



HAL
open science

Short-term plant legacy alters the resistance and resilience of soil microbial communities exposed to heat disturbance in a Mediterranean calcareous soil

Ana Beatriz de Oliveira, Amélie A. M. Cantarel, Marie Seiller, Alessandro Florio, Annette Berard, Philippe Hinsinger, Edith Le Cadre-Barthélemy

► 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-02365828>

Submitted on 21 Dec 2021

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.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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.**

3 Ana Beatriz de Oliveira^a, Amélie A. M. Cantarel^b, Marie Seiller^a, Alessandro Florio^b, Annette
4 Bérard^c, Philippe Hinsinger^d, Edith Le Cadre^{a,1}

5 ^aUMR SAS, AGROCAMPUS OUEST, INRA, bâtiment 13, 65 rue de Saint-Brieuc CS 84215,
6 35042, Rennes Cedex, France. E-mails: ana-beatriz.de-oliveira@inra.fr;
7 marie.seiller@agrocampus-ouest.fr

8 ^bLaboratoire d'Ecologie Microbienne LEM, UMR INRA 1418, UMR CNRS 5557, INRA,
9 CNRS, Université Lyon1, Université de Lyon, bâtiment G. Mendel, 43 boulevard du 11
10 novembre 1918, F-69622 Villeurbanne Cedex, France. E-mails: amelie.cantarel@univ-
11 lyon1.fr; alessandro.florio@univ-lyon1.fr

12 ^cUMR EMMAH, INRA, 228 route de l'Aérodrome, Domaine Saint-Paul – Site Agroparc, CS
13 40509, 84914, Avignon Cedex 9, France. E-mail: annette.berard@inra.fr

14 ^dUMR Eco&Sols, Univ Montpellier, INRA, CIRAD, IRD, Montpellier SupAgro, Bâtiment
15 12, 2 Place Viala, 34060, Montpellier Cedex 2, France. E-mail: philippe.hinsinger@inra.fr

16

17 ¹ corresponding author :

18 Bâtiment 8, UMR Sol Agro hydrosystème Spatialisation, 65 rue de Saint Brieuc, CS
19 84 215, 35 042 Rennes cedex, France

20 E-mail: edith.lecadre@agrocampus-ouest.fr

21 Tel.: +33 (0)2 23 48 54 74

22

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 Mauguio
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⁻¹, fine silt 200 g kg⁻¹, coarse silt 233 g kg⁻¹, fine sand 156 g kg⁻¹ and coarse sand 118 g kg⁻¹),
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-dm³), 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⁻¹, silt 454
133 g kg⁻¹, sand 343 g kg⁻¹, total CaCO₃ 39 g kg⁻¹, pH_w 8.3, CEC_{Metson} 15 cmol₊ kg⁻¹, organic C
134 10.4 g kg⁻¹, total N 0.95 g kg⁻¹, ammonium (N-NH₄⁺) 20 mg kg⁻¹, nitrate (N-NO₃⁻) 39 mg kg⁻¹.
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-dm³ 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-cm³ 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 NH₄⁺ and NO₃⁻ 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-cm³ of NaOH was back-titrated with 0.1 M HCl, after adding 2.5-cm³ of BaCl₂
172 solution (30 %) to precipitate the Na₂CO₃ issued from soil CO₂ respiration. Results of BR
173 were expressed in µg C-CO₂ day⁻¹ g⁻¹ 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⁻¹ dry soil). A CO₂ accumulation kinetic was measured using a gas chromatograph
176 (µGC R3000, SRA instruments, France) at 28°C. SIR was expressed in µg C-CO₂ h⁻¹ g⁻¹ of
177 dry soil. NEA was measured with 3 g of equivalent dry soil according to Patra et al. (2005).
178 Briefly, a (NH₄)₂SO₄ solution (50 µg N-NH₄⁺ g⁻¹ 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 NO₂⁻ + NO₃⁻ production was measured every two hours using a photometer
181 (Smartchem 200, AMS Alliance, France). NEA was expressed in µg of N-(NO₂⁻+NO₃⁻) h⁻¹ g⁻¹
182 of dry soil. Finally, soil ammonium (N-NH₄⁺) and soil nitrate (N-NO₃⁻) 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-NH₄⁺ and mg N-NO₃⁻ kg⁻¹ 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_i and C_i 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, $N\text{-NO}_3^-$ and $N\text{-NH}_4^+$ 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-NH₄⁺, N-
251 NO₃⁻ measurements for all sampling dates and stress conditions) explained 74.2% of the total
252 variance (Fig. 1). BR, N-NH₄⁺, NEA and N-NO₃⁻ 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 N-NO_3^- concentrations in soils, and
326 negatively to 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 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-NH₄⁺ 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 ¹⁵N 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 O₂
437 consumption by mineralization during the incubation period could be pointed out. A O₂
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

490 **References**

491 Anderson, J. P. E., and Domsch, K. H. (1978). A physiological method for the quantitative
492 measurement of microbial biomass in soils. *Soil Biology & Biochemistry* **10**, 215-221.

