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### ► To cite this version:

Ana Beatriz de Oliveira, Amélie A. M. Cantarel, Marie Seiller, Alessandro Florio, Annette Berard, et al.. Short-term plant legacy alters the resistance and resilience of soil microbial communities exposed to heat disturbance in a Mediterranean calcareous soil. *Ecological Indicators*, 2020, 108, pp.105740. 10.1016/j.ecolind.2019.105740 . hal-02365828

HAL Id: hal-02365828

<https://institut-agro-rennes-angers.hal.science/hal-02365828v1>

Submitted on 21 Dec 2021

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1 **Title: Short-term plant legacy alters the resistance and resilience of soil microbial**  
2 **communities exposed to heat disturbance in a Mediterranean calcareous soil.**

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23

24 **Abstract**

25 Plant legacy is a concept representing the effects exerted by plants on soil once they are no  
26 longer growing. We hypothesized that plant species and mixture (intercropping) would induce  
27 different short-term legacy effects impacting carbon and nitrogen-related soil microbial  
28 activities and resistance and resilience after a heat disturbance. A microcosm experiment was  
29 conducted using a calcareous Mediterranean soil conditioned by a complete vegetative cycle  
30 in a greenhouse with four planting modalities (W = monoculture of Wheat (*Triticum aestivum*  
31 L.); L = monoculture of white Lupin (*Lupinus albus* L.); WL = both species intercropped; U =  
32 unplanted soil). Half of microcosms were incubated at 28°C (C = control conditions) whereas  
33 the remaining half were exposed at 48°C for 2 days (S = stress conditions), with an  
34 immediately return to control conditions. Microcosms were destructively sampled at 2, 7, 16  
35 and 28 days (T2, T7, T16, T28) after the end of the heat disturbance and the following soil  
36 measurements were performed: Basal Respiration (BR), Substrate-Induced Respiration (SIR),  
37 Nitrification Enzyme Activity (NEA) and N mineral concentrations. Our results demonstrated  
38 that monocultures and intercropping promoted different legacy effects under control  
39 conditions especially for SIR. WL soils presented lower values of SIR than L and higher than  
40 W soils. For SIR, W and WL soils conferred greater resistance to the heat stress, whereas L  
41 and WL soils conferred higher resilience at T28. For NEA, no differences between soils were  
42 observed for resistance to heat stress, but at T16, soils having WL legacy were more resilient  
43 than L soils, but comparable to those having W legacy. Our results highlight that a short-term  
44 legacy effect is measurable but greatly differs between C- and N-related microbial activities.  
45 We estimated that intercropping had modified ability of soil microorganisms to face heat  
46 stress, suggesting that plant legacy effect has to be considered to mitigate extreme climatic  
47 events in Mediterranean soils.

48 **Keywords:** climate change; soil respiration; nitrification; crop mixture; plant soil feedback,  
49 heat stress.

## 50 **1. Introduction**

51 Substantial legacy effects on soil properties are induced by plants and can persist after  
52 the disappearance of those plants that were responsible for these effects (van Der Putten et al.,  
53 2013) . Plant legacy effects have been pointed out to influence carbon storage in soils (Lange  
54 et al., 2015), succession in plant communities (van der Putten et al., 2016) or plant invasions  
55 (Bailey and Schweitzer, 2016). Legacy effects of plants on soils are largely driven by shifts in  
56 soil microbial communities. Indeed, plant roots can directly alter soil microbial communities  
57 by releasing carbon compounds named rhizodeposits (Eisenhauer et al., 2017; Hunter et al.,  
58 2014; Kuzyakov and Blagodatskaya, 2015). Rhizodeposits include a wide range of organic  
59 compounds including specific root exudates that shape microbial communities in root  
60 vicinity, *i.e.* the rhizosphere microbiome (Dennis et al., 2010; Li et al., 2018). Furthermore,  
61 composition of rhizodeposits is known to be plant specific (Hunter et al., 2014; Li et al.,  
62 2018), which suggests that a single species will affect differently soil microbial communities  
63 compared to a plant mixture (Chen et al., 2019; Eisenhauer et al., 2017; Tang et al., 2014;  
64 Tang et al., 2016). Plants are also able to alter soil microbial communities indirectly by  
65 modifying the abiotic properties of the soil, *i.e.* pH, nutrient availability, moisture or soil  
66 structure (Hinsinger et al., 2009). Several plant legacies were characterized in grasslands  
67 (Grman and Suding, 2010; Lange et al., 2015; Strecker et al., 2016; Zak et al., 2003), but  
68 debated in agroecosystems while recent publication pointed out its implication in shaping soil  
69 microbiome (Li et al., 2018). In agroecosystems, the frequency of perturbations due to annual  
70 practices and the low plant diversity barely support comparison to natural ecosystems.  
71 However, some agronomic practices such as intercropping are in rupture with conventional  
72 agriculture, as it consists to grow two or more species or genotypes in the same field during a  
73 significant period of their life cycle (Brooker et al., 2015). Intercropping is of great interest in  
74 many countries as a solution to replace synthetic inputs by ecological processes (Brooker et

75 al., 2015; Doré et al., 2011; Malézieux et al., 2009). Long-term legacy effects of intercropping  
76 have been estimated by Cong et al. (2015), but within 1-year timespan authors are reluctant to  
77 expect a significant effect on soil communities. However, Pivato et al. (2017) estimated that  
78 after only 12 weeks of bi-species culture, N cycling-related microbial communities were  
79 strongly affected especially at low N level compared to each species in monoculture. Grman  
80 and Suding (2010) also estimated a short-term legacy effect pointing out the multiple drivers  
81 defining both plant legacy duration and intensity. Given the crucial role of soil microbial  
82 activities in ecosystem functioning, these plant legacy effects can contribute to agroecosystem  
83 stability in the context of climate change and particularly during extreme events. Several  
84 studies have been focused on resistance (capacity to withstand change) and resilience  
85 (capacity for recovering of function) of C-related soil microbial activities to environmental  
86 disturbances (Bérard et al., 2012; Bérard et al., 2011; Chaer et al., 2009; Guillot et al., 2019;  
87 Hamdi et al., 2011; Mooshammer et al., 2017; Orwin and Wardle, 2005). However, studies  
88 about stability of more specialized soil microbial communities like nitrifiers are scarce  
89 (Mooshammer et al., 2017; Thion and Prosser, 2014; Wertz et al., 2007). Yet there are  
90 reasons to argue that plants are able to shape soil related N-cycle communities (Zak et al.,  
91 2003). Our rationale is to consider that diverse plant species yield different legacies,  
92 ultimately contributing to alleviate effects of extreme climatic events as heat waves in  
93 agroecosystems. We hypothesized that intercropped species promote a (i) different legacy  
94 effect on soil microbial processes in comparison to single species, and accordingly (ii)  
95 influence the resistance and resilience of soils. We focused on nitrification and respiration  
96 microbial processes (potential nitrification, substrate-induced respiration and basal  
97 respiration) as key processes of the N and C cycles in environmental and plant productivity  
98 issues. To achieve this goal, we generated a novel plant legacy in a calcareous cambisol,  
99 through a greenhouse experiment conducted with a soil collected in a Mediterranean site near

100 Montpellier, France. Soil was collected 3 months after a pea crop, sieved at 1-cm to be  
101 homogenized and ridded of any coarse organic material, then air dried and stored at ambient  
102 temperature before use. In 2017, soil was mixed with perlite and conditioned by one complete  
103 vegetative cycle of two crop species grown as single crops (monocultures), (i) white lupin  
104 (*Lupinus albus* L.) and (ii) bread wheat (*Triticum aestivum* L.), as well as the mixture of both  
105 species (intercrop). Finally, we calculated the resistance and resilience indices (*sensu* Orwin  
106 and Wardle (2004)) after a heat disturbance.

