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REGULAR ARTICLE

Endogeic earthworms modify soil phosphorus, plant growth and interactions in a legume–cereal intercrop

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Abstract
Background and aims Intercropping of legumes and cereals appears as an alternative agricultural practice to decrease the use of chemical fertilizers while maintaining high yields. A better understanding of the biotic and abiotic factors determining interactions between plants in such associations is required. Our study aimed to analyse the effect of earthworms on the legume–cereal interactions with a focus on the modifications induced by earthworms on the forms of soil phosphorus (P).
Methods In a glasshouse experiment we investigated the effect of an endogeic earthworm (*Allolobophora chlorotica*) on the plant biomass and on N and P acquisition by durum wheat (*Triticum turgidum durum* L.) and chickpea *(Cicer arietinum* L.) either grown alone or intercropped. The modifications of the different organic and inorganic P forms in the bulk soil were measured.
Results There was no overyielding of the intercrop in the absence of earthworms. Earthworms had a strong influence on biomass and resource allocation between roots and shoots whereas no modification was observed in terms of total biomass production and P acquisition. Earthworms changed the interaction between the intercropped species mainly by reducing the competition for nutrients. Facilitation (positive plant–plant interactions) was only observed for the root biomass and P acquisition in the presence of earthworms. Earthworms decreased the amount of organic P extracted with NaOH (Po NaOH), while they increased the water soluble inorganic P (Pi H_2O) content.
Conclusions In this experiment, earthworms could be seen as "troubleshooter" in plant–plant interaction as they reduced the competition between the intercropped species. Our study brings new insights into how earthworms affect plant growth and the P cycle.

Keywords Soil fauna · Nitrogen · Chickpea · Durum wheat · Resource allocation · Competition

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Introduction

The so-called ecological intensification of agriculture aims to replace the extensive use of synthetic fertilizers and pesticides by practices that take a better advantage of biological processes. To achieve such an agricultural transition, there is a great need to bring together agronomic and ecological knowledge in order to develop efficient alternative agricultural practices such as intercropping (Bennett et al. 2012; Bommarco et al. 2012). Intercropping stands for the growth of two or more crops together in the same field during at least a portion of the growing season in order to promote positive interactions, i.e. facilitation, between them (Vandermeer 1992; Zhang and Li 2003). Mixing crops could enhance pest protection or resource use efficiency. According to this theory, resources necessary for plant growth, such as light, water or nutrients are more efficiently absorbed and converted into crop biomass by the intercrop as a result of the niche differentiation of each component species of the intercrop (Vandermeer 1992; Wang et al. 2007). Legumes are often more phosphorus (P) use efficient as a result of (i) organic anion excretion and subsequent release of inorganic P from mineral constituents; (ii) production of enzymes that transform organic P into inorganic P. These two mechanisms can thus facilitate P acquisition in intercropped legumes and non-legume species (Cu et al. 2005; Li et al. 2008; Hinsinger et al. 2011a, b; Betencourt et al. 2012). Intercropping can significantly enhance the yield (i.e. overyielding) of the intercrop compared to the yield of each component species grown alone, especially under low P conditions (Li et al. 2007).

However, intercropping does not systemically lead to overyielding. This practice cannot be the single solution but must be used in concert with other agroecological practices. Among them, reducing tillage and increasing soil organic matter inputs are known to enhance soil biodiversity and biological activity (McLaughlin and Mineau 1995; Giller et al. 1997) with, ultimately, a positive impact on soil fertility (Altieri and Nicholls 2005). Those practices are also likely to favour earthworms that as ecosystem engineers are able to influence greatly their physical and biological environment (Lavelle 1996; Blanchart et al. 1999; Lavelle et al. 2006).

Regarding the effect of earthworms, most published studies reported an increase in shoot biomass of plants in their presence (review in Scheu 2003; Brown et al. 2004; Blouin et al. 2013). A large panel of mechanisms has been described in earthworm–plant interactions: changes in soil structure and water regime (Logsdon and Linden 1992), mineralization of nutrients and especially nitrogen (Postma-Blaauw et al. 2006), hormone-like effects (Muscolo et al. 1999), dispersal of growth stimulating microorganisms and of microorganisms antagonistic to root pathogens (Clapperton et al. 2001). In addition, a recent review by Chapuis-Lardy et al. (2011) highlighted the role of earthworms in P cycling. In comparison to bulk soil, earthworm casts are characterized by higher P contents, especially water-soluble or available inorganic P (Pi) (Le Bayon and Binet 2006; Kuczak et al. 2006), which have demonstrated positive effects on plant growth. Earthworm-processed soils have thus the potential for higher P availabilities than non-ingested soils, but the role of earthworm activities in the acquisition of P by growing plants (i.e. P bioavailability) needs to be further studied. In addition, the compilation of experimental results by Scheu (2003) shows a high occurrence of non-significant or negative effect of earthworms on legume biomass. This trend, corroborated by recent field (Eisenhauer et al. 2009) and laboratory (Eisenhauer and Scheu 2008; Laossi et al. 2009) studies, indicates that different functional groups of plants can exhibit different responses to earthworm presence thus changing the interaction between the different plant species that are co-occurring in natural ecosystems or in intercrop-based agroecosystems.

