# An on-site sample preparation approach for plant eco-metabolomics and its application to agroecosystems in East Africa

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Mass spectrometry-based plant metabolomics is frequently used to identify novel naturally occurring molecules or study the effect of specific treatments on a plant's metabolism. Reliable sample handling is required to avoid artifacts, which is why most protocols mandate immediate shock freezing of plant tissue in liquid nitrogen and an uninterrupted cooling chain to preserve labile molecules. However, the logistical challenges of acquiring liquid nitrogen and establishing an uninterrupted cooling chain make this approach infeasible for some studies. Especially for research on environmental or ecological systems, permanent cooling can pose a challenge, which is why frequently dried leaf tissue is used instead. While this approach works for stable molecules, the drying process has a significant impact on the overall metabolite profile.

In preparation for a metabolomic study of an agroecosystem in sub-Saharan Africa, we screened ten extraction and storage approaches for plant metabolites retrieved from maize leaf tissue across two cropping seasons to find a method which can be implemented for large sample quantities under logistically challenging conditions. The methods were evaluated across a two-month storage period and directly compared to samples obtained from shock-frozen leaf tissue. We show that our on-site liquid-liquid extraction protocol provides a good compromise between sample replicability, extraction efficiency, material logistics, and metabolite profile stability and is a viable alternative for metabolomics analyses of environmental or ecological systems.

Our on-site sample preparation was then used to study neighbourhood effects in a push-pull intercropping system. The samples were collected from farmer fields in Kenya, Rwanda, Ethiopia, and Uganda, and extracted on-site before shipment to Switzerland for mass spectrometry measurements. Our approach could differentiate maize plants grown under push-pull systems from plants grown with conventional agricultural practices and molecular classification is currently ongoing.

We demonstrated the feasibility of using an on-site liquid-liquid extraction protocol for plant metabolites from maize leaf tissue. We conclude that our method provides a reliable alternative for logistically challenging conditions regarding sample quality and stability as well as ecological scalability. This protocol allowed us to identify differences in the metabolite profiles of maize plants grown under different agricultural practices, highlighting the potential of this method for ecological studies.