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Split-root system for root exudate collection to study plant-plant interactions

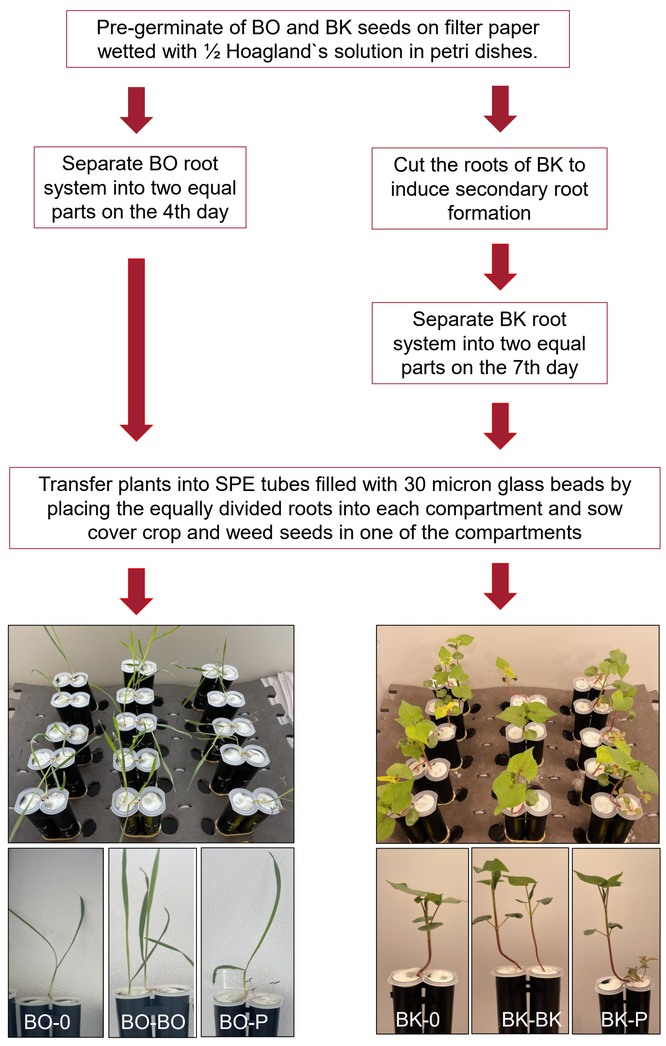
Çağla Görkem Eroğlu[1], Alexandra Bennett[2], Aurélie Gfeller[1], Judith Wirth[1], Stephan Hann[2], Markus Puschenreiter[3]

[1]Herbology in Field Crops, Plant Production Systems, Agroscope, 1260 Nyon, Switzerland ; www.agroscope.ch, [2]Department of Chemistry, Institute of Analytical Chemistry, University of Natural Resources and Life Sciences, 1190, Vienna, Austria, [3]University of Natural Resources and Life Sciences, Vienna, Department of Forest and Soil Sciences, Institute of Soil Research, 3430 Tulln, Austria

Introduction

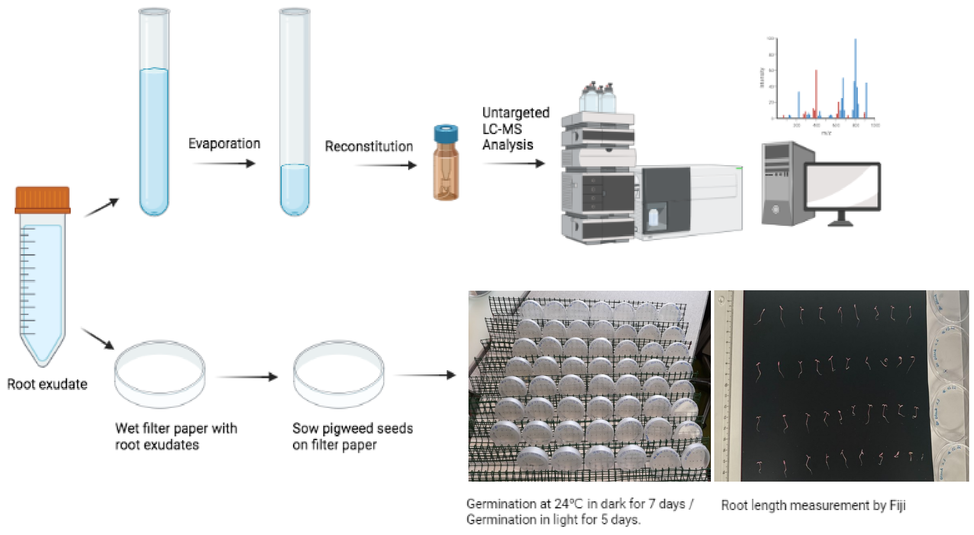
Above- and below-ground interactions of plants with their neighbors involve complex mechanisms. Through root exudation, plants regulate soil microbiota, change soil properties, and influence germination, growth, and survival of neighboring plants by potentially detecting and recognising their neighbours. Split root systems enable treating separate parts of a single root system differently and collection of root exudates from independent compartments. In the present study, a split-root system using non-complex soil-free media with minimized root damage is established to elucidate inter- and intra-specific below-ground interactions and successfully used for root exudate analysis and assessment of physiological characteristics of roots to provide insights into the below-ground cover crop (Fagopyrum esculentum (BK), Avena strigosa (BO) – weed (Amarantus retroflexus, P) interactions.

