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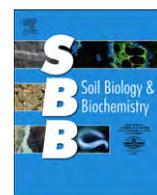
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## Short communication

## Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses

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## ABSTRACT

Legumes are prized for their seed protein and lipid mass fractions. Since legumes spend up to 4–16% of photosynthesis on each of the rhizobial and arbuscular mycorrhizal (AM) fungal symbioses, it might be expected that positive responses in yield due to rhizobial and AM symbioses are accompanied by decreases in seed protein and lipid mass fractions due to a photosynthate (C) limitation. We performed a meta-analysis of 348 data points from published studies with 12 legume species to test whether yield, harvest index, and seed protein and lipid mass fractions are affected by symbioses. There was a significant increase in yield due to rhizobial inoculation (16% in the field; 59% in pot experiments). There were no responses of yield to AM fungi and rhizobial + AM fungi inoculations in the field (presumably because an AM fungi-free control cannot be ensured), but significant responses in pots (45% with AM fungi; 44% with rhizobial + AM fungi). Rhizobial inoculation improved seed protein mass fraction by 7% in the field; AM fungi increased this parameter by 14% in pots. There were no discernable effects of symbioses on seed lipid mass fraction. Rhizobial symbioses in the field increased harvest index (+5%), but AM fungi did not affect harvest index. In conclusion, increases in yield due to symbioses also resulted in increases in seed protein and constant lipid mass fractions, indicating that legumes are not C-limited under symbiotic conditions.

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Theories on the regulation of rhizobial and arbuscular mycorrhizal (AM) fungal symbioses are based on the assumption that photosynthates (C) are a limiting resource for plants. Thus, growth and activity of both the microsymbiont and the host plant would be limited by the C competition between partners (e.g. Treseder and Allen, 2002; Kiers and Denison, 2008). Given C limitation, plants should support microbial growth if the benefits from the symbioses (increased carbon fixation as a consequence of increased nutrient acquisition) are greater than the C costs invested in the symbiotic partners; and accordingly, plants should suppress symbiotic associations if the benefits are smaller than the C costs (Koide and Elliot, 1989; Kiers and Denison, 2008). The cost: benefit and the C limitation theories presume that plants will suppress symbioses whenever the microsymbiont lacks effectiveness in the supply of nutrients. However, we recently showed that the C costs of the rhizobial and AM fungal symbioses for legumes are compensated by increases in the rates of leaf photosynthesis, independent of

nutritional benefits (Kaschuk et al., 2009b). This physiological feedback is termed sink stimulation of photosynthesis by the C costs of rhizobial and AM fungal symbioses, and agrees with the argument that plants have a photosynthetic capacity larger than their own C requirements, but are usually limited by soil nutrients (Fitter, 1991; Millard et al., 2007).

Probably due to the absence of C limitation, plants invest C in rhizobial and AM symbioses whenever it is needed (responding to a feedback resulting from small phloem N and P concentrations in rhizobial (Parsons et al., 1993) and AM symbioses (Valentine and Kleinert, 2007), respectively), even if there is no benefit from this. Indeed, some studies have shown that growth of symbiotic legumes is not determined by C limitation, but rather a limitation in the effectiveness of rhizobial (and AM fungal strains) to supply nutrients (e.g. Skot et al., 1986; Twary and Heichel, 1991). The non-specific and frequently inefficient rhizobial and AM symbioses observed (e.g. rhizobia – Michiels et al., 1998; AM symbioses – Klironomos, 2003) suggest that plants largely are unable to restrict colonization by ineffective rhizobial and AM fungal strains. Therefore, since symbiotic plants are not limited by C availability, inoculation with effective rhizobial and mycorrhizal strains would make legumes produce more seeds than their counterpart fertilized plants.

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Legumes are valuable crops for food, fodder, green manure and biofuel. Although some legumes may store large mass fractions of starch in their seeds, legumes are generally prized for their protein-rich biomass and seed protein and lipid mass fractions (e.g. Smartt, 1990). Both lipid and protein accumulation in seeds is very costly due to the high C requirements for biosynthesis (cf. Penning de Vries et al., 1974). Under the concept of C limitation, one would expect that accumulation of protein in seeds of nodulated and mycorrhizal legumes would be traded off against lipid mass fractions. However, because of sink stimulation of photosynthesis, symbiotic plants could support larger yields with higher seed protein mass fractions and no difference in the lipid mass fraction.

We performed a meta-analysis of 348 data points gathered from 52 studies with 12 legume species [often referred to in literature as grain legumes, pulse or oilseed crops; Smartt, 1990] to test whether yield, harvest index (the ratio of seeds to above-ground biomass) and seed protein and lipid mass fractions are affected by symbioses. The meta-analysis consisted of a calculation of the response ratio ( $R$ ) from a control in relation to a treatment value in a large number of pot and field experiments. Differences in the magnitude of response in field and pot experiments could be expected because

responses in pots are obtained against non-symbiotic (fertilized) treatments, whereas responses in the field are obtained from enhancement of colonization or replacement by more effective strains against indigenous less effective strains (Lekberg and Koide, 2005). Based on the variation around the mean of each value, a confidence interval indicates that a given response is reliable at a given probability using the method of Gurevitch and Hedges (2001).

