Bud development and shoot morphology in relation to crown location

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Abstract. Plant architecture is shaped by endogenous growth processes interacting with the local environment. The current study investigated crown development in young black alder trees, assessing the effects of local light conditions and branch height on individual bud mass and contents. In addition, we examined the characteristics of parent shoots [the cross-sectional area (CSA) of stem and total leaf area, shoot length, the number of nodes, the number and total mass of buds per shoot] and leaf–stem as well as bud–stem allometry, as several recent studies link bud development to hydraulic architecture. We sampled shoots from top branches and two lower-crown locations: one subjected to deep shade and the other resembling the upper branches in light availability. Sampling was carried out three times between mid-July and late October, spanning from the early stages of bud growth to dormancy. Individual bud mass and shoot characteristics varied in response to light conditions, whereas leaf–stem allometry depended on branch height, most likely compensating for the increasing length of hydraulic pathways. Despite the differences in individual bud mass, the number of preformed leaves varied little across the crown, indicating that the plasticity in shoot characteristics was mainly achieved by neoformation. The relationship between total bud mass and stem CSA scaled similarly across crown locations. However, scaling slopes gradually decreased throughout the sampling period, driven by bud rather than by stem growth. This suggests that the allometry of total bud mass and CSA of stem is regulated locally, instead of resulting from crown-level processes.

Keywords: Allometry; architecture; bud mass; current-year shoot; light; neoformation; preformation; vertical crown gradient.

Introduction

Tree crowns develop by forming semi-autonomous repeated elements: buds, metamers, shoots and branches (White 1979; Maillette 1982a, b; Barthélémy and Caraglio 2007; Kawamura 2010). In young, fast-growing trees, bud production is prolific at the top of the crown, whereas lower branches degrade, as buds are fewer, prone to higher mortality rates and tend to produce short, low-vigour shoots adjusted to leaf display rather than crown expansion (Maillette 1982a; Jones and Harper 1987a, b; Kimura et al. 1998; Kull and Tulva 2002; Remphrey et al. 2002). Unequal rates of bud production promote a rapid gain in height over crown width (Maillette 1982b), allowing young trees to escape from unfavourable, shaded environments (Henry and Aarssen 2001).

Plant architecture is shaped by endogenous processes that interact with the local environment (Barthélémy and Caraglio 2007; Kawamura 2010). Local conditions vary...
widely within the crown of a tree. Most obviously, the upper and outer parts of a crown receive more light, but suffer also from greater heat and moisture stress and stronger winds than the lower branches or the interior of a crown (Ninemets and Valladares 2004). Light is a crucial factor affecting bud characteristics: buds formed in shade are smaller, exhibit lower probabilities of bud break in the following spring (Sanz-Pérez and Castro-Díez 2010) and produce shoots consisting of fewer nodes (Kimura et al. 1998). Besides organ initiation, light availability also influences the anatomical development of incipient leaves (Eschrich et al. 1989; Uemura et al. 2000). Accordingly, contrasting light conditions contribute to determining the differing rates of bud production, mortality and the characteristics of the future shoots in the upper versus lower crown (Jones and Harper 1987a, b; Eschrich et al. 1989; Kimura et al. 1998; Uemura et al. 2000; Kull and Tulva 2002).

However, differences in bud characteristics are also frequently attributed to the position within the architecture of a plant (e.g. Barthélémy and Caraglio 2007). Along a parent shoot, buds positioned distally are larger, contain a greater number of preformed organs and display higher probabilities of bud break than basal buds (Gill 1971; Remphrey and Davidson 1994; Sabatier and Barthélémy 2001; Gordon et al. 2006; Alla et al. 2013b). The mechanisms implicated in determining bud characteristics include hormonal signalling (Cline 2000; Djennane et al. 2014) and resource competition among buds (Clínez et al. 2009; Mason et al. 2014), but several recent studies also highlight the importance of vascular connections between buds and stem. The number of leaf primordia found in overwintering buds is correlated to xylem area at the base of the parent shoot as well as to the hydraulic conductance of the vascular pathway leading to the buds (Cochard et al. 2005). Furthermore, the vascular differentiation of embryonic shoots is a key step in the sequence of events leading to bud break in spring (de Fay et al. 2000; Sutinen et al. 2012), and buds failing to establish a vascular connection to the stem remain dormant or abort (Han et al. 2007; Lauri et al. 2008). Unlike current-year shoots, the contribution of these endogenous mechanisms is less clear at the crown level, as potential topological effects are confounded by environmental heterogeneity.

Developmental and functional interdependencies among shoot components lead to coordinated growth in response to prevailing conditions. Due to both mechanical and hydraulic reasons, the leaf area supported by a shoot depends on the cross-sectional area (CSA) of stem (Preston and Ackerly 2003; Normand et al. 2008). Consequently, leaf–stem allometry responds to environmental gradients across habitats (Preston and Ackerly 2003; Westoby and Wright 2003; Sun et al. 2006) and seasonal changes in precipitation (Bucci et al. 2005). The leaf area supported by a given stem area also decreases as trees grow taller, to overcome the hydraulic stress imposed by the increasing length of resource transport pathways and gravity (McDowell et al. 2002). In contrast, studies investigating the allometry of current-year shoots rarely involve buds. Alla et al. (2011) have examined the scaling of apical bud mass and stem CSA, concluding that bud–stem relationships are influenced by environmental factors, but bud–stem allometry along the vertical crown gradient and especially for axillary buds remains unexplored.

The current study investigated crown development in young black alder trees. We examined individual bud mass and contents, the characteristics of current-year shoots, and leaf–stem and bud–stem allometry. Sampling was carried out in three crown locations to assess the effects of two underlying factors: light availability and branch height. The characteristics of parent shoots were expected to respond mainly to light conditions, and improved light availability was also expected to increase individual bud mass and the number of leaf primordia per bud. However, due to the potential link between bud development and hydraulic architecture, we hypothesized that similarly to leaf–stem allometry, the relationship between total bud mass and stem CSA would mainly respond to branch height. To gain further insight, we also examined the allometry of individual bud mass and the CSA of stem. Sampling was carried out three times between mid-July and late October, to investigate whether the effects of crown location on individual bud mass and on bud–stem allometry vary throughout the period of rapid bud development.

