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# Supplementary Materials for

## Reproducible growth of *Brachypodium* in EcoFAB 2.0 reveals that nitrogen form and starvation modulate root exudation

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

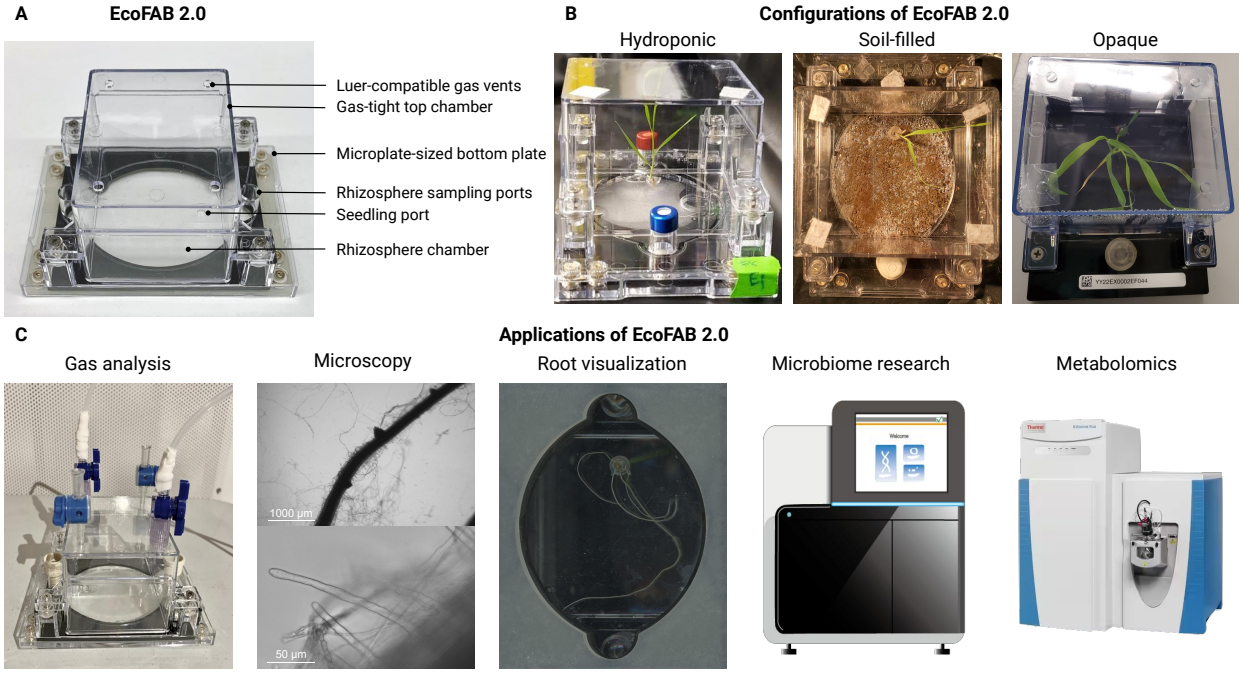


**Fig. S1: EcoFAB 2.0 devices can accommodate a wide diversity of plant research.** (**A**) Empty EcoFAB 2.0 and its main functional parts. (**B**) Configurations of EcoFAB 2.0 for *B. distachyon* growth include (from left): EcoFAB 2.0 filled with liquid medium for hydroponic plant growth (used in this study), soil-filled setup, and a version of EcoFAB 2.0 with opaque black root chamber that allows light-sensitive rhizosphere experiments. (**C**) Examples of possible applications include (from left): gas analysis, microscopy of roots (scale bars represent 1000 µm and 50 µm), root system visualization, study of microbiomes by 16S sequencing, and metabolomics on growth medium (used in this study).

