Arabidopsis plant-soil feedbacks mediated by maize benzoxazinoids

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INTRODUCTION

Plants mediate their immediate soil environment, including their root microbiota through root exudates. This leads to so-called plant-soil feedbacks, where the altered soil biota modulates the fitness of a second plant generation. Maize and other grass crop species produce and exude the secondary metabolites `benzoxazinoids´ (BXs) into the soil, which can have attracting or repelling properties towards microorganisms.[1,2] Hu and colleagues (2018) showed that a conditioning of soil with wild-type maize plants compared to the mutant *bx1*, which is defective in the BX biosynthesis pathway, lead to differences in the soil and root microbiota.[3] Furthermore, a second generation of wild-type maize plants displayed differences in growth and defense when grown on these two conditioned soils.[3] Thus, BXs shape the soil in a meaningful way that affects the fitness of the next plant generation. However, the underlying mechanisms are still largely unknown. Therefore, we want to lay the basis for understanding these BX-mediated plant-soil feedbacks by asking two main questions:

- How does a non-BX exuding plant species respond as a feedback plant on BX-conditioned soils?
- Can *Arabidopsis thaliana* be used as a model plant to further investigate BX-driven plant-soil feedbacks?

METHODS

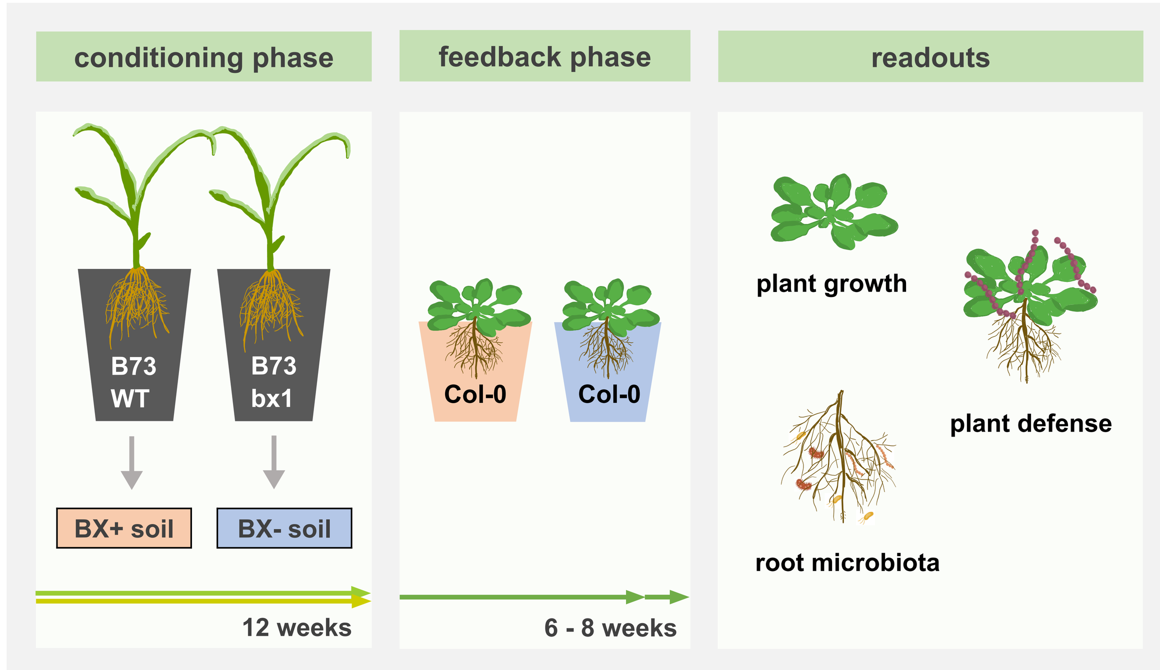

Figure 1 **Overview of the study system**
During the conditioning phase, B73 wild-type and B73 *bx1* mutant maize plants were grown in field soil collected from Changins, Switzerland, in a climate chamber for twelve weeks. The soil from the pots was collected, rendering BX+ soil and BX– soil respectively. The differently coloured arrows represent two independent soil conditioning experiments. In the feedback phase, the response plant *Arabidopsis thaliana* (Col-0) was grown on BX+ and BX– soils. As readouts, we measured the shoot area and shoot biomass to characterize plant growth and analysed the root microbiota compositions. To characterize plant defense, we challenged *Arabidopsis* with *Botrytis cinerea* and analysed the lesion size of the infections.

Conditioning phase: To manipulate the soil microbiota, B73 wild-type maize and non-BX producing *bx1* mutant maize were grown in agricultural soil under controlled conditions in a climate chamber for twelve weeks. The soil from the pots was harvested and pooled, resulting in BX+ and BX– soil respectively.
Feedback phase: The harvested soils were homogenized, amended with 20% autoclaved sand and used to grow *Arabidopsis thaliana* (Col-0) plants for six to eight weeks under short day conditions in climate chambers. To control the soil moisture, we regulary watered pots by weight or by water volume.
Readouts: To determine plant growth, we measured the shoot area and shoot biomass. The root microbiota composition was determined by amplicon sequencing of the 16S rRNA gene to identify bacteria and a segment of the ITS spacer region to determine fungi. We assessed plant defense by infecting the leaves with the necrotrophic fungal pathogen *Botrytis cinerea* and quantifying the lesion area three days post infection.