- 493 Avrahami, S., and Conrad, R. (2003). Patterns of community change among ammonia
 494 oxidizers in meadow soils upon long-term incubation at different temperatures. *Appl.*
 495 *Environ. Microbiol.* **69**, 6152-6164.
- 496 Bailey, J. K., and Schweitzer, J. A. (2016). The rise of plant–soil feedback in ecology and
 497 evolution. *Functional Ecology* **30**, 1030-1031.
- 498 Bérard, A., Ben Sassi, M., Kaisermann, A., and Renault, P. (2015). Soil microbial community
 499 responses to heat wave components: drought and high temperature. *Climate Research*
 500 **66**, 243-264.
- 501 Bérard, A., Ben Sassi, M., Renault, P., and Gros, R. (2012). Severe drought-induced
 502 community tolerance to heat wave. An experimental study on soil microbial processes.
 503 *Journal of Soils and Sediments* **12**, 513-518.
- 504 Bérard, A., Bouchet, T., Sévenier, G., Pablo, A. L., and Gros, R. (2011). Resilience of soil
 505 microbial communities impacted by severe drought and high temperature in the
 506 context of Mediterranean heat waves. *European Journal of Soil Biology* **47**, 333-342.
- 507 Brooker, R. W., Bennett, A. E., Cong, W.-F., Daniell, T. J., George, T. S., Hallett, P. D.,
 508 Hawes, C., Iannetta, P. P. M., Jones, H. G., Karley, A. J., Li, L., McKenzie, B. M.,
 509 Pakeman, R. J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C. A., Zhang,
 510 C., Zhang, F., Zhang, J., and White, P. J. (2015). Improving intercropping: a synthesis
 511 of research in agronomy, plant physiology and ecology. *New Phytologist* **206**, 107-
 512 117.
- 513 Cantarel, A. A. M., Pommier, T., Desclos-Theveniau, M., Diquélou, S., Schloter, M., and
 514 Poly, F. (2015). Using plant traits to explain plant–microbe relationships involved in
 515 nitrogen acquisition. *Ecology* **96**(3), 788-799.
- 516 Chaer, G., Fernandes, M., Myrold, D., and Bottomley, P. (2009). Comparative resistance and
 517 resilience of soil microbial communities and enzyme activities in adjacent native
 518 forest and agricultural soils. *Microb Ecol* **58**, 414-24.
- 519 Chen, C., Chen, H. Y., Chen, X., and Huang, Z. (2019). Meta-analysis shows positive effects
 520 of plant diversity on microbial biomass and respiration. *Nature communications* **10**,
 521 1332.
- 522 Cong, W. F., Hoffland, E., Li, L., Six, J., Sun, J. H., Bao, X. G., Zhang, F. S., and Van Der
 523 Werf, W. (2015). Intercropping enhances soil carbon and nitrogen. *Glob Chang Biol*
 524 **21**, 1715-26.
- 525 de Vries, F. T., Liiri, M. E., Bjørnlund, L., Setälä, H. M., Christensen, S., and Bardgett, R. D.
 526 (2012). Legacy effects of drought on plant growth and the soil food web. *Oecologia*
 527 **170**, 821-833.
- 528 Dennis, P. G., Miller, A. J., and Hirsch, P. R. (2010). Are root exudates more important than
 529 other sources of rhizodeposits in structuring rhizosphere bacterial communities?
 530 *FEMS microbiology ecology* **72**, 313-327.
- 531 Dissanayaka, D. M. S., Maruyama, H., Masuda, G., and Wasaki, J. (2015). Interspecific
 532 facilitation of P acquisition in intercropping of maize with white lupin in two
 533 contrasting soils as influenced by different rates and forms of P supply. *Plant and Soil*
 534 **390**, 223-236.
- 535 Doré, T., Makowski, D., Malézieux, E., Munier-Jolain, N., Tchamitchian, M., and Tittone,
 536 P. (2011). Facing up to the paradigm of ecological intensification in agronomy:

- 537 revisiting methods, concepts and knowledge. *European Journal of Agronomy* **34**, 197-
538 210.
- 539 Duchene, O., Vian, J.-F., and Celette, F. (2017). Intercropping with legume for agroecological
540 cropping systems: Complementarity and facilitation processes and the importance of
541 soil microorganisms. A review. *Agriculture, Ecosystems & Environment* **240**, 148-
542 161.
- 543 Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M. P., and
544 Mommer, L. (2017). Root biomass and exudates link plant diversity with soil bacterial
545 and fungal biomass. *Scientific reports* **7**, 44641.
- 546 Erguder, T. H., Boon, N., Wittebolle, L., Marzorati, M., and Verstraete, W. (2009).
547 Environmental factors shaping the ecological niches of ammonia-oxidizing archaea.
548 *FEMS microbiology reviews* **33**, 855-869.
- 549 Fierer, N., and Schimel, J. P. (2002). Effects of drying–rewetting frequency on soil carbon
550 and nitrogen transformations. *Soil Biology and Biochemistry* **34**, 777-787.
- 551 Griffiths, B. S., and Philippot, L. (2013). Insights into the resistance and resilience of the soil
552 microbial community. *FEMS Microbiol Rev* **37**, 112-29.
- 553 Grman, E., and Suding, K. N. (2010). Within - year soil legacies contribute to strong priority
554 effects of exotics on native California grassland communities. *Restoration Ecology* **18**,
555 664-670.
- 556 Guillot, E., Hinsinger, P., Dufour, L., Roy, J., and Bertrand, I. (2019). With or without trees:
557 Resistance and resilience of soil microbial communities to drought and heat stress in a
558 Mediterranean agroforestry system. *Soil Biology & Biochemistry* **129**, 122-135.
- 559 Hamdi, S., Chevallier, T., Ben Aïssa, N., Ben Hammouda, M., Gallali, T., Chotte, J.-L., and
560 Bernoux, M. (2011). Short-term temperature dependence of heterotrophic soil
561 respiration after one-month of pre-incubation at different temperatures. *Soil Biology
562 and Biochemistry* **43**, 1752-1758.
- 563 Hinsinger, P., Bengough, A. G., Vetterlein, D., and Young, I. M. (2009). Rhizosphere:
564 biophysics, biogeochemistry and ecological relevance. *Plant and soil* **321**, 117-152.
- 565 Hunter, P. J., Teakle, G., and Bending, G. D. (2014). Root traits and microbial community
566 interactions in relation to phosphorus availability and acquisition, with particular
567 reference to Brassica. *Frontiers in plant science* **5**, 27.
- 568 Kumar, A., and Verma, J. P. (2018). Does plant—Microbe interaction confer stress tolerance
569 in plants: A review? *Microbiological research* **207**, 41-52.
- 570 Kuzyakov, Y., and Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil:
571 concept & review. *Soil Biology and Biochemistry* **83**, 184-199.
- 572 Kuzyakov, Y., and Xu, X. (2013). Competition between roots and microorganisms for
573 nitrogen: mechanisms and ecological relevance. *New Phytologist* **198**, 656-669.
- 574 Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-
575 Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C.,
576 Trumbore, S. E., and Gleixner, G. (2015). Plant diversity increases soil microbial
577 activity and soil carbon storage. *Nature Communications* **6**.
- 578 Le Roux, X., Schmid, B., Poly, F., Barnard, R. L., Niklaus, P. A., Guillaumaud, N., Habekost,
579 M., Oelmann, Y., Philippot, L., Salles, J. F., Schloter, M., Steinbeiss, S., and Weigelt,
580 A. (2013). Soil environmental conditions and microbial build-up mediate the effect of

