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Duration of the conditioning phase affects the results of plant-soil feedback experiments via soil chemical properties

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## 1 **Abstract**

2 Plant-soil feedback (PSF) is a fundamental mechanism explaining plant community composition.  
3 Two-phase experiments, i.e. conditioning and feedback, represent a common methodology to  
4 study PSF. The duration of the conditioning phase varies among studies and the PSF observed is  
5 often explained by its biotic component. Little is known about the temporal variation of PSF and  
6 its abiotic component. As early life stages are crucial for plant establishment, we grew *Rorippa*  
7 *austriaca* in soil conditioned over 2, 4, 6 or 8 weeks by a conspecific or a co-occurring species,  
8 *Agrostis capillaris*. For each conditioning duration, we analysed the soil chemical properties and  
9 the direction and intensity of intra- or inter-specific feedbacks. With increasing duration, the  
10 negative intra- and inter-specific feedbacks became stronger and weaker, respectively. The  
11 interspecific feedback was more negative than the intraspecific at 2 weeks and this reversed  
12 thereafter. The Mg content decreased with conditioning duration whatever the conditioning  
13 species was. With increasing duration, conditioning by *R. austriaca* strongly decreased pH, while  
14 *A. capillaris* did not affect pH. The K and P contents were not affected by the conditioning  
15 duration and were higher in *R. austriaca* soil than in *A. capillaris* soil. Our results suggest that  
16 not only conditioning species but also duration of conditioning phase may affect the magnitude of  
17 PSF. The changes in soil chemical properties linked to the conditioning species or the  
18 conditioning phase duration may drive the feedbacks by affecting plant growth directly or via the  
19 interacting microbial communities.

20

## 21 **Keywords**

22 Conditioning, plant-soil feedback indices, negative feedback, native species, Brassicaceae

23

24

## 25 **Introduction**

26 Plant-soil feedback (PSF) represents the abiotic and biotic changes brought to the soil by the  
27 plant that alter subsequent plant growth (Kulmatiski et al. 2008). By releasing diverse carbon-  
28 derived compounds from the roots (Bais et al. 2006) or selectively depleting the soil nutrients  
29 (Schenk 2006), the plant modifies the soil chemical properties (Hemrová et al. 2016). The biotic  
30 component of the soil affected by the plant consists of soil communities including symbiotic  
31 microbes, pathogens and mutualists (Reynolds et al. 2003; Stinson et al. 2006; Mangla et al.  
32 2008; Lankau 2013). Their abundance (Hendriks et al. 2015) and composition (Klironomos 2002;  
33 Bezemer et al. 2006; Teste et al. 2017) depend on plant species.

34         These changes of soil properties caused by the plant will, in turn, alter the growth of the  
35 individual itself or other individuals of the same species (called intraspecific feedback) or that of  
36 another species (called interspecific feedback) (van der Putten et al. 2013). Both intra- and inter-  
37 specific feedbacks could have neutral, negative or positive effects on plant performance. In the  
38 field, the intra- and inter-specific feedbacks can be observed by assessing plant traits related to  
39 performance (Bukowski and Petermann 2014) or abundance (Hemrová et al. 2016) of a plant  
40 species growing near conspecific individuals and heterospecifics, respectively. The feedback  
41 through abiotic changes can indirectly affect the growth of neighbouring plants by modifying the  
42 availability of soil resources (Craine and Dybzinski 2013; Trinder et al. 2013) or the soil  
43 communities (Ranjard and Richaume 2001) or directly by the release of allelopathic substances  
44 (Kruse et al. 2000; van Grunsven et al. 2007). The feedback through biotic changes was  
45 previously demonstrated for invasive species that modify the abundance and composition of  
46 symbiotic communities interacting with local species (Hawkes et al. 2006). This may lead to  
47 negative effects on growth of native species (Stinson et al. 2006). Consequently, the PSF can  
48 impact plant abundance (Klironomos 2002; Hemrová et al. 2016), diversity (Klironomos 2002),

49 invasion potential (Dostálek et al. 2016), plant persistence (Bever 2003) and succession dynamics  
50 (Kardol et al. 2007; Fry et al. 2017). Thus, it is a fundamental ecological mechanism explaining  
51 the composition and dynamic of plant communities (Kardol et al. 2013).

52         The strength and direction of the feedback (Kardol et al. 2013) may vary according to the  
53 developmental stage of the plant inducing the changes in the soil. Indeed, the plant's acquisition  
54 and allocation of resources depends on developmental stage (Kardol et al. 2013). Thus, the  
55 amount and composition of carbon compounds released into the soil (Kuzyakov and Domanski  
56 2000; Bardgett et al. 2005) and level of nutrient intake (Schenk 2006) change over plant life. The  
57 developmental stage of the plant (Bardgett et al. 2005; Philippot et al. 2013; Balasooriya et al.  
58 2014) can therefore also affect the microbial communities. For example, Mougél et al. (2006)  
59 showed that the intensity of symbiotic associations was higher at the flowering stage than at the  
60 vegetative stages for the legume *Medicago truncatula*. In line with this, Houlden et al. (2008) and  
61 Micallef et al. (2009) found that bacterial diversity and activity was more important in the  
62 rhizosphere of mature plants than near seedlings.

63         In order to study the PSF in the greenhouse, it is necessary to simulate the two phases that  
64 compose it. Firstly, the conditioning phase during which a plant modifies the abiotic and biotic  
65 components of the soil around its root system. Secondly, the feedback phase where the altered  
66 soil properties affect the growth of conspecific or heterospecific individual. Use of two-phase  
67 experiments, consisting of conditioning and feedback phase, is a common technique to study the  
68 intensity of PSF both at the intra- and inter-specific level (e.g., Kulmatiski et al. 2008; Kulmatiski  
69 and Kardol 2008; Brinkman et al. 2010). In these experiments, soil is cultivated for a given  
70 species for a set period of time during the conditioning phase. Afterwards, the plant is harvested  
71 and the same or another plant species is then planted into this conditioned soil to realize the

72 feedback phase. The plant performance in the conditioned soil is then compared to the  
73 performance in the unconditioned soil to assess the feedback intensity.