### Methods

#### Study system

The current study was conducted in a small stand of black alder (Alnus glutinosa (L.) Gaertn.) trees, located on former agricultural land in Rõka village, south-eastern Estonia (58°15′N, 27°18′E; 50 m above sea level). The soil in the study area is Endogleyic Planosol (Hansen et al. 2013). Long-term mean temperature is +16.9 °C in July and −5.4 °C in February; mean annual precipitation amounts to 637 mm. Black alder is a light-demanding deciduous tree species, preferring wet habitats. The stand consists of even-aged trees, planted in a 2 × 2 m grid in 2007. By the time of sampling in 2013, trees had reached the height of 6–7 m and formed a closed canopy.

#### Sampling

Sampling was carried out on five trees growing at the edge of the stand. The edge follows NW–SE direction,
facing an open field to the west of the stand. On average, sample trees measured 7.1 ± 0.7 m in height (± SD) and had the diameter of 8.2 ± 1.7 cm at 1.3 m from ground level.

Current-year shoots were collected from three crown locations: lower-crown branches facing the interior or the edge of the stand (LI and LE, respectively), and upper-crown (U) branches facing the edge. Sampling was carried out three times. Shoots were first collected during the early stages of bud development (July); further sampling was carried out near the end of the growing season (August) and finally, dormant shoots were collected after leaf drop (October). At any given sampling date, one lateral branch per crown location was selected from each sample tree. Selected branches were 5 year olds in the lower crown and 2–3 year olds in the upper crown, issuing from the trunk at the height of ≏1.5 m (LI and LE branches) and 4 m (U branches), respectively (Table 1).

In July and August, 10 randomly selected shoots were harvested per branch. However, a few of these shoots were later found to carry mainly dead buds (discernible by ready abscission) and were discarded, resulting in slightly reduced sample sizes (Table 1). In October, eight shoots, each carrying viable buds, were sampled per branch. Harvested shoots were stored in sealed plastic bags at +5 °C for 2–5 days until further measurements.

The sampled crown locations were exposed to differing light environments. Light availability was quantified using hemispherical photography in the following summer. Four photographs per location, covering both the periphery and the interior of the crown, were taken from each sample tree, using a horizontally levelled digital camera (Coolpix 950, Nikon) equipped with a fish-eye lens (LC-ER1, Nikon). Photographs were taken above branches located ~0.5 m higher than the branches sampled in the previous summer. As trees had gained height, new branches corresponded to the same relative position and degree of shading within the canopy as the previous summer sample branches. Photographs were analysed using the WinScanopy software (version 2001a, Regent Instruments Inc.) to estimate the percentage of light transmitted through the canopy (i.e. total site factor). On average, U branches received nearly four times the light of LI branches, but only ~40 % more light than LE branches (Table 1). Thus, the sampling design included a steep (U versus LI) as well as a shallow (U versus LE) vertical light gradient. By comparing the three crown locations, we were therefore able to assess the effects of two underlying factors: similar values for LI and LE branches but different for U branches were attributed to the difference in branch height, whereas similar values for LE and U branches but different for LI branches were presumably related to light availability.

**Measured variables**

The characteristics determined for sampled shoots included the CSA of stem, shoot length, the number of nodes, leaf area, the number of buds and total bud mass per shoot. In addition, a bud was sampled from each shoot to assess individual bud mass and contents (i.e. the number of leaf primordia per bud).

The CSA of stem was calculated based on stem diameter, assuming that the cross-section is circular. Diameter was measured with a digital calliper at shoot base (the mean of two perpendicular measurements, precision 0.01 mm). Shoot length was measured with a ruler (precision 1 mm). In July and August, the leaf area per shoot was obtained by summing the areas of individual leaves. Leaf areas were predicted based on leaf length × width, using a linear regression (\(\log_{e}(\text{area}) = -5.31 + 1.04 \times \log_{e}(\text{length} \times \text{width}), R^2 = 99.3 \%, P < 0.001, n = 60\)). During field sampling in August, leaves for the model were randomly selected from each crown location, but the data were pooled, since leaf shape was similar across the crown (data not shown). The lengths and widths of leaf blades were determined using a ruler (precision 1 mm), and the areas were measured to the nearest 0.01 cm\(^2\) using an optical area meter (LI-3100C, LI-COR, Inc., Lincoln, NE, USA).

Buds were then carefully cut from the stems below the first bud scale. In July, buds were dried at 60 °C for 48 h and weighed to determine total bud mass per shoot (precision 0.1 mg); subsequently, a bud was selected

![Table 1. The heights of sample branches (mean ± SD, n = 5), sample sizes (the total number of shoots sampled per crown location with the number of shoots per branch in brackets) and the percentage of light transmitted through the canopy (TSF, total site factor; mean ± SD, n = 20). LI, lower-crown, interior-facing branches; LE, lower-crown, edge-facing branches; U, upper crown.]
randomly from each shoot and weighed to assess individual bud mass. The number of leaf primordia was not determined in July, because buds were still small and their contents could not be reliably identified. In August and October, sample buds were set aside before drying, weighed and dissected under a stereomicroscope (×16–40 magnification) to count the number of true leaf primordia. In black alder, each leaf primordium is flanked by two stipules, and the outer stipules function as bud scales; consequently, the number of true leaf primordia and the total number of organs are highly correlated. The dry mass of dissected buds (i.e. individual bud mass) was predicted based on fresh mass, using linear regressions fitted on buds that were additionally collected during field sampling ($R^2 = 96.1–98.8\%$, $P < 0.001$, $n = 16–23$ for each combination of location and sampling date; data not shown). Thus, in August and October, total bud mass per shoot was obtained by adding the dry mass of the sample bud to the rest of the buds on a given shoot.