Durum wheat (*Triticum turgidum durum* L.) and chickpea (*Cicer arietinum* L.) have been exhibiting positive interactions (i.e. facilitation) when intercropped (Betencourt et al. 2012). In the present work, we wanted to assess how such an intercropping system could be impacted by earthworm activity in a greenhouse experiment.

We addressed this question in the present work by means of an experimental approach in greenhouse. Chickpea and durum wheat were grown in pots either in pure stand or intercropped, with and without earthworms. Our hypotheses are: (H1) earthworms affect plant growth and root–shoot ratio, (H2) earthworms change plant–plant interactions by favouring durum wheat, (H3) overyielding if measured is more important in presence of earthworms than in their absence, (H4) earthworms increase the amount of available P, and thus the amount of P acquired by plants (i.e. P bioavailability).

Materials and methods

Soil, plants and earthworms

A calcareous Cambisol (26 % clay, 18 % fine silt, 29 % coarse silt, 16 % fine sand, 11 % coarse sand) taken from the INRA experimental station of Mauguio (South of

France 03°59′03″E, 43°37′14″N, 12 m above sea level) was used in this study. We collected the topsoil layer (0–10 cm depth), air-dried and sieved the soil at 2 mm. It exhibited a low organic carbon content (14 mg $C.g^{-1}$ soil), a C:N ratio around 11, and a substantial level of Olsen P (30 $mg.kg^{-1}$ soil) which is typical for this type of soil under cultivation. Earthworms of the species *Allolobophora chlorotica* (Savigny) were the most abundant earthworms sampled from the plot where soil was taken (60 $ind.m^{-2}$ at spring 2011, Blanchart unpublished data). This species belongs to the endogeic geophagous ecological category (Bouché 1977). The plants used in the study were durum wheat (*Triticum turgidum durum* L., var. LA1823) and chickpea *(Cicer arietinum* L.*,* var. Daisy).

Experiment

Plastic containers (1.5-dm^3 volume) were filled with 1,200±1 g of soil. The containers were lined with a thin plastic film so as to facilitate soil collection at the end of the experiment, while preventing drainage. Deionized water was added in order to reach 80 % of the field capacity, i.e. water content equal to 15 % for the sieved soil. Water content was controlled and adjusted by weight regularly all over the experiment.

Seeds were pre-germinated for 72 h and then sown on 28th January 2010 in the containers. Since durum wheat and chickpea has contrasted sizes, they could not be sawn at equal density. We used a proportional substitution experimental design (Snaydon 1991; Jolliffe 2000; Connolly et al. 2001) in which the two intercropped plant species do not have the same density as when grown alone. We referred to densities used in the field for the cultivation of each species. Density of chickpea was two plants per container in pure culture and one plant per container in mixed culture. Density of durum wheat was six plants per container in pure culture and three plants per container in mixed culture. Each container with chickpea was inoculated with 1.5 cm^3 of a solution containing the specific strain *Rhizobium cicerii*. At the beginning of the experiment, 18 mg of urea (CH_4N_2O) were added to each container. This intermediate level of nitrogen was chosen in order to avoid a deficiency for the wheat without inhibiting chickpea nodulation and nitrogen fixation (Dreyfus and Dommergues 1980). The four plant treatments, i.e. (i) no plants, (ii) pure culture of chickpea, (iii) pure culture of durum wheat, and (iv) mixed culture of chickpea and durum wheat, were conducted with and without earthworms. We chose to add eight earthworms per container, i.e. a density corresponding to 114 individuals per m^2, which is about twice the field population density (60 $ind.m^{-2}$). This was high but realistic since (Schmidt and Curry 1999) recorded earthworm population densities above 1,000 per m^2 in a wheat–clover intercropping system.

Each of the 8 treatments was replicated 5 times, yielding a total of 40 containers that were arranged on 5 different shelves in a greenhouse. Each of the five replicates per treatment combination was kept in one of five shelves, with containers distributed randomly within shelves and position changed every week according to a completely randomized block design. The experiment lasted 46 days and at harvest chickpea was at the beginning flowering stage, while durum wheat was at advanced tillering stage. At harvest, the soil was gently, manually disaggregated. Earthworms were removed, washed and weighed; shoots and roots of each species were separated (according to their colour and shape) and oven-dried at 60 °C during 48 h before being analysed. After homogenization of soil, an aliquot was sampled for further analyses.

