Supplementary Information:

Metabolic footprint of epiphytic bacteria on *Arabidopsis thaliana* leaves

Florian Ryffel[a], Eric JN Helfrich[a], Patrick Kiefer[a], Lindsay Peyriga[b,c,d], Jean-Charles Portais[b,c,d], Jörn Piel[a], Julia A Vorholt[a,1]

Summary:

The supplementary information includes eight supplementary figures.

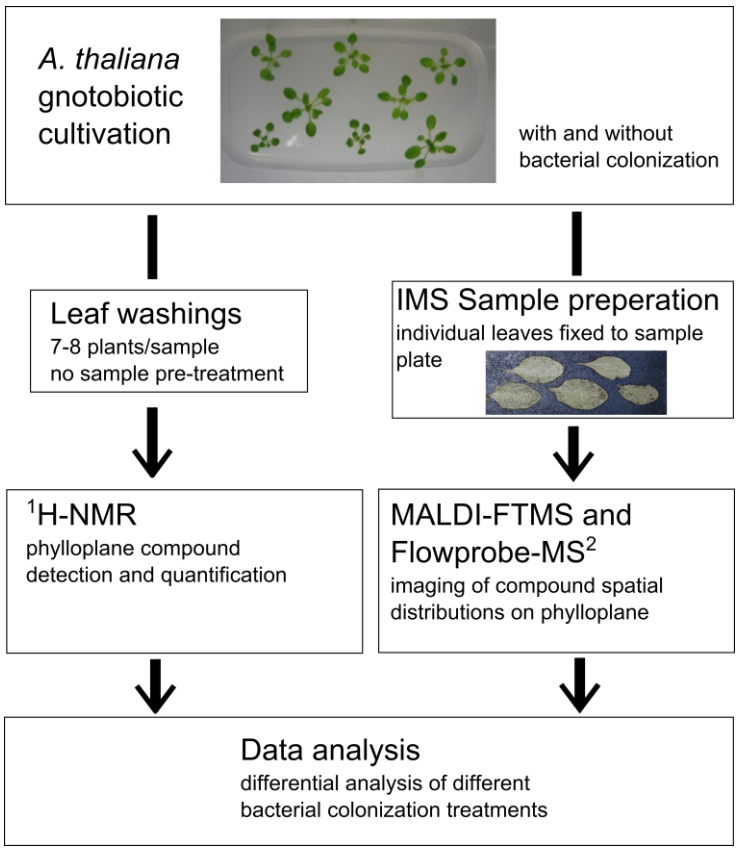

Figure S1: Schematic representation of the experimental rationale used for this study.

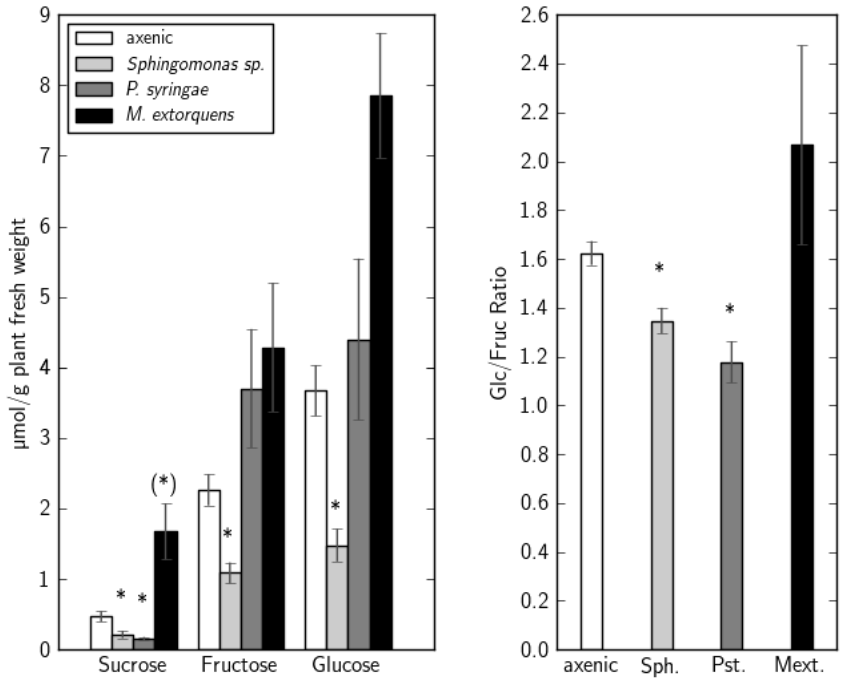

Figure S2: Potential Substrate Quantities determined by ^{1}H-NMR from a second independent experiment. (Analogous to Figure 1).