74         The conditioning is therefore a fundamental phase of the experiment allowing the  
75 establishment of the PSF of interest and its duration could affect the direction and intensity of the  
76 resulting feedback (Kardol et al. 2013). Several previous studies assessed the effect of time in the  
77 PSF experiments. Some, e.g. Hawkes et al. (2013), focused on the duration of the feedback phase  
78 and not of the conditioning phase. Studies exploring the duration of the conditioning phase did so  
79 in the field through the study of species, generally invasive, introduced for a different period of  
80 time in a new area (Diez et al. 2010; Dostál et al. 2013; Speek et al. 2015). They have shown that  
81 the longer the conditioning time, i.e. the residence time since the non-native species was  
82 established in a new area, the stronger the negative intraspecific feedback, i.e. effect of non-  
83 native species on conspecifics, (Diez et al. 2010; Speek et al. 2015) and the weaker the  
84 interspecific feedback, i.e. effect of non-native species on native ones, (Dostál et al. 2013). Such  
85 studies were performed with long durations, i.e. at least 10 years of conditioning, which allows  
86 the establishment of all the mechanisms that shape the plant communities. In greenhouse  
87 experiments, different studies are using different length of the conditioning phase lasting from six  
88 weeks (Speek et al. 2015) to 96 weeks (Bezemer et al. 2006; Münzbergová and Šurinová 2015).  
89 However, no studies assessed the effect of variation of the conditioning duration, over short  
90 durations, on feedback intensity and direction. Indeed, short durations correspond to the crucial  
91 step in the individual plant establishment and further plant performance (van der Heijden 2004).

92         Synthetic studies characterising mechanisms responsible for PSF and their dynamics  
93 across short temporal scales highlighted the need to understand the links between plant  
94 performances and the shifts in abiotic soil conditions (Kulmatiski and Kardol 2008; Kardol et al.  
95 2013). However, the majority of studies about PSF observed the direction and magnitude of

96 feedback without characterizing the mechanisms (Diez et al. 2010; Dostál et al. 2013; Hawkes et  
97 al. 2013; Speek et al. 2015). When exploring the mechanisms responsible, most studies focused  
98 on the biotic component of PSF (Micallef et al. 2009) and specifically the symbionts (Klironomos  
99 2002; Reinhart et al. 2005; Kuebbing et al. 2015). Finally, the studies including the abiotic  
100 component of PSF did not take into account the dynamical aspect of PSF and thus the changes in  
101 the intensity of PSF over time (Bezemer et al. 2006; Hendriks et al. 2013; Münzbergová and  
102 Šurinová 2015; Dostálek et al. 2016). To our knowledge, no studies assessed the changes of soil  
103 chemical characteristics across time during the conditioning phase and explored, how these  
104 abiotic changes may affect the PSF in the feedback phase. Little is thus known about the temporal  
105 changes in soil properties that could play a fundamental role in the explanation of PSF by acting  
106 directly or indirectly, via microbes.

107         The aim of this study was to assess the effect of the conditioning duration on the PSF and  
108 its abiotic component. To do this, we used a two-phase experiment and modulated the duration of  
109 the conditioning phase. We chose a potentially invasive species, *Rorippa austriaca* and a co-  
110 occurring grass species, *Agrostis capillaris* as the models. In its native range, *R. austriaca* is  
111 known to have a negative intraspecific feedback and also a negative interspecific feedback on *A.*  
112 *capillaris* (Dostálek et al. 2016). Dostálek et al. (2016) hypothesised that these negative  
113 feedbacks could be linked to modifications of soil nutrients but they did not study them. Thus, we  
114 conditioned soil by *R. austriaca* or *A. capillaris*, for 2, 4, 6 or 8 weeks and measured chemical  
115 characteristics of the soil after the conditioning phase. Within the second phase, i.e. feedback, we  
116 grew *R. austriaca* in the conditioned soils for 8 weeks, and evaluated the direction and intensity  
117 of intra- or inter-specific feedbacks. Specifically, we wanted to answer the following questions:  
118 1) What is the effect of duration of the conditioning phase on the results of PSF experiments?, 2)

119 Does the effect of time depend on the conditioning species?, 3) Can we explain the effect of time  
120 by changes in the chemical composition of the soil?

121 We predicted, that the negative intraspecific feedback previously observed by Dostálek et  
122 al. (2016) could become stronger with the increase in the conditioning duration. Indeed, if the  
123 negative feedback is due to modification of soil nutrients (Dostálek et al. 2016) as previously  
124 found for other species (Gómez-Aparicio and Canham 2008), we hypothesised that these changes  
125 could be accentuated with time. As both species co-exist in the field, we expected also a negative  
126 feedback of *A. capillaris* on *R. austriaca*. This could prevent *A. capillaris* from being  
127 outcompeted by *R. austriaca*. Finally, we anticipated that the negative feedback could be  
128 mediated by depletion in soil nutrients (Kourtev et al. 2003; Meisner et al. 2012).