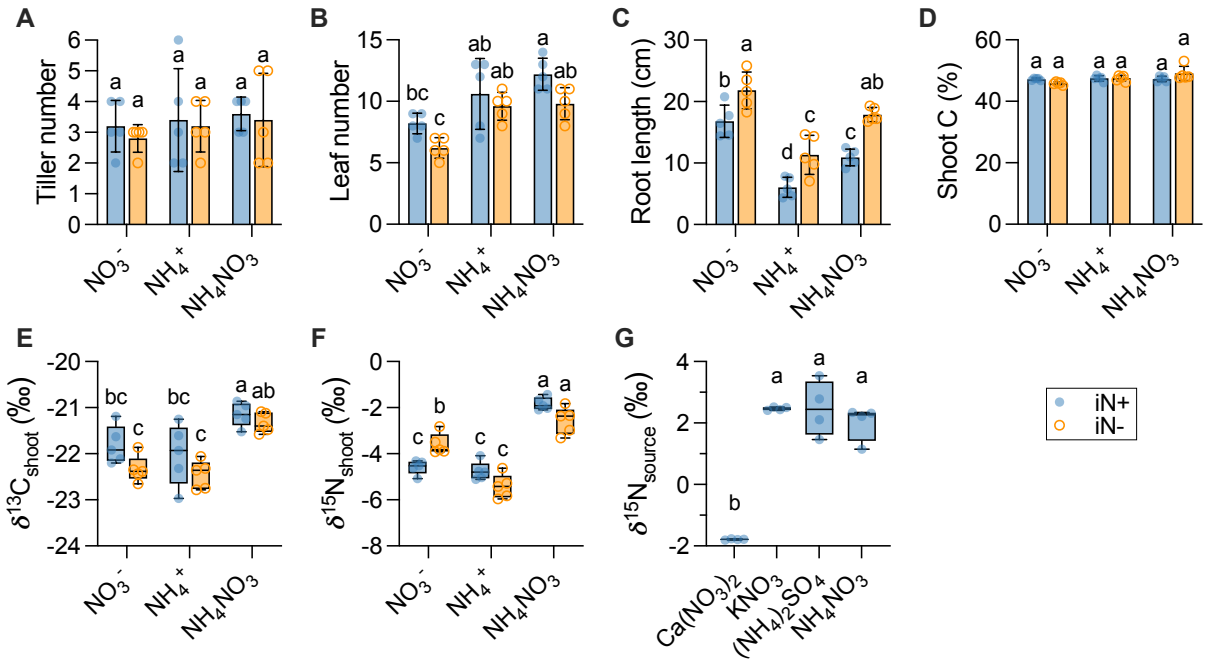


**Fig. S2: Multivariate analysis of plant growth and nutrient dynamics.** (**A**) total number of tillers (primary and secondary). (**B**) Total leaf number (fully developed and emerging). (**C**) Maximum root length. (**D**) Total carbon in dry shoot weight (% w/w). (**E**) Carbon stable isotope ratios of shoots. (**F**) Shoots N stable isotope ratios. (**G**) N stable isotope ratios of iN sources from the iN+ growth media. Different letters indicate statistically significant differences (two-way ANOVA with post hoc Tukey's HSD test; $n = 5$; $p \leq 0.05$). Bars show mean ±SD. Box plots show all points, hinges extend from the 25th to 75th percentiles, the middle line indicates the median, and the whiskers extend to min and max values. The iN+ control treatments are labeled by blue color and the iN- treatments by orange.