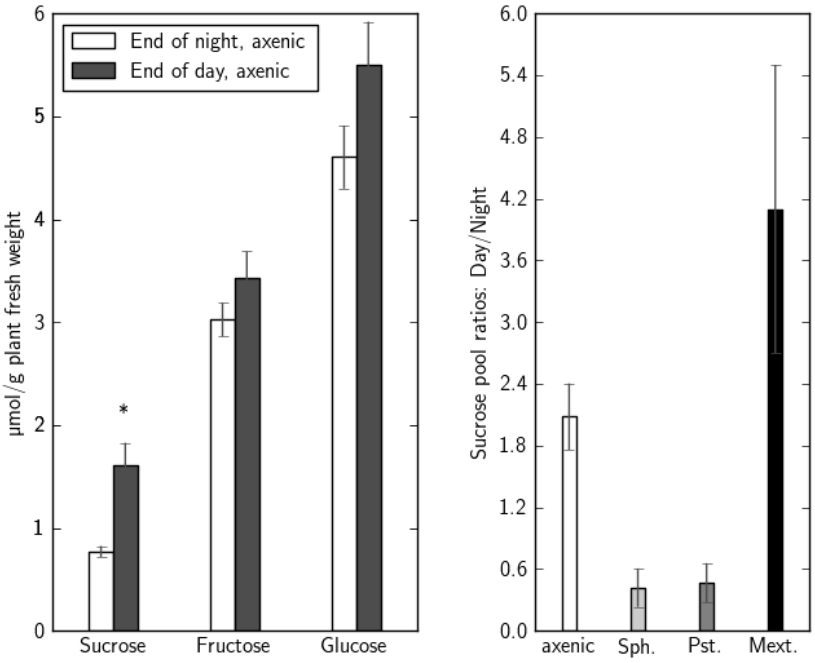

Figure S3: End of day vs. end of night changes in plant photo-assimilate concentrations on axenic plants (left). This accumulation effect is diminished on plants colonized by potentially glycolytic bacterial strains, shown as the ratio of sucrose levels measured at day- over night-time (right). The abbreviations used here are the same as in Figure 1.

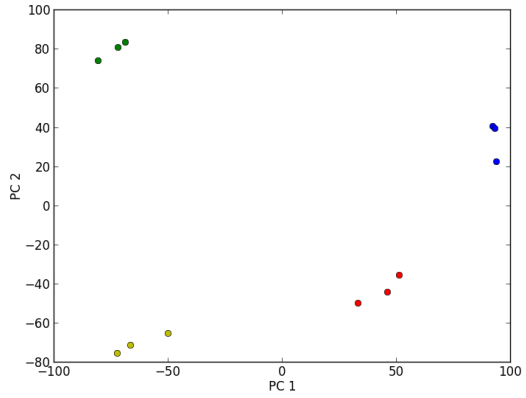

Figure S4: Scatter plot showing principal component analysis (PCA) of the MALDI-IMS data set used for pairwise distance analysis shown in Figure 2. Points have treatment-wise coloring as follows: Blue, axenic plants; red, *M. extroquens* colonization; yellow, *S. melonis* colonization; green, *P. syringae* infection. PCA gave separation of axenic and *M. extorquens* colonization from *S. melonis* colonization and *P. syringae* infection with the first principal component (PC1) and clear separation of all treatments together with PC2.

