## *New Phytologist* Supporting Information

**Article title:** Legacy effects of premature defoliation in response to an extreme drought event modulate phytochemical profiles with subtle consequences for leaf herbivory in European beech

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Supporting Information

**Table S1.** Location and characteristics of drought stressed and control plots in the study sites in the regions of Baselland and Zurich/Aargau.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Site | Region | Plot | Community | CHX coordinate | CHY coordinate | Elevation (m a.s.l.) |
| BL1 | Baselland | drought stressed | Muttenz | 615991 | 265491 | 275 |
| BL1 | Baselland | control | Muttenz | 615963 | 265499 | 276 |
| BL2 | Baselland | control | Birsfelden | 615373 | 265546 | 274 |
| BL2 | Baselland | drought stressed | Muttenz | 615482 | 265705 | 273 |
| BL3 | Baselland | control | Muttenz | 615753 | 265853 | 273 |
| BL3 | Baselland | drought stressed | Muttenz | 615760 | 265836 | 275 |
| BL4 | Baselland | drought stressed | Birsfelden | 615427 | 265500 | 273 |
| BL4 | Baselland | control | Muttenz | 615439 | 265738 | 274 |
| ZH1 | Zurich/Aargau | drought stressed | Affoltern am Albis | 677234 | 237919 | 579 |
| ZH1 | Zurich/Aargau | control | Affoltern am Albis | 677163 | 237872 | 565 |
| ZH2 | Zurich/Aargau | drought stressed | Affoltern am Albis | 676914 | 238547 | 580 |
| ZH2 | Zurich/Aargau | control | Affoltern am Albis | 677036 | 238133 | 576 |
| ZH3 | Zurich/Aargau | drought stressed | Affoltern am Albis | 677071 | 238091 | 576 |
| ZH3 | Zurich/Aargau | control | Hedingen | 676937 | 238514 | 583 |
| ZH4 | Zurich/Aargau | drought stressed | Affoltern am Albis | 677167 | 237987 | 573 |
| ZH4 | Zurich/Aargau | control | Affoltern am Albis | 677315 | 238000 | 589 |

**Table S2**

Most influential compounds in the partial least squares discriminant analysis models of drought stressed *F. sylvatica* in comparison to control trees one or two years after the drought. All compound levels (peak areas) differ significantly between drought stressed and control trees (p ≤ 0.05). Compounds are ordered by the change in compound levels of previously drought stressed trees relative to compound levels in control trees in descending order (see also Appendix 1: Fig. S3). Compound ID levels are categorized as follows: 1 = confirmed structure with reference standard, 2 = probable structure based on identification via spectral databases or manual interpretation of MS/MS data, 3 = tentative candidate at the class level, 4 = unequivocal molecular formula, 5 = only exact mass measurable.