### Statistical analysis

Analyses were carried out using the statistics software R (R Development Core Team 2014).

The effects of crown location and sampling month on shoot and bud characteristics were analysed using mixed-effects modelling: random effects included sample tree and branch (nested within tree). Continuous variables were tested by mixed-effects analysis of variance, using package nlme (Pinheiro et al. 2014), and counts were tested using mixed-effects Poisson regression implemented in package lme4 (Bates et al. 2014). When necessary, continuous variables were transformed, and non-significant interactions between the fixed effects were dropped. $P$-values for the fixed effects were based on type III sums of squares and were obtained using conditional $F$-tests (ANOVARs) or Wald $\chi^2$ tests (Poisson regressions), the latter of which were carried out using package car (Fox and Weisberg 2011).

![Figure 1](image.png)

**Figure 1.** Mean individual bud mass (A) and mean number of leaf primordia per bud (B) in relation to crown location and sampling month. Error bars denote 95 % CI. Different letters indicate significant differences among crown locations (within each month), and different numbers indicate significant differences across sampling months (within each location). LI, lower-crown, interior-facing branches; LE, lower-crown, edge-facing branches; U, upper crown.

### Table 2

The effects of crown location and sampling month on individual bud mass and contents. Individual bud mass (square-root-transformed) was analysed using mixed-effects ANOVA and the number of leaf primordia by mixed-effects Poisson regression (nested random effects: sample tree and branch).

| Variable                  | Fixed effect | Sampling month |
|---------------------------|--------------|----------------|
| Individual bud mass       | $F(2, 36) = 24.4$ | $F(2, 36) = 239.7$ |
|                           | $P < 0.001$  | $P < 0.001$    |
| Number of primordia       | $\chi^2(1) = 2.5$ | $\chi^2(1) = 2.5$ |
|                           | $P = 0.4$    | $P = 0.1$      |
Tests for the effects of crown location and sampling month were followed by pair-wise comparisons of group means. To this end, new one-way mixed-effects models were fitted for each dependent variable, containing the combination of crown location and sampling month as the fixed effect. Pair-wise comparisons were then carried out using simultaneous inference procedures implemented in package multcomp (Hothorn et al. 2008). Comparisons were set up among crown locations within each month and among sampling months within each location, as other possible pair-wise comparisons conveyed little useful meaning. Differences were deemed significant at \( P < 0.05 \).

Leaf area and stem CSA, total bud mass and stem CSA, and individual bud mass and stem CSA are related allometrically, approximating to a power law: \( y = a \times x^b \). The relationships between shoot components were therefore linearized using \( \log_{10}(y) - \log_{10}(x) \) transformation. Linear mixed-effects models fitted for \( \log_{10}(\text{leaf area}), \log_{10}(\text{total bud mass}) \) and \( \log_{10}(\text{individual bud mass}) \) indicated that the variance not captured by fixed effects (\( \log_{10}(\text{stem CSA}) \), crown location and sampling month) was mainly accounted for by individual shoots (i.e. residual variance). The random effects of sample tree and branch contributed only 18.5, 11.9 and 18.2 % depending on the model (data not shown). Therefore, we decided to drop tree and branch effects when analysing shoot allometry, and instead pooled the data within each combination of crown location and sampling month.

Log–log-transformation puts variables on a multiplicative scale, and consequently, the slope of a bivariate relationship characterizes the scaling of the two variables relative to one another (i.e. slope = 1 means that if \( x \) is doubled, \( y \) is doubled as well, slope = 2 means that if \( x \) is the doubled, \( y \) is quadrupled, etc.). The relationships were fitted in each group (i.e. each combination of location and month) using standardized major axis (SMA) estimation.

We relied on pair-wise comparisons to investigate whether the allometries scale differently across crown locations or sampling months. Using a test based on the likelihood ratio (LR) statistic, groups were first tested for a common slope, followed by pair-wise comparisons, if the global test was deemed significant. Again, comparisons were carried out among crown locations within each month and among sampling months within each location. The \( P \)-values of pair-wise comparisons were adjusted using Bonferroni correction, and differences were deemed significant at \( P < 0.05 \). If slopes were homogeneous, differences in \( y \)-intercepts were investigated, using a test based on the Wald statistic (W). Similarly to slopes, a global test was carried out, followed by pair-wise comparisons. Standardized major axis estimation and tests for slopes and intercepts were performed using the functions implemented in package smatr (Warton et al. 2012); the underlying methodology is described in more detail by Warton et al. (2006). Pearson’s correlations coefficients between the leaf area and stem CSA or bud mass and stem CSA were calculated based on \( \log_{10} \)-transformed variables.

### Results

#### Individual bud mass and contents

Individual bud mass depended significantly on crown location and sampling month (Table 2). On average, LI buds had lower mass than both LE and U buds, and pair-wise comparisons revealed that the differences were significant in July and October, although not in August (Fig. 1A). In contrast, pair-wise comparisons showed that mean individual bud mass was similar in LE and U branches throughout the study. Between July and August, mean individual bud mass increased significantly in all three locations, and the further mass increase between August and October was significant in LE and U branches. Despite the differences in individual bud mass, the average

| Variable          | Fixed effect | Crown location | Sampling month |
|-------------------|--------------|----------------|----------------|
| CSA of stem       | \( F(2, \, 36) = 63.4 \) | \( P < 0.001 \) | \( F(2, \, 36) = 9.5 \) | \( P = 0.001 \) |
| Shoot length      | \( F(2, \, 36) = 40.8 \) | \( P < 0.001 \) | \( F(2, \, 36) = 3.2 \) | \( P = 0.05 \) |
| Number of nodes   | \( \chi^2(2) = 100.1 \) | \( P < 0.001 \) | \( \chi^2(2) = 15.4 \) | \( P < 0.001 \) |
| Total leaf area   | \( F(2, \, 22) = 9.4 \) | \( P = 0.001 \) | \( F(1, \, 22) = 1.2 \) | \( P = 0.3 \) |
| Number of buds    | \( \chi^2(2) = 132.5 \) | \( P < 0.001 \) | \( \chi^2(2) = 10.9 \) | \( P = 0.005 \) |
| Total bud mass    | \( F(2, \, 36) = 68.0 \) | \( P < 0.001 \) | \( F(2, \, 36) = 157.7 \) | \( P < 0.001 \) |
number of leaf primordia was unaffected by crown location, and sample buds contained a similar number of leaf primordia in August and October (Table 2, Fig. 1B).

