Oecologia (2020) 192:893–907
https://doi.org/10.1007/s00442-020-04622-y

PHYSIOLOGICAL ECOLOGY – ORIGINAL RESEARCH

To compete or defend: linking functional trait variation with life-history tradeoffs in a foundation tree species

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Received: 13 March 2019 / Accepted: 8 February 2020 / Published online: 14 February 2020

Abstract

Although chemical deterrents to herbivory often exact costs in terms of plant growth, the manner in which those costs arise, and their physiological relationship to other functional traits, remain unclear. In the absence of appreciable herbivory, we examined interrelationships among chemical defense levels and other foliar functional traits (e.g., light-saturated photosynthesis, specific leaf area, nitrogen concentration) as co-determinants of tree growth and, by extension, competitive ability in high-density populations comprising 16 genotypes of *Populus tremuloides*. Across genotypes, concentrations of chemical defenses were not significantly related to other leaf functional traits, but levels of the salicinoid phenolic glycosides (SPGs) salicin, salicortin and tremulacin were each negatively correlated with relative mass growth (RMG) of aboveground woody tissue ($P \leq 0.001$). RMG, in turn, underpinned 77% of the genotypic variation in relative height growth (our index of competitive ability). RMG was also positively related to light-saturated photosynthesis ($P \leq 0.001$), which, together with the three SPGs, explained 86% of genotypic RMG variation ($P \leq 0.001$). Moreover, results of a carbon balance simulation indicated that costs of resource allocation to SPGs, reaching nearly a third of annual crown photosynthesis, were likely mediated by substantial metabolic turnover, particularly for salicin. The lack of discernible links between foliar defense allocation and other (measured) functional traits, and the illustrated potential of metabolic turnover to reconcile influences of SPG allocation on RMG, shed additional light on fundamental physiological mechanisms underlying evolutionary tradeoffs between chemical defense investment and competitive ability in a foundation tree species.

Keywords Competition · Functional traits · Growth · Salicinoid phenolic glycosides · Photosynthesis

Introduction

Despite the pervasive threat to plant fitness posed by herbivores, considerable phenotypic variation exists, within as well as across plant species, in levels of chemical deterrents to phytophagy. Theories regarding this variable expression are based on a fundamental premise that defense compound production exacts a metabolic or allocation cost, resulting in tradeoffs between growth and resistance to herbivory (Herms and Mattson 1992; Stamp 2003; Züst and Agrawal 2017). Numerous studies have documented the existence of growth-defense tradeoffs in diverse taxa and have further revealed that the magnitude of costs can vary substantially among genotypes and environments (Koricheva 2002; Viola et al. 2010; Cipollini et al. 2014).

Although the existence of chemical defense costs is well supported empirically, the manner in which they influence plant carbon (C) balance—particularly in relation to other functional traits governing C and other resource acquisition, growth and fitness—remains largely unresolved (Züst and Agrawal 2017). To illustrate the potential importance of this knowledge gap, Agrawal (2011) argued, based on the results of evolutionary models, that the actual costs of defense allocation could be completely obscured if phenotypic variation in resource acquisition (e.g., photosynthetic performance) overshadowed that in allocation to defense. Accordingly, an improved understanding of interrelationships among defenses and other leaf biochemical, structural and/or physiological traits, particularly those governing

Communicated by Fernando Valladares.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00442-020-04622-y) contains supplementary material, which is available to authorized users.

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acquisition of resources such as C, may shed light on the causes underlying inconsistent findings with respect to the existence and/or magnitude of defense costs (e.g., Simms and Rausher 1989; Koricheva 2002; Cipollini et al. 2014).

Regarding the foliar functional traits mediating variation in plant C gain, a growing body of evidence (e.g., Kaelke et al. 2001; Kruger and Volin 2006; Dillaway and Kruger 2014) indicates that, particularly among tree species, C gain is often closely and positively correlated with photosynthesis per unit leaf mass (A_{mass}). In turn, A_{mass} variation is governed by a number of other leaf traits, including stomatal conductance (g_s), leaf nitrogen concentration (N_{mass}), and specific leaf area (SLA). In the context of these functional traits, which are principal components of the leaf economics spectrum (Wright et al. 2004), synthesis of constitutive chemical defenses may negatively influence plant C and biomass gain through one or more physiological mechanisms. First, in lieu of additional leaf area production, accumulation of foliar defensive compounds could decrease SLA and, consequently, mass-based photosynthetic capacity (Wright et al. 2004). Second, photosynthetic capacity may also decline owing to the diversion of nutrients and key metabolic intermediates from the photosynthetic apparatus to enzymes involved in defense compound synthesis (Jones and Hartley 1999). This constraint would be manifested, for example, by a decline in photosynthetic nitrogen use-efficiency (PNUE) (Feng et al. 2009). Third, regardless of where metabolically expensive, C-rich defenses accumulate in the plant, their synthesis and subsequent metabolism can accelerate C loss rates (Zangerl et al. 1997), thereby lowering the efficiency with which assimilated C is used to produce biomass. This likelihood is especially pronounced in the case of labile compounds that undergo substantial metabolic turnover (Neilson et al. 2013), which frequently plays an important role in models predicting costs of allocation to chemical defenses (e.g., Coley et al. 1985; Mooney and Gulmon 1982).

We contend that these potential consequences, their interrelationships, and their implications for growth and competitive ability have not been adequately explored. Accordingly, in high-density populations comprising 16 genotypes (genets) of trembling aspen (*Populus tremuloides* Michx.), we examined correlations among chemical defense levels and other foliar functional traits (e.g., light-saturated A_{mass}, SLA, N_{mass}, PNUE), and assessed their respective roles as co-determinants of tree biomass and height growth in the absence of appreciable herbivory. Here we assume that, in high-density stands, relative height growth governs future access to light and is, therefore, the foremost metric of competitive ability for a fast-growing, early successional, shade-intolerant tree species.

Trembling aspen is an appealing model system, as it has the broadest geographic range of all North American tree species, and, correspondingly, exhibits striking genetic variation in many phenotypic traits, including those associated with physiology, growth and defense (Lindroth and St. Clair 2013). Chemical defenses in aspen consist primarily of phenolic compounds derived from the phenylpropanoid pathway. The signature secondary metabolites of aspen comprise a suite of four salicinoid phenolic glycosides (SPGs): salicin, salicortin, tremuloidin, and tremulacin (Lindroth and St. Clair 2013). Of these, salicortin and tremulacin occur in high concentrations (1–8% of leaf dry mass each, occasionally attaining 15%, Hemming and Lindroth 1999; Donaldson et al. 2006a). In aspen and its congeners, a growing body of evidence suggests that SPGs exhibit considerable turnover in leaves and other organs (Kleiner et al. 1999; Massad et al. 2014). Although moderately inducible, expression of these phenolics is largely constitutive in nature, and constitutive genotypic variation greatly exceeds herbivore-induced plasticity (Rubert-Nason et al. 2015). Condensed tannins (CTs) are also abundant, with foliar levels ranging between 1–25% of leaf dry mass (Hemming and Lindroth 1999; Donaldson et al. 2006b). In aspen, however, SPGs appear to be much more effective than CTs as herbivore deterrents, especially against lepidopteran defoliators (Lindroth and St. Clair 2013).

Focusing, then, on the implications of allocation to aspen's suite of secondary metabolites, we tested the following interrelated hypotheses, which collectively map the mechanistic connections between competitive ability, growth and leaf functional traits—including levels of foliar chemical defenses—in the absence of substantive aboveground herbivore damage:

1. Across our 16 aspen genotypes, variation in mass growth of aboveground woody tissue—a principal determinant of tree height growth and competitive ability—is positively related to light-saturated A_{mass}.
2. In turn, genotypic variation in light-saturated A_{mass} and associated leaf traits (SLA, N_{mass} and PNUE) is negatively related to foliar SPG and/or CT concentration.
3. Alternatively, genotypic variation in mass growth of aboveground woody tissue is directly and negatively related to foliar SPG and/or CT concentrations, owing to hypothetical losses of assimilated C associated with appreciable metabolic turnover of defense compounds.