|  |  |  |  |
| --- | --- | --- | --- |
| **Primary metabolites** |  |  |  |
| **First year after the drought** |  |  |  |
| **Compound** | **Mean control (C).**  **(peak area)** | **Mean drought stressed (D).**  **(peak area)** | **Change (%) D relative to C** |
| Coutaric-acid | 0.13±0.07 | 1.27±0.42 | 975 |
| Aspartic-acid | 0.35±0.09 | 0.60±0.08 | 170 |
| Maltose | 0.57±0.08 | 0.85±0.09 | 149 |
| D-Mannitol | 1.20±0.10 | 1.61±0.17 | 134 |
| Erythrono-1\_4-lactone | 0.84±0.03 | 1.01±0.06 | 120 |
| Beta-Sitosterol | 4.69±0.17 | 5.25±0.16 | 112 |
| Quinic-acid | 349.29±20.72 | 291.36±19.62 | 83 |
| Citric-acid | 7.50±0.60 | 6.01±0.45 | 80 |
| Galacturonic-acid | 0.07±0.02 | 0.01 ±0.01 | 15 |
| **Second year after the drought** |  |  |  |
| **Compound** | **Mean control (C).**  **(peak area)** | **Mean drought stressed (D).**  **(peak area)** | **Change (%) D relative to C** |
| Alpha-Linolenic-acid | 0.42±0.03 | 0.51±0.03 | 123 |
| Beta-Sitosterol | 0.42±0.02 | 0.36±0.02 | 86 |
| L-Glutamic-acid | 0.21±0.02 | 0.14±0.01 | 64 |
| L-Serine | 0.0585±0.008 | 0.035±0.004 | 61 |
| L-Phenylalanine | 0.015±0.003 | 0.008±0.001 | 56 |
| Oleanoic-acid | 0.022±0.004 | 0.012±0.002 | 54 |
| Mucic-acid | 0.009±0.002 | 0.004±0.001 | 50 |
| L-Valine | 0.014±0.003 | 0.007±0.001 | 46 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Specialized metabolites** |  |  |  |  |  |  |
| **First year after the drought** |  |  |  |  |  |  |
| **Compound** | **Mean control (C).**  **(peak area)** | **Mean drought stressed (D).**  **(peak area)** | **Change (%) D relative to C** | **Ret. time**  **(min)** | **Mass**  **(m/z)** | **ID level** |
| Unknown phenolic compound 1 | 2.32±1.01 | 7.79±2.22 | 235 | 3.54 | 563.1401 | 3 |
| Kaempferol 3-(2''-P-Coumaryl-Alpha-L-Arabinopyranoside) | 6.7±1.69 | 12.31±2.01 | 84 | 6.56 | 563.1189 | 2 |
| Quercetin-rhamnoside | 15.59±3.09 | 25.69±3.93 | 65 | 4.80 | 447.0927 | 2 |
| Unknown phenolic compound 5 | 25.79±2.89 | 36.89±3.88 | 43 | 3.82 | 371.0979 | 3 |
| 3-caffeoylquinic acid | 81.95±8.14 | 114.72±10.22 | 40 | 2.71 | 353.0874 | 2 |
| Quercetin-glucoside | 30.04±2.51 | 39.31±3.2 | 31 | 4.41 | 463.0877 | 2 |
| Kaempferol-glucoside | 85.11±6.51 | 107.82±7.84 | 27 | 4.74 | 447.0926 | 2 |
| Dimer of epicatechin | 80.76±5.7 | 100.11±7.2 | 24 | 3.13 | 577.1346 | 2 |
| Catechin | 187.27±10.19 | 224.01±10.83 | 20 | 3.28 | 289.0714 | 1 |
| Unknown compound 3 | 175.91±7.79 | 152.57±7.56 | -13 | 6.32 | 763.3168 | 4 |
| Astragalin-2''-O-(4-Methoxy-E-cinnamoyl), 6''-O-(4-hydroxy-E-cinnamoyl) | 53.28±3.47 | 42.83±2.5 | -20 | 7.94 | 753.1805 | 2 |
| Digalactosylmonoacylglycerol (18:3/0:0) | 65.58±4.8 | 50.51±3.16 | -23 | 8.11 | 721.3638 | 2 |
| Unknown compound 4 | 18.1±0.96 | 13.87±0.85 | -23 | 6.43 | 733.3059 | 4 |
| Monogalactosylmonacylglycerol (18:3/0:0) | 47.83±3.95 | 36.52±3.06 | -24 | 8.66 | 559.3115 | 2 |
| Trihydroxylated fatty acid | 25.95±1.88 | 19.6±1.43 | -24 | 6.48 | 327.2173 | 2 |
| Monogalactosylmonacylglycerol (18:3/0:0) | 32.04±3.22 | 23.72±2.55 | -26 | 8.78 | 559.3116 | 2 |
| Isomer of astragalin-2''-O-(4-Methoxy-E-cinnamoyl), 6''-O-(4-hydroxy-E-cinnamoyl) | 25.77±1.96 | 17.79±1.13 | -31 | 8.04 | 753.1805 | 2 |
| Fatty acid (18:3) glucose | 20.73±2.19 | 14.16±1.46 | -32 | 9.01 | 485.2751 | 2 |
| Digalactosylmonoacylglycerol (18:3/0:0) | 21.59±2.42 | 14.72±1.56 | -32 | 8.22 | 721.3638 | 2 |
| Unknown compound 2 | 16.94±2.18 | 11.13±1.27 | -34 | 4.85 | 427.1603 | 4 |
| Fatty acid derivative | 94.73±9.17 | 61.97±9.97 | -35 | 6.86 | 227.1284 | 3 |
| Oxygentated fatty acid | 10.51±1.36 | 6.38±0.82 | -39 | 9.43 | 293.2114 | 3 |
| Phosphatidylglycerol (16:0/0:0) | 8.63±0.75 | 4.92±0.76 | -43 | 7.77 | 483.2720 | 2 |
| Gallocatechin-catechin | 12.18±2.15 | 6.65±0.88 | -45 | 3.09 | 593.1292 | 2 |
| Unknown compound 5 | 22.9±2.58 | 12.28±2.06 | -46 | 9.87 | 723.4307 | 5 |
| Unknown compound 6 | 15.42±1.89 | 7.34±1.47 | -52 | 9.26 | 885.4827 | 5 |
| 11-Hydroxy-9,10-Dihydrojasmonic Acid 11-Beta-D-Glucoside | 14.35±2.73 | 5.74±0.71 | -60 | 5.60 | 389.1812 | 2 |
| **Second year after the drought** |  |  |  |  |  |  |
| **Compound** | **Mean control (C).**  **(peak area)** | **Mean drought stressed (D).**  **(peak area)** | **Change (%) D relative to C** | **Ret. time**  **(min)** | **Mass**  **(m/z)** | **ID level** |
| Unknown phenolic compound 1 | 1.45±0.55 | 8.19±2.06 | 465 | 3.54 | 563.1401 | 3 |
| Dimer of catechin | 22.67±1.77 | 41.08±4.29 | 81 | 2.94 | 577.1344 | 2 |
| Dimer of epicatechin | 73.07±4.68 | 113.41±6.03 | 55 | 3.13 | 577.1346 | 2 |
| Epicatechin-epiafzelechin | 28.32±3.46 | 43.61±4.18 | 54 | 3.57 | 561.1397 | 2 |
| Isomer of daglesioside III | 18.06±1.44 | 27.63±2.15 | 53 | 7.67 | 709.1547 | 2 |
| Tannin | 8.88±0.69 | 13.49±0.77 | 52 | 3.69 | 577.1343 | 3 |
| Daglesioside III | 47.36±3.7 | 69.27±4.78 | 46 | 7.57 | 709.1547 | 2 |
| Proanthocyanidin C1 | 47.57±2.92 | 64.63±3.04 | 36 | 3.24 | 865.1968 | 2 |
| Monogalactosylmonacylglycerol (18:3/0:0) | 17.85±1.56 | 23.36±1.61 | 31 | 8.78 | 559.3116 | 2 |
| Tannin | 21.21±1.03 | 26.88±1.04 | 27 | 3.89 | 577.1341 | 3 |
| Oxygentated fatty acid | 14.49±1.62 | 17.82±2.02 | 23 | 8.73 | 293.2116 | 3 |
| Catechin | 222.66±8.24 | 268.24±8.43 | 20 | 3.28 | 289.0714 | 1 |
| 5-caffeoylquinic acid | 75.68±4.69 | 58.12±5.5 | -23 | 2.76 | 707.1816 | 2 |
| Unknown phenolic compound 2 | 33.34±2.24 | 25.53±2.26 | -23 | 3.60 | 193.0504 | 3 |
| Astragalin-2'',4''-Bis-O-(4-hydroxycinnamoyl) | 23.37±1.68 | 17.51±1.68 | -25 | 4.20 | 739.1653 | 2 |
| Unknown phenolic compound 3 | 22.59±1.29 | 16.33±1.37 | -28 | 4.44 | 451.1028 | 3 |
| Astragalin-2''-O-(4-Methoxy-E-cinnamoyl), 6''-O-(4-hydroxy-E-cinnamoyl) | 45.48±2.39 | 32.72±1.73 | -28 | 7.94 | 753.1805 | 2 |
| 2/4-O-trans-caffeoyl-threonic acid | 121.28±11.55 | 81.36±8.69 | -33 | 2.86 | 595.1301 | 2 |
| Unknown phenolic compound 4 | 12.03±0.8 | 7.83±0.74 | -35 | 2.42 | 511.1089 | 3 |
| 1-caffeoylquinic acid | 87.02±5.03 | 55.36±5.3 | -36 | 3.17 | 353.0874 | 2 |
| Isomer of astragalin-2''-O-(4-Methoxy-E-cinnamoyl), 6''-O-(4-hydroxy-E-cinnamoyl) | 19.07±1.18 | 11.75±0.69 | -38 | 8.04 | 753.1805 | 2 |
| Astragalin- 6''-O-(4-Hydroxy-3-methoxycinnamoyl), 3''-O-(4-hydroxycinnamoyl) | 16.05±1.13 | 9.36±0.88 | -42 | 7.72 | 769.1753 | 2 |
| Unknown compound 1 | 24.48±2.4 | 13.96±1.6 | -43 | 3.02 | 305.0663 | 4 |
| Gallocatechin-catechin | 34.29±3.63 | 18.45±2 | -46 | 3.09 | 593.1292 | 2 |
| Glycosylated phenolic compound | 7.68±0.7 | 3.92±0.42 | -49 | 3.99 | 479.0825 | 3 |
| Unknown compound 2 | 16.43±2.36 | 7.43±0.59 | -55 | 4.85 | 427.1603 | 4 |

**Methods S1-Analysis of primary and specialized metabolome**

For the analysis of primary metabolites, polar low-molecular-weight metabolites were extracted and derivatized according to a modified method from Erxleben et al. (2012). For each sample, approximately 10-20 mg of homogenized leaf powder was weighed into a pre-frozen 2 mL round-bottom Eppendorf tube and for each mg of sample, 50 µL of extraction medium cooled to -80 °C was added (90 vol.% Methanol, 10 vol.% Milli-Q water, and 1 µg/mL Phenyl-ß-D-glucopyranoside as an internal standard for quality control and internal normalization). 50 μL aliquots of the supernatant were dried under vacuum in 1.5 ml microfuge tubes after centrifugation. Dried extracts were methoximated by adding 20 μL of a 20 mg mL-1 solution of methoxyamine hydrochloride in anhydrous pyridine and incubated at 28 °C for 90 min with shaking at 1400 RPM. For trimethylsilylation, 70 μL of N-methyl-N- (trimethylsilyl) trifluoroacetamide (MSTFA; Sigma) was transferred to each tube and incubated at 37 °C for 30 min with 1400 RPM shaking. An n-alkane retention index calibration mixture was created by adding 10 µL of n-alkane mix [n-Alkane- Mix 16 (C10-C40 even), Cat.-No.:14640, concentration: 50 µg ml-1 in n-hexane; Neochema, Germany] to 40 µL of n-hexane. After short vortex, reaction mixtures were centrifuged at 14,000 g, 20 °C for 2 min and then 30 μL of supernatant were transferred to amber GC-MS vials with low volume inserts and screw top seals (Agilent Technologies, Palo Alto, CA, USA) for GC-MS analysis. Additionally, 30 µL of each sample was pooled into one quality control sample which was separated into 30 µL aliquots, which were used for column equilibration as well as quality controls. Derivatized metabolite samples were analyzed on an Agilent GC/MSD system comprised of an Agilent GC 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) fitted with a GERSTEL MultiPurpose Sampler (MPS2-XL, GERSTEL, Mülheim, Germany) and 5975C Inert XL EI/CI MSD quadrupole MS detector (Agilent Technologies, Palo Alto, CA, USA). The capillary column used was HP-5MS 5% Phenyl Methyl Silox, length: 60 m, diameter: 0.25 mm, film thickness: 0.25 µm (Agilent Technologies, Palo Alto, CA, USA). GC-MS run conditions were set up according to Erxleben et al. (2012) with some slight adaptations. The GC column oven was held at the initial temperature of 80 °C for 3 min and then to 325 °C at 5 °C min-1 before being held at 325 °C for 14 min. Total run time was 66 min. Inlet temperature was 230 °C, transfer line temperature was 280 °C and MS source temperature was 230 °C. Samples were injected in a randomized order with n-hexane blank as the first and n-alkane standard as the second injection. These samples were followed by 7 consecutive injections of separate aliquots of the pooled sample. Then, after each 6th sample injection a pooled sample was injected as a quality control. The raw data files were processed with the free AMDIS (automated mass spectral deconvolution and identification system, version 2.69) software supplied by NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA). After peak filtering and deconvolution, Kováts’ retention indices were calculated from the n-alkane standard residence times and applied to all samples. A compound matrix was generated in the SpectConnect software, where spectral mass features were matched and grouped. The compounds were then identified by comparison to the NIST spectral library based on retention indices (RI) and mass spectra similarities of the fragments. For identification, the deviation of the retention index had to be below 5 % and spectral similarity above 75 %. The relative quantification of the identified metabolites peaks was achieved by calculating the area of the ion signal and normalizing to the internal standard. Metabolites detected not in all replicates were discarded

