Soil Biol. Biochem.* 37, 1718-1725. DOI:
524 10.1016/j.soilbio.2005.02.006

525 Chaves, N., Escudero, J.C., 1999. Variation of flavonoid synthesis induced by ecological
526 factors. In: Inderjit, Dakshini, K.M.M., Foy, C.L. (Eds.), *Principles and practices in plant*
527 *ecology*. CRC Press, Boca Raton, pp 267-285.

528 Chomel, M., Fernandez, C., Bousquet-Mélou, A., Gers, C., Monnier, Y., Santonja, M.,
529 Gauquelin, T., Gros, R., Lecareux, C., Baldy, V., 2014. Secondary metabolites of *Pinus*
530 *halepensis* alter decomposer organisms and litter decomposition during afforestation of
531 abandoned agricultural zones. *J. Ecol.* 102, 411-424. DOI: 10.1111/1365-2745.12205

532 Chou, C.H., Fu, C.Y., Li, S.Y., Wang, Y.F., 1998. Allelopathic potential of *Acacia confusa* and
533 related species in Taiwan. *J. Chem. Ecol.* 24, 2131-2150. DOI: 10.1023/A:1020745928453

534 Chou, C.H., 1999. Roles of allelopathy in plant biodiversity and sustainable agriculture. *Crit.*
535 *Rev. Plant Sci.* 18, 609-636. DOI: 10.1080/07352689991309414

536 Coleman, D.C., Crossley, J.D.A., Hendrix, P.F., 2004. *Fundamentals of soil ecology*. Elsevier
537 Academic Press, San Diego, CA, USA.

538 Das, S., Joy, V.C., 2009. Chemical quality impacts of tropical forest tree leaf litters on the
539 growth and fecundity of soil Collembola. *Eur. J. Soil Biol.* 45, 448-454. DOI:
540 10.1016/j.ejsobi.2009.09.001

541 Fernandez, C., Monnier, Y., Santonja, M., Gallet, C., Weston, L.A., Prévosto, B., Saunier, A.,
542 Baldy, V., Bousquet-Mélou, A., 2016. The impact of competition and allelopathy on the
543 trade-off between plant defense and growth in two contrasting tree species. *Front. Plant Sci.*
544 7. DOI: 10.3389/fpls.2016.00594

545 Fernandez, C., Santonja, M., Gros, R., Monnier, Y., Chomel, M., Baldy, V., Bousquet-Mélou,
546 A., 2013. Allelochemicals of *Pinus halepensis* as drivers of biodiversity in Mediterranean
547 open mosaic habitats during the colonization stage of secondary succession. *J. Chem. Ecol.*
548 39, 298-311. DOI: 10.1007/s10886-013-0239-6

549 Fernandez, C., Monnier, Y., Ormeño, E., Baldy, V., Greff, S., Pasqualini, V., Mévy, J.P.,
550 Bousquet-Mélou, A., 2009. Variations in allelochemical composition of leachates of
551 different organs and maturity stages of *Pinus halepensis*. *J. Chem. Ecol.* 35, 970-979. DOI:
552 10.1007/s10886-009-9667-8

553 Fernandez, C., Voiriot, S., Mévy, J.-P., Vila, B., Ormeño, E., Dupouyet, S., Bousquet-Mélou,
554 A., 2008. Regeneration failure of *Pinus halepensis* Mill.: The role of autotoxicity and some
555 abiotic environmental parameters. *For. Ecol. Manag.* 255, 2928-2936. DOI:
556 10.1016/j.foreco.2008.01.072

557 Fernandez, C., Lelong, B., Vila, B., Mévy, J.P., Robles, C., Greff, S., Dupouyet S., Bousquet-
558 Mélou, A. 2006. Potential allelopathic effect of *Pinus halepensis* in the secondary
559 succession: an experimental approach. *Chemoecology* 16, 97-105. DOI: 10.1007/s00049-
560 006-0334-z

561 Filser, J., 2002. The role of Collembola in carbon and nitrogen cycling in soil. *Pedobiologia* 46,
562 234-245. DOI: 10.1016/S0031-4056(04)70139-0

563 Garbaye, J., 2013. La symbiose mycorhizienne: Une association entre les plantes et les
564 champignons. Editions Quae, Paris.