Methods

Aspen populations

In the fall of 2010, a set of 18 populations, each comprising 16 trembling aspen genotypes, was established at the Arlington Agricultural Research Station (AARS), University of Wisconsin (43.3° N 89.3° W). The 16 genotypes, which

have been studied extensively (e.g., Donaldson et al. 2006a, b; Stevens et al. 2008; Keefover-Ring et al. 2015; Holeski et al. 2016; Cope et al. 2019), were originally collected from natural aspen stands located in different areas of Wisconsin, and were confirmed to be unique by microsatellite analysis (Cole 2005). Ramets were micropropagated (Donaldson et al. 2006a, b) in early 2010, and then grown individually in small pots containing soilless growth media (Metro-Mix 350, SunGro Horticulture, Agawam, MA, USA). In October 2010, ramets were transplanted to a common garden at AARS, which had been plowed, disked and overlain with landscape cloth. The garden was underlain by a comparatively fertile, silt loam soil (Huntsville series, mesic Cumulic Hapludoll).

Within a given 5 m × 5 m population, one ramet from each of the 16 genotypes was randomly assigned to a location on a 0.5 m × 0.5 m grid in each of four interior (2 m × 2 m) quarters. The resulting 64-tree, 4 m × 4 m square (including a total of four trees per genotype across the four quarters) was surrounded by an additional set of 36 perimeter trees (drawn from surplus ramets), for a total of 100 trees per population. The planting density (40,000 trees ha^{-1}) was intended to mimic the relatively intense competition often observed in newly established aspen stands of seed origin (e.g., Romme et al. 1995).

The populations were hand weeded, while the 2 m inter-population buffers were periodically mowed. Initially, nine of the 18 populations were protected from mammalian browsers. In the unprotected populations, browsing was modest and confined mostly to perimeter trees. Hence, at the onset of the present study (2013), measures of tree growth and associated traits did not differ significantly between protected and unprotected populations (Keefover-Ring, Kruger and Lindroth, unpubl. data). Tree mortality prior to the 2013 growing season was less than 2% across the 18 populations. During the growing season (May 15 through September 30) of 2013, air temperature averaged 18.2 °C (0.6 °C higher than the average between 1981 and 2010), and total precipitation was 466 mm (4 mm above the average between 1981 and 2010) (AWON 2015).

Functional trait measurements

In late June 2013, we collected 15–25 mature leaves from throughout the crown of every tree in all 18 populations. Leaves were excised at the base of the lamina, placed on ice and transported to the laboratory, where they were freeze-dried and stored at − 20 °C until chemical analysis. The dried leaves were milled with 30 3-mm steel beads in 20-mL plastic scintillation vials, and then assayed for phenolic glycoside (SPG) concentrations using ultra high-performance liquid chromatography (UHPLC), as described by Rubert-Nason et al. (2018). Salicin standard was obtained from Sigma-Aldrich, while salicortin and tremulacin standards were purified from aspen tissue in our laboratory. We quantified condensed tannin (CT) concentrations using the acid butanol assay described by Porter et al. (1985), with purified CTs from aspen as a standard. Leaf nitrogen concentration (N_{mass}) was determined using a Thermo Finnigan Flash 1112 elemental analyzer (Thermo Finnigan, San Jose, CA, USA).

On five separate dates, beginning in late July and extending through late August, light-saturated photosynthesis and stomatal conductance were measured on a mature, sunlit leaf from each of the 16 genotypes using a LI-6400 portable photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). Assessments, conducted in five randomly chosen populations (one per date), were confined to the upper third of the crown on trees with a "neutral" competitive status at the beginning of 2013 (see explanation below). During measurements, which occurred between 0800 and 1200 h CDT, photosynthetic photon flux, CO_2 partial pressure in the reference chamber and block temperature were maintained at 2000 µmol m^{-2} s^{-1}, 40 Pa and 25 °C, respectively. On a given date, following gas exchange assessments, all 16 leaves were harvested, measured for fresh area with a LI-3100 leaf area meter (Li-Cor Biosciences), oven-dried at 70 °C for at least 96 h, and weighed. These data (total of five leaves per genotype) were used to determine specific leaf area (SLA, m^2 kg^{-1}) and, ultimately, light-saturated photosynthesis per unit leaf mass (A_{sat}).

We note that, based on ocular estimates, the areal extent of leaf damage (e.g., from herbivores and pathogens) observed on foliage sampled during the aforementioned campaigns rarely exceeded 10%, and was typically less than 5%. Furthermore, we did not detect genotypic differences in damage levels. Correspondingly, folivore activity was negligible in our aspen populations during the 2013 growing season.

Measurement of aboveground woody growth and tree competitive ability

In the fall of 2012 and 2013, following leaf senescence, we measured the total height (H, from soil surface to apical bud) and basal stem diameter (D, at 2 cm above soil surface) of every tree in all populations. Additionally, in the winter of 2013–2014, we harvested, oven-dried (at 70 °C for 21 days) and weighed the stem and branches of 2–3 randomly selected trees per genotype in each of nine populations (total = 22–27 trees per genotype). Based on these data, we used multiple linear regression to develop, for each genotype, an allometric equation relating dry mass of the stem and branches to a stem volume index (D^2H, cm^3). Highly significant and precise models were generated for all genotypes ($P < 0.0001$, $r^2 \geq 0.9$, RMSE < 5% of overall variable range, see Online Resource 1), and the resulting polynomial equations were

used to estimate aboveground woody mass (M) for every tree at the end of 2012 and 2013. With those data, we calculated relative mass growth (RMG) of aboveground woody tissue for every tree as $\ln(M_{2013}) - \ln(M_{2012})$.

Our index of a tree's competitive ability was its relative height growth (RHG) during the 2013 growing season. An individual's RHG was calculated as $\ln(H_{2013}) - \ln(H_{2012})$. Among all of the trees in the common garden, only a subset was included in our growth analyses, based on tree competitive status at the beginning of the 2013 growing season. Namely, trees were included only if their initial competitive status was "neutral," meaning that their crowns were in a codominant position and their heights at the beginning of 2013 were within ± 0.5 m of the average height of the four nearest neighbors. Perimeter trees, as well as those in the adjacent row (i.e., fourth row from each population center), were excluded. The resulting subset included 15–33 individuals per genotype, for a total of 386 trees, sampled from 10–17 populations. Among these "neutral" trees, averages for initial height, basal diameter and aboveground woody mass were 3.9 m, 3.3 cm and 317 g, respectively.

Statistical analyses

Significance of observed trait variation among genotypes was assessed with analysis of variance (ANOVA), treating genotype as a fixed effect and population as a random (block) effect. Interrelationships among functional traits were examined with linear regression and path analysis. When necessary, variables were transformed (e.g., with a square root or ln) prior to analysis to minimize heteroscedasticity in residuals. Unless stated otherwise, ANOVAs, correlations and regressions were considered significant when $P \leq 0.05$. Analyses of mixed- and fixed-effects linear models were performed using JMP Pro v. 11 statistical software (SAS Institute Inc., 2013), while path analysis was performed with Stata 14 Data Analysis Software (StataCorp, 2015).

Mass-balance simulation of aboveground woody growth

To further examine the role of resource allocation to SPGs (salicin, salicortin, tremulacin) as a co-determinant of RMG, we constructed a dual time-step simulation of tree C gain and allocation during the 2013 growing season (May 15 through September 30). The principal intent of this modeling exercise was to characterize the pattern of C investment in SPG metabolism that would quantitatively reconcile observed relationships between aboveground woody growth and leaf SPG concentration, given the observed genotypic values (means) for RMG and measured leaf traits, and operating under the assumption that the magnitude of C allocation to chemical defenses scales monotonically with genotypic variation in leaf SPG levels. We note that CTs did not exert a significant influence on growth or leaf traits governing photosynthesis (see "Results"), and, thus, they were omitted in this simulation, which focused exclusively on the role of SPGs.

Key output from our simulation (Fig. 1), based on a growth model presented by Coley et al. (1985), includes a set of derived coefficients (e.g., a and b) in an expression quantifying the relative deflection in C that would otherwise have been allocated to aboveground woody growth, where relative deflection $= a[\mathrm{SPG}]^b$. A numerical optimization program (Excel Solver) identifies the set of coefficient values that afford the closest possible match between predicted and observed genotype means for relative mass growth (RMG) of aboveground woody tissue. Based on those coefficient values, the simulation then generates genotypic estimates of net metabolic turnover and fractional C allocation for the three SPGs.