129

## 130 **Material and Methods**

### 131 *Studied species*

132 *Rorippa austriaca* is a polycarpic herbaceous perennial plant species with a semi rosette growth  
133 form and relatively deep storage roots (Oberdorfer 1990). It reproduces by clonal growth with  
134 lateral roots, i.e. can regenerate from root fragments (Dietz et al. 2002). It is an obligate  
135 outcrossing species (Bleeker and Matthies 2005) able to reproduce via seeds, but the seed  
136 production is very limited and most of the reproduction is vegetative (Dietz et al. 2002). The  
137 species occupies diverse habitats comprising soils with high or low level of nutrients such as  
138 loamy and sandy soils, respectively (Dostálek et al. 2016). *R. austriaca* can be found in early  
139 successional habitats as well as in habitats with high cover of other vegetation (Dietz et al. 2002).  
140 The species is native to western Asia and south-eastern Europe (Jonsell 1973) including our study  
141 area in the Czech Republic. In the past few decades, it started expanding and has become  
142 invasive in Germany and Netherlands (Bleeker 2003; Tamis et al. 2005; Macel et al. 2017) and



143 further in North America (Müller 2009). For this study, we used 25 clones of *R. austriaca*  
144 collected from five populations, i.e. five clones per population, from the native range of the  
145 species in the Czech Republic. The five clones per population were considered as five replicates  
146 for each population, further called the 25 *R. austriaca* clones. These were collected for a previous  
147 study (see Dostálek et al. 2016). The plants were cultivated in the experimental garden of the  
148 Institute of Botany, Academy of Sciences of the Czech Republic since 2011.

149 *Agrostis capillaris* is a species commonly co-occurring with *R. austriaca* and was used as  
150 a second model. *A. capillaris* is a rhizomatous perennial grass often forming dense, almost  
151 monospecific stands (Ruemmele 2003). It reproduces clonally from short rhizomes and stolons or  
152 via seeds through cross-pollination (Rajasekar et al. 2007). It mainly occurs on nutrient-poor soils  
153 (Newbery et al. 1995). The native range of the species is similar to that of *R. austriaca* and the  
154 species is now distributed worldwide (Rajasekar et al. 2007).

155 As the establishment from root fragments is a common way of reproduction of *R.*  
156 *austriaca* after disturbance, we used root fragments of *R. austriaca* for our experiment. In  
157 contrast, *A. capillaris* produces high quantity of viable seeds and we thus used seeds for this  
158 species. *R. austriaca* is naturally a bigger species than *A. capillaris* (personal observation).  
159 Consequently, thanks to a previous experiment conducted with the same species (see Dostálek et  
160 al. 2016), we adapted the number of seeds of *A. capillaris* added in each pot in order to obtain  
161 approximately equivalent biomasses per pot, over the growth, compared to *R. austriaca*. This  
162 aimed to limit the fact that the effects of both conditioning plants on soil are mainly due to a  
163 difference of biomass.

164 *A. capillaris* belongs to Poaceae known for their facultative interactions with arbuscular  
165 mycorrhizal fungi (AMF) (Javaid 2008; Dostálek et al. 2013), while *R. austriaca* belongs to

166 Brassicaceae that are not AMF-dependent (Müller 2009). The symbiotic status of both species  
167 could play a role in the explanation of feedbacks.

168

### 169 *Experimental design*

170 We used a standard two-phase experiment, consisting of conditioning and feedback phase, as  
171 suggested by Brinkman et al. (2010). For the conditioning phase, we used square pots (16 × 16 ×  
172 16 cm large) filled with a 1:1 mixture of common garden soil and sand. The common garden soil  
173 was a compost naturally containing soil microorganisms. This mixture was previously analysed  
174 and contained 0.7% C, 0.07% N and P in amount of 23 g/1000 g of soil dry weight (data not  
175 published). When setting up the experiment, in April 2013, 225 pots were prepared and filled  
176 with the substrate. We used one root fragment (4 cm long and 0.2-0.4 cm in diameter, planted 1  
177 cm deep) from each of the 25 *R. austriaca* clones and planted it into one of the pots at the  
178 beginning of the experiment. These 25 pots corresponded to the conditioning duration of 8  
179 weeks. The other pots, without plants, were kept in the same environmental and watering  
180 conditions as the pots for 8 weeks. In the same way, we planted 25 *R. austriaca* clones into these  
181 empty pots after 2, 4 and 6 weeks. Eight weeks after planting the first fragments, the conditioning  
182 phase was terminated. In this way, we obtained soils conditioned by *R. austriaca* (further referred  
183 to as *R. austriaca* soil) for 2, 4, 6 and 8 weeks. At each time period, we also sowed 25 pots with  
184 seeds of *A. capillaris* (0.1 g of seeds per pot, i.e. about 100 seeds, from a local seed provider  
185 [www.plantanaturalis.com](http://www.plantanaturalis.com)) and thus also obtained soils conditioned by *A. capillaris* (further  
186 referred to as *A. capillaris* soil) for 2, 4, 6 and 8 weeks. The remaining 25 pots were left without  
187 any plants as controls. All the pots, including the empty ones, were left in the experimental  
188 garden during the 8 weeks, regularly watered and weeded.

189           When terminating the conditioning phase of the experiment, in June 2013, all the plants  
190 including their roots were carefully removed from the soil. The soil from the 25 pots conditioned  
191 by the same species for the same period of time or unconditioned pots was mixed. While mixing  
192 was criticized by Reinhart and Rinella (2016), Cahill et al. (2017) suggested that the soil must be  
193 mixed if we aimed to study the overall effect of the species *R. austriaca* and *A. capillaris* in their  
194 native range rather than studying the effect of the population or the genotype selected. Moreover,  
195 the growth of the plants was uneven with large or small plants for *R. austriaca* and many or few  
196 seedlings for *A. capillaris*. Thus, by mixing the soil we ensured that the soil used for the feedback  
197 phase experienced the same intensity of conditioning. In this way, we obtained 9 soil mixtures  
198 (soil conditioned by *A. capillaris* for 2, 4, 6 and 8 weeks, soil conditioned by *R. austriaca* for 2,  
199 4, 6 and 8 weeks and unconditioned soil). From each soil mixture, we took three soil samples of  
200 10 g for subsequent soil analysis (see below).