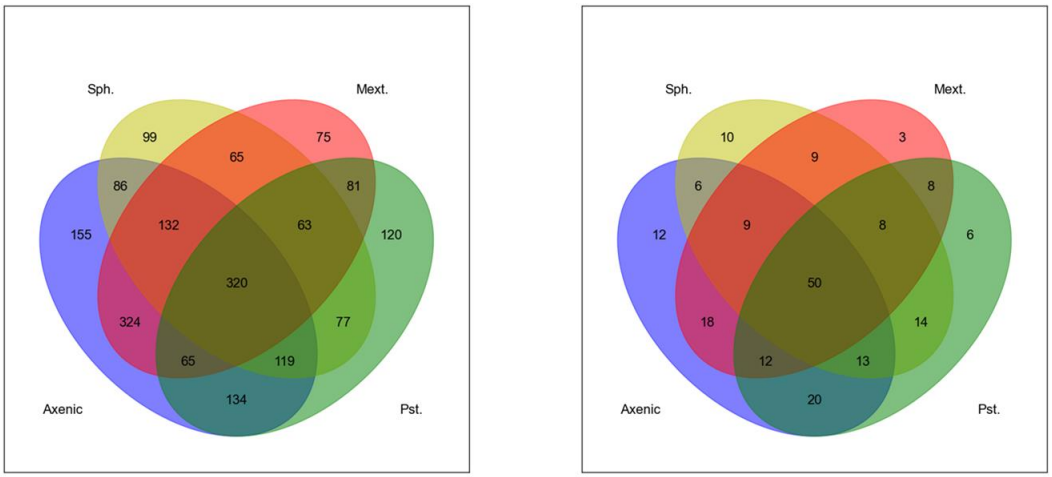

Figure S5: Venn diagram of all phylloplane MALDI-IMS features (left), and the ones putatively identified by exact molecular mass database queries (right), showing shared vs. colonization-specific phylloplane mass features and putative compounds, respectively. The source files for these diagrams can be downloaded from massive.ucsd.edu (see Material and methods).