Soil and plant analyses

Phosphorus cycle being complex, it can be abundant in soil but poorly available because of its immobilization in adsorbed, mineral, or organic P forms. We thus measured five different organic or inorganic P forms in soil to have a broad picture of how plants and earthworms influenced P cycle in our experiment. Twenty grams of soil from each treatment were oven-dried for water content measurement. Soil available P was measured on fresh soil samples using three chemical extractants: H_2O, $NaHCO_3$ 0.5 M (pH 8.5), and NaOH 0.1 M in order to assess the different P pools (Olsen et al. 1954; Bowman and Cole 1978; Tiessen and Moir 1993). After shaking, the extract was centrifuged for 10 min at 14,000 g and then filtered at 45 μm. We used the following soil/solution ratios: 1/10 for water and 2 h of shaking, 1/20 for $NaHCO_3$ and 30 min of shaking, 1/30 for NaOH and 16 h of shaking. An aliquot of the $NaHCO_3$ and NaOH extracts was mineralized in presence of HCl 12N (extract/acid ratio= 1/1) during 16 h at 110 °C (Ali et al. 2009). We used the malachite green method to measure inorganic P in the extracts (Ohno and Zibilske 1991) and a spectrophotometer (visible, Elx808, Dialab, Germany) microplate reader. Organic P (Po) concentrations were determined as a difference between total P and Pi obtained from mineralized extracts.

After 48 h in the oven at 60 °C, plants (shoots and roots separately) were weighed and then ground. Total N and C contents in plant biomass were measured by using a CHN microanalyser (Fisons/Carlo Erba NA 2000, Milan, Italy), while P content in plants was measured using the vanadomolybdate ammonium procedure (Murphy and Riley 1962).

Data analyses

Effects of earthworms and intercropping on total biomass production per container were tested using an ANOVA model for randomized block design. Earthworm and intercropping were considered as fixed factors and block as random factor. Then, in order to analyse variables measured on each species, a correction factor was applied to account for the substitutive design. The biomass production for each species as well as nutrient acquisition obtained in pure stand treatments were divided by 2 to be directly comparable to results obtained in intercropping treatments. The differences in biomass production, N accumulation and P accumulation were analyzed using ANOVA models for randomized complete block design. Those three variables were analyzed at the whole plant level and also for roots and for shoots separately. Plant species (chickpea or durum wheat), earthworms (presence or absence) and intercropping (pure stand or intercropped) were considered as fixed factors and blocks were considered as random factors. For those analyses we assumed that block by factor interactions were null and were pooled in residuals (Quinn and Keough 2002). Data were Arcsin or power transformed prior to analyses in order to achieve parametric assumption. The differences in shoot: root ratio were analyzed using two way ANOVA on each species separately where earthworms and intercropping were considered as fixed factors and block as random factor.

The Relative Interaction Index (RII) was calculated for each container with intercropped plants using Armas et al. (2004) formula:

$$RII = \frac{B_{inter} - B_{pure}}{B_{inter} + B_{pure}}$$

Where B_{pure} is the mean biomass of an individual plant in pure stand and B_{inter} is the biomass of individual plants in intercropping. RII values are comprised between −1 and 1, negative values corresponding to competition and positive ones to facilitation. The RII was calculated for biomass, N accumulation and P accumulation. We tested the data for normality and homoscedasticity using the Shapiro–Wilk and Bartlett Tests, respectively. As in most cases variances were not homogeneous we decided to use the non-parametric Mann–Whitney test to test for effect of earthworms on RII for each plant species. Finally the effect of earthworms on soil P forms was assessed by a student test performed for each P form. All statistical analyses were performed using R software version 3.0.1 (R Development Core Team 2013).

Results

The final biomass of earthworms was in average 70 % of the initial biomass. Over the 8 earthworms added initially, we recorded only 5.9 individuals per container on average at the end of the experiment. This decrease was mainly due to earthworms that escaped from containers during the study.

At the level of the container, there was no significant effect of intercropping (p=0.58) and earthworms (p=0.35) on total biomass production (data not shown). Consequently further result presentation focuses on intercropping and earthworm effects on above- and belowground parts of wheat and chickpea separately.