Materials and Methods



BO and BK grown in split root systems. From left to the right BO w/o (B0-0), BO neighbouring BO (BO-BO), BO neighbouring P (BO-P) and BK w/o neighbour (B0-0), BK neighbouring BK (BK-BK), BK neighbouring P (BK-P).

- Place the tubes with 2-week old plants on the manifold connected to vacum pump set it to 585 mmHg. Add 30 ml of extraction solution (95% MeOH, 0.05% CH_2O_2) and vacuum off for a minute.
- Wash the roots, scan & analyse using WinRHIZO.



Results and Discussion

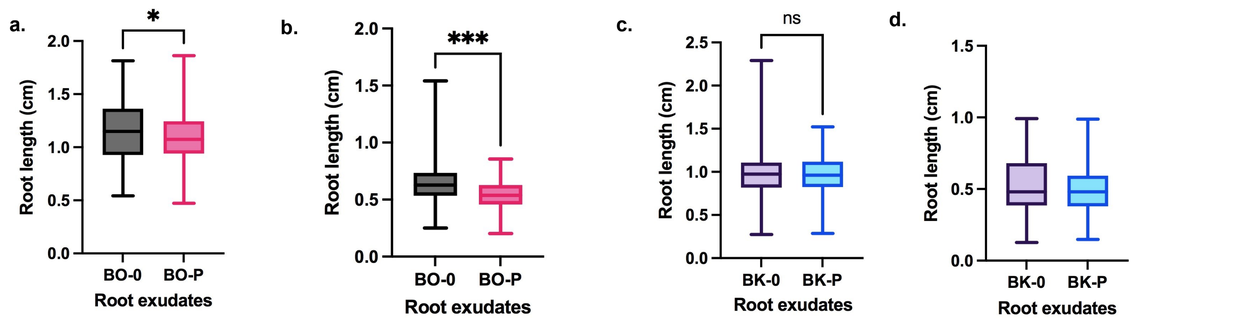


Figure 1: Root length of pigweed seedlings treated with BO-0 and BO-P root exudates and grown in light conditions for 7 days (a), grown in dark conditions for 5 days (b), treated with BK-0 and BK-P root exudates and grown in light conditions for 7 days (c), grown in dark conditions for 5 days (d) , t test, n= 120 ≤n≤ 190, P < 0.05, 0.12 nonsignificant (ns), 0.033 (*), 0.002 (**), < 0.001 (***),

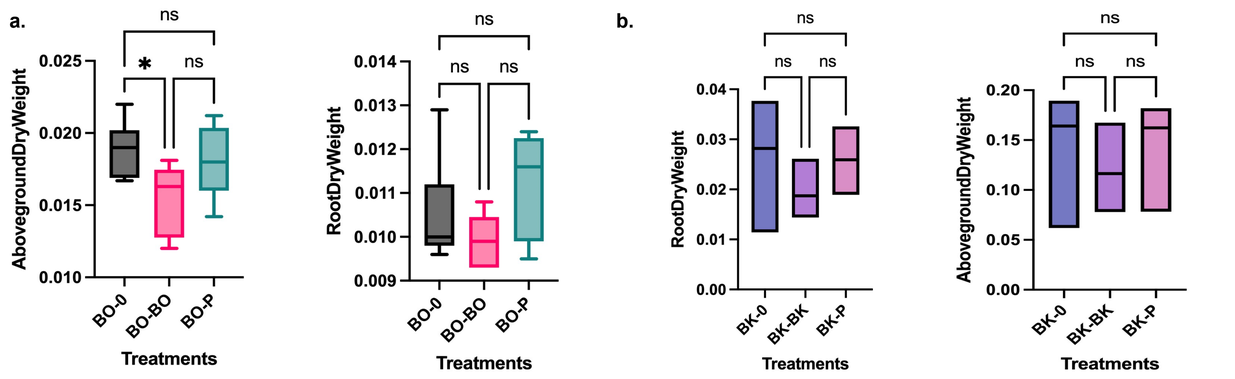


Figure 2: Aboveground and root dry weight of BO-0, BO-BO and BO-P (a), and BK-0, BK-BK and BK-P (b) grown in split root systems. One way ANOVA with Tukey as post hoc test family-wise alpha and confidence level 0.05 (95% confidence level). n= 3 - 5, P < 0.05, 0.12 nonsignificant (ns), 0.033 (*), 0.002 (**), < 0.001 (***),