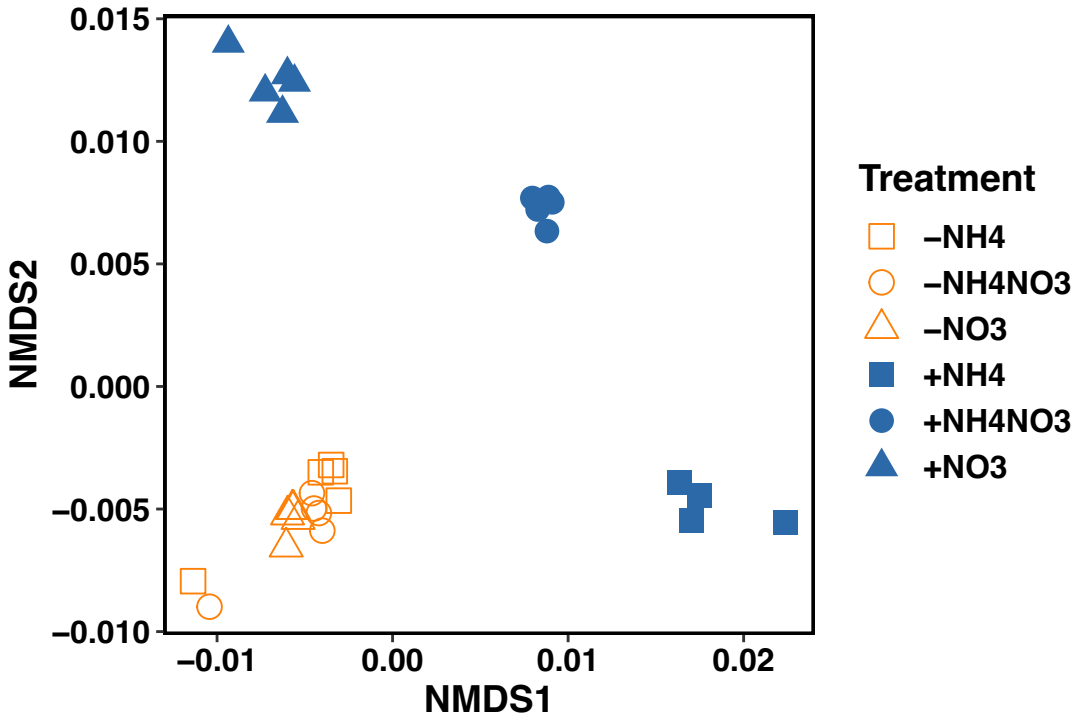


**Fig. S3: NMDS plot for *B. distachyon* root exudate features using raw peak height data (filtered features, *n* = 2065).** Each biological replicate across iN treatments is represented with a symbol. The Blue symbols represent iN+ treatments, while the orange symbols represent plants grown in iN- medium.