565 Gavinet, J., Prévosto, B., Fernandez, C., 2016. Do shrubs facilitate oak seedling establishment
566 in Mediterranean pine forest understory? *For. Ecol. Manag.* 381, 289-296. DOI:
567 10.1016/j.foreco.2016.09.045

568 Gavinet, J., Prévosto, B., Bousquet-Mélou, A., Gros, R., Quer, E., Baldy, V., Fernandez, C.,
569 2018. Do litter-mediated plant-soil feedbacks influence Mediterranean oak regeneration? A
570 two-year pot experiment. *Plant Soil* 430, 59-71. DOI: 10.1007/s11104-018-3711-9

571 Gessner, M.O., Chauvet, E., 1993. Ergosterol-to-biomass conversion factors for aquatic
572 hyphomycetes. *Appl. Environ. Microbiol.* 59, 502-507.

573 Hashoum, H., Santonja, M., Gauquelin, T., Saatkamp, A., Gavinet, J., Fernandez, C., Bousquet-
574 Mélou, A. 2017. Biotic interactions in a Mediterranean oak forest: role of allelopathy along
575 phenological development of woody species. *Eur. J. For. Res.* 136, 699-710. DOI:
576 10.1007/s10342-017-1066-z

577 Hopkins, S.P., 1997. *The Biology of Springtails (Insects: Collembolan)*. Oxford University
578 Press, NY, USA.

579 Hortal, S., Bastida, F., Armas, C., Lozano, Y.M., Moreno, J.L., Garcia, C., Pugnaire, F.I., 2013.
580 Soil microbial community under a nurse-plant species changes in composition, biomass and
581 activity as the nurse grows. *Soil Biol. Biochem.* 64, 139-146. DOI :
582 10.1016/j.soilbio.2013.04.018

583 IFN, 2014. Résultats d'inventaire forestier. Tome zonage écoforestier Méditerranée. Les
584 résultats des campagnes d'inventaire 2009 à 2013. Inventaire Forestier National.

585 Inderjit, 1996. Plant phenolics in allelopathy. *Bot. Rev.* 62, 186-202.

586 Inderjit, 2005. Soil microorganisms: An important determinant of allelopathic activity. *Plant*
587 *Soil* 274, 227-236. DOI: 10.1007/s11104-004-0159-x

588 Kaundun, S.S., Lebreton, P., Fady, B., 1998. Geographical variability of *Pinus halepensis* Mill.
589 as revealed by foliar flavonoids. *Biochem. Syst. Ecol.* 26:83-96.

590 Kaur, H., Kaur, R., Kaur, S., Baldwin, I.T., Inderjit, 2009. Taking ecological function seriously:
591 soil microbial communities can obviate allelopathic effects of released metabolites. *Plos One*
592 4e4700. DOI: 10.1371/journal.pone.0004700

593 Le Roux, P.C., Shaw, J.D., Chown, S.L., 2013. Ontogenetic shifts in plant interactions vary
594 with environmental severity and affect population structure. *New Phytol.* 200, 241-250.
595 DOI: 10.1111/nph.12349

596 Mallik, A.U., 2008. Allelopathy in forested ecosystems. In: Zeng, RS; Mallik, AU; Luo, SM
597 (Eds.), *Allelopathy in sustainable agriculture and forestry*, pp. 363-386.

598 Mallik, A., Zhu, H., 1995. Overcoming allelopathic growth-inhibition by mycorrhizal
599 inoculation. In: Inderjit, Dakshini, K.M.M, Einhellig, F.A. (Eds.), *Allelopathy: organisms,*
600 *processes, and applications.* Amer Chemical Soc, Washington, pp 39-57.

