Oikos

O18427

Fujita, Y., Robroek, B. J. M., de Ruiter, P. C., Heil, G. W. and Wassen, M. J. 2010. Increased N affects P uptake of eight grassland species: the role of root surface phosphatase activity. – Oikos 119: 1665–1673.

Appendix 1

Adsorption capacity of quartz sand

The amount of P uptake was low even with orthophosphate treatments; the highest P uptake was approximately 50% of the total P supply. Therefore it was suspected that some of the supplied P was adsorbed to the quartz sand. To test the magnitude of the adsorption, we performed an additional lab experiment to check adsorption capacity of the quartz sand for orthophosphate. We monitored the phosphorus adsorption capacity of 50 g of quartz sand in 100 ml of KH_2PO_4 solution for one week. Although adsorption was ignorable when P concentration of the solution was high (50 mg P l^{-1}), considerable amount of adsorption (ca 45% and 30%) was observed after one week when P concentration was low (0.5 mg P l^{-1}) and intermediate (5 mg P l^{-1}), respectively. The concentration of P in the solution used in our bound-P experiment was 2.6 mg P l^{-1}.

Although the observed high percentages of P adsorption in the extra lab experiment were considerable, the effects of adsorption on the results of our fertilization experiments may be questioned, since plants may take up P quickly after fertilization before P will be adsorbed. Güsewell (2005) showed that *Carex* species took up around 80% of P from solution in seven hours when N:P supply ratio was larger than 5. In addition, percentage of N uptake relative to supplied N was also low in our experiment, ranging from 3% to 66%. This indicates that transportation of solution to roots in general, rather than adsorption of P to sand, was a major problem for plants in taking up nutrients.

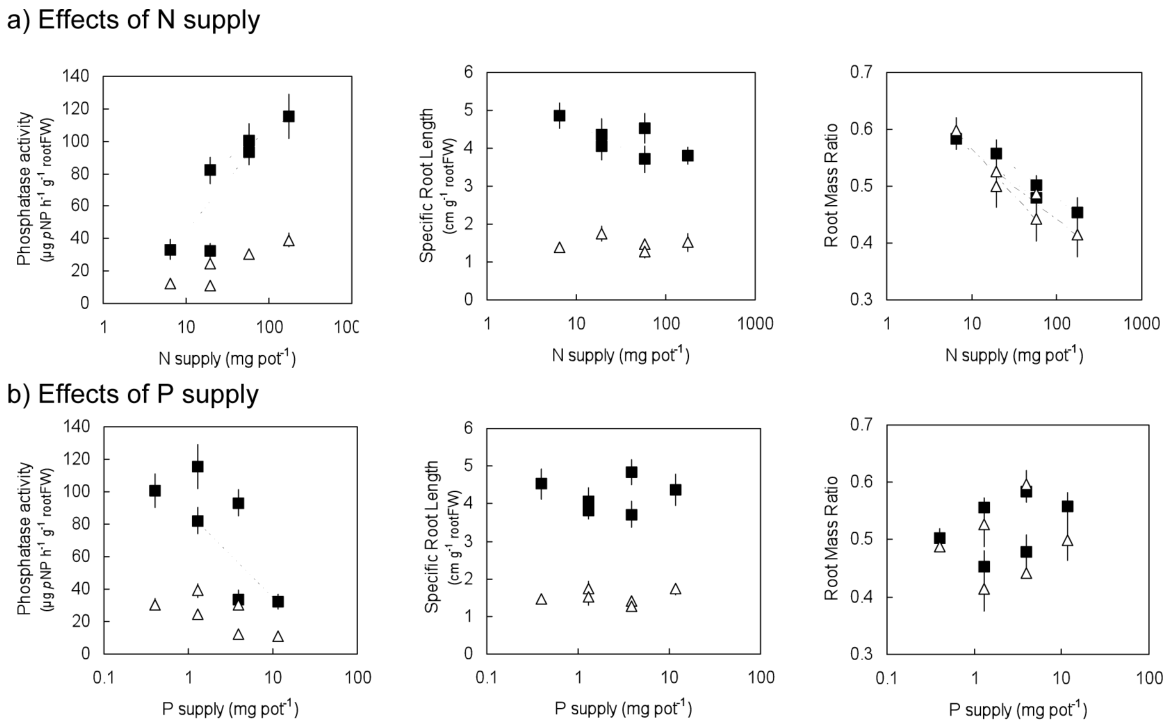

Figure A1. Effects of N supply (a) and P supply (b) on phosphatase activity ($Pase_{root}$), specific root length (SRL), and root mass ratio (RMR) are shown for grasses and herbs. Closed squares represent grasses, while open triangles represent herbs. Treatments are connected with dotted lines when supply of the other nutrient was the same. Average values and standard errors are shown.

