Experiment 3 - Data S3

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Experiment 3 – BX-feedbacks on wheat on growth and physiology

The third feedback experiment was performed with wheat, again, with a randomized design testing the factor conditioning (BX+ or BX-). The experiment was conducted with a greenhouse-conditioned batch of Changins soil and a field-conditioned batch of Reckenholz soil (Table S1). Because of fewer amounts of the Changins soil, this part of the experiment was performed in 0.5 L pots (16 experimental units = 2 BX conditions * 8 replicates; see Data S3 for experimental design). The other part of the experiment with Reckenholz soil was conducted with 1L pots (20 experimental units = 2 BX conditions * 10 replicates; Data S3). Because of the differences in pot size and how the soils were conditioned, we do not compare the two soils in this experiment. We grew the two wheat cultivars 'Drifter', a hard winter wheat cultivar, and 'Fiorina', a spring wheat currently used by Swiss farmers. Six seeds were sowed per pot and two weeks after seeds germinated the number of plants was reduced to 5 for plants growing in the bigger pots, and reduced to 4 for the ones in smaller pots. Growth conditions in the greenhouse included a light period from 8:00 to 22:00, 70% relative humidity and temperatures of 22°C (day) and 18°C (night). Fertilization was done by mixing NPK granules (Osmocote exact, Standard 5-6M; 15-9-12 NPK, 2MgO and micronutrient; 4g / kg soil) into the soils before filling the pots. Plants were uniformly watered with tap water when needed. Chlorophyll content was measured at 3, 4, 5 and 6 weeks after sowing. The experiment was harvested at 6 weeks (plants were before flowering) as follows: the second fully unfolded leaves were cut, immediately wrapped in aluminium paper, shock-frozen in liquid nitrogen and then stored at -80° until hormone analysis. The remaining aboveground shoots were cut and recorded as fresh and dry weight (after drying in an oven at 60°C for 72h) biomass per pot.

Table 1: Number of replicates

	BX+	BX-
Changins_Drifter	7	8
Changins_Fiorina	7	7
Reckenholz_Drifter	10	10
Reckenholz_Fiorina	10	9

Feedback experiment with wheat (Drifter and Fiorina) (*wheat_line* variable) grown on Changins and eckenholz field soil (*soil* variable) conditioned with maize B73 (BX+ soil) and B73(*bx1*) (BX- soil) (*BX_condition* variable).

Wheat biomass

Table 2: ANOVA on wheat shoot fresh weight (FW) and dry weight (DW). Model = response variable ~ BX_condition * soil * wheat_line

	Sum Sq	Pr(>F)	Sum Sq	Pr(>F)
1	**FW**		**DW**	
BX_condition	1.36378	0.00771	0.00107	0.00586
soil	3.69271	3e-05	0.16062	0
wheat_line	2.56254	0.00036	0.00094	0.00964
BX_condition:soil	0.00367	0.88677	1e-04	0.37746
BX_condition:wheat_line	0.00348	0.88963	1e-04	0.38835
soil:wheat_line	0.00498	0.86822	0	0.8964
BX_condition:soil:wheat_line	0.00167	0.92339	0	0.97473
Residuals	10.76128	NA	0.00785	NA

Table 3: t.test on wheat shoot fresh weight

soil_wheat_line	mean in BX+	mean in BX-	p.value
Changins_Drifter	2.491	2.756	0.3156
Changins_Fiorina	2.08	2.351	0.1568
Reckenholz_Drifter	2.942	3.218	0.1785
Reckenholz_Fiorina	2.545	2.868	0.1006

Table 4: t.test on wheat shoot dry weight

soil_wheat_line	mean in BX+	mean in BX-	p.value
Changins_Drifter	0.3407	0.3453	0.6044
Changins_Fiorina	0.35	0.35	1
Reckenholz_Drifter	0.2394	0.2492	0.03061
Reckenholz_Fiorina	0.2496	0.2544	0.2446