201           To set up the feedback phase, each soil mixture was filled into 25 pots (16 × 16 × 16 cm  
202 large) in June 2013. One root fragment of each of the 25 *R. austriaca* clones (4 cm long and 0.2-  
203 0.4 cm in diameter) was planted into one pot for each soil mixture, resulting in 25 *R. austriaca*  
204 clones × 9 soil mixtures, i.e. 225 pots in total. We only used a single species in the feedback  
205 phase as we primarily attempted to study different durations of the conditioning phase and  
206 wanted to keep our experiment within reasonable limits. We selected *R. austriaca* for the  
207 feedback phase as it has previously shown stronger intraspecific feedback than *A. capillaris*  
208 (Dostálek et al. 2016). The plants were grown for 8 weeks in the experimental garden. The pots  
209 were regularly watered and weeded. At the end of the feedback phase, in August 2013, all the  
210 plants were harvested. Above- and below-ground parts of the plants were separated, dried to a  
211 constant weight and weighted.

212

213 *Chemical composition of the soil*

214 To test if the differences in plant growth in soils conditioned for a different period of time could  
215 be explained by differences in their chemical composition, we analysed pH(H<sub>2</sub>O) and the content  
216 of total nitrogen (N), total carbon (C), calcium (Ca), magnesium (Mg), potassium (K) and  
217 exchangeable phosphorus (P) in the soils. Note that we only measured total content of N and not  
218 the available N as our analyses were done from air dried soil and the latter would not be reliable.  
219 The three soil samples from each mixture (see above) were sieved through a 2-mm sieve and  
220 analysed, i.e. 27 measures in total (9 soil mixtures × 3 samples per mixture). We analysed the soil  
221 after the conditioning and not after the feedback phase as we attempted to use conditions at the  
222 end of the conditioning phase, i.e. at the start of the feedback phase, as explanation of plant  
223 growth in the feedback phase. The analyses were done in the Analytical Lab of the Institute of  
224 Botany, Academy of Sciences of the Czech Republic. The methods used for the analyses are  
225 described in detail in Raabová et al. (2008).

226

227 *Data analysis*

228 To assess the growth response of the plant to the conditioned soil, we calculated the PSF index  
229 for each individual plant collected at the end of the experiment. We used formula of Petermann et  
230 al. (2008):  $\ln(\chi/s)$ , where  $\chi$  is the biomass of a plant growing in a soil conditioned by a certain  
231 treatment and  $s$  is the mean biomass of plants growing in unconditioned soil of the same  
232 treatment. An index value greater than zero indicates a positive feedback, while a negative value  
233 indicates a negative feedback. The PSF indices were calculated separately for above- and below-  
234 ground biomass. We performed t-tests to check if the PSF for each treatment differed from zero.  
235 We used generalized linear models to explain the PSF indices by taking the conditioning species  
236 (*R. austriaca* or *A. capillaris*), the duration of the conditioning phase (2, 4, 6 or 8 weeks), the

237 population of *R. austriaca* cultivated in the feedback phase (five populations each represented by  
238 five clones, see above) and their interactions as independent variables. The pair-wise differences  
239 in the duration of the conditioning phase were analysed for each conditioning species separately  
240 with Tukey-Kramer HSD tests.

241 We performed redundancy analyses (RDA) to describe changes in all the chemicals  
242 together. Redundancy analysis is a constrained ordination, based on multivariate linear models,  
243 determining how much of the variation in the set of dependent variables (the chemical properties)  
244 can be explained by the set of independent variables (species, duration and their interaction)  
245 (Ramette 2007). Because the chemical properties were measured in different units, they were  
246 centred and standardized, i.e. scaled by their standard deviations, before the analysis. To test if  
247 the chemical properties were affected by the species, the duration or their interaction, we used  
248 permutation tests with 999 permutations. We also analysed separately each chemical element.  
249 Thus, the differences in soil chemical properties after conditioning phase were tested with two-  
250 way ANOVA using the pH(H<sub>2</sub>O) and nutrient contents (N, C, Ca, Mg, K, P) as dependent  
251 variables and the conditioning species, the conditioning duration and their interaction as  
252 independent variables. To take into account the multiple testing, we used the Bonferroni  
253 correction and considered significant the *p*-values < 0.007 (standard *p*-value 0.05 divided by  
254 seven, which is number of variables expressing soil chemical properties). This correction allows  
255 to correct the *p*-values and avoid type I error (i.e. incorrect rejection of the null hypothesis). We  
256 used it because the same null hypothesis, i.e. no differences between both conditioned soils, was  
257 repeatedly used, with two-way ANOVA, on seven soil chemicals (Cabin and Mitchell 2000).

258 The relationship between the PSF indices and each chemical element was tested by  
259 generalized linear model using the PSF indices as dependent variables and the chemical  
260 properties, the species, the duration and their interactions as independent variables.

261 Normality and variance equality of the dependent variables were tested by Shapiro-Wilk  
262 and Bartlett tests, respectively. The conditioning duration was used as a continuous variable in all  
263 above described tests. The statistical analyses were performed using the statistical software JMP  
264 7 (SAS Institute, Cary, NC) and R version 3.3.2 (R Development Core Team, R Foundation for  
265 Statistical Computing, Vienna, Austria) with an alpha level of 0.05.