The spreadsheet-based (Excel) model is calibrated with observed genotype means for leaf traits (Online Resources 2 and 3), local solar radiation and air temperature data retrieved from the Automated Weather Observation Network (AWON), ancillary data collected from the populations in 2013 and 2014, and information (data and formulas) regarding leaf and stem tissue metabolism and chemistry, tissue construction costs, C allocation patterns, etc., drawn from the literature. In Online Resource 4, we provide calibration values for parameters in the crown photosynthesis and C allocation submodels along with additional notes and caveats regarding model structure. The simulation itself is contained in Online Resource 5 (Excel workbook). Below, we provide detail on the structure and calibration of two submodels: crown photosynthesis and C allocation.

Crown photosynthesis submodel

The core of this subroutine is a model (1) characterizing the response of leaf photosynthesis (A_{leaf}, nmol g^{-1} s^{-1}) to variation in incident light intensity (Hanson et al. 1987). The model is driven by photosynthetic photon flux (PPF, μmol m^{-2} s^{-1}) and three intrinsic parameters (Table A1): light-saturated photosynthesis (A_{sat}, nmol g^{-1} s^{-1}), daytime respiration of a darkened leaf (R_{d}, nmol g^{-1} s^{-1}), and light compensation PPF (LCP, μmol m^{-2} s^{-1}).

$$A_{\mathrm{leaf}} = A_{\mathrm{sat}}\left[1 - \left(1 - R_{\mathrm{d}}/A_{\mathrm{sat}}\right)^{\mathrm{1\text{-}PPF/LCP}}\right] \quad (1)$$

While A_{sat} is assumed to be relatively insensitive to temperature under current levels of atmospheric CO_2 (e.g., Volin et al. 2002), LCP is assigned a temperature-normalized value (Table A1) and an activation energy ($E = 51.2$ kJ mol^{-1} K^{-1}), which, when incorporated in the Arrhenius equation, allows LCP to double with a 10 °C increase in leaf temperature

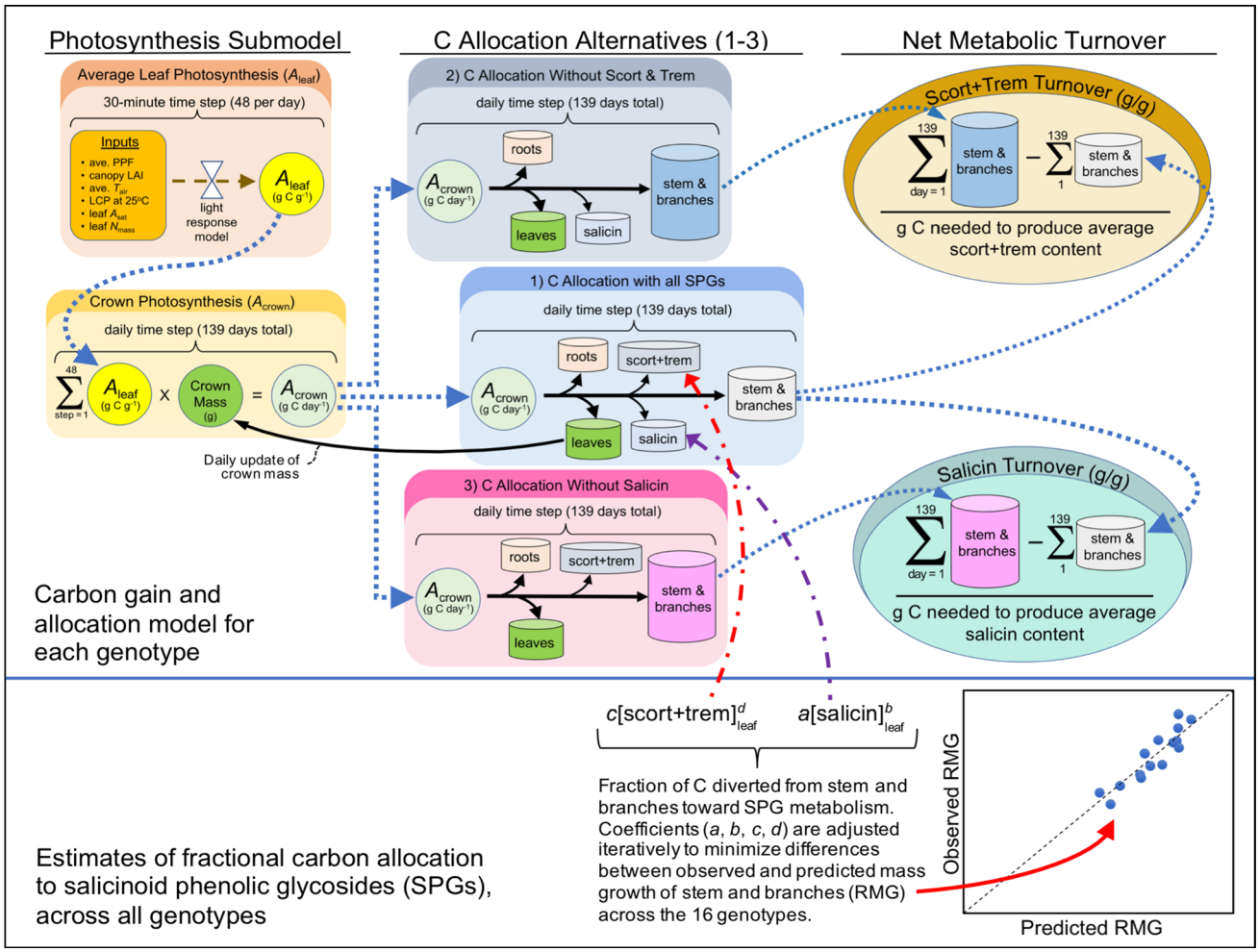

Fig. 1 Overall structure of our carbon (C) gain and allocation model (Online Resource 5), which comprises submodels for crown photosynthesis, C allocation and net metabolic turnover of salicinoid phenolic glycosides (SPGs), including salicin and the combination of salicortin and tremulacin (scort + trem). Resulting allocation and metabolic turnover estimates (Figs. 6 and 7) characterize patterns of C investment in SPG metabolism that would quantitatively reconcile the apparent influence of leaf SPG investment on relative mass growth (RMG) of aboveground woody tissue. Using outputs from three alternative C allocation scenarios (1–3), net metabolic turnover is calculated as seasonal C flux to SPG metabolism divided by the amount of C used to produce the growing season average for aboveground (leaf and bark) SPG content. The numerator depends on fractional C allocation to SPGs, which, in turn, is a function of foliar SPG concentrations [in brackets]. Coefficient values for fractional SPG allocation formulas (a, b, c, d) are generated using Solver in Excel, based on minimization of the sum of squared differences between observed and predicted values for RMG across the 16 aspen genotypes. We note that C flux to stem and branches in C allocation alternative 1 is the principal driver of predicted genotypic variation in RMG. Solid black arrows indicate C fluxes, whereas other arrows track the roles of key model parameters. For the sake of clarity, the feedback (solid black arrow) from C allocation alternatives 2 and 3 to crown mass is not included. *PPF* photosynthetic photon flux, T_{air} air temperature, A_{sat} light-saturated photosynthesis, LAI leaf area index, N_{mass} nitrogen concentration, *LCP* light compensation PPF. This figure is available in color in the online version of the journal

(assumed here to equal air temperature). This is a commonly adopted characterization of metabolic temperature sensitivity that is often consistent with observed leaf behavior (e.g., Lewis et al. 1999; Zufferey et al. 2000; Dillaway and Kruger 2011). Leaf R_d is then calculated as a function of LCP.