107

## 108 **2. Material and methods**

### 109 **2.1. Soil origin and greenhouse experiment before heat stress experiment**

110 Soil originated from a plot of the INRA experimental station localized at Manguio  
111 (3°59'6"E, 43°37'13"N, 12 m altitude) in French littoral Mediterranean region. The soil was a  
112 calcareous cambisol with a clay loam texture according to USDA classification (clay 294 g  
113 kg<sup>-1</sup>, fine silt 200 g kg<sup>-1</sup>, coarse silt 233 g kg<sup>-1</sup>, fine sand 156 g kg<sup>-1</sup> and coarse sand 118 g kg<sup>-1</sup>),  
114 which had developed on an alluvial calcareous parent material, typical of the South East  
115 plains of Montpellier. The regional climate is typically Mediterranean with surface regularly  
116 exposed to drying-rewetting cycles due to irregular rain events mainly concentrated in  
117 Autumn and Spring. The annual average rainfall at this site during the growing season, from  
118 September to June, over the last 20 years was 598 mm. Recorded average soil temperatures at  
119 10 cm depth since 2012 demonstrated that plot had never experienced temperatures higher  
120 than 38.4 °C. During the four months before soil sampling, 3 cycles of drought were recorded  
121 (*i.e.* 20 days with cumulative rainfall lower than 2mm, according to Tang et al. (2014)), while  
122 maximal air temperatures ranged from 22.8 °C (in September) to 34.7 °C (in August). The last  
123 precipitation episode (4.8 mm) preceding soil sampling occurred 5 days earlier. Soil was  
124 collected (0-15 cm depth) 3 months after pea harvest, air dried and sieved at 1-cm to

125 homogenize and remove coarse organic material, then finally stored at ambient temperature  
126 before use to preserve inherent soil quality (Salomé et al., 2014).

127 A pot experiment was then conducted in a greenhouse (Centre Mondial de  
128 l'Innovation – Roullier Group – Saint-Malo, France) from September to December 2017 with  
129 the soil previously described. Before being conditioned in the pots (4-dm<sup>3</sup>), the soil was re-  
130 sieved with a 4-mm mesh to remove any organic material and mixed with perlite to allow  
131 better conditions for plant root development. Pots were filled with substrate composed of 2 kg  
132 of soil and 0.2 kg of perlite. The characteristics of the substrate were clay 202 g kg<sup>-1</sup>, silt 454  
133 g kg<sup>-1</sup>, sand 343 g kg<sup>-1</sup>, total CaCO<sub>3</sub> 39 g kg<sup>-1</sup>, pH<sub>w</sub> 8.3, CEC<sub>Metson</sub> 15 cmol<sub>+</sub> kg<sup>-1</sup>, organic C  
134 10.4 g kg<sup>-1</sup>, total N 0.95 g kg<sup>-1</sup>, ammonium (N-NH<sub>4</sub><sup>+</sup>) 20 mg kg<sup>-1</sup>, nitrate (N-NO<sub>3</sub><sup>-</sup>) 39 mg kg<sup>-1</sup>.  
135 The WHC of the substrate corresponded to 28% of gravimetric humidity. For simplicity,  
136 substrate will be named herein as soil. The planting modalities of this experiment were: (i)  
137 monoculture of wheat (W) (*Triticum aestivum* L. cv. Lennox) at a density of 4 plants per pot,  
138 (ii) monoculture of white lupin (L) (*Lupinus albus* L. cv. Feodora) at a density of 2 plants per  
139 pot, (iii) intercropping of both species (WL) at half of the density for each corresponding  
140 monoculture (2 wheat and one lupin plants), and (iv) an unplanted soil (U). Each planted and  
141 unplanted treatments was replicated 4 times. At maturity, plants (shoots and roots) were  
142 harvested and the soil was carefully separated from the roots by gently shaking followed by  
143 hand sorting and then kept at 4°C in the dark until microcosm experiment (16 weeks).

144

## 145 **2.2. Heat stress microcosm experiment**

146 A total of 128 microcosms were prepared (4 replicates x 4 planting treatments x 2  
147 temperatures x 4 sampling dates) containing 50 g of equivalent dry soil from greenhouse  
148 experiment, and placed in 1-dm<sup>3</sup> glass jars hermetically sealed by a rubber gasket. Soils of  
149 microcosms were rehydrated at 100% of the water holding capacity and pre incubated at 12°C



150 for 5 days in order to ensure uniform initial experimental conditions. Afterwards, half of the  
151 microcosms (64 samples) were incubated at 28°C and the remaining half at 48°C,  
152 corresponding to control (C) and heat stress (S) conditions, respectively. All microcosms were  
153 placed into darkness in laboratory incubators (KBWF 720, Binder, Germany) with a precision  
154 of 0.4 °C. In order to i) avoid any drying-rewetting cycles during the experiment altering the  
155 C and N cycles including nitrification (Fierer and Schimel, 2002) and ii) ensure precise  
156 estimation of nitrification potential assays conducted with soil slurries (see section 2.3), we  
157 kept soil moisture at 100% WHC. To ensure no variations of water regime in microcosms, a  
158 20-cm<sup>3</sup> polypropylene container filled with water was placed in each sealed microcosm.  
159 Microcosms were regularly weighted to check any soil moisture changes. After two days of  
160 heat disturbance at 48°C, the stressed microcosms were transferred back at 28°C. Each  
161 microcosm contained an 1M NaOH trap for basal respiration determination (see section 2.3).  
162 At each sampling date (T2, T7, T16 and T28 corresponding to 2, 7, 16 and 28 days after the  
163 end of the heat stress), samples were immediately split into 4 subsamples for all subsequent  
164 soil analyses (substrate-induced respiration (SIR), nitrification enzyme activity (NEA), soil  
165 NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> measurements, and soil water content). Measurements of SIR and NEA were  
166 performed on fresh soils within 2 days after sampling, whereas soil samples for mineral N  
167 measurements were frozen (-20°C) until analysis.

168

### 169 **2.3. Soil microbial and chemical analyses**

170 Basal respiration (BR) traps were collected and replaced at each sampling date. An  
171 aliquot of 1-cm<sup>3</sup> of NaOH was back-titrated with 0.1 M HCl, after adding 2.5-cm<sup>3</sup> of BaCl<sub>2</sub>  
172 solution (30 %) to precipitate the Na<sub>2</sub>CO<sub>3</sub> issued from soil CO<sub>2</sub> respiration. Results of BR  
173 were expressed in µg C-CO<sub>2</sub> day<sup>-1</sup> g<sup>-1</sup> of dry soil. SIR measurements were performed on 10 g  
174 of equivalent dry soil according to Patra et al. (2005), in presence of glucose (1.2 mg C-

175 glucose g<sup>-1</sup> dry soil). A CO<sub>2</sub> accumulation kinetic was measured using a gas chromatograph  
176 (µGC R3000, SRA instruments, France) at 28°C. SIR was expressed in µg C-CO<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup> of  
177 dry soil. NEA was measured with 3 g of equivalent dry soil according to Patra et al. (2005).  
178 Briefly, a (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution (50 µg N-NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> dry soil) was added to each soil sample.  
179 Samples were placed at 28°C, shaken at 140 rpm to ensure aerobic conditions during 10  
180 hours, and NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> production was measured every two hours using a photometer  
181 (Smartchem 200, AMS Alliance, France). NEA was expressed in µg of N-(NO<sub>2</sub><sup>-</sup>+NO<sub>3</sub><sup>-</sup>) h<sup>-1</sup> g<sup>-1</sup>  
182 of dry soil. Finally, soil ammonium (N-NH<sub>4</sub><sup>+</sup>) and soil nitrate (N-NO<sub>3</sub><sup>-</sup>) were extracted from a  
183 soil:solution ratio of 1:2 with 1M KCl solution after a 30 min of contact at 140 rpm, followed  
184 by 30 min of rest. Supernatant were filtered at 0.45 µm and mineral N concentrations were  
185 estimated with an Analytical Discrete Multi-Chemistry Analyzer (AQ2, SEAL Analytical,  
186 UK). Soil mineral N content were expressed as mg N-NH<sub>4</sub><sup>+</sup> and mg N-NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> of dry soil.