266

## 267 **Results**

### 268 *Direction and intensity of PSF*

269 The PSF indices were calculated from the above- and below-ground biomass that were on  
270 average of (mean  $\pm$  SE)  $0.43 \pm 0.25$  and  $0.91 \pm 0.60$  g, respectively, for *R. austriaca*, and  $0.62 \pm$   
271  $0.42$  and  $1.31 \pm 0.64$  g, respectively, for *A. capillaris* (Online Resource 1). Both PSF indices were  
272 affected by the interaction between the conditioning duration and the conditioning species (Table  
273 1). After 2 weeks of conditioning, the PSF for aboveground biomass was significantly more  
274 negative in *A. capillaris* soil than in *R. austriaca* soil, while this trend was reversed after 6 weeks  
275 of conditioning (Fig. 1a). For belowground biomass, the PSF index in *R. austriaca* soil, from 4  
276 up to 8 weeks of conditioning, was significantly more negative compared with *A. capillaris* soil  
277 (Fig. 1b). Concerning the effect of the conditioning species alone, both PSF indices for  
278 aboveground and belowground biomass were affected (Table 1, Fig. 1). The feedbacks were  
279 more negative in *R. austriaca* soil compared to *A. capillaris* soil. The conditioning duration alone  
280 had a significant effect on the PSF for belowground biomass (Table 1). Such a result was  
281 explained by the PSF in *R. austriaca* soil, because the duration did not affect the PSF in *A.*  
282 *capillaris* soil (Fig. 1b).

283 The PSF indices were also affected by the population of *R. austriaca* cultivated during the  
284 feedback phase (Table 1). The PSF for aboveground was significantly positive for one population

285 but negative for the others (Online Resource 2). The PSF for belowground was neutral or  
286 significantly negative depending on the population (Online Resource 2).

287

### 288 *Soil chemical properties*

289 The RDA analysis revealed that the conditioning species as well as the conditioning duration and  
290 their interaction had a significant effect on soil chemistry (all  $p$ -values  $\leq 0.003$ ). The conditioning  
291 species, conditioning duration and their interaction explained 25%, 19% and 13% of the total  
292 variance, respectively.

293 When tested separately, the conditioning species significantly affected the pH and K and  
294 P contents, while the conditioning duration significantly affected the pH and Mg content (Table  
295 2). The total N, total C and Ca contents were not significantly affected by any of the independent  
296 variables tested (Table 2). *R. austriaca* soil was characterized by lower pH (*R. austriaca*:  $8.03 \pm$   
297  $0.03$ , *A. capillaris*:  $8.16 \pm 0.01$ , mean  $\pm$  SE) and higher K and P contents (*R. austriaca* soil: K:  
298  $126.8 \pm 3.5$  mg kg<sup>-1</sup>, P:  $48.4 \pm 0.4$  mg kg<sup>-1</sup>, *A. capillaris* soil: K:  $99.5 \pm 4.1$  mg kg<sup>-1</sup>, P:  $45.4 \pm 0.7$   
299 mg kg<sup>-1</sup>, mean  $\pm$  SE) compared to *A. capillaris* soil. Globally, the pH and Mg content decreased  
300 with time (Fig. 2). The interaction between the conditioning species and the conditioning duration  
301 significantly affected the pH (Table 2). With the increase in the conditioning duration, pH  
302 strongly decreased in *R. austriaca* soil compared to the control and *A. capillaris* soil (Fig. 2a).

303

### 304 *Link between PSF and soil chemistry*

305 The PSF indices for aboveground and belowground biomass were marginally positively  
306 correlated with the Mg content (above- and below-ground:  $R^2 = 0.04$ ,  $\chi^2 = 3.72$ ,  $p = 0.05$  and  $R^2 =$   
307  $0.09$ ,  $\chi^2 = 3.74$ ,  $p = 0.05$ , Online Resource 3, see also Fig. 2b), but were not correlated with any  
308 other chemical soil properties ( $p > 0.06$  in all cases).

309

## 310 **Discussion**

311 We demonstrated that the duration of the conditioning phase modified the magnitude of PSF to a  
312 greater or lesser extent depending on the conditioning species. Furthermore, some changes of soil  
313 chemical characteristics were linked to the species and the duration of the conditioning phase.

314

### 315 *Intra- and inter-specific PSF and conditioning duration*

316 The negative feedback on *R. austriaca* biomass during the feedback phase in *R. austriaca* soil  
317 was consistent with a previous study showing that *R. austriaca* from its native range inhibited its  
318 own growth (Dostálek et al. 2016; Macel et al. 2017). Macel et al. (2017) hypothesised that in its  
319 native range, *R. austriaca* massively invested in defence and in production of allelopathic  
320 substances to the detriment of its growth. In addition, we demonstrated a neutral or negative  
321 feedback on *R. austriaca* in *A. capillaris* soil. Although *A. capillaris* does not improve and  
322 sometimes even restricts the growth of *R. austriaca*, the two species co-exist in the field  
323 (Dostálek et al. 2016). The PSF indices were more negative after intraspecific than after  
324 interspecific conditioning. This is in line with other studies showing that intraspecific feedback  
325 tends to be more negative than interspecific, at least in the native species (van Grunsven et al.  
326 2007; Kulmatiski et al. 2008; Hendriks et al. 2013, 2015; Hemrová et al. 2016). This  
327 phenomenon may explain maintenance of species diversity in plant communities (Bever 2003).  
328 Indeed, by inhibiting their own growth, this allows other species, including subordinate ones  
329 (Kardol et al. 2006), to settle in the same range, promoting plant coexistence (Kulmatiski et al.  
330 2008; Kulmatiski and Kardol 2008).