Using the light-response model (1), crown-level averages for A_{leaf} are estimated for each of the 16 aspen genotypes during every 30-min interval throughout the diel cycle from May 15 through September 30. To account for heterogeneity in crown light environment, every 30-min mean comprises an average of A_{leaf} calculated for upper and lower halves of the crown. These averages are based on a 30-min mean for incident PPF (calculated from local solar radiation data, AWON 2015) that is attenuated, for each half, using the Beer-Lambert law, calibrated with a canopy-level value for leaf area index (LAI) and an extinction coefficient (k) = −0.1 and −0.3 for the upper and lower crown halves, respectively. Those two k values reflect the distribution of transmittance

values observed during a vertical assessment of canopy light attenuation in the aspen populations during September 2014. That assessment, conducted under diffuse light conditions, entailed ~ 2400 instantaneous, spatially explicit measurements of PPF at 50-cm height intervals using a 20-sensor array, which was inserted into the canopy at arbitrarily chosen locations within the interior of a given population. Each of those PPF measures was then converted to a transmittance based on simultaneously recorded values from a proximal open-sky PPF sensor. During the A_{leaf} simulation, when the 30-min average for open-sky PPF falls to 0, the aforementioned A_{mass} model is superseded by an estimate of nighttime leaf respiration (R_n, Online Resource 4) derived from (1) a temperature-normalized prediction based on leaf nitrogen concentration (Dillaway and Kruger 2011), and (2) an adjustment of that predicted value to account for variation in leaf (air) temperature using the Arrhenius equation (where, again, $E = 51.2$ kJ mol^{-1} K^{-1}).

For each genotype, a daily, crown-level estimate of C assimilation (A_{crown}) is then calculated by integrating average A_{leaf} ($-R_n$) through a 24-h period and multiplying that integral by crown foliar mass. For all genotypes, the latter is initially set (on May 15) to 60 g, based on a global allometric relationship between total leaf mass and an index of woody stem volume [the square of basal stem diameter (D^2) × total stem height (H)] generated from data collected in 2014 (Helm, Kruger, Keefover-Ring and Lindroth, unpubl. data), with measures of initial D^2H in 2013. That initial value of crown foliar mass is continually augmented, through the first 61 days of the growing season (until simulated budset on July 15), owing to daily C allocation to new leaf construction (see explanation below). Daily A_{crown} estimates are then used as inputs to the C allocation submodel.

C allocation submodel

On a daily basis, throughout the growing season (May 15–September 30), A_{crown} is allocated to various processes using a hierarchical allocation scheme (e.g., Schippers et al. 2015). The highest priorities are allocation to belowground processes (e.g., coarse root production, fine root production and turnover, maintenance respiration, mycorrhizae, exudation), production of new foliage and maintenance respiration in aboveground woody tissue (Online Resource 4). The remaining assimilated C is then allocated either to metabolism associated with the synthesis and turnover of SPGs or biomass accumulation (other than bark SPGs) in stem and branches. The manner in which C is partitioned between these two processes is explained below.

Based on the growth model proposed by Coley et al. (1985), which incorporates costs of foliar chemical defenses, we rely on a mass-balance approach to estimate the proportion of assimilated C invested in synthesis and turnover of three SPGs—salicin, salicortin and tremulacin. Specifically, we use the SOLVER tool in Excel to determine, via minimization of the sums of squares of differences between observed and estimated genotype means for relative mass growth of aboveground woody tissue (RMG), values of the coefficients a, b, c and d used to quantify the metabolic costs of investment in salicin and the combination of salicortin and tremulacin, where, expressed as a fraction of C assimilation, cost $= a[\text{salicin}]^b + c[\text{salicortin} + \text{tremulacin}]^d$ (g g^{-1}). Specifically, on a daily basis, C allocation to construction of aboveground woody tissue (stem and branches) is adjusted downward by multiplying the available assimilated C (after allocation belowground, to new foliage, and to stem plus branch maintenance respiration) by $(1 - a[\text{salicin}]^b + c[\text{salicortin} + \text{tremulacin}]^d)$, where the same coefficient values (a, b, c and d) are applied to all genotypes (assuming constant cost per unit allocation to defense).

Next, for each SPG variable ([salicin] and [salicortin + tremulacin]), we (1) predict the increase in aboveground woody mass that would have occurred if no C was allocated to its synthesis/turnover, (2) multiply that differential by the estimated costs of stem and branch construction (Online Resource 4), and (3) divide the resulting product by the seasonal total for A_{crown}, yielding an estimate of fractional C allocation to synthesis/turnover of that SPG. We also use the product of step 2 to quantify net metabolic turnover, expressed as "production equivalents" by dividing that value by the seasonal average content of each SPG in the crown (based on the measured SPG levels in foliage), and adjusting the quotient to account for theoretical construction costs (CC) for each SPG (Poorter 1994). Specifically, CC (g C [g biomass]$^{-1}$) is calculated based on the C concentration ([C]) of a given SPG, where $CC = 0.4(5.08[C] - 1.04)$. Thus, the estimated CC for salicin, salicortin and tremulacin is 0.69, 0.73 and 0.83 g C [g biomass]$^{-1}$, based on a [C] of 0.545, 0.566 and 0.614, respectively. In addition, we generate an alternative estimate of "production equivalents" based on the assumption that SPGs are also present in the bark of aspen stems and branches, and that, for all genotypes, bark SPG levels resemble those in foliage (Palo 1984, Massad et al. 2014, Keefover-Ring et al. 2015—adjusting bulk stem concentrations for the estimated proportion of stem mass in bark). We acknowledge that, given the evidence at hand, the latter assumption may be more valid for the more complex SPGs (salicortin and tremulacin) than for salicin. An additional assumption is that bark comprises 30% of total dry mass in stem and branches, based on a stem diameter at breast height that averaged roughly 2 cm during the 2013 growing season (Tullus et al. 2009).

Model calibration with estimates of uncertainty

Several model parameters are calibrated either with genotype means for measured traits (e.g., A_{sat}) or estimates derived from those traits (e.g., leaf nighttime respiration, R_n). In the case of five parameters, however, we lack data from the aspen populations in 2013, and, instead, calibrate them with values from relevant literature or ancillary data from other years (Online Resource 4). Given the absence of information regarding the magnitude of genotypic variation in these parameters across our populations in 2013, and the potential for that variation to exert an important influence on simulation results, we incorporate a reasonable level of uncertainty in parameter calibration. Namely, for each simulation run, a value for each of the five parameters is randomly sampled from a Gaussian distribution [using function NORMINV(RAND(), $\overline{X}$, SD)] in which, except for CC of aboveground woody tissue, the coefficient of variation (CV) = 10% of the mean (Online Resource 4). For the CC of aboveground woody tissue, we used a CV = 1%, as studies (e.g., Kaakinen et al. 2004) have shown that, at least in a common garden, stem chemical composition (i.e., stem [C] and [N]) varies only slightly among aspen genotypes. For a given genotype, the resulting uncertainty in our simulation outcomes is portrayed as a standard deviation.

Results

Genotypic trait variation

Across the 16 aspen genotypes, we observed considerable variation in several leaf functional traits (> 0.4-fold range, $P \leq 0.001$, Online Resources 2 and 3), including light-saturated photosynthesis (A_{sat}), stomatal conductance (g_s), photosynthetic nitrogen use-efficiency (PNUE = A_{sat}/N_{mass}), and specific leaf area (SLA), as well as concentrations of condensed tannins (CTs) and each of the measured salicinoid phenolic glycosides (SPGs). A substantial range (> 0.75-fold, $P \leq 0.001$, Online Resource 6) was also observed in relative mass growth (RMG) of aboveground woody tissue and relative height growth (RHG). Particularly for measures of leaf chemistry and morphology, observed trait variation within a genotype was relatively low, and heritability across genotypes was high (Online Resources 2, 3 and 6).

Functional trait interrelationships

Among leaf traits, A_{sat} was positively related to N_{mass}, PNUE, g_s and SLA ($P \leq 0.006$), while PNUE was positively correlated with g_s and SLA ($P \leq 0.004$, Fig. 2). None of the aforementioned leaf traits were significantly correlated with levels of chemical defenses (CTs or any of the SPGs, Fig. 2). Among defensive chemicals, however, CT concentration was negatively related to the combined concentrations of salicortin and tremulacin ($P = 0.011$).

RMG was positively correlated ($P \leq 0.05$) with several leaf functional traits, including A_{sat}, N_{mass}, PNUE, and SLA, and negatively correlated with levels of salicin and salicortin + tremulacin (Fig. 3). We employed path analysis to assess the hypothetical roles of measured leaf traits as co-determinants of variation in RMG (Fig. 4), which again was positively related to A_{sat} ($P \leq 0.001$), and negatively related to both [salicin] ($P \leq 0.001$) and [salicortin + tremulacin] ($P \leq 0.001$). Standardized (β) coefficients indicated that, in terms of magnitude, the relative influences of the two SPG variables on RMG were similar to one another and to that of A_{sat}. Additionally, analysis verified the presumed existence of a strong and positive relationship between RMG and RHG ($\beta = 0.88$, $P \leq 0.001$).