**Shoot characteristics**

The characteristics of current-year shoots differed significantly across crown locations, and with the exception

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Figure 2. Mean values of shoot characteristics in relation to crown location and sampling month: CSA of stem (A), shoot length (B), number of nodes per shoot (C), total leaf area per shoot (D), number of buds per shoot (E) and total bud mass per shoot (F). Error bars denote 95% CI. Different letters indicate significant differences among crown locations (within each month), and different numbers indicate significant differences across sampling months (within each location). LI, lower-crown, interior-facing branches; LE, lower-crown, edge-facing branches; U, upper crown.
of total leaf area, depended also on sampling month (Table 3). Pair-wise comparisons showed that LI shoots had significantly smaller stem CSA than both LE or U shoots throughout the study period (Fig. 2A). The shoots from LI branches were also shorter than LE or U shoots, although the difference between LI and LE shoots was non-significant in August (Fig. 2B). Nevertheless, LI shoots consisted of significantly fewer nodes than both LE and U shoots throughout the study (Fig. 2C), and LI shoots also tended to support a smaller total leaf area (Fig. 2D).

In contrast, the differences between LE and U shoots were mostly non-significant (Fig. 2A–D). In July, shoots had almost the same stem CSA and the number of nodes in both locations, but unlike lower-crown shoots, U shoots continued growing in late summer, so that significant increases were detected for both variables (Fig. 2A and C). Consequently, U shoots sampled in October had significantly greater stem CSA than LE shoots, although the number of nodes was still deemed similar by pair-wise comparisons. Besides differences in other shoot characteristics, LI shoots also produced significantly fewer buds than LE or U shoots, while bud number was similar in the two latter locations throughout the study (Fig. 2E). Although sampling month had a significant effect on the number of buds (Table 3), pair-wise comparisons could not detect any differences in bud number in relation to sampling date (Fig. 2E). However, depending on the location, total bud mass per shoot increased 5–8 times during the study period. Total bud mass increased significantly in all three locations between July and August, and tended to further increase between August and October (Fig. 2F). Nevertheless, total bud mass in LI shoots remained significantly lower than elsewhere throughout the study, whereas the differences between LE and U shoots were non-significant.

**Leaf–stem allometry**

Total leaf area was highly correlated to stem CSA in all six groups (Table 4). The relationship between the two variables scaled similarly regardless of crown location or sampling month; however, the intercepts were non-homogeneous (Table 4). In general, upper-crown shoots

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**Table 4.** The allometry between total leaf area per shoot and the CSA of stem. Leaf–stem relationships were fitted using SMA estimation. As the relationships scaled similarly across each combination of crown location and sampling month (LR(5) = 1.74, *P* = 0.9), they were fitted with a common slope (presented with 95 % CI) across all groups. However, the intercepts were non-homogeneous (W(5) = 97.7, *P* < 0.001), so the global test was followed by pair-wise comparisons. Different letters denote significantly different intercepts among crown locations (within each month), and different numbers denote significant differences between sampling months (within each location). Correlation coefficients (*r*) are significant at *P* < 0.001. LI, lower-crown, interior-facing branches; LE, lower-crown, edge-facing branches; U, upper crown.

| Sampling month | Crown location | LI | LE | U |
|----------------|----------------|----|----|----|
| Common slope   |                | 1.75 (1.65...1.86) |
| July           | Intercept      | −1.11 a1 | −1.21 a1 | −1.34 b1 |
|                | *r*            | 0.733 | 0.878 | 0.889 |
| August         | Intercept      | −1.25 a2 | −1.37 a2 | −1.47 b2 |
|                | *r*            | 0.815 | 0.890 | 0.940 |

**Figure 3.** The allometry between the total leaf area per shoot and CSA of stem in mid-July (A) and late August (B). The lines represent SMA regressions, fitted with a common slope (1.75) across crown locations and sampling months. Note the log10-transformed axes. LI, lower-crown, interior-facing branches; LE, lower-crown, edge-facing branches; U, upper crown.
supported a smaller total leaf area at a given stem CSA than lower-crown shoots, and the total leaf area per given stem CSA was reduced between July and August. Pair-wise comparisons revealed that the intercepts for U shoots differed significantly from LI and LE shoots both in July and in August, and intercepts also differed between sampling months within each location (Table 4, Fig. 3A and B).

### Bud–stem allometry

Total bud mass per shoot and stem CSA were also highly correlated (Table 5). Testing for a common slope indicated that the scaling relationship differed across the nine groups (Table 5). Pair-wise comparisons revealed that slopes were similar among crown locations within each month. However, slopes tended to vary across sampling months: although significant differences were detected only for U shoots, slopes followed a similar, decreasing trend in all three crown locations. Thus, the data were pooled across crown locations, and the slopes fitted for each sampling month differed significantly from one another (Table 5, Fig. 4). Although the slopes remained positive (i.e. shoots with greater stem CSA had greater total bud mass in each sampling month), they gradually decreased towards the end of the study period (i.e. total bud mass increased at a faster rate with increasing stem CSA in shoots that were sampled earlier in the season). Testing for a common slope revealed that the allometry of individual bud mass and stem CSA scaled differently across the nine groups (Table 6). However, the scaling slopes did not follow any apparent pattern across crown locations or sampling months, and the correlations between individual bud mass and stem CSA were weak and mostly non-significant, except in July (Table 6). Pooled across crown locations, the correlations between individual bud mass and stem CSA were significant in each sampling month, and the relationships were positive (i.e. shoots with greater stem CSA had larger buds; Table 6). The slope fitted on pooled data was significantly steeper in July than in August or October, resulting mainly from the fact that the slope fitted for LI shoots in July was particularly steep (Table 6, Fig. 5).