Plant biomass and nutrient acquisition

Mean total biomass (dry weight) of wheat and chickpea were respectively 0.92 and 1.04 g in control treatments, *i.e.* pure stand without earthworms. The total plant biomass did not change with respect to intercropping or presence of earthworms (Table 1). Nevertheless, earthworm presence as well as intercropping significantly reduced root production (p=0.009 and p=0.003 respectively), for example chickpea roots decreased by 42 % in presence of earthworms (Fig. 1). For durum wheat, the shoot: root ratio increased in presence of earthworms (p=0.004) but did not change in intercropping compared to pure stand (p=0.8) (Table 2). For chickpea, the earthworms increased significantly the shoot: root ratio (p=0.03). However, the intercropping affected the shoot: root ratio only in the absence of earthworms, hence intercropping had no significant direct effect on

Table 1 Results of ANOVA to test for the effects of Earthworms, intercropping and plant species on the belowground biomass, the aboveground biomass and the whole plant biomass

Source of variance	Belowground biomass			Aboveground biomass			Total biomass		
	Df	*F*-value	*p*-value	Df	*F*-value	*p*-value	Df	*F*-value	*p*-value
Between blocks:									
Block	4	–	–	4	–	–	4	–	–
Within blocks:									
Intercropping (I)	1	0.880	0.356	1	0.363	0.552	1	0.545	0.467
Earthworm (E)	1	7.773	0.009**	1	1.219	0.279	1	0.619	0.438
Species (Sp)	1	10.587	0.003**	1	40.256	<0.001***	1	5.435	0.027*
I × E	1	2.217	0.148	1	0.054	0.817	1	0.116	0.736
I × Sp	1	0.477	0.496	1	0.068	0.796	1	0.032	0.859
E × Sp	1	0.017	0.897	1	0.017	0.896	1	0.266	0.610
I × E × Sp	1	0.990	0.328	1	0.898	0.351	1	0.005	0.942
Residuals	28	–	–	28	–	–	28	–	–

The significance of effects, or interactions of effects is shown by asterisks: *$p<0.05$, **$p<0.01$, ***$p<0.001$

the shoot: root ratio of chickpea ($p=0.5$), but the interaction between earthworms and intercropping was significant ($p=0.02$).

Chickpea accumulated more nitrogen than durum wheat (Fig. 1). Nitrogen acquisition was positively influenced by earthworms. Chickpea accumulated significantly more N from soil; for example in pure stand the presence of earthworms increased by 17 % the total N acquired by chickpea (Table 3). Amount of nodules produced by chickpea was highly variable (between 0.12 and 2.7 % of root biomass), no earthworm effect on nodulation could be detected; we thus assume that the proportion of nitrogen coming from fixation was also low. In pure stand without earthworms, more N was accumulated in shoots than in roots, with a factor of 2.4 and 3.8 respectively for durum wheat and chickpea. As for biomass, earthworms globally decreased the N amount in roots and increased the N amount in shoots, leading to an increase of the shoot: root ratio for N acquisition (data not shown).

Phosphorus acquisition at the whole plant level was not significantly different between durum wheat and chickpea (Fig. 1). Moreover phosphorus acquisition did not change with respect to intercropping or presence of earthworms (Table 4). However at the shoot level, earthworms increased shoot P by 27 % in average across all treatments ($p=0.01$). At the root level neither earthworms nor intercropping affected P acquisition. ($p=0.15$ and $p=0.12$ respectively). As for biomass and N, earthworms increased the shoot: root ratio for P (data not shown).

Effects of earthworms on plant interactions

The values of RII indices indicate the strength of the interaction between plants (which can be positive or negative). In many cases the RII was close to 0, indicating an interspecific competition similar to intraspecific competition (Fig. 2). Regarding biomass production, durum wheat was slightly negatively, although not significantly, affected by the presence of chickpea in the intercropping. However chickpea exhibited a strong competition at the root level when intercropped with durum wheat; this negative effect, significant at $p=0.057$, disappeared in the presence of earthworms that brought up the RII for root to positive values. Concerning N acquisition, the RII for durum wheat tended to be positive and the RII for chickpea tended to be negative (Fig. 2). Without earthworms RII for P uptake was negative for both plant species: −0.09 for durum wheat and −0.17 for chickpea. Earthworm presence strongly increased the RII for P acquisition for all plant parts of chickpea. Values of RII were therefore not significantly different from 0, i.e. the null interaction value.

