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Short communication

Flowering phenology of a herbaceous species (*Poa annua*) is regulated by soil Collembola

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The role of soil organisms as possible driver of flowering has never been investigated. We hypothesized that Collembola (microarthropods) will change plant allocation to reproductive modes by changing soil nutrient availability. Individual seedlings of *Poa annua* were planted in microcosms, in the presence or absence of Collembola. Collembola affected biotic (fungal biomass) and abiotic ($N - NO_3^-$, P_2O_5) soil properties and some morphological (number of leaves, root biomass) and chemical (C:N, K, Mg, N) traits of *P. annua*. As a result, flowering of *P. annua* was promoted by the presence of Collembola. This provides experimental evidence that soil microarthropods can affect the reproduction strategy and phenology of a plant.

The belowground compartment is a fundamental driver of the aboveground system and therefore of ecosystem fate and properties (De Deyn et al., 2003; Wardle et al., 2004; Wagg et al., 2014). Collembola are among the most abundant microarthropods in the rhizosphere of plants (Coleman et al., 2004). Several studies have highlighted a strong influence of various Collembolan species on plant roots either indirectly by regulating soil nutrient availability (Bardgett and Chan, 1999; Filser, 2002; Ngosong et al., 2014) or directly by exerting rhizophagous pressure (Endlweber et al., 2009). Nevertheless, the consequences of such impacts on resource allocation for plant reproduction and on flowering phenology have apparently never been investigated. A small change in reproductive success or flowering time can affect plant fitness (e.g. Galloway and Burgess, 2012; Weis et al., 2015), and consequently might alter interactions with populations of pollinators, seed dispersers, or floral herbivores. The role of biotic interactions in shaping plant flowering phenology has been demonstrated for pollinators and herbivores (Elzinga et al., 2007). Therefore, in the present study, we tested in a microcosm experiment whether the presence of a natural assemblage of Collembola affected plant growth and sexual reproduction (flowering) of *Poa annua* L.

In a microcosm experiment, two treatments were established corresponding to the presence ("Coll.") or absence ("None") of a natural assemblage of Collembola. Each treatment was replicated 15 times in microcosms (plastic pots 10 × 9 × 9 cm) where individuals of *P. annua* were grown. The soil was a rendosol (Organic Matter content = 6.17%; pH = 7.79) collected from a chalk grassland (1°7′30″E, 49°22′22″N) in Normandy, France (further details are given in Supplemental information). A part of the soil was defaunated by repeated deep-freezing and thawing (see Supplemental information). The 30 microcosms were meshed (250 μm) to prevent any escape of microarthropods and were filled with a mixture of defaunated soil and sand (5:1). From the remaining part of the collected soil, microorganisms (fungi and bacteria) and Collembola were extracted and re-inoculated in order to set the two different treatments. Microorganisms were re-inoculated in all microcosms prior to Collembola by adding "soil extract" (see Supplemental information). Re-inoculation of microorganisms was followed, two weeks later, by Collembola addition into the 15 "Coll" microcosms. Collembola were extracted from collected soil using a Berlese–Tullgren apparatus (see Supplemental information). The extraction of Collembola in microcosms at the end of the experiment, allowed us to confirm that (i) no contamination was observed in the treatment without Collembola, and that (ii) collembolan assemblages, in terms of abundance, were maintained until the end of the experiment, even if inevitably compositional

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Table 1
Effect of Collembolan inoculation on soil properties after 10 weeks of *Poa annua* growth. Data are means ± SE. Statistical significance was set at $p < 0.05$.

Soil properties:	N	None	Coll.	Statistical test	
				T or Z	P value
Cmic (mgC g^{-1} dw)	10	300.1 ± 20.2	315.6 ± 18.3	−0.570[a]	0.575
Ergosterol (μg g^{-1} dw)	10	1.84 ± 0.08	2.45 ± 0.12	−4.166[a]	**<0.001**
Fungal:bacterial ratio	10	0.63 ± 0.03	0.79 ± 0.03	−3.228[a]	**0.004**
$N-NH_4^+$ (μg g^{-1} dw)	10	0.097 ± 0.007	0.109 ± 0.013	−0.189[b]	0.853
$N-NO_3^-$ (μg g^{-1} dw)	10	0.063 ± 0.002	0.100 ± 0.011	−3.175[b]	**<0.001**
P_2O_5 (μg g^{-1} dw)	10	34.7 ± 1.6	53.0 ± 2.5	−6.153[a]	**<0.001**
pH	10	7.78 ± 0.03	7.76 ± 0.02	0.600[a]	0.555

The p value in bold indicates a significant effect of Collembola inoculation on the soil variable.
[a] Student's t test.
[b] Mann–Whitney non-parametric test.

changes appeared (see Supplemental information and supplemental Table 1). Basically we observed a shift from a community dominated by *Folsomia quadrioculata* (Tullberg 1871) at the start and finally dominated by *Parisotoma notabilis* (Schäffer 1896). However both species belong to the same ecological group (i.e. hemiedaphic species).