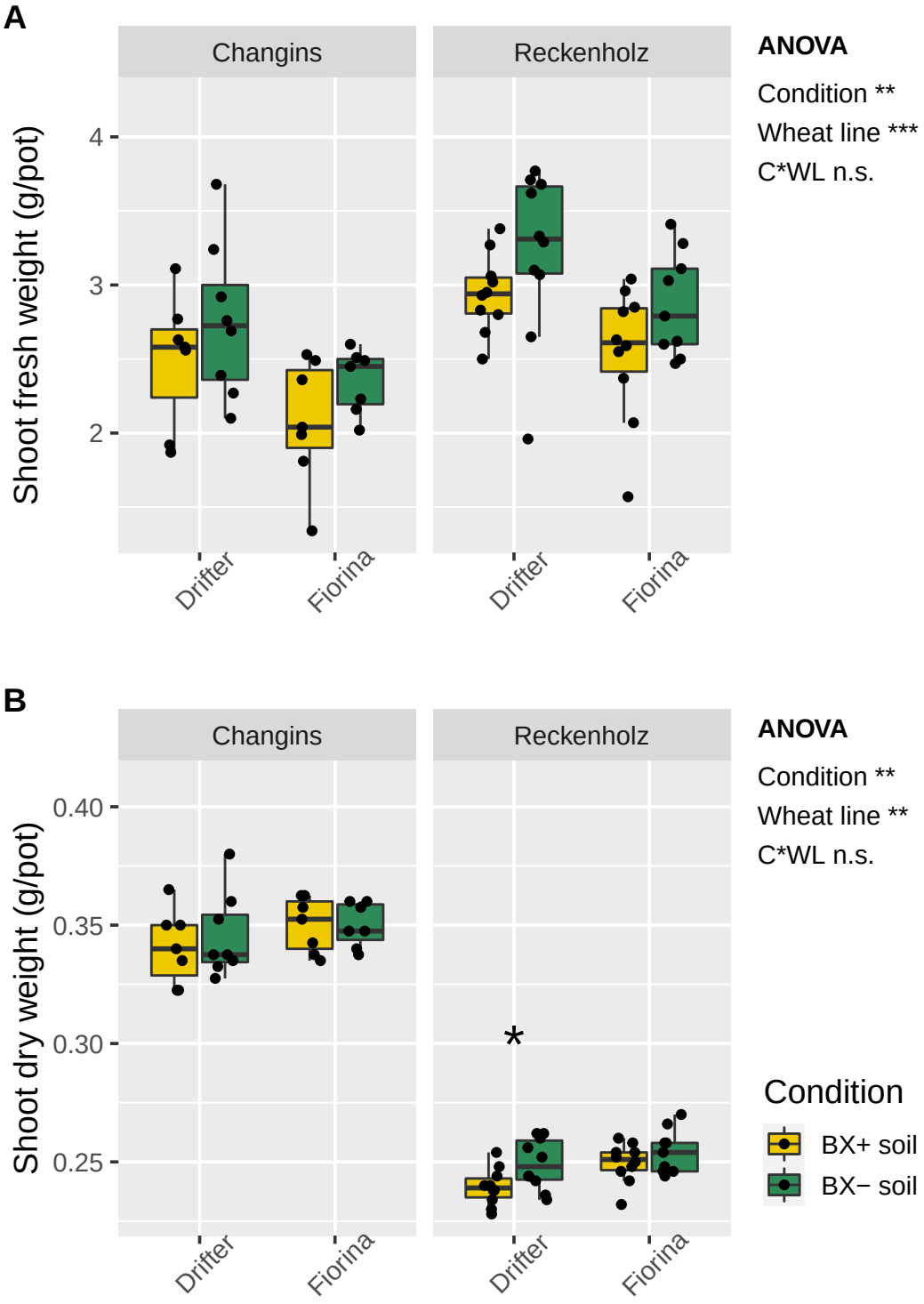


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Figure 3 | BX-feedback on wheat growth
Wheat plants were grown on 'BX+' and 'BX-' variants of Changins and Reckenholz soils. Shoot biomass was measured as (A) fresh and (B) dry weight for the two wheat lines Drifter and Fiorina after 6 weeks of growth. The ANOVA results (model: ~ condition (C) * wheat line (WL)) are reported next to the Figure and the pair-wise T-test results inside the panels (Significance code: P < 0.001 ***; P < 0.01 **, P < 0.05 *; not

significant = ‘n.s.’).