331 The magnitude of intraspecific and interspecific feedbacks was also affected by the  
332 duration of the conditioning phase. In soil conditioned by *R. austriaca*, the PSF indices tended to



333 become more negative, up to a maximum at six weeks. These results highlight the fact that *R.*  
334 *austriaca* inhibited its own growth to a lesser extent after two weeks than during the rest of its  
335 development. This weaker negative feedback at the beginning of growth was already noticed in  
336 studies about the ability of *R. austriaca* to become invasive in other areas (Bleeker and Matthies  
337 2005; Meisner et al. 2012; Dostálek et al. 2016; Macel et al. 2017). Moreover, at six weeks, the  
338 strong negative intraspecific feedback was particularly visible at the belowground biomass level.  
339 It has been demonstrated that the capacity of *R. austriaca* to become invasive is due to its high  
340 ability to regenerate clonally (Dietz et al. 2002), thanks to higher investment in root growth in  
341 invasive ranges compared to native ones (Meisner et al. 2012; Dostálek et al. 2016).  
342 Consequently, we can suppose that the negative feedback on belowground biomass could prevent  
343 *R. austriaca* in its native range from becoming dominant in the plant community.

344         In soil conditioned by *A. capillaris*, the effect of conditioning duration was visible by  
345 comparing the PSF indices between two weeks and the other durations. The most striking effect  
346 was the strong reduction of *R. austriaca* aboveground biomass at two weeks. We can hypothesise  
347 that this could be due to presence of AMF symbionts interacting with *A. capillaris*. Indeed, *A.*  
348 *capillaris* associates with AMF (Sudová et al. 2008), which play an important role during the first  
349 stages of plant growth (Ehlers 2011). Conversely, *R. austriaca* belongs to the Brassicaceae and is  
350 not associated to AMF (Müller 2009). Thus, in *A. capillaris* soil, the *R. austriaca* growth could  
351 be restricted by the presence of AMF that reduced the soil resources available for *R. austriaca*.  
352 Indeed, some studies demonstrated that the mycorrhizal symbionts can colonize Brassicaceae  
353 without a mutual interaction (Regvar et al. 2003), which could lead to a cost for the plant  
354 (Cipollini and Cipollini 2016).

355         The different effect of time after conditioning by the two species may be partly caused by  
356 the fact that the conditioning species were added in a different form, i.e. root fragments or seeds.

357 Since the plant developmental stage played a role in the dynamic of PSF (Kardol et al. 2013), we  
358 can wonder if such differences between intra- and inter-specific feedbacks could result from  
359 distinct developmental stages rather than effect of the conditioning species. Although we cannot  
360 exclude such a hypothesis, we assumed that the differences between species prevailed to the  
361 effect of developmental stage. As a supporting evidence, our results highlighted the fact that the  
362 conditioning duration mainly affected intraspecific feedback. Indeed, as the PSF is considered as  
363 species-specific (Cortois et al. 2016), we can suppose that the physiological changes of the plant  
364 during its growth could affect the soil abiotic and biotic factors in a way affecting itself more than  
365 another plant species. In addition, such a bias could only be minimized by beginning this  
366 experiment with two species at a same developmental stage because the plant developmental  
367 rhythm (Baxendale et al. 2014) and the soil abiotic and biotic changes occurring during plant  
368 development are species-specific (Bezemer et al. 2006). Moreover, the effect of different form of  
369 addition could be related to the fact that the two species achieved different biomass in different  
370 time periods. Thus, *R. austriaca* is naturally a bigger species than *A. capillaris* and we assumed  
371 that the plant will affect the soil proportionally to its biomass. Indeed, Schwinning and Weiner  
372 (1998) emphasized that plant size proportionally influenced nutrient uptake by the plant.  
373 However, we had, in reality, one *R. austriaca* individual or many *A. capillaris* seedlings in a pot  
374 resulting in comparable above- and below-ground biomass per pot for the two species. By this  
375 way, we minimized the fact that the soil modifications could be biomass-dependent (see Dostálek  
376 et al. (2016) for identical method). Nevertheless, these aspects should be kept in mind and the  
377 comparison between intra- and inter-specific feedback should be considered carefully in our  
378 experiment.

379 We also noticed that the populations of *R. austriaca*, which had distinct genotypes  
380 (Dostálek et al. 2016), differed in their PSF during the feedback phase. Several previous studies

381 suggested that the PSF could also be genotype-specific (Schweitzer et al. 2008; Hovatter et al.  
382 2013; Haney et al. 2015; Pieterse et al. 2016). This could result from the strong link between  
383 plant, soil and microbes, which led to a tight local adaptation of genotypes (Rengel and  
384 Marschner 2005; Pánková et al. 2008; Pregitzer et al. 2013; Pánková et al. 2014). Consequently,  
385 the regulation of soil nutrient pool and soil processes over plant growth is genotype-specific  
386 (Pregitzer et al. 2013). This could explain the distinct feedbacks, in term of magnitude and  
387 direction, between genotypes of *R. austriaca* even if they grew in the same conditioned soil.

388

### 389 *Relationship between PSF and soil chemical properties*

390 In our experiment, we did not measure the soil biotic mechanisms. We were therefore not able to  
391 distinguish if the observed feedbacks were mediated by the abiotic or biotic changes of the  
392 conditioned soils. However, we discussed the potential consequences of the abiotic changes on  
393 the feedbacks and hypothesised how they could affect the PSF through biotic changes.

394 As each species can specifically affect the soil properties, we looked for explanations of  
395 the PSF indices in the soil chemistry modified during the conditioning phase. The most striking  
396 result was a strong decrease in pH in *R. austriaca* soil. Soil acidification by plants is a known  
397 process (Ehrenfeld et al. 2005) that could disturb their own growth and that of other plants in a  
398 direct or indirect way. For example, mosses in the genus *Sphagnum* that firstly establish on an  
399 area, strongly acidify the soil, which prevents the establishment of vascular plant competitors  
400 (van Breemen 1995). However, the effect of pH on plant growth could also be indirect when  
401 mediated by soil communities as pH is one of the most important factor determining composition  
402 of bacterial communities (Fierer and Jackson 2006; Lauber et al. 2008; Rousk et al. 2010a,  
403 2010b). Thus, some studies have shown that a low pH reduced soil nutrient mineralization by  
404 microbes and so reduced the amount of nutrients available for the plants (Ehrenfeld et al. 2001;

405 Jones and Jacobsen 2005; Rooney et al. 2013). In our study, the negative intraspecific feedback  
406 experienced by *R. austriaca* in *R. austriaca* soil could be linked to the strong decrease in pH.

