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ORIGINAL ARTICLE

Impacts of bud set and lammas phenology on root:shoot biomass partitioning and carbon gain physiology in poplar

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Abstract

Key message **Timing of bud set and occurrence of lammas in trees can alter growth partitioning (i.e., root:shoot ratios), while only bud set effectively modifies carbon gain by increasing photosynthesis-related physiological traits.**

Abstract Bud set and lammas (second bud flushing) phenology may strongly influence growth, physiology, and biomass in trees. To test effects of these phenological events, 54 individuals from 16 genotypes of black cottonwood poplar (*Populus trichocarpa*) were grown in a potted trial under greenhouse conditions (with extended daylengths promoting growth), followed by open-air cultivation (with natural daylengths promoting bud set and/or lammas). Trees were monitored for phenology, repeatedly measured for photosynthesis-related traits, harvested for biomass, and assessed for growth partitioning (separating above- and belowground parts). We grouped trees by phenology for comparisons: (1) trees with early summer bud set, (2) trees with early summer bud set that underwent lammas, (3) trees with late summer bud set (August), and (4) trees with bud set occurring in autumn (September). We found that bud set timing positively affected growth partitioning where earlier bud set resulted in shorter trees with higher root:shoot biomass ratios (by increasing root mass). Lammas growth altered these ratios by significantly increasing shoot growth relative to belowground growth. Trees with bud set occurring in late summer also had higher root:shoot biomass ratios (by increasing root mass) compared to trees setting bud in autumn. Occurrence of bud set coincided with modified physiology of the existing canopy where photosynthesis-related traits were enhanced relative to trees still actively growing. These physiological changes were unaltered by occurrence of lammas. This suggests that bud set prompts a significant, coordinated mechanism of higher carbon gain physiology and belowground biomass accumulation in plants within a "post-bud set" phase.

Keywords Biomass accumulation · Lammas growth · Photoperiod · Photosynthesis · Post-bud set · Seasonality

Introduction

Seasonal events of the canopy cycle (phenology) play a central and critical role in the adaptation of plants across the landscape (Lechowicz 1984), and altering the timing of these periodical phenomena may have dramatic impacts on plant growth and survivability (Wareing 1948). In poplars (*Populus* L.), phenology is strongly linked with geography (Pauley and Perry 1954; Farmer 1993; Luquez et al. 2008; Keller et al. 2011; McKown et al. 2014); this connection is often latitudinal in nature, primarily reflecting adaptation to local photoperiodicity and annual seasonal temperatures (Wareing 1948; Kalcsits et al. 2009; Rohde et al. 2011; Soolanayakanahally et al. 2013; McKown et al. 2014; Guy 2014). The precise dates of phenological events (i.e., bud

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break, leaf flushing, bud set, leaf senescence and leaf drop; Fig. 1a) are plastic to some extent and can shift from year to year depending on temperature conditions and/or secondary environmental stressors, such as drought or disease. Nevertheless, phenological traits are relatively consistent and result in repeatable year-to-year patterns, such as genotype or population rankings, observed across poplar collections grown within common gardens (Luquez et al. 2008; Keller et al. 2011; Rohde et al. 2011; Olson et al. 2013; Soolanayakanahally et al. 2013; McKown et al. 2014).

In particular, the timings of annual phenological events can have profound physiological effects on growth and carbon gain in poplars (Wareing 1948; Cannell and Willett 1976; Riemenschneider et al. 1992; Farmer 1993; Soolanayakanahally et al. 2013). Within a yearly cycle, leaf emergence in the spring to leaf drop in the autumn demarcates the overall duration of the green canopy, defining the potential for growth and overall carbon gain (Fig. 1a). Other seasonal events, such as new leaf production and stem height growth, can also influence plant growth and determine overall canopy size (Fig. 1b). These events precede the mid-seasonal phenomenon of "bud set" (Fig. 1c), where signals are perceived by the terminal meristem to develop a bud through formation of leaf primordia surrounded by protective bud scales, generally heralding the cessation of height growth (Rohde et al. 2011; Cooke et al. 2012). Bud set occurs in a progressive fashion and is usually initiated in response to photoperiodic

cues (i.e., lengthening night time period) but may also occur in response to biotic and abiotic stressors (e.g., heat, disease and/or drought) (Kalcsits et al. 2009; Rohde et al. 2011). In both *P. balsamifera* L. and *P. trichocarpa* Torr. & A. Gray, early or premature bud set has been observed where a mismatch exists between the environment and the genotype, primarily due to an inappropriate photoperiod (Soolanayakanahally et al. 2013; McKown et al. 2014). The main effect of shifting bud set timing forward (via photoperiodic mismatch or stress cues) has been noted as a reduction in height of the individual. The period following bud formation (i.e., a "post-bud set" phase) is interpreted as potentially reallocating carbon and other plant resources from height growth and canopy development (branch formation, leaf production) to increasing stem diameter and/or root growth (Cannell and Willett 1976; Soolanayakanahally et al. 2013). In addition, *P. trichocarpa* trees transitioning from active growth to the post-bud set phase have shown general population-wide patterns of increasing photosynthetic rate and stomatal conductance in leaves, supported by an overall increase in leaf nitrogen and chlorophyll content within the static canopy (i.e., changes occurring within the existing leaves) (McKown et al. 2013).