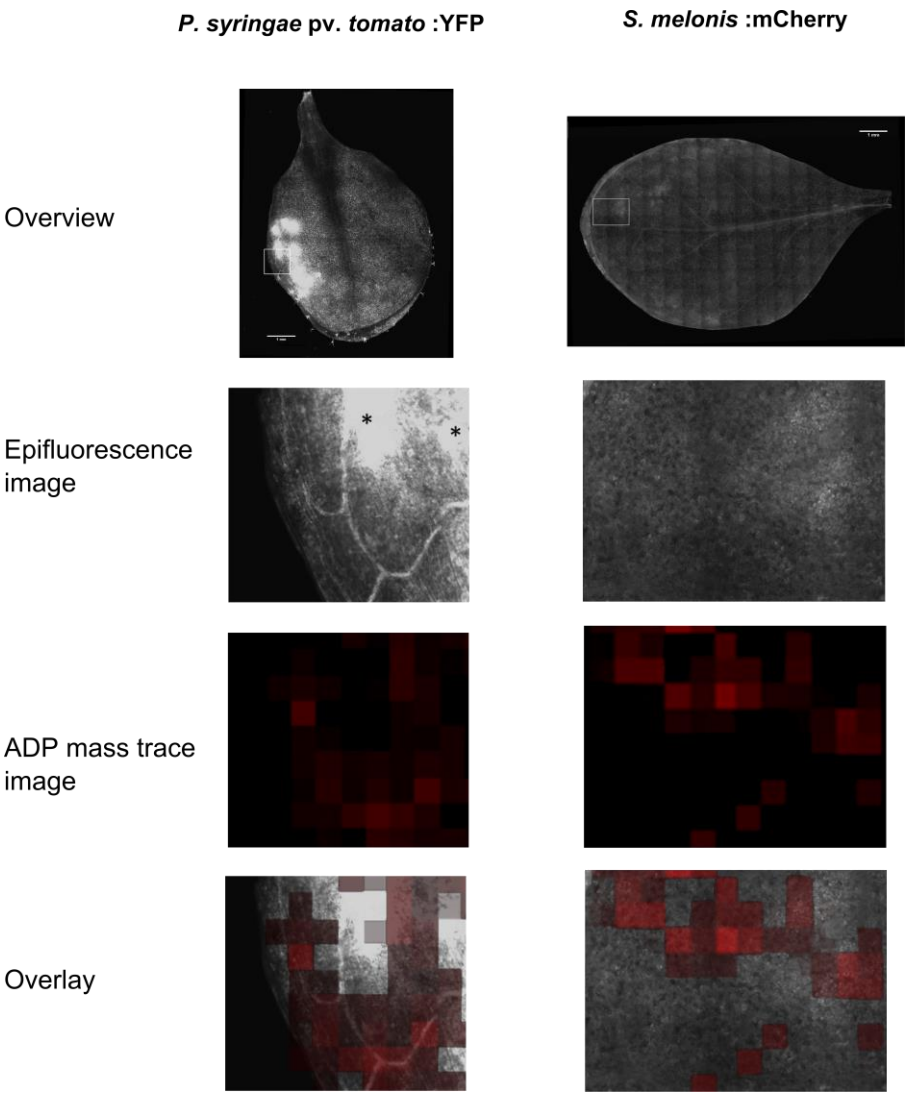

Figure S6: Overlay of reporter strain emitted fluorescence image obtained by whole-leaf epifluorescence microscopy and phylloplane MALDI-IMS generated nucleotide mass trace image (ADP $[M+ H]^+$ *m/z*: 428.0367). The regions framed in the whole-leaf epifluorescence experiments shown in the top row are magnified in the second row, with the corresponding regions from the MALDI-MS measurement of the same leaf in the third row. Each pixel (i.e., scan) of the MALDI-MS mass trace image is 100 µm wide, which serves as a size indicator for the microscopic image in the overlay shown in the bottom row. A monochromatic color scale was used to represent mass spectrometric intensity values (light red pixel encode high intensity measurements of ADP, zero intensity pixels were removed). Note the areas of epiphyte reporter strain colonization- predominantly in epidermal grooves or near leaf vascular tissues- which show co-localized ADP signals. The strain names plus the chromosomally integrated reporter genes used for the epifluorescence experiments shown, are given above the respective columns. Asterisk in the epifluorescence image panels indicate auto-fluorescent lesions elicited by *P. syringae* infection, which show no ADP Signal.