Soil phosphorus forms

There was no significant difference between plant species and no effect of intercropping for any of the measured P pools (Appendix Figure A1, Appendix Tables A1, A2). It would have required to sample the

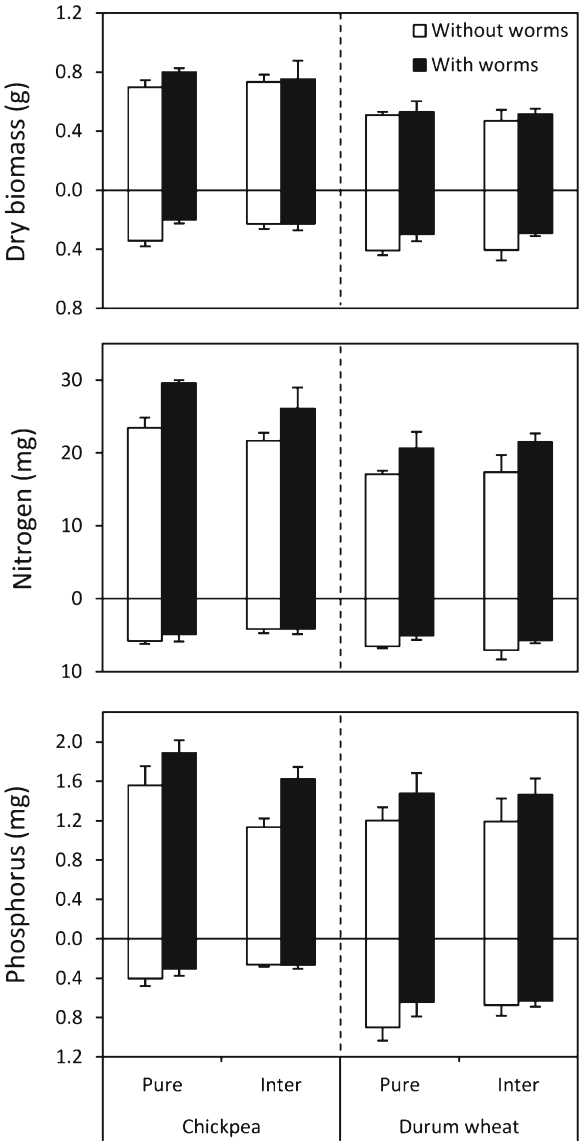

Fig. 1 Effects of earthworms and intercropping (i) on the above-ground (*values above 0*) and belowground (*values below 0*) biomass production in durum wheat and chickpea, (ii) on nitrogen accumulation in the aboveground and belowground parts of durum wheat and chickpea, and (iii) on phosphorus accumulation in the aboveground and belowground parts of durum wheat and chickpea. Means and standard errors (n=5)

rhizosphere instead of the bulk soil to see significant changes induced by plant roots. Therefore, the following concerns only the effect of the earthworms in the unplanted pots. Two out of five forms of soil P were significantly impacted by the presence of earthworms (Fig. 3). In the unplanted soil, earthworms slightly increased available P, namely an 8 % increase of water extractable P, i.e. inorganic phosphorus extracted with

Table 2 Effects of earthworm and intercropping on biomass allocation (shoot: root ratios) in chickpea and durum wheat

Plant species	Culture	Without earthworms	With earthworms
Chickpea	Pure stand	2.2±0.3^{bc}	4.2±0.4^{a}
	Intercropped	3.5±0.5^{a}	3.4±0.2^{ab}
Durum wheat	Pure stand	1.3±0.1^{x}	1.9±0.2^{y}
	Intercropped	1.3±0.2^{x}	1.8±0.1^{xy}

Differences in shoot: root ratios were tested separately for the two species using ANOVA. Tukey's post hoc tests were performed to evaluate pairwise differences (different lower case letters indicate significant differences means)

pure H_2O (PiH_2O). Conversely, organic phosphorus extracted with NaOH (Po NaOH) drastically decreased from 20.1 to 11.7 mg.kg^{-1} in the presence of earthworms and absence of plants (unplanted soil). Earthworms did not significantly affect Pi extracted with $NaHCO_3$, NaOH and the organic form of P (Po) extracted with $NaHCO_3$.

Discussion

Effect of earthworms on plant growth in pure stand

Earthworms simultaneously reduced root biomass and increased shoot biomass without modifying the total biomass production. Earthworm effect thus consisted basically in a modification of biomass allocation. Such results are in agreement with those of Kreuzer et al. (2004), Partsch et al. (2006) and Laossi et al. (2010), while several other experimental studies found no effect of earthworms on biomass allocation (Scheu et al. 1999; Laossi et al. 2009). Wurst et al. (2005), Eisenhauer and Scheu (2008) and Puga-Freitas et al. (2012) even found a stimulation of root growth. The contrasting results of experimental works indicated that site-specific and species-specific effects are of great importance and make difficult the establishment of a comprehensive framework about earthworm–plant interactions. However, it stresses the importance to measure the effect of earthworm on both shoot and root biomass in order to avoid misleading interpretation. An increase in shoot biomass is interesting in any case on an agronomic point of view, but if this increase is due to an allocation shift or to a net increase of carbon fixed by photosynthesis is important to the understanding of