For the untargeted analysis of specialized metabolites we extracted 30 mg of leaf powder in Eppendorf tubes containing 1500 μl of methanol:water:formic acid, 80:19.5:0.5, v/v . We added 8-10 glass beads to each tube. The mixture was shaken in a ball mill for 3 min., centrifuged (3 min., 13′000×g) and the supernatant was collected. Samples were analyzed by ultra-high-performance liquid chromatography - quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS) using an Acquity UPLC™ coupled to a Synapt G2 MS (Waters, Milford, USA) as described in (Eisenring et al. 2018) with minor modifications. The system was controlled by Masslynx v.4.1. An Acquity UPLC™ HSS T3 column (100 mm × 2.1 mm, 1.8 μm) maintained at 25 °C was used at a flow rate of 0.5 ml/min. Mobile phases consisted of H2O + 0.05% formic acid (solvent A) and acetonitrile + 0.05% formic acid (solvent B). A segmented gradient from 0 to 40% B in 6.0 min and then from 40-100% in 4 min was applied. The injection volume was 2.0 μl. MS detection was performed in negative electrospray ionization over a mass range of 85–1200 Da. The MSE mode, in which data are acquired alternatively at low and high collision energies, was employed. Leucine-enkephalin was used as an internal calibrant to ensure accurate mass measurements. Quality control samples were prepared by pooling aliquots of all samples (control and treated). Peak picking was performed in Markerlynx XS. The obtained list of markers (signals that can either be specific compounds or analytical artefacts), characterized by their retention time and mass-to-charge ratio, was normalized to unit norm [i.e. to the total integrated area per sample (Glauser et al. 2013)]) .

**Methods S2- Herbivory assessments**

We distinguished between mines of the weevil *Orchestes fagi* and those of the larvae of the lepidopteran genera *Phyllonorycter* (all barcoded individuals were *P. maestingella*) and *Stigmella* in the assessment but pooled them for the analyses. In addition, we noted the presence of galls induced by gall midges (i.e., *Mikiola fagi*, *Hartigiola annulipes*, *Phegomyia fagicola*) and gall mites (i.e., *Aceria nervisequa* (leaf upperside), *Aceria nervisequa faginea* (leaf underside), and *Acalitus stenaspis* (rolled leaf edge), and other more sessile insect herbivores (i.e., *Phyllaphis fagi* and spuners such as *Carcina quercana*) for each leaf. We pooled all gall midges (gall midge total) and all gall mites (gall mites total) for further analyses, and of the other more sessile herbivores, we included only the woolly beech aphid *Phyllaphis fagi,* as this was the only herbivore that was sufficiently abundant to be analyzed. Doubtful cases of *P. fagi* were stored in the fridge and subsequently either verified or sent for molecular identification of individuals by barcoding.

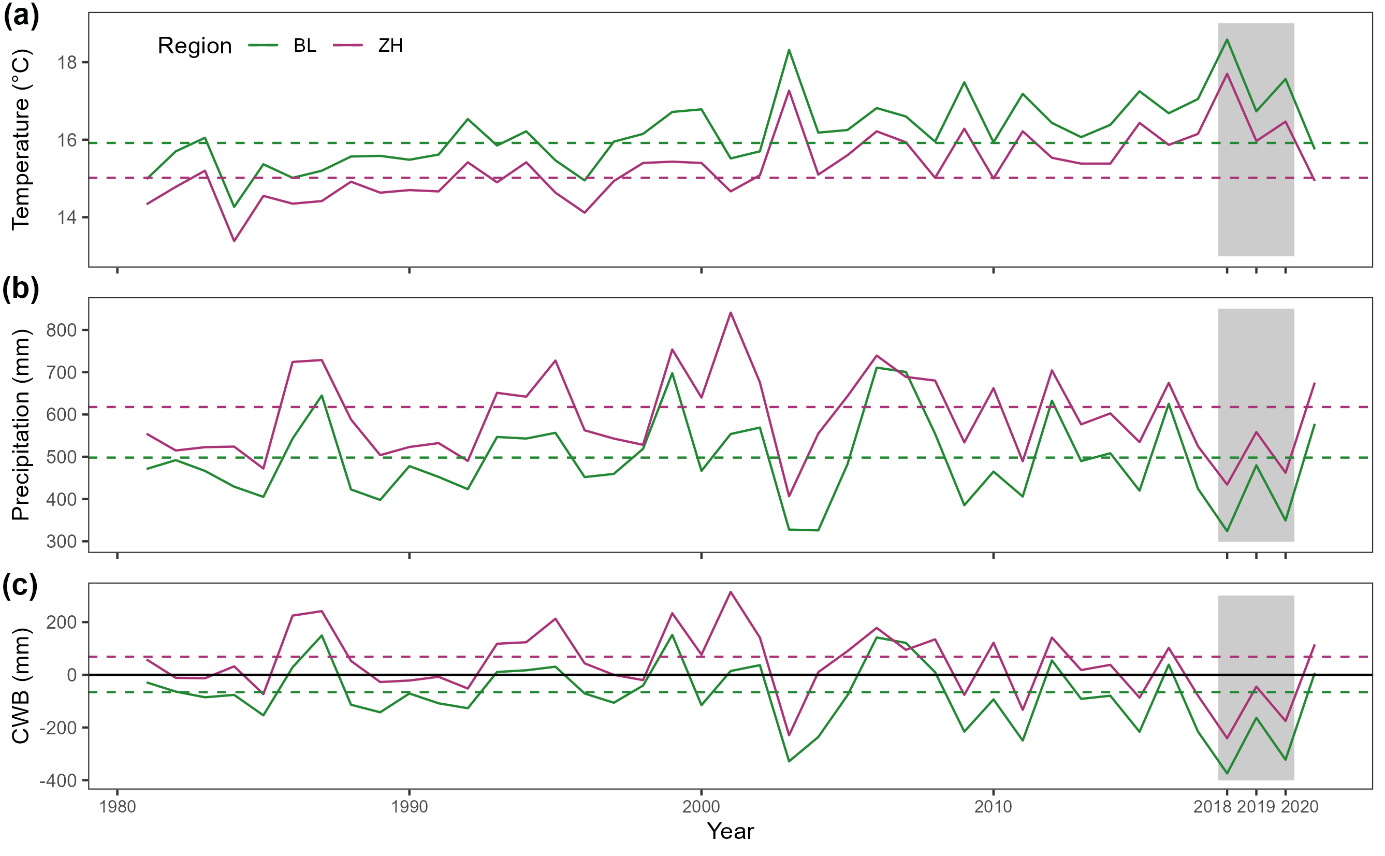
**Methods S3-Statistical analysis in R**

