




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

Michael Eisenring¹ , Arthur Gessler^{2,3} , Esther R. Frei^{3,4,5} , Gaétan Glauser⁶, Bernd Kammerer⁷ , Maurice Moor¹, Anouchka Perret-Gentil¹, Thomas Wohlgemuth³  and Martin M. Gossner^{1,2} 

¹Forest Health & Biotic Interactions, Swiss Federal Research Institute WSL, Birmensdorf, 8903, Switzerland; ²Department of Environmental Systems Science, Institute of Terrestrial Ecosystems, ETH Zurich, Zürich, 8092, Switzerland; ³Forest Dynamics, Swiss Federal Research Institute WSL, Birmensdorf, 8903, Switzerland; ⁴WSL Institute for Snow and Avalanche Research SLF, Flüelastrasse 11, Davos, 7260, Switzerland; ⁵Climate Change and Extremes in Alpine Regions Research Centre CERC, Davos, 7260, Switzerland; ⁶Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Neuchâtel, 2000, Switzerland; ⁷Core Facility Metabolomics, Albert-Ludwigs-University Freiburg, Freiburg, 79014, Germany

Summary

Author for correspondence:
Michael Eisenring
Email: michael.eisenring@wsl.ch

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- Extreme droughts can have long-lasting effects on forest community dynamics and species interactions. Yet, our understanding of how drought legacy modulates ecological relationships is just unfolding. We tested the hypothesis that leaf chemistry and herbivory show long-term responses to premature defoliation caused by an extreme drought event in European beech (*Fagus sylvatica* L.).
- For two consecutive years after the extreme European summer drought in 2018, we collected leaves from the upper and lower canopy of adjacently growing drought-stressed and unstressed trees. Leaf chemistry was analyzed and leaf damage by different herbivore-feeding guilds was quantified.
- We found that drought had lasting impacts on leaf nutrients and on specialized metabolomic profiles. However, drought did not affect the primary metabolome. Drought-related phytochemical changes affected damage of leaf-chewing herbivores whereas damage caused by other herbivore-feeding guilds was largely unaffected. Drought legacy effects on phytochemistry and herbivory were often weaker than between-year or between-canopy strata variability.
- Our findings suggest that a single extreme drought event bears the potential to long-lastingly affect tree–herbivore interactions. Drought legacy effects likely become more important in modulating tree–herbivore interactions since drought frequency and severity are projected to globally increase in the coming decades.

Introduction

Around the world, forest ecosystems are increasingly challenged by combined effects of heat and drought (Hammond *et al.*, 2022). Projected climate change scenarios suggest that the occurrence and intensity of future drought events will increase, exacerbating the situation for forests (Touma *et al.*, 2015; Samaniego *et al.*, 2018; UNCCD, 2022). Droughts can affect forest ecosystems directly, for example by impairing tree physiology and metabolic functions (Aranda *et al.*, 2012; Niinemets, 2016; Hartmann *et al.*, 2018; Salmon *et al.*, 2019), or indirectly by modulating the relationship between trees and other organisms via tree physiological and metabolomic changes (Stultz *et al.*, 2009; Anderegg *et al.*, 2015; Gely *et al.*, 2020).

Insect herbivores are important ecosystem drivers (Soliveres *et al.*, 2016) as they shape plant community dynamics

(Borgström *et al.*, 2016; Tamburini *et al.*, 2018), provide food for higher trophic levels (Nyffeler *et al.*, 2018) and affect energy and nutrient cycling through consumption of plant biomass (Belovsky & Slade, 2000; Chapman *et al.*, 2003). Consequently, drought effects on plant–herbivore interactions may entail far-reaching ecosystem-level consequences posing a challenge to forest health and biodiversity conservation. Yet, despite a large body of research on plant–herbivore interactions, a conclusive framework on how different herbivore-feeding guilds correspond to plants impacted by drought is still lacking (Gely *et al.*, 2020). Moreover, while in particular immediate drought effects on insect herbivores have been explored (Huberty & Denno, 2004; Jactel *et al.*, 2012) it remains unclear how drought stress affects the interaction between mature trees and herbivores in the long term (but see Rouault *et al.*, 2006). Addressing this knowledge gap is important, as drought legacy effects on tree–herbivore

interactions may be highly relevant from an ecosystem-level perspective (Kannenberg *et al.*, 2020; Müller & Bahn, 2022).

Severe drought stress can impair water transfer within trees by inducing vessel embolism (Bréda *et al.*, 2006; Rennenberg *et al.*, 2006), which is indicated, for example in European beech by premature defoliation (see Guan *et al.*, 2022). Drought recovery is a metabolomic costly process and may take several years (Huang *et al.*, 2018). During this time previously drought-stressed trees likely differ in their phytochemistry from unstressed trees (Gessler *et al.*, 2017). Moreover, different tree species can vastly vary in their drought coping and recovery strategies (Ruehr *et al.*, 2019). Exploring drought legacy effects on tree–herbivore interactions is further complicated by the fact that drought impacts may vary across crown strata. For example, Richter *et al.* (2022) showed that drought affects the microclimatic gradient along the vertical dimension of forest canopies and these changes depend on the tree species. Yet, a constant increase of temperature with tree height was observed for most species (Richter *et al.*, 2022). Consequently, drought effects on herbivores may be more accentuated in the upper, sun-exposed stratum than in the lower crown.

The enormous diversity of tree-feeding herbivore species and their often vastly different feeding strategies make it challenging to generalize herbivore responses to drought. However, herbivores of the same feeding guild tend to show similar immediate responses to drought (Koricheva *et al.*, 1998; Huberty & Denno, 2004). Sapsuckers and miners often perform better when feeding on moderately drought-stressed than on unstressed plants (Koricheva *et al.*, 1998; Gely *et al.*, 2020). By contrast, the performance of chewing and gall-forming insects often decreases with increasing drought stress of host plants (Koricheva *et al.*, 1998; Huberty & Denno, 2004; Gutbrodt *et al.*, 2011).

Feeding guild-specific differences in responses to drought are likely the result of drought-induced changes in phytochemical defenses and leaf nutrient levels (He & Dijkstra, 2014; Jamieson *et al.*, 2017). Because of their specialized feeding mode, it has been hypothesized that sapsuckers and miners benefit from increasing nitrogen levels when feeding on moderately drought-stressed plants while avoiding defensive compounds (Gely *et al.*, 2020). Consequently, these feeding guilds may only be minimally affected by drought-related changes in defense chemistry. By contrast, externally feeding chewing and scraping herbivores feed less selectively than suckers and miners. Hence, the positive effects of plant nutritional quality on drought-stressed plants may be offset by drought-induced changes in defense chemistry (Gely *et al.*, 2020).

European beech (*Fagus sylvatica* L.) is currently the most dominant broad-leaved tree species in Central European forests, hosting a well-characterized herbivore community (Brändle & Brandl, 2001; Durrant *et al.*, 2016). Several studies have documented distinct insect herbivory patterns across vertical and horizontal spatial scales in beech (Gossner *et al.*, 2014; Mangels *et al.*, 2015), which is likely explained by microclimatic variation among strata.

In addition, beech–herbivore interactions are mediated by specialized leaf metabolites. Besides mono- and sesquiterpenes beech

specialized metabolite profiles are dominated by phenolics, many of which have known effects on insect communities and herbivory (Petrakis *et al.*, 2011; Packham *et al.*, 2012; Rehman *et al.*, 2012). Moreover, studies suggest short-term consequences of drought stress for beech primary chemistry (Pflug *et al.*, 2018; Zang *et al.*, 2021). However, how drought stress may affect the relationship between phytochemistry and herbivory in beech, in the long run, remains unclear.

In 2018, Central Europe experienced one of the most severe summer droughts ever recorded and beech particularly suffered from this drought (Schuldt *et al.*, 2020). In late summer 2018, several beech trees in many forest stands had already prematurely shed their leaves. Premature defoliation in beech is a clear indicator for severe drought stress (Frei *et al.*, 2022) and therewith associated xylem embolism since the leaf petiole xylem is much more resistant to embolisms than the stem xylem in this species (Guan *et al.*, 2022). Interestingly, prematurely defoliated, that is drought-stressed, trees grew in many cases adjacent to nondefoliated healthy looking beech trees (Frei *et al.*, 2022). The observed differences in drought stress were attributed to the small-scale variation in competition for resources (Klesse *et al.*, 2022) and allowed a clear differentiation between strongly drought-affected, symptomatic trees in 2018 and nonsymptomatic trees which remained below a xylem cavitation threshold leading to defoliation.

In the present study, we explore the legacy effects of the 2018 extreme drought summer on beech–herbivore interactions across two regions in Switzerland over 2 yr using a natural experimental set up containing sites with heavily drought-stressed trees (symptomatic, that is with premature defoliation in 2018) growing next to non or only minimally stressed beech (nonsymptomatic in 2018) established by Wohlgemuth *et al.* (2020). We tested the following hypotheses: (1) Previously drought-stressed and unstressed beech differ in their phytochemical profile. (2) Herbivore-feeding guilds differ in their preference for previously drought-stressed trees, that is leaf damage by sapsuckers and miners is higher, while leaf damage by chewing, scraping and gall-forming herbivores is lower on previously drought-stressed than on unstressed beech. (3) Drought legacy effects on phytochemistry and herbivory will be more pronounced in the upper, sun-exposed crown than in the lower crown; and (4) more pronounced in the first year than in the second year after the drought. (5) Drought legacy effects on leaf chemistry explain variations in leaf damage.

Materials and Methods

Experimental overview

In August 2018, we identified several forest sites in Switzerland with beech trees (*F. sylvatica* L.) displaying premature leaf discoloration and shedding as a result of the extreme 2018 summer drought (Frei *et al.*, 2022). In beech, premature defoliation is a sign of twig and stem xylem cavitation as the leaf petiole xylem has a higher resistance to cavitation compared to the twig and stem xylem (Guan *et al.*, 2022). Thus, we differentiated between

symptomatic trees with visible drought stress signs and nonsymptomatic trees, exposed to no or lower stress impacts.