187

#### 188 **2.4. Percentage change relative to control**

189 In order to investigate recovery rate, percentage change of values in stressed samples  
190 relative to those in the controls, at each sampling date, were calculated as follows (Bérard et  
191 al., 2011; Chaer et al., 2009):

192

$$193 \text{ relative change (\%)} = \left[ \left( \frac{S_i}{C_i} \right) - 1 \right] \times 100 \quad (1)$$

194

195 where S<sub>i</sub> and C<sub>i</sub> are the variable values in the stressed and control soil samples, respectively,  
196 at sampling date i (*i.e.* T2, T7, T16 or T28).

197

## 198 2.5. Soil microbial resistance and resilience indices

199 To investigate only potential differences among soils with specific plant legacy (W, L,  
200 WL), unplanted (U) soil was not considered for calculation of resistance and resilience indices  
201 (see section 3.3). Resistance (RS) and resilience (RL) of soil microbial activities to a heat  
202 stress were assessed by the following indices (Orwin and Wardle, 2004):

203

$$204 \quad RS = 1 - \frac{2 \times |D_2|}{C_2 + |D_2|} \quad (2)$$

205

206 where  $D_2$  is the difference between the values of the response variable (BR, SIR or NEA) in  
207 control ( $C_2$ ) and stressed conditions ( $S_2$ ), 2 days after the end of the disturbance;

208

$$209 \quad RL = \frac{2 \times |D_2|}{|D_2| + |D_x|} - 1 \quad (3)$$

210

211 where  $D_x$  is the difference between the control and disturbed soil on 7, 16 or 28 days after the  
212 end of the disturbance.

213 Both indices are bounded by  $-1$  and  $+1$ , where an index of  $1$  indicates full resistance  
214 (no effect of the disturbance on the response variable) or full resilience (same values of the  
215 response variable in control and stressed conditions). For resistance, an index value of  $0$   
216 indicates either  $100\%$  decrease or increase in the response variable compared to control, while  
217 a negative index represents a modification greater than  $100\%$  in the response variable  
218 compared to the control (*i.e.* the soil has low resistance). In regard to resilience, an index of  $0$   
219 indicates that the disturbed soil has either not recovered after the end of the disturbance (*i.e.*  
220  $D_x = D_2$ ) or that the stressed soil is different to the control by the same amount, but in the  
221 opposite direction (*i.e.*  $D_x = -D_2$ ). A resilience index between  $0$  and  $1$  indicates that the

222 response variable has not fully recovered, whereas a negative index value indicates that the  
223 absolute value of  $D_x$  is higher than the absolute value of  $D_2$  (*i.e.* the soil has low resilience).

224

## 225 **2.6. Statistical analyses**

226 The effects of planting legacy (*i.e.* both planted (W, L, WL) and unplanted (U)  
227 modalities) and heat stress on all soil variables were tested for each sampling date by two-way  
228 ANOVA (planting legacy x heat stress). Afterwards, one-way ANOVA was performed in  
229 absence of interaction to test differences among treatments. The effect of specific plant legacy  
230 (*i.e.* only planted modalities – W, L, WL) on resistance and resilience indices, as well as the  
231 effect of planting legacy on percentages of change in microbial activities relative to controls  
232 were also tested by one-way ANOVA, within each sampling date. Significant differences  
233 between means were tested by Tukey's multiple comparison tests ( $p < 0.05$ ). Normality of  
234 residuals and homogeneity of variance were tested by the Shapiro and Levene's tests,  
235 respectively. When necessary, data for microbial activities were squared root-transformed.  
236 Principal component analysis (PCA) were performed with all chemical and microbial soil  
237 variables. Within a PCA, coordinates of the individuals on each axis were used for testing  
238 differences between groups (*i.e.* levels of a factor) by one-way ANOVA or Kruskal-Wallis  
239 tests in case of non-normality. Tukey's multiple comparison tests ( $p < 0.05$ ) or Wilcoxon rank  
240 sum tests ( $p < 0.05$ ) were then performed accordingly. Finally, a redundancy analysis (RDA)  
241 was performed to assess the effect of all soil microbial and chemical variables (SIR, NEA,  
242 BR,  $N-NO_3^-$  and  $N-NH_4^+$  contents) on resistance (at T2) and resilience (at T16) indices.  
243 Statistical analyses were performed with R software v. 3.5.1 (R Core Team, 2018). Packages  
244 used for the ANOVA, Tukey's test, PCA and RDA analysis were: *car*, *agricolae*,  
245 *FactoMineR* and *vegan*, respectively.

246

### 247 **3. Results**

#### 248 **3.1. Contrasted effects of planting and heat stress on microbial activities and soil** 249 **mineral N content**

250 The first two axes of the PCA performed on full data set (BR, SIR, NEA, N-NH<sub>4</sub><sup>+</sup>, N-  
251 NO<sub>3</sub><sup>-</sup> measurements for all sampling dates and stress conditions) explained 74.2% of the total  
252 variance (Fig. 1). BR, N-NH<sub>4</sub><sup>+</sup>, NEA and N-NO<sub>3</sub><sup>-</sup> are the major variables contributing to the  
253 first axis while SIR was strongly and negatively correlated to the second axis (Table A.1).  
254 Both planting legacy (W, L, WL, U) and sampling dates combined with control (C) and stress  
255 (S) conditions had a significant impact on values of samples (Kruskal-Wallis  $p < 0.001$ ; Table  
256 A.2 and A.3). Along axes 1 and 2, unplanted soil was significantly different from those with  
257 W, L and WL legacies (Wilcoxon test,  $p < 0.001$ ), with centroids showing negative and  
258 positive values, respectively (Fig. 1a). Considering sampling dates combined with control and  
259 stress conditions (Fig. 1b), earlier dates were significantly distinct between control and heat  
260 stress. However, at these earlier dates, the unplanted stressed soils were closer to their  
261 controls, suggesting a greater resistance to heat stress. Interestingly, during the period of  
262 resilience, the trajectories of the control and stressed soils evolved along the first axis, and at  
263 the later dates (T16, T28) stressed and control soils were not significantly different from each  
264 other. At all dates combined, a clear difference was observed between stress and control  
265 conditions along the second axis (Wilcoxon test,  $p < 0.001$ ), with all centroids of the stressed  
266 samples showing positive values, whereas centroids of controls showed negative values.

267

#### 268 **3.2. Legacy effect of planting on soil C- and N- related activities in response to heat** 269 **stress**

270 Planting legacy was significant for BR values at the first two dates of measure ( $p <$   
271  $0.001$ ; Table 1 and Fig C.1). At these dates, L soils clearly differed from unplanted soils, with  
272 L soils showing values 1.9-fold greater, when combining control and stress conditions. The  
273 planting legacy was still significant at T16, but no effect was observed at T28. An important  
274 effect of heat stress was also observed on BR values, particularly at T2 and T7 ( $p < 0.01$  and  $p$   
275  $< 0.001$ , respectively). At the two first dates, stressed soils presented values 1.35-fold greater  
276 than control soils on average. At T16 the difference between stressed and control soils was  
277 lower, whereas no significant difference between stressed and control soils was observed at  
278 T28.

279 In regard to SIR, planting legacy ( $p < 0.001$ ), heat stress ( $p < 0.001$ ) and planting x  
280 stress interaction ( $p < 0.01$  at T28 and  $p < 0.001$  at other dates) had a strong effect on SIR  
281 values (Table 1 and Fig. C.2). Significant differences between planting legacies of control  
282 soils were observed at all sampling dates. However, heat stress alleviated these differences.  
283 For instance, at T2 (control), SIR means ranked as follows:  $L > WL > W = U$ , whereas no  
284 differences were noticed after stress. One should note that L soils exhibited the greatest SIR  
285 values, while unplanted soils presented the lowest. Conversely to BR, stressed conditions  
286 exhibited a dramatic decrease of SIR activities compared to the controls, whatever the  
287 sampling date. The most important difference was observed at the first date, when stressed  
288 soils were 3.6-fold lower than controls. The L soils were particularly altered at the first date  
289 because SIR activity were 5-fold lower than controls. This difference between stress and  
290 control conditions was also present at the other dates, but decreased with time.