Onset of the post-bud set phase can be circumvented or delayed by development of late-season shoots: (1) lammas shoots, which are derived from terminal buds (Fig. 1d, Suppl. Fig. 1), and/or (2) proleptic shoots, occurring from expansion of lateral buds below a terminal bud (Kozlowski and Pallardy 1997). Under normal circumstances, a bud enters dormancy and does not flush until experiencing a chilling period and perceiving growth signals in the following spring; however, lammas phenology disrupts the progressive nature of bud set as the terminal meristem undergoes a second, mid-seasonal flushing of the bud to resume growth (Fig. 1d, Suppl. Fig. 1). This often occurs in response to photoperiodicity, fluctuating temperatures, insect and/or disease damage (Wareing 1948; Grudzinskaja 1960; Cooke et al. 2012; Soolanayakanahally et al. 2013). Lammas growth in trees is widespread and found across diverse phylogenetic families, such as Pinaceae (Adams and Bastien 1994; Mboyi and Lee 1999), Fagaceae (Wareing 1954; Taylor and Dobson 1989; Alaoui-Sossé et al. 1996), Rosaceae (Beikircher and Mayr 2013) and Salicaceae (Critchfield 1960; Soolanayakanahally et al. 2013). While the genetics underlying lammas behavior are not well understood, lammas is presumed to reflect a reassessment of environmental cues by the plant (Wareing 1948; Adams and Bastien 1994), and may provide a "margin of safety" in the case of premature bud set by acting to increase plant biomass, as height growth and leaf development can be resumed (Soolanayakanahally et al. 2013). Duration of growth due to a second flushing is
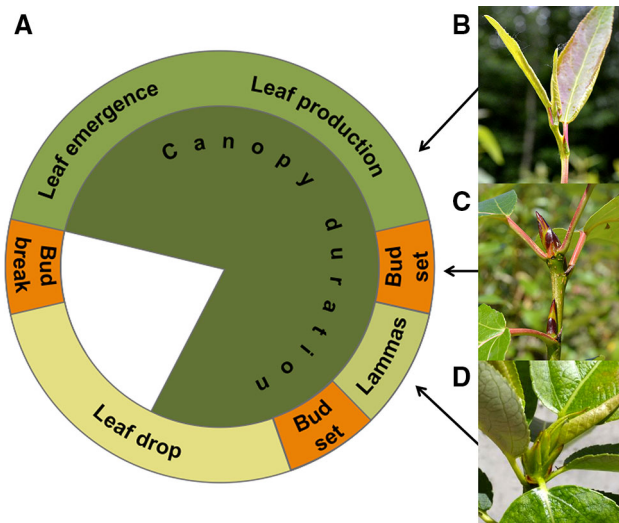
Fig. 1 **a** Diagram indicating the annual progressive and cyclical nature of phenological events occurring within the tree canopy, and duration of the green canopy period in *Populus trichocarpa*. Timing of lammas (mid-seasonal flushing) between first and second bud set indicated where it might occur. **b** Terminal meristem under active growth indicating leaf production. **c** Formation of a terminal bud. **d** Lammas, or second, mid-seasonal flushing of the terminal meristem

generally limited, however, and lammas is often rapidly followed by a final, terminal bud set (Soolanayakanahally et al. 2013). In both *P. balsamifera* and *P. trichocarpa*, lammas behavior was observed in trees planted within common gardens outside their native ranges (Soolanayakanahally et al. 2013; McKown et al. 2013; Suppl. Fig. 2), but the phenomenon also occurs in wild trees within their native environments (Suppl. Fig. 1).

In this study, we investigated the effects of lammas and timing of bud set on biomass accumulation, above- vs. belowground growth partitioning, and carbon gain physiology in *P. trichocarpa*. While early or premature bud set is predicted to curtail height growth, we hypothesized that a plant with early bud set would shift growth and allocate resources towards greater root development (cf. Cannell and Willett 1976; Soolanayakanahally et al. 2013), resulting in altered root:shoot biomass ratios compared to trees setting bud at a later date. We also assessed the effects of lammas growth in *P. trichocarpa* to determine whether any changes in biomass and/or above- vs. belowground growth partitioning accompanied a second flushing state. We hypothesized that lammas growth would increase total biomass and modify root:shoot ratios as the plant would reallocate resources primarily towards stem height growth after a second flushing. We also assessed carbon gain traits

in leaves in conjunction with the onset of bud set and/or lammas growth. We predicted that changes in leaf physiology (such as higher photosynthetic rates, cf. McKown et al. 2013) would occur in combination with increasing shoot growth (following lammas) or higher root growth (following bud set). In assessing the effects of bud set and lammas phenology on biomass accumulation, growth partitioning and carbon gain physiology, we aimed to understand how these important, natural phenomena might play a role in tree survival within a variable environment.