We selected four sites located in the region Zurich/Aargau and four sites in the region Baselland. At each site, we chose a plot with drought-stressed beech trees, that is trees exhibiting premature defoliation (henceforth: drought-stressed), and, in close proximity, a control plot with beech trees that grew under comparable conditions but showed no signs of severe drought stress, that is no premature defoliation (henceforth: control; mean distance between drought-stressed and control plots: 199 m; Table S1). In each of the 16 plots we selected three mature beeches comparable in age and size (i.e. a total of 24 drought-stressed and 24 control beeches). We recorded the position of each tree using a GNSS receiver (GeoXH 6000 DGNSS; Trimble Navigation, Sunnyvale, CA, USA). Postprocessed coordinates achieved a horizontal precision of 0.1–2.0 m. For each tree, we determined distance to the forest edge (which may affect herbivory) based on tree coordinates and a forest mask from the Swiss National Forest Inventory (Waser *et al.*, 2015). Leaves for phytochemistry and herbivory measurements were sampled in summer 2019 and 2020. Compared to 2018 lower temperatures, higher cumulative levels of precipitation and climatic water balance, that is precipitation minus potential evapotranspiration, were measured in 2019 and 2020 as well as in the 14 previous years (2004–2017) (Fig. S1) we sampled leaves from one branch of the upper, sun-exposed and one branch of the lower, shaded crown of all trees. Thus, the experimental design for evaluating the effects of the summer drought, time since the drought event and crown stratum on herbivory and leaf chemistry was a 2 (plots/drought treatments) \times 2 (years since drought) \times 2 (crown strata) \times 2 (regions) \times 4 (sites) split-plot. With three sampled trees per plot this resulted in 96 branches collected in 2019. In 2020, due to forest sanitary measures, we were unable to collect data from seven drought-stressed trees and the six most proximate control trees, reducing the number of analyzed branches to 70.

Crown transparency assessment

In July 2019, 1 yr after the drought, the crown transparency of all trees was estimated as the percentage of leaf loss compared to a reference tree with a healthy, fully foliated crown using a photo guide with species-specific reference standards (Eichhorn *et al.*, 2016; Frei *et al.*, 2022). Crown transparency is known to increase under (and in years after) drought (Strand, 1997; Seidling, 2007). Continued increased defoliation can, therefore, be used as a proxy for drought legacy effects and high defoliation in the long term is generally related to mortality risk (Hunziker *et al.*, 2022).

Leaf sampling

Leaves were collected in late June/early July 2019 and 2020. From each tree, we randomly selected a branch in the sun-exposed crown (upper quarter of the tree crown between 20 and 25 m height, henceforth: upper stratum) and the shaded

crown (lower quarter of the tree crown between 3 and 5 m height, henceforth: lower stratum). Branches were harvested using either a crossbow and rope to break down branches (for details see Gossner *et al.*, 2014) or telescopic shears to cut branches (only lower crown). The branches were selected to have at least 100 fully developed leaves. Thirty fully developed leaves without signs of herbivory were randomly selected from each branch and used for phytochemical analyses. Moreover, we randomly sampled 50 fully developed leaves, starting from the branch tip, for herbivory assessments. Leaves for phytochemistry were kept on dry ice in the field and then stored at -20°C until further processing. Leaves for herbivory assessments were put in plastic bags with a piece of wet sponge cloth and stored in cooling boxes in the field. In the lab, the leaves were stored in a fridge at 4°C until analyzed.

Phytochemical analyses

Leaves for phytochemical analyses were pooled by branches, lyophilized, and then ground using a micro impact mill with a 1 mm mesh screen (Culatti AG, Steinerberg, Switzerland). A subsample of the ground material was used for the analysis of leaf fiber (reported as the total amount of cellulose and lignin) and lignin content. Both constituents contribute to leaf toughness and are associated with herbivore-feeding deterrence (Choong, 1996; Liu *et al.*, 2018). Fiber and lignin were quantified gravimetrically using sequential extraction in a hot acid detergent solution in an Ankom 200 Digestor and then incubated in 72% H_2SO_4 (Rowland & Roberts, 1994). The remaining ground leaf material was pulverized in a ball mill and used for all additional phytochemical analyses.

Leaf nitrogen concentration, a proxy for leaf protein content and, therefore, an important nutrient for herbivores (Awmack & Leather, 2002), was measured via a thermal combustion/elementary analysis (TC/EA) system (isoEAth; Firma Sercon, Crewe, UK) coupled to an IRMS (HS2022; Firma Sercon). Finally, the leaf primary metabolome, encompassing compounds that are required for plant development and growth (Ferne & Pichersky, 2015), and the specialized metabolome, encompassing mainly compounds that are involved in plant stress responses (Hartmann, 2007), were analyzed. The untargeted analysis of the primary metabolome was performed with gas chromatography-mass spectrometry (GC-MS) according to Jansen *et al.* (2014), the specialized metabolome was analyzed by ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS) as described in Eisenring *et al.* (2018) with minor modifications. Detailed extraction and analytical methods can be found in Methods S1.

Herbivory assessments

We assessed leaf herbivory 1–3 d after field collection. On each leaf, we estimated the proportion of leaf area affected by chewers, scrapers, suckers and miners using templates of beech leaves with defined damage areas (details in Methods S2). In addition, we

noted the presence of galls induced by gall midges and gall mites and the woolly beech aphid *Phyllaphis fagi* (L.), as this was the only herbivore that was sufficiently abundant to be analyzed.

Statistical analyses

Analyses were conducted with R 4.2.2 (R Core Team, 2023). A detailed description of the R-packages used for each analysis can be found in Methods S3.

The impact of drought stress (early leaf senescence due to drought /no signs of drought stress) and region (Zurich/Basel-land) on tree crown transparency 1 yr after the drought was analyzed in a linear model (LM). Effects on nitrogen, fiber and lignin were analyzed in linear mixed effects models (LMMs) with drought stress, year since drought stress, crown stratum, all possible two-way interactions as fixed effects and individual trees as random effects.

The effects of drought stress, crown stratum and year since drought on the primary and specialized metabolome were explored by subjecting the primary and specialized metabolome data from the first and second year after the drought to a principal component analysis (PCA). The first two principal components (PC), representing integrative measures of metabolomic variation, were then used as variables in piecewise structural equation models (see below). PC may not always reflect the variation, that is relevant for separating groups of ecological interest (Scott & Crone, 2021). Therefore, the primary and specialized metabolome of upper and lower crown leaves of drought-stressed and control trees were also compared using partial least squares discriminant analysis (PLS-DA). The latter is a supervised multivariate analysis optimized to summarize (metabolomic) variables in a way that best explains differences among response categories (i.e. maximizing of co-variation between predictor variables and response categories). The models were tuned using 10-fold cross-validation on five PLS-DA components. Since PLS-DA is prone to overfitting, it is important to validate the models before the score plots can be reliably interpreted (Hervé *et al.*, 2018). We validated all tuned PLS-DA models via permutation tests (1000 permutations) using the 'B : W ratio' as suggested by Bijlsma *et al.* (2006). The primary and specialized metabolomic features that best discriminated between drought treatments were identified for both years. To do so, variable influence on projection (VIP) scores were calculated in validated PLS-DA models (as mentioned in the previous section) discriminating between drought-stressed and control trees. For both years, the 50 most influential features were selected and submitted to Student's *t*-tests. Features that differed significantly between control and drought-stressed trees were then identified based on their exact mass and MS/MS fragment properties and based on the comparison with existing databases.

Herbivore damage from the different feeding guilds and the presence of *P. fagi* were analyzed separately on the branch level in two steps. In a first step, we distinguished between leaves with or without feeding guild-specific damage. The probability of finding leaf damage (presence/absence of damage) by each feeding guild was modeled using generalized linear mixed models (GLMMs)

with binomial distribution. In a second step, we calculated the average proportional leaf damage (i.e. leaf area consumed) separately for each feeding guild per branch except for gall midges, gall mites and *P. fagi*, for which only presence/absence data was available. The proportional data (i.e. observations of the open interval (0, 1)) was analyzed in GLMMs with beta distribution (Douma & Weedon, 2019). In all models we explored the impact of the fixed effects drought stress (yes /no), crown stratum (upper/lower), year since the drought event (first/second) and all possible two-way interactions between these effects on herbivore damage and the presence of *P. fagi*. Initially, we included distance to the forest edge (which may affect herbivore communities) and individual trees nested in region as random intercepts in all models. Based on likelihood ratio tests we removed region from all and distance from the forest edge from all but two models (GLMM binomial model for sucker and miner). Individual trees were kept as random intercepts in all models.

Piecewise structural equation models (SEMs) (Lefcheck, 2016) were used to disentangle the direct and indirect impacts (via changes in nitrogen, fiber, lignin, variation in composite primary and specialized phytochemical profiles (PCA axes)) of drought stress, crown stratum and year since the drought event on the probability of leaf damage by each feeding guild (see Methods S4).

Finally, we explored the relationship between individual specialized metabolites that best discriminated between drought treatments and herbivore damage patterns. The relationship between the probability of leaf damage for each feeding guild and all specialized metabolites that did not show signs of multicollinearity was modeled in GLMMs with binomial distribution with selected metabolites, crown stratum, and year since the drought effect as fixed effects, individual trees as random effects and damage probabilities as response variables. The relationship between proportional leaf damage (consumed leaf area) for each feeding guild and specialized metabolites was explored with partial least squares regression (PLSR; can deal with highly collinear explanatory variables) and metabolites that were most strongly associated with variation in proportional leaf damage were identified. Metabolites with variable influence on projection (VIP) scores > 1 were subjected to GLMMs with beta distribution with the selected metabolites, crown stratum, and year since the drought effect as fixed effects, individual trees as random effects and herbivore damage levels as response variables. Based on likelihood ratio tests we decided not to include region and distance from the forest edge in any model as they did not improve the model fit.

Results

Drought legacy effects on crown transparency

Trees with premature leaf discoloration and shedding due to drought stress in 2018 had a > 15% higher crown transparency ($P < 0.001$) in the year following the drought event than the control trees (Fig. 1). Crown transparency levels were not affected by regional differences.