291 A very strong effect of planting legacy on NEA values was observed, whatever the  
292 sampling date ( $p < 0.001$ ; Table 1 and Fig. C.3). Unplanted soils exhibited the greatest values  
293 compared to planted soils (2.2- to 3.8-fold greater than W, L and WL). The heat stress effect  
294 on NEA was particularly observed at T2 and T7 ( $p < 0.001$ ). At these dates, a significant

295 decrease in NEA values was observed between controls and stressed soils, but to a lesser  
296 extent than that observed for SIR. At T16, heat stress had no effect on NEA, whereas at T28,  
297 a significant effect of heat stress ( $p < 0.05$ ) and interaction of heat stress and planting legacy  
298 ( $p < 0.001$ ) were observed. An interesting dynamic was observed from T16 to T28, as NEA  
299 values increased particularly for stressed (S) soils, and to a lesser extent for control (C) soils.  
300 Indeed, at T28, there was a final ranking with L values becoming closer to U values ( $U_C >$   
301  $U_S = L_S \geq L_C = W_S = WL_S > WL_C = W_C$ ).

302

### 303 **3.3. Resistance and resilience of soil microbial activities**

304 Recovery rates are illustrated in Fig. 2. Just after heat disturbance (T2), SIR activity  
305 was severely altered (-70%, on average) compared to NEA (-46%) and BR (+36%). The most  
306 important decrease at T2 was observed for L soils, with a reduction of 79% in SIR relative to  
307 control samples. At this date, L soil also exhibited the largest increase in BR (+59%) relative  
308 to control samples. Conversely, for NEA, T2 showed the lowest decrease for the unplanted  
309 soil (-15%). Focusing on the other dates for SIR, significant differences between percentages  
310 of change were only observed at T7, as follows:  $U > W = WL > L$  (Table B.1). For NEA in  
311 T16, percentages of change for WL and L, but not for W soils, switched from negative to  
312 positive. Significant differences between values were observed for NEA at T28. The W soils  
313 exhibited greater NEA values in stressed soils relative to controls and compared to WL and L  
314 (Table B.1).

315 Indices of resistance (RS) and resilience (RL) are presented in Table 2. Significant  
316 differences in resistance between soils were only observed for SIR ( $p < 0.05$ ). The W soils  
317 exhibited the highest RS index, which was significantly different from L, but not from WL.  
318 For resilience indices, significant differences between soils for NEA at T16 ( $p < 0.01$ ) and for  
319 SIR at T28 ( $p < 0.05$ ) were observed. The RL indices for NEA of both WL and W soils were

320 greater than that of L soils. The RL indices of L soils were negative at T7, T16, and T28,  
321 meaning a very low resilience of NEA. For SIR at T28, the highest RL indices were found for  
322 L and WL soils.

323 The ordination plots of RDA performed on resistance (RS), at T2, and resilience (RL),  
324 only at T16, indices clearly discriminated the unplanted soil (Fig. 3 a,b). The resistance  
325 indices of NEA were positively associated to NEA and  $\text{N-NO}_3^-$  concentrations in soils, and  
326 negatively to  $\text{N-NH}_4^+$  contents. The RS indices of SIR were negatively associated to BR.  
327 Considering resilience indices of NEA, these latter were negatively linked to NEA and N-  
328  $\text{NO}_3^-$ , whereas BR resilience indices were negatively linked to SIR and RL of SIR. Finally,  
329 considering soils with only specific plant legacies (W, L, WL), L soils slightly distinguished  
330 from the other soils on RDA plots for resistance, and strongly differed from the other soils for  
331 resilience. Thus, these results confirmed percentages of change (Fig. 2) and RS and RL  
332 indices (Table 2). The L soils were clearly different, being characterized by lower RS of SIR  
333 and RL of NEA (Fig. 3 a,b).

334

## 335 **4. Discussion**

### 336 **4.1. Legacy effect of plants on soil microbial activities under control conditions**

337 Marked differences were observed between unplanted and planted soils, suggesting  
338 that plants were responsible for a legacy effect. Unplanted soils always exhibited greater  
339 values of NEA than those for W, L and WL. As plants are able to shape N-related microbial  
340 communities (Moreau et al., 2015; Pivato et al., 2017), it occurred that all planted treatments  
341 had a negative legacy effect on the activity of nitrifiers. This negative legacy effect may be  
342 attributed to the competition between plants and microorganisms for N (Cantarel et al., 2015;  
343 Kuzyakov and Xu, 2013; Xu et al., 2011). According to Schimel and Bennett (2004) plants  
344 are effective competitors for ammonium which is also the substrate for nitrifiers, reducing



345 their abundance and activity. Some plant morphological and/or physiological characteristics  
346 can also affect the diversity of nitrifiers (Cantarel et al., 2015; Legay et al., 2014; Pommier et  
347 al., 2018), or inhibit nitrification (Subbarao et al., 2012). According to these recent results,  
348 one should hypothesize that nitrifiers community is shaped by the environment (Erguder et  
349 al., 2009; Prosser and Nicol, 2012; Zhalnina et al., 2012), or by plant N-use strategy (Thion et  
350 al., 2016). Thereby, we can reasonably suggest a plant legacy effect on nitrification. A great  
351 contrast between unplanted and planted soils was also observed for BR and SIR values, with  
352 lower values of U compared to W, L and WL. This is consistent with Orwin and Wardle  
353 (2005), who observed lower values of soil BR and SIR for bare soil compared to the planted  
354 grass treatments. As large amounts of C-compounds can be released by roots as rhizodeposits,  
355 e.g. sugars, organic acids, amino acids, mucilage, root or border cells (Kuzyakov and  
356 Blagodatskaya, 2015; Philippot et al., 2013), an increase in microbial biomass and activities  
357 (Tang et al., 2014) is expected in planted compared to unplanted soils.

358         Considering only planted soils, we also observed different legacy effects among the  
359 modalities (W, L, WL), which is consistent with our first hypothesis. Indeed, except for the  
360 first sampling date, greater values of NEA were observed for L soil, compared to W and WL,  
361 *i.e.* a stronger legacy effect for lupin than wheat. Le Roux et al. (2013) also reported a  
362 positive relationship between the presence of legumes and NEA, which was explained by the  
363 build-up of nitrifying communities in response to greater N-NH<sub>4</sub><sup>+</sup> availability in soils,  
364 probably due to the release of N derived from Rhizodeposition (NdfR) by legumes (Duchene  
365 et al., 2017). In addition, legumes are usually considered less competitive for soil mineral N  
366 compared to cereals, which could suggest a higher availability of mineral N in soils under  
367 legumes (Mallarino and Wedin, 1990). L soils were also remarkable regarding to C-related  
368 microbial variables, showing the greatest values of SIR whatever the date of measurement, as  
369 well as the highest values of BR at T2 and T7. White lupin is well known to exude large

370 quantities of organic anions, like citrate (Dissanayaka et al., 2015; Veneklaas et al., 2003;  
371 Wang et al., 2010), compared to cereals and other legumes (Wang et al., 2016). Such exudates  
372 may have promoted SIR and BR in our study. This positive legacy effect of white lupin on C-  
373 related microbial variables compared to intercropped wheat and white lupin is however not in  
374 agreement with the commonly observed positive effect of plant diversity on soil microbial  
375 biomass and respiration (Chen et al., 2019; Eisenhauer et al., 2017; Tang et al., 2014).  
376 Accordingly, one should note that legume legacies may be further investigated in  
377 agroecosystems in order to decipher their potential role in rotation or in intercropping.