601 Marčetić, M., Božić, D., Milenković, M., Malešević, N., Radulović, S., Kovačević, N., 2013.
602 Antimicrobial, antioxidant and anti-inflammatory activity of young shoots of the smoke tree,
603 *Cotinus coggygria* Scop. *Phytother. Res.* 27, 1658-1663. DOI: 10.1002/ptr.4919

604 Matić, S, Stanić, S, Solujić, S, Milosević, T, Niciforović, N, (2011) Biological properties of the
605 *C. coggygria* methanol extract. *Period. Biol.* 113, 87-92.

606 Mazliak, P., 1982. *Physiologie Végétale: Croissance et Développement.* Hermann, Paris.

607 Meiners, S.J., Kong, C.H., Ladwig, L.M., Pisula, N.L., Lang, K.A., 2012. Developing an
608 ecological context for allelopathy. *Plant Ecol.* 213, 1861-1867. DOI: 10.1007/s11258-012-
609 0078-5.

610 Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000.
611 Biodiversity hotspots for conservation priorities. *Nature* 403, 853-858. DOI:
612 10.1038/35002501

613 Nilsson, M., 1994. Separation of allelopathy and resource competition by the boreal dwarf
614 shrub *Empetrum-Hermaphroditum* Hagerup. *Oecologia* 98, 1-7. DOI: 10.1007/BF00326083

615 Nilsson, M.C., Zackrisson, O., Sterner, O., Wallstedt, A., 2000. Characterisation of the
616 differential interference effects of two boreal dwarf shrub species. *Oecologia* 123, 122-128.
617 DOI: 10.1007/s004420050997

618 Novaković M, Vucković I, Janacković P, Soković M, Filipović A, Tesević V, Milosavljević S
619 (2007). Chemical composition, antibacterial and antifungal activity of the essential oils of
620 *C. coggygria* from Serbia. *J. Serb. Chem. Soc.* 72, 1045-1051.

621 Petranka, J.W., McPherson, J.K., 1979. The role of *Rhus Copallina* in the dynamics of the
622 forest-prairie ecotone in North-Central Oklahoma. *Ecology* 60, 956-965. DOI:
623 10.2307/1936864

624 Poinso-balaguer, N., Racon, L., Sadaka, N., Lepetit, J., 1993. Effects of tannin compounds on
625 two species of Collembola. *Eur. J. Soil Biol.* 29:13-16.

626 Prévosto, B., Ripert, C., Ostermeyer, R., 2013. Éclaircir est-il suffisant pour favoriser la
627 régénération de taillis de chêne blanc? Retour sur un dispositif expérimental installé il y a
628 27 ans en forêt domaniale de Lure (Alpes-de-Haute-Provence). *For. Méditerranéenne* 34, 3-
629 12.

630 Quézel, P., Médail, F., 2003. *Ecologie et biogéographie des forêts du bassin méditerranéen*,
631 Elsevier, Paris.

632 R Development Core Team, 2017. *R: A language and environment for statistical computing*. R
633 Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>

634 Rey Benayas, J.M., Navarro, J., Espigares, T., Nicolau, J.M., Zavala, M.A., 2005. Effects of
635 artificial shading and weed mowing in reforestation of Mediterranean abandoned cropland
636 with contrasting *Quercus* species. *For. Ecol. Manag.* 212, 302-314. DOI:
637 10.1016/j.foreco.2005.03.032

638 Rose, S.L., Perry, D.A., Pilz, D., Schoeneberger, M.M., 1983. Allelopathic effects of litter on
639 the growth and colonization of mycorrhizal fungi. *J. Chem. Ecol.* 9, 1153-1162. DOI:
640 10.1007/BF00982218

641 Santonja, M., Baldy, V., Fernandez, C., Balesdent, J., Gauquelin, T., 2015. Potential shift in
642 plant communities with climate change in a Mediterranean Oak forest: consequence on
643 nutrients and secondary metabolites release during litter decomposition. *Ecosystems* 18,
644 1253-1268. DOI: 10.1007/s10021-015-9896-3