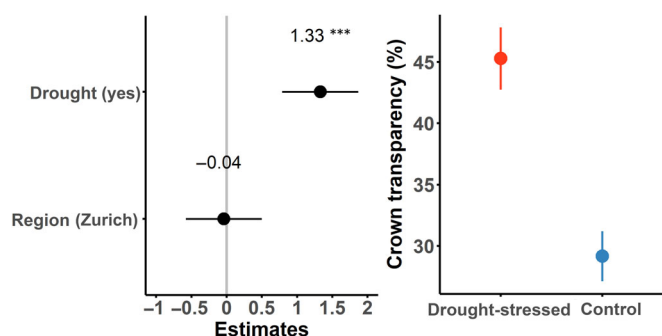


Fig. 1 Estimate and line plot illustrating the effects of previous drought stress (2018) and region on *Fagus sylvatica* crown transparency assessed 1 yr after the drought (2019). *P*-values were calculated with linear models. *P*-values in the estimate plots: ***, < 0.001. The line plot represents the predicted trait values (mean \pm 1SE).

Drought legacy effects on leaf chemistry

Leaf nitrogen was affected by drought, but drought effects varied between the years postdrought and between crown strata (Fig. 2a). We found that in the first year after the drought, drought-stressed trees had 5% higher nitrogen values than control trees. In agreement with our hypothesis (4), we found that in the second year after the drought; however, nitrogen levels were minimally lower in drought-stressed than in control trees (drought \times year interaction, Fig. 2a). Nitrogen levels also differed between strata in previously drought-stressed trees but not in control trees (drought \times stratum interaction Fig. 2a). In the lower stratum, leaf nitrogen levels were 8% higher in drought-stressed trees whereas in the upper stratum nitrogen levels showed the opposite pattern. When averaged across years and crown strata, leaf nitrogen concentrations were minimally (< 1%) yet significantly higher in previously drought-stressed trees (Fig. 2a). This finding is consistent with hypothesis (1) stating that drought stress leads to differences in the phytochemical profile. In support of hypothesis (1) also fiber levels were affected by drought with leaf fiber concentrations being on average 4% lower in previously drought-stressed trees (Fig. 2b). However, nitrogen and fiber levels were mainly determined by factors other than drought. Variations in nitrogen levels were most strongly associated with interannual differences (13% differences between years, Fig. 2a) while fiber levels were mainly affected by differences between the stratum (6.5% differences between crown strata, Fig. 2b). Leaf lignin levels were not affected by any of the explored variables (Fig. S2).

The first two PCA score plots explained 59.4% (year 1) and 52.1% (year 2) of the variance in the primary metabolome and 26.6% and 27.3% of the variance in the secondary metabolome in the first 2 yr after the drought event (Fig. S3). Using a PLS-DA we did not find any separation among treatment groups with respect to their overall primary metabolome profile (Figs 2c, S2). Potential differences between previous drought stress and nonstressed trees were mainly related to mostly subtle changes in amino-, organic- and fatty acid levels (Table S2; Fig. S4).

By contrast, PLS-DA score plots on the specialized metabolome profile revealed a clear clustering of previously drought-stressed and

unstressed leaves and a weaker but still present separation among upper and lower stratum leaves (Figs 2d, S2). These findings support hypothesis (1) as they indicate that previous drought stress can modulate the specialized phytochemical leaf profile in a long-lasting way. Drought-related changes in the specialized metabolome profiles could mainly be attributed to 41 different compounds quantified in this study (Table S2; Fig. 3). Previous drought stress was associated with the increase in phenolic compounds, which in the most extreme case increased by > 200% in drought-stressed trees compared to controls. Moreover, an increase of flavanols and flavonols was observed in both years after the drought. In the first year after the drought, previously drought-stressed trees had lower levels of fatty acids and lipids.

The specialized metabolome score plots do not indicate a more distinct separation between phytochemical profiles of drought-stressed and control leaves in the first (Fig. S2) compared to the second year after the drought (Fig. 2d), contradicting hypothesis (4). Moreover, we found a weak separation of lower and upper leaf chemistry within a drought treatment group which may indicate that upper and lower stratum leaf specialized metabolites are differently affected by drought.

Drought legacy effects on herbivory

Drought legacy effects had no or only subtle effects on herbivory patterns of most feeding guilds (Figs 4, S5). The most pronounced drought effects were observed for damage caused by chewing herbivores (Fig. 4a). The probability of observing chewing damage was similar for drought-stressed and control trees in the first year after the drought (Fig. 4a). However, in the second year, leaves from previously drought-stressed trees were damaged with a 10% lower probability than leaves from control trees (drought \times year interaction, Fig. 4a). This result supports our hypothesis (2) of lower chewing damage on previously drought-stressed trees. However, our findings do not align with hypothesis (4) as drought effects were more pronounced in the second than in the first year after the drought event. Lower crown leaves from previously drought-stressed trees were on average 9% less likely damaged than lower crown leaves from control trees (drought \times stratum interaction, Fig. 4a). No differences between drought stress treatments were found in the upper crown. These results contradict our hypothesis (3) of more pronounced drought legacy effects in the upper than in the lower crown.

We predicted that besides chewing damage also the probability of scraper damage and the number of galls would be lower on previously drought-stressed plants (hypothesis 2). However, neither scraper, gall midge nor gall mite damage (Figs 4d, S5) corresponded to drought legacy effects. The probability of finding sucker damage did not differ between drought-stressed and control trees in the 2 yr after the drought (no significant drought \times year interaction effects, Fig. 4b). However, previous drought stress affected the probability of finding sucker damage in the lower and upper crown stratum differently (significant drought \times stratum interaction, Fig. 4b). Sucker damage probability was 4% higher in lower stratum leaves but 3% smaller in upper stratum leaves on previously drought-stressed trees, when compared

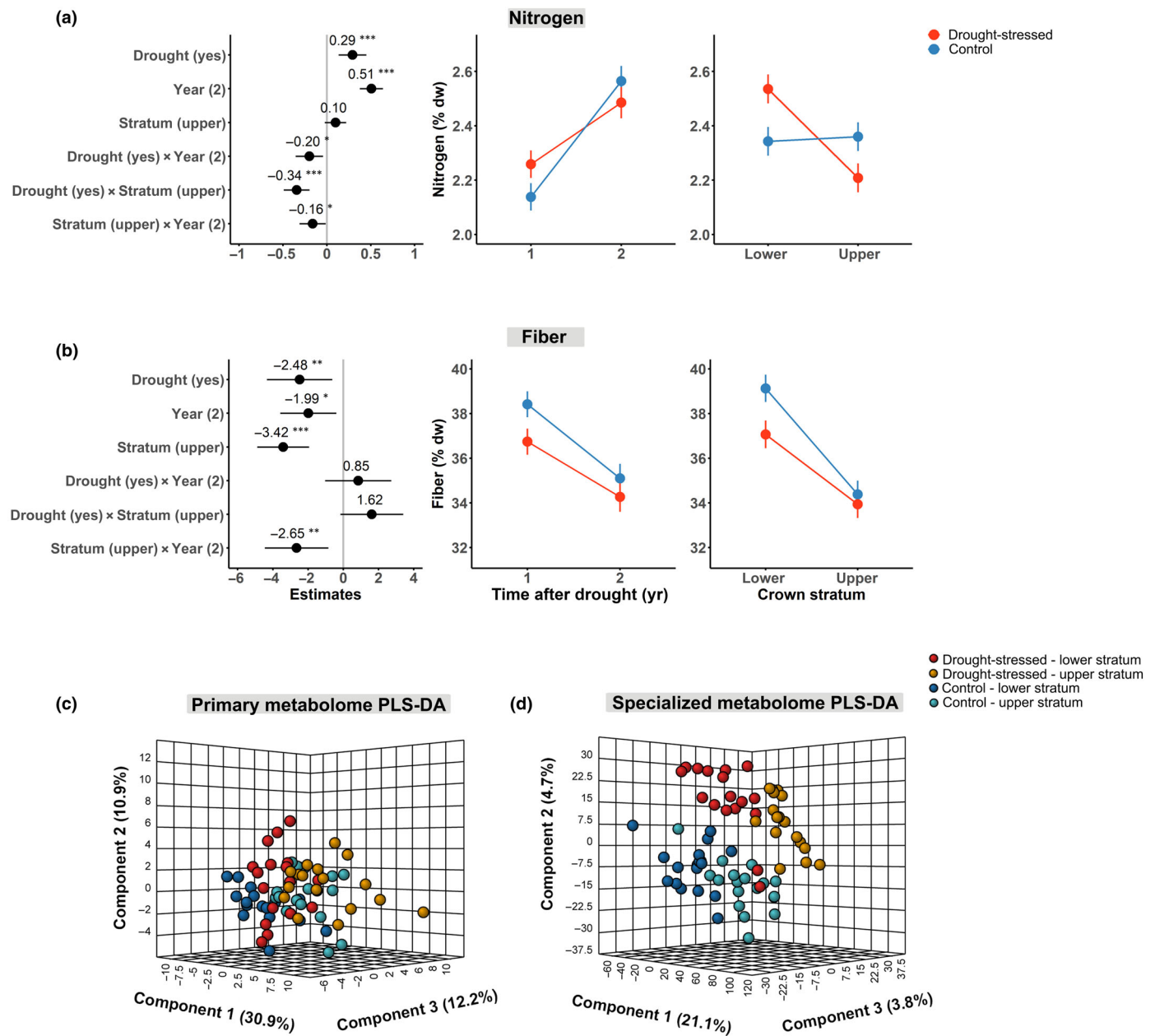


Fig. 2 Estimate and line plots (predicted mean \pm 1SE) illustrate the effects of previous drought stress, year, region, crown stratum and their two-way interactions on (a) leaf nitrogen and (b) leaf fiber concentrations of *Fagus sylvatica*. *P*-values were calculated via linear mixed effects models. *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 (c) the primary *F. sylvatica* metabolome and (d) the specialized metabolome of leaves from the upper and lower crown stratum of previously drought-stressed and control trees for the second year after the drought. Each dot represents a leaf sample of 30 pooled leaves. Results are shown for leaves collected 2 yr after the drought event. Score plots for the first year after the drought are shown in Supporting Information Fig. S2.

to control trees. Similarly, the probability for finding the sucking *P. fagi* was higher in the upper crown of drought-stressed than of control trees (Fig. S5). Miner damage probabilities were not affected by drought stress. Overall, our results on sucker and miner damage do not align with hypothesis (2) posing that leaf damage of suckers and miners is generally higher on previously drought-stressed trees.