A single seedling of *P. annua* was transplanted into each microcosm. All of them were incubated in a climate chamber (temperature: 20 °C; daily light/dark 16 h/8 h) with soil moisture kept to 60% of the soil water holding capacity during the experiment. The experiment was stopped after 10 weeks, when more than 80% of *P. annua* were flowering in the Collembola treatment. Several plant traits were measured. Root parameters (root length, total root projected area and number of root tips) were assessed using an image analysis software. Shoot and root were oven dried at 65 °C for 48 h to obtain root biomass (R), shoot biomass (S) and S:R ratio. The Chlorophyll Content Index (CCI) was measured on fresh leaves. Finally carbon, nitrogen, potassium and magnesium content of shoots were measured (see Supplemental information). Soil properties were also investigated at the end of the experimentation and included fungal biomass (i.e. ergosterol concentration), Microbial carbon biomass (Cmic), mineral N ($N-NO_3^-$ and $N-NH_4^+$), phosphorus content (P_2O_5), pH H_2O (see Supplemental information for methods). Univariate analyses were performed to detect significant effects of Collembola on soil and plant. Student's t tests were used for parametric data, whereas Mann–Whitney U tests were chosen for non-parametric data. All statistical analyses were conducted with Statistica software (Statsoft, Inc., V10).

The presence of Collembolan assemblages led to a significant increase of soil $N-NO_3^-$ (+59%) and P_2O_5 (+53%) after 3 months compared to the control treatment, but did not affect $N-NH_4^+$ content (Table 1). In parallel, the fungal biomass (ergosterol) also significantly increased in the presence of Collembola (+39%, Table 1). Since there were no changes in microbial biomass between treatments (Table 1), the fungal:bacterial biomass ratio was also higher in the presence of Collembola (+25%, Table 1). Past experiments have shown that Collembola stimulate soil fungi either directly by browsing or indirectly by dispersing propagules and excreting nutrient-rich excreta (Bardgett et al., 1993; Crowther et al., 2012). Additionally, several studies suggest that these Collembola – microorganisms interactions resulted in increased rates of soil nutrient mineralization through the grazing activities of the Collembola (Ineson et al., 1982; Cole et al., 2004; Crowther et al., 2012).

The increase in soil N was associated with an increase in shoot N content (Table 2) and with a parallel decrease in the C:N ratio ($T = 2.181$, $p = 0.038$, Fig. 1C). Shoots of *P. annua* had significantly more leaves and more ramets when growing with Collembola (Table 2) but shoot height and biomass were not different between our treatments. There was no difference in root morphology between treatments beside root biomass which was 2.4 fold higher in the presence of Collembola (Table 2) conversely to the study of Scheu et al. (1999) that used two collembolan species. As a consequence, the shoot:root ratio was lower with Collembola ($Z = 2.67$ and $p = 0.006$, Fig. 1B). Plants generally invest less in their roots when soil nutrient is rich (Reynolds and D'Antonio, 1996; but see Hodge, 2009). This unexpected result might also reflect a feeding pressure of Collembola on *P. annua* roots, leading to compensatory growth of the roots (Endlweber et al., 2009).

The effect of Collembola was evident in plant reproduction (Fig. 1A). After 10 weeks of monitoring, flower emergence was 6.5-fold higher in the presence of Collembola ($Z = -3.54$, $P = 0.0002$). Specifically, 86.7% of *P. annua* were flowering when growing with Collembola, *versus* 13.33% in their absence. The onset of flowering was promoted by 2 weeks (14 days) by Collembola. Although flowering phenology strongly responds to climatic factors, other biotic and abiotic soil properties mediated by Collembola can act as a stimuli. Recently, Wagner et al. (2014) demonstrated that the flowering phenology of two herb species was slightly shifted (about

Table 2
Effect of Collembolan inoculation on plant (*Poa annua) parameters* after 10 weeks of experimentation. Data are means ± SE. Statistical significance was set at $p < 0.05$.

	N	None	Coll.	Statistical test	
				T or Z value	P Value
Shoot morphology					
Shoot height (cm)	15	27.15 ± 1.0	27.93 ± 0.6	−0.648[a]	0.522
No. of leaves	15	20.33 ± 1.9	25.73 ± 1.8	−2.083[a]	**0.046**
No. of ramets	15	2.93 ± 0.3	4.47 ± 0.2	−3.027[b]	**0.002**
Shoot biomass (mg)	15	100.3 ± 11.5	117.4 ± 11.1	−1.071[a]	0.293
Root morphology					
Root biomass (mg)	15	1399 ± 304.6	3310 ± 660.9	−5.641[b]	**0.008**
Root length (cm)	15	110.3 ± 27.2	157.4 ± 37	−1.209[b]	0.247
Root projected area (cm^2)	10	1.2 ± 0.3	2.1 ± 0.6	−1.361[b]	0.190
Number of root tips	10	867.6 ± 248.3	1181 ± 217.7	−1.361[b]	0.190
Plant chemistry (aerial part)					
N content (%)	15	2.6 ± 0.3	3.2 ± 0.9	−1.679[a]	0.097
C content (%)	15	38.2 ± 0.1	37.74 ± 0.2	1.099[b]	0.285
Mg content (mg g^{-1})	9	1.8 ± 0.1	1.5 ± 0.1	2.175[b]	**0.045**
Plant physiology					
Chlorophyll content index (CCI)	15	1.37 ± 0.04	1.27 ± 0.03	2.342[a]	**0.027**

The p value in bold indicates a significant effect of Collembola inoculation on the soil variable.
[a] Student's t test.
[b] Mann–Whitney non-parametric test.