Wheat chlorophyll content (SPAD values)

Table 5: Linear mixed effect model on chlorophyll content, measured at week 3, 4, 5 and 6. Model: SPAD ~ week * BX_condition * soil * wheat_line, random=pot

	numDF	denDF	F-value	p-value
(Intercept)	1	180	68367	0
week	3	180	183.2	0
BX_condition	1	60	8.286	0.005529
soil	1	60	138.2	0
wheat_line	1	60	41.93	1.948e-08
week:BX_condition	3	180	4.054	0.0081
week:soil	3	180	15.43	5.631e-09
BX_condition:soil	1	60	3.288	0.07479
week:wheat_line	3	180	18.48	1.702e-10
BX_condition:wheat_line	1	60	0.0553	0.8149
soil:wheat_line	1	60	2.891	0.09424

Table 6: ANOVA on chlorophyll content for each week. Model: SPAD ~ BX_condition * soil * wheat_line (continued below)

	Sum Sq	Pr(>F)	Sum Sq
1	**3 weeks**		**4 weeks**
BX_condition	2.48529	0.34051	107.75529
soil	13.6889	0.0278	499.36726
wheat_line	91.47675	0	663.49628
BX_condition:soil	0.19668	0.78787	46.32785
BX_condition:wheat_line	9.39158	0.06669	50.5555
soil:wheat_line	0.5857	0.6426	55.48283
BX_condition:soil:wheat_line	1.81668	0.41464	0.11526
Residuals	161.53399	NA	557.54457

Table 7: Table continues below

	Pr(>F)	Sum Sq	Pr(>F)
1		**5 weeks**	
BX_condition	0.00118	10.64132	0.15817
soil	0	447.11101	0
wheat_line	0	41.69144	0.00635
BX_condition:soil	0.0293	7.16076	0.24571
BX_condition:wheat_line	0.02305	1.31057	0.61784
soil:wheat_line	0.0175	4.95697	0.33329
BX_condition:soil:wheat_line	0.91169	0.98663	0.66502
Residuals	NA	312.63114	NA

	Sum Sq	Pr(>F)
1	**6 weeks**	

	Sum Sq	Pr(>F)
BX_condition	13.81504	0.19889
soil	194.29624	1e-05
wheat_line	8.71827	0.30623
BX_condition:soil	0.06962	0.92683
BX_condition:wheat_line	1.77515	0.64314
soil:wheat_line	23.67912	0.09417
BX_condition:soil:wheat_line	10.24939	0.26763
Residuals	491.18347	NA

Table 9: t.test on chlorophyll at week 4

soil_wheat_line	mean in BX+	mean in BX-	p.value
Changins_Drifter	39.95	37.04	0.06404
Changins_Fiorina	34.1	27.79	0.01543
Reckenholz_Drifter	42.77	43.3	0.6133
Reckenholz_Fiorina	40.41	37.87	0.08241

Table 10: t.test on chlorophyll at week 5

soil_wheat_line	mean in BX+	mean in BX-	p.value
Changins_Drifter	45.13	43.19	0.06911
Changins_Fiorina	43.64	42.78	0.4436
Reckenholz_Drifter	49.96	49.77	0.8489
Reckenholz_Fiorina	47.87	47.78	0.9495