645 Santonja, M., Fernandez, C., Gers, C., Proffit, M., Gauquelin, T., Reiter, I., Cramer, W., Baldy,
646 V., 2017. Plant litter mixture partly mitigates the negative effects of extended drought on
647 soil biota and litter decomposition in a Mediterranean oak forest. *J. Ecol.* 105, 801-815. DOI:
648 10.1111/1365-2745.12711

649 Santonja, M., Foucault, Q., Rancon, A., Baldy, V., Gauquelin, T., Fernandez, C., Mirleau, P.,
650 2018. Contrasting responses of bacterial and fungal communities to plant litter diversity in
651 a Mediterranean oak forest. *Soil Biol. Biochem.* 125, 27-36. DOI:
652 10.1016/j.soilbio.2018.06.020

653 Scognamiglio, M., D'Abrosca, B., Esposito, A., Pacifico, S., Monaco, P., Fiorentino, A., 2013.
654 Plant growth inhibitors: allelopathic role or phytotoxic effects? Focus on Mediterranean
655 biomes. *Phytochem. Rev.* 12, 803-830. DOI: 10.1007/s11101-013-9281-9

656 Seastedt, T.R. (1984) The role of microarthropods in decomposition and mineralization
657 processes. *Annu. Rev. Entomol.* 29, 25-46. DOI: 10.1146/annurev.en.29.010184.000325

658 Singleton, V.L., Rossi, J.A.J., 1965. Colorimetry of total phenolics with phosphomolybdic-
659 phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144-158.

660 Soliveres, S., DeSoto, L., Maestre, F.T., Olano, J.M., 2010. Spatio-temporal heterogeneity in
661 abiotic factors modulate multiple ontogenetic shifts between competition and facilitation.
662 *Perspect. Plant Ecol. Evol. Syst.* 12, 227-234. DOI: 10.1016/j.ppees.2010.02.003

663 Souto, C., Chiapusio, G., Pellissier, F., 2000a. Relationships between phenolics and soil
664 microorganisms in spruce forests: significance for natural regeneration. *J. Chem. Ecol.* 26,
665 2025-2034. DOI: 10.1023/A:1005504029243

666 Souto, C., Pellissier, F., Chiapusio, G., 2000b. Allelopathic effects of humus phenolics on
667 growth and respiration of mycorrhizal fungi. *J. Chem. Ecol.* 26, 2015-2023. DOI:
668 10.1023/A:1005551912405

669 Trevors, J.T., 1996. Sterilization and inhibition of microbial activity in soil. *J. Microbiol. Meth.*
670 26, 53-59. DOI: 10.1016/0167-7012(96)00843-3

671 Valianou, L., Stathopoulou, K., Karapanagiotis, I., Magiatis, P., Pavlidou, E., Skaltsounis, A.L.,
672 Chryssoulakis, Y., 2009. Phytochemical analysis of young fustic (*Cotinus coggygria*
673 heartwood) and identification of isolated colourants in historical textiles. *Anal. Bioanal.*
674 *Chem.* 394, 871-882. DOI: 10.1007/s00216-009-2767-z

675 Vilà, M., Sardans, J., 1999. Plant competition in mediterranean-type vegetation. *J. Veg. Sci.* 10,
676 281-294. DOI: 10.2307/3237150

677 Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Putten, W.H. van der, Wall, D.H.,
678 2004. Ecological Linkages between aboveground and belowground biota. *Science* 304,
679 1629-1633. DOI: 10.1126/science.1094875

680 Wardle, D.A., Nilsson, M.C., Gallet, C., Zackrisson, O., 1998. An ecosystem-level perspective
681 of allelopathy. *Biol. Rev.* 73, 305-319. DOI: 10.1017/S0006323198005192