- 581 plant diversity on soil nitrifying and denitrifying enzyme activities in temperate
582 grasslands. *PLoS One* **8**, e61069.
- 583 Legay, N., Baxendale, C., Grigulis, K., Krainer, U., Kastl, E., Schloter, M., Bardgett, R. D.,
584 Arnoldi, C., Bahn, M., and Dumont, M. (2014). Contribution of above-and below-
585 ground plant traits to the structure and function of grassland soil microbial
586 communities. *Annals of botany* **114**, 1011-1021.
- 587 Li, X., Jousset, A., de Boer, W., Carrión, V. J., Zhang, T., Wang, X., and Kuramae, E. E.
588 (2018). Legacy of land use history determines reprogramming of plant physiology by
589 soil microbiome. *The ISME journal*, 1.
- 590 Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H.,
591 Rapidel, B., Tourdonnet, S., and Valantin-Morison, M. (2009). Mixing plant species in
592 cropping systems: concepts, tools and models. A review. *Agronomy for Sustainable*
593 *Development* **29**, 43-62.
- 594 Mallarino, A., and Wedin, W. (1990). Effect of species and proportion of legume on herbage
595 yield and nitrogen concentration of legume - grass mixtures. *Grass and Forage*
596 *Science* **45**, 393-402.
- 597 Mathieu, O., Henault, C., Leveque, J., Baujard, E., Milloux, M. J., and Andreux, F. (2006).
598 Quantifying the contribution of nitrification and denitrification to the nitrous oxide
599 flux using ¹⁵N tracers. *Environ Pollut* **144**, 933-40.
- 600 Mooshammer, M., Hofhansl, F., Frank, A. H., Wanek, W., Hämmerle, I., Leitner, S.,
601 Schneckner, J., Wild, B., Watzka, M., and Keiblinger, K. M. (2017). Decoupling of
602 microbial carbon, nitrogen, and phosphorus cycling in response to extreme
603 temperature events. *Science advances* **3**, e1602781.
- 604 Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejicek, A., Strbik, F.,
605 Philippot, L., and Mougél, C. (2015). Plant traits related to nitrogen uptake influence
606 plant-microbe competition. *Ecology* **96(8)**, 2300-2310.
- 607 Orwin, K. H., and Wardle, D. A. (2004). New indices for quantifying the resistance and
608 resilience of soil biota to exogenous disturbances. *Soil Biology and Biochemistry* **36**,
609 1907-1912.
- 610 Orwin, K. H., and Wardle, D. A. (2005). Plant Species Composition Effects on Belowground
611 Properties and the Resistance and Resilience of the Soil Microflora to a Drying
612 Disturbance. *Plant and Soil* **278**, 205-221.
- 613 Patra, A., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S., Loiseau, P., Louault,
614 F., Mahmood, S., Nazaret, S., and Philippot, L. (2005). Effects of grazing on microbial
615 functional groups involved in soil N dynamics. *Ecological Monographs* **75**, 65-80.
- 616 Philippot, L., Raaijmakers, J. M., Lemanceau, P., and van der Putten, W. H. (2013). Going
617 back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* **11**,
618 789-99.
- 619 Pivato, B., Bru, D., Busset, H., Deau, F., Matejicek, A., Philippot, L., and Moreau, D. (2017).
620 Positive effects of plant association on rhizosphere microbial communities depend on
621 plant species involved and soil nitrogen level. *Soil Biology and Biochemistry* **114**, 1-4.
- 622 Pommier, T., Cantarel, A. A., Grigulis, K., Lavorel, S., Legay, N., Baxendale, C., Bardgett, R.
623 D., Bahn, M., Poly, F., and Clément, J. C. (2018). The added value of including key

- 624 microbial traits to determine nitrogen - related ecosystem services in managed
625 grasslands. *Journal of applied ecology* **55**, 49-58.
- 626 Prosser, J. I., and Nicol, G. W. (2012). Archaeal and bacterial ammonia-oxidisers in soil: the
627 quest for niche specialisation and differentiation. *Trends Microbiol* **20**, 523-31.
- 628 R Core Team (2018). R: A Language and Environment for Statistical Computing. R
629 Foundation for Statistical Computing, Vienna, Austria.
- 630 Robertson, G. P., and Groffman, P. M. (2015). Nitrogen Transformations. In "Soil
631 microbiology, ecology and biochemistry" (E. A. Paul, ed.), pp. 421-446. Academic
632 Press, Burlington, Massachusetts, USA.
- 633 Salomé, C., Coll, P., Lardo, E., Villenave, C., Blanchart, E., Hinsinger, P., Marsden, C., and
634 Le Cadre, E. (2014). Relevance of use-invariant soil properties to assess soil quality of
635 vulnerable ecosystems: The case of Mediterranean vineyards. *Ecological Indicators*
636 **43**, 83-93.
- 637 Schimel, J. P., and Bennett, J. (2004). Nitrogen mineralization: challenges of a changing
638 paradigm. *Ecology* **85**, 591-602.
- 639 Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Burgmann, H., Huber, D. H.,
640 Langenheder, S., Lennon, J. T., Martiny, J. B., Matulich, K. L., Schmidt, T. M., and
641 Handelsman, J. (2012). Fundamentals of microbial community resistance and
642 resilience. *Front Microbiol* **3**, 417.
- 643 Strecker, T., Macé, O. G., Scheu, S., and Eisenhauer, N. (2016). Functional composition of
644 plant communities determines the spatial and temporal stability of soil microbial
645 properties in a long-term plant diversity experiment. *Oikos* **125**, 1743-1754.
- 646 Subbarao, G., Sahrawat, K., Nakahara, K., Ishikawa, T., Kishii, M., Rao, I., Hash, C., George,
647 T., Rao, P. S., and Nardi, P. (2012). Biological nitrification inhibition—a novel
648 strategy to regulate nitrification in agricultural systems. In "Advances in agronomy",
649 Vol. 114, pp. 249-302. Elsevier.
- 650 Tang, X., Bernard, L., Brauman, A., Daufresne, T., Deleporte, P., Desclaux, D., Souche, G.,
651 Placella, S. A., and Hinsinger, P. (2014). Increase in microbial biomass and
652 phosphorus availability in the rhizosphere of intercropped cereal and legumes under
653 field conditions. *Soil Biology and Biochemistry* **75**, 86-93.
- 654 Tang, X., Placella, S. A., Daydé, F., Bernard, L., Robin, A., Journet, E.-P., Justes, E., and
655 Hinsinger, P. (2016). Phosphorus availability and microbial community in the
656 rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. *Plant and*
657 *Soil* **407**, 119-134.
- 658 Taylor, A. E., Giguere, A. T., Zoebelin, C. M., Myrold, D. D., and Bottomley, P. J. (2017).
659 Modeling of soil nitrification responses to temperature reveals thermodynamic
660 differences between ammonia-oxidizing activity of archaea and bacteria. *ISME J* **11**,
661 896-908.
- 662 Thion, C., and Prosser, J. I. (2014). Differential response of nonadapted ammonia-oxidising
663 archaea and bacteria to drying-rewetting stress. *FEMS Microbiol Ecol* **90**, 380-9.
- 664 Thion, C. E., Poirel, J. D., Cornulier, T., De Vries, F. T., Bardgett, R. D., and Prosser, J. I.
665 (2016). Plant nitrogen-use strategy as a driver of rhizosphere archaeal and bacterial
666 ammonia oxidiser abundance. *FEMS Microbiol Ecol* **92**.