### Discussion

In the current study, we investigated crown development in young black alder trees. Identifying the factors that regulate bud and shoot growth provides an insight into the mechanisms determining plant architecture, which is a key component of plant growth modelling, a method used widely in the fields of environmental sciences, agriculture and forestry (Fourcaud et al. 2008).

We found that individual bud mass was determined by local light conditions rather than by branch height, as...
upper- and lower-crown buds differed little in the absence of a steep light gradient. Similarly, it has been shown that artificially shading the top branches induces bud characteristics similar to those found further down the crown (Jones and Harper 1987a, b; Kimura et al. 1998; Uemura et al. 2000). As defoliation leads to a decrease in bud size (Marcelis-van Acker 1994), it is likely that light availability promotes bud development through assimilate supply. However, light also acts as a developmental cue: both the intensity and quality of light affect bud outgrowth in rose, and the light signal is perceived by buds, not by the shoot (Girault et al. 2008).

Nevertheless, in the current study, the average number of leaf primordia per bud varied little across the crown. Similarly, Gordon et al. (2006) and Taugourdeau and Sabatier (2010) found that light availability had little effect on the number of preformed leaves in peach and walnut, respectively. In contrast, Kimura et al. (1998) report that shoot morphology in beech is largely determined by previous-year light conditions, presumably via preformation in the bud. In general, larger buds contain a greater number of preformed organs (e.g. Remphrey and Davidson 1994; Cochard et al. 2005; Lauri et al. 2008). However, the current study found that although higher light availability increased individual bud mass, the number of leaf primordia per bud was unaffected, suggesting that the initiation and growth of leaf primordia were decoupled. Likewise, Henry et al. (2011) observed that bud elongation in rose was highly diminished in response to sugar starvation during bud outgrowth, whereas organogenesis remained constant. According to Gordon et al. (2006), preformation in peach is little affected by a range of both exogenous and endogenous factors (e.g. light availability, drought, tree carbohydrate status), and plasticity in crown architecture is mostly achieved via neoformation, by initiating new organs that extend during the same growing season. Thus, the limited impact of light availability on organ initiation within the bud may represent a more widespread strategy among species relying on neoformed growth (Guédon et al. 2006).

Although leaf–stem allometry was affected by branch height, shoot characteristics corresponded mainly with local light availability, consistent with previous studies (Kimura et al. 1998; Kull and Tulva 2002; Osada et al. 2004). Upper-crown shoots and well-lit lower-crown shoots had greater stem CSA, they were longer and consisted of a greater number of nodes than the shaded lower-crown shoots. These shoots also produced more leaves and buds and consequently supported a greater total leaf area and total bud mass per shoot. Most likely, light availability stimulated the different aspects of shoot growth via assimilate supply. Unlike both lower-crown locations, however, upper-crown shoots exhibited late-summer growth. Similarly, a greater extent of neoformation in the upper versus lower crown was observed by Davidson and Remphrey (1994). In the current study, light availability was slightly better in the upper crown than in the well-lit lower branches, and it remains

Figure 4. The allometry between total bud mass per shoot and the CSA of stem in mid-July (A), late August (B) and late October (C). The lines represent SMA regressions (grey lines represent fits for data that were pooled across crown locations). Note the log10-transformed axes.
unknown whether comparable light conditions would trigger late-summer growth in the lower crown. Goulet et al. (2000) observed that, depending on species, differences in shoot growth either correspond with local light availability, or the effect of light availability is modulated by branch height, so that only upper-crown shoots are capable of utilizing the more favourable conditions.

The scaling slopes of leaf–stem allometry remain constant in a wide range of circumstances, whereas the intercepts vary, reflecting an adjustment to hydraulic or mechanical stress (McDowell et al. 2002; Preston and Ackerly 2003; Westoby and Wright 2003; Bucci et al. 2005; Sun et al. 2006; Normand et al. 2008). Similarly, upper-crown shoots supported a smaller total leaf area per given stem CSA in the current study, most likely compensating for the hydraulic limitation imposed by the longer resource transport pathways (c.f. McDowell et al. 2002). In addition, total leaf area per given stem CSA was reduced in all three crown locations between mid-July and late August. The change was driven by leaf shedding rather than radial growth, as the latter was restricted to upper-crown shoots. In black alder, leaf shedding commonly starts in the inner parts of the crown as early as July, while new leaves are continuously produced in the periphery of the crown (Eschenbach and Kappen 1996).

Unlike leaf–stem relationships, allometric studies rarely involve buds. Nevertheless, climatic conditions, namely temperature and rainfall, are known to modulate bud–stem allometry, so that steeper scaling slopes are found in milder climate (Alla et al. 2011). The mechanisms linking bud growth and stems have remained unclear. Cochard et al. (2005) hypothesize that enhanced bud development in shoots with greater xylem area is associated with improved hydraulic supply. Supporting this notion, meristem vigour decreases as trees mature, manifested in the production of small, low-vigour shoots. According to grafting studies, reduced shoot vigour is related to increasing tree height and possibly involves hydraulic constraints (Bond et al. 2007). Nevertheless, the present study found that branch height as well as local light conditions had little effect on the allometry of total bud mass and the CSA of stem. Homogeneous scaling slopes may reflect a limited height difference between upper and lower branches: although the sampling locations were sufficiently far apart to influence leaf–stem allometry, developing buds are far less susceptible to water shortage than leaves (Barigah et al. 2013). Alternatively, the mechanisms regulating bud–stem allometry may act locally.