Yield significantly increased due to rhizobial inoculation ( $R = 1.16$ , or an increase of 16%; and  $R = 1.59$ , or an increase of 59%, in field and pot experiments, respectively). Although the responses of yield to AM fungal and rhizobial + AM fungal inoculation was not significant in field experiments (probably because it is difficult to ensure an AM fungi-free control in the field), the responses of yield to AM fungal and rhizobial + AM fungal inoculations were highly significant in pot experiments (increases of 45 and 44%, due to AM fungal and rhizobial + AM fungal, respectively) (Table 1).

Next to the observed increases in yield, the meta-analysis shows that rhizobial inoculation improved grain protein mass fraction by 7% ( $R = 1.07$ ) under field conditions; in the case of AM fungal inoculation, the increase in protein reached 14% in pot experiments

**Table 1**  
Meta-analysis of the effects of inoculation of rhizobial and/or AM fungal species on the yield and harvest index (ratio seed:above-ground biomass) of several legumes in pot and field experiments.

	Experiment	Yield			Harvest index			Grain protein		
		$R$	95%CI	$n$	$R$	95%CI	$n$	$R$	95%CI	$n$
<b>Rhizobia</b>										
<i>Arachis hypogaea</i>	Field/pot	1.10	1.00–1.21	13						
<i>Cajanus cajan</i>	Pot	1.19	0.49–2.86	1	0.99	0.76–1.24	1			
<i>Cicer arietinum</i>	Field	1.16*	1.09–1.24	31	1.03 <sup>ns</sup>	0.98–1.08	31	1.06 <sup>ns</sup>	1.01–1.11	14
<i>C. arietinum</i>	Pot	1.63	1.51–1.75	25	1.00	0.95–1.05	25	1.02	0.92–1.12	4
<i>Glycine max</i>	Field	1.41	1.21–1.63	21	1.19	1.05–1.35	12	1.09	1.05–1.13	9
<i>Lens culinaris</i>	Pot	2.13	1.52–2.99	9	1.00	0.72–1.31	9			
<i>Phaseolus vulgaris</i>	Field	0.97	0.92–1.02	32	0.95	0.88–1.04	8	1.04	0.99–1.10	30
<i>Pisum sativum</i>	Field/pot	1.27	1.17–1.37	39	1.07	1.02–1.11	39	1.07	1.01–1.13	8
<i>Vicia faba</i>	Field	1.20	0.88–1.63	3	0.97	0.81–1.15	3	1.03	0.86–1.23	3
<i>Vicia sativa</i>	Field	1.06	0.99–1.14	14	0.99	0.94–1.05	14			
<i>Vigna radiata</i>	Field/pot	1.33	1.25–1.41	14	1.11	1.04–1.19	14			
<i>Vigna umbellata</i>	Pot	1.16	0.91–1.43	1	0.88	0.66–1.10	1			
Average	Field	1.16*	1.13–1.20	154	1.05 <sup>ns</sup>	1.02–1.08	120	1.07 <sup>ns</sup>	1.04–1.10	69
Average	Pot	1.59	1.50–1.69	49	1.02	0.97–1.06	48	1.03	0.90–1.17	5
<b>AM fungi</b>										
<i>C. arietinum</i>	Field/pot	1.17	1.04–1.32	4	1.04	0.86–1.25	4			
<i>G. max</i>	Field/pot	1.46	1.31–1.61	54	1.05	1.02–1.09	54	1.06	1.00–1.12	8
<i>L. culinaris</i>	Field/pot	1.19	0.86–1.65	11	0.87	0.53–1.43	11			
<i>P. vulgaris</i>	Field	0.94	0.75–1.14	6	0.99	0.88–1.11	6	0.95	0.83–1.08	6
<i>P. sativum</i>	Field	1.04 <sup>ns</sup>	0.57–1.90	4	1.05 <sup>ns</sup>	0.84–1.30	4	0.95*	0.85–1.06	4
<i>P. sativum</i>	Pot	1.53	1.17–2.01	10	1.04	0.94–1.15	10	1.15	1.08–1.22	8
<i>V. faba</i>	Field	1.12	0.91–1.39	8	1.04	0.94–1.16	8	1.03	0.96–1.11	6
<i>V. umbellata</i>	Pot	1.59	0.47–5.38	2	0.99	0.53–1.88	2			
Average	Field	1.09*	0.96–1.24	28	1.02 <sup>ns</sup>	0.92–1.13	28	0.98*	0.93–1.04	18
Average	Pot	1.45	1.34–1.56	71	1.03	0.96–1.09	71	1.14	1.07–1.21	15
<b>Rhizobia +AM fungi</b>										
<i>C. arietinum</i>	Pot	2.16	1.78–2.60	1	1.74	1.42–2.10	1	1.76	1.43–2.12	1
<i>G. max</i>	Field/pot	1.31	1.13–1.52	3	1.34	1.12–1.60	3			
<i>L. culinaris</i>	Pot	1.58	1.22–2.04	18	0.60	0.47–0.75	18			
<i>P. vulgaris</i>	Field	1.00	0.84–1.19	6	0.91	0.76–1.09	6	0.89	0.76–1.03	6
<i>P. sativum</i>	Pot	1.28	1.13–1.44	20	1.04	0.95–1.14	20			
<i>V. faba</i>	Field	1.19	0.89–1.59	6	1.09	0.95–1.25	6	1.14	0.97–1.34	6
<i>V. umbellata</i>	Pot	2.02	1.66–2.42	1	0.91	0.69–1.15	1			
Average	Field	1.12*	0.93–1.34	14	1.04 <sup>ns</sup>	0.82–1.32	14	1.02	0.92–1.13	14
Average	Pot	1.44	1.30–1.58	41	0.82	0.72–0.93	41	1.76	1.43–2.12	1