682 Zeng, R.S., Mallik, A.U., 2006. Selected ectomycorrhizal fungi of black spruce (*Picea*
683 *mariana*) can detoxify phenolic compounds of *Kalmia angustifolia*. *J. Chem. Ecol.* 32, 1473-
684 1489. DOI: 10.1007/s10886-006-9063-6

685

686 **Table 1:** Germination and growth parameters of the 5 target species in the control treatment (watered with deionized water) depending on soil
 687 type. Data are means \pm standard errors (n = 15 boxes for germination parameters, n = 35-150 seedlings for growth parameters). Different letters
 688 indicate significant differences between species with a>b>c>d ($P<0.05$).

689

	Autoclaved soil				Natural soil			
	Germination rate (%)	Germination velocity	Root growth (cm)	Shoot growth (cm)	Germination rate (%)	Germination velocity	Root growth (cm)	Shoot growth (cm)
<i>Lactuca sativa</i>	85.3 \pm 1.6a	43.1 \pm 0.6a	2.42 \pm 0.08b	3.04 \pm 0.10b	79.3 \pm 1.6a	44.1 \pm 0.0a	2.04 \pm 0.07b	3.44 \pm 0.11b
<i>Linum narbonense</i>	74.3 \pm 2.0b	8.2 \pm 0.2d	6.01 \pm 0.13a	5.40 \pm 0.08a	75.3 \pm 7.2a	8.3 \pm 0.3c	6.13 \pm 0.11a	5.62 \pm 0.09a
<i>Satureja montana</i>	82.0 \pm 2.8a	22.9 \pm 0.9b	2.27 \pm 0.04b	2.16 \pm 0.03c	74.0 \pm 3.2a	23.1 \pm 0.9b	2.31 \pm 0.05b	2.57 \pm 0.04c
<i>Silene nutans</i>	47.7 \pm 3.0c	15.8 \pm 0.7bc	2.16 \pm 0.09b	2.49 \pm 0.05c	55.3 \pm 3.9b	24.3 \pm 1.2b	2.61 \pm 0.07b	2.65 \pm 0.06c
<i>Verbascum pulverulentum</i>	13.7 \pm 2.7d	16.2 \pm 1.5c	1.02 \pm 0.04c	0.99 \pm 0.03d	34.0 \pm 3.5c	19.7 \pm 0.9b	1.41 \pm 0.03c	1.18 \pm 0.03d

690

691

692 **Table 2:** Results of ANOVAs testing the effects of *Cotinus coggygia* extracts, sampling date,
693 and their interactions on oak sapling root, stem and leaf biomasses and mycorrhization rate. *F*-
694 values and associated *P*-values (***) ($P < 0.001$) are indicated. Significant results are highlighted
695 in bold.

696

697

	<i>Cotinus</i> d.f.=1	Date d.f.=2	<i>Cotinus</i> x Date d.f.=2
Root biomass	0.5	33.3***	0.4
Stem biomass	0.1	11.3***	0.8
Leaf biomass	0.1	2.9	1.1
Mycorrhization rate	0.2	78.2***	1.3

698

699

700 **Table 3.** Saprophytic fungal biomass (expressed as $\mu\text{g.g}^{-1}$ soil DM) and abundances of the
 701 different mesofauna groups (expressed as nb ind.g⁻¹ soil DM) according to *Cotinus coggygia*
 702 extract concentrations. Values are means \pm standard errors; n= 5. Different letters indicate
 703 significant differences between leachate treatments with a>b (Kruskal-Wallis test, $P<0.05$).