Each new generation of buds is formed in mid-summer, followed by a period of rapid bud development in late summer and fall (Eschrich et al. 1989; Alla et al. 2013a). Similarly, both individual and total bud mass increased multiple times during the current study, but allometries

| Sampling month | Crown location | Pooled |
|----------------|----------------|--------|
|                | LI             | LE     | U     | Pooled |
| July           | Slope          | 2.31 (1.73 … 3.08) a1 | 0.93 (0.75 … 1.16) b1 | 1.17 (0.92 … 1.49) b1 | 1.51 (1.33 … 1.72) I |
|                | Intercept      | −4.25  | −3.08 | −3.34 | −3.64 |
|                | r              | 0.538*** | 0.656*** | 0.565*** | 0.657*** |
| August         | Slope          | 0.80 (0.56 … 1.13) a2 | 0.72 (0.54 … 0.96) a1 | 0.93 (0.71 … 1.23) a12 | 0.77 (0.66 … 0.91) 2 |
|                | Intercept      | −2.52  | −2.47 | −2.79 | −2.55 |
|                | r              | 0.083 ns | 0.262 ns | 0.477** | 0.389*** |
| October        | Slope          | 1.14 (0.84 … 1.55) a2 | 0.79 (0.57 … 1.09) ab1 | 0.53 (0.39 … 0.73) b2 | 0.68 (0.58 … 0.80) 2 |
|                | Intercept      | −2.54  | −2.31 | −2.20 | −2.27 |
|                | r              | 0.359* | 0.114 ns | 0.214 ns | 0.380*** |

Table 6. The allometry between individual bud mass and CSA of stem. Bud–stem relationships were fitted using SMA estimation. Slopes are presented with 95 % CI. As the slopes fitted for each combination of crown location and sampling month were significantly different (LR(8) = 52.4, P < 0.001), the global test was followed by pair-wise comparisons. Different letters denote significant differences among crown locations (within each month), and numbers denote significant differences among sampling months (within each crown location). Bud–stem relationships were also fitted on data pooled across crown locations within each month, and significant differences among sampling months are denoted by different numbers. Significance levels for correlation coefficients (r): ***P < 0.001; **P < 0.01; *P < 0.05; ns P > 0.05. LI, lower-crown, interior-facing branches; LE, lower-crown, edge-facing branches; U, upper crown.
revealed that total bud mass increased disproportionally less in thicker shoots regardless of crown location. As late-summer growth was restricted to the upper crown, the CSA of stem and bud number remained the same in lower-crown shoots throughout the study, so changes in the allometry of total bud mass and stem CSA were mainly driven by the growth of individual buds. The allometry of individual bud mass and stem CSA revealed that larger shoots carried larger buds, but slopes also tended to decrease across sampling months. However, unlike the clear pattern for total bud mass, the difference mainly stems from a particularly steep slope fitted for shaded lower-crown shoots in July. In late summer, shoot and bud abscission were relatively common in shaded parts of the crown, and the fact that poorly-developed shoots were eliminated later in the season may explain the anomalous slope. Unlike total bud mass, however, the correlations between individual bud mass and stem CSA were relatively strong in July, but mostly weak on later sampling dates. Gill (1971) found that initially, bud size varies little along a parent shoot, but inequalities become evident in late summer, so increasing differences may also be responsible for the lower correlation of individual bud mass and the CSA of stem in the current study.

Within a branching zone, well-developed buds are characterized by higher xylem hydraulic conductance in spring (Lauri et al. 2008), so the differences in bud size and contents may be linked to vascular differentiation. A possible mechanism connecting the two processes involves auxin signalling (Sachs et al. 1993). Auxins are synthesized in young, developing leaves, but low auxin levels are necessary for maintaining leaf initiation and growth (Domagalska and Leyser 2011), so for bud growth to continue, excess auxin needs to be exported. Via positive feedback, auxin transport is canalized into specialized files of cells, which may later differentiate into vascular strands (Berleth and Mattsson 2000). As auxin sources compete for a common transport pathway to the root (Domagalska and Leyser 2011), competition among adjacent buds may also be responsible for the changes in the allometry of total bud mass and stem CSA that were observed in the current study. However, other explanations are possible as well: for example, buds supported by thicker shoots may have reached their final size earlier.

**Figure 5.** The allometry between individual bud mass and CSA of stem in mid-July (A), late August (B) and late October (C). The lines represent SMA regressions (grey lines represent fits for data that were pooled across crown locations). Note the log10-transformed axes.

**Conclusions**

In young black alder trees, crown development is influenced by local environment as well as endogenous processes. Bud size in the upper versus lower crown depended mainly on light availability; however, the number of preformed leaf primordia per bud varied little across the crown. Consequently, the plasticity in shoot size and bud production, driven by local light availability, is mostly achieved by neoformation. Unlike shoot characteristics, leaf–stem allometry depended on branch height, most likely reflecting hydraulic stress due to longer resource transport pathways. In contrast, bud–stem allometry was unaffected by branch height, but scaling slopes varied throughout the sampling period. Varying scaling was driven...
by bud growth and suggests that bud–stem allometry is regulated locally rather than at the crown level.

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Contributions by the Authors
M.K. developed the sampling protocol, collected and analysed the data, and wrote the paper. A.S. conceived the study and provided feedback during manuscript writing.

Conflict of Interest Statement
None declared.

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Literature Cited
Alla AQ, Camarero JJ, Rivera P, Montserrat-Martí G. 2011. Variant allometric scaling relationships between bud size and secondary shoot growth in Quercus faginea: implications for the climatic modulation of canopy growth. Annals of Forest Science 68: 1245–1254.

Alla AQ, Camarero JJ, Montserrat-Martí G. 2013a. Seasonal and inter-annual variability of bud development as related to climate in two coexisting Mediterranean Quercus species. Annals of Botany 111: 261–270.