Table 3 Results of ANOVA to test for the effects of earthworms, intercropping and plant species on nitrogen accumulated in the belowground biomass, the aboveground biomass and the whole plant biomass

Source of variance	Belowground nitrogen			Aboveground nitrogen			Total nitrogen		
	Df	*F*-value	*p*-value	Df	*F*-value	*p*-value	Df	*F*-value	*p*-value
Between blocks:									
Block	4	–	–	4	–	–	4	–	–
Within blocks:									
Intercropping (I)	1	0.433	0.516	1	0.710	0.406	1	0.719	0.404
Earthworm (E)	1	2.960	0.096	1	13.981	<0.001***	1	5.406	0.028
Species (Sp)	1	6.285	0.018*	1	24.485	<0.001***	1	8.915	0.006*
I × E	1	0.365	0.550	1	0.046	0.832	1	0.001	0.976
I × Sp	1	2.725	0.110	1	1.688	0.203	1	2.498	0.125
E × Sp	1	0.725	0.402	1	0.286	0.596	1	0.638	0.431
I × E × Sp	1	0.068	0.797	1	0.258	0.615	1	0.068	0.796
Residuals	28	–	–	28	–	–	28	–	–

The significance of effects, or interactions of effects is shown by asterisks: *$p<0.05$, **$p<0.01$, ***$p<0.001$

plant–earthworm interaction mechanisms and nutrient flux in agroecosystem.

Contrary to our first hypothesis, biomass allocation of chickpea was more strongly affected than that of durum wheat. The shift in biomass allocation could be due to trophic interactions between earthworms and plants. According to the optimal allocation theory, plants preferentially invest resources in organs that are responsible for the acquisition of the limiting resource, e.g. light, water or nutrients (Tilman 1988; Aikio and Markkola 2002). The decrease in root biomass and the increase in biomass allocation to aboveground parts in the presence of earthworms might indicate that earthworms increased soil nutrient availability, water supply being maintained at a non-limiting level throughout the pot experiment. Another possible explanation of the change in allocation involves non-trophic interactions. For instance, Jana et al. (2010) showed that the intimate contact of earthworms with roots was responsible for an increased transcription of stress signalling genes that

Table 4 Results of ANOVA to test for the effects of Earthworms, intercropping and plant species on phosphorus accumulated in the belowground biomass, the aboveground biomass and the whole plant biomass

Source of variance	Belowground phosphorus			Aboveground phosphorus			Total phosphorus		
	Df	*F*-value	*p*-value	Df	*F*-value	*p*-value	Df	*F*-value	*p*-value
Between blocks:									
Block	4	–	–	4	–	–	4	–	–
Within blocks:									
Intercropping (I)	1	2.145	0.154	1	1.841	0.1857	1	2.624	0.116
Earthworm (E)	1	2.576	0.120	1	7.303	0.0116*	1	1.918	0.177
Species (Sp)	1	50.191	<0.001***	1	3.092	0.0896	1	1.132	0.297
I × E	1	2.153	0.153	1	0.203	0.6555	1	0.475	0.496
I × Sp	1	0.346	0.561	1	1.437	0.2406	1	0.785	0.383
E × Sp	1	0.002	0.963	1	0.203	0.6554	1	0.482	0.493
I × E × Sp	1	0.00	0.983	1	0.073	0.7897	1	0.006	0.939
Residuals	28	–	–	28	–	–	28	–	–

The significance of effects, or interactions of effects is shown by asterisks: *$p<0.05$, **$p<0.01$, ***$p<0.001$