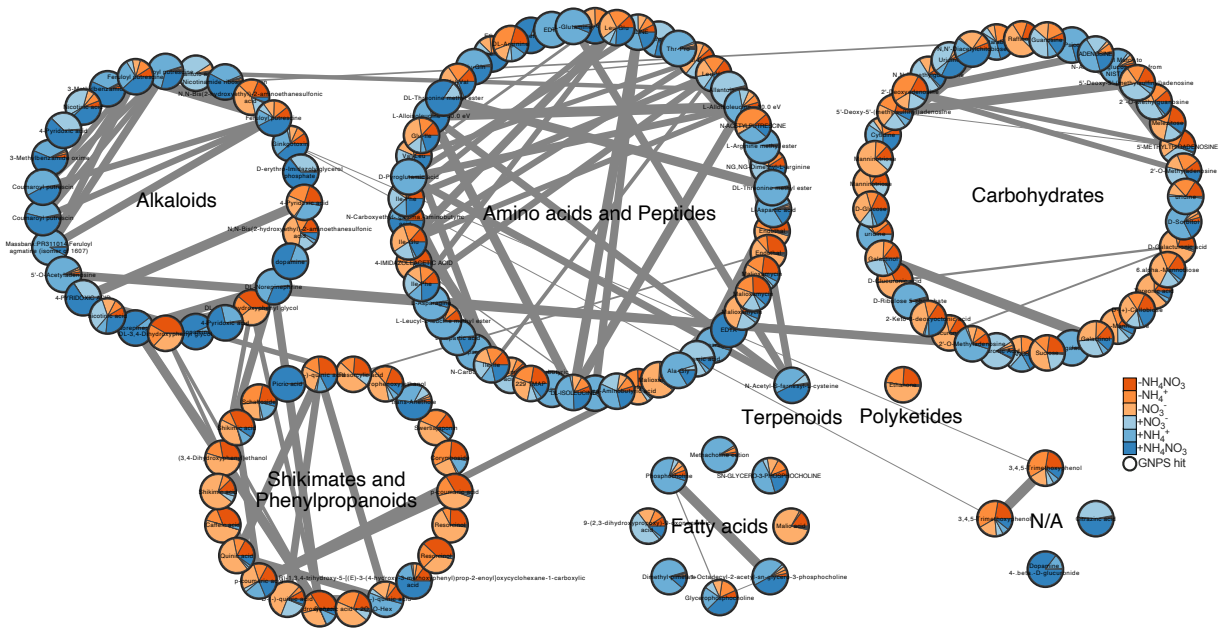


**Fig. S4: Sub-network of grouped annotated features.** The features were grouped into biosynthetic pathways by NPClassifier. Pie charts show relative mean peak heights for individual iN- treatments (shades of orange) and iN+ treatments (shades of blue). The black borders indicate GNPS annotation with MQScore > 0.7 (*n*=155). The network shows merged features from positive and negative polarities of polar metabolite analysis in root exudates of *B. distachyon* Bd21-3 at week 5.

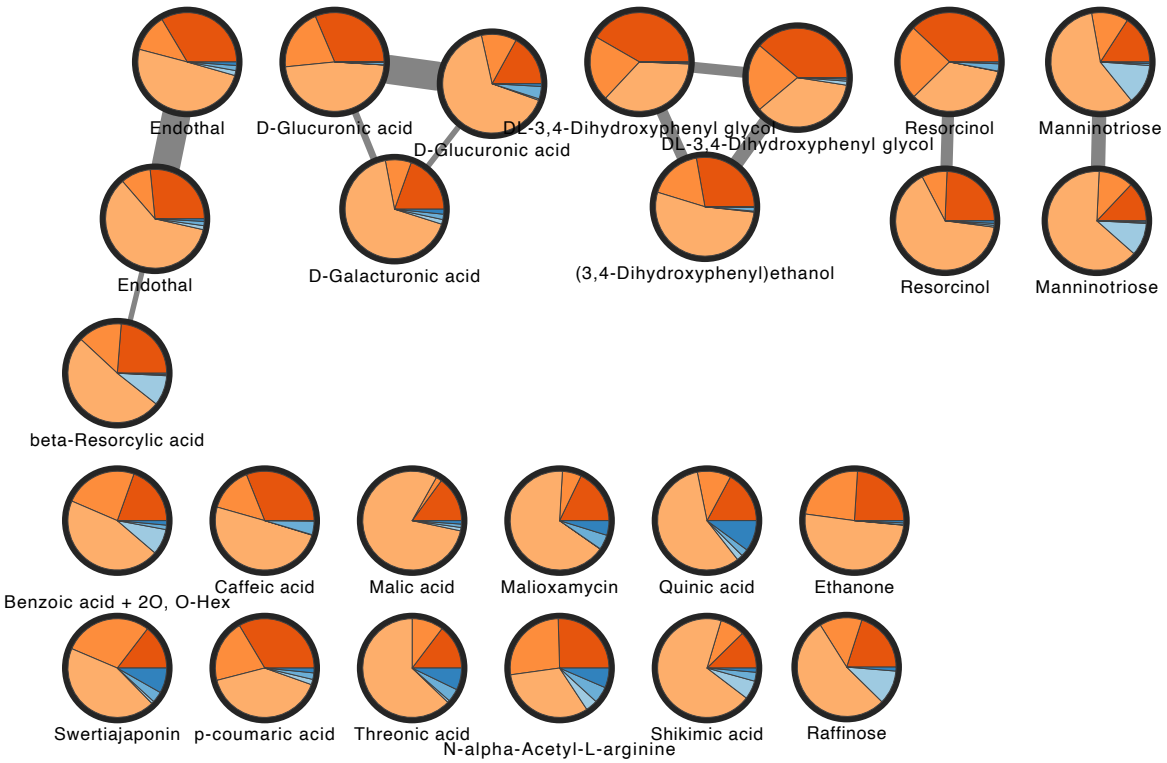


**Fig. S5. Sub-network of annotated features abundant in N-deficient root exudates.** The network shows features significantly increasing > 5-fold in iN- treatments (*n*=15) relative to the iN+ treatments (*n*=14) (t-test, *p*-value ≤ 0.5). Pie charts show relative mean peak heights for individual iN- treatments (shades of orange) and iN+ treatments (shades of blue). The black borders indicate GNPS annotation with MQScore > 0.7. The network shows merged features from positive and negative polarities of polar metabolite analysis in root exudates of *B. distachyon* Bd21-3 at week 5.