Materials and methods

Tree materials and cultivation

Materials used in this study originated from wild *P. trichocarpa* genotypes collected by the British Columbia Ministry of Forests, Lands & Natural Resource Operations (FLNRO) (Xie et al. 2009). Numerous genotypes were outplanted as young trees in June 2008 within a "clone bank" at Totem Field, University of British Columbia, Vancouver, British Columbia and subjected to yearly coppicing treatment. Dormant/quiescent whip cuttings were taken in January 2012 (selected genotypes listed in Table 1) and
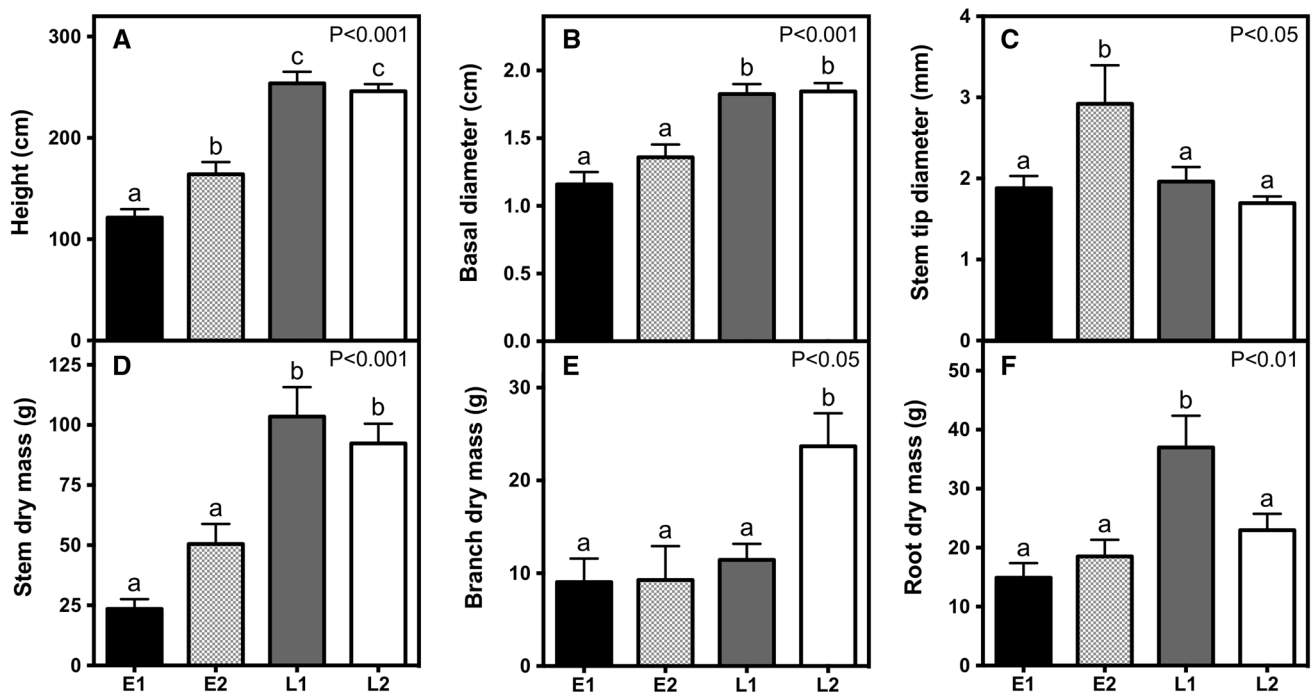
Fig. 2 General growth and biomass measures (±standard error) of potted *Populus trichocarpa* trees distinguished by timing of bud set and presence of lammas. **a** Final height (cm). **b** Basal diameter (cm). **c** Stem tip diameter (mm). **d** Stem dry biomass (g). **e** Total branch dry biomass (g). **f** Total root dry biomass (g). Phenological groups (*E1*, *E2*, *L1*, *L2*) are tested using linear mixed effects modeling and

compared with differences in Least Squares Mean tests (full details and model-estimated means in Suppl. Table 3). *E1* (*black bars*) trees with early bud set without lammas; *E2* (*pattern bars*) trees with early bud set that underwent lammas; *L1* (*dark gray bars*) trees with bud set occurring in August; *L2* (*white bars*) trees with bud set occurring in September

Table 1 Origin of *Populus trichocarpa* genotypes from British Columbia, Canada used in the potted growth and physiology trial

FLNRO code[a]	River drainage	Latitude (°N)	Longitude (°W)	Elevation (m)	N[b]
BULF11-5	Bulkley[c]	54.55	126.83	561	3
FNYI28-2	Fanny Bay	49.52	124.85	46	3
HAZH10-5	Hazelton[c]	55.22	127.67	238	4
ISKA6-1	Iskut[c]	56.93	130.33	317	4
KTSG10-5	Kitseguecla[c]	55.10	127.92	213	4
MCGR15-4	McGregor[c]	54.18	122.0	579	4
QAUS16-7	Australian[c]	52.72	122.47	442	4
SHEL15-4	Shelley[c]	54.03	122.60	564	4
SKNN10-4	Skeena[c]	54.58	128.42	61	3
SKNP10-2	Skeena[c]	54.55	128.47	61	3
SKNP10-4	Skeena[c]	54.55	128.47	61	4
SKNR10-1	Skeena[c]	54.68	128.35	244	4
SLMC28-1	Salmon	50.27	125.83	30	1
SLMD28-1	Salmon	50.28	125.87	30	2
SQMA25-1	Squamish	49.87	123.23	61	4
TOBB23-5	Toba	50.57	124.08	73	3

[a] Codes provided by the British Columbia Ministry of Forests, Lands and Natural Resource Operations (FLNRO)

[b] Number of plants per genotype in the potted growth trial

[c] Accessions originating from this location underwent lammas in Totem Field, Vancouver, Canada

whips collected from all trees were considered "leader" shoots with terminal buds (due to resprouting in *P. trichocarpa* following coppicing). For our experiments, *P. trichocarpa* genotypes were selected from this larger collection to represent a range of latitudes ($\sim$7.2°) within British Columbia and diversity in phenological behavior (Table 1). Specifically, genotypes from variable latitudes were chosen as they were known to have differing photoperiodic responses under the summertime daylength conditions at 49 °N (Vancouver, BC), resulting in bud set occurring across a range of dates (i.e., northern genotypes setting bud before southern genotypes, cf. McKown et al. 2014). Many northern trees were also selected because only a few genotypes (from 54 °N and higher) were observed to lammas under the photoperiodic conditions at 49 °N in any given year (McKown et al. 2013; Table 1, Suppl. Figs. 1, 2). With this diversity, we aimed to track the effects of timing of bud set and/or lammas on biomass and physiology under similar growing conditions for all individuals.