407 The other noticeable effect of the conditioning duration was a decrease in Mg content  
408 occurring independently of the conditioning species. This could result from the use of this  
409 element by the plants during their growth, through mineral weathering directly by plant or via  
410 soil microbes that provide plant with Mg (Bolou-Bi et al. 2010). Moreover, we found a positive  
411 relationship between the Mg content and the PSF indices in the feedback phase. As Mg is an  
412 essential nutrient for plant metabolism (Bolou-Bi et al. 2010; Verbruggen and Hermans 2013),  
413 the Mg decrease in the soil may partly explain the observed negative intra- and inter-specific  
414 feedbacks.

415 Concerning the absence of significant changes in soil N over the experiment, it is  
416 important to highlight that we only measured the total N content. This means that we had no  
417 indication about mineral N concentration or N mineralization rate. Consequently, we did not  
418 measure the N availability for plant in our conditioned soils. Indeed, we could hypothesise that  
419 the negative intra- and inter-specific feedbacks could be mediated by a depletion in soil nutrients  
420 over time, specifically N, one of the most limiting element for plant growth (LeBauer and  
421 Treseder 2008). In line with this, previous studies have shown that invasive species may increase,  
422 decrease or not affect the soil extractable N content which can negatively or positively affect the  
423 intra- and inter-specific feedbacks (Ehrenfeld et al. 2001; Ehrenfeld 2003; Meisner et al. 2012).

424 Finally, the other effects on soil chemical properties depended only on the conditioning  
425 species. Thus, the higher K content in *R. austriaca* soil was in accordance with the fact that *R.*  
426 *austriaca* is a forb. Indeed, the forbs are highly dependent on K and enrich the soil in that  
427 element (Bezemer et al. 2006). Potassium content is generally higher in forb monocultures than  
428 in grass monocultures (Bezemer et al. 2006), which is congruent with our results. Soil

429 conditioned by *A. capillaris* had lower P content than *R. austriaca* soil. This was in accordance  
430 with our previous hypothesis about the presence of AMF that could negatively affect the growth  
431 of *R. austriaca* in the feedback phase.

432 Our aim was to identify the link between soil chemical properties of the conditioned soil  
433 and the PSF in the feedback phase with different conditioning durations. We noticed a negative  
434 relationship between the conditioning duration and the Mg content. In our study, the low number  
435 of samples used for the measurement of the chemical properties (three by conditioning duration)  
436 comparatively to the samples used for the evaluation of PSF indices (25 by conditioning  
437 duration) may be responsible for the absence of significant correlation between PSF and  
438 chemistry. Another explanation for the absence of correlation between PSF and chemistry could  
439 result from more complex relationships involving direct effect of soil chemistry on PSF or  
440 indirect effect via other soil properties (Ehrenfeld et al. 2005). Indeed, a direct link between soil  
441 chemical properties and PSF is hard to identify because the chemical properties interact in  
442 complex pathways with the physical properties and the soil microbial communities (Ehrenfeld et  
443 al. 2005). In addition, a delay can occur between the cause at the level of the soil chemical  
444 composition and the response at the plant growth level, making the relationship more complex to  
445 identify. For example, allelochemical components accumulate in soil which contributes to direct  
446 negative feedback (Lankau 2010). Nevertheless, some microbes can progressively develop and  
447 degrade these allelochemicals (Lankau 2010) or make them more toxic (Inderjit 2005).  
448 Consequently, the results of PSF experiments may change according to the chosen timing.

449 Our findings demonstrated that the conditioning duration may influence the magnitude of  
450 the PSFs. The conditioning species has also to be considered, because it interacted with the  
451 conditioning duration. The soil chemical characteristics can be put in parallel with the PSFs even  
452 though only marginally significant relationships have been demonstrated. Thus, future PSF

453 studies using two-phase experiments should consider the duration of the conditioning phase.  
454 Therefore, they should adapt the conditioning duration to the stage of plant growth of interest.  
455 Further studies coupling abiotic soil properties and molecular analyses of soil microbial  
456 communities over time could bring complementary information to explain the change in  
457 magnitude of intra- and inter-specific feedbacks. Our findings may be relevant for studies dealing  
458 with the mechanisms determining the invasive potential of exotic species depending on the  
459 duration of growth of the native species in field conditions. It may also be important for studies  
460 exploring species replacement along successional series.

461

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723 nutritional imbalance in plants. Plant Soil 368:87-99. doi: 10.1007/s11104-013-1589-0

724 **Table 1** Results of generalized linear models explaining the plant-soil feedback (PSF) indices  
 725 depending on the duration of the conditioning phase (Duration), the conditioning species  
 726 (Species), the population of *Rorippa austriaca* cultivated in the feedback phase (Population) and  
 727 their interactions

Source of variation	<i>df</i>	PSF index aboveground biomass		PSF index belowground biomass	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Species	1	17.99	<0.001	22.77	<0.001
Duration	1	2.02	0.155	6.22	0.013
Species × Duration	1	26.10	<0.001	12.74	<0.001
Population	4	180.55	<0.001	23.35	<0.001
Population × Species	4	4.58	0.333	7.58	0.108
Population × Duration	4	7.60	0.107	3.32	0.506
Population × Species × Duration	4	5.78	0.216	5.03	0.284