Year and stratum had in most cases a stronger effect on damage probabilities than drought legacy effects (Figs 4, S5). Interestingly,

chewing damage probabilities were often conversely affected by year and stratum than damage by the other feeding guilds. For example, the probability of finding chewing damage was 23% lower in the second than in the first year after the drought when averaged across upper and lower stratum of drought-stressed and control trees (Fig. 4a). The average probability of finding sucker and miner damage, however, was 35% and 12% higher in the second year than in the first year (Fig. 4b,c). Similarly, the probability of finding chewer damage was 14% higher in the upper than in

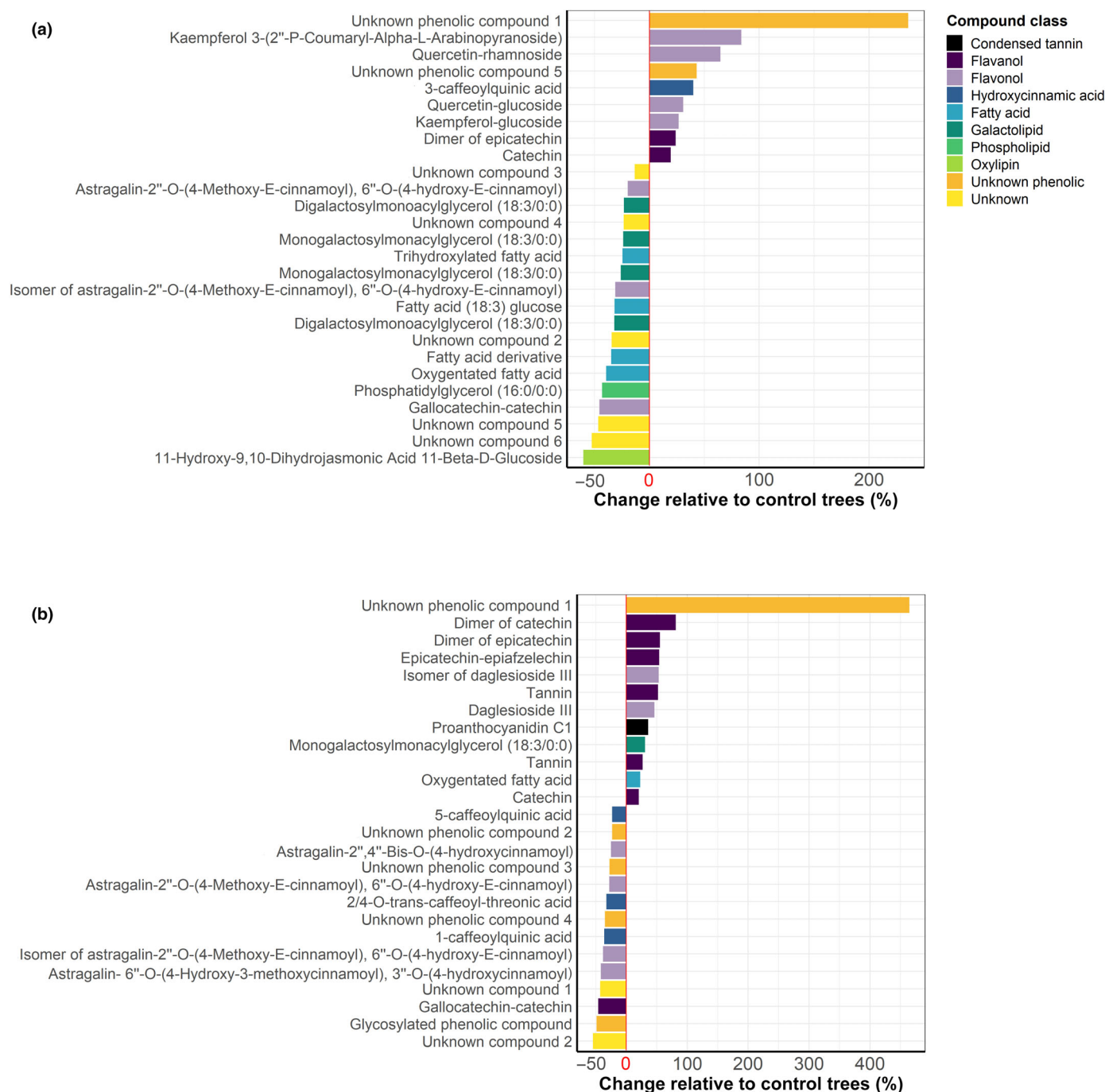


Fig. 3 Change in *Fagus sylvatica* compound levels (peak areas) of the most influential specialized metabolites in partial least squares discriminant analysis models of drought-stressed trees in comparison to control trees (a) 1 yr or (b) 2 yr after the drought. All compound levels differed significantly between drought-stressed and control trees ($P \leq 0.05$). A detailed list of all compounds can be found in Supporting Information Table S2.

the lower stratum when averaged across years and across drought-stressed and control trees (Fig. 4a). However, sucker damage and gall mites occurred each on average with a 4% lower probability in the upper stratum (Fig. 4b,d).

While drought legacy affected the probability of leaf damage in several cases, the amount of leaf area consumed was not affected by drought (Fig. S6). However, the consumed leaf area varied among sampling years and crown strata.

Phytochemistry-mediated drought legacy effects on herbivory

We found that drought and drought \times year interactions had an impact on individual phytochemical compounds as well as phytochemical profiles, which in turn affected damage probabilities of all feeding guilds (Figs 5, S7), supporting hypothesis (5) posing that drought legacy effects on leaf chemistry explain variations in

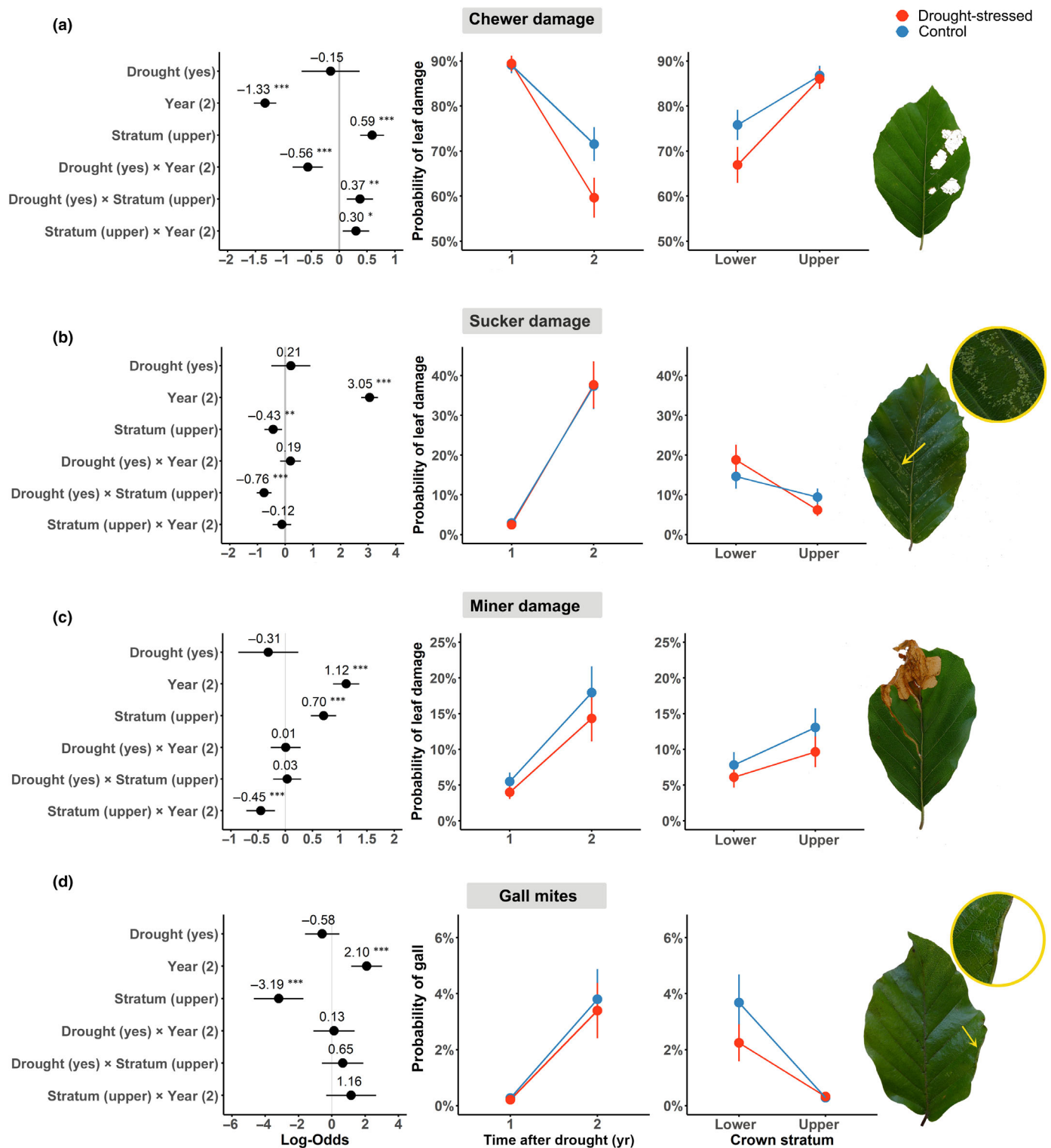


Fig. 4 Estimate and line plots illustrating the effects of previous drought stress, year since the drought event, crown stratum and their two-way interactions on the probability of finding *Fagus sylvatica* leaves with (a) chewing damage, (b) sucker damage, (c) miner damage or (d) gall mites. Leaf images 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 ± 1SE).

leaf damage. However, drought-mediated phytochemical effects on damage probabilities were in most cases weak as indicated by either the low estimated effect sizes of individual specialized

metabolites (Fig. S8) or the low standardized path coefficients (i.e. thin arrows connecting phytochemical traits and damage probabilities; Figs S7).