704

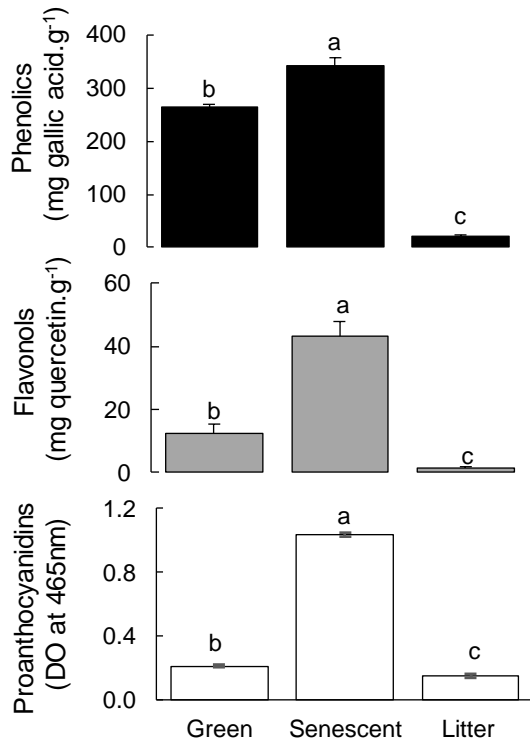
Leachate concentration	Saprophytic fungal biomass	Collembola abundance	Oribatida abundance	Predatory Acari abundance
0	116.89 \pm 8.26 a	0.07 \pm 0.01 a	0.22 \pm 0.03 a	0.07 \pm 0.01 a
2.5	129.49 \pm 7.37 a	0.08 \pm 0.04 ab	0.31 \pm 0.06 a	0.08 \pm 0.01 a
5	133.46 \pm 10.29 a	0.02 \pm 0.00 b	0.21 \pm 0.04 a	0.08 \pm 0.01 a

705

706

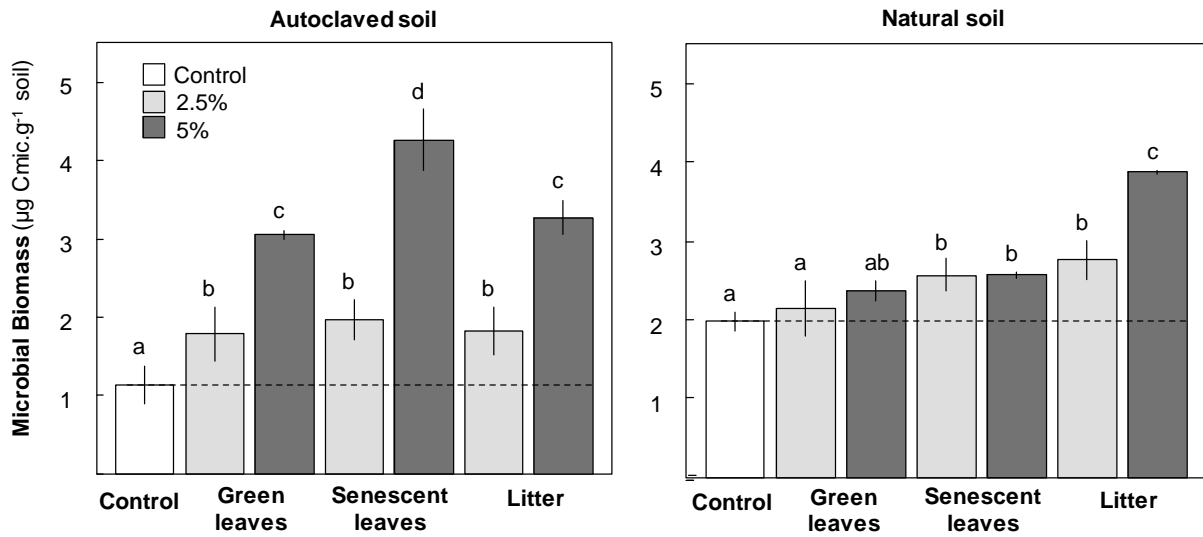
707 **Figure 1:** Chemical composition of *Cotinus coggygia* aqueous extracts of green leaves,
708 senescent leaves and leaf litter. Values are means \pm standard errors; n= 6. Different letters
709 indicate significant differences between leaf types with a>b>c (Kruskal-Wallis test, $P<0.05$).