Regression models of aboveground woody growth

Based on the relationships highlighted above, we regressed RMG against an additive combination of individual leaf traits. After accounting for potential nonlinearity in trait relationships by ln-transforming all variables, the following combination of A_{sat}, [salicin] and [salicortin + tremulacin] explained 86% of the variation in RMG ($P \leq 0.001$, Fig. 5a):

$$\ln(\mathrm{RMG}) = 0.7 \ln\left(A_{sat}\right) - 0.145 \ln[\mathrm{salicin}] - 0.221 \ln[\mathrm{salicortin} + \mathrm{tremulacin}] - 3.65 \quad (2)$$

Mass-balance simulation of aboveground woody growth

Although the model (2) above related growth to foliar traits, it was not useful for estimating costs or C allocation associated with chemical defenses. In particular, generation of credible reference values for growth in the absence of defense allocation was precluded by the fact that the model yielded unreasonably high growth estimates (e.g., exceeded observed values by at least ten fold) at SPG levels below the observed ranges (data not shown). Thus, to further examine the role of resource allocation to SPGs as a co-determinant of aboveground woody growth, we relied on the dual time-step simulation of tree C gain and allocation during the 2013 growing season (Fig. 1; model calibration detailed in Online Resource 4; actual simulation provided in Online Resource 5).

In the process of producing a close fit between observed and simulated RMG ($r^2 = 0.83$, $P \leq 0.001$, Fig. 5b) that resembled the trend in Fig. 5a, our simulation yielded several noteworthy results. First, to generate the trend in

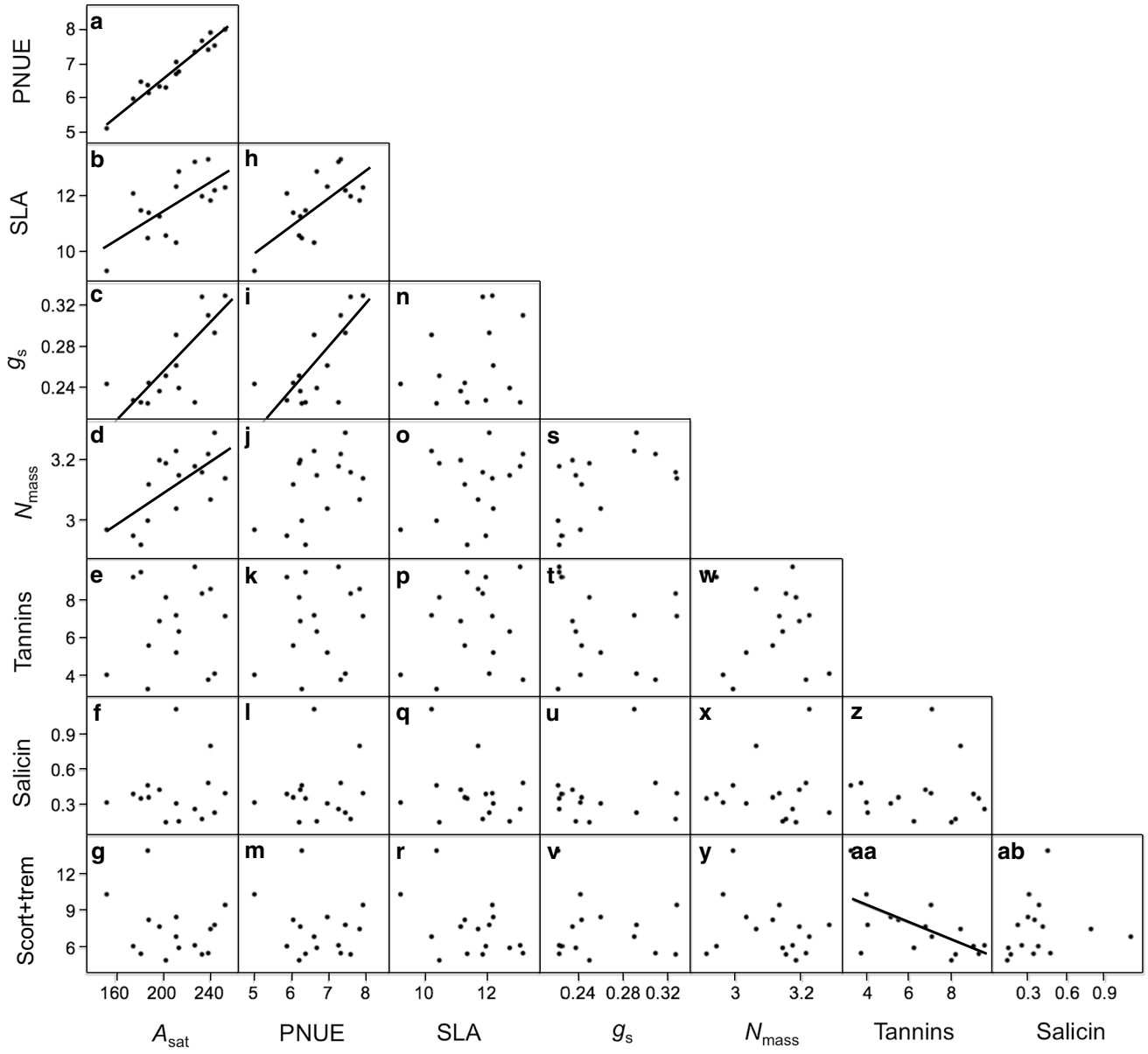

Fig. 2 Relationships among leaf traits, including light-saturated photosynthesis (A_{sat}, nmol g^{-1} s^{-1}, panels **a**–**g**), photosynthetic nitrogen-use efficiency (PNUE, calculated as A_{sat}/N_{mass}, μmol [g^{-1} N] s^{-1}, panels **a**, **h**–**m**), specific leaf area (SLA, m^2 kg^{-1}, panels **b**, **h**, **n**–**r**), stomatal conductance (g_s, mol m^{-2} s^{-1}, panels **e**, **i**, **n**, **s**–**v**), as well as foliar concentrations (%) of nitrogen (N_{mass}, panels **d**, **j**, **o**, **s**, **w**–**y**), condensed tannins (panels **e**, **k**, **p**, **t**, **w**, **z**, **aa**), salicin (panels **f**, **l**, **q**, **u**, **x**, **z**, **ab**) and the combination of salicortin and tremulacin (scort + trem, panels **g**, **m**, **r**, **v**, **y**, **aa**, **ab**). The latter two were combined because they were closely correlated with one another ($r = 0.97$, data not shown). Data are genotype means ($n = 16$). Trend lines indicate significant correlations ($P \leq 0.05$)

Fig. 5b, the simulation solved for four coefficients (*a*, *b*, *c*, *d*) in an expression characterizing the relative amount (decimal fraction) of C allocated to aboveground woody growth, where allocation = 1 – (*a*[salicin]^b + *c*[salicortin + tremulacin]^d), with SPG levels expressed in g g^{-1}. When the simulation was calibrated with the values for inputs provided in Online Resource 4, the derived values for *a*, *b*, *c*, and *d* were 2.36, 0.54, 1.75 and 1.18, respectively.