- 667 van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami,
668 T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N.,
669 Van de Voorde, T. F. J., Wardle, D. A., and Hutchings, M. (2013). Plant-soil
670 feedbacks: the past, the present and future challenges. *Journal of Ecology* **101**, 265-
671 276.
- 672 van der Putten, W. H., Bradford, M. A., Pernilla Brinkman, E., van de Voorde, T. F., and
673 Veen, G. (2016). Where, when and how plant–soil feedback matters in a changing
674 world. *Functional Ecology* **30**, 1109-1121.
- 675 Veneklaas, E. J., Stevens, J., Cawthray, G. R., Turner, S., Grigg, A. M., and Lambers, H.
676 (2003). Chickpea and white lupin rhizosphere carboxylates vary with soil properties
677 and enhance phosphorus uptake. *Plant and Soil* **248**, 187-197.
- 678 Wang, B. L., Tang, X. Y., Cheng, L. Y., Zhang, A. Z., Zhang, W. H., Zhang, F. S., Liu, J. Q.,
679 Cao, Y., Allan, D. L., Vance, C. P., and Shen, J. B. (2010). Nitric oxide is involved in
680 phosphorus deficiency-induced cluster-root development and citrate exudation in
681 white lupin. *New Phytologist* **187**, 1112-1123.
- 682 Wang, X., Tang, C., Severi, J., Butterly, C. R., and Baldock, J. A. (2016). Rhizosphere
683 priming effect on soil organic carbon decomposition under plant species differing in
684 soil acidification and root exudation. *New Phytologist* **211**, 864-873.
- 685 Wertz, S., Degrange, V., Prosser, J. I., Poly, F., Commeaux, C., Guillaumaud, N., and Le
686 Roux, X. (2007). Decline of soil microbial diversity does not influence the resistance
687 and resilience of key soil microbial functional groups following a model disturbance.
688 *Environ Microbiol* **9**, 2211-9.
- 689 Xu, X., Ouyang, H., Richter, A., Wanek, W., Cao, G., and Kuzyakov, Y. (2011). Spatio-
690 temporal variations determine plant–microbe competition for inorganic nitrogen in an
691 alpine meadow. *Journal of Ecology* **99**, 563-571.
- 692 Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., and Tilman, D. (2003). Plant
693 diversity, soil microbial communities, and ecosystem function: are there any links?
694 *Ecology* **84**, 2042-2050.
- 695 Zhalnina, K., de Quadros, P. D., Camargo, F. A., and Triplett, E. W. (2012). Drivers of
696 archaeal ammonia-oxidizing communities in soil. *Front Microbiol* **3**, 210.
- 697 Zhang, N. N., Sun, Y. M., Wang, E. T., Yang, J. S., Yuan, H. L., and Scow, K. M. (2015).
698 Effects of intercropping and Rhizobial inoculation on the ammonia-oxidizing
699 microorganisms in rhizospheres of maize and faba bean plants. *Applied Soil Ecology*
700 **85**, 76-85.
- 701 Zhang, W., Liang, C., Kao-Kniffin, J., He, H., Xie, H., and Zhang, X. (2017). Effects of
702 drying and wetting cycles on the transformations of extraneous inorganic N to soil
703 microbial residues. *Scientific reports* **7**, 9477.
- 704