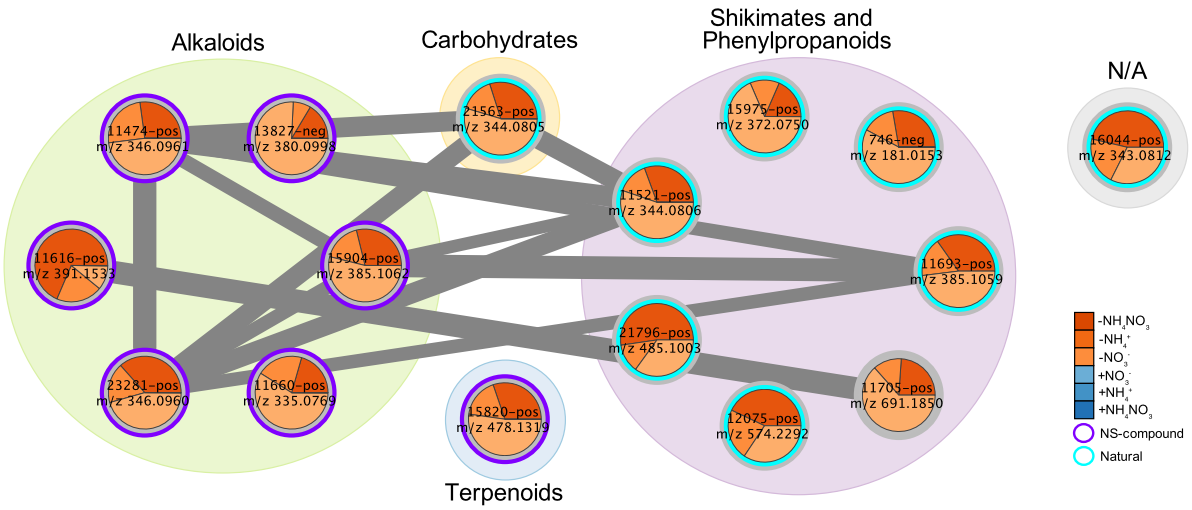


**Fig. S6: GNPS analog search (Analog_MQScore > 0.7) for subnetwork of top iN-upregulated features.** The features are grouped based on the analog hit classification (biosynthetic pathways by NPClassifier). Pie charts show average peak intensities of iN- treatments (orange gradient) and iN+ treatments (blue gradient). The cyan node border fill (Natural) primarily shows putative shikimate and phenylpropanoid analog compounds that are naturally found in plants (see references in Table S8) and the purple node borders (NS-compound) show putative alkaloid hits analogous to nitrogen-sulfur-compounds such as MES buffer that was in the growth media. Features without a hit to neither NS-compound nor natural plant compound were left in gray.