710



711

712 **Figure 2:** Influence of *Cotinus coggygia* extracts on microbial biomass of autoclaved (left) or
713 natural soils (right). Dashed lines represent the mean value of control samples. Different letters
714 denote differences between treatments with $a < b < c$ (post-hoc Tukey tests, $P < 0.05$). Values are
715 means \pm standard errors; $n = 4$.
716

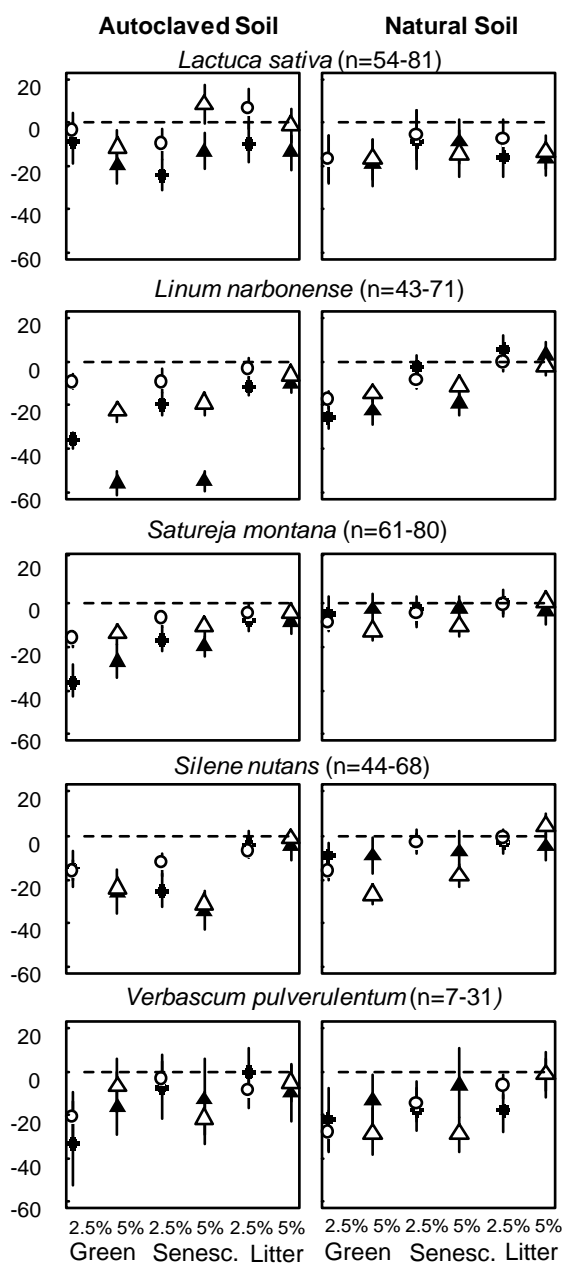


717

718

719 **Figure 2:** Relative Allelopathic Effects of *Cotinus coggygia* aqueous extracts on herbaceous
 720 species root (filled symbols) and shoot (open symbols) lengths. Extracts were prepared with
 721 different leaf types (green, senescent and litter) at two concentrations (2.5% and 5% dry matter
 722 of plant material, figured by circles and triangles, respectively) and the target species were
 723 grown either on autoclaved (left) or natural soils (right). Error bars are bootstrapped confidence
 724 intervals, number of seedlings (n) are indicated for each species.