Whips were stored at 4 °C in a cold chamber until late January 2012, then trimmed to 25 cm and planted into Ray Leach conical tubes ("cone-tainers") with a potting soil mixture of 70 % peat moss and 30 % Perlite (West-Creek Farms, BC, Canada). Cuttings were flushed within a greenhouse setting and watered twice daily with bench flooding. After 4 weeks in cone-tainers, established cuttings with leaves and well-developed roots were replanted into 1-L pots. Plants were watered daily and fertilized weekly with a low-dose, all-purpose fertilizer water [15-5-15 N-P-K plus micronutrients (Plant Products Co., ON, Canada)]. To promote active growth and prevent premature bud set in the northern genotypes, plants were grown under a 21-h photoperiod with a minimum photosynthetic photon flux density (PPFD) of 677 μmol m^{-2} s^{-1} and maintained at $\sim$20 °C. After 12 weeks of growth, healthy, well-established saplings from 16 genotypes (with replicates = 54 plants total) were replanted into larger 3 gal ($\sim$11 L) pots in late May, and maintained under the 21-h photoperiod. In late June (after the summer solstice), all plants were moved to an outdoor area of the greenhouse with natural lighting only (max. 16 h light) to permit normal phenological responses to the naturally changing photoperiod and lengthening night.

Phenology, physiology and growth

From late July to late September 2012, all 54 trees were monitored for seasonal canopy events of leaf production, bud set and lammas. Specifically, we checked the terminal meristem on the main stem for production of new leaves indicating active growth (Fig. 1b), appearance of a green bud (early stage of bud set, cf. Rohde et al. 2011), or formation of a red-brown bud (late stage of bud set, cf. Rohde et al. 2011) (Fig. 1c). We also assessed trees for occurrence and duration of lammas phenology (i.e., second/mid-season flushing of the terminal bud) (Fig. 1d,

Suppl. Fig. 1). These observations were used to form natural phenological groups among the individual trees (see "Results", Suppl. Table 1).

Trees were sampled for five physiological traits over a 3-week time course. This included three photosynthesis-related gas exchange-based traits: maximum photosynthetic rate (A_{max}; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}), and instantaneous water-use efficiency (WUE; µmol CO_2 mmol^{-1} H_2O) as determined by photosynthetic rate over transpiration under constant vapor pressure deficit (VPD). We also assessed leaf chlorophyll levels using the chlorophyll content index (CCI; unit-less), and leaf mass per unit area (LMA; mg cm^{-2}) as a physiological trait that accounts for the dry mass investment in leaves per unit of light-capture area. All traits were measured once per week throughout mid-late August (with sampling separated by 7 days) to coincide with active growth and/or bud set and/or lammas depending on the tree. We chose the first three fully developed, upper canopy leaves on the main stems and continued to measure these same leaves repeatedly across all weeks. For each physiological trait, an average value was calculated per sampled leaf and used to determine a mean trait value for each individual at each time point (Suppl. Table 2).

Within each genotype, we split replicated ramets randomly into two groups and measured two out of three replicates (where possible) for gas exchange-based traits and chlorophyll measures. All remaining trees were assessed for LMA (due to the destructive nature of tissue sampling, these were not used for gas exchange). We used 15 genotypes (28 trees total) spanning the geographical range of genotypes selected and all phenological stages (see below) for gas exchange-based traits. Measurements were taken between 0900 and 1500 h using either a LI-COR 6400 or LI-COR 6400 XT portable infrared gas exchange system (LI-COR Biosciences, Lincoln, NE, USA). The sampled leaf was gently placed within the LI-COR chamber set at a photosynthetic photon flux density of 2000 µmol m^{-2} s^{-1}, 400 µmol CO_2 mol^{-1} air, and 25 °C. After stabilizing at a VPD of 1.45–1.55 kPa, intercellular CO_2 to ambient CO_2 ratios (C_i/C_a) of 0.6–0.9, and with steady rates of A_{max} and g_s, three readings were recorded for each leaf separated by 20–30 s each. Four CCI measurements were taken on these same leaves using a CCM-200 plus SPAD chlorophyll meter (Opti-Sciences, Inc., Hudson, NH, USA). LMA was measured in 13 genotypes (20 trees total) spanning the geographical range of genotypes selected and all phenological stages. Using a hand-held punch, we repeatedly sampled two small discs of leaf tissue (total 61 mm^2) over the same time period. Samples were oven dried at 60 °C for 48 h and weighed to determine dry mass values.

Following an 8-month cultivation period (February–September), we quantified multiple traits relating to biomass accumulation and growth partitioning (Suppl. Table 1). General stem growth was assessed by measuring total tree height (H; cm). Trees that underwent lammas were measured for the additional height growth and the number of leaves produced from the second flushing. We measured the diameter of the stem at the soil level (D; cm) and tip (1 cm below the terminal bud) and calculated size ratios using total tree height and basal diameter (i.e., $H{:}D$ ratios; cm cm^{-1}). Trees were then defoliated, and the aboveground biomass was harvested and separated into the main stem and branches. We carefully removed the roots from the soil matrix and retained as much of the fine root mass as possible with gentle sifting and washing in water. Stems, branches, and roots were dried in individual paper bags at 60 °C for 2 weeks to ensure full removal of all water content and weighed to obtain dry masses. These values were also used to calculate total biomass (g) and root:shoot biomass ratios (g g^{-1}).

Statistics

Comparisons of growth, biomass and physiology among individual trees were tested based on their phenological status in August and we defined comparative groups according to the general timing of bud set observed (see "Results"). Trees with bud set occurring in early summer prior to August ("E") were distinguished by whether or not lammas was observed (E1 = no lammas, E2 = occurrence of lammas). Trees with bud set occurring later in the season ("L") were distinguished by whether bud set was observed during or after the physiological measurement period (L1 = bud set occurring in August, L2 = bud set occurring in September).