LMs were fitted using Base-R, LMMs and GLMMs with binomial probability distribution were fitted using the “lme4” package (1.1-27.1, Bates et al. (2015)). GLMMs with beta distribution were fitted using the package “glmmTMB” (1.1.2.3, Brooks et al. (2017)). Model estimates were plotted using “sjPlot”(2.8.14 Lüdecke (2023)). PCA and PLS-DA were conducted using the “MetaboAnalystR” package (3.2.0, (Pang et al. 2020)). Prior to analyses non-informative markers (i.e. near-constant values throughout treatment conditions) were detected based on the interqantile range and removed. Data were then mean-centered, and Pareto scaled. PLSR was achieved with the mixOmics package (version 6.22.0, (Rohart et al. 2017)). Piecewise SEMs were constructed using the “piecewiseSEM” package (version 2.3.0, (Lefcheck 2016)). Model selection was achieved with the “MuMIn” package (version 1.47.5, (Barton 2023). Model assumptions were tested with the DHARMa package (version 0.4.6, (Hartig 2022)). Data was transformed if needed. Multicollinearity issues were explored with the performance package (version 0.10.2, (Lüdecke et al. 2021)) and co-variates were removed if necessary. Observation level random effects were used to account for overdispersion (Harrison 2014). The plotted model means (± SE) were predicted with the package “emmeans”(1.8.5 Lenth (2023)).

**Methods S4-Piecewise structural equation models (SEMs)**

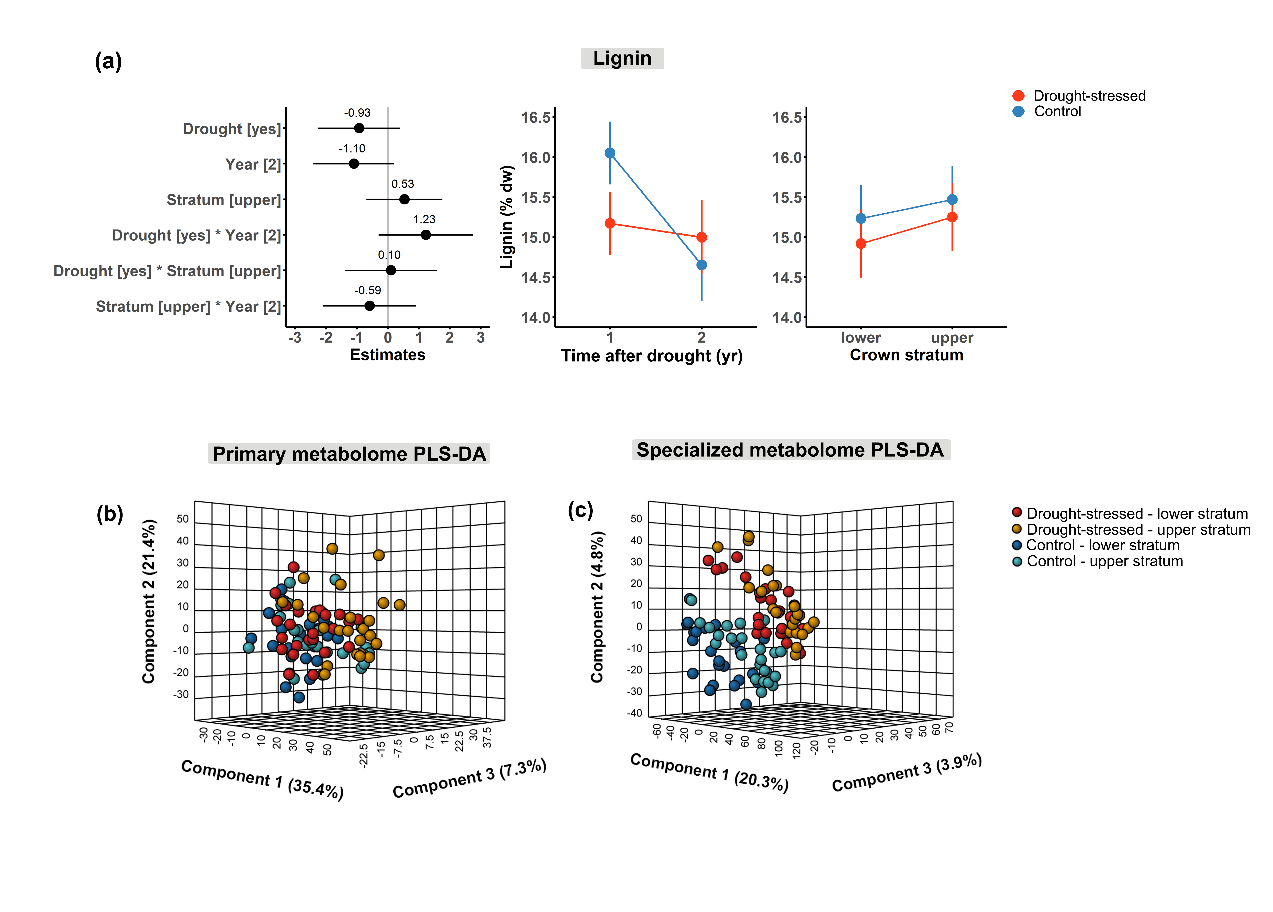
We reduced the number of explanatory variables for each SEM on leaf damage probabilities in two steps. First, models were fitted with all combinations including all possible 2-way interactions among drought stress, crown stratum and year since the drought event. All continuous variables were standardized (scaled to zero mean and unit variance). Models were then ranked according to Akaikes information criterion corrected for small sample sizes (AICc) and models selected based on two criteria; (i) models with a Δ AICc (from the lowest AICc obtained) value of ≤6 were kept (Richards 2005), (ii) variables were selected that had a relative importance of ≥0.5. The selected variables were then included in the final leaf damage model that was used in the SEM. It is currently not possible to analyze GLMMs with beta distribution in a piecewise SEM framework. Therefore, all beta distribution models were substituted by LMMs in the SEM analysis. A comparison among all beta distribution models and LMMs revealed that both model types produced very similar model summaries.

**Figure S1**

Mean growing season (April to September) air temperature (a), growing season precipitation sum (b), and climatic water balance (CWB), i.e. precipitation minus potential evapotranspiration (c) for the period 1981–2021 for two sampling regions Baselland (BL, green line) and Zurich (ZH, purple line) in Switzerland. Horizontal dashed lines indicate the corresponding value for the climate norm period 1981–2010. and grey shading is the observation period of this study (2018–2020). Climate data derived from © MeteoSwiss