RESULTS

Plant Growth

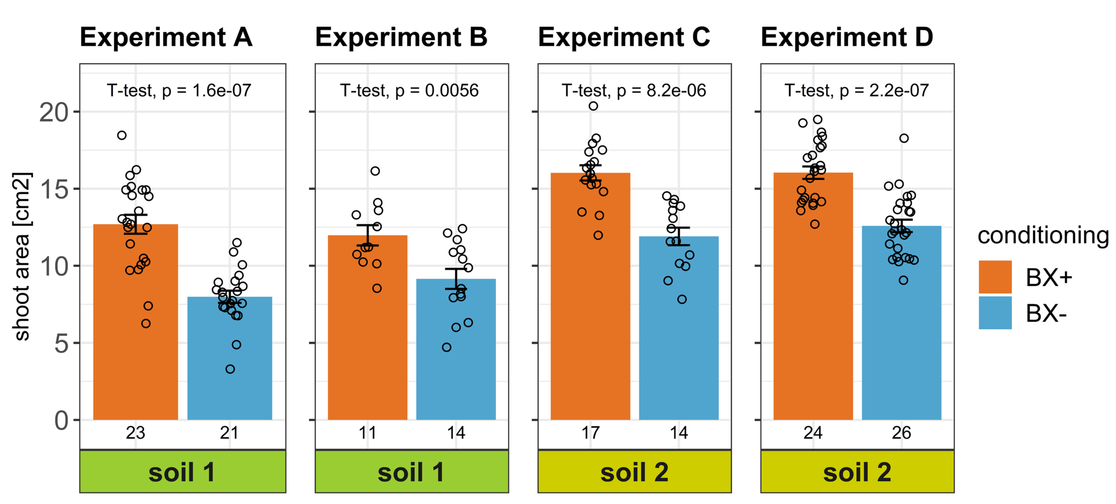

Figure 2 **Shoot area**
Shoot area of *Arabidopsis* from four independent experiments grown on soil from two separate conditioning experiments, indicated with the differently coloured labels at the bottom. The error bars represent the standard error of the mean. A two-sided student's t-test is shown on top and the sample size is indicated below each bar.

Root Microbiota

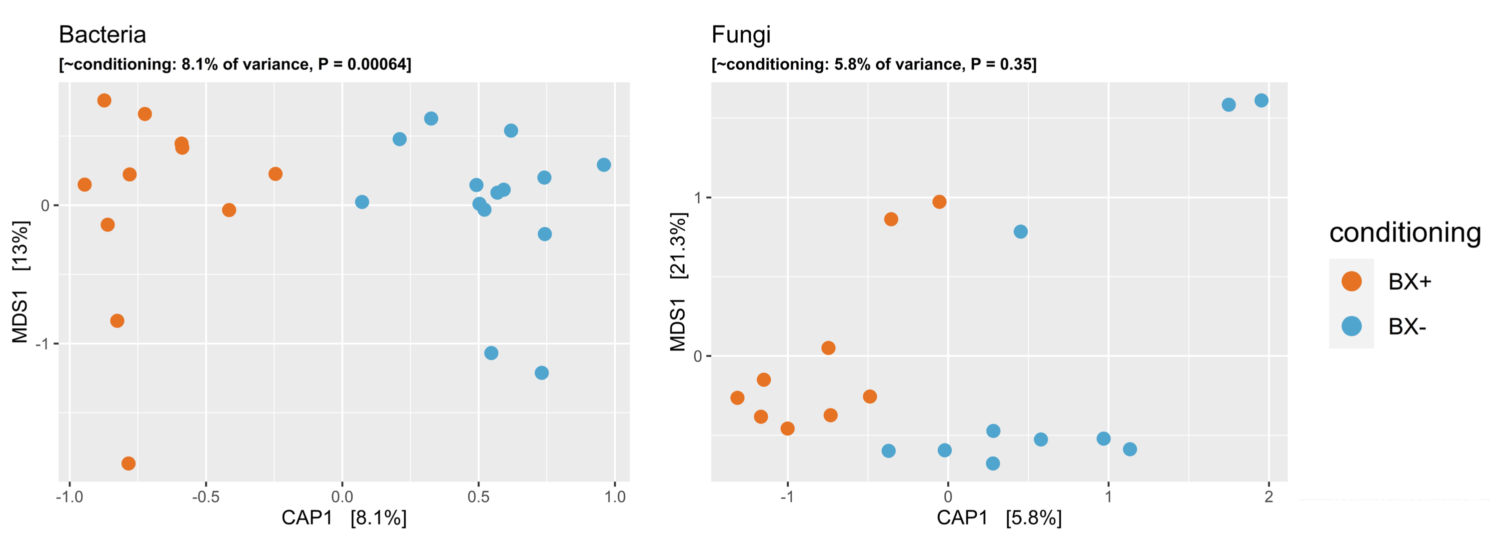

Figure 4 **Beta diversity of microbial community compositions**
The beta diversity represents the differences in communities between two treatments. In this case, the bacterial and fungal community differences between *Arabidopsis* roots grown in BX+ soil and roots from BX- soil are depicted. Canonical Analysis of Principal Coordinates (CAP) were performed using Bray-Curtis distances and constrained for the explanatory variable 'conditioning'. An ANOVA-like permutation test was performed, and the fraction of the variance explained by the conditioning together with the model significance is displayed above the plots. The CAP1 axis shows the proportion of total variance explained by the constrained explanatory variable.