Alla AQ, Camarero JJ, Palacio S, Montserrat-Martí G. 2013b. Revisiting the fate of buds: size and position drive bud mortality and bursting in two coexisting Mediterranean Quercus species with contrasting leaf habit. Trees – Structure and Function 27: 1375–1386.

Barigha TS, Bonhomme M, Lopez D, Traore A, Douris M, Venisse J-S, Cochard H, Bodel E. 2013. Modulation of bud survival in Populus nigra sprouts in response to water stress-induced embolism. Tree Physiology 33: 261–274.

Barthélemy D, Caraglio Y. 2007. Plant architecture: a dynamic, multi-level and comprehensive approach to plant form, structure and ontogeny. Annals of Botany 99: 375–407.

Bates D, Maechler M, Bolker B, Walker S. 2014. lme4: linear mixed-effects models using Eigen and S4. Vienna, Austria: R Foundation for Statistical Computing. R package version 1.1-7.

Berleth T, Mattsson J. 2000. Vascular development: tracing signals along veins. Current Opinion in Plant Biology 3: 406–411.

Bond BJ, Czaromski NM, Cooper C, Day ME, Greenwood MS. 2007. Developmental decline in height growth in Douglas-fir. Tree Physiology 27: 441–453.

Bucci SJ, Goldstein G, Meinerz FC, Franco AC, Campanello P, Scholz FG. 2005. Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in Neotropical savanna trees. Trees – Structure and Function 19: 296–304.

Cline MG. 2000. Execution of the auxin replacement apical dominance experiment in temperate woody species. American Journal of Botany 87: 182–190.

Cline MG, Bhove N, Harrington CA. 2009. The possible roles of nutrient deprivation and auxin repression in apical control. Trees – Structure and Function 23: 489–500.

Cochard H, Coste S, Chanson B, Guelh JM, Nicolini E. 2005. Hydraulic architecture correlates with bud organogenesis and primary shoot growth in beech (Fagus sylvatica). Tree Physiology 25: 1545–1552.

Davidson CG, Remphrey WR. 1994. Shoot neoformation in clones of Fraxinus pennsylvanica in relation to genotype, site and pruning treatments. Trees – Structure and Function 8: 205–212.

de Fays E, Vacher V, Humbert F. 2000. Water-related phenomena in winter buds and twigs of Picea abies L. (Karst.) until bud-burst: a biological, histological and NMR Study. Annals of Botany 86: 1097–1107.

Djennane S, Hibrand-Saint Oyant L, Kawamura K, Lalanne D, Laffaire M, Thouroude T, Chalain S, Sakr S, Boumaza R, Foucher F, Leduc N. 2014. Impacts of light and temperature on shoot branching gradient and expression of strigolactone synthesis and signalling genes in rose. Plant, Cell and Environment 37: 742–757.

Domagalska MA, Leyser O. 2011. Signal integration in the control of shoot branching. Nature Reviews Molecular Cell Biology 12: 211–221.

Eschbach, A, Kappen L. 1996. Leaf area index determination in an older forest: a comparison of three methods. Journal of Experimental Botany 47: 1457–1462.

Eschrich W, Burchardt R, Essihamah S. 1989. The induction of sun and shade leaves of the European beech (Fagus sylvatica L.): anatomical studies. Trees – Structure and Function 3: 1–10.

Fournoud T, Zhang X, Stokes A, Lambers H, Körner C. 2008. Plant growth modelling and applications: the increasing importance of plant architecture in growth models. Annals of Botany 101: 1053–1063.

Fox J, Weisberg S. 2011. An R Companion to applied regression, 2nd edn. Thousand Oaks, CA: Sage.

Gill AM. 1971. The formation, growth and fate of buds of Fraxinus americana L. in central Mass. Harvard Forest Paper 20: 1–16.

Girault T, Bergougoux N, Combes D, Viemont J-D, Leduc N. 2008. Light controls shoot meristem organogenic activity and leaf primordia growth during bud burst in Rosa sp. Plant, Cell and Environment 31: 1534–1544.

Gordon D, Damiano C, DeJong TM. 2006. Precambrian in vegetative buds of Prunus persica: factors influencing number of leaf primordia in overwintering buds. Tree Physiology 26: 537–544.

Goulet J, Messier C, Nikinmaa E. 2000. Effect of branch position and light availability on shoot growth of understory sugar maple and yellow birch sapling. Canadian Journal of Botany 78: 1077–1085.
Guédon Y, Puntieri JG, Sabatier S, Barthélémé D. 2006. Relative extents of preformation and neoformation in tree shoots: analysis by a deconvolution method. Annals of Botany 98:835 – 844.

Hansen R, Mander Ü, Soosaar K, Maddison M, Löhmus K, Kupper P, Kanal A, Söber J. 2013. Greenhouse gas fluxes in an open air humidity manipulation experiment. Landscape Ecology 28:637 – 649.

Henry C, Robot A, Laloi M, Mortreau E, Sigogne M, Leduc N, Lemoine R, Sakr S, Vian A, Pelleschi-Travier S. 2011. Regulation of RhSUC2, a sucrose transporter, is correlated with the light control of bud burst in Rosa sp. Plant, Cell and Environment 34:1776 – 1789.

Henry HAL, Aarssen LW. 2001. Inter- and intraspecific relationships between shade tolerance and shade avoidance in temperate trees. Oikos 93:477 – 487.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346 – 363.

Jones M, Harper JL. 1987a. The influence of neighbours on the growth of trees I: the demography of buds in Betula pendula. Proceedings of the Royal Society B: Biological Sciences 232:1 – 18.

Jones M, Harper JL. 1987b. The influence of neighbours on the growth of trees: II. The fate of buds on long and short shoots in Betula pendula. Proceedings of the Royal Society B: Biological Sciences 232:19 – 33.