As a manifestation of the derived coefficient values (*a*, *b*, *c*, *d*), simulation output indicated that a substantial amount of C was allocated to SPG metabolism (Figs. 6a and 7a), owing in large part to considerable metabolic turnover (Figs. 6b and 7b). Our estimate of net metabolic turnover, calculated as the quotient of seasonal C flux to SPG metabolism (g C $tree^{-1}$) and the amount of C used to produce the growing-season average for aboveground (leaf

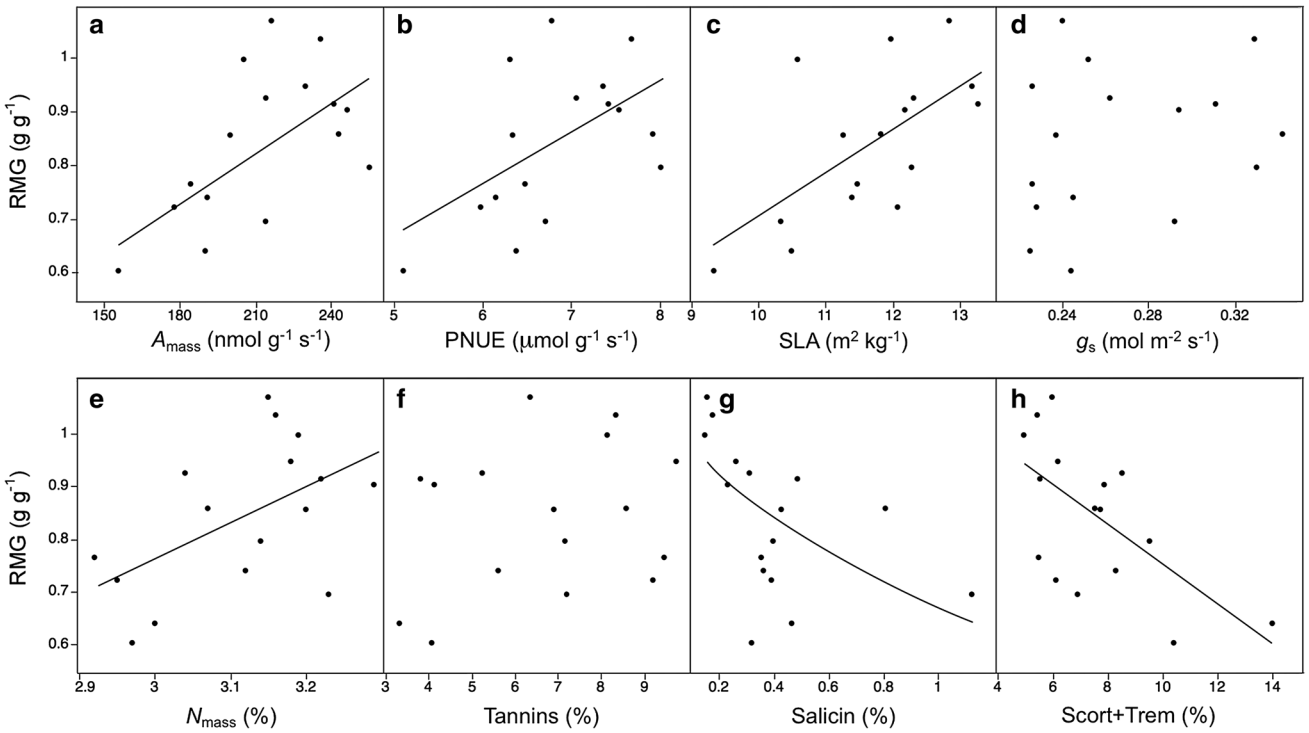

Fig. 3 Relationships between relative mass growth (RMG) of aboveground woody tissue and leaf functional traits, including **a** light-saturated photosynthesis (A_{sat}, nmol g^{-1} s^{-1}), **b** photosynthetic nitrogen-use efficiency (PNUE, calculated as A_{sat}/N_{mass}, μmol [g^{-1} N] s^{-1}), **c** specific leaf area (SLA, m^2 kg^{-1}), **d** stomatal conductance (g_s, mol m^{-2} s^{-1}), as well as foliar concentrations (%) of **e** nitrogen (N_{mass}), **f** condensed tannins, **g** salicin and **h** the combination of salicortin and tremulacin (scort + trem). The latter two were combined because they were closely correlated with one another ($r = 0.97$, data not shown). Data are genotype means ($n = 16$). Trend lines indicate significant correlations ($P \leq 0.05$). In panel **g**, the trendline also reflects natural log transformation of salicin

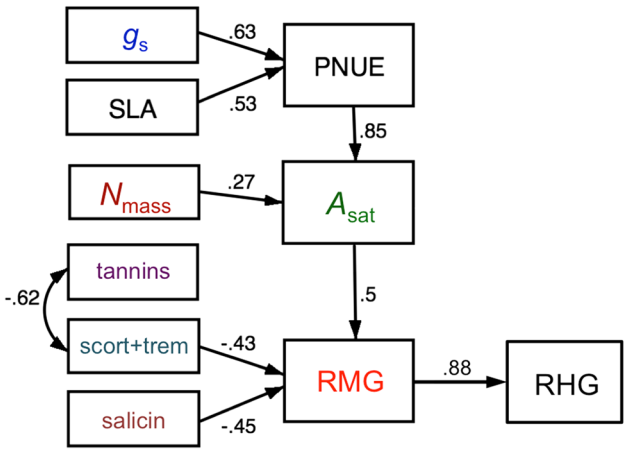

Fig. 4 Path diagrams illustrating interrelationships among leaf traits as co-determinants of relative mass growth (RMG) of aboveground woody tissue, and RMG as a determinant of relative height growth (RHG). Exogenous leaf traits include stomatal conductance (g_s), specific leaf area (SLA), and concentrations of nitrogen (N_{mass}), condensed tannins, salicin, and the combination of salicortin and tremulacin (scort + trem). Endogenous leaf traits include photosynthetic nitrogen-use efficiency (PNUE) and light-saturated photosynthesis (A_{sat}). Arrows indicate significant correlations ($P \leq 0.05$), which are accompanied by their standardized (β) coefficients. Analysis is based on $n = 16$ genotype means for each variable

and bark) SPG content (based on SPG construction costs; Poorter 1994), was especially high and dynamic in the case of salicin. For example, assuming that SPGs were produced in foliage and bark (Palo 1984; Massad et al. 2014), estimated salicin turnover, which declined from 1.5 to 0.6 g [g^{-1} salicin] d^{-1} with increasing foliar [salicin], exceeded that of salicortin + tremulacin by 33-fold on average. As a result, C allocation to salicin surpassed that to higher-order SPGs, despite the fact that foliar concentrations of the latter were typically at least 20-fold higher than those of the former (Figs. 6b and 7b). Consequently, across genotypes, estimates of the total fraction of assimilated C allocated to overall SPG metabolism ranged from 13–32%.

Discussion

Results of path analysis, a regression-based growth model and a mass-balance simulation of tree C gain all support our basic premise that, in the absence of appreciable aboveground herbivore damage, allocation to SPG metabolism is indeed costly for a woody perennial amidst intense intraspecific competition. Our study joins an expanding body of work indicating that costs of allocation to chemical defenses,

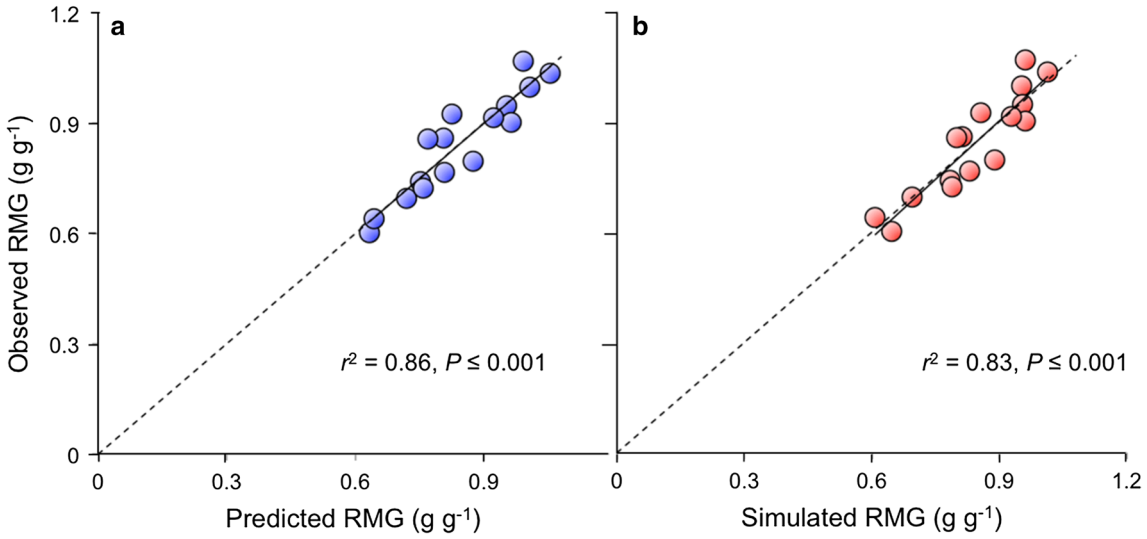

Fig. 5 Observed genotype means ($n = 16$) for relative mass growth (RMG) of aboveground woody tissue, plotted against **a** predicted and **b** simulated values. In **a**, predictions were generated with a model in which all variables were first ln-transformed: $\ln(\mathrm{RMG}) = 0.7\ln(A_{sat}) - 0.145\ln[\text{salicin}] - 0.221\ln[\text{salicortin} + \text{tremulacin}] - 3.65$, where A_{sat} is light-saturated photosynthesis (nmol g^{-1} s^{-1}), and leaf [salicin] and [salicortin + tremulacin] are expressed in %. In **b**, values are outputs from a mass-balance simulation of tree C assimilation and allocation (Online Resource 5). In neither case does the regression slope differ significantly from unity, and neither intercept is significant. Also included is a (dashed) 1:1 line