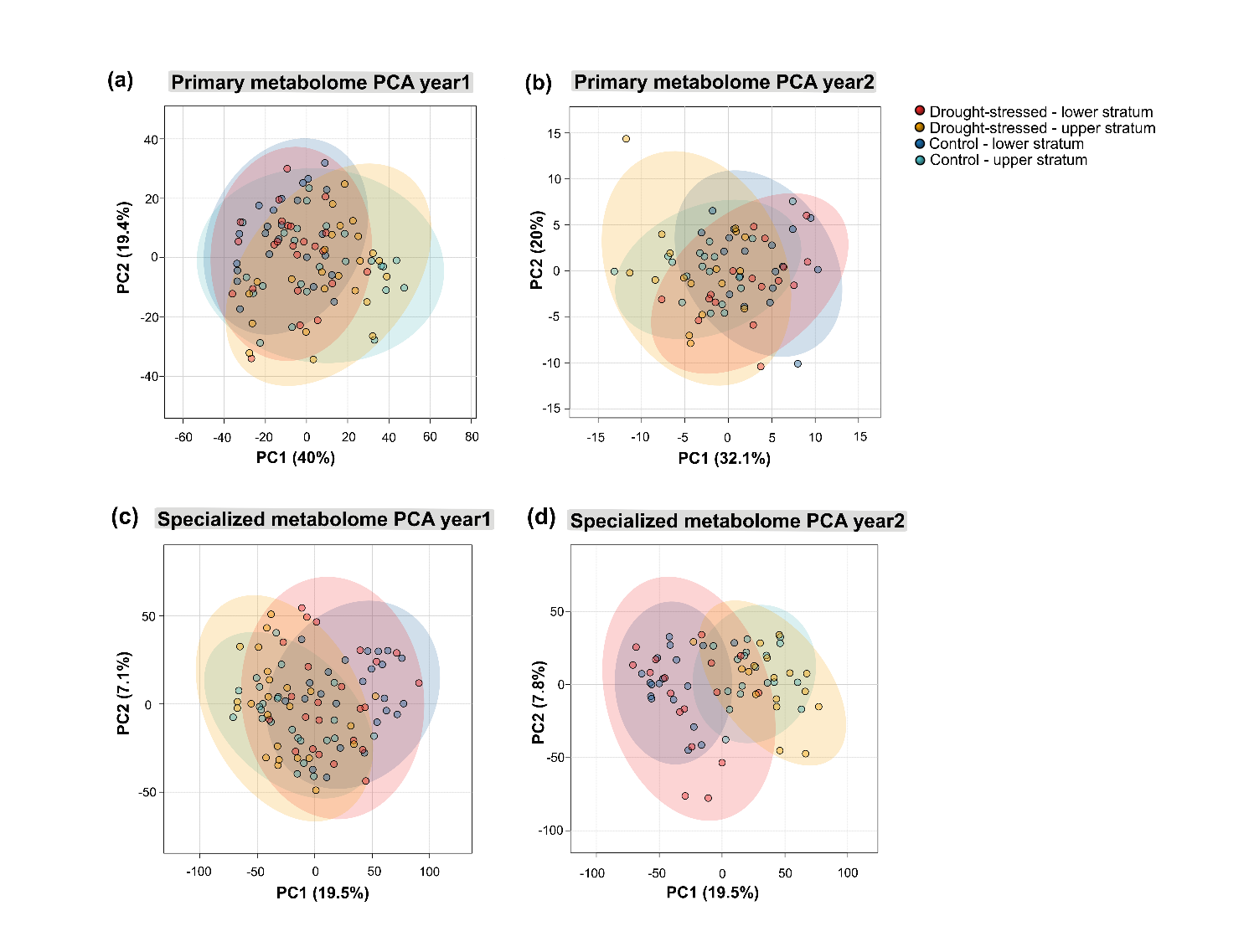
**Figure S2**

Estimate and line plots (predicted mean ± 1 SE) in (a) illustrate the effects of previous drought stress, region, crown stratum and their two-way interactions on leaf lignin concentrations of *Fagus sylvatica*. P-values were calculated via linear mixed effects models. P-values in the estimate plots: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001. dw=dry weight. Score plots of a partial least squares discriminant analysis of (b) the primary *F. sylvatica* metabolome and (c) the secondary metabolome of leaves from the upper and lower crown stratum of previously drought stressed and control trees for the first year after the drought. Each dot represents a leaf sample of 30 pooled leaves. Results are shown for leaves collected one year after the drought event. Score plots for the second year after the drought are shown in Fig. 2.



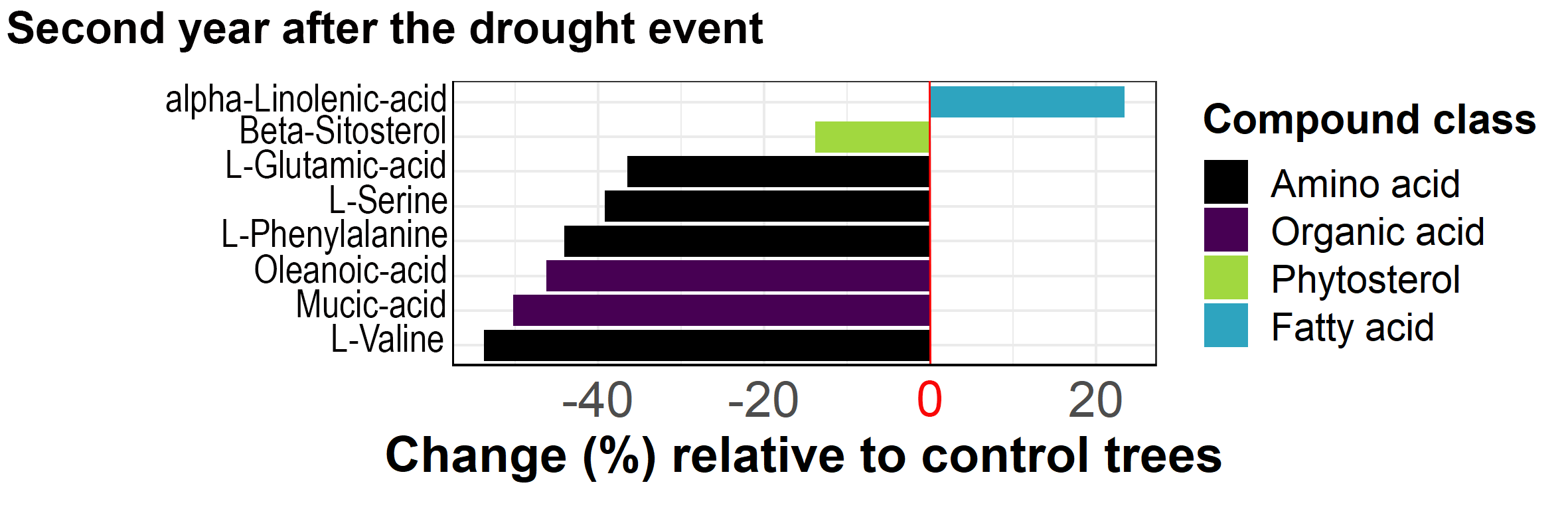
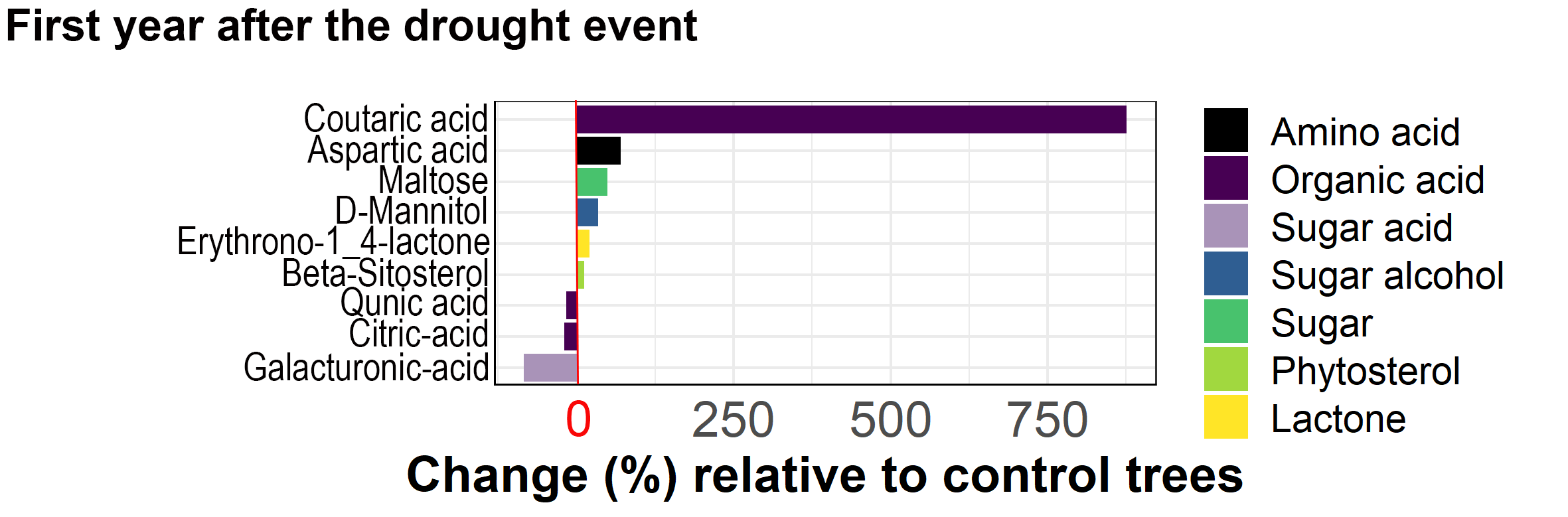
**Figure S3**

Principal component analysis score plot of (a) the primary *F. sylvatica* metabolome in the first and (b) the second year after the drought event, (c) the specialized metabolome in the first and (b) the second year after the drought event. Leaves from the upper and lower crown stratum of previously drought stressed and control trees were analyzed separately. Each dot represents a leaf sample of 30 pooled leaves.