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-NO₃⁻) and ammonium (N-NH₄⁺). 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-NO₃⁻)
723 and ammonium (N-NH₄⁺). 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)).

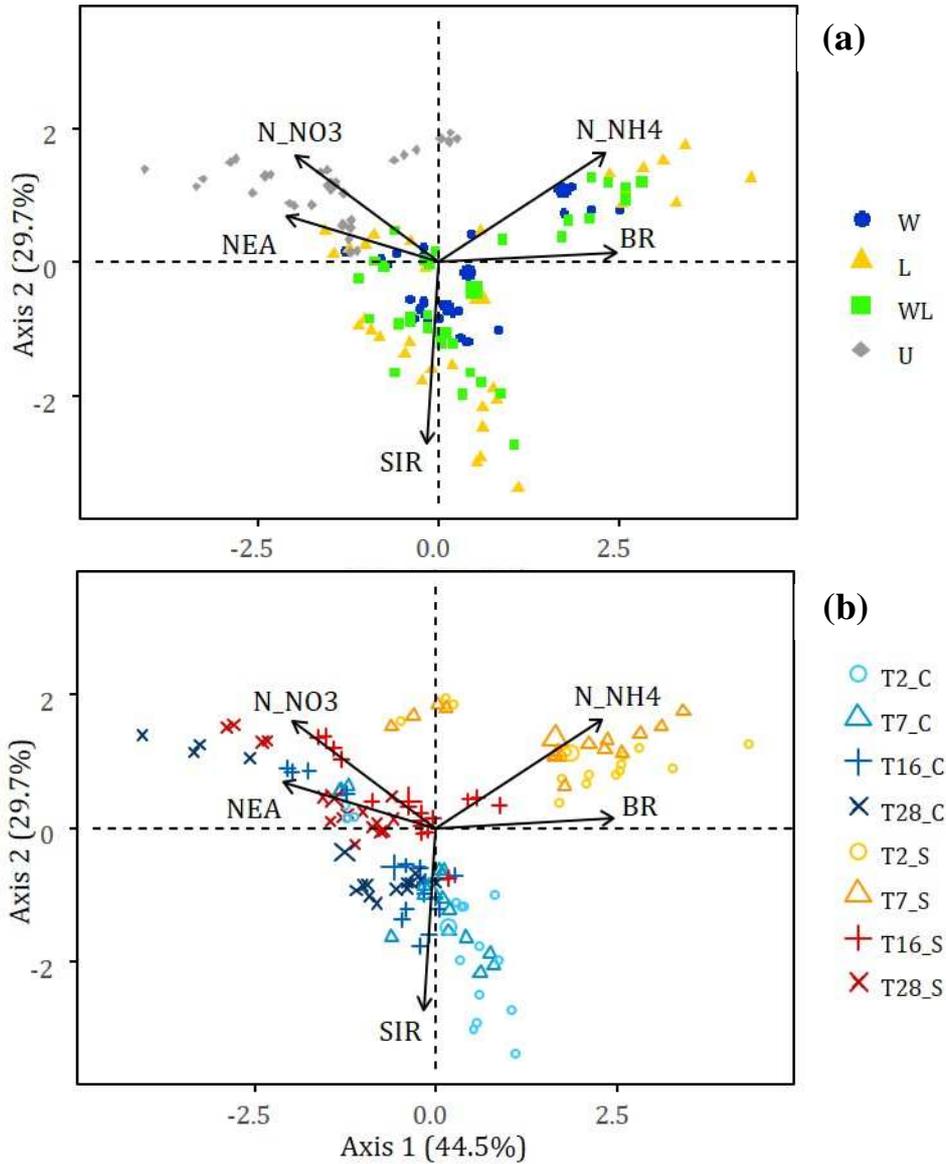


Fig. 1. Principal component analysis (PCA) performed on basal respiration (BR), substrate-induced respiration (SIR), nitrification enzyme activity (NEA) and soil mineral N content as nitrate (N-NO_3^-) and ammonium (N-NH_4^+). Symbols stand for planting legacy (W – wheat, L – lupin, WL – wheat + lupin, 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.