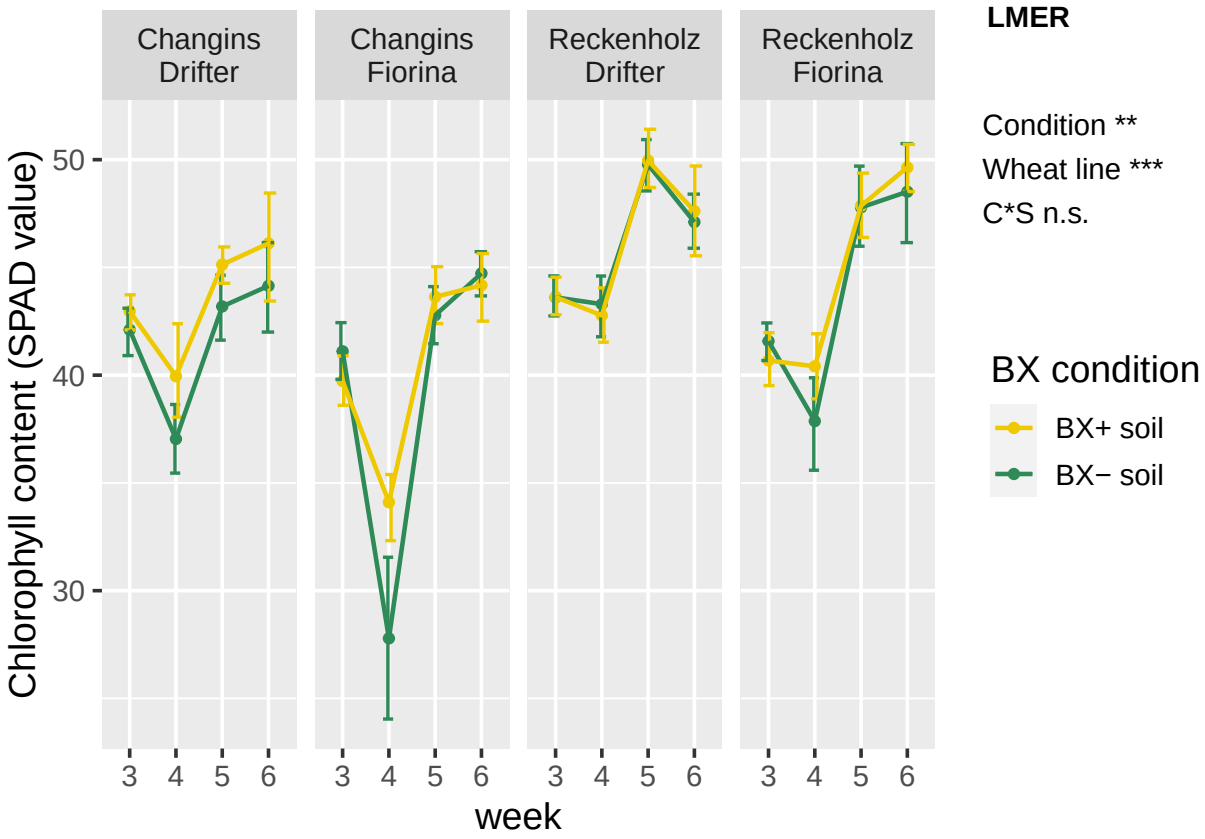


Figure S2 | BX-effect on wheat leaf chlorophyll content during growth
Wheat plants from cultivar Drifter and Fiorina were grown on 'BX+' and 'BX-' variants of Changins and Reckenholz soil. Mean chlorophyll content (SPAD values) per pot was measured from the 3rd to the 6th week of plant growth. The LME results for condition and wheat line variables (model: ~ week * soil and pot size * condition (C) * wheat line (WL), random factor = pot) are reported next to the figure (significance code: $P < 0.001$ ***; $P < 0.01$ **, $P < 0.05$ *; 'n.s.' = not significant).

Wheat hormones

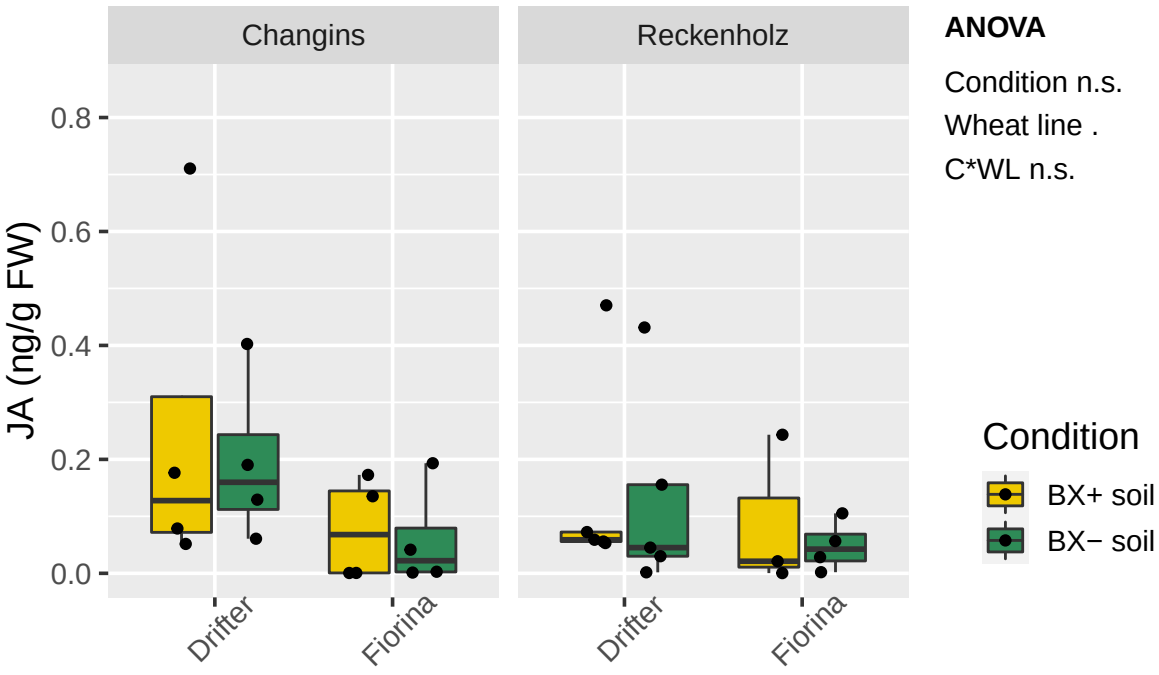
Table 11: ANOVA on plant hormone concentration of jasmonic acid (JA), salicylic acid (SA), abscicic acid (ABA), Jasmonic acid -Isoleucine (JA-Ile), Oxo-phytodienoic acid (OPDA) at 6 weeks. Model = response variable ~ BX_condition * soil * wheat_line (continued below)