Plant Defense

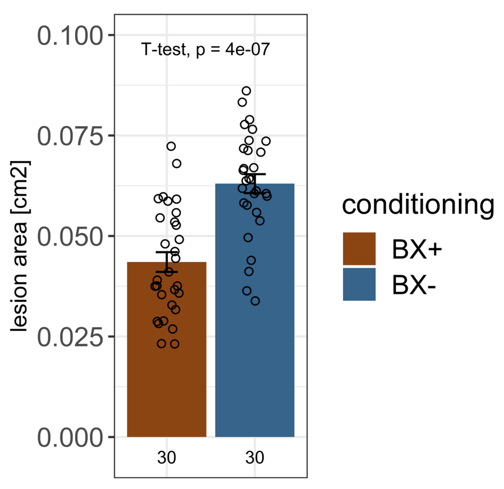

Figure 3 **Lesion area after leaf infection with *Botrytis cinerea***
Three week old *Arabidopsis* plants were infected with droplets of *Botrytis cinerea* spore suspension on three source leaves each. The lesion area three days post infection is depicted, where smaller lesion area represents better plant defense. The error bars represent the standard error of the mean. A two-sided student's t-test is shown on top and the sample size is indicated below each bar.

Data: Henry Janse van Rensburg

Summary

Increased shoot area on BX+ soil compared to BX– soil
- On two independently conditioned soils

Smaller lesion size after *Botrytis cinerea* infection on BX+ soil
- Better plant defense against necrotrophic pathogens on BX+ soil?

Different *Arabidopsis* root microbiota between the conditionings
- Bacteria are more differentiated than fungal communities

***Arabidopsis* (Col-0) displays elevated growth and defense on BX+ soil and is thus a model to further study BX-driven plant-soil feedbacks.**

FUTURE RESEARCH DIRECTIONS

Microbial vs. chemical ?
Disentangle the microbes as the biotic soil part from the BXs as the chemical soil part to assess their individual effects on *Arabidopsis* growth and defense.
- Role of BX+ microbiota in growth promotion?
- Allelopathic effects of BXs in soil on *Arabidopsis*?

Defense ?
Further study defense of *Arabidopsis* on BX-conditioned soils against aboveground or belowground pathogens such as *Botrytis cinerea, Pseudomonas syringae* and *Pythium ultimum.*
- Role of the microbiota and the soil chemicals in defense?

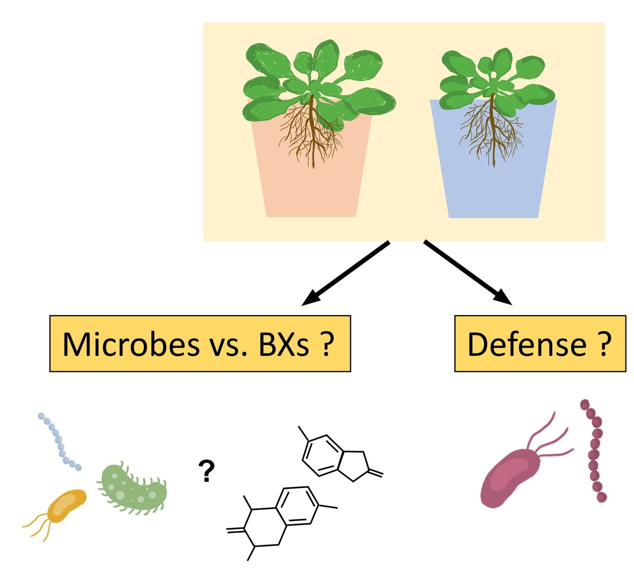

Figure 5 **Future research directions**
We aim to further investigate the role of the microbes versus the BX compounds in the soil on *Arabidopsis* growth and defense. We also want to test BX-dependent defense responses of *Arabidopsis* against different plant pathogens.

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REFERENCES

1 Neal AL, Ahmad S, Gordon-Weeks R, Ton J. Benzoxazinoids in Root Exudates of Maize Attract *Pseudomonas putida* to the Rhizosphere. *PLoS ONE* 7(4), e35498 (2012).
2 Niemeyer, H.M. Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one: key defense chemicals of cereals. *Agric Food Chem* 57, 1677–1696 (2009).
3 Hu, L., Robert, C.A.M., Cadot, S. *et al.* Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat Commun* 9, 2738 (2018).