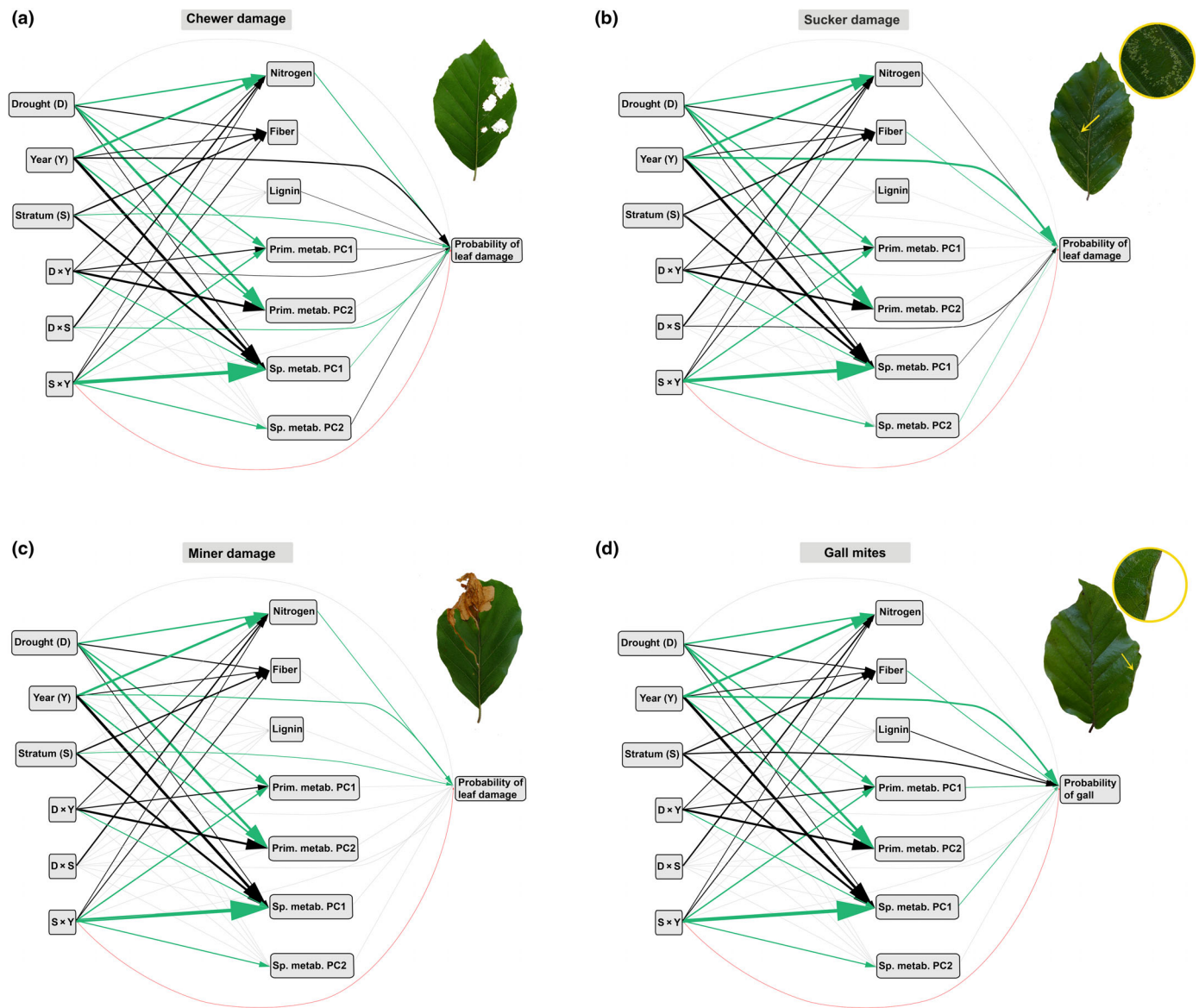


Fig. 5 Results from piecewise structural equation models (SEM) on the relations of the probability of observing (a) chewer damage, (b) sucker damage (c) miner damage, (d) gall mite galls and drought stress, year since the drought event, crown stratum and their two-way interactions as well as phytochemistry (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)). 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 are shown in red. Results of the piecewise SEM including standardized estimates are listed in Supporting Information Table S3. Leaf images represent examples of feeding guild-specific damage patterns.

Leaf nitrogen levels, that were on average slightly higher in previously drought-stressed trees, but effect strength depended on stratum and year, increased the probability of leaf damage by chewers and miners and the occurrence of the beech aphid *P. fagi* (Figs 5a,c, S7). By contrast, damage probabilities by suckers other than *P. fagi* and gall midges were negatively affected by nitrogen (Figs 5b, S7). Fiber concentrations, that were lower in drought-stressed trees, were positively related to the occurrence of sucker damage and gall mites (Fig. 5b,d). Among the 41 specialized metabolites that were most influential for discriminating between drought treatments, only 11 different specialized metabolites from six compound classes significantly yet weakly correlated

with damage probabilities (Fig. S8). About half of these compounds were positively related to damage probabilities.

Variation in the primary metabolomic profile affected the probability of scraper damage and the occurrence of gall midges (Fig. S7). Drought-driven changes in the specialized metabolomic profile (PC1) differentially affected the probability of chewer and sucker damage (Fig. 5a,b). Finally, gall mite gall and *P. fagi* occurrence were affected by both, changes in the primary and specialized metabolomic profile (Figs 5d, S7).

Drought-driven variation in phytochemistry had only a minimal effect on the amount of leaf damage. Only five out of 41 specialized metabolites that were relevant for explaining differences

in leaf chemistry between drought-stressed and unstressed trees affected leaf damage (Fig. S9). Moreover, only leaf miner damage was affected by changes in leaf nitrogen levels (Fig. S10). Changes in the phytochemical profiles did not affect leaf damage quantity of any feeding guild (Fig. S10).

Discussion

Drought events are expected to have long-lasting consequences for community dynamics (Müller & Bahn, 2022). Yet, an in-depth understanding of how drought may shape species interactions in the long term is just emerging. Our findings provide novel insights by showing that drought stress can have long-lasting effects on nitrogen, fiber and specialized metabolite phytochemical profiles in beech leaves. Drought and drought-associated phytochemical changes mediated leaf damage probabilities of leaf-chewing herbivores. Yet, no or rather subtle drought legacy effects were found on leaf damage caused by other feeding guilds. Drought legacy effects on phytochemistry and herbivory have often been blurred by year, canopy strata, or possible differential responses among feeding guilds. Our study helps to better disentangle how these factors individually and in interaction with drought legacy effects can influence tree–herbivore interactions.

Extreme drought affects leaf chemistry over 2 yr

A key finding of our study was that drought effects on the total beech specialized metabolome were still detectable 2 yr post-drought supporting hypothesis (1). However, in contrast to our hypothesis (4) the detected legacy effects were similarly pronounced in both years. Numerous studies, for example on eucalyptus (McKiernan *et al.*, 2016), poplar (Eisenring *et al.*, 2022) or oak (Rivas-Ubach *et al.*, 2014) reported short-term drought-induced changes in tree specialized metabolite levels. Specialized metabolites are reported to be also involved in long-term drought recovery processes. Yet, most recovery studies on specialized metabolites have only been followed up upon for a few weeks after the drought event (McKiernan *et al.*, 2016; Almeida *et al.*, 2020; Du *et al.*, 2021). Ongoing recovery from drought may also explain the differences in the specialized metabolome in our case. If this was true, drought recovery in beech would take place at least over 2 yr. Because European beech sheds its leaves every year, the annual initiation and maintenance of the drought recovery process may be a systemic rather than a leaf-localized response. Alternatively, the metabolomic differences observed may be the result of postdrought acclimation ('drought memory effect') (Walter *et al.*, 2011; Gessler *et al.*, 2020). Severe drought stress can lead to long-term transcriptional (Ding *et al.*, 2012) and therewith associated metabolomic reprogramming (Menezes-Silva *et al.*, 2017), which may, in our case, explain the long-lasting differences in the specialized metabolome between previously drought-stressed and control trees.

In agreement with hypothesis (1) also leaf fiber and nitrogen levels were significantly affected by drought stress. Fiber levels were on average lower in previously drought-stressed beech than

in control beech. Studies on woody species show that leaf toughness (a proxy for leaf lignin content) is associated with drought tolerance (Savi *et al.*, 2017; Fichtner *et al.*, 2020). These findings are in alignment with studies on herbaceous grassland plant species showing that leaf fiber levels can increase under drought stress (Jiang *et al.*, 2012; Deléglise *et al.*, 2015). During drought recovery, however, plants can overcompensate for the drought-caused increase in fiber levels and the latter can drop below initial levels of the prestressed state (Deléglise *et al.*, 2015). Whether the observed reduction of leaf fiber levels in beech in response to previous drought stress is also the result of drought recovery-associated overcompensation remains to be tested.

Leaf nitrogen levels were not only modulated by drought treatment effects but also by the crown stratum. Drought effects led to increased nitrogen levels in the lower crown while it reduced nitrogen levels in the upper crown. Our findings broadly correspond with Cano *et al.* (2013) who measured higher nitrogen levels in the lower than in the upper crown in moderately drought-stressed beech. Leaf nitrogen content is tightly linked to the leaf photosynthetic machinery (Björkman, 1968) and its distribution optimized within canopies to increase CO₂ assimilation (Milcu *et al.*, 2017). Hence, Cano *et al.* (2013) explained within-crown changes in leaf nitrogen levels with the observed changes in photosynthesis levels between crown levels. Our findings add to the results by Cano *et al.* (2013) by suggesting that a drought event can have a long-lasting effect on tree leaf nitrogen levels and possibly also on photosynthesis activity. However, the ramifications of such drought legacy effects seem to vary among crown strata.