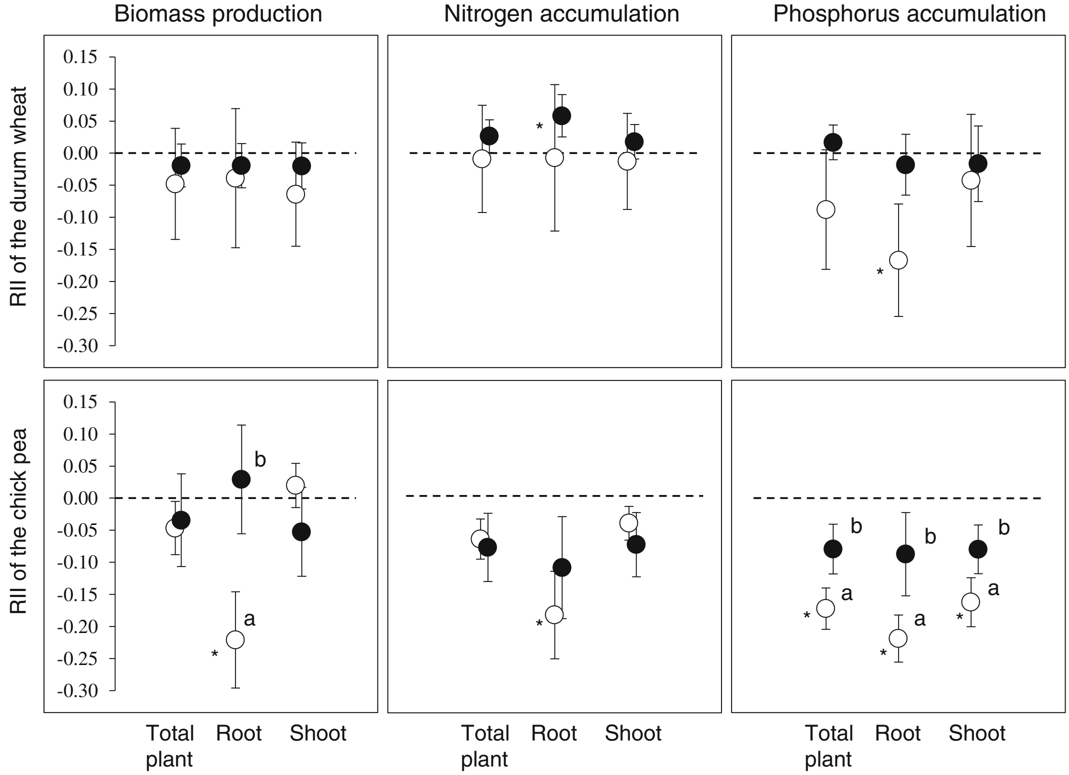

Fig. 2 Effect of earthworms on interactions between durum wheat and chickpea for biomass production and accumulation of N and P. RII was calculated by using plant performance in pure stand and in intercropping according to Armas et al. (2004); it is thus a way to compare the relative importance of inter- versus intraspecific competition. To calculate RII without earthworms (*white circles*), plant performance in pure stand and in intercropping without earthworms was used. To calculate RII with earthworms (*black circles*), the performance of plant in pure stand and in intercropping with earthworms was used. *Stars* indicate that a value is significantly different from 0, i.e. the null interaction (*dashed line*). *Letter* indicates that the treatment with earthworms is significantly different from the treatment without earthworms

could reduce root growth. In addition, auxin-like effect was found in casts by Muscolo et al. (1999) and earthworms-mediated hormones have been shown to impact plant growth (Puga-Freitas et al. 2012). High concentration of auxin has a negative impact on root elongation and could be an explanation for the root biomass reduction observed in the presence of earthworms.

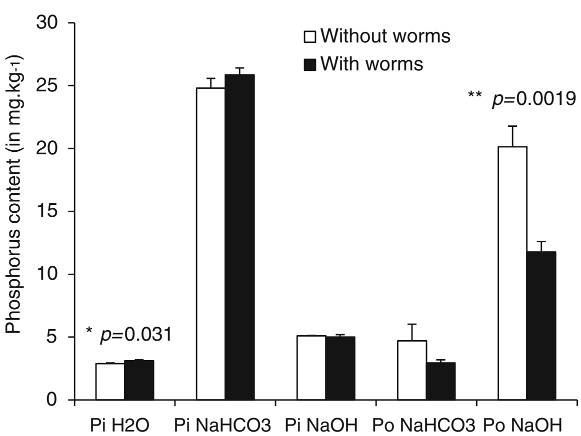

Fig. 3 Effect of earthworms on soil phosphorus measured after five different extractions of soil from pots without plant. *P*-values refer to significance of earthworm effect tested by student test

Effect of intercropping on plant growth

In our study, chickpea root production was reduced in the presence of durum wheat. Cereals have a fibrous root system characterized by an important production of fine roots. As a consequence, they are more competitive than legumes for N uptake from soil (Schmidt and Curry 1999). This reduction of chickpea roots had further consequences on N and P content of the chickpea intercropped without earthworms. The success of a cereal/legume intercropping system depends on the maintenance of an optimum balance between the two crops (Jones and Clements 1993) that was obviously not achieved in our case. The overyielding of the cereal/legume intercrops has been shown to be largely depending on N fertilization and/or soil N status: if these are high, N_2 fixation by the legume component may be limited and the cereal may outcompete the legume, while not achieving better growth than when grown alone, as shown in previous field experiments (Corre-Hellou et al. 2006; Bedoussac and Justes 2010). For P, there are much less references. Li et al. (2007) suggested that greater overyielding of cereal/legume intercrops was achieved in field experiments at low fertilizer P addition, in line with the stress gradient hypothesis that stipulates that facilitation shall be greater than competition when the stress (e.g. nutrient deficiency) increases (Bertness and Callaway 1994). Betencourt et al. (2012) verified this stress-gradient hypothesis in a pot experiment with durum wheat and chickpea. Overyielding was observed in durum wheat intercropped with chickpea in a low P soil, while no such effect was observed at high P content. In the soil used for the present experiment, considering its high N and P status as related to past fertilizer history and preceding crop (legume), conditions were rather favouring competition between the intercropped species, which might explain why we found no evidence of facilitation.

