msGBS: A new high-throughput approach to quantify relative species abundance in root samples of multi-species plant communities

Niels Wagemaker[1], Liesje Mommer[2], Eric Visser[1], Alexandra Weigelt[3], Thomas Gurp[4], Maarten Postuma[5], Annemiek Smit-Tiekstra[6], and Hans de Kroon[1]

[1]Radboud University Nijmegen
[2]WUR
[3]Leipzig University
[4]Naktuinbouw
[5]Wageningen Universiteit
[6]Radboud Universiteit Nijmegen

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Abstract

Plant interactions are as important belowground as aboveground. Belowground plant interactions are however inherently difficult to quantify, as roots of different species are difficult to disentangle. Although for a couple of decades molecular techniques have been successfully applied to quantify root abundance, root identification and quantification in multi-species plant communities remains particularly challenging. Here we present a novel methodology, multi-species Genotyping By Sequencing (msGBS), as a next step to tackle this challenge. First, a multi-species meta-reference database containing thousands of gDNA clusters per species is created from GBS derived High Throughput Sequencing (HTS) reads. Second, GBS derived HTS reads from multi-species root samples are mapped to this meta-reference which, after a filter procedure to increase the taxonomic resolution, allows the parallel quantification of multiple species. The msGBS signal of 111 mock-mixture root samples, with up to 8 plant species per sample, was used to calculate the within-species abundance. Optional subsequent calibration yielded the across-species abundance. The within- and across-species abundances highly correlated (R2 range 0.72-0.94 and 0.85-0.98, respectively) to the biomass-based species abundance. Compared to a qPCR based method which was previously used to analyze the same set of samples, msGBS provided similar results. Additional data on 11 congener species groups within 105 natural field root samples showed high taxonomic resolution of the method. msGBS is highly scalable in terms of sensitivity and species numbers within samples, which is a major advantage compared to the qPCR method and advances our tools to reveal the hidden belowground interactions.

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