

Supplementary material for

Nitrogen and phosphorus availability affect wheat carbon allocation pathways: rhizodeposition and mycorrhizal symbiosis

Bahareh Bicharanloo^{A,B}, Milad Bagheri Shirvan^A, Claudia Keitel^A, and Feike A. Dijkstra^A

^ASydney Institute of Agriculture, School of Life and Environmental Sciences, The University of Sydney, Camden, NSW 2570, Australia.

^BCorresponding author: bahareh.bicharanloo@sydney.edu.au

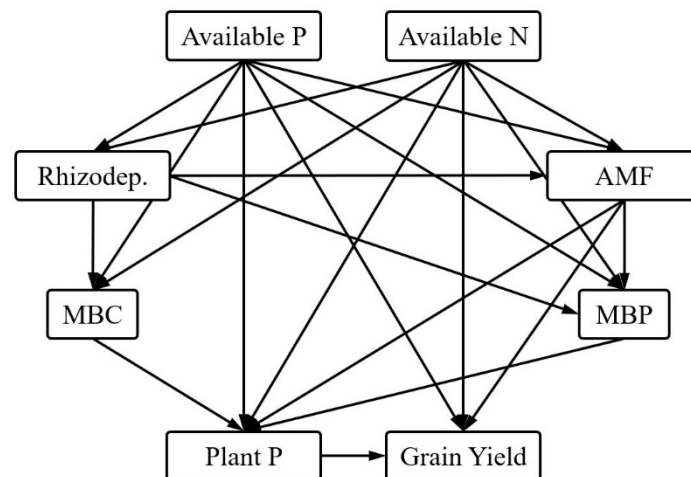


Fig. S1. Structural equation model containing all a priori hypothetical pathways. (Abbreviations: Available P: Soil available phosphorus; Available N: Soil available nitrogen; Rhizodep.: Rhizodeposition; AMF: Arbuscular mycorrhizal fungi colonization; MBC: microbial biomass carbon; MBP: microbial biomass phosphorus; Plant P: Total phosphorus content in aboveground wheat plant part; Grain yield: Grain weight of wheat).

Table S1. Possible priori hypothetical pathways between all of variables in priori model

Arrows indicate the direction of the path

Pathways	References
Available N/P → Rhizodeposition	Gregory <i>et al.</i> 1995; Henry <i>et al.</i> 2005; Chowdhury <i>et al.</i> 2014
Available N/P → Mycorrhizal colonization	Konvalinková <i>et al.</i> 2017; Suri <i>et al.</i> 2011; Bakhshandeh <i>et al.</i> 2017
Available P → Microbial biomass C	Shibahara and Inubushi 1997
Available P → Microbial biomass P	Sugito <i>et al.</i> 2010
Available P → Plant phosphorus	Romer and Schilling 1986
Available P → Grain yield	Takahashi and Anwar 2007; Xin-kai <i>et al.</i> 2012
Available N → Microbial biomass C	Treseder 2008
Available N → Microbial biomass P	Shen <i>et al.</i> 2016
Available N → Plant phosphorus	Mehta <i>et al.</i> 1963
Available N → Grain yield	Zhang <i>et al.</i> 2017; Bashirov 2009
Rhizodeposition → Mycorrhizal colonization	Bécard and Piché 1989, Waters <i>et al.</i> 2017
Rhizodeposition → Microbial biomass C	Nguyen 2009
Rhizodeposition → Microbial biomass P	Spohn <i>et al.</i> 2013
Mycorrhizal colonisation → Microbial biomass P	Toljander <i>et al.</i> 2007
Mycorrhizal colonisation → Plant phosphorus	Campos <i>et al.</i> 2018
Mycorrhizal colonisation → Grain yield	Bakhshandeh <i>et al.</i> , 2017
Microbial biomass C → Plant phosphorus	Spohn and Kuzyakov 2013
Microbial biomass P → Plant phosphorus	Sugito <i>et al.</i> 2010
Plant phosphorus → Grain yield	Sandana and Pinochet 2014

Table S2. Effect of genotype (G), phosphorus (P) and nitrogen (N) fertiliser on root biomass, specific root length (SRL), root diameter (RD), proportion of fine (0.01–0.2 mm), intermediate (0.2–1 mm) and coarse (1–2 mm) diameter roots at tillering stage (mean ± s.e.m.)