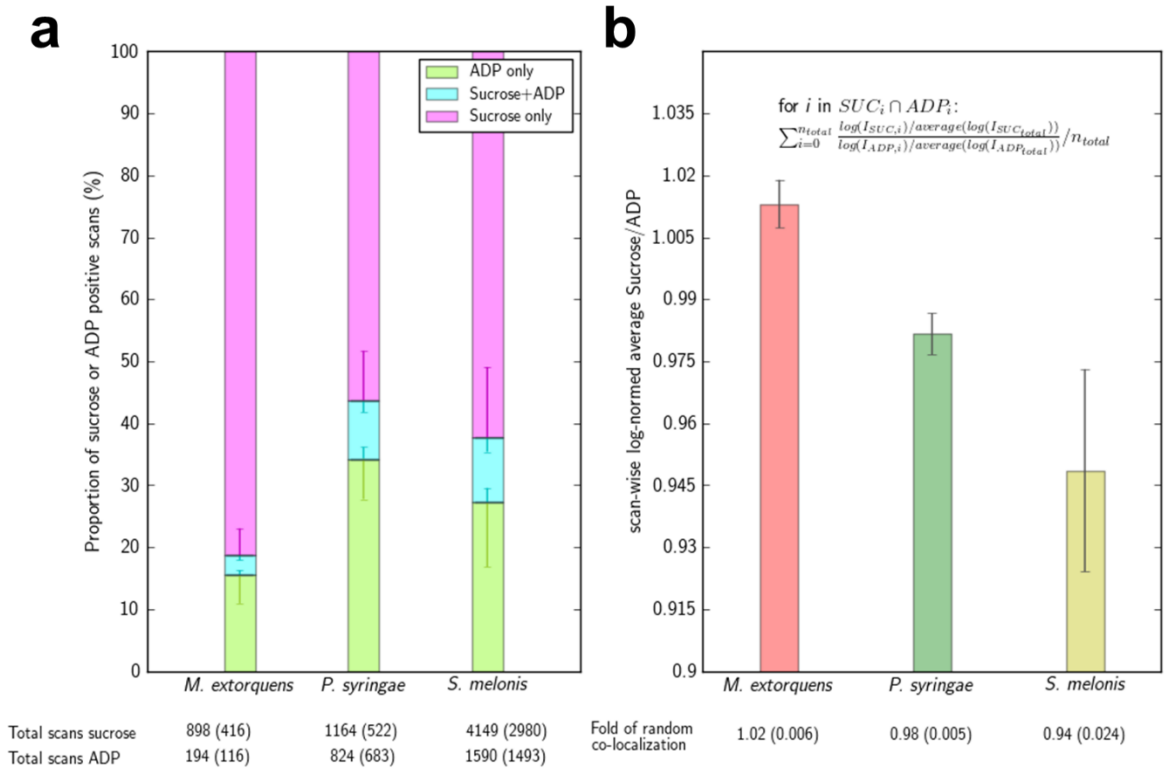

Figure S7: Quantifications of the MALDI-MS based bacterial colonization and substrate co-localization experiment. (**a**) Percental proportion plot of sucrose and/or ADP positive scans of a minimum of five MALDI-FTMS phylloplane imaging data sets per plant colonization treatment shows a variable number ADP- or sucrose-positive scans, yet a reproducible proportion of co-localizing ADP and sucrose (i.e., ADP and sucrose in the same scan) for each treatment. The table below the bar plot gives the respective qualitative scan counts with standard deviations in brackets. (**b**) Average-normed Sucrose over ADP ratios for the three epiphytic colonization treatments calculated as a sum of such ratios for all scans of a measurement individually ("scan-wise"). The simplified formula applied is given at the top. Because the mass spectrometry used here is not absolutely quantitative, we log-normalized all ion counts. The fold values of the measured ratios relative to test ratios of randomly joined ADP and sucrose intensities (corresponding to random co-localization) with the corresponding standard errors of the mean are listed in the table below.

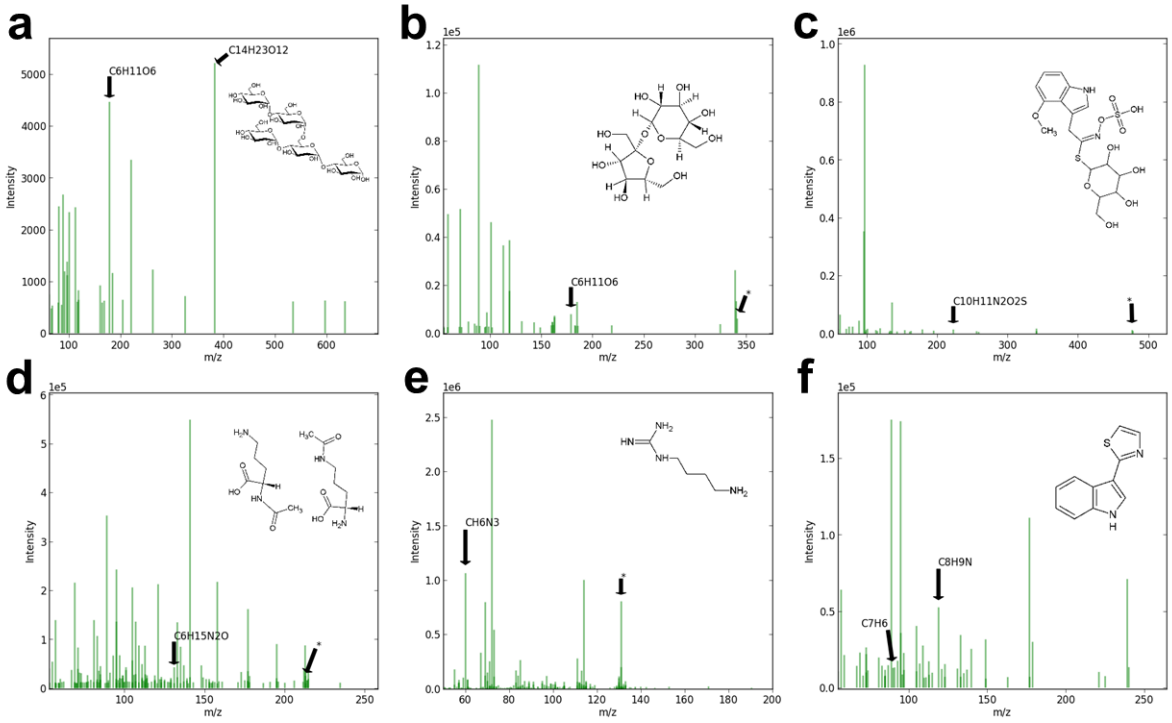

Figure S8: Exemplary fragment spectra of phylloplane compounds derived from Flowprobe-MS^2 measurements. The parent masses were identified as follows: (**a**) Pentaose (amylopectin); (**b**) sucrose; (**c**) methoxy-glucobrassicin; (**d**) N-acetyl-ornithine; (**e**) agmatine and (**f**) camalexin. The fragment spectra were matched to deposited reference spectra or, in the case of pentaose (amylopectin) and camalexin, predicted by substructure analysis using 'massbank'. Arrows indicate peaks with indicative substructure annotations, and asterisks denote parent mass signals. The respective chemical structures are given for illustration purposes. Note that N-acetyl-ornithine can be present in both the N-α- and N-γ-acetylated isomeric forms. The spectra files used for this figure can be downloaded under massive.ucsd.edu (see Material and methods).