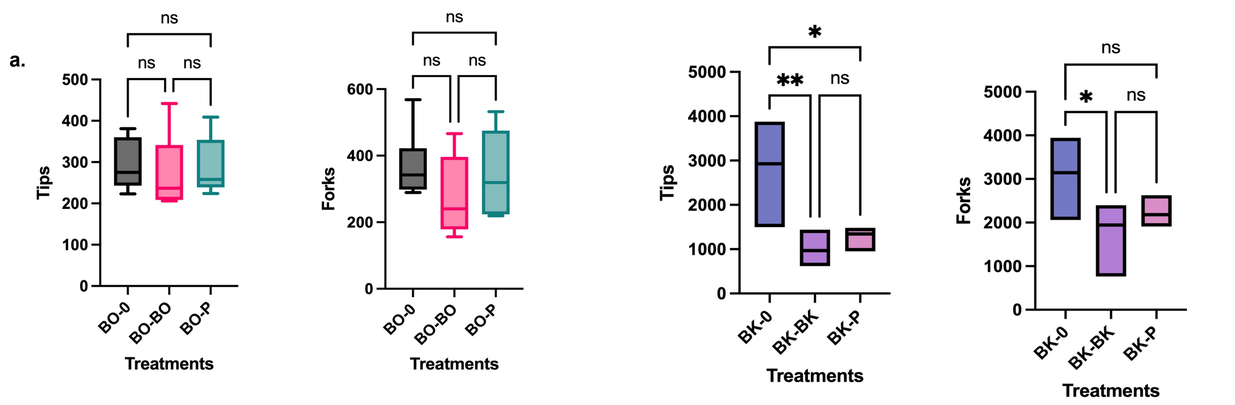


Figure 3: Number of root tips and forks of BO-0, BO-BO and BO-P (a), and BK-0, BK-BK and BK-P (b) grown in split root systems measured by WinRHIZO image analysis system 2021. One way ANOVA with Tukey as post hoc test family-wise alpha and confidence level 0.05 (95% confidence level). n= 3 - 5, P < 0.05, 0.12 nonsignificant (ns), 0.033 (*), 0.002 (**), < 0.001 (***),

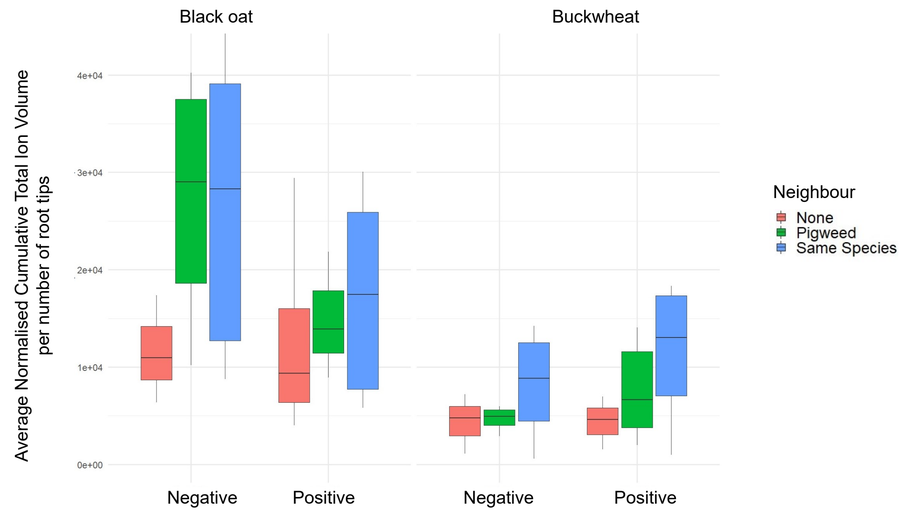


Figure 4: Box plot showing the average total ion volume (M+H or M-H signal plus all of its isotopologues and adducts aggregated into one signal) which has been normalized to quality control, internal standard, and number of root tips for every compound.

Summary

- Our methodological approach enabled differential cover crop root exudate collection from plants grown in presence of intra- and inter-specific neighbours.
- Biological tests were performed using root exudates of cover crops growing alone (BO-0 and BK-0) and in presence of P (BO-P and BK-P). P seeds treated with BO-P root exudates had significantly shorter seedling root length in comparison to seedlings treated with BO-0 root exudates. While performing biological tests in dark conditions resulted in elevated root growth suppression with BO root exudates no difference was observed with BK root exudates.
- Presence neighbouring BK significantly changed the number of root tips and forks in BK and presence of P significantly changed the number of root forks in BK. No significant difference was observed in root tips and forks of BO.
- Average normalized cumulative total ion volume per number of tips were higher in presence of neighbours, the effect was more visible in BO than BK.