In contrast to our results on the specialized metabolome, leaf nitrogen and fiber content, PLS-DA revealed no pronounced drought legacy effects on the total primary metabolome. Primary metabolites are traditionally classified as highly conserved traits that are central for basic plant functions such as development and growth but that are less involved in plant–environment interactions (Ferne & Pichersky, 2015; Maeda, 2019). It has been shown for Scots pine (*Pinus sylvestris* L.) that leaf carbohydrate concentrations are kept constant over different long-term water availability regimes indicating that trees can keep levels of central primary metabolites homeostatic (Schönbeck *et al.*, 2018). By contrast, observations on beech (Peuke *et al.*, 2002; Aranda *et al.*, 2018; Pflug *et al.*, 2018) and other plants (Chan *et al.*, 2013; Tschapinski *et al.*, 2019) challenge the traditional classification of primary metabolites being highly conserved as studies found drought-induced changes in the primary metabolites. However, studies on beech leaves (Zang *et al.*, 2014; Pflug *et al.*, 2018) indicate that many drought-related changes of primary metabolites are restored within a few days after the drought (Leuschner, 2020). Our results are in line with these previous findings by showing that even an extreme drought event does not have a long-lasting impact on the leaf primary metabolomic profile in beech. The observed changes in leaf nitrogen contents might be seen as indicative for changes in photosynthetic capacity (Evans, 1989) and enzyme abundance and activity in general. This might also have affected metabolic fluxes through various pathways. Our approach, however, assesses the change in metabolic abundance and thus metabolite pools. As the primary

metabolites are mainly metabolic pathway intermediates, their pools not necessarily change with altered metabolic flux rates while the end products in the secondary metabolism can become affected.

Phytochemistry mediates drought legacy effects on leaf-chewing herbivores

Drought legacy effects had an impact on the probability of herbivore-caused leaf damage. Yet, the magnitude and direction of drought on leaf damage differed strongly among feeding guilds. The most pronounced effect was observed for the probability of chewer damage that was reduced on drought-stressed trees. By contrast, sucker or miner damage were only minimally affected by previous drought stress. While these results are broadly consistent with hypothesis (2) they do not align with the hypothesized positive effect of moderate drought stress on sap sucker damage. In fact, neither the probability nor the quantity of any feeding guild-associated leaf damage increased on previously drought-stressed trees.

Herbivore-feeding guild-specific differences in drought responses are often explained by phytochemical differences between drought-stressed and nonstressed plants (Jamieson *et al.*, 2017; Gely *et al.*, 2020). It has been proposed that sapsuckers are mainly affected by drought-related changes in nutritional compounds, leaf miners are affected by changes in plant nutrients and leaf toughness, whereas leaf-chewing insects (chewers and scrapers) and gall inducers are affected by changes in plant nutrients, leaf toughness and defensive metabolites (Gely *et al.*, 2020). In alignment with ecological theory and our hypothesis (5) stating that drought legacy effects on leaf chemistry explain variations in leaf damage, we found that at least damage probabilities of suckers and chewers are differentially affected by drought-related changes in phytochemistry. While chewer and sucker damage were oppositely affected by drought effects in the first and the second year after the drought event (drought \times year interaction) and by drought effects on the upper and lower canopy (drought \times stratum effects), they also showed opposite responses to drought-related changes in nitrogen and variation in the specialized phytochemical profile (variation in specialized metabolome captured via PC1), suggesting that these two feeding guilds show contrasting responses to drought-induced phytochemical changes.

However, we could not identify individual compound classes that would explain differences in leaf damage among herbivore-feeding guilds. Moreover, we found that about half of all specialized metabolites were positively related to damage probabilities and the amount of leaf damage. Traditionally, specialized metabolites are associated with protective functions against both biotic stressors (Hartmann, 2007). As such one would assume that herbivore damage should be negatively related to specialized compound levels. However, specialized metabolites are often multifunctional (Erb & Kliebenstein, 2020), and several studies have shown that specialized metabolites can increase herbivore performance or damage (Richards *et al.*, 2012; Hu *et al.*, 2018; Gossner *et al.*, 2023). Taken together, our results suggest that

herbivory in European beech is determined by a suite of phytochemicals rather than by some individual flagship defense compounds. Drought legacy effects can alter leaf phytochemical profiles and consequently have the potential to also modulate leaf herbivory patterns.

Drought effects on chemistry and herbivory vary between crown strata and postdrought years

In several cases, drought effects on phytochemistry and herbivory varied between the upper and the lower tree crown stratum. However, in contrast to our hypothesis (3) we found not a single case in which drought effects on herbivory or phytochemistry were more pronounced in the upper crown than in the lower crown. Instead, we found that fiber levels and chewing damage were less pronounced in drought-stressed trees in the lower stratum, while drought effects in the upper stratum did not differ. Leaves in the upper tree crowns are often exposed to harsher, environmental conditions such as higher temperatures (De Frenne *et al.*, 2021), higher vapor pressure deficit (Barker, 1996), increased windspeed (Sellier & Fourcaud, 2009) or reduced sap-flow rates (Richter *et al.*, 2022) than lower crown regions, which ultimately affects transpiration and rapid water loss. Hence, upper-crown leaves that are already adapted to harsh environmental conditions may be less responsive to drought stress than leaves from the lower crown.

Our study did also not support hypothesis (4) posing that drought legacy effects on phytochemistry or herbivory are more pronounced in the first year than in the second year after the drought. In the case of chewing damage probabilities, we found that drought effects were even more pronounced in the second than in the first year after the drought. In summary, our results suggest that stratum and interannual effects, both of which can strongly affect phytochemistry (Osier *et al.*, 2000; Jamieson *et al.*, 2015; Eisenring *et al.*, 2021), herbivore populations and herbivory (Meiners *et al.*, 2000; Seifert *et al.*, 2020), confound the studied 2018 drought legacy effects in beech.

Conclusion

Our study demonstrates that an extreme drought event can alter phytochemical profiles of beech leaves, that is organs that are newly produced annually, for at least 2 yr postdrought. These drought legacy effects on beech chemistry have the potential to affect damage probabilities of leaf-chewing herbivores and thus possibly also species communities and forest health. Moreover, drought frequency and severity are projected to increase in many regions in the 21st century (Touma *et al.*, 2015; Samaniego *et al.*, 2018; UNCCD, 2022). In addition, successive drought events are expected to have more detrimental effects on trees and forest ecosystems than initial droughts (Anderegg *et al.*, 2020). Therefore, drought legacy effects likely gain in relevance in modulating tree–herbivore interactions and thus forest ecosystem dynamics in the near future.

As such, the findings of the present study also bear relevance for forest management strategies. Besides focusing on measures

that address the short-term consequences of drought events on herbivore and potential pest populations, it will become increasingly relevant to also consider closer monitoring of herbivore population dynamics over several years following a drought event to manage delayed negative impacts at an early stage.

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
Competing interests

None declared.

Author contributions

TW, MMG and AG secured funding. TW, ERF, AG and MMG designed the study. MMG, ERF, MM and APG collected field data and assessed herbivore damage. BK, GG, ME and APG performed chemical analysis. ME and MMG performed statistical analysis. ME wrote the first draft of the manuscript. All authors contributed to revising the manuscript and gave final approval for publication.

ORCID

Michael Eisenring  <https://orcid.org/0000-0002-4756-7982>
 Esther R. Frei  <https://orcid.org/0000-0003-1910-7900>
 Arthur Gessler  <https://orcid.org/0000-0002-1910-9589>
 Martin M. Gossner  <https://orcid.org/0000-0003-1516-6364>
 Bernd Kammerer  <https://orcid.org/0000-0002-4631-8853>
 Thomas Wohlgemuth  <https://orcid.org/0000-0002-4623-0894>

Data availability

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References

Almeida T, Pinto G, Correia B, Gonçalves S, Meijón M, Escandón M. 2020. In-depth analysis of the *Quercus suber* metabolome under drought stress and recovery reveals potential key metabolic players. *Plant Science* 299: 110606.

- Anderegg WR, Hicke JA, Fisher RA, Allen CD, Aukema J, Bentz B, Hood S, Lichstein JW, Macalady AK, McDowell N *et al.* 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* 208: 674–683.
- Anderegg WR, Trugman AT, Badgley G, Konings AG, Shaw J. 2020. Divergent forest sensitivity to repeated extreme droughts. *Nature Climate Change* 10: 1091–1095.
- Aranda I, Gil-Pelegrín E, Gascó A, Guevara M, Cano J, Miguel MD, Ramírez-Valiente J, Peguero-Pina J, Perdiguero P, Soto A *et al.* 2012. Drought response in forest trees: from the species to the gene. In: Aroca R, ed. *Plant responses to drought stress*. Berlin, Heidelberg, Germany: Springer, 293–333.
- Aranda I, Sanchez-Gomez D, Cadahia E, de Simón BF. 2018. Ecophysiological and metabolic response patterns to drought under controlled condition in open-pollinated maternal families from a *Fagus sylvatica* L. population. *Environmental and Experimental Botany* 150: 209–221.
- Awmack CS, Leather SR. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47: 817–844.
- Barker MG. 1996. Vertical profiles in a Brunei rain forest: I. Microclimate associated with a canopy tree. *Journal of Tropical Forest Science* 12: 505–519.
- Belovsky G, Slade J. 2000. Insect herbivory accelerates nutrient cycling and increases plant production. *Proceedings of the National Academy of Sciences, USA* 97: 14412–14417.
- Bijlsma S, Bobeldijk I, Verheij ER, Ramaker R, Kochhar S, Macdonald IA, Van Ommen B, Smilde AK. 2006. Large-scale human metabolomics studies: a strategy for data (pre-) processing and validation. *Analytical Chemistry* 78: 567–574.
- Björkman O. 1968. Carboxydismutase activity in shade-adapted and sun-adapted species of higher plants. *Physiologia Plantarum* 21: 1–10.
- Borgström P, Strengbom J, Viketof M, Bommarco R. 2016. Aboveground insect herbivory increases plant competitive asymmetry, while belowground herbivory mitigates the effect. *PeerJ* 4: e1867.
- Brändle M, Brandl R. 2001. Species richness of insects and mites on trees: expanding Southwood. *Journal of Animal Ecology* 70: 491–504.
- Bréda N, Huc R, Granier A, Dreyer E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* 63: 625–644.
- Cano FJ, Sánchez-Gómez D, Rodríguez-Calcerrada J, Warren CR, Gil L, Aranda I. 2013. Effects of drought on mesophyll conductance and photosynthetic limitations at different tree canopy layers. *Plant, Cell & Environment* 36: 1961–1980.
- Chan KX, Wirtz M, Phua SY, Estavillo GM, Pogson BJ. 2013. Balancing metabolites in drought: the sulfur assimilation conundrum. *Trends in Plant Science* 18: 18–29.
- Chapman SK, Hart SC, Cobb NS, Whitham TG, Koch GW. 2003. Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. *Ecology* 84: 2867–2876.
- Choong M. 1996. What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. *Functional Ecology* 10: 668–674.
- De Frenne P, Lenoir J, Luoto M, Scheffers BR, Zellweger F, Aalto J, Ashcroft MB, Christiansen DM, Decocq G, De Pauw K. 2021. Forest microclimates and climate change: importance, drivers and future research agenda. *Global Change Biology* 27: 2279–2297.
- Deléglise C, Meisser M, Mosimann E, Spiegelberger T, Signarbieux C, Jeangros B, Buttler A. 2015. Drought-induced shifts in plants traits, yields and nutritive value under realistic grazing and mowing managements in a mountain grassland. *Agriculture, Ecosystems & Environment* 213: 94–104.
- Ding Y, Fromm M, Avramova Z. 2012. Multiple exposures to drought 'train' transcriptional responses in *Arabidopsis*. *Nature Communications* 3: 1–9.
- Douma JC, Weedon JT. 2019. Analysing continuous proportions in ecology and evolution: A practical introduction to beta and Dirichlet regression. *Methods in Ecology and Evolution* 10: 1412–1430.
- Du B, Kruse J, Winkler JB, Alfarraj S, Albasher G, Schnitzler J-P, Ache P, Hedrich R, Rennenberg H. 2021. Metabolic responses of date palm (*Phoenix dactylifera* L.) leaves to drought differ in summer and winter climate. *Tree Physiology* 41: 1685–1700.
- Durrant TH, de Rigo D, Caudullo G. 2016. *Fagus sylvatica* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayaz J, de Rigo D,