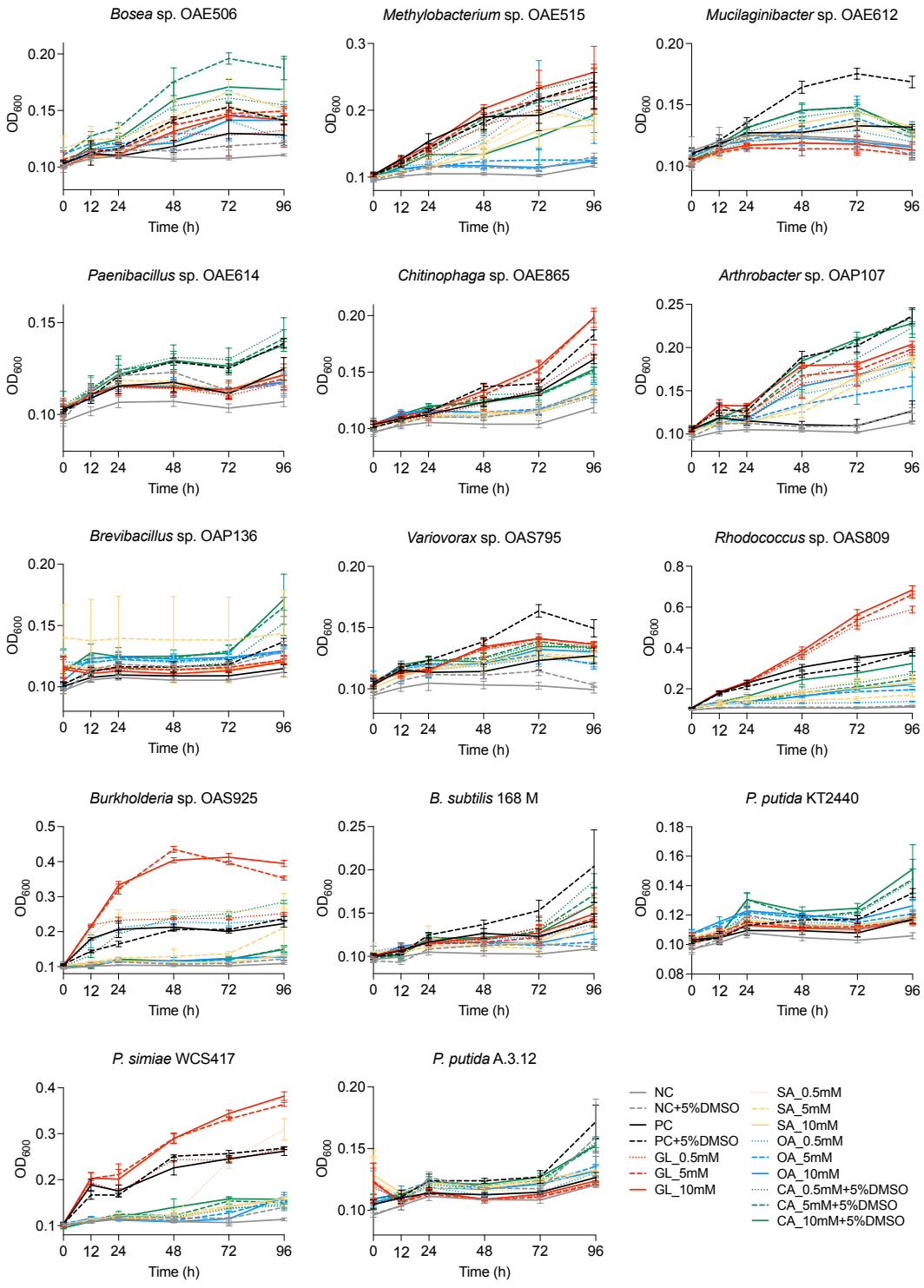


**Fig. S7: Growth curves shown as raw $OD_{600}$ for 14 soil bacterial isolates in different carbon sources and concentrations.** Bacteria grew in buffered 0.1x R2A medium supplemented with glucose (GL, red), shikimic acid (SA, yellow), oxalic acid (OA, blue), and *p*-coumaric acid (CA, green). Dotted, dashed, and full lines represent 0.5 mM, 5 mM, and 10 mM concentrations, respectively. Error bars show mean±SD ($n$=6). Negative controls (NC, gray) are uninoculated media, and positive controls (PC, black) are inoculated with the bacteria.

**Table S1: LCMS parameters and Metabolite extraction internal standards mix.**

**Table S2: Molecular network metadata node table.**

**Stable S3: All GNPS annotations for features with top annotation MQ score > 0.7.**

**Table S4: Negative and positive polarity alignment of filtered features.**

**Table S5: Statistically significant differences (ANOVA with post hoc Tukey HSD test, $p \leq 0.01$) between peak heights of features in plant exudate samples.**

**Table S6: Selection of differentially produced features and verification against library references.**

**Table S7: Using CANOPUS for determination of class and formula of the top iN-upregulated unknown features.**

**Table S8: GNPS analog search of the top iN- upregulated unknown features - top hits with analog cosine >0.7.**

**Table S9: Coefficient of variation (CV) summary of all data.**

**File S1: Positive mode MZMine2 parameters.**

**File S2: Negative mode MZMine2 parameters.**

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