measured as growth losses, are often most conspicuous under resource limitation (Osier and Lindroth 2006; Donaldson et al. 2006a, b; Sampedro et al. 2011; Cipollini and Lieurance 2012; Moreira et al. 2015). Moreover, in our high-density populations, the close coupling of height and mass growth allows us to clearly illustrate the negative implications of chemical defense allocation for competitive ability. An unambiguous link between the latter two life-history traits has seldom been demonstrated empirically (Noitsakis and Jacquard 1992; Zavala et al. 2004; Viola et al. 2010; Kempel et al. 2011; Ballhorn et al. 2014), especially in a woody perennial (Cipollini et al. 2014).

Contrary to our second hypothesis, the costs of allocation to SPGs are not attributable to detrimental influences on other key growth determinants (e.g., light-saturated photosynthesis, SLA, leaf nutrient status, or photosynthetic nutrient-use efficiency). This finding is consistent with results of previous work on aspen (Donaldson et al. 2006a; Stevens et al. 2008), but negative correlations between leaf photosynthesis and chemical defense levels have been reported in other tree species (e.g., Matsuki and Koike 2006; Massad et al. 2012; Sumbele et al. 2012). Other growth analyses (Goodger et al. 2006; Züst et al. 2015) have revealed a negative relationship between net assimilation rate (NAR) and foliar defense levels. Unfortunately, however, NAR encompasses both leaf C gain and plant C loss (expressed per unit leaf area), and, thus, the implications of defense allocation remain somewhat ambiguous in those analyses.

Arguably, to date, the most compelling elucidation of mechanisms underlying defense costs has resulted from studies of altered growth and metabolism associated with damage-induced defense synthesis. Campos et al. (2016) concluded that, in *Arabidopsis*, growth declines accompanying glucosinate and anthocyanin production resulted not from C losses owing to defense synthesis or turnover, but, rather, from coincident, hormone-mediated decreases in leaf area growth. Conversely, Zangerl et al. (1997) demonstrated that, in wild parsnip, post-damage increases in leaf dark respiration, and, correspondingly, declines in plant C gain, were closely coupled with changes in leaf furanocoumarin levels. While we do not explicitly consider the influence of SPG metabolism on tissue respiration in our analysis, we assume that it is the principal means by which assimilated C is lost as a result of allocation to chemical defenses.

In our study, the independent roles of SPGs and other growth co-determinants imply that phenotypic variation in resource acquisition traits such as photosynthesis could at least partially obscure the costs of allocation to defense (Agrawal 2011). Resolving the roles of defense costs in the context of variation in other growth co-determinants may help explain some of the numerous circumstances in which costs have not been detected (e.g., Price et al. 1989; Simms and Rausher 1989; Ridenour et al. 2008; Harding et al. 2009; Sampedro et al. 2011). In the same vein, we suspect that the existence of costs may be most evident when plants are grown in resource-limited environments primarily because

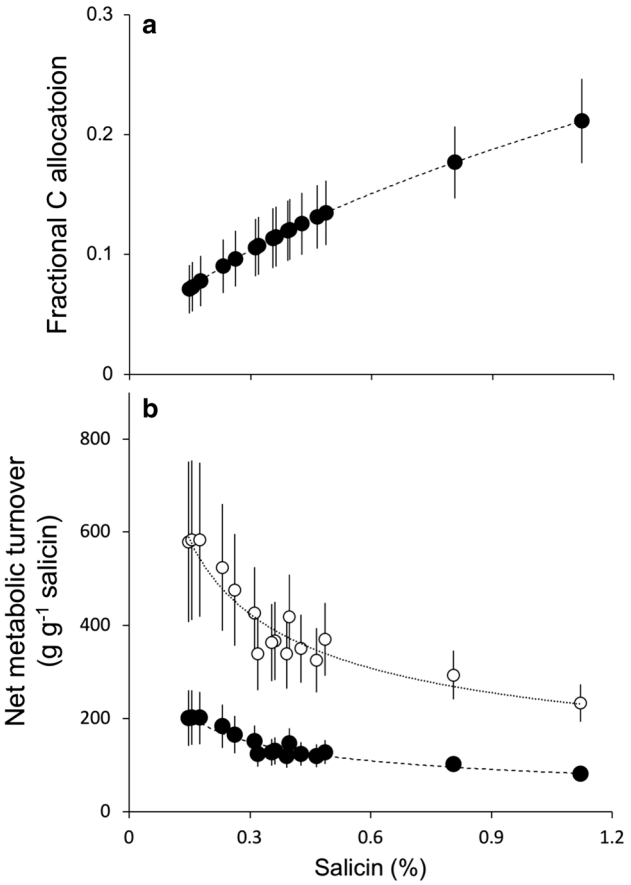

Fig. 6 Plots depicting variation in C allocation to salicin metabolism, as a function of leaf [salicin], across 16 aspen genotypes. Y axes include **a** the estimated fraction of assimilated C allocated to salicin metabolism in foliage and bark of stems and branches; and **b** net metabolic turnover, representing the total amount of C allocated to salicin metabolism, expressed as salicin production equivalents, based on the estimate that 0.69 g of C is required to produce 1 g of salicin. In **b**, turnover was estimated based on the assumption that salicin was produced only in foliage (open symbols), or, alternatively, that salicin was also produced in stem and branch bark (closed symbols). Genotype values are based on a simulation of tree C gain and allocation, and each represents a mean of results from 100 simulations wherein values for a subset of parameters were randomly sampled from a Gaussian distribution (Online Resource 4). Means are accompanied by standard deviations indicating levels of uncertainty across the 100 simulations. All trends are significant ($P \leq 0.001$, $n = 16$)

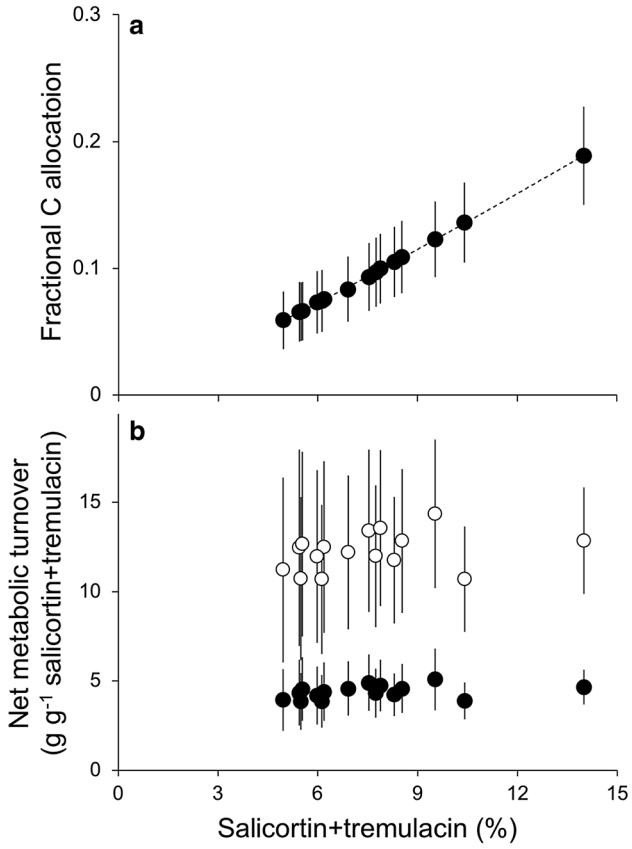

Fig. 7 Plots depicting variation in C allocation to salicortin and tremulacin metabolism, as a function of leaf [salicortin + tremulacin], across 16 aspen genotypes. Y axes include **a** the estimated fraction of assimilated C allocated to salicortin and tremulacin metabolism in foliage and bark of stems and branches; and **b** net metabolic turnover, representing the total amount of C allocated to salicortin and tremulacin metabolism, expressed as salicortin/tremulacin production equivalents, based on the estimate that 0.77 g of C is required to produce 1 g of salicortin and tremulacin. In **b**, turnover was estimated based on the assumption that SPGs were produced only in foliage (open symbols), or, alternatively, that they were also produced in stem and branch bark (closed symbols). Genotype values are based on a simulation of tree C gain and allocation, and each represents a mean of results from 100 simulations wherein values for a subset of parameters were randomly sampled from a Gaussian distribution (Online Resource 4). Means are accompanied by standard deviations indicating levels of uncertainty across the 100 simulations. The trend in **a** is significant ($P \leq 0.001$, $n = 16$)