- Caudullo G, Houston Durrant T, Mauri A, eds. *European atlas of forest tree species*. Luxembourg, Europe: Publication Office, EU, 94–95.
- Eichhorn J, Roskams P, Potoëic N, Timmermann V, Ferretti M, Mues V. 2016. *Part IV: visual assessment of crown condition and damaging agents, manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests*. Eberswalde, Germany: Thünen Institute of Forest Ecosystems.
- Eisenring M, Glauser G, Meissle M, Romeis J. 2018. Differential impact of herbivores from three feeding guilds on systemic secondary metabolite induction, phytohormone levels and plant-mediated herbivore interactions. *Journal of Chemical Ecology* 44: 1178–1189.
- Eisenring M, Lindroth RL, Flansburg A, Giezendanner N, Mock KE, Kruger EL. 2022. Genotypic variation rather than ploidy level determines functional trait expression in a foundation tree species in the presence and absence of environmental stress. *Annals of Botany* 131: 229–242.
- Eisenring M, Unsicker SB, Lindroth RL. 2021. Spatial, genetic and biotic factors shape within-crown leaf trait variation and herbivore performance in a foundation tree species. *Functional Ecology* 35: 54–66.
- Erb M, Kliebenstein DJ. 2020. Plant secondary metabolites as defenses, regulators and primary metabolites—the blurred functional trichotomy. *Plant Physiology* 184: 39–52.
- Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78: 9–19.
- Fernie AR, Pichersky E. 2015. Focus issue on metabolism: metabolites, metabolites everywhere. *Plant Physiology* 169: 1421–1423.
- Fichtner A, Schnabel F, Bruelheide H, Kunz M, Mausolf K, Schuldt A, Härdtle W, von Oheimb G. 2020. Neighbourhood diversity mitigates drought impacts on tree growth. *Journal of Ecology* 108: 865–875.
- Frei ER, Gossner MM, Vitasse Y, Queloz V, Dubach V, Gessler A, Ginzler C, Hagedorn F, Meusburger K, Moor M *et al.* 2022. European beech dieback after premature leaf senescence during the 2018 drought in northern Switzerland. *Plant Biology* 24: 1132–1145.
- Gely C, Laurance SG, Stork NE. 2020. How do herbivorous insects respond to drought stress in trees? *Biological Reviews* 95: 434–448.
- Gessler A, Bottero A, Marshall J, Arend M. 2020. The way back: recovery of trees from drought and its implication for acclimation. *New Phytologist* 228: 1704–1709.
- Gessler A, Schaub M, McDowell NG. 2017. The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* 214: 513–520.
- Gossner MM, Pašalić E, Lange M, Lange P, Boch S, Hessenmöller D, Müller J, Socher SA, Fischer M, Schulze E-D *et al.* 2014. Differential responses of herbivores and herbivory to management in temperate European beech. *PLoS ONE* 9: e104876.
- Gossner MM, Perret-Gentil A, Britt E, Queloz V, Gaetan G, Ladd T, Row AD, Cleary M, Liziniwicz M, Nielsen LR *et al.* 2023. A glimmer of hope – ash genotypes with increased resistance to ash dieback pathogen show cross-resistance to emerald ash borer. *New Phytologist* 240: 1219–1232.
- Guan X, Werner J, Cao KF, Pereira L, Kaack L, McAdam S, Jansen S. 2022. Stem and leaf xylem of angiosperm trees experiences minimal embolism in temperate forests during two consecutive summers with moderate drought. *Plant Biology* 24: 1208–1223.
- Gutbrodt B, Mody K, Dorn S. 2011. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos* 120: 1732–1740.
- Hammond WM, Williams AP, Abatzoglou JT, Adams HD, Klein T, López R, Sáenz-Romero C, Hartmann H, Breshears DD, Allen CD. 2022. Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. *Nature Communications* 13: 1761.
- Hartmann H, Moura CF, Anderegg WR, Ruehr NK, Salmon Y, Allen CD, Arndt SK, Breshears DD, Davi H, Galbraith D *et al.* 2018. Research frontiers for improving our understanding of drought-induced tree and forest mortality. *New Phytologist* 218: 15–28.
- Hartmann T. 2007. From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry* 68: 2831–2846.
- He M, Dijkstra FA. 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. *New Phytologist* 204: 924–931.
- Hervé MR, Nicolè F, Lê Cao K-A. 2018. Multivariate analysis of multiple datasets: a practical guide for chemical ecology. *Journal of Chemical Ecology* 44: 215–234.
- Hu L, Mateo P, Ye M, Zhang X, Berset JD, Handrick V, Radisch D, Grabe V, Köllner TG, Gershenzon J *et al.* 2018. Plant iron acquisition strategy exploited by an insect herbivore. *Science* 361: 694–697.
- Huang M, Wang X, Keenan TF, Piao S. 2018. Drought timing influences the legacy of tree growth recovery. *Global Change Biology* 24: 3546–3559.
- Huberty AF, Denno RF. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* 85: 1383–1398.
- Hunziker S, Begert M, Scherrer SC, Rigling A, Gessler A. 2022. Below average midsummer to early autumn precipitation evolved into the main driver of sudden Scots pine vitality decline in the Swiss Rhône valley. *Frontiers in Forests and Global Change* 5: 874100.
- Jactel H, Petit J, Desprez-Loustau ML, Delzon S, Piou D, Battisti A, Koricheva J. 2012. Drought effects on damage by forest insects and pathogens: a meta-analysis. *Global Change Biology* 18: 267–276.
- Jamieson MA, Burkle LA, Manson JS, Runyon JB, Trowbridge AM, Zientek J. 2017. Global change effects on plant–insect interactions: the role of phytochemistry. *Current Opinion in Insect Science* 23: 70–80.
- Jamieson MA, Schwartzberg EG, Raffa KF, Reich PB, Lindroth RL. 2015. Experimental climate warming alters aspen and birch phytochemistry and performance traits for an outbreak insect herbivore. *Global Change Biology* 21: 2698–2710.
- Jansen K, Du B, Kayler Z, Siegwolf R, Ensminger I, Rennenberg H, Kammerer B, Jaeger C, Schaub M, Kreuzwieser J *et al.* 2014. Douglas-fir seedlings exhibit metabolic responses to increased temperature and atmospheric drought. *PLoS ONE* 9: e114165.
- Jiang Y, Yao Y, Wang Y. 2012. Physiological response, cell wall components, and gene expression of switchgrass under short-term drought stress and recovery. *Crop Science* 52: 2718–2727.
- Kannenber SA, Schwalm CR, Anderegg WR. 2020. Ghosts of the past: how drought legacy effects shape forest functioning and carbon cycling. *Ecology Letters* 23: 891–901.
- Klesse S, Wohlgemuth T, Meusburger K, Vitasse Y, Von Arx G, Lévesque M, Neycken A, Braun S, Dubach V, Gessler A *et al.* 2022. Long-term soil water limitation and previous tree vigor drive local variability of drought-induced crown dieback in *Fagus sylvatica*. *Science of the Total Environment* 851: 157926.
- Koricheva J, Larsson S, Haukioja E. 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. *Annual Review of Entomology* 43: 195–216.
- Lefcheck JS. 2016. piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7: 573–579.
- Leuschner C. 2020. Drought response of European beech (*Fagus sylvatica* L.)—a review. *Perspectives in Plant Ecology, Evolution and Systematics* 47: 125576.
- Liu Q, Luo L, Zheng L. 2018. Lignins: biosynthesis and biological functions in plants. *International Journal of Molecular Sciences* 19: 335.
- Maeda HA. 2019. Evolutionary diversification of primary metabolism and its contribution to plant chemical diversity. *Frontiers in Plant Science* 10: 881.
- Mangels J, Blüthgen N, Frank K, Grassein F, Hilpert A, Mody K. 2015. Tree species composition and harvest intensity affect herbivore density and leaf damage on beech, *Fagus sylvatica*, in different landscape contexts. *PLoS ONE* 10: e0126140.
- McKiernan AB, Potts BM, Brodrick TJ, Hovenden MJ, Davies NW, McAdam SA, Ross JJ, Rodemann T, O'Reilly-Wapstra JM. 2016. Responses to mild water deficit and rewatering differ among secondary metabolites but are similar among provenances within *Eucalyptus* species. *Tree Physiology* 36: 133–147.
- Meiners S, Handel S, Pickett S. 2000. Tree seedling establishment under insect herbivory: edge effects and inter-annual variation. *Plant Ecology* 151: 161–170.
- Menezes-Silva PE, Sanglard LM, Ávila RT, Morais LE, Martins SC, Nobres P, Patreze CM, Ferreira MA, Araújo WL, Fernie AR *et al.* 2017. Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. *Journal of Experimental Botany* 68: 4309–4322.
- Milcu A, Gessler A, Roscher C, Rose L, Kayler Z, Bachmann D, Pirhofer-Walzl K, Zavadlav S, Galiano L, Buchmann T *et al.* 2017. Top canopy nitrogen