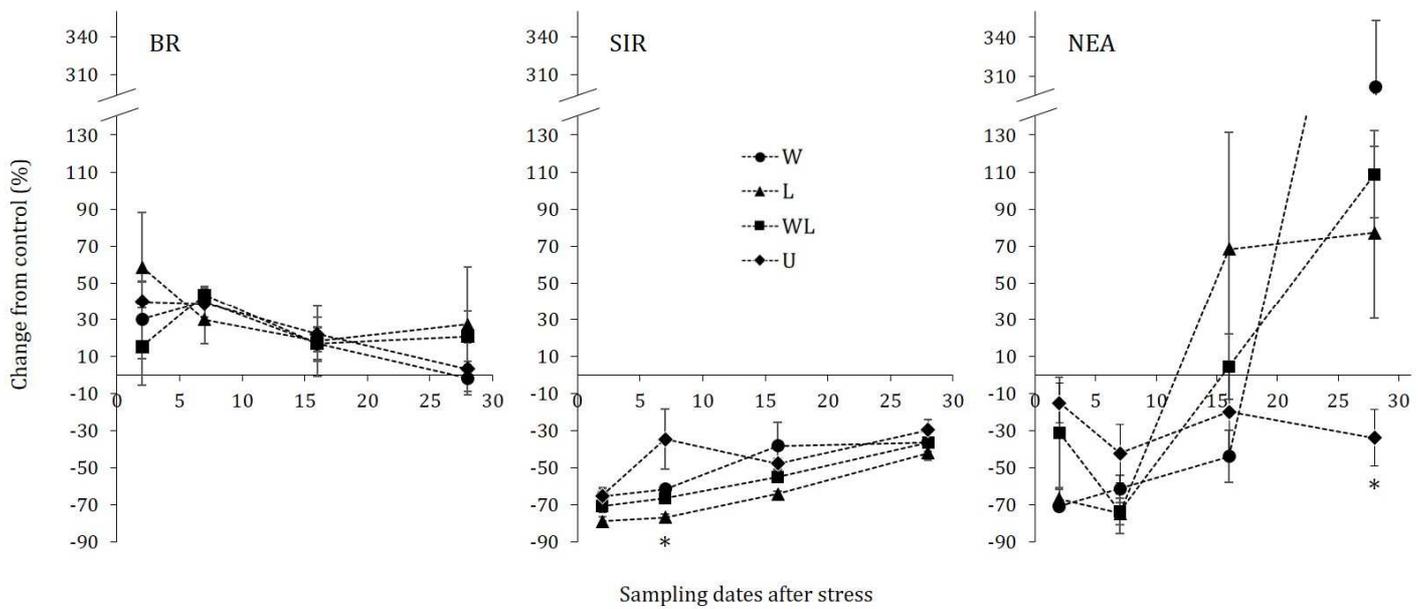


Fig. 2. Percentages of change in microbial activities (BR – basal respiration, SIR – substrate-induced respiration, NEA – nitrification enzyme activity) relative to controls along all sampling dates after the end of the heat stress (T2, T7, T16, T28). Symbols represent planting legacy (W – wheat, L – 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 ($p < 0.05$)).