725

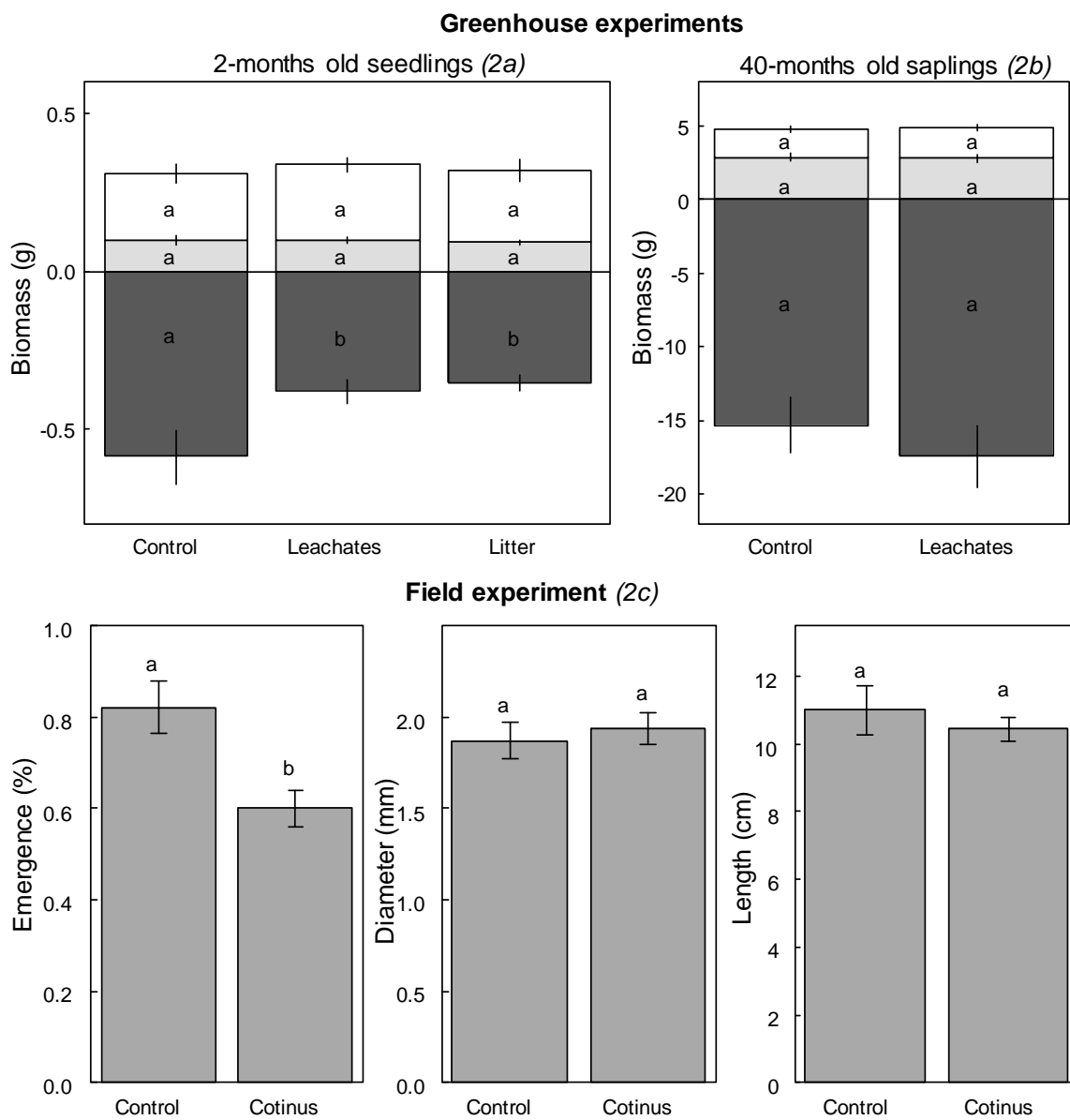


726

727

728 **Figure 4:** Influence of *Cotinus coggygia* on downy oak seedlings development. Upper panel:
 729 greenhouse experiments showing the influence of allelochemicals on oak leaf (white), stem
 730 (grey) and root (dark grey) biomasses. Lower panel: field experiment showing the influence of
 731 *Cotinus coggygia* on seedling emergence, diameter and length. Different letters indicate
 732 differences between treatments for each biomass compartment. Values are means \pm standard
 733 errors; n= 10 for greenhouse experiments, n= 30 – 50 for field experiment.

734



735

736