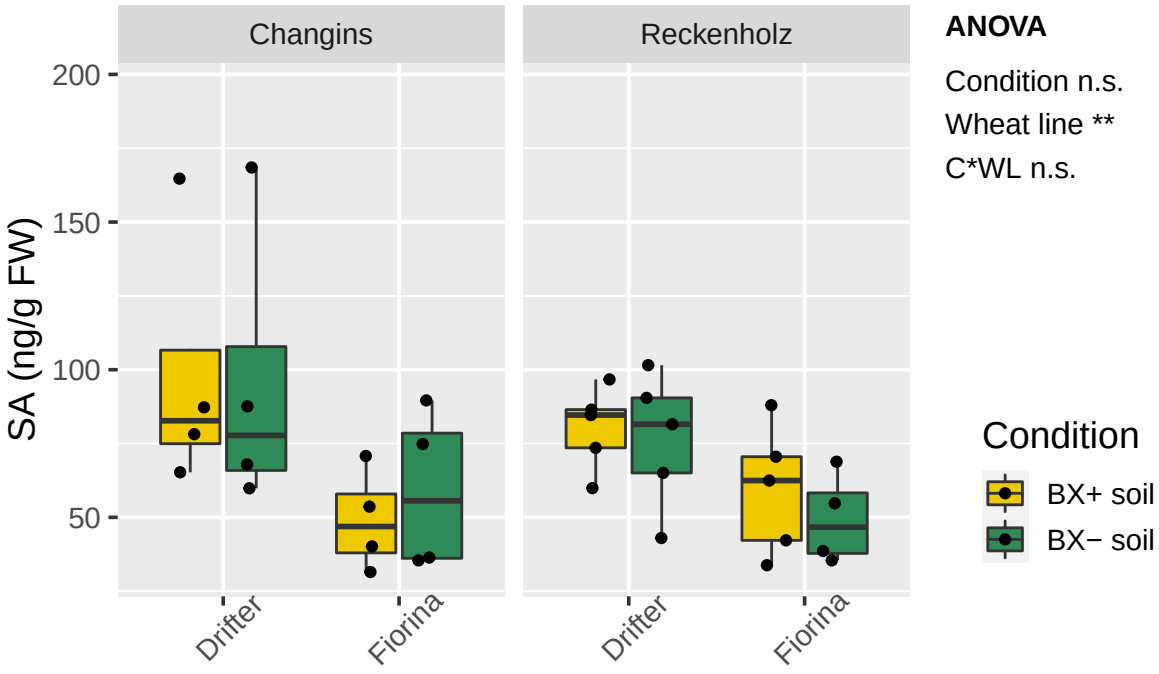
	Sum Sq	Pr(>F)	Sum Sq	Pr(>F)
1	**JA**		**SA**	
BX_condition	0.00921	0.57225	10.68026	0.9099
soil	0.01194	0.52056	630.14286	0.38799
wheat_line	0.10299	0.06712	9261.80816	0.00231
BX_condition:soil	0.00075	0.87131	255.88446	0.58068
BX_condition:wheat_line	0.00014	0.94398	9.58033	0.91465
soil:wheat_line	0.01483	0.47437	844.03539	0.31888
BX_condition:soil:wheat_line	0.00261	0.76332	194.73448	0.62965
Residuals	0.70275	NA	22098.89587	NA