Kawamura K. 2010. A conceptual framework for the study of modularity in plant functional groups: an allometric scaling relationship. Oikos 119:1167 – 1175.

Koestner B, Magnani F, Marshall J, Meinzer F, Phillips N, Ryan M, Whitehead D. 2002. The relationship between tree height and leaf area: sapwood area ratio. Oecologia 132:12 – 20.

Kukk and Söber — Bud development and shoot morphology in relation to crown location

Lauri P-E´, Bourdel G, Trottier C, Cochard H. 2008. Apple shoot architecture: evidence for strong variability of bud size and composition along a branching zone. Tree Physiology 28:197 – 208.

McDowell N, Barnard H, Bond B, Hinckley T, Hubbard R, Ishii H, Köstner B, Magnani F, Marshall J, Meinzer F, Phillips N, Ryan M, Whitehead D. 2002. The relationship between tree height and leaf area: sapwood area ratio. Oecologia 132:12 – 20.

Maillette L. 1982. Structural dynamics of silver birch. I. The fates of buds. Journal of Applied Ecology 19:203 – 218.

Mailliet L. 1982a. Structural dynamics of silver birch. II. The fate of buds. Journal of Applied Ecology 19:219 – 238.

Mailliet L. 1982b. Structural dynamics of silver birch. III. A matrix model of the bud population. Journal of Applied Ecology 19:219 – 238.

Marcelis-van Acker CAM. 1994. Effect of assimilate supply on development and growth potential of axillary buds in roses. Annals of Botany 73:415 – 420.

Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA. 2014. Sugar demand, not auxin, is the initial regulator of apical dominance. Proceedings of the National Academy of Sciences of the USA 111:6092 – 6097.

McDowell N, Barnard H, Bond B, Hinckley T, Hubbard R, Ishii H, Köstner B, Magnani F, Marshall J, Meinzer F, Phillips N, Ryan M, Whitehead D. 2002. The relationship between tree height and leaf area: sapwood area ratio. Oecologia 132:12 – 20.

Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA. 2014. Sugar demand, not auxin, is the initial regulator of apical dominance. Proceedings of the National Academy of Sciences of the USA 111:6092 – 6097.

McDowell N, Barnard H, Bond B, Hinckley T, Hubbard R, Ishii H, Köstner B, Magnani F, Marshall J, Meinzer F, Phillips N, Ryan M, Whitehead D. 2002. The relationship between tree height and leaf area: sapwood area ratio. Oecologia 132:12 – 20.

Niinemets Ü, Valladares F. 2004. Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. Plant Biology 6:254 – 268.

Normand F, Bissery C, Damour G, Lauri P-E. 2008. Hydraulic and mechanical stem properties affect leaf–stem allometry in mango cultivars. New Phytologist 178:590 – 602.

Osada N, Toteno R, Mori A, Takeda H. 2004. Changes in crown development patterns and current-year shoot structure with light environment and tree height in Fagus crenata (Fagaceae). American Journal of Botany 91:1981 – 1989.

Pinheiro J, Bates D, DebRoy S, Sarkar D; the R Development Core Team. 2014. nlme: linear and nonlinear mixed effects models. Vienna, Austria: R Foundation for Statistical Computing. R package version 3.1-117.

Preston KA, Ackery DD. 2003. Hydraulic architecture and the evolution of shoot allometry in contrasting climates. American Journal of Botany 90:1502 – 1512.

Preston KA, Ackery DD. 2003. Hydraulic architecture and the evolution of shoot allometry in contrasting climates. American Journal of Botany 90:1502 – 1512.

R Development Core Team. 2014. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Remphrey WR, Davidson CG. 1994. Shoot preformation in clones of Fraxinus pennsylvanica in relation to site and year of bud formation. Trees – Structure and Function 8:126 – 131.

Remphrey WR, Bartlett GA, Davidson CG. 2002. Shoot morphology and fate of buds in relation to crown location in young Fraxinus pennsylvanica var. subintegerrima. Canadian Journal of Botany 80:1274 – 1282.

Sabatier S, Barthélémé D. 2001. Bud structure in relation to shoot morphology and position on the vegetative annual shoots of Juglans regia L. (Juglandaceae). Annals of Botany 87:117 – 123.

Sachs T, Novoplansky A, Cohen D. 1993. Plants as competing populations of redundant organs. Plant, Cell and Environment 16:765 – 770.

Sanz-Pérez V, Castro-Diez P. 2010. Summer water stress and shade alter bud size and budburst date in three mediterranean Quercus species. Trees – Structure and Function 24:89 – 97.

Sun S, Jin D, Shi P. 2006. The leaf size–twig size spectrum of temperate woody species along an altitudinal gradient: an invariant allometric scaling relationship. Annals of Botany 97:97 – 107.

Sutinen S, Portanen J, Viherä-Aarnio A, Hätikönens, R. 2012. Development and growth of primordial shoots in Norway spruce buds before visible bud burst in relation to time and temperature in the field. Tree Physiology 32:987 – 997.

Taugourdeau O, Sabatier S. 2010. Limited plasticity of shoot preformation in response to light by understory saplings of common walnut (Juglans regia). AoB PLANTS 2010: plq022; doi:10.1093/aobpla/plq022.

Uemura A, Ishida A, Nakano T, Terashima I, Tanabe H, Matsumoto Y. 2000. Acclimation of leaf characteristics of Fagus species to previous-year and current-year solar irradiances. Tree Physiology 20:945 – 951.

Worton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. Biological Reviews 81:259 – 291.

Worton DI, Duursma RA, Falster DS, Taskinen S. 2012. smatr 3—an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution 3:257 – 259.

Westoby M, Wright IJ. 2003. The leaf size–twig size spectrum and its relationship to other important spectra of variation among species. Oecologia 135:621 – 628.

White J. 1979. The plant as a metapopulation. Annual Review of Ecology and Systematics 10:109 – 145.