Table A1. Field distribution pattern of eight species in N and P limited grasslands. A total of 381 field sites from Dutch, Belgium, German and Polish grasslands were classified into either N, co, or P limited sites based on biomass N:P ratio of plant community (<13.5, 13.5 to 16 and >16, respectively). For the eight species used in the experiment, number of sites of occurrence in each type of nutrient limitation was shown. Species were considered to have affinity to N or P limited sites when they had significantly more frequent occurrence in N or P limited sites tested by χ^2-test. Positive or negative associations to the limitation type were shown as + or –, respectively. Significant levels of the chi-square test were shown as; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant.

	Species	No. of sites of occurrence (n = 381)			Affinity to N limited sites	Affinity to P limited sites
		N limited	Co limited	P limited		
Grass	*Molinia caerulea*	10	16	72	–***	+***
Grass	*Festuca ovina*	4	1	8	–*	ns
Grass	*Agrostis capillaris*	23	6	14	ns	ns
Grass	*Festuca rubra*	84	12	38	ns	ns
Grass	*Alopecurus pratensis*	34	0	0	+***	–*
Herb	*Succisa pratensis*	4	4	36	–***	+***
Herb	*Lycopus europaeus*	25	3	18	ns	ns
Herb	*Lychnis flos-cuculi*	66	12	5	+**	–**

Table A2. Composition of nutrient solution.

(a) Amount of N and P

Treatment		N (mg pot^{-1})	P (mg pot^{-1})
Bound-P experiment			
<P source>	<N level>		
orthophosphate	low	19.4 [*1]	1.3 [*3]
	high	174.3 [*2]	1.3 [*3]
bound-P	low	19.4 [*1]	1.3 [*4]
	high	174.3 [*2]	1.3 [*4]
Stoichiometry experiment			
<Supply level>	<N:P ratio>		
low	1.7	6.5 [*1]	3.9 [*3]
	15	19.4 [*1]	1.3 [*3]
	135	58.1 [*1]	0.4 [*3]
high	1.7	19.4 [*1]	11.6 [*3]
	15	58.1 [*1]	3.9 [*3]
	135	174.3 [*2]	1.3 [*3]

(b) Amount of other elements

Element	mg pot^{-1}	Compound
Mg	25.64	$MgSO_4$ $7H_2O$
S	37.11	$MgSO_4$ $7H_2O$, $FeSO_4$ $7H_2O$, $CuSO_4$ $5H_2O$, $MnSO_4$ $4H_2O$, $ZnSO_4$ $7H_2O$
Fe	5.13	$FeSO_4$ $7H_2O$
Cu	0.01	$CuSO_4$ $5H_2O$
B	0.24	H_3BO_3
Mn	0.55	$MnSO_4$ $4H_2O$
Mo	0.01	Na_2MoO_4 $2H_2O$
Zn	0.03	$ZnSO_4$ $7H_2O$
Ca	79.76	$CaCl_2$[*5],$Ca(NO_3)_2$ $4H_2O$[*6]
K	332.39	KCl[*5], KNO_3, KH_2PO_4

[*1] supplied as KNO_3. [*2] supplied as KNO_3 and $Ca(NO_3)_2$ in order to provide the same amount of potassium for all treatments. The amount of calcium associated with the $Ca(NO_3)_2$ was subtracted from $CaCl_2$, so that the same amount of calcium was supplied for all treatments. [*3] supplied as KH_2PO_4. [*4] supplied as a mixture of organic and inorganic P compounds. For the organic P compound we used inositol hexaphosphate ($C_6H_{18}O_{24}P_6$), since phytates typically comprise the largest fraction of organic P in natural soils (Dalal 1977). Because organic P typically comprise 30–65 % of total P (Harrison 1987) and phytates comprise ca 50% of total organic P (Dalal 1977) in natural soils, we supplied 20% of our bound-P supply as inositol hexaphosphate. For the inorganic P compound we chose an acid-labile and an alkaline-labile compounds: calcium phosphate ($Ca_{10}(PO_4)_6(OH)_2$) and aluminum phosphate ($AlPO_4$), respectively. To resemble natural soils (Scheffer and Schachtschabel 1970), we supplied more calcium phosphate than aluminum phosphate (50% and 30% of our bound-P supply, respectively). [*5] not for the treatments of high supply level × N:P ratio135 and orthophosphate × high N level. [*6] supplied only for the treatments of high supply level × N:P ratio135 and orthophosphate × high N level.

References

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