We tested growth and biomass traits among all trees using a step-wise process. We assessed models considering different factors contributing to variance in a given trait, including "latitude" as a proxy for population structure and ecological differentiation (which can have strong effects on traits, cf. McKown et al. 2014), "group" defining our phenological categories, and "genotype" to account for ramet replication. We used linear mixed effects modeling with the *lme4* package (Bates et al. 2015) in the base R package (R Core Team; http://www.R-project.org) to test for the additive and interactive contributions of fixed effects ("latitude", "group") and random effects ("genotype" to account for ramet replication) to trait variance. Significance of the fixed-effect term "latitude" was assessed using a likelihood ratio test (LRT) with the 'anova' function in the *lmerTest* package in R (Kuznetsova et al. 2016). As the fixed effect of "latitude" was not a significant factor in any model for biomass or growth traits,

we removed this term. We then used the 'differences in Least Squares Means' function in *lmerTest* to compare model-estimated means of growth and biomass traits between our phenological groups (E1, E2, L1, L2).

For physiological traits measured repeatedly over 3 weeks, we ran separate tests assessing (1) the effects of lammas on physiology among early bud set trees (E1 vs. E2), and (2) the effects of onset of bud set among actively growing trees (L1 vs. L2). We pursued separate analyses as sample sizes were lower in our repeated measures (compared to above), and similar within, but not between, "E" and "L" groups. We used a mixed effects modeling approach with the *lme4* package in R (described above), and considered the additive and interactive contributions of fixed effects ("latitude", "group", "week") and random effects ("genotype") to physiological trait variance. We tested the significance of the fixed-effect term "latitude" with LRT using *lmerTest* (as described above) and maintained this fixed effect in models where it was a significant factor for a given physiological trait. We then compared model-estimated means between our phenological groups (E1 vs. E2, L1 vs. L2) across weeks using the differences in Least Squares Means function in *lmerTest*.

Results

The individuals grown and measured during our study showed a range in timings of phenological events. By monitoring trees over the span of the late summer period, we were able to distinguish four distinct groups based on timing of terminal bud formation and subsequent terminal bud behavior. These four groups were further used to compare effects of bud set timing and/or occurrence of lammas on growth, biomass accumulation and physiology in *P. trichocarpa*.

At the outset of our physiological measurements in August, we found that 20 individuals had undergone "early" bud set where a fully formed and matured terminal bud (i.e., red bud stage) was already present indicating that termination of height growth and initiation of bud formation had occurred several weeks earlier. These individuals were from eight genotypes originating from a wide range of latitudes (49.9–56.7 °N) and representing eight different river drainage localities within British Columbia (Suppl. Table 1). Only two genotypes (ISKA6-1 and SKNP10-4, originating from 56.7 to 54.6 °N, respectively) had set bud in all individuals. Among trees with early bud set, 10 out of 20 individuals (E1) remained fully bud set (indicating a relatively dormant terminal bud) while 10 out of 20 individuals (E2) underwent lammas growth (i.e., second, mid-seasonal bud flushing and resumed height growth/leaf production). These individuals were from four genotypes

originating from one southern (49.9 °N) and three northern localities (55.1–56.7 °N) (Suppl. Table 1). We found that trees that underwent lammas grew an additional 18 cm, on average, and were qualitatively thicker at the stem tip. In addition, lammas growth produced nine leaves, on average (including both pre-formed and neo-formed leaves) (Suppl. Table 1). These leaves tended to have a different morphology compared to those produced earlier in the growing season (i.e., lammas leaves were lighter in color and thinner). After a further 2–3 weeks of lammas-induced growth, the terminal meristem of these individuals developed a new bud, which then proceeded to dormancy and did not flush again.

All other trees were actively growing at the start of physiological monitoring in August. We broadly termed these as "later" bud set to distinguish them from trees with earlier bud set dates (see above). Trees with later bud set were from 14 genotypes (34 individuals) originating from 11 different river drainage localities within British Columbia and represented a wide range of latitudes (49.9–55.2 °N) (Suppl. Table 1). Among these trees, 15 out of 34 individuals (L1) showed initial stages of bud set (i.e., the development of a green bud) by the third week of monitoring in August, while the remaining 19 out of 34 individuals (L2) did not set bud until mid-late September.

Effects of phenology on growth partitioning and biomass accumulation

We tested for biomass and growth differences among our phenological groupings using linear mixed effects modeling. We included "latitude" co-ordinates as a covariate in our analyses; however, this term was not a significant factor for any model and was subsequently removed (Suppl. Table 3). We found that height for all trees was directly determined by timing of bud set, and trees with earlier bud set were less than half the height of trees with later bud set (Fig. 2a). Lammas growth (E2) resulted in significantly taller trees (by 35 %, on average) than their counterparts without lammas (E1), reflecting the period of resumed height growth. By comparison, trees setting bud in August (L1) were not significantly different in height to those setting bud later in September (L2). There was a general pattern of increasing basal diameter from earlier to later bud set trees; however, there were no differences among early bud set trees (E1, E2) or among later bud set trees (L1, L2) (Fig. 2b). This resulted in relatively little change to the allometric relationship between stem height and diameter (i.e., H:D ratio) among our groups (Suppl. Fig. 3). Stem tip diameters did not show the same pattern as basal stem diameters, and trees that underwent lammas had significantly thicker stem tips (1.5× greater) compared to trees within other groups (Fig. 2c). The average dry

mass of stems followed the trend observed in tree heights, and trees with later bud set had up to 70 % greater stem dry mass, on average, than trees with earlier bud set (Fig. 2d). Total dry mass of branches was greatest in trees setting bud latest (L2) compared to all other groups (Fig. 2e). No clear pattern was observed between timing of bud set and root development (as assessed by weight), and trees with bud set occurring in August (L1) had the greatest root dry mass, on average, compared to all other groups (Fig. 2f). Notably, root dry mass differed by 40 %, on average, between the tree groups with later bud set (L1, L2).