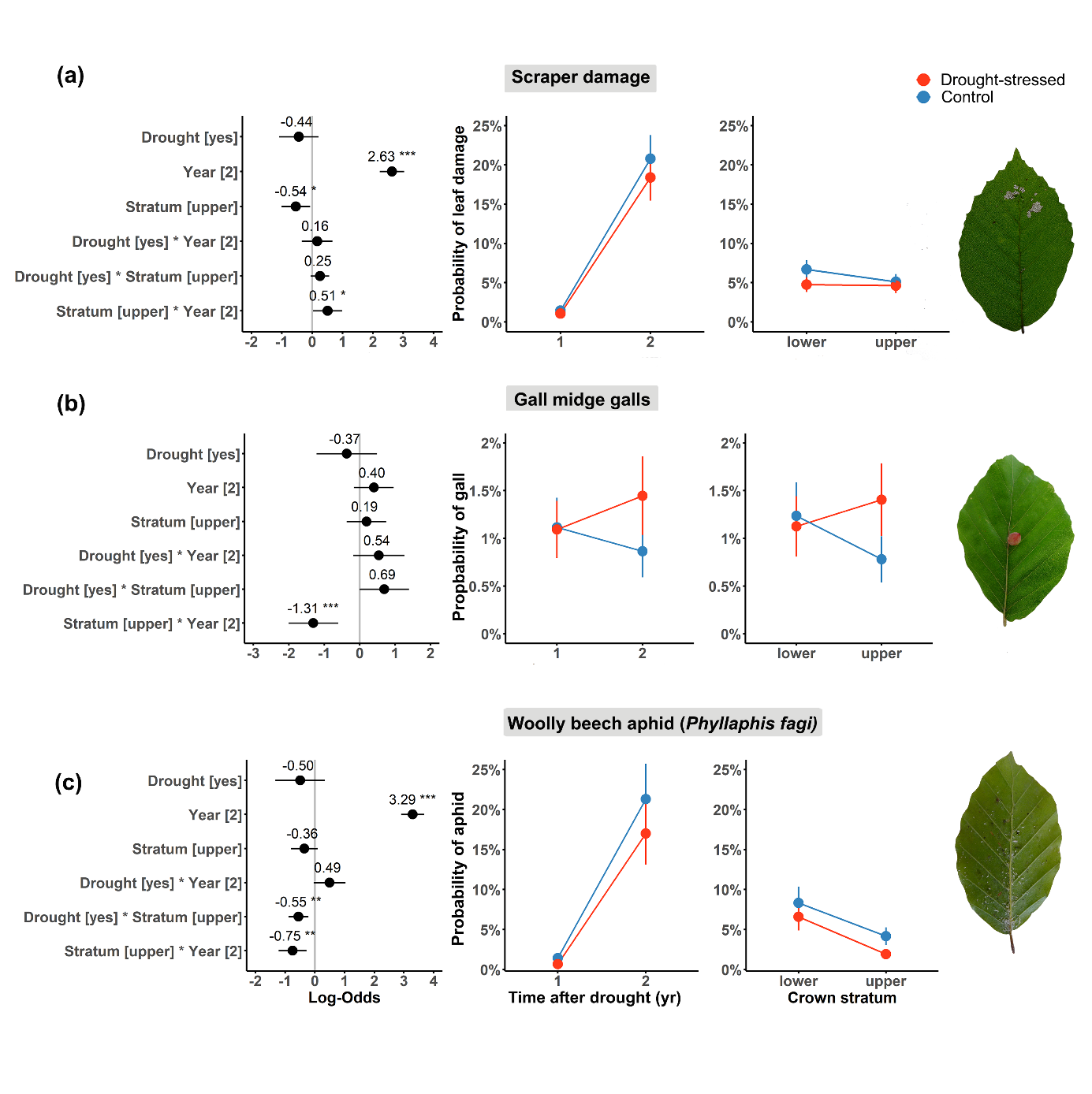
**Figure S4**

Change in *F. sylvatica* compound levels (peak areas) of the most influential primary metabolites in PLS-DA models of drought stressed trees in comparison to control trees in the first of the second year after the drought event. All compound levels differed significantly between drought stressed and control trees (p ≤ 0.05). A detailed list of all compounds can be found in Table S2