Notes for clarification:

1. The meta-analysis was performed according to Gurevitch and Hedges (2001).

2. ' $R$ ' is the response ratio, '95%CI' is the confidence intervals at  $P < 0.95\%$  for the  $R$  to be valid, and ' $n$ ' is the number of data points. If  $R$  and the lower 95%CI are larger than 1, then the response is significantly positive; if  $R$  and the upper 95%CI are smaller than 1, then the response is significantly negative.

3. 'Chi-square' test was applied to compare the responses of field and pot experiments within a given legume species. The symbol \* indicates that the differences between pot and field are significantly different at  $P < 0.05$  and 'ns' that the differences are not significant. The responses within a given legume species were presented separately when there was a significant difference between field and pot experiments; otherwise, an average response including both field and pot experiment was presented.

4. References for the meta-analysis are given in Supplementary Material.

(Table 1). Our meta-analysis reveals that the mass fraction of lipids was increased by just 1% in peanut (*Arachis hypogaea* L.) and soybean [*Glycine max* L. (Merrill)] due to rhizobial inoculation (not shown in the table: peanut:  $R = 1.01$ ; 95%CI = 0.99–1.04;  $n = 7$ ; soybean:  $R = 1.01$ ; 95%CI = 0.89–1.06;  $n = 5$ ), none of which were statistically significant. This outcome seems inconsistent with the fact that there is a genetically-determined trade off between the accumulation of protein and lipids in grain legumes (Brim and Burton, 1979). Consequently, if protein and lipid accumulation was limited by C, it would be not expected that lipid mass fraction would remain the same when protein mass fraction increase.

In none of the studies considered in Table 1 were rates of photosynthesis measured directly. An indirect indicator for a possible sink stimulation by rhizobial and AM fungal inoculation (above the C costs) is an increased harvest index, meaning that more seeds were produced with the same amount of photosynthetic biomass. Table 1 shows that harvest index was only increased significantly by rhizobial inoculation under field conditions ( $R = 1.05$  or a 5% increase), whereas AM fungal inoculation, either with or without rhizobia inoculation, did not affect harvest index. Lack of response in the harvest index with mycorrhizal plants suggests that sink stimulation of photosynthesis (Kaschuk et al., 2009b) in principle only compensates the C costs of AM fungal symbioses, and that further increases in plant productivity are explained by indirect effects of sink stimulation.

Similarly to symbioses, microbe pathogen infections may also stimulate the rates of leaf photosynthesis by changing C sink–source relations (e.g. Paul and Foyer, 2001). However, only sink stimulation of photosynthesis by rhizobial and mycorrhizal fungal C costs favour plant growth, particularly because these symbionts do not reduce productive leaf area. Initially, photosynthetic capacity increases due to carbon sink strength of symbioses, regardless of increased leaf nutrient concentrations (Brown and Bethlenfalvay, 1988; Kaschuk et al., 2009b). At full canopy (flowering) plants stimulated by symbioses achieve their maximum rates, and thus growth can be maximized. During grain filling, sink stimulation of photosynthesis associated with N supply from  $N_2$  fixation delays the degradation of photosynthetic enzymes and postpones leaf senescence (Abu-Shakra et al., 1978; Kaschuk et al., 2009a). Against the belief that symbioses may constrain plant productivity by limiting C availability, our meta-analysis shows that inoculation with effective rhizobial and AM fungal strains results in increased yield without compromising grain quality. We conclude that sink stimulation of photosynthesis not only compensates the C costs of symbioses, but also favours grain production.

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#### Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.soilbio.2009.10.017.

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