We found that the total biomass accumulation of the plant clearly reflected patterns in stem growth and the timing of bud set (Fig. 3a). In general, an earlier bud set date without lammas (E1) resulted in a threefold reduction in total dry biomass of the plant compared to trees with a later bud set date (L1, L2). Lammas growth (E2) partially moderated these effects by increasing total biomass (up to 50 %) compared to early bud set trees without lammas (E1). Nevertheless, trees that underwent lammas growth still had a twofold reduction in total dry mass of the plant compared to trees with a later bud set date. Notably, trees setting bud within the late summer or autumn period (L1, L2) were not significantly different in total biomass. By comparison, our assessments considering growth partitioning (i.e., using root:shoot biomass ratios) found a significant pattern change between our comparative groups (Fig. 3b). Values for root:shoot biomass ratios did not necessarily mirror either the patterns observed in stem mass (Fig. 2d) or root dry mass (Fig. 2f) but did reflect a timeline of bud set. This ratio was significantly higher (1.7× greater) in early bud set trees (E1) compared to their counterparts with lammas (E2). It was also significantly higher (1.5× greater) in trees setting bud in August (L1)

compared to trees setting bud in September (L2). Root:-shoot biomass ratios were highest in trees with the earliest bud set dates (E1) and lowest in trees setting bud the latest (L2), while ratios in lammas trees (E2) and trees setting bud in August (L1) were intermediate (and equivalent). This overall pattern was unaltered with the inclusion of branches with stem dry mass in calculating the growth partitioning ratios (Suppl. Fig. 4).
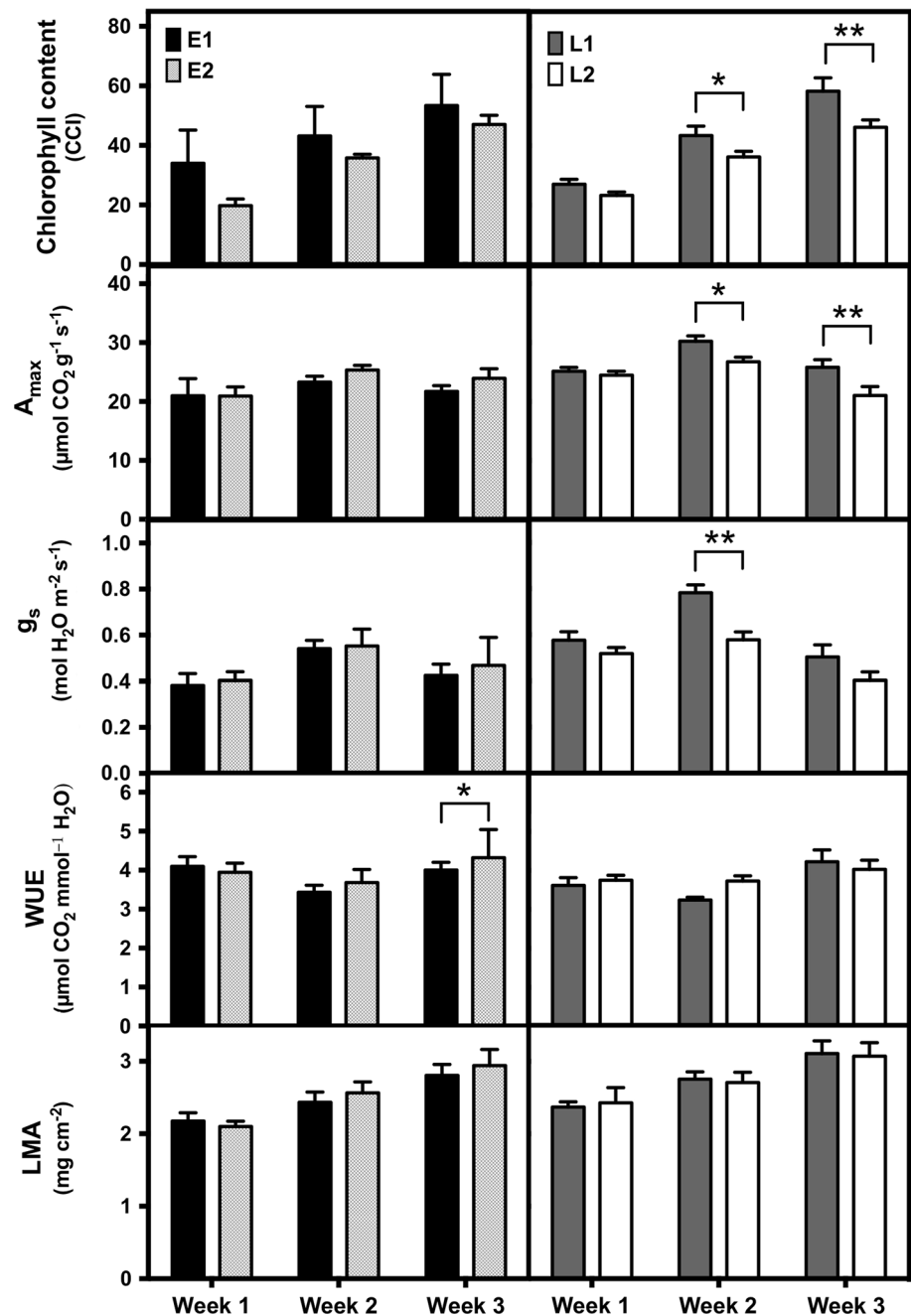
Effects of phenology on physiology