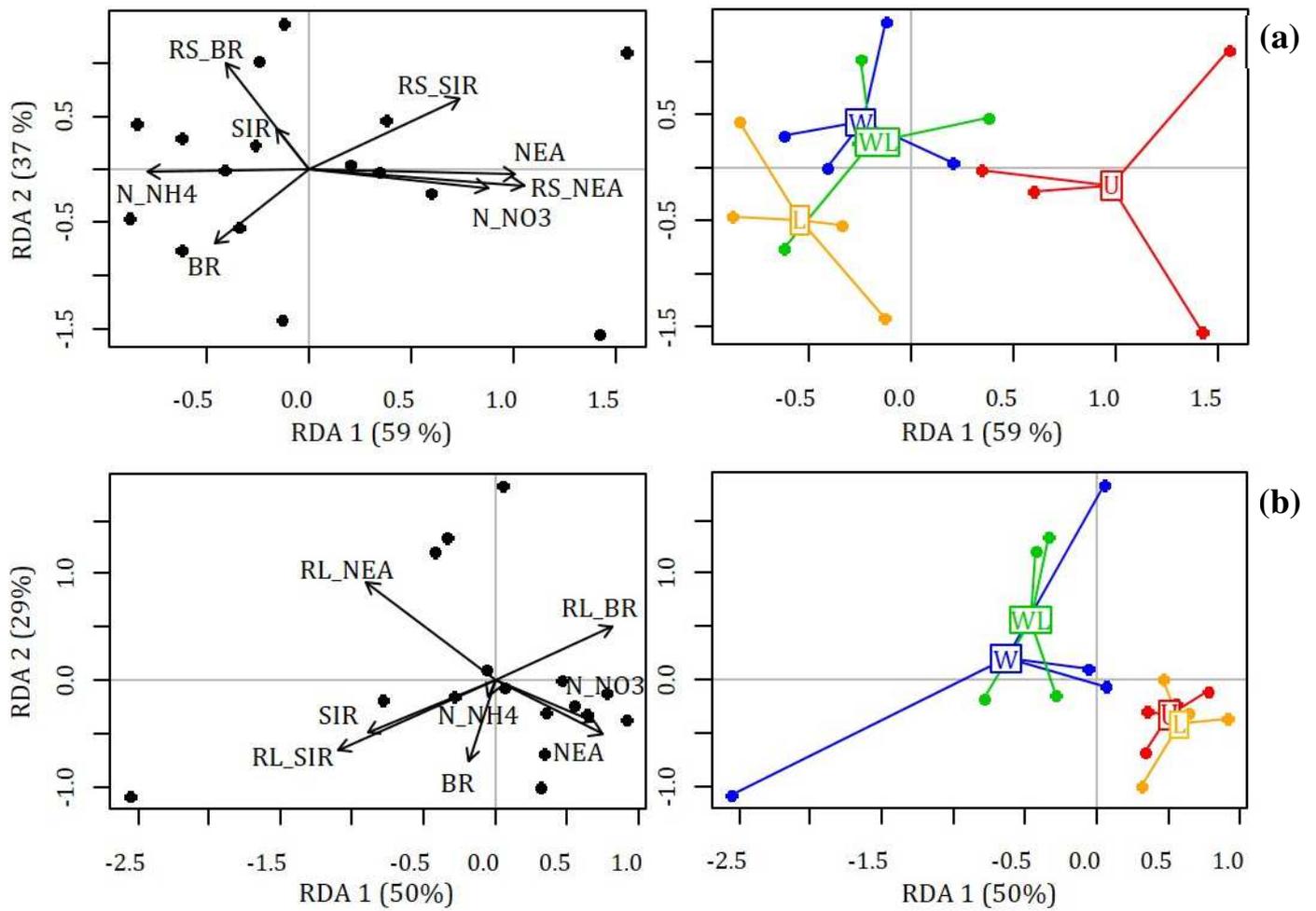


Fig. 3. Redundancy analysis (RDA) based on resistance (RS) (a) or resilience (RL) (b) indices of basal respiration (BR), substrate-induced respiration (SIR) and nitrification enzyme activity (NEA), constrained by BR, SIR, NEA, as well as soil mineral N content as nitrate (N-NO_3^-) and ammonium (N-NH_4^+). 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^B</u>	<u>Stress^A</u>	<u>Control^B</u>	<u>Stress^A</u>	<u>Control^B</u>	<u>Stress^A</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	5.77 c	2.01 d	4.33 c	1.65 e	4.21 bc	2.64 de	4.94 bc	3.15 de			
	L	12.42 a	2.59 d	9.11 a	2.10 e	7.69 a	2.75 de	6.92 a	4.00 cd			
	WL	9.24 b	2.78 d	6.56 b	2.20 de	5.38 b	2.41 de	5.38 b	3.43 de			
	U	4.98 c	1.72 d	3.50 cd	1.71 e	3.47 cd	1.81 e	3.82 d	2.67 e			
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^A</u>	<u>Stress^B</u>	<u>Control^A</u>	<u>Stress^B</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	0.65 c	2.69 bc
	L	0.54 b	0.17 b	B	0.57 ab	0.12 b	B	0.74 b	1.12 ab	B	2.83 bc	4.66 b
	WL	0.42 b	0.28 b	B	0.40 b	0.11 b	B	0.43 b	0.45 ab	B	1.28 c	2.63 bc
	U	1.44 a	1.21 a	A	0.91 a	0.49 a	A	1.99 a	1.36 a	A	8.63 a	5.38 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	0.21 a	0.16	0.47	0.35 b
	L	0.11 b	0.16	0.32	0.54 a
	WL	0.17 ab	0.19	0.37	0.53 a
<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	0.24 a	- 0.63
	L	0.20	-0.03	- 0.58 b	- 0.48
	WL	0.29	- 0.10	0.46 a	- 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 ($p < 0.05$)).