Effect of earthworms on plant growth in intercropping

In our experiment, earthworms could be seen as "competition troubleshooters" between wheat and chickpea as they reduced belowground competition in intercropping. This was confirmed by a modification of biomass production of plants grown individually, earthworms were able to modify plant–plant interactions (Wurst et al. 2005; Eisenhauer and Scheu 2008) and the resulting biomass production of an intercropping system. By separating roots of individual plant species, we could identify an especially strong competition at the root level between durum wheat and chickpea. Belowground competition between root systems requires an overlap of the rhizospheres of the intercropped species (Casper and Jackson 1997; Raynaud et al. 2008). When burrowing and feeding on soil, earthworms may have disrupted root contacts between durum wheat and chickpea (Schmidt and Curry 1999), thus reducing the competition in intercropping. It is also clear that the decrease of root biomass in the presence of earthworm played an important role in reducing competition among the two intercropped species.

Earthworms induced a shift in biomass allocation without affecting total biomass and nutrient uptake. With less roots, plant were able to acquire at least the same amount of nutrients from soil reinforcing the assumption that, earthworm effect on plant interaction might be mediated by an increase in nutrient availability in soil. Even if our soil could be considered as rich in nutrients, they were not necessary bioavailable, especially the phosphorus. We recorded a slight increase of available P forms in the bulk soil in the presence of earthworms, in agreement with previous results (Milleret et al. 2009). It has been previously shown that endogeic earthworms, by enhancing P availability in their casts (Le Bayon and Binet 2006) are able to influence P dynamics in soil. As there was no P leaching in our experiment, we identify three possible mechanisms responsible for enhancing P availability that can be mediated by either physico-chemical or biological processes. (1) Chapuis-Lardy et al. (2009) demonstrated that tropical endogeic earthworms (*Pontoscolex corethrurus*) increase P availability by increasing P desorption in their casts, as induced by the competition between mucus organic compounds and P for the same adsorption sites (Lopez-Hernandez et al. 1993). (2) The second explanation involves microorganisms, which can solubilize unavailable inorganic and P forms. If such microbial populations are stimulated in earthworm casts it can increase available P (Richardson and Simpson 2011). (3) Finally, the supply of fresh carbon via mucus deposition, could have induced a priming effect, that enhance the mineralization of soil organic matter (and organic P) leading to the release of inorganic P (Bernard et al. 2012). Those three mechanisms could explain the decrease of the organic P pool in presence of earthworms; however, the amount of P recovered in the inorganic available pools was around ten times smaller than the amount lost from the organic P pool. We do not know the fate of the remaining organic P (which was not recovered in the measured inorganic P pools). This substantial amount of P could have been recovered in the inorganic P pools that we did not measure, especially in the acid soluble P pool, which was the dominant pool of P in this calcareous soil. This pool was so large (more than half the total soil P) that we did not expect it to change significantly; hence we did not measure it. Alternatively, P could have been immobilized in the microbial biomass as we measured P on soil extracts that had been filtered at 0.45 μm (thus preventing the inclusion of microbial cell). We did not measure this particular pool of P. It might also have ended up in earthworm biomass as microorganisms constitute a significant part of their diet (Curry and Schmidt 2007). The challenge is now to study simultaneously interactions between plants, microorganisms, and soil fauna and understand the mechanisms that are involved in order to develop new agricultural practices to take better advantage of positive interactions between organisms.

Conclusion

Earthworms mitigated the effect of competition by reducing the root competition experienced by chickpea intercropped with durum wheat. In this particular experiment, earthworms could thus be seen as "troubleshooters" in plant–plant interactions. However, this must be interpreted with caution due to the small scale of the experiment, the high N and P status of the soil used in the experiment, and the short duration of the experiment. Despite the lack of mechanistic explanation for the observed effects, we recorded an increase in available inorganic P pool (Pi H2O) concomitant to a decrease in organic recalcitrant P pool (Po NaOH) that could have influenced plant growth. The fate of a large amount of P from the organic pool remains unknown, which calls for further research, especially on the integration of microorganisms involved in the relation between earthworms and plants. Finally, we advocate that plant–earthworm interactions must be taken into account for developing new intercropping systems because soil biological activity will be enhanced in agroecological agricultural practices.

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