We used linear mixed effects modeling in our physiological study with repeated measures to determine trait differences among our phenological groupings. We included latitude as a covariate, and removed the term from the model where it was not a significant factor (Suppl. Table 4). In all trees commencing the physiological time course study with a fully formed terminal bud (i.e., E1, E2), we measured and compared traits in leaves produced before the first bud set event. Our results indicated that there were few differences in leaf physiology between trees with bud set (E1) or trees that underwent lammas (E2) at any given time point (Fig. 4). In general, there was an increase over time in the chlorophyll content index and LMA in both groups, but no clear time-related patterns in gas exchange-based traits were observed. Models for WUE included latitude as a significant covariate and we found that WUE was significantly higher in the third week of measurements in trees that underwent lammas (E2) compared to trees with bud set (E1). No other significant trait differences were found between groups. We noted that although A_{max} was not significant at any time point, trees that underwent lammas (E2) had 10 % higher photosynthetic rates, on average, in
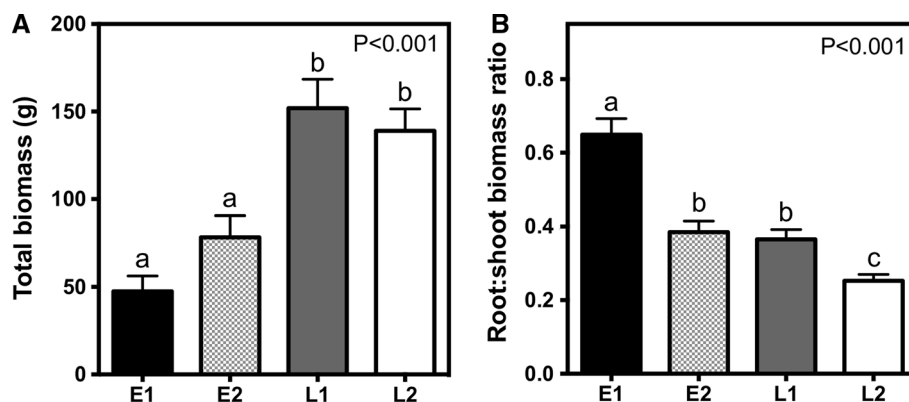
Fig. 3 Biomass measures (±standard error) of potted *Populus trichocarpa* trees distinguished by timing of bud set and presence of lammas. **a** Total biomass (g). **b** Ratio of root vs. shoot dry biomass. Phenological groups (*E1, E2, L1, L2*) are tested using linear mixed effects modeling and compared with differences in Least Squares

Mean tests (full details and model-estimated means in Suppl. Table 3). *E1* (*black bars*) trees with early bud set without lammas; *E2* (*pattern bars*) trees with early bud set that underwent lammas; *L1* (*dark gray bars*) trees with bud set occurring in August; *L2* (*white bars*) trees with bud set occurring in September

Discussion

Biomass and physiological modifications related to the timing of bud set

Modifying the timing of bud set in *P. trichocarpa* had clear impacts on total biomass accumulation (Fig. 3a) and signaled a significant change in growth partitioning (i.e., above- vs. belowground biomass differences) relating directly to phenology (Fig. 3b). Not unexpectedly, trees setting bud in the earlier part of the summer had severely curtailed height growth compared to those setting bud in later summer and/or early autumn. Similar patterns have been observed in common garden growth trials of *P. trichocarpa* (Cannell and Willett 1976; McKown et al. 2014) and its sister-species, *P. balsamifera* (Soolanayakanahally et al. 2013). Within our study, we determined that trees with a later occurrence of bud set had up to 70 % greater aboveground biomass accumulation compared to trees with earlier bud set. This related only partly to changes in aboveground growth where trees with earlier bud set had shorter stems, less stem dry mass, and limited growth of the tree canopy (i.e., fewer leaves, less branch dry mass). As the leaf canopy remained green following bud set (in all trees), we anticipated continued carbon fixation and that plant resources might be allocated to increasing root growth (cf. Cannell and Willett 1976; Soolanayakanahally et al. 2013). Trees with the earliest occurrence of bud set (E1) showed a very high amount of root growth relative to their stem height, and had root dry masses similar to trees setting bud much later in the season (L2) (Fig. 2f). This observation was notable as the canopy was much smaller (i.e., fewer leaves) on trees with earlier bud set, indicating that the amount of root investment was considerable relative to the overall capacity of the canopy. These changes in root dry mass did not alter patterns of total biomass accumulation but were clearly reflected in the root:shoot biomass ratios, which were much higher in trees with earlier bud set.

Other consequences of bud set timing to growth partitioning were outlined by the two groups of "later" bud set trees (L1, L2) where obvious changes to the stem (such as height and diameter) were not necessarily observed. Trees setting bud approximately 1 month earlier in August (L1) tended to have equivalent stem mass (relative to their height) while trees setting bud later in September (L2) tended to have greater canopy development (as determined by branch dry mass). By contrast, trees setting bud in August (L1) had substantially greater root development (by mass) than those that continued height growth into the autumn (L2). The change in growth partitioning did not alter overall biomass accumulation substantially (Fig. 3a)

but significantly modified root:shoot biomass ratios (Fig. 3b). This clear difference between L1 vs. L2 trees underscored the shift in resource allocation to root growth following the onset of bud set.