378

#### 379 **4.2. Effects of experimental conditions on C-related microbial activities: Basal** 380 **Respiration and Substrate-Induced Respiration**

381 Amongst response variables, SIR was the most affected by heat stress (T2). Samples  
382 affected by stress presented reductions varying from 65% to 79% relative to controls. As SIR  
383 may be considered as a proxy of the active microbial biomass (Anderson and Domsch, 1978;  
384 Orwin and Wardle, 2005), these results suggest a dramatic decrease in microbial biomass by  
385 the disturbance. The mortality of temperature-sensitive microbes has been observed in other  
386 studies where heat and combined heat-drought stresses were applied (Bérard et al., 2011;  
387 Guillot et al., 2019; Hamdi et al., 2011). The effect of temperature on SIR activities was also  
388 evident when comparing the heat stress responses of planted soils. In our study, L soils  
389 presented the highest values of SIR activity under control conditions, but after heat stress, SIR  
390 activity decreased to levels comparable to the other treatments. Then, we concluded that an  
391 important microbial biomass in L soils did not confer a better resistance to the heat  
392 disturbance. This finding was consistent with Guillot et al. (2019), who observed that  
393 Mediterranean soils exhibiting the highest microbial biomass were not systematically

394 associated with a higher resistance. Conversely, W soils which exhibited the lowest values of  
395 SIR, presented the greatest resistance index (Table 2), which suggests a greater proportion of  
396 more heat-tolerant microbes within this modality. A weaker plant legacy effect on C-related  
397 microbial activities due to inherent quality of this type of soil (Salomé et al., 2014) was  
398 expected. However, recent studies pointed out that combined heat and drought stresses were  
399 able to significantly alter the microbial biomass (Bérard et al., 2011; Guillot et al., 2019), or  
400 acclimate of soil communities to heat waves (Bérard et al., 2012) in Mediterranean soils. In  
401 our study, the plant-induced effects may be partly due to a soil microbes protection by plant  
402 liberation of exopolysaccharides (Kumar and Verma, 2018). Soils with L and WL legacies  
403 presented the highest resilience indices and recovery rates for SIR. The rapid progression of L  
404 and WL soils over time reduced final differences between these soils and W at T28 (Fig. 2).  
405 Despite strongly affected by heat stress, the better resilience of L and WL suggest that heat  
406 tolerant microbes rapidly proliferated. The greater resilience of L soils could be attributed to a  
407 greater C availability derived from death of the most sensitive microorganisms (Bérard et al.,  
408 2015), but also by transformation of soil organic matter as induced by the elevated  
409 temperature (Bérard et al., 2011; Hamdi et al., 2011). It should be noted that a positive  
410 priming effect due to lupin legacy may also contribute to C availability in L soils conferring a  
411 greater resilience of L and WL (Wang et al., 2016).

412

### 413 **4.3. Effects of experimental conditions on N-related microbial activities: Nitrification**

#### 414 **Enzyme Activity (NEA)**

415 NEA was affected by heat stress, but to a lesser extent than SIR, while comparable to  
416 BR (Fig. 2). Both temperature and moisture are considered as major environmental factors  
417 affecting microbial activities and structure. Considerable debate stands in literature about  
418 optimal temperature for soil nitrifiers (Taylor et al., 2017) with scarce studies dealing with the

419 effect of nitrification rate at temperatures higher than 40°C. Indigenous nitrifiers have  
420 different optimal temperatures depending on native region (Avrahami and Conrad, 2003).  
421 Albeit > 40°C temperatures may be experienced in Mediterranean regions, the duration of  
422 continuous heat stress, *i.e.* 2 days, is not reproducible under Mediterranean field conditions.  
423 French regulation for Mediterranean region defines severe heat waves as a period lasting 2-3  
424 days, and characterized by elevated temperature > 35°C and > 21°C during day and night  
425 respectively. Thus, night cooling decreases temperature stress even during severe heat waves,  
426 which was not the case during our experiment. Then, 2 days on continuous applied 48°C  
427 temperature have to be considered as a stress while not fully realistic of field conditions. One  
428 should note our moisture content of soils (100% of WHC) during the experiment. Nitrification  
429 rate is affected by drying-rewetting cycles (Fierer and Schimel, 2002) such as mineralization  
430 (Zhang et al., 2017). Fierer and Schimel (2002) depicted an increase of nitrification potentials  
431 in frequently drying-rewetting stress treatments. In their experiment, they do not succeed to  
432 attribute any changes of nitrification rates when soil moisture increased from 35% to 50%  
433 WHC. Mathieu et al. (2006) estimated no significant differences of nitrification rates using  
434 <sup>15</sup>N tracer under 150% WHC (saturated) and 75% of WHC (unsaturated conditions) in soil  
435 incubations with 2 mm aggregates. We thus assumed that our uncommon WHC did not alter  
436 potential nitrification rates. However, possible restricted oxygen availability due to O<sub>2</sub>  
437 consumption by mineralization during the incubation period could be pointed out. A O<sub>2</sub>  
438 restriction may finally alter nitrifier's community growth rate and activity at earlier dates of  
439 our experiment. As nitrification potentials are estimated under shaking to provide aeration,  
440 oxygen limitation at earlier dates (T2 and T7) may have been less pronounced than at T16.  
441 One should note that our goal was to test if planted treatments conferred different legacy  
442 effects on potential NEA in response to a heat disturbance. Despite discussed limitations of  
443 our experiment, we discussed significant differences while keeping in mind potential

444 limitations of field transposition of our results. We demonstrated that WL legacy exhibited  
445 comparable resistance and resilience than those with W legacy. The WL soil was the less  
446 affected by the heat stress initially (T2), with a reduction by only 31% in NEA relative to the  
447 control (Fig. 2), but this trend was not reflected on resistance indices, probably due to the high  
448 variability among replicates. However, in terms of resilience, at T16, significant differences  
449 between indices were verified, with WL and W soils exhibiting the highest resilience indices.  
450 Pivato et al. (2017) estimated a greater abundance of nitrifiers in soils from plant associations,  
451 whereas Le Roux et al. (2013) and Zhang et al. (2015) reported a negative effect of plant  
452 richness on the abundance of ammonia oxidizers. These latter articles suggested that plant  
453 identity rather than plant diversity can affect nitrifier community abundances. Considering  
454 gene-based associated groups of nitrifiers namely Ammonia Oxidizing Archaea and Bacteria  
455 (AOA and AOB), Thion et al. (2016) estimated greater AOA abundance in the rhizosphere of  
456 exploitative grass species, like wheat. Moreover, Taylor et al. (2017) considered that AOA  
457 nitrifiers are more tolerant to higher temperatures. Thus, better resilience in planting  
458 modalities containing wheat could be attributed to these published results. Finally, one should  
459 also note the interesting NEA dynamic observed at later stages in our study. A strong increase  
460 in NEA activities of all soils was observed at T28, compared to T16, for both control and heat  
461 stress conditions (Table 1). Such increase in NEA at this final date could be explained by a  
462 “boost” in the activities of nitrifiers after 30 days of incubation in optimal conditions. Since  
463 the growth rates of nitrifiers are recognized to be relatively slow (Robertson and Groffman,  
464 2015; Wertz et al., 2007), the interpretation of NEA resilience is then highly dependent of the  
465 final experimental dates. Accordingly, one should note that individual properties of  
466 microorganisms like dormancy, plasticity and growth rate (Shade et al., 2012) are also  
467 important components of resistance and resilience of soil microbial communities.

468

## 469 **Conclusions**

470           Adaptation to climate change of Mediterranean soils regularly exposed to heat  
471 waves may be possible through plant legacy effects. Intercropping confers more stability of  
472 soil microbial activities compared to single crop species when both C and N are investigated  
473 together due to individual benefits of each plant type. Finally, additional studies are essential  
474 to investigate if, i) prolonged plant presence will allow a more persistent effect on soils, and if  
475 ii) longer period of incubation would be able to detect full recovery of soil microbial activities  
476 over time. These results can provide useful information for designing cropping systems, in  
477 particular crop rotations and/or intercropping to capitalize on different legacy effects of  
478 various crops.