- allocation linked to increased grassland carbon uptake in stands of varying species richness. *Scientific Reports* 7: 8392.
- Müller LM, Bahn M. 2022. Drought legacies and ecosystem responses to subsequent drought. *Global Change Biology* 28: 5086–5103.
- Niinimets Ü. 2016. Uncovering the hidden facets of drought stress: secondary metabolites make the difference. *Tree Physiology* 36: 129–132.
- Nyffeler M, Şekerciöğlu ÇH, Whelan CJ. 2018. Insectivorous birds consume an estimated 400–500 million tons of prey annually. *The Science of Nature* 105: 1–13.
- Osier TL, Hwang S-Y, Lindroth RL. 2000. Within-and between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones. *Biochemical Systematics and Ecology* 28: 197–208.
- Packham JR, Thomas PA, Atkinson MD, Degen T. 2012. Biological flora of the British Isles: *Fagus sylvatica*. *Journal of Ecology* 100: 1557–1608.
- Petrakis PV, Spanos K, Feest A, Daskalidou E. 2011. Phenols in leaves and bark of *Fagus sylvatica* as determinants of insect occurrences. *International Journal of Molecular Sciences* 12: 2769–2782.
- Peuke A, Schraml C, Hartung W, Rennenberg H. 2002. Identification of drought-sensitive beech ecotypes by physiological parameters. *New Phytologist* 154: 373–387.
- Pflug EE, Buchmann N, Siegwolf RT, Schaub M, Rigling A, Arend M. 2018. Resilient leaf physiological response of European beech (*Fagus sylvatica* L.) to summer drought and drought release. *Frontiers in Plant Science* 9: 187.
- R Core Team. 2023. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL <http://www.r-project.org/>.
- Rehman F, Khan FA, Badruddin SMA. 2012. Role of Phenolics in plant defense against insect herbivory. In: Khemani LD, Srivastava MM, Srivastava S, eds. *Chemistry of phytopotentials: health, energy and environmental perspectives*. Berlin Heidelberg, Germany: Springer, 309–313.
- Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal R, Gessler A. 2006. Physiological responses of forest trees to heat and drought. *Plant Biology* 21: 556–571.
- Richards LA, Lampert EC, Bowers MD, Dodson CD, Smilanich AM, Dyer LA. 2012. Synergistic effects of iridoid glycosides on the survival, development and immune response of a specialist caterpillar, *Junonia coenia* (Nymphalidae). *Journal of Chemical Ecology* 38: 1276–1284.
- Richter R, Ballasus H, Engelmann R, Zielhofer C, Sanaei A, Wirth C. 2022. Tree species matter for forest microclimate regulation during the drought year 2018: disentangling environmental drivers and biotic drivers. *Scientific Reports* 12: 17559.
- Rivas-Ubach A, Gargallo-Garriga A, Sardans J, Oravec M, Mateu-Castell L, Pérez-Trujillo M, Parella T, Ogaya R, Urban O, Peñuelas J. 2014. Drought enhances folivory by shifting foliar metabolomes in *Quercus ilex* trees. *New Phytologist* 202: 874–885.
- Rouault G, Candau J-N, Lieutier F, Nageleisen L-M, Martin J-C, Warzee N. 2006. Effects of drought and heat on forest insect populations in relation to the 2003 drought in Western Europe. *Annals of Forest Science* 63: 613–624.
- Rowland A, Roberts J. 1994. Lignin and cellulose fractionation in decomposition studies using acid-detergent fibre methods. *Communications in Soil Science and Plant Analysis* 25: 269–277.
- Ruehr NK, Grote R, Mayr S, Arneth A. 2019. Beyond the extreme: recovery of carbon and water relations in woody plants following heat and drought stress. *Tree Physiology* 39: 1285–1299.
- Salmon Y, Dietrich L, Sevanto S, Hölttä T, Dannoura M, Epron D. 2019. Drought impacts on tree phloem: from cell-level responses to ecological significance. *Tree Physiology* 39: 173–191.
- Samaniego L, Thober S, Kumar R, Wanders N, Rakovec O, Pan M, Zink M, Sheffield J, Wood EF, Marx A. 2018. Anthropogenic warming exacerbates European soil moisture droughts. *Nature Climate Change* 8: 421–426.
- Savi T, Love VL, Dal Borgo A, Martellos S, Nardini A. 2017. Morpho-anatomical and physiological traits in saplings of drought-tolerant Mediterranean woody species. *Trees* 31: 1137–1148.
- Schönbeck L, Gessler A, Hoch G, McDowell NG, Rigling A, Schaub M, Li MH. 2018. Homeostatic levels of nonstructural carbohydrates after 13 yr of drought and irrigation in *Pinus sylvestris*. *New Phytologist* 219: 1314–1324.
- Schuldt B, Buras A, Arend M, Vitasse Y, Beierkuhnlein C, Damm A, Gharun M, Grams TEE, Hauck M, Hajek P *et al.* 2020. A first assessment of the impact of the extreme 2018 summer drought on Central European forests. *Basic and Applied Ecology* 45: 86–103.
- Scott ER, Crone EE. 2021. Using the right tool for the job: the difference between unsupervised and supervised analyses of multivariate ecological data. *Oecologia* 196: 13–25.
- Seidling W. 2007. Signals of summer drought in crown condition data from the German Level I network. *European Journal of Forest Research* 126: 529–544.
- Seifert CL, Lamarre GP, Volf M, Jorge LR, Miller SE, Wagner DL, Anderson-Teixeira KJ, Novotný V. 2020. Vertical stratification of a temperate forest caterpillar community in eastern North America. *Oecologia* 192: 501–514.
- Sellier D, Fourcaud T. 2009. Crown structure and wood properties: influence on tree sway and response to high winds. *American Journal of Botany* 96: 885–896.
- Soliveres S, van der Plas F, Manning P, Prati D, Gossner MM, Renner SC, Alt F, Arndt H, Baumgartner V, Binkenstein J *et al.* 2016. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature* 536: 456–459.
- Shultz CM, Gehring CA, Whitham TG. 2009. Deadly combination of genes and drought: increased mortality of herbivore-resistant trees in a foundation species. *Global Change Biology* 15: 1949–1961.
- Strand G. 1997. Effects of early summer drought on the crown density of Norway spruce. *Forestry: An International Journal of Forest Research* 70: 157–160.
- Tamburini G, Dani E, Bommarco R, Marini L. 2018. Effect of insect herbivory on plant community dynamics under contrasting water availability levels. *Journal of Ecology* 106: 1819–1828.
- Touma D, Ashfaq M, Nayak MA, Kao S-C, Diffenbaugh NS. 2015. A multi-model and multi-index evaluation of drought characteristics in the 21st century. *Journal of Hydrology* 526: 196–207.
- Tschaplinski TJ, Abraham PE, Jawdy SS, Gunter LE, Martin MZ, Engle NL, Yang X, Tuskan GA. 2019. The nature of the progression of drought stress drives differential metabolomic responses in *Populus deltoides*. *Annals of Botany* 124: 617–626.
- UNCCD. 2022. *Drought in numbers 2022- restoration for readiness and resilience*. Côte d'Ivoire: UNCCD.
- Walter J, Nagy L, Hein R, Rascher U, Beierkuhnlein C, Willner E, Jentsch A. 2011. Do plants remember drought? Hints towards a drought-memory in grasses. *Environmental and Experimental Botany* 71: 34–40.
- Waser LT, Fischer C, Wang Z, Ginzler C. 2015. Wall-to-wall forest mapping based on digital surface models from image-based point clouds and a NFI forest definition. *Forests* 6: 4510–4528.
- Wohlgemuth T, Kistler M, Aymon C, Hagedorn F, Gessler A, Gossner MM, Quézel V, Vögeli I, Wasem U, Vitasse Y *et al.* 2020. Früher Laubfall der Buche während der Sommertrockenheit 2018: resistenz oder Schwächesyndrom? *Schweizerische Zeitschrift Für Forstwesen* 171: 257–269.
- Zang U, Goisser M, Grams TE, Häberle K-H, Matyssek R, Matzner E, Borken W. 2014. Fate of recently fixed carbon in European beech (*Fagus sylvatica*) saplings during drought and subsequent recovery. *Tree Physiology* 34: 29–38.
- Zang U, Goisser M, Meyer N, Häberle K-H, Borken W. 2021. Chemical and morphological response of beech saplings (*Fagus sylvatica* L.) to an experimental soil drought gradient. *Forest Ecology and Management* 498: 119569.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Long-term climatic data from study regions.

Fig. S2 Drought, stratum and annual effects on lignin as well as primary and specialized metabolome.

Fig. S3 Principal component analysis score plots of primary and specialized metabolome.

Fig. S4 Most influential compounds selected via partial least squares discriminant analysis.

Fig. S5 Drought, crown stratum and annual effects on damage probability.

Fig. S6 Drought, crown stratum and annual effects on damage quantities.

Fig. S7 Piecewise structural equation models on relationship between drought, crown stratum annual effects chemistry and damage probabilities.

Fig. S8 The effect of most influential specialized metabolites on damage probabilities.

Fig. S9 The effect of most influential specialized metabolites on damage quantities.

Fig. S10 Piecewise structural equation models on relationship between drought, crown stratum annual effects chemistry and damage quantities.

Methods S1 Analysis of primary and specialized metabolome.

Methods S2 Herbivory assessments.

Methods S3 Statistical analyses in R.

Methods S4 Piecewise structural equation models.

Table S1 Location and characteristics of drought-stressed and control plots in the study sites.

Table S2 Most influential compounds in the partial least squares discriminant analysis models of drought-stressed trees in comparison to control trees 1 or 2 yr after the drought.

Table S3 Results of the piecewise structural equation models including standardized estimates.

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