In addition to modifications in root:shoot biomass ratios, physiological differences within leaves of the existing canopy were observed with the onset of bud set (L1) compared to active growth (L2). The occurrence of bud set was paralleled by a significantly greater amount of chlorophyll within the individual leaf and higher gas exchange traits (photosynthesis, stomatal conductance) compared to trees still within an active growing phase. Notably, these physiological changes were superimposed on leaf age, which often shows a decrease in leaf photosynthetic capacity over time (Wilson et al. 2001). By comparison, LMA showed high variability across time but there were no differences between groups, suggesting that LMA may be less informative about the effects of phenology on leaf carbon gain physiology.

Many of the trees within our study set bud earlier in the summer primarily due to photoperiodic mismatch; however, it posits that under natural circumstances, an unfavorable environment or damage causing early bud set might be mitigated by increased belowground resources (e.g., as a mechanism to support enhanced growth in the following spring season). Cannell and Willett (1976) noted that root:shoot "imbalances" relating to early bud set were corrected through growth in subsequent spring and summer seasons across different tree species. Nevertheless, the shift to increasing root growth following bud set is likely the normal mechanism, even when bud set occurs within an appropriate timeframe. Time-trial studies of shoot and root mass in *P. balsamifera* have suggested a similar phenomenon occurs within this species relating specifically to the timing of bud set (R. Soolanayakanahally, pers.comm.). The onset of bud set and the shift towards root growth is accompanied by a significant transition in carbon gain physiological traits (within the existing canopy). This strongly supports the post-bud set period as a phase of enhanced carbon gain per unit leaf area through increases in leaf chlorophyll content and photosynthesis within pre-existing leaves. Based on these observations, we consider "post-bud set" to describe a phenomenon where phenology (specifically, bud set) triggers a coordinated shift towards a combined mechanism of greater carbon gain (per unit leaf area) and belowground investment within the plant. It is unlikely that the duration of this period (or the activities occurring within it) is identical from year to year (e.g., an extended summer vs. early winter); rather, this phase exists to increase resources in support of future events, such as winter survival, disease resistance and/or rapid spring growth.

Lammas affects biomass accumulation but not necessarily physiological traits

Trees that underwent lammas (E2) had greater above-ground biomass, reflecting an increased overall stem size (height, diameter, mass), compared to their early bud set counterparts without lammas (E1). In other tree species, lammas growth has been noted to increase height growth by 25 % (e.g., Adams and Bastien 1994; Mboyi and Lee 1999). Tree stem tips in *P. trichocarpa* were also significantly thicker following lammas growth (Fig. 2c; see Suppl. Fig. 1). A similar phenomenon was observed in *Malus* (Rosaceae) where stem/branch thickening occurred with lammas growth through altered stem anatomy and the development of greater layers of pith and cortical parenchyma layers, while other tissues (such as xylem) were relatively unchanged (Beikircher and Mayr 2013). In *P. trichocarpa*, we also found that leaves developing after lammas bud flushing were visually different in appearance (primarily thinner and lighter in color) compared to those of the pre-existing canopy. Critchfield (1960) observed similar morphological changes in leaves from lammas shoots of *P. trichocarpa* and attributed this to a shortened embryonic and/or development period.

Nevertheless, resuming a short period of stem growth due to lammas clearly shifted root:shoot biomass ratios in *P. trichocarpa* trees, resulting in ratios that were significantly lower than early bud set trees without lammas (E1), and equivalent to trees setting bud for the first time in August (L1). In comparison to changes in aboveground growth, lammas phenology did not significantly increase or reduce overall root dry mass (relative to E1 trees) suggesting that trees that underwent lammas were maintaining a similar "rate" of root investment alongside the increased height growth. The sustained investment in roots is plausible as a taller stem is favorable for light competition within natural environments but requires both belowground stabilization (against wind and other stem bending forces) and resource support for the following spring (Cannell and Willett 1976).

We detected few significant changes to leaf physiology in trees that underwent lammas (specifically within pre-existing canopy leaves, not leaves produced by lammas growth). Within the Fagaceae, there is evidence that some qualities in leaves produced by lammas growth may differ from pre-existing canopy leaves, such as sucrose content in *Quercus* (Alaoui-Sossé et al. 1996) and general photosynthetic output in *Fagus* (Taylor and Dobson 1989). In *P. trichocarpa*, there was a small increase in overall photosynthetic rates among trees undergoing lammas growth (despite relatively lower levels of leaf chlorophyll) and a change in water-use efficiency, but in general, physiological trait differences were not significant. While this may

be partly related to our reduced sample size, it is also likely that the physiological shift observed following bud set (see above) remains largely unchanged within the existing canopy even if lammas occurs.

Conclusions

While both bud set and lammas produced predictable patterns in stem growth and total biomass accumulation, root:shoot biomass ratios were specifically modified by timing of bud set (altering root mass growth) and/or occurrence of lammas (altering stem growth). We suggest that the activities of enhanced photosynthesis and root biomass accumulation following the onset of bud set (and into the post-bud set phase) are a coordinated mechanism for carbon gain. In the case of an early bud set date (due to either photoperiodic mismatch or other phenomena; e.g., E1 trees), we consider that extra carbon might support growth under later, more favorable conditions, whereas in the case of an extended summer (e.g., L1 trees), additional resources would be accumulated for future events. In addition, we propose that lammas phenology specifically moderates the negative effects of premature bud set by adding to plant height growth but does not supersede the general mechanism of increasing belowground biomass or photosynthesis in leaves during the phase induced following bud set.

Compliance with ethical standards

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

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