479

## 480 **Acknowledgements**

481           This work was supported by Centre Mondial de l'Innovation - CMI (Roullier Group).  
482 Authors warmly acknowledge Dr. Jean-Claude Yvin and Dr. Mustapha Arkoun from CMI for  
483 their support during this project. Authors want to thank Mrs. Béatrice Trinkler for her  
484 technical support during incubation period and for soil chemical analyses. We also thank the  
485 “Activités Microbiennes dans l'Environnement” platform (AME platform, Université Lyon1,  
486 UMR 5557) and particularly Mr. Jonathan Gervaix for supervising and measuring SIR and  
487 NEA microbial activities with Ms. Marie Seiller. We are also grateful to the anonymous  
488 reviewers for their comments improving our article.

489

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705 **Figure and table captions**

706

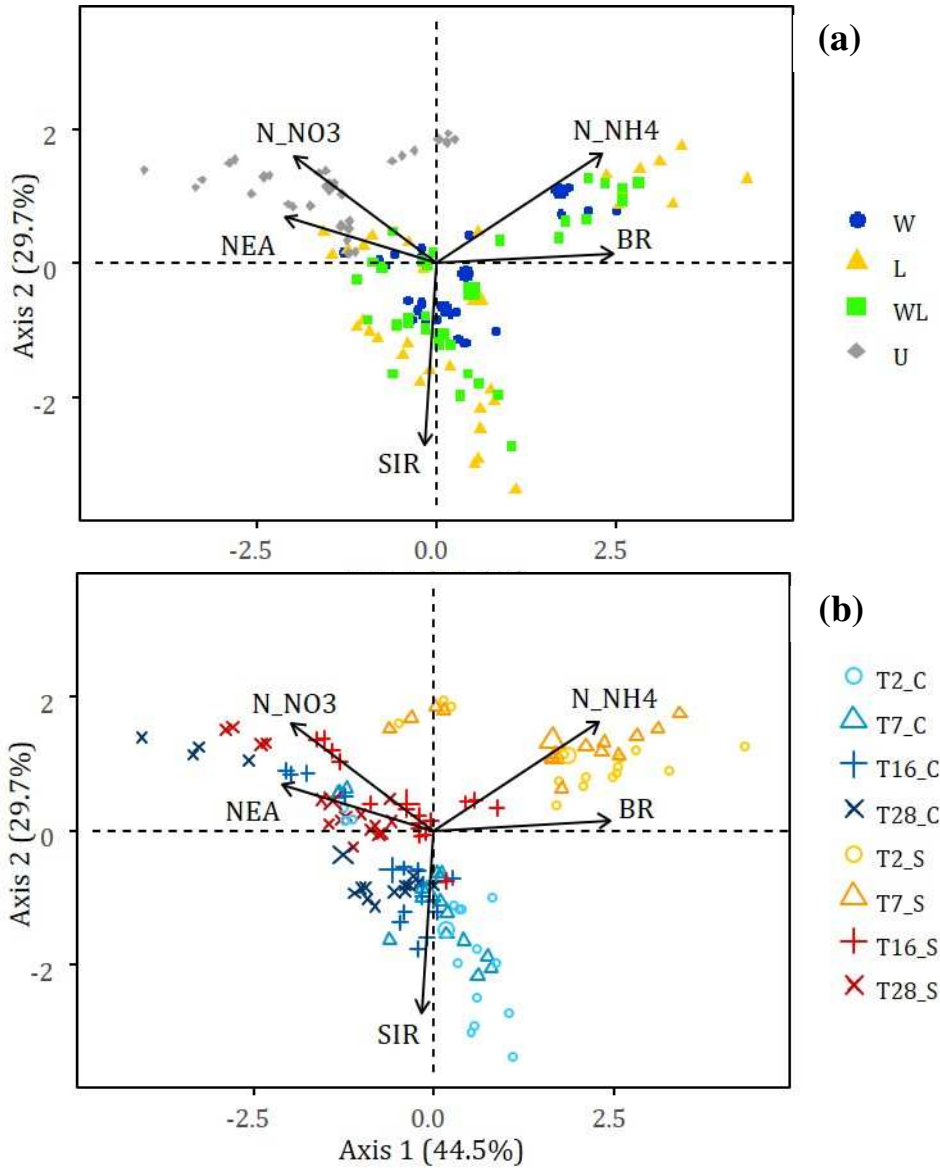
707 **Fig. 1.** Principal component analysis (PCA) performed on basal respiration (BR), substrate-  
708 induced respiration (SIR), nitrification enzyme activity (NEA) and soil mineral N content as  
709 nitrate (N-NO<sub>3</sub><sup>-</sup>) and ammonium (N-NH<sub>4</sub><sup>+</sup>). Symbols stand for planting legacy (W – wheat, L  
710 – lupin, WL – wheat + lupin, U – unplanted) (a) and sampling dates (T2, T7, T16 and T28 –  
711 2, 7, 16 and 28 days after the end of the heat stress) within control (C) and heat stress (S)  
712 conditions (b). Centroids are represented by larger symbols.

713 **Fig. 2.** Percentages of change in microbial activities (BR – basal respiration, SIR – substrate-  
714 induced respiration, NEA – nitrification enzyme activity) relative to controls along all  
715 sampling dates after the end of the heat stress (T2, T7, T16, T28). Symbols represent planting  
716 legacy (W – wheat, L – white lupin, WL – wheat + lupin, U – unplanted). Vertical bars  
717 indicate standard errors (n=4). Asterisks indicate significant differences between planting  
718 legacies within a sampling date (one-way ANOVA, followed by a Tukey post hoc test ( $p <$   
719 0.05)).

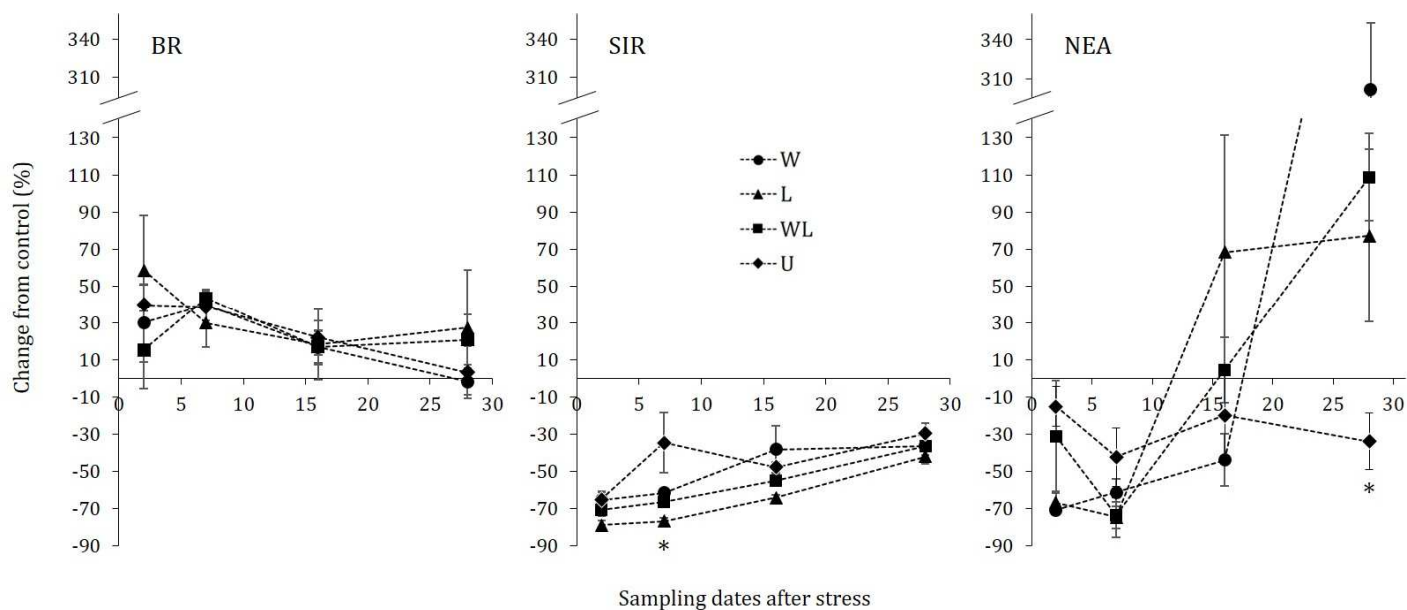
720 **Fig. 3.** Redundancy analysis (RDA) based on resistance (RS) (a) or resilience (RL) (b) indices  
721 of basal respiration (BR), substrate-induced respiration (SIR) and nitrification enzyme activity  
722 (NEA), constrained by BR, SIR, NEA, as well as soil mineral N content as nitrate (N-NO<sub>3</sub><sup>-</sup>)  
723 and ammonium (N-NH<sub>4</sub><sup>+</sup>). Resistance indices were calculated for T2, whereas resilience  
724 indices were calculated for T16 (2 and 16 days after the end of the heat stress). Groups of  
725 planting legacies are represented by W (wheat) in blue, L (lupin) in orange, WL (wheat +  
726 lupin) in green and U (unplanted) in red. The RDA was performed on center-reduced matrix.

