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A Novel Workflow for Deep Metabolome Annotation and Interpretation in *Lemna minor*

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Lemna minor of the Lemnaceae (aka duckweeds) family is a superior model for investigating plant-microbe interactions. We designed a project, which aims to quantify the effects of a synthetic bacterial community across the largest genotype $\times$ environmental stress matrix (6,480 combinations) examined in a plant-microbe study to date. Multi-omics (metabolome, transcriptome, ionome, and phenome) studies will be applied to understand how microbes affect stress resilience and growth performance of 30 genome-resolved *L. minor* genotypes. In this report, we present a novel metabolomics workflow for deep metabolome annotation and interpretation in *L. minor*. The workflow integrates complementary LC-MS data processing, statistical prioritization of biologically relevant features, multi-layer metabolite annotation, confidence-level labeling, and pathway-based interpretation. Metabolite annotation combines MS/MS spectral library matching, fragmentation-based annotation, and spectral similarity or metabolic relationship-based approaches. These results are harmonized into an automated confidence-ranking system spanning confirmed structures to unknown features. As a case study, the workflow was applied to two *L. minor* genotypes with contrasting growth performance. Our analysis detected thousands of metabolic features, including a subset of high-confidence annotations. Initial analyses identified broad metabolic differences between the two model genotypes involving amino acid and nitrogen, phenylpropanoid/flavonoid, carbon and energy, lipid, and pigment-associated metabolism. Although the biological interpretation is ongoing, we can conclude that the novel workflow offers a promising approach to automate deep, evidence-based metabolome annotation, as well as providing a flexible foundation for future research into duckweed metabolomics and the plant-microbiome relationship.

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References

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