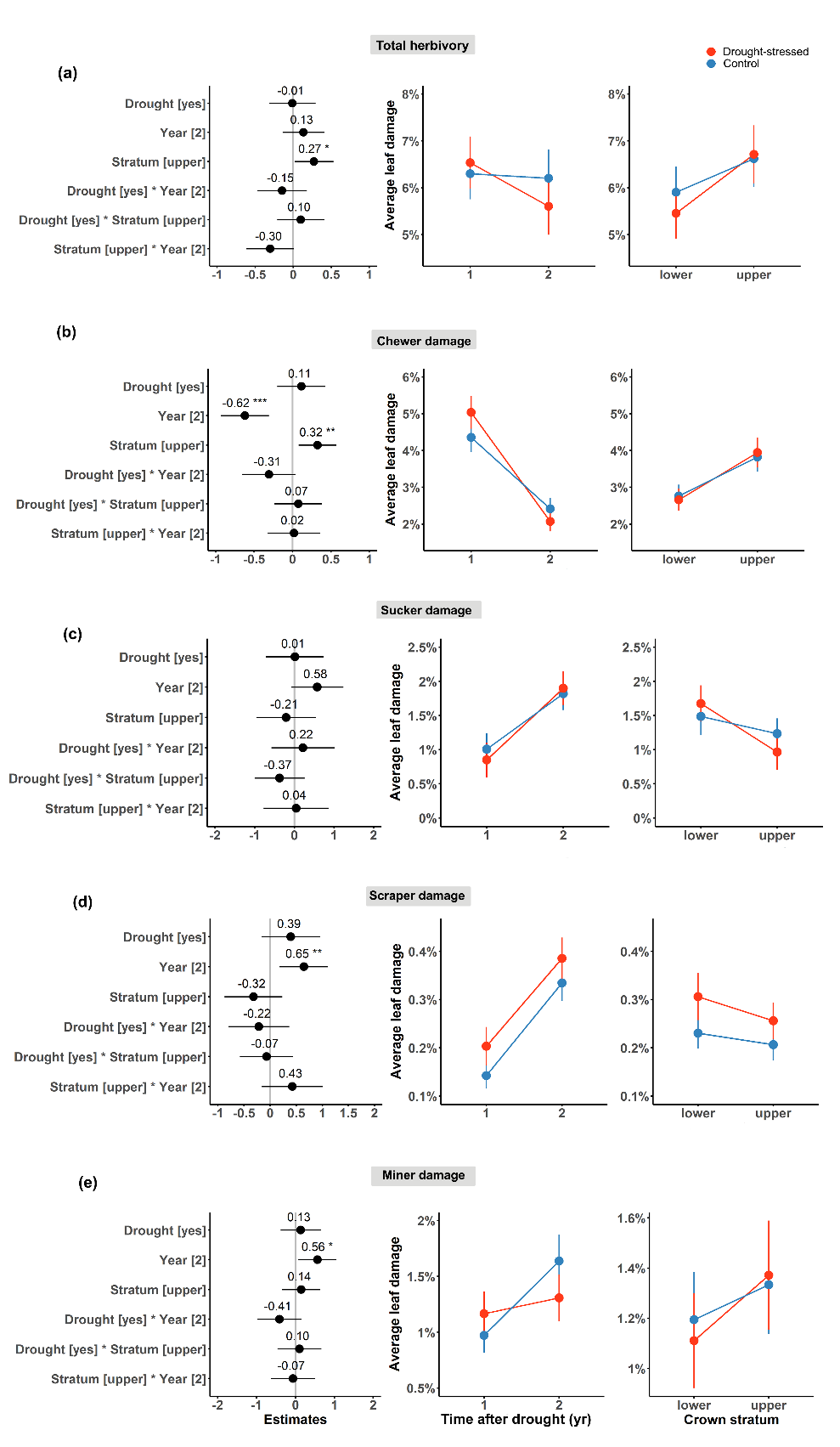


**Figure S5**

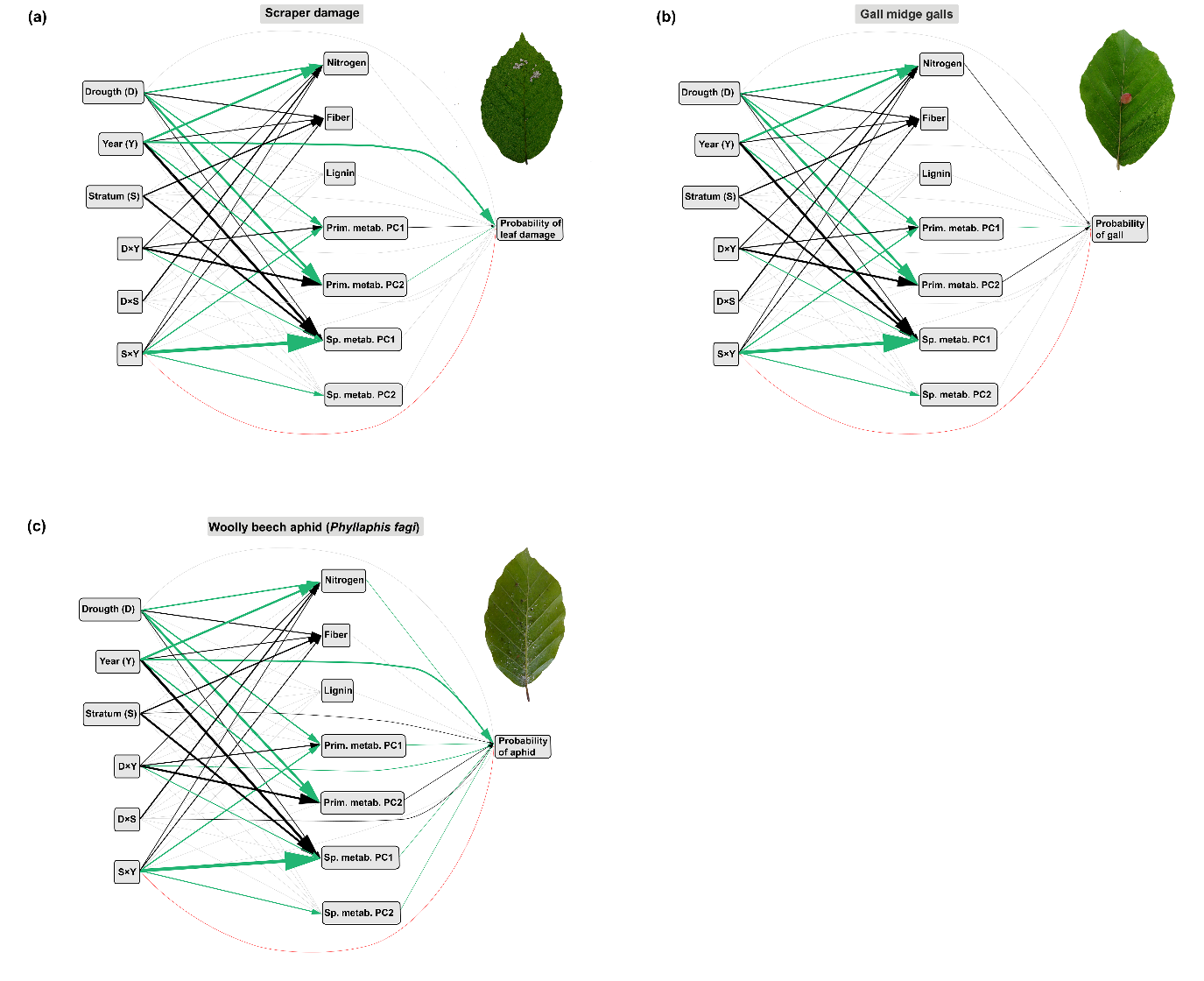
Estimate and line plots illustrating the effects of previous drought stress, year since the drought event, crown stratum and their two-way interactions on the probability of finding *F. sylvatica* leaves with (a) scraper damage, (b) gall midge galls, (c) the aphid *P. fagi*. Leaf images represent examples of feeding-guild specific damage patterns. P-values were calculated via generalized linear mixed models with binomial distribution. P-values in the estimate plots: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001. Line plots represent predicted trait values (mean ± 1 SE).

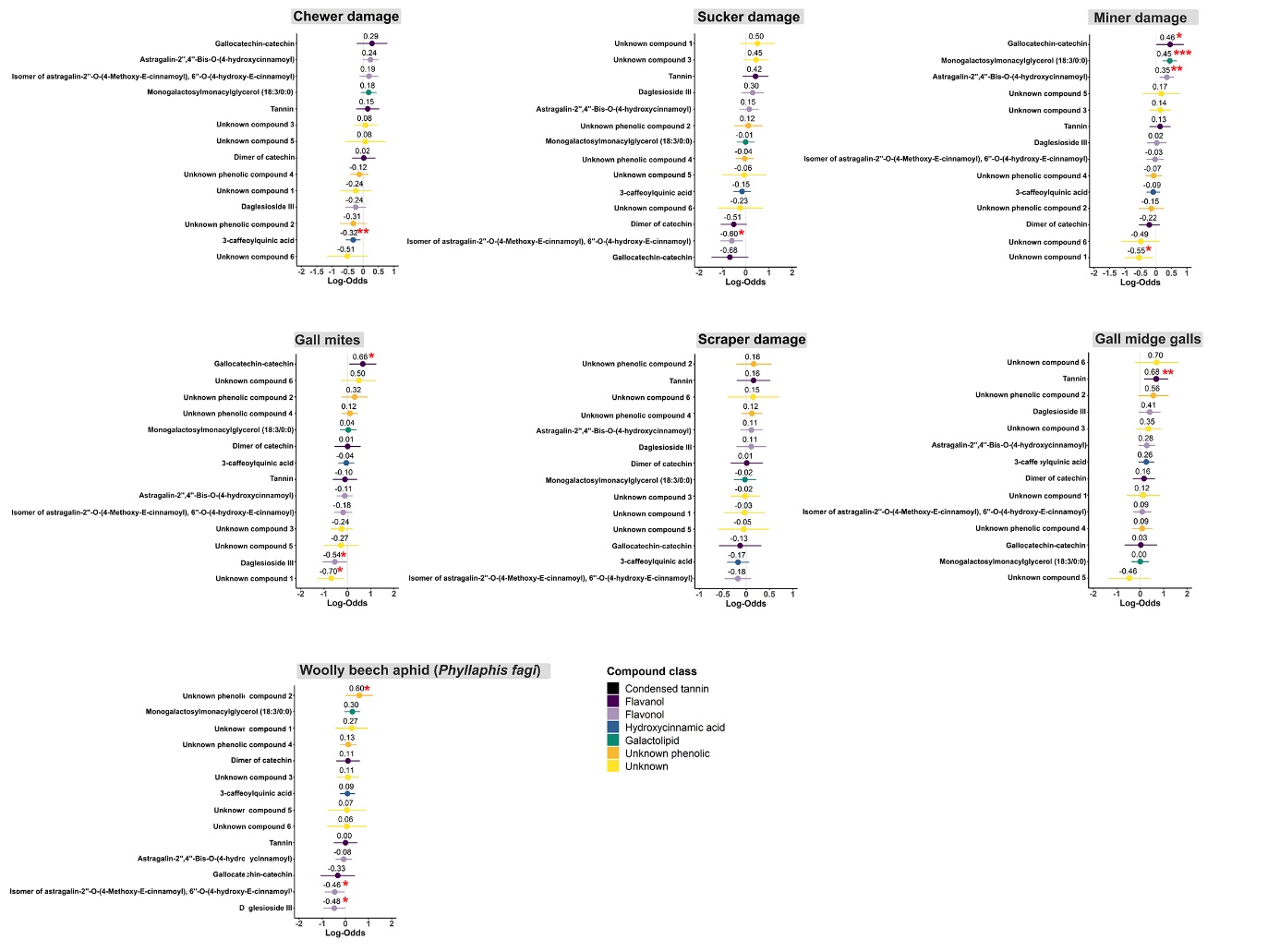
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**Figure S6**