727 **Table 1.** Basal respiration (BR – in  $\mu\text{g C-CO}_2 \text{ day}^{-1} \text{ g}^{-1}$  dry soil), substrate-induced respiration  
728 (SIR – in  $\mu\text{g C-CO}_2 \text{ h}^{-1} \text{ g}^{-1}$  dry soil) and nitrification enzyme activity (NEA – in  $\mu\text{g N-NO}_2^- +$   
729  $\text{N-NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  dry soil) of each planting legacy (W – wheat, L – lupin, WL – wheat+lupin, U  
730 – unplanted), within control and stress conditions, for each sampling date after the end of the  
731 heat stress (T2, T7, T16, T28). Values are the means of four replicates (n=4). Thresholds of  
732 probability of the two-way ANOVA (Planting x Stress) are indicated at the top of each  
733 variable-related data set. When significant interaction between factors (Planting x Stress) is  
734 present, different bold letters represent significant differences between 8 means (i.e. control  
735 and heat stress conditions combined; Tukey post hoc test ( $p <$  0.05)). Different lowercase  
736 letters represent significant differences within a single column, whereas different uppercase  
737 letters represent either significant differences between stress conditions (Control vs Stress), or  
738 between planting legacies with control and stress conditions combined (Tukey post hoc test ( $p$   
739  $<$  0.05)).

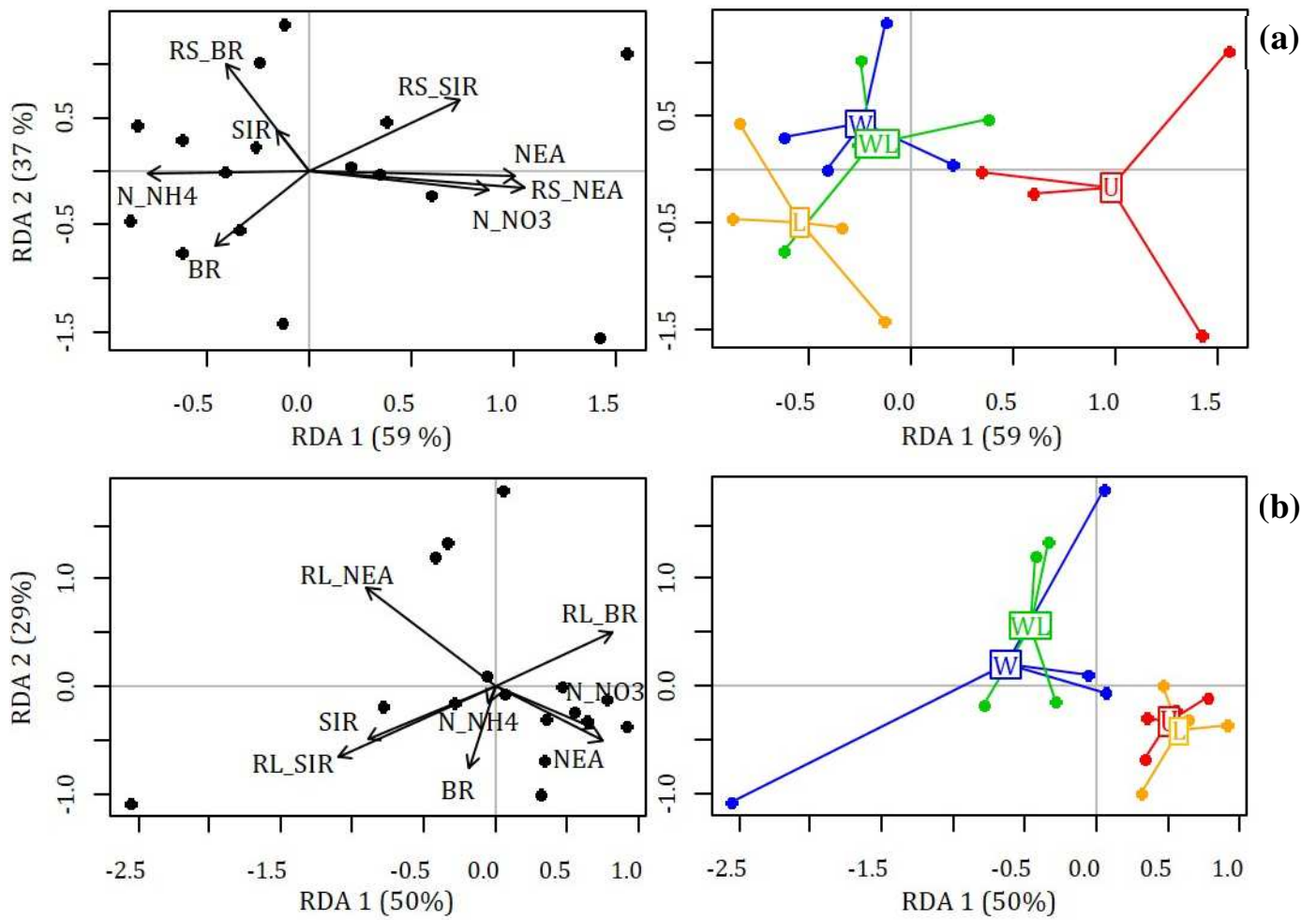
740 **Table 2.** Indices of resistance and resilience based on basal respiration (BR), substrate-  
741 induced respiration (SIR) and nitrification enzyme activity (NEA) for each specific plant  
742 legacy (W – wheat, L – lupin, WL – wheat+lupin). Resistance indices were calculated on T2  
743 date (2 days after the end of the heat stress), whereas resilience indices were calculated on T7,  
744 T16 and T28 dates of measure (7, 16 and 28 days after the end of the heat stress). Values are  
745 the means of four replicates (n=4). Probabilities of the one-way ANOVA are indicated at the  
746 bottom of each variable-related data set. Different letters represent significant differences  
747 within a column (Tukey post hoc test ( $p <$  0.05)).



**Fig. 1.** Principal component analysis (PCA) performed on basal respiration (BR), substrate-induced respiration (SIR), nitrification enzyme activity (NEA) and soil mineral N content as nitrate (N-NO<sub>3</sub><sup>-</sup>) and ammonium (N-NH<sub>4</sub><sup>+</sup>). Symbols stand for planting legacy (W – wheat, L – lupin, WL – wheat + lupin, U – unplanted) (a) and sampling dates (T2, T7, T16 and T28 – 2, 7, 16 and 28 days after the end of the heat stress) within control (C) and heat stress (S) conditions (b). Centroids are represented by larger symbols.



**Fig. 2.** Percentages of change in microbial activities (BR – basal respiration, SIR – substrate-induced respiration, NEA – nitrification enzyme activity) relative to controls along all sampling dates after the end of the heat stress (T2, T7, T16, T28). Symbols represent planting legacy (W – wheat, L – white lupin, WL – wheat + lupin, U – unplanted). Vertical bars indicate standard errors (n=4). Asterisks indicate significant differences between planting legacies within a sampling date (one-way ANOVA, followed by a Tukey post hoc test ( $p < 0.05$ )).