those constraints dampen variation in rates of resource acquisition (Osier and Lindroth 2006). In our dense populations, for instance, the partial shade and restrictions to root growth imposed by neighbors may have suppressed phenotypic differences in crown nutrient status, water use and photosynthetic performance (e.g., Medhurst and Beadle 2005).

Outputs from our mass-balance simulation of tree C balance indicate that maintenance of even modest SPG levels in aboveground tissues entails considerable losses of assimilated C. At the extreme among our aspen genotypes, nearly one-third of the C assimilated by a tree may be lost as a consequence of chemical defense investments. Moreover, particularly in the case of salicin, sizable losses hypothetically equate with rapid metabolic turnover. Although considerable uncertainty remains with respect to the magnitude of metabolic turnover for many defense compounds (e.g., Gershenzon 1994; Ruuhola and Julkunen-Tiitto 2000), our results generally align with those of two studies (Kleiner et al. 1999; Massad et al. 2014) demonstrating the lability of foliar and bark SPGs in members of the genus *Populus*. Contrary to the implications of our simulation

output, however, Massad et al. (2014) did not find that turnover differed much between salicin and salicortin. We acknowledge that our calculation of net metabolic turnover, expressed as "production equivalents," is likely an overestimate, as it does not account for the potentially large and variable costs linked to coincident synthesis and turnover of the associated metabolic infrastructure, especially proteins (Scheurwater et al. 2000).

In any case, metabolic turnover is a common denominator in long-standing models that quantify defense costs (Coley et al. 1985; Mooney and Gulmon 1982; Fagerström 1989). From a mass-balance standpoint, the consequences of SPG investments in our study are difficult to reconcile without invoking turnover, since, in its absence, estimated C loss stemming from a one-time synthesis of SPGs in new leaf and bark tissues averages about 5 g C per tree, which represents less than 1% of annual crown photosynthesis (Online Resource 5). In other words, when SPGs are components of leaf and bark, and their presence has no discernible impact on other measured growth determinants, their synthesis, without turnover, would only slightly inflate the overall costs of tissue construction (Poorter 1994). We speculate that such is the case for foliar condensed tannins, which are thought to be relatively stable (e.g., Reichardt et al. 1991), and, correspondingly, do not appear to exert a strong influence on aspen tree growth in our highly competitive setting. This reasoning is contradicted, however, by results of recent research linking declining growth rate with the accumulation of condensed tannins in aspen (Donaldson et al. 2006a; Cole et al. 2016). The discrepancy in results, between studies involving some of the same genotypes, may stem in large part from differences in the environmental conditions under which the trees were grown, the treatments (e.g., defoliation and fertilization) to which they were subjected (Osier and Lindroth 2006), and ontogenetic shifts in expression of SPGs and condensed tannins as trees age (Donaldson et al. 2006b).

Estimates of fractional C allocation to SPGs, derived from our simulation of tree C balance, afford the opportunity to predict the apparently independent costs, with respect to plant biomass growth, incurred by maintaining individual defense compounds. In principle, this capability, coupled with the efficacies of different SPGs as deterrents to herbivory (Donaldson and Lindroth 2007), could inform models forecasting the combined influences of competition and herbivory on the composition, structure and function of early-successional forests. We note that C allocation estimates underlying our cost predictions do not hinge, per se, on temporal stability in tissue defense levels across the growing season, but they are based on the assumption that costs scale monotonically with genotypic variation in leaf defense levels sampled at one point in time (late June). We did not assess the validity of either related assumption in our populations, but data in Osier et al. (2000) indicate that, for a given SPG, concentrations measured in late June correlate closely with growing season averages across ten aspen genotypes.

Another outcome of our simulation is the nonlinear (convex) relationship between fractional C allocation and foliar levels for salicin (Fig. 6a), which differs somewhat from the linear trend for the two higher-order SPGs (Fig. 7a). The mechanistic model of defense costs devised by Fagerström (1989) provides two possible rationales for this contrast. The first is a decline in salicin's net turnover rate with increases in its leaf concentration (Fig. 6b), whereas turnover does not vary consistently with foliar concentration for the higher-order SPGs (Fig. 7b). The second is a negative relationship between salicin's marginal construction cost (g C lost [g^{-1} salicin produced]) and its foliar concentration. We have no data with which to further evaluate either possibility, but we point to the assertion by Ruuhola and Julkunen-Tiitto (2000) that salicin may be a key intermediate (e.g., precursor for higher-order phenolic glycosides, but see Babst et al. 2010), lying at the nexus of several metabolic pathways. Such a role may result in complex, concentration-dependent dynamics in salicin metabolism. Alternatively, this pattern may reflect, in part, a relatively high "fixed" (rather than marginal or scaled) cost of the enzymatic machinery associated with salicin synthesis and turnover.

While the aim of our present work is not to test prevailing models concerning the evolution of defense against herbivory (reviewed by Cipollini et al. 2014), our findings have implications for some of their basic tenets. In particular, we see clear evidence of a growth-defense tradeoff in a source-limited system—i.e., where tree biomass accumulation is limited by C assimilation capacity (A_{sat}). This result is inconsistent with predictions from the modeling framework outlined by Herms and Mattson (1992), where tradeoffs emerge primarily in sink-limited systems. Additionally, in our simulation of tree C balance, allocation to chemical defenses does not necessitate a decline in new leaf production, and the latter is not a primary driver of growth variation, at least within a growing season. Our approach is based on a hierarchical allocation model that prioritizes allocation to foliage as well as root production and maintenance (Schippers et al. 2015). Thus, defense costs are borne solely by aboveground woody tissue, which is a lower allocation priority. As a result, during the simulated growing season, relative C allocation to foliage versus stem and branches actually increases modestly with a rise in leaf SPG concentrations (data not shown). A similar pattern was observed in a study on hybrid aspen (Haikio et al. 2009), wherein the slower-growing, more heavily defended phenotypes also possessed a comparatively high foliage mass for

a given aboveground woody mass. Of course, in the long run, defense costs would inevitably impinge on leaf production, which scales positively with (live) stem mass in aspen (Bond-Lamberty et al. 2002).

In sum, this study constitutes the first in-depth examination of the interplay between intraspecific competition and the physiological underpinnings of growth-defense tradeoffs in a woody plant system. As such, these findings inform our understanding of the ecological and evolutionary factors that interact to shape key life-history characteristics in a foundation tree species. These characteristics, subjected to selective forces such as competition and herbivory, govern evolutionary trajectories of aspen populations, determine the genetic composition of forest stands, and produce genetic mosaics of ecosystem function at the landscape scale (Madritch et al. 2009).

Acknowledgements Financial support for this study was provided by the National Science Foundation (grants DEB-0841609 and DEB-1456592) and the USDA McIntire-Stennis Program (grant WIS01842). We are grateful for the invaluable technical assistance provided by Andrew Helm, Kennedy Rubert-Nason, and Daniel Reeves.

Author contribution statement RLL and ELK secured funding, and, with KKR, designed the study. LMH and KKR initiated and maintained the study. ELK, LMH and KKR collected and processed data. ELK conducted data analyses and simulations. ELK generated the first manuscript draft, and all authors contributed substantially to revisions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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