Estimate and line plots illustrating the effects of previous drought stress, year since the drought event, crown stratum and their two-way interactions on the amount of *F. sylvatica* leaf damage (leaves with no leaf damage were excluded) caused by (a) all feeding guilds (excluding gallers and *P.fagi*), (b) chewers, (c) suckers, (d) scrapers and (e) miners. P -values were calculated via generalized linear mixed models with beta distribution. p-values in the estimate plots: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001. Line plots represent predicted trait values (mean ± 1 SE).

**Figure S7**

****Results from piecewise structural equation models (SEM) on the relations of the probability of observing (a) scraper damage, (b) gall midge galls (c) wooly beech aphids and drought stress, year since the drought event, crown stratum and their two-way interactions as well as phytochemistry (including nitrogen, fiber, lignin and the coordinates of the first two principal components [PC] of a principal component analysis on the primary metabolome [Prim. metab] and specialized metabolome [Sp. metab]) and. Positive paths in green, negative in black, non-significant in gray. Thickness of paths represent standardized effect sizes. Paths that were removed due to multicollinearity issues in red. Results of the piecewise SEM including standardized estimates are listed in Table S3. Leaf images represent examples of feeding-guild specific damage patterns.



**Figure S8**

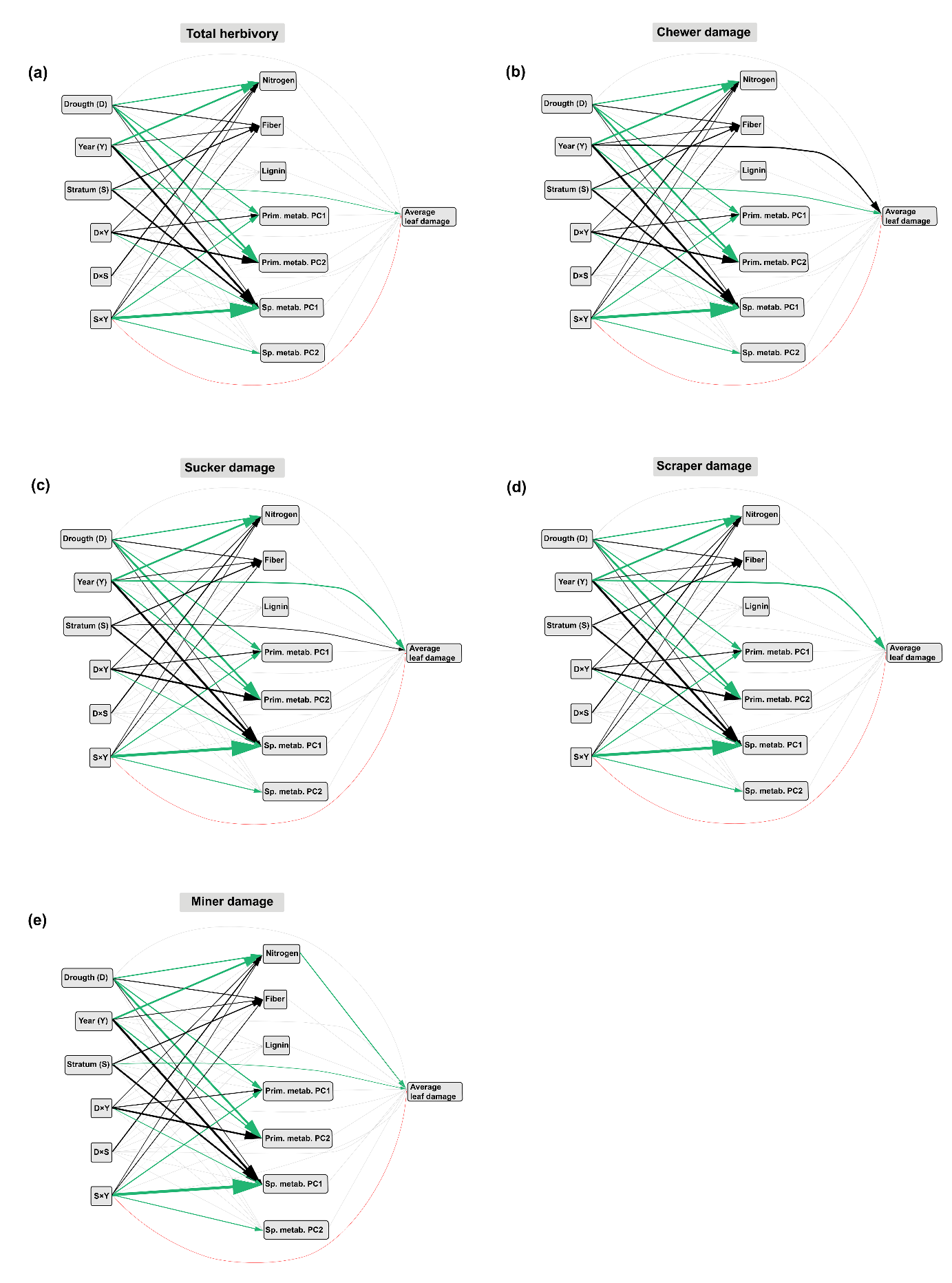
The effect of specialized *F. sylvatica* metabolites that are most relevant for explaining the differences between previously drought stressed and non-stressed trees (selected via partial least square discriminant analysis) on damage probabilities. The number of tested compounds was reduced to avoid multicollinearity issues. P-values in the estimate plots: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001

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**Figure S9**

The effect of specialized *F. sylvatica* metabolites that are most relevant for explaining the differences between previously drought stressed and non-stressed trees (selected via partial least squares discriminant analysis) on damage quantities. The tested compounds were selected via partial least squares regression. P-values in the estimate plots: \* < 0.05, \*\* < 0.01

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**Figure S10**

Results from piecewise structural equation models (SEM) on the relations of the amount of damage caused by (a) all studied feeding guilds, (b) chewers, (c) suckers, (d) scrapers, (e) miners and drought stress, year since the drought event, crown stratum and their two-way interactions as well as phytochemistry (including nitrogen, fiber, lignin and the coordinates of the first two principal components [PC] of a principal component analysis on the primary metabolome [Prim. metab] and specialized metabolome [Sp. metab]) and. Positive paths in green, negative in black, nonsignificant in gray. Thickness of paths represent standardized effect sizes. Paths that were removed due to multicollinearity issues in red. Results of the piecewise SEM including standardized estimates are listed in Table S4.

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