**Fig. 3.** Redundancy analysis (RDA) based on resistance (RS) (a) or resilience (RL) (b) indices of basal respiration (BR), substrate-induced respiration (SIR) and nitrification enzyme activity (NEA), constrained by BR, SIR, NEA, as well as soil mineral N content as nitrate ( $\text{N-NO}_3^-$ ) and ammonium ( $\text{N-NH}_4^+$ ). Resistance indices were calculated for T2, whereas resilience indices were calculated for T16 (2 and 16 days after the end of the heat stress). Groups of planting legacies are represented by W (wheat) in blue, L (lupin) in orange, WL (wheat + lupin) in green and U (unplanted) in red. The RDA was performed on center-reduced matrix.

Variable	Factor	T2		T7		T16		T28				
BR	Planting legacy	< 0.001***		< 0.001***		< 0.05*		ns				
	Heat stress	< 0.01**		< 0.001***		< 0.05*		ns				
	Planting x Stress	ns		ns		ns		ns				
			<u>Control<sup>B</sup></u>	<u>Stress<sup>A</sup></u>	<u>Control<sup>B</sup></u>	<u>Stress<sup>A</sup></u>	<u>Control<sup>B</sup></u>	<u>Stress<sup>A</sup></u>	<u>Control</u>	<u>Stress</u>		
	W	32.82 ab	41.39 ab	BC	21.52 b	29.79 bc	B	20.18 a	22.73 a	AB	20.07 a	19.31 a
	L	41.61 a	63.79 a	A	37.91 a	48.14 a	A	21.79 a	24.76 a	A	20.19 a	23.88 a
	WL	39.32 a	43.41 ab	AB	21.56 b	38.46 ab	B	20.99 a	24.37 a	A	15.78 a	18.37 a
	U	23.73 b	33.23 b	C	18.63 b	25.30 c	B	16.17 a	19.21 a	B	16.50 a	16.12 a
	Planting legacy	< 0.001***		< 0.001***		< 0.001***		< 0.001***				
	Heat stress	< 0.001***		< 0.001***		< 0.001***		< 0.001***				
Planting x Stress	< 0.001***		< 0.001***		< 0.001***		< 0.01**					
SIR		<u>Control</u>	<u>Stress</u>	<u>Control</u>	<u>Stress</u>	<u>Control</u>	<u>Stress</u>	<u>Control</u>	<u>Stress</u>			
	W	<b>5.77 c</b>	<b>2.01 d</b>	<b>4.33 c</b>	<b>1.65 e</b>	<b>4.21 bc</b>	<b>2.64 de</b>	<b>4.94 bc</b>	<b>3.15 de</b>			
	L	<b>12.42 a</b>	<b>2.59 d</b>	<b>9.11 a</b>	<b>2.10 e</b>	<b>7.69 a</b>	<b>2.75 de</b>	<b>6.92 a</b>	<b>4.00 cd</b>			
	WL	<b>9.24 b</b>	<b>2.78 d</b>	<b>6.56 b</b>	<b>2.20 de</b>	<b>5.38 b</b>	<b>2.41 de</b>	<b>5.38 b</b>	<b>3.43 de</b>			
	U	<b>4.98 c</b>	<b>1.72 d</b>	<b>3.50 cd</b>	<b>1.71 e</b>	<b>3.47 cd</b>	<b>1.81 e</b>	<b>3.82 d</b>	<b>2.67 e</b>			
NEA	Planting legacy	< 0.001***		< 0.001***		< 0.001***		< 0.001***				
	Heat stress	< 0.001***		< 0.001***		ns		< 0.05*				
	Planting x Stress	ns		ns		ns		< 0.001***				
			<u>Control<sup>A</sup></u>	<u>Stress<sup>B</sup></u>	<u>Control<sup>A</sup></u>	<u>Stress<sup>B</sup></u>	<u>Control</u>	<u>Stress</u>	<u>Control</u>	<u>Stress</u>		
	W	0.54 b	0.16 b	B	0.40 b	0.15 b	B	0.59 b	0.30 b	B	<b>0.65 c</b>	<b>2.69 bc</b>
	L	0.54 b	0.17 b	B	0.57 ab	0.12 b	B	0.74 b	1.12 ab	B	<b>2.83 bc</b>	<b>4.66 b</b>
	WL	0.42 b	0.28 b	B	0.40 b	0.11 b	B	0.43 b	0.45 ab	B	<b>1.28 c</b>	<b>2.63 bc</b>
	U	1.44 a	1.21 a	A	0.91 a	0.49 a	A	1.99 a	1.36 a	A	<b>8.63 a</b>	<b>5.38 b</b>
	Planting legacy	< 0.001***		< 0.001***		< 0.001***		< 0.001***				
	Heat stress	< 0.001***		< 0.001***		ns		< 0.05*				
Planting x Stress	ns		ns		ns		< 0.001***					

**Table 1.** Basal respiration (BR – in  $\mu\text{g C-CO}_2 \text{ day}^{-1} \text{ g}^{-1}$  dry soil), substrate-induced respiration (SIR – in  $\mu\text{g C-CO}_2 \text{ h}^{-1} \text{ g}^{-1}$  dry soil) and nitrification enzyme activity (NEA – in  $\mu\text{g N-NO}_2^- + \text{N-NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  dry soil) of each planting legacy (W – wheat, L – lupin, WL – wheat+lupin, U – unplanted), within control and stress conditions, for each sampling date after the end of the heat stress (T2, T7, T16, T28). Values are the means of four replicates (n=4). Thresholds of probability of the two-way ANOVA (Planting x Stress) are indicated at the top of each variable-related data set. When significant interaction between factors (Planting x Stress) is present, different bold letters represent significant differences between 8 means (i.e. control and heat stress conditions combined; Tukey post hoc test ( $p < 0.05$ )). Different lowercase letters represent significant differences within a single column, whereas different uppercase letters represent either significant differences between stress conditions (Control vs Stress), or between planting legacies with control and stress conditions combined (Tukey post hoc test ( $p < 0.05$ )).



Variable	Plant legacy	T2 (Resistance)	T7	T16	T28
BR	W	0.52	- 0.00	0.27	0.48
	L	0.34	0.31	0.55	0.27
	WL	0.60	- 0.22	0.20	0.26
<i>p-value (one-way ANOVA)</i>		<i>0.61</i>	<i>0.31</i>	<i>0.61</i>	<i>0.86</i>
SIR	W	<b>0.21 a</b>	0.16	0.47	<b>0.35 b</b>
	L	<b>0.11 b</b>	0.16	0.32	<b>0.54 a</b>
	WL	<b>0.17 ab</b>	0.19	0.37	<b>0.53 a</b>
<i>p-value (one-way ANOVA)</i>		<i>0.05*</i>	<i>0.87</i>	<i>0.63</i>	<i>0.04*</i>
NEA	W	0.17	0.21	<b>0.24 a</b>	- 0.63
	L	0.20	-0.03	<b>- 0.58 b</b>	- 0.48
	WL	0.29	- 0.10	<b>0.46 a</b>	- 0.68
<i>p-value (one-way ANOVA)</i>		<i>0.34</i>	<i>0.16</i>	<i>0.008**</i>	<i>0.56</i>

**Table 2.** Indices of resistance and resilience based on basal respiration (BR), substrate-induced respiration (SIR) and nitrification enzyme activity (NEA) for each specific plant legacy (W – wheat, L – lupin, WL – wheat+lupin). Resistance indices were calculated on T2 date (2 days after the end of the heat stress), whereas resilience indices were calculated on T7, T16 and T28 dates of measure (7, 16 and 28 days after the end of the heat stress). Values are the means of four replicates (n=4). Probabilities of the one-way ANOVA are indicated at the bottom of each variable-related data set. Different letters represent significant differences within a column (Tukey post hoc test ( $p < 0.05$ )).