728

729 **Table 2** Results of two-way ANOVA examining the effects of conditioning species (Species),  
 730 duration of the conditioning phase (Duration) and their interaction on soil chemical properties. To  
 731 take into account multiple testing, Bonferroni correction was applied and significant *p*-values  
 732 adjusted: < 0.007, \*\* *P* < 0.001, \* *P* ≤ 0.007, n.s. non significant with *P* > 0.007

Source of variation	<i>df</i>	pH(H <sub>2</sub> O)		total N (%)		total C (%)		Ca (mg kg <sup>-1</sup> )		Mg (mg kg <sup>-1</sup> )		K (mg kg <sup>-1</sup> )		EXCHANGEABLE P (mg kg <sup>-1</sup> )	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Species	1	57.1	**	4.8	n.s.	2.5	n.s.	4.1	n.s.	5.0	n.s.	31.7	**	19.2	**
Duration	1	44.5	**	7.2	n.s.	7.3	n.s.	7.2	n.s.	14.8	*	1.3	n.s.	1.7	n.s.
Species × Duration	1	33.9	**	7.2	n.s.	7.3	n.s.	3.1	n.s.	0.1	n.s.	5.9	n.s.	5.9	n.s.

733

734 **Figure legends:**

735

736 **Fig. 1** The effect of the conditioning species (*Agrostis capillaris* or *Rorippa austriaca*) and the  
737 duration of the conditioning phase on the plant-soil feedback (PSF) indices for a) aboveground  
738 and b) belowground biomass. Bars represent means  $\pm$  SE ( $17 \leq n \leq 25$ ). The filled triangles  
739 indicate that the PSF significantly differs from zero after *t*-tests. Asterisks indicate that the PSF  
740 indices are significantly different between the two conditioning species, after *t*-tests performed  
741 for each duration separately. Significant differences in the PSF indices between the four durations  
742 of the conditioning phase within *A. capillaris* and within *R. austriaca* are indicated by different  
743 uppercase and lowercase letters, respectively, after Tukey-Kramer HSD tests

744

745 **Fig. 2** The effect of the conditioning species (*Agrostis capillaris* or *Rorippa austriaca*) and the  
746 duration of the conditioning phase on a) pH and b) Mg content. The grey and black dotted lines  
747 indicate the mean and the standard error, respectively, for the control soil without plant. Bars  
748 represent means  $\pm$  SE ( $n = 3$ ). The empty triangles indicate that the value is significantly different  
749 from the control soil without plant after *t*-tests. Asterisks indicate that the values of the chemical  
750 element measured are significantly different between the two conditioning species, after *t*-tests  
751 performed for each duration separately. Significant differences in the plant-soil feedback indices  
752 between the four durations of the conditioning phase within *A. capillaris* and within *R. austriaca*  
753 are indicated by different uppercase and lowercase letters, respectively, after Tukey-Kramer HSD  
754 tests

Fig. 1

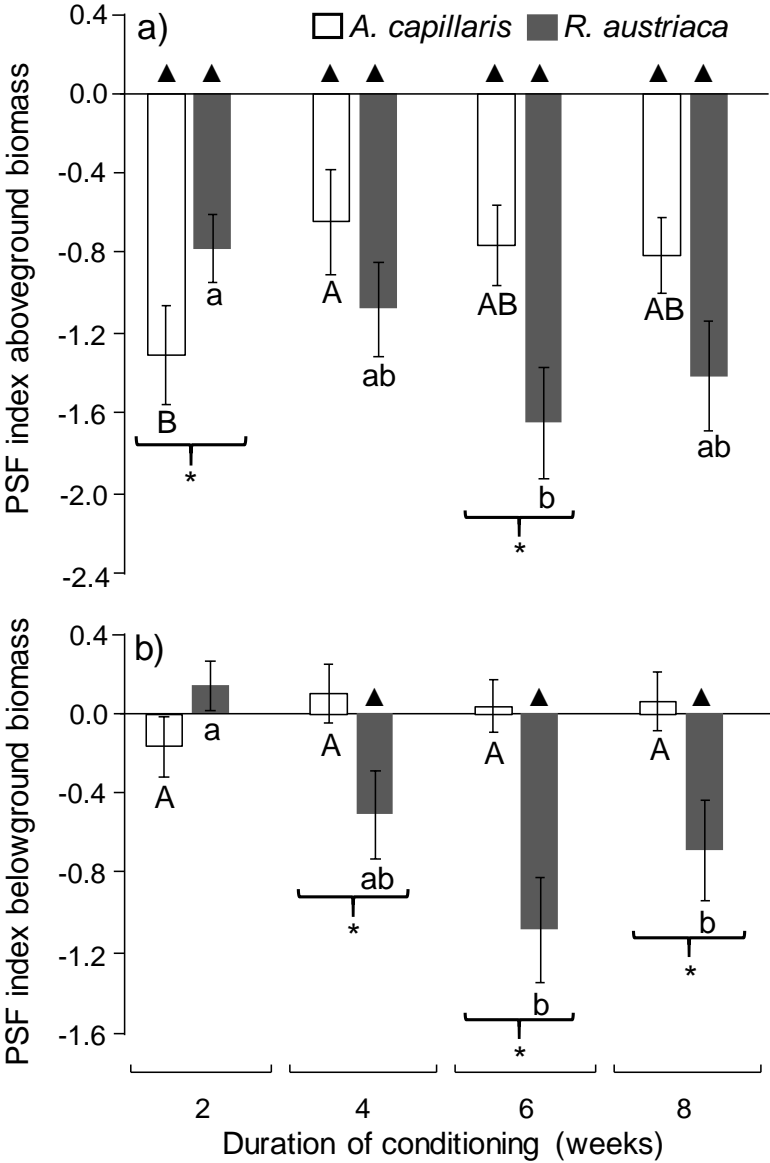


Fig. 2