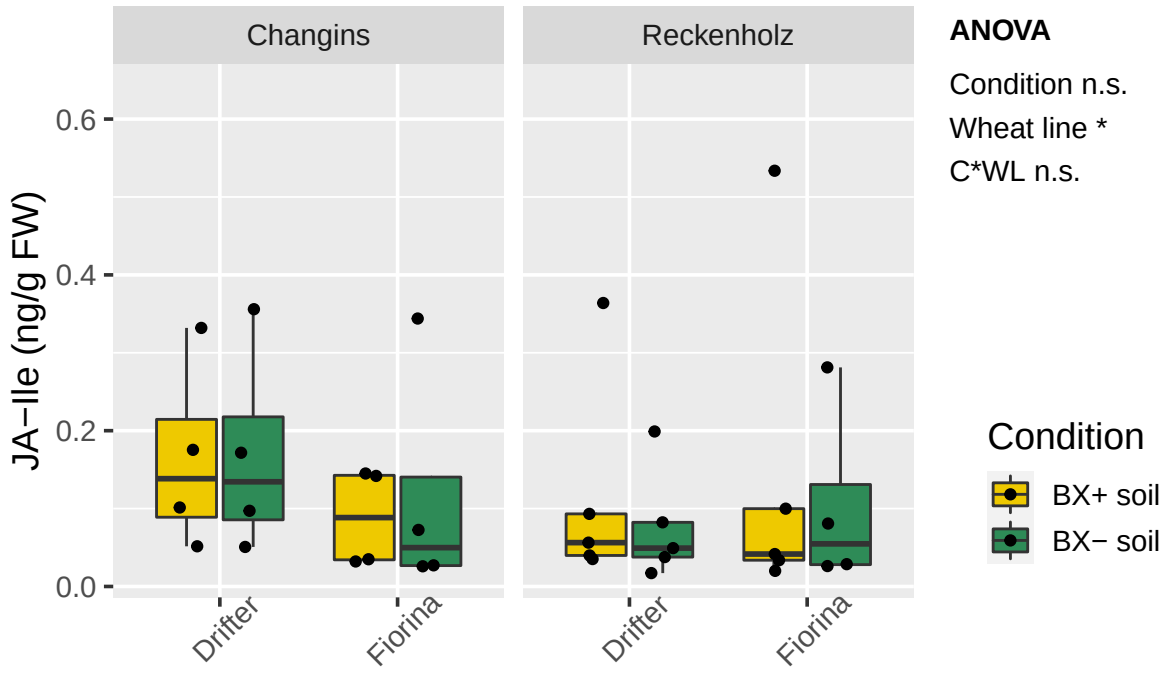
Table 12: Table continues below

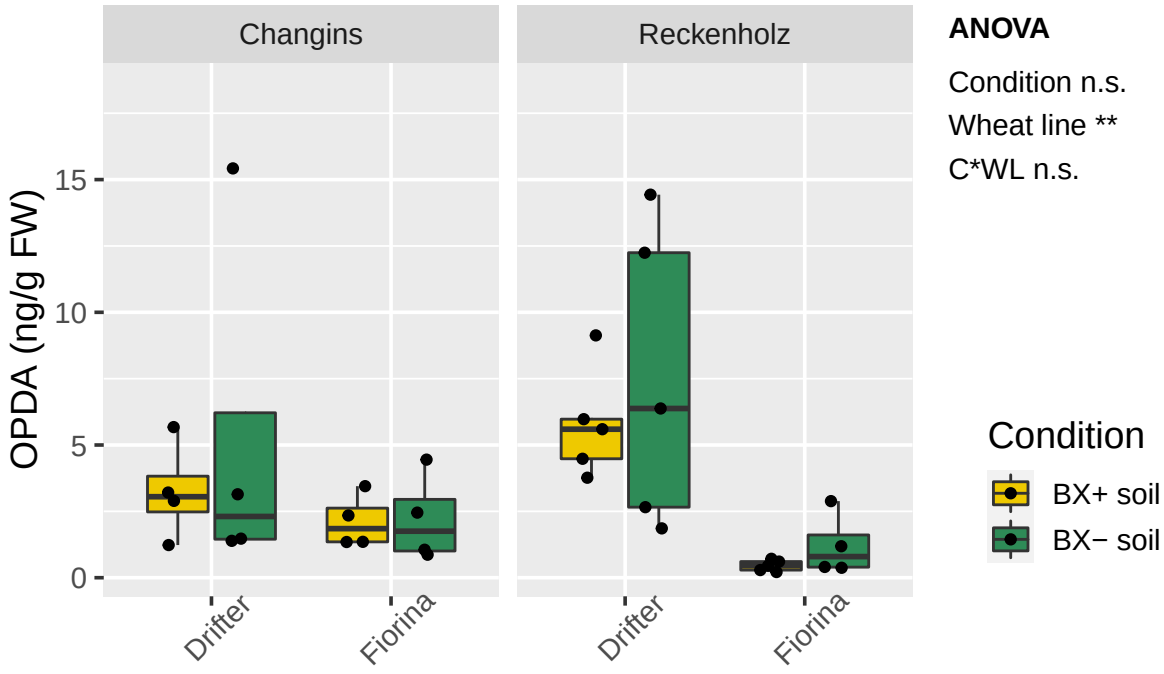
	Sum Sq	Pr(>F)	Sum Sq	Pr(>F)
1	**ABA**		**JA-Ile**	
BX_condition	389.38682	0.17559	0.00196	0.751
soil	585.02674	0.09968	0.00492	0.61569
wheat_line	1568.02115	0.00952	0.00161	0.77389
BX_condition:soil	88.62059	0.51259	0.00773	0.52966
BX_condition:wheat_line	53.80542	0.60933	0.00015	0.92994
soil:wheat_line	927.01652	0.041	0.01832	0.33585
BX_condition:soil:wheat_line	62.11938	0.58308	0.00037	0.89067
Residuals	5433.89471	NA	0.51503	NA

	Sum Sq	Pr(>F)
1	**OPDA**	
BX_condition	15.83568	0.2481
soil	3.98107	0.55885
wheat_line	148.15451	0.00123
BX_condition:soil	0.15742	0.90718
BX_condition:wheat_line	3.93018	0.56135
soil:wheat_line	29.49155	0.11881
BX_condition:soil:wheat_line	0.60266	0.8196
Residuals	306.81563	NA


Changins
Reckenholz
JA (ng/g FW)
0.8
0.6
0.4
0.2
0.0
Drifter
Fiorina
Drifter
Fiorina
ANOVA
Condition n.s.
Wheat line .
C*WL n.s.
Condition
BX+ soil
BX− soil


Changins
Reckenholz
SA (ng/g FW)
200
150
100
50
Drifter
Fiorina
Drifter
Fiorina
ANOVA
Condition n.s.
Wheat line **
C*WL n.s.
Condition
BX+ soil
BX− soil


Changins
Reckenholz
JA−Ile (ng/g FW)
0.6
0.4
0.2
0.0
Drifter
Fiorina
Drifter
Fiorina
ANOVA
Condition n.s.
Wheat line *
C*WL n.s.
Condition
BX+ soil
BX− soil


Changins
Reckenholz
OPDA (ng/g FW)
15
10
5
0
Drifter
Fiorina
Drifter
Fiorina
ANOVA
Condition n.s.
Wheat line **
C*WL n.s.
Condition
BX+ soil
BX− soil