ANOVA *P*-values are in bold when *P* < 0.05 and different letters in each column indicate significant differences among G treatments (*post hoc* Tukey's HSD test results are only shown for significant main or interactive effects of G)

Genotype	Phosphorus levels (kg ha ⁻¹)	Nitrogen levels (kg ha ⁻¹)	SRL (m g ⁻¹)	Average diameter (mm)	Proportion of fine diameter roots (0.01 – 0.2 mm)	Proportion of intermediate diameter roots (0.2 – 1 mm)	Proportion of coarse diameter roots (1 – 2 mm)
249	40	100	65.1 ± 27	0.27 ± 0.021	0.430 ± 0.045 b	0.560 ± 0.043 a	0.011 ± 0.005
	40	25	130.0 ± 41	0.24 ± 0.006	0.529 ± 0.025 b	0.468 ± 0.025 a	0.003 ± 0.001
	10	100	92.3 ± 41	0.26 ± 0.017	0.484 ± 0.013 b	0.500 ± 0.015 a	0.016 ± 0.011
	10	25	161.3 ± 29	0.24 ± 0.005	0.532 ± 0.009 b	0.461 ± 0.011 a	0.007 ± 0.002
IAW2013	40	100	93.1 ± 32	0.24 ± 0.010	0.515 ± 0.019 a	0.479 ± 0.019 b	0.006 ± 0.002
	40	25	81.3 ± 25	0.23 ± 0.004	0.570 ± 0.012 a	0.427 ± 0.011 b	0.003 ± 0.002
	10	100	179.3 ± 28	0.23 ± 0.003	0.559 ± 0.023 a	0.435 ± 0.024 b	0.006 ± 0.002
	10	25	103.5 ± 27	0.24 ± 0.012	0.561 ± 0.026 a	0.427 ± 0.028 b	0.013 ± 0.006
Scout	40	100	171.2 ± 50	0.25 ± 0.006	0.485 ± 0.004 b	0.511 ± 0.007 a	0.004 ± 0.003
	40	25	148.2 ± 22	0.24 ± 0.002	0.501 ± 0.011 b	0.495 ± 0.012 a	0.003 ± 0.002
	10	100	118.4 ± 54	0.25 ± 0.007	0.494 ± 0.016 b	0.500 ± 0.017 a	0.005 ± 0.001
	10	25	155.6 ± 31	0.25 ± 0.003	0.496 ± 0.016 b	0.499 ± 0.017 a	0.005 ± 0.001
Suntop	40	100	75.9 ± 28	0.25 ± 0.011	0.509 ± 0.054 ab	0.483 ± 0.055 ab	0.008 ± 0.003
	40	25	161.1 ± 21	0.24 ± 0.005	0.517 ± 0.021 ab	0.474 ± 0.020 ab	0.008 ± 0.002
	10	100	91.6 ± 38	0.24 ± 0.003	0.525 ± 0.024 ab	0.468 ± 0.028 ab	0.007 ± 0.004
	10	25	121.7 ± 57	0.24 ± 0.011	0.544 ± 0.030 ab	0.448 ± 0.029 ab	0.008 ± 0.004
ANOVA <i>p</i> values							
G			0.4355	0.1589	0.0058	0.0066	0.5010
P			0.5098	0.7802	0.1824	0.1341	0.2149
N			0.2387	0.0751	0.0172	0.0263	0.4704
G*P			0.4248	0.8763	0.8991	0.8789	0.7459
G*N			0.1377	0.3004	0.2629	0.4129	0.3033
P*N			0.7102	0.3232	0.2863	0.3423	0.5634
G*P*N			0.6072	0.6907	0.7762	0.8146	0.8086

Table S3. Effect of genotype (G), phosphorus (P) and nitrogen (N) fertiliser on $\delta^{13}\text{C}$ root, $\delta^{13}\text{C}$ soil and $\delta^{13}\text{C}$ MB at ripening stage (mean \pm s.e.m.)

ANOVA *P*-values are in bold when *P* < 0.05 and different letters in each column indicate significant differences among G treatments based on *post hoc* Tukey's HSD test

Genotype	Phosphorus levels (kg ha ⁻¹)	Nitrogen levels (kg ha ⁻¹)	$\delta^{13}\text{C}$ Roots (‰)	$\delta^{13}\text{C}$ Soil (‰)	$\delta^{13}\text{C}$ MB (‰)
249	40	100	-37.49 \pm 0.55 ab	-23.16 \pm 0.14 b	-26.78 \pm 0.28 b
	40	25	-37.84 \pm 0.24 ab	-23.25 \pm 0.06 b	-28.08 \pm 1.13 b
	10	100	-37.60 \pm 0.34 ab	-23.36 \pm 0.16 b	-29.87 \pm 2.66 b
	10	25	-37.49 \pm 0.26 ab	-23.44 \pm 0.30 b	-27.69 \pm 0.27 b
IAW2013	40	100	-37.66 \pm 0.62 b	-22.94 \pm 0.03 a	-25.14 \pm 0.83 a
	40	25	-38.57 \pm 0.20 b	-22.92 \pm 0.11 a	-24.57 \pm 0.27 a
	10	100	-38.72 \pm 0.97 b	-23.04 \pm 0.03 a	-23.05 \pm 2.09 a
	10	25	-37.89 \pm 0.35 b	-22.90 \pm 0.08 a	-24.48 \pm 0.49 a
Scout	40	100	-36.80 \pm 0.19 a	-23.20 \pm 0.10 ab	-24.87 \pm 0.21 a
	40	25	-37.03 \pm 0.32 a	-23.13 \pm 0.12 ab	-24.12 \pm 0.23 a
	10	100	-36.92 \pm 0.93 a	-22.97 \pm 0.13 ab	-23.06 \pm 1.70 a
	10	25	-37.59 \pm 0.32 a	-23.03 \pm 0.10 ab	-27.28 \pm 1.47 a
Suntop	40	100	-36.37 \pm 0.48 a	-23.18 \pm 0.18 ab	-23.97 \pm 1.21 a
	40	25	-35.79 \pm 0.41 a	-22.82 \pm 0.16 ab	-25.06 \pm 0.65 a
	10	100	-37.05 \pm 0.19 a	-23.02 \pm 0.06 ab	-22.56 \pm 0.55 a
	10	25	-37.24 \pm 1.09 a	-23.23 \pm 0.17 ab	-22.94 \pm 1.35 a
ANOVA <i>p</i> values					
G			0.001	0.006	<0.0001
P			0.2	0.5	0.8
N			0.7	0.7	0.3
G×P			0.5	0.2	0.2
G×N			0.9	0.8	0.6
P×N			0.7	0.3	0.6
G×P×N			0.3	0.3	0.1

References

- Bakhshandeh S, Corneo PE, Mariotte P, Kertesz MA and Dijkstra FA (2017) Effect of crop rotation on mycorrhizal colonization and wheat yield under different fertilizer treatments. *Agriculture, Ecosystems & Environment* **247**, 130-136.
- Bashirov VV (2009) Correlation study between soil nutrient indices and yield of wheat and barley in the Ganjabasar region of Azerbaijan. *International Journal of Soil Science* **4(4)**, 114-122.
- Bécard G, Piché Y (1989) Fungal growth stimulation by CO₂ and root exudates in vesicular-arbuscular mycorrhizal symbiosis. *Applied and Environmental Microbiology* **55**, 2320-2325.
- Campos P, Borie F, Comejo P, Lopez-Raez JA, Lopez-Garcla A, Seguel A (2018) Phosphorus acquisition efficiency related to root traits: Is mycorrhizal symbiosis a key factor to wheat and barley cropping? *Frontiers in Plant Science* **9**, 752.
- Chowdhury S, Farrell M, Bolan N (2014) Photoassimilated carbon allocation in a wheat plant-soil system as affected by soil fertility and land-use history. *Plant and Soil* **383**, 173-189.
- Gregory PJ, Palta JA and Batts GR (1995) Root systems and root:mass ratio-carbon allocation under current and projected atmospheric conditions in arable crops. *Plant and Soil* **187**, 221-228.
- Henry F, Nguyen C, Paterson E, Sim A and Robin C (2005) How does nitrogen availability alter rhizodeposition in *Lolium multiflorum* Lam. during vegetative growth? *Plant and Soil* **269**, 181-191.
- Konvalinková T, Püschel D, Řezáčová V, Gryndlerová H and Jansa J (2017) Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization. *Plant and Soil* **419**, 319-333.
- Mehta KM, Puntamkar SS, Kalamkar VG (1963) Study on uptake of nutrients by wheat as influenced by nitrogen and phosphorus fertilization. *Soil Science and Plant Nutrition* **9(5)**, 29-34.
- Nguyen C (2009) Rhizodeposition of Organic C by Plant: Mechanisms and Controls. In 'Sustainable Agriculture'. (Eds Lichtfouse E., Navarrete M., Debaeke P., Véronique S., Alberola C.) pp. 97-123.(Springer Netherlands).
- Romer W, and Schilling G (1986) Phosphorus requirements of the wheat plant in various stages of its life cycle. *Plant and Soil* **91**, 221-229.
- Sandana P, Pinochet D (2014) Grain yield and phosphorus use efficiency of wheat and pea in a high yielding environment. *Journal of Soil Science and Plant Nutrition* **14**, 973-986.
- Shibahara F, and Inubushi K (1997) Effects of organic matter application on microbial biomass and available nutrients in various types of paddy soils. *Soil Science and Plant Nutrition* **43(1)**, 191-203.
- Shen P, Murphy DV, George SJ, Lapis-Gaza H, Xu M and Gleeson DB (2016) Increasing the size of the microbial biomass altered bacterial community structure which enhances plant phosphorus uptake. *PLoS ONE* **11**, e0166062.
- Spohn M, Ermak A, Kuzyakov Y (2013) Microbial gross organic phosphorus mineralization can be stimulated by root exudates – A ³³P isotopic dilution study. *Soil Biology and Biochemistry* **65**, 254-263.
- Spohn M, Kuzyakov Y (2013) Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology and Biochemistry* **61**, 69-75.
- Sugito T, Yoshida K, Takebe M, Shinano T, Toyota K (2010) Soil microbial biomass phosphorus as an indicator of phosphorus availability in a Gleyic Andosol. *Soil Science & Plant Nutrition* **56(3)**, 390-398.

- Suri VK, Choudhary AK, Chander G, Verma TS (2011) Influence of vesicular arbuscular mycorrhizal fungi and applied phosphorus on root colonization in wheat and plant nutrient dynamics in a phosphorus-deficient acid alfisol of Western Himalayas. *Communications in Soil Science and Plant Analysis* 42, 1177-1186.
- Takahashi S, Anwar MR (2007) Wheat grain yield, phosphorus uptake and soil phosphorus fraction after 23 years of annual fertilizer application to an Andosol. *Field Crop Research* 101. doi: 10.1016/j.fcr.2006.11.003
- Toljander JF, Lindahl BD, Paul LR, Elfstrand M, Finlay RD (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol Ecology* 61, 295-304.
- Treseder KK (2013) The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil* 371, 1-13.
- Waters MT, Gutjahr C, Bennett T, Nelson DC (2017) Strigolactone signaling and evolution. *Annual Review of Plant Biology* 68, 291-322.
- Zhang M, Wang H, Yi Y, Ding J, Zhu M, Li C, Guo W, Feng C, Zhu X (2017) Effect of nitrogen levels and nitrogen ratios on lodging resistance and yield potential of winter wheat (*Triticum aestivum* L.). *PLoS ONE* 12(11), e0187543. doi: 10.1371/journal.pone.0187543
- Xin-kai Z, Chun-yan L, Zong-qing J, Lian-lian H, Chao-nian F, Wen-shan G, Yong-xin P (2012) Responses of phosphorus use efficiency, grain yield, and quality to phosphorus application amount of weak-gluten wheat. *Journal of Integrative Agriculture* 11(7), 1103-1110.