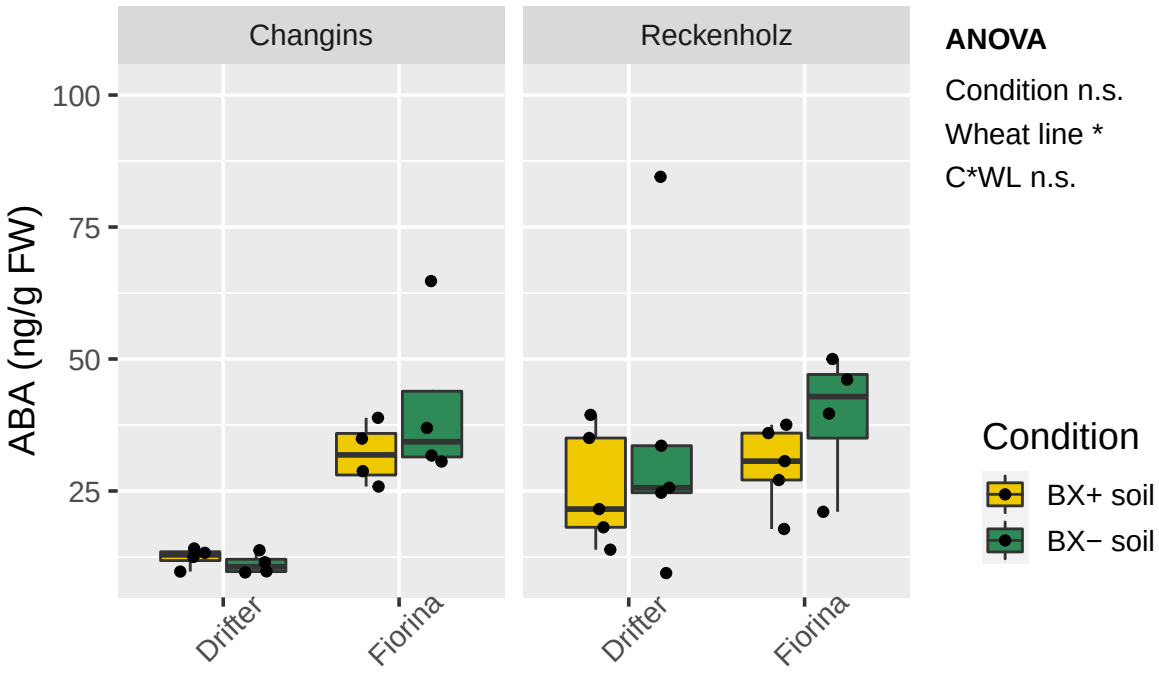


Figure S3 | BX-effect on wheat hormone concentration
Wheat plants from cultivar Drifter and Fiorina were grown on 'BX+' and 'BX-' variants of Changins and Reckenholz soil. Leaf concentration of (A) jasmonic acid (JA), (B) salicylic acid (SA), (C) jasmonic acid – isoleucine (JA-Ile), (D) oxophytodienoic acid and (E) abscicic acid (ABA) were measured in 6 week old plants. The ANOVA results for condition and wheat line (model: ~ soil and pot size * condition (C) * wheat line (WL)) are reported next to the figure (significance code: $P < 0.001$ ***; $P < 0.01$ **, $P < 0.05$ *; $P < 0.1$ '.'; 'n.s.' = not significant).

Descriptive statistics

Table 14: Mean and standard deviation (sd) of measures. Dr = Drifter, Fio = Fiorina, Ch = Changins, Re = Reckenholz (continued below)

	mean BX+ Dr Ch	sd BX+ Dr Ch	mean BX- Dr Ch
wheat shoot DW (g)	0.3407	0.01559	0.3453
wheat shoot FW (g)	2.491	0.4481	2.756
spad week4	39.95	3.149	37.04

Table 15: Table continues below

	sd BX- Dr Ch	percent diff Dr Ch	mean BX+ Fio Ch
wheat shoot DW (g)	0.01765	1.34	0.35
wheat shoot FW (g)	0.5237	10.09	2.08
spad week4	2.42	-7.57	34.1

Table 16: Table continues below

	sd BX+ Fio Ch	mean BX- Fio Ch	sd BX- Fio Ch
wheat shoot DW (g)	0.01164	0.35	0.009354
wheat shoot FW (g)	0.4241	2.351	0.2149
spad week4	2.316	27.79	5.45

Table 17: Table continues below

	percent diff Fio Ch	mean BX+ Dr Re	sd BX+ Dr Re
wheat shoot DW (g)	0	0.2394	0.007891
wheat shoot FW (g)	12.25	2.942	0.2617
spad week4	-20.41	42.77	2.185

Table 18: Table continues below

	mean BX- Dr Re	sd BX- Dr Re	percent diff Dr Re
wheat shoot DW (g)	0.2492	0.01059	4.01
wheat shoot FW (g)	3.218	0.5657	8.96
spad week4	43.3	2.375	1.22

Table 19: Table continues below

	mean BX+ Fio Re	sd BX+ Fio Re	mean BX- Fio Re
wheat shoot DW (g)	0.2496	0.008208	0.2544
wheat shoot FW (g)	2.545	0.4475	2.868

	mean BX+ Fio Re	sd BX+ Fio Re	mean BX- Fio Re
spad week4	40.41	2.506	37.87

	sd BX- Fio Re	percent diff Fio Re
wheat shoot DW (g)	0.009315	1.92
wheat shoot FW (g)	0.3503